

# The Elucidation of Hydrogen Sulfide Signalling Through Persulfidation

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### THE THESIS PRESENTED IS TO OBTAIN THE QUALIFICATION OF

# DOCTOR OF THE UNIVERSITY OF BORDEAUX

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Specialisation: Biochemistry

# The Elucidation of Hydrogen Sulfide

# Signalling Through Persulfidation

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Date Defended: 17<sup>th</sup> December 2019

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# ABSTRACT

#### L'Élucidation de la Signalisation du Sulfure d'Hydrogène par Persulfidation

Le sulfure d'hydrogène (H<sub>2</sub>S), auparavant considéré comme un gaz toxique, est aujourd'hui reconnu comme gazotransmetteur. De nombreuses études ont révélé le rôle de l' H<sub>2</sub>S en tant que molécule de signalisation redox contrôlant d'importantes fonctions physiologiques et pathologiques. Le mécanisme sous-jacent proposé pour expliquer ses effets est la persulfidation (R-SSH, aussi connue sous le nom de S-sulfhydration), une modification post-traductionnelle oxydative des thiols de résidus cystéines. La persulfidation des protéines est restée sous-étudiée en raison de son instabilité et de sa réactivité chimique similaire à celle d'autres modifications de la cystéine, faisant d'elle une modification très difficile à marquer sélectivement. De là, nous avons développé une nouvelle méthode chimiosélective en deux étapes, aisément adaptable à des applications diverses, pour la détection et le marquage des protéines persulfidées, connue sous le nom de méthode "Dimedone-switch". Nous avons confirmé la cinétique et la sélectivité de la méthode, tout en montrant que la persulfidation des protéines est une modification et aussi contrôlée par l'H<sub>2</sub>S produit dans les voies de transsulfuration et de catabolisme de la cystéine. Nous avons adapté la méthode à une détection directe sur gel à différents organismes-modèles, à la microscopie à fluorescence, à une approche de antibody microarray et à l'analyse protéomique par spectrométrie de masse.

Par la suite, nous avons étudié le rôle de l' H<sub>2</sub>S dans la signalisation redox via la persulfidation. Pour cela, nous avons étudié l'interconnexion entre R-SSH et les modifications séquentielles des thiols de cystéines, à savoir la sulfenylation (R-SOH), la sulfinylation (R-SO<sub>2</sub>H) et la sulfonylation (R-SO<sub>3</sub>H), formées lors de l'exposition au stress oxydatif (espèces réactives à l'oxygène). Nos études ont montré une corrélation directe entre R-SSH et ces modifications de manière temporelle et dose-dépendante. Nous avons observé un net décalage de phase dans la réponse entre les deux modifications de cystéines, R-SSH et R-SOH, qui mettent en évidence la présence de "vagues de protection" par la persulfidation des protéines. Couplés à des études mécanistiques montrant la réduction efficace de R-SSH par le système thiorédoxine, ces résultats suggèrent que la persulfidation des protéines est la voie principale par laquelle les acides sulféniques sont reconvertis en thiols originaux, et donc éliminés lors du stress oxydatif. A ce titre, nous avons proposé un mécanisme général (potentiel vestige des temps anciens où la vie a émergé et proliféré dans un environnement riche en H<sub>2</sub>S) dans lequel la persulfidation figure une boucle de sauvetage face à l'hyper-oxydation des cystéines et au dommage cellulaire oxydatif subséquent.

De plus, dans le but de faire la lumière sur l'intérêt biologique de cette protection naturelle des persulfides, nous avons exploré une possible corrélation entre les niveaux de persulfides et le vieillissement. En nous appuyant sur la capacité des persulfides à piéger les oxydants qui s'accumulent, nous avons mené une série d'études visant à obtenir une meilleure compréhension du rôle de la voie de transsulfuration dans la résistance au stress et sur la durée de vie. Nous avons observé une corrélation directe entre la capacité à produire des persulfides et la résistance au stress oxydant, ainsi qu'une diminution de la persulfidation au cours du vieillissement chez *C. elegans*, le rat et les cellules humaines.

**Mots clés**: Sulfure d'hydrogène, Persulfidation (*S*-sulfhydration), *S*-Sulfenylation, Oxydation de thiol, Modification post-traductionnelle oxidative, Signalisation Redox, Cystéine, Protéomique.

#### The Elucidation of Hydrogen Sulfide Signalling through Persulfidation

Hydrogen sulfide ( $H_2S$ ), originally considered a toxic gas, is now a recognised gasotransmitter. Numerous studies have revealed the role of  $H_2S$  as a redox signalling molecule that controls important physiological/pathophysiological functions. The underlying mechanism postulated to serve as an explanation of these effects is protein persulfidation (R-SSH, also known as S-sulfhydration), an oxidative posttranslational modification of cysteine thiols. Protein persulfidation has remained understudied due to its instability and chemical reactivity similar to other cysteine modifications, making it very difficult to selectively label. Herein, we developed a novel, versatile, two-step chemoselective method for the detection and labelling of protein persulfides, called the Dimedoneswitch method. We confirmed the method's kinetics and selectivity and showed that protein persulfidation is an evolutionarily conserved modification controlled by  $H_2S$  generated by transsulfuration pathway and cysteine catabolism. We adapted the method for direct in-gel detection in different model organisms, fluorescence microscopy, antibody microarray approach and proteomic analysis by mass spectrometry.

Next, we studied the role of  $H_2S$  in redox signalling through persulfidation. To do this we investigated the interconnection between R-SSH and the sequential modifications of cysteine thiols, sulfenylation (R-SOH), sulfinylation (R-SO<sub>2</sub>H) and sulfonylation (R-SO<sub>3</sub>H), formed when exposed to oxidative stress (reactive oxygen species). Our studies showed a direct correlation between R-SSH and these modifications in a time- and dose- dependent manner. We observed a clear phase shifted response between the two cysteine modifications, R-SSH and R-SOH, revealing the presence of 'protective waves' of protein persulfidation. Coupled with mechanistic studies showing the efficient reduction of R-SSH by the thioredoxin system, these results suggest that protein persulfidation is the main pathway by which sulfenic acids are resolved under oxidative stress. As such, we proposed a general mechanism (potentially an evolutionary remnant of the times when life emerged and flourished in a  $H_2S$  environment) in which persulfidation represents a rescue loop from cysteine overoxidation and subsequent oxidative cellular damage.

Furthermore, in order to shed light on the biological relevance of this protective nature of persulfides, we explored a possible correlation between persulfide levels and aging. This was explored through a range of studies, from the persulfide's chemical ability to scavenge the build-up of oxidants, to gaining a better understanding of the role of transsulfuration pathway in stress resistance and lifespan. We observed a direct correlation between ability to make persulfides and oxidative stress resistance, and a decrease in persulfidation with aging, in *C. elegans*, rats and human cells.

**Keywords**: Hydrogen sulfide, Persulfidation (*S*-sulfhydration), *S*-Sulfenylation, Thiol oxidation, Oxidative posttranslational modification, Redox Signalling, Cysteine, Proteomics.

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# LIST OF ABBREVIATIONS

	Name
=	3-Mercaptopyruvate
=	Alanine aminotransferase
=	S-adenosylmethionine
=	Aminooxyacetic acid
=	Antioxidant response element
=	Activating transcription factor 4
=	Adenosine triphosphate
=	Aquaporin
=	$\beta$ -cyano-L-alanine
=	N-(6-(Biotinamido)hexyl)-3'-(2'-pyridyldithio)-propionamide
=	Bovine serum albumin
=	Benzo[c][1,2]thiazine-based sulfenic acid probe
=	Caenorhabditis elegans
=	Calcium ions
=	Cysteine aminotransferase
=	Cystathionine $\beta$ -synthase
=	Carbon monoxide
=	Coenzyme Q
=	Caloric Restriction
=	Cystathionine $\gamma$ -lyase
=	Copper(I)-catalysed azide-alkyne cycloaddition
=	Cysteine
=	Cystine
=	Cysteine persulfide
=	Cytochrome c
=	Diallyl disulfide
=	D-amino acid oxidase
=	Diallyl sulfide
=	Diallyl trisulfide
=	5,5-Dimethyl-1,3-cyclohexadione
=	Parkinson's disease protein 7
=	N,N-dimethyl-p-phenylenediamine
=	Deoxyribonucleic acid
=	Dietary Restriction
=	Bacterial thiol disulfide oxidoreductase A
=	1,4-Dithiothreitol
=	Escherichia coli
=	Electron donating group
=	Epidermal Growth Factor
=	Endothelial nitric oxide synthase
=	Endoplasmic Reticulum

ETC	=	Electron Transport Chain
ETHE1, PDO	=	Persulfide dioxygenase
EWG	=	Electron withdrawing group
FAD	=	Flavin adenine dinucleotide
FDNB	=	1-Fluoro-2,4-dinitrobenzene
GAPDH	=	Glyceraldehyde 3-phosphate dehydrogenase
GC	=	Gas chromatography
GPx	=	Glutathione peroxidase
GR	=	Glutathione reductase
Grx	=	Glutaredoxin
GSH (GSSH)	=	Glutathione (persulfide)
GSSG	=	Oxidised glutathione
GYY4137	=	Morpholin-4-ium 4-methoxyphenyl(morpholino) phosphine-dithioate
$H_2O_2$	=	Hydrogen Peroxide
H <sub>2</sub> S	=	Hydrogen sulfide
Hcy (-SSH)	=	Homocysteine (persulfide)
HNO	=	Nitroxyl
HS⁻	=	Hydrosulfide anion
HS•	=	Sulfanyl
HSA	=	Human serum albumin
Htt	=	Huntingtin Protein
I/R	=	Ischemia/reperfusion
IAA	=	Iodoacetamide
K <sup>+</sup>	=	Potassium ions
K <sub>ATP</sub>	=	ATP-dependent potassium channel
Keap1	=	Kelch-like ECH-associated protein 1
LMW	=	Low molecular weight
MB+	=	Methylene blue
MBB	=	Monobromobimane
Me <sub>2</sub> S	=	Dimethylsulfide
MEF	=	Mouse embryonic fibroblasts
MeSH	=	Methanethiol
MMP-7	=	Matrilysin
MMTS	=	S-Methylmethanwthiosulfonate
MnSOD	=	Manganese superoxide dismutase
MS	=	Mass spectrometry
MSBT	=	Methylsulfonyl benzothiazole
Msr	=	Methionine Sulfoxide Reductase
MST, MPST	=	3-Mercaptopyruvate sulfurtransferase
Na <sub>2</sub> S	=	Sodium sulfide
NAC	=	<i>N</i> -Acetylcysteine
NADH	=	Nicotinamide adenine dinucleotide
NADPH	=	Nicotinamide adenine dinucleotide phosphate
NaSH	=	Sodium hydrogen sulfide
NBD-Cl	=	4-chloro-7-nitrobenzo-2-oxa-1,3-diazole

NEM	=	N-ethylmaleimide
NF-κB	=	Nuclear factor κΒ
nmc-PSSH	=	N-Methoxycarbonyl penicillamine persulfide
NMDA	=	N-Methyl-D-aspartate
NO	=	Nitric oxide
NO•	=	Nitric oxide radical
Nrf2	=	Nuclear factor erythroid-derived 2-like 2
NSAID	=	Non-steroidal anti-inflammatory drug
O <sub>2</sub> •-	=	Superoxide
OhrR	=	Organic hydroperoxide resistance transcriptional regulator
ONOO-	=	Peroxynitrite
Orp1	=	Oxysterol-binding protein-related protein 1
oxPTM	=	Oxidative posttranslational modification
OxyR	=	Hydrogen peroxide-inducible genes activator
PD	=	Parkinson's Disease
PDGF	=	Platelet-derived growth factor
PERK	=	Phosphorylated extracellular signal-related kinase
PG, PAG	=	L-Propargylglycine
PIP <sub>2</sub>	=	Phosphatidylinositol-4,5-bisphosphate
PLP	=	Pyridoxal 5'-phosphate
PRD	=	Piperidine-2,4-dione- based sulfenic acid probe
Prx	=	Peroxiredoxin
PTEN	=	Phosphatase and tensin homolog
PTP	=	Protein tyrosine phosphatase
PYD	=	Pyrrolidine-2,4-dione- based sulfenic acid probe
QTRP	=	Quantitative thiol reactivity profiling
R-C(O)SS-R'	=	Perthiol-group
R-N₃	=	Azide group
R-NO <sub>2</sub>	=	Nitro group
R-(S) <sub>4</sub> -R'	=	Tetrasulfide group
R-SeOH	=	Selenic Acid
R-SG	=	S-Glutathionylation
$R_2S=S$	=	Thiosulfoxide
R-SH	=	Thiol
R-SNO	=	S-Nitrosothiol
$R-SO_2H$ , $R-SO_2^-$	=	Sulfinic Acid
R-SO₃H, R-SO₃⁻	=	Sulfonic Acid
R-SOH	=	Sulfenic Acid
R-SSH, R-SS⁻	=	Persulfide
R-SSO₂H	=	Perthiosulfinic acid
R-SSO₃H	=	Perthiosulfonic acid
R-SSOH	=	Perthiosulfenic acid
RAGE	=	Receptor of advanced glycation end products
Redox	=	Reduction oxidation
Rho, TST	=	Rhodanese

RNA	=	Ribonucleic acid
RNS	=	Reactive nitrogen species
ROS	=	Reactive oxygen species
RS•	=	Thiyl Radical
$RS_n$ or $HS_n$	=	Polysulfides
RSS	=	Reactive sulfur species
RTK	=	Receptor tyrosine kinase
S <sup>2-</sup>	=	Sulfide anion
$S_2O_3^{2-}$	=	Thiosulfate
S. cerevisiae	=	Saccharomyces cerevisiae
SATO	=	S-Aroylthiooxime
SCA3	=	Spinocerebellar ataxia 3
SHP2	=	Src homology region 2 domain-containing phosphatase-2
SNL	=	Sulfinic acid Nitroso Ligation
$S_n O_n^{2-}$	=	Polythionates
SO3 <sup>2-</sup>	=	Sulfite
SO4 <sup>2-</sup>	=	Sulfate
SOD	=	Superoxide dismutase
Sp1	=	Specificity protein 1
SQR	=	Sulfide quinone oxidoreductase
Srx	=	Sulfiredoxin
TCA	=	Tricarboxylic acid
TCEP	=	Tris(2-carboxyethyl)phosphine hydrochloride
TD	=	Thiazolidin-4-one1,1-dioxide sulfenic acid probe
TGF-β	=	Transforming growth factor $\beta$
TMT	=	Thiol S-methyltransferase
ТР	=	Transsulfuration Pathway
TPP <sup>+</sup>	=	Triphenylphosphonium cation
TRP	=	Transient receptor potential
Trx	=	Thioredoxin
TrxR	=	Thioredoxin Reductase
VEGFR	=	Vascular endothelial growth factor receptor
ZF	=	Zinc finger

**CHAPTER 1: Introduction** 

### 1.1 General Properties of Hydrogen Sulfide (H<sub>2</sub>S)

Hydrogen sulfide (H<sub>2</sub>S) is a small colourless gas that has sparked large controversy over the past decade. Before the discovery that eukaryotes synthesise H<sub>2</sub>S and the recognition that it has a physiological purpose, for hundreds of years, H<sub>2</sub>S was viewed solely as a toxic gas released into the atmosphere by volcanic eruptions and utilised by bacteria and microbes. However, it was H<sub>2</sub>S that was used to synthesise the building blocks of life such as RNA, lipids and nucleic acids and early life forms thrived in an  $H_2S$ -rich environment for hundreds of millions of years.<sup>1</sup> The recognition of the physiological importance of H<sub>2</sub>S started to evolve from the first report in 1996, by Abe and Kimura, which identified that H<sub>2</sub>S is a neurological modulator in the brain.<sup>2</sup> This initiated a wave of research demonstrating a wide range of biological roles and effects of H<sub>2</sub>S, such as: a smooth muscle relaxation, regulation of inflammation, protection against myocardial ischemic damage, induction of a suspended-like animation state in mice among others.<sup>2-9</sup> H<sub>2</sub>S has been recognised as a member of the group of molecule endogenously-produced small signalling known agents as 'gasotransmitters', alongside nitric oxide (NO) and carbon monoxide (CO),<sup>10</sup> with a growing body of evidence linking it to various signalling pathways.<sup>8,11</sup> In spite of this growth in interest in H<sub>2</sub>S and its biological effects in recent years, the ways in which this gasotransmitter relays its signal to control all those physiological processes is not yet well understood.

#### 1.1.1 Physiochemical Properties of H<sub>2</sub>S

 $H_2S$  is a toxic and flammable gas, with a characteristic smell of rotten eggs. It is a water-soluble gas, which remains in equilibrium with its gas phase when dissolved in a solvent. It is soluble up to 120 mM at 20°C and 80 mM at 37°C in water, and 600 mM at 20°C in absolute ethanol. Consequently, its high solubility, coupled with its inability to form hydrogen bonds and its slightly hydrophobic nature allows it to freely permeate across biological membranes and act as a paracrine-signalling molecule. It has been suggested, however, that membranes may partially impede the diffusion of

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 $H_2S$ , resulting in local aggregation in compartments where it is produced.<sup>12</sup>  $H_2S$  is a weak acid and ionises instantly in aqueous solution existing in fast equilibrium (dependent on pH) between hydrogen sulfide, hydrosulfide anion and sulfide anion ( $H_2S/HS^-/S^{2-}$ ) species.

$$H_2 S \rightleftharpoons H^+ + HS^-$$
(1)  
$$HS^- \rightleftharpoons 2H^+ + S^{2-}$$
(2)

In aqueous solutions, its  $pK_{a1}$  is 6.9 and  $pK_{a2} \ge 17$ , suggesting that, at physiological pH and at 37 °C, it primarily exists (81%) in its anionic deprotonated form, HS<sup>-</sup>, with negligible amounts of S<sup>2-.8</sup> Sulfur has six valence electrons and an empty 3d orbital allowing it to exist in a range of oxidation states (-2 to +6). It is known that H<sub>2</sub>S is a reducing agent, with a standard two-electron reduction potential of -0.23 V at pH 7.0, similar to that of cysteine (Cys) and glutathione (GSH) redox couples.<sup>8</sup> Even though it is thermodynamically unfavourable for H<sub>2</sub>S and HS<sup>-</sup> to react with O<sub>2</sub>, under aerobic conditions H<sub>2</sub>S solutions have a tendency for autoxidation, similar to solutions of other thiols (R-SH, such as Cys or GSH).<sup>13</sup> This reaction is most likely facilitated by the traces of metal ions which could act as catalysts leading to the formation of a range of sulfur species, sulfite (SO<sub>3</sub><sup>2-</sup>), sulfate (SO<sub>4</sub><sup>2-</sup>), thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>), polythionates (S<sub>n</sub>O<sub>n</sub><sup>2-</sup>, n≥2), and polysulfides (S<sub>n</sub><sup>2-</sup>, n≥2), and other oxidised mixed polysulfide species.<sup>8</sup> The term 'H<sub>2</sub>S' in this thesis is used to denote the gas and the mixture of species (H<sub>2</sub>S and HS<sup>-</sup>) in aqueous solution, unless otherwise specified.

The chemical reactivity of H<sub>2</sub>S in biological systems, through which it has been suggested that it can relay its signalling properties, can be classified into three types of reactions (Fig.1): a) binding to metal centres of proteins, reducing them or allowing for catalysis in sulfide oxidation chemistry; b) cross-talk with or scavenging of reactive oxygen species (ROS)/reactive nitrogen species (RNS) which can lead to the formation of other signalling molecules; and c) the oxidative posttranslational modification (oxPTM) of cysteines, called persulfidation (RSSH; also known as *S*-sulfhydration), which is described in further detail in Section 1.5.



Figure 1 - Reactivity of H<sub>2</sub>S in biological systems.

#### 1.1.2 Inorganic Sources and Donors of $H_2S$

 $H_2S$ -releasing compounds (i.e. donors) have become increasingly important in the understanding of the biological mechanisms and functions of  $H_2S$ . In order to further study the physiological importance of  $H_2S$ , donors with variable triggers and rates of release have become essential. However, due to the wide range of available donors, from inorganic to synthetic, with very different releasing mechanisms, conflicting results arise in the literature. This is partly due to the different releasing capabilities of each donor used and the uncontrolled by-products, with unclear biological effects, which may lead to disparate results. As such, this section provides a brief overview on the types of  $H_2S$ -releasing agents available and their possible limitations.

#### 1.1.2.1 – Sulfide Salts

The most common types of H<sub>2</sub>S donors used in biological studies are sulfide salts, such as sodium hydrogen sulfide (NaSH) and sodium sulfide (Na<sub>2</sub>S). These salts are usually used in their hydrated forms (NaSH•xH<sub>2</sub>O or Na<sub>2</sub>S•xH<sub>2</sub>O) or anhydrous Na<sub>2</sub>S. They have been employed over the past decade as H<sub>2</sub>S equivalents toward the understanding of the signalling/physiological roles of H<sub>2</sub>S and used to investigate the therapeutic potential of exogenous H<sub>2</sub>S delivery.<sup>14</sup>

Sulfide salts hydrolyse instantly to give  $H_2S$ ; therefore, they cannot be considered as donors of  $H_2S$  inasmuch as a source of  $H_2S$ . It is important to note, moreover, that the use of these salts carries certain caveats. For instance, the question of the purity of the sulfide salt requires caution, considering that impurities such as

elemental sulfur, polysulfides and other oxidation products may be present. In addition, as a result of their highly hygroscopic nature, their degree of hydration is not accurately accounted for, thereby making it difficult to calculate the concentration of H<sub>2</sub>S. Finally, when these salts have been used in the literature to investigate the effects of H<sub>2</sub>S, high doses of salts have been used, causing supraphysiological concentrations of H<sub>2</sub>S.<sup>8,15</sup> Although useful for the proof-of-concept studies, these sources of H<sub>2</sub>S do not reflect the tightly regulated, endogenously produced rates of H<sub>2</sub>S.

#### 1.1.2.2 – Naturally Occurring H<sub>2</sub>S Donors

For hundreds of years garlic has been recognised as a natural product with beneficial effects against different diseases. Studies of the active ingredients in garlic have shown that organosulfur compounds from garlic release H<sub>2</sub>S and generate low molecular weight (LMW) persulfides (Figure 2A).<sup>16–20</sup>



Figure 2 - Naturally occurring H<sub>2</sub>S donors from garlic (A) and mechanism of low molecular weight (LMW) persulfide formation (B). (A) Due to its instability allicin transforms into diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS) and ajoene. (B) When reacting with glutathione (GSH) DADS generate H<sub>2</sub>S and glutathione persulfide (GSSH).

However, recent studies have proposed that sulfane sulfur compounds (such as persulfides, polysulfides etc.), derived from  $H_2S$ , can help to explain the biological effects of  $H_2S$ . Sulfane sulfurs (sometimes abbreviated as S<sup>0</sup>) refer to sulfur atoms covalently bonded to 2 or more sulfur atoms (RS(**S**)<sub>n</sub>SR), or to 1 sulfur atom and an ionisable hydrogen.<sup>8</sup> Additionally, the active principles from garlic are also very electrophilic and could modify Cys residues directly. Therefore, caution should be taken when using these substances as  $H_2S$  donors.<sup>14</sup>

#### 1.1.2.3 – Synthetic H<sub>2</sub>S Donors

The development of novel H<sub>2</sub>S donors is currently a rapidly growing field. As promising biological tools with therapeutic potential, several classes of synthetic H<sub>2</sub>S donors have been published. These donors demonstrate different mechanisms of release and unlike sulfide salts, they exhibit a slow(er) -release of H<sub>2</sub>S, mimicking physiological H<sub>2</sub>S production. In this section, the main H<sub>2</sub>S donors have been classified according to their mechanism of H<sub>2</sub>S release: (i) thiol-triggered release; (ii) hydrolysis-triggered release; (iii) light- or ROS-triggered release; and (iv) bicarbonate-triggered release (Fig. 3).

#### Thiol-triggered H<sub>2</sub>S release

Since free thiols are abundant in the cells, thiol-triggered donors (Fig. 3A) are designed to release H<sub>2</sub>S through thiol exchange, following nucleophilic addition. The first reported class of thiol-activated donors with controllable H<sub>2</sub>S release rates were *N*-(benzoylthio)benzamides, published by Zhao *et al.*.<sup>21</sup> These donors have an *N*-mercapto template (*N*-SH), with an acylated thiol group for enhanced stability (Fig. 3A). The proposed thiol-triggered mechanism of H<sub>2</sub>S release was established in the presence of thiols, such as *N*-acetylcysteine (NAC) and GSH. They also displayed tuneable release rates with respect to structural modifications (electron withdrawing/donating groups, EWG/EDG).<sup>14</sup> These donors have been evaluated in cell studies where they prevented methylglycoxal-induced cell damage and

dysfunction, and in animal models where they displayed cardioprotective effects in myocardial ischemia/reperfusion (I/R) injury.<sup>22,23</sup>

In perthiol-based donors (R-C(O)SS-R'), first reported by Xian *et al.* Fig. 3A,<sup>24</sup> an acyl group was also used as a protecting group but this time for the unstable *S*-SH moiety, enhancing its stability and H<sub>2</sub>S-releasing capacity.<sup>14</sup> Analogous to *N*-SH and *S*-SH donors are dithioperoxyanhydrides donors, reported by Galardon and co-workers (Fig. 3A).<sup>25</sup> However, it is important to note that both types of donors result in the formation of mixed disulfides, which could cause alternative protein modifications and signalling.<sup>8</sup> Nevertheless, penicillamine-perthiols have shown protective effects towards myocardial I/R injury,<sup>24</sup> and dithioperoxyanhydrides a concentration-dependent vasorelaxation of pre-contracted rat aortic rings.<sup>14</sup>

In addition to these donors, tetrasulfide donors (R-(S)<sub>4</sub>-R'), <sup>26,27</sup> arylthioamides, <sup>14,28</sup> and *S*-aroylthiooximes (SATOs)<sup>29</sup> have shown thiol-dependent H<sub>2</sub>S release but have not yet found broad application as experimental tools (Fig. 3A).



Figure 3 – Structures of commonly used H<sub>2</sub>S donors. (A) Structural scaffolds of thiol-triggered H<sub>2</sub>S donors. (B) Hydrolysis-triggered scaffolds and donors. (C) Structures of light- and ROS- triggered H<sub>2</sub>S donors. (D) H<sub>2</sub>S release by bicarbonate-triggered H<sub>2</sub>S donors.

#### Hydrolysis-triggered H<sub>2</sub>S release

Widely used H<sub>2</sub>S donors are 2,4-Bis(4-methoxyphenyl)-1,3,2,4-dithiadiphosphetane-2,4-disulfide derivatives (Lawesson's reagent, Fig 3B), which have been reported to spontaneously hydrolyse in aqueous solutions, releasing H<sub>2</sub>S.<sup>30</sup> Lawesson's reagent has been proven to regulate ion channels, anti-inflammatory effects and to be beneficial in reducing severity of colitis in rats.<sup>31</sup> However, due to the insolubility of this reagent and uncontrolled hydrolysis and release of H<sub>2</sub>S in solution, its applicability is limited. On the contrary, one of the most commonly used commercially available H<sub>2</sub>S donors is a water-soluble derivative of the Lawesson's reagent known as GYY4137 (morpholin-4-ium 4-methoxyphenyl(morpholino) phosphine-dithioate) (Fig 3B).<sup>32</sup> It has been reported that upon *in vitro* hydrolysis, GYY4137 releases H<sub>2</sub>S more slowly than a sulfide salt, and its rate of release is pH- and temperature-dependent (the more acidic the pH or the higher the temperature, the greater the release).<sup>28,33</sup> GYY has been reported to have vasorelaxatory, anti-hypertensive, anti-inflammatory and anticancer properties.<sup>8,34</sup> Analogues of GYY4137 have been developed with different H<sub>2</sub>S release rates and biological applications, such as in I/R injury where the pH of the tissue has been suggested to drop.<sup>35,36</sup> (Fig 3B)

Dithiolethiones represent another class of hydrolysis-triggered H<sub>2</sub>S donors<sup>9,37</sup> (Fig 3B), and the H<sub>2</sub>S releasing moiety is commonly conjugated to pharmacologically active components such as non-steroidal anti-inflammatory drugs (NSAIDs),<sup>9,14</sup> adenosine,<sup>38</sup> or even to triphenylphosphonium cations (TPP<sup>+</sup>) as mitochondrial anchors.<sup>39-41</sup> The mitochondria-targeted analogue, known as AP39, has shown at low nanomolar concentrations, compartmental specificity, an increase in protein persulfide levels,<sup>42</sup> and antioxidative properties suppressing mitochondrial cell death cascades.<sup>41,43</sup>

#### Other classes of H<sub>2</sub>S donors

In addition to those widely used classes of H<sub>2</sub>S-releasing compounds, light-triggered and ROS-triggered H<sub>2</sub>S donors were developed to allow for a more specific triggering mechanism (Fig. 3C).<sup>44–48</sup>

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Finally, thioaminoacids, such as thioglycine and thiovaline, have been reported to react with bicarbonate at mild pH, while simultaneously releasing their respective N-carboxyanhydride amino acid and H<sub>2</sub>S (Fig. 3D).<sup>49</sup>

#### 1.1.3 Methods for $H_2S$ Measurement

Endogenous levels of H<sub>2</sub>S have been investigated over the past decade, with reported values ranging from undetectable to >500  $\mu$ M. Complex biological samples contain labile sulfur compounds that release H<sub>2</sub>S upon certain chemical treatments.<sup>50,51</sup> Furthermore, acidic pH liberates H<sub>2</sub>S from iron sulfur clusters, which constitute the acid-labile sulfur pool, while the addition of reductants, such as 1,4-dithiothreitol (DTT), liberates H<sub>2</sub>S from sulfane sulfur compounds, particularly from persulfides, polysulfides and elemental sulfur. Alkaline conditions also result in H<sub>2</sub>S release from various other sulfur-containing species, particularly thiols and disulfides. All these potential artefacts have contributed to estimates of H<sub>2</sub>S concentrations in biological samples varying by five orders of magnitude.

Before H<sub>2</sub>S became recognised as a physiological mediator, essentially all measurements of H<sub>2</sub>S in blood either failed to detect it, or produced extremely low values, consistent with the fact that H<sub>2</sub>S cannot be detected by its odour from a wound or when a patient has their blood drawn. Since 2000, however, the reported concentrations of H<sub>2</sub>S in blood have risen to an average of ~50  $\mu$ M.<sup>52</sup> In tissues, measurements performed with gas chromatography coupled with chemiluminiscence detection have revealed that basal H<sub>2</sub>S levels are quite low. According to one study, the basal H<sub>2</sub>S level is ~10-15 nM in murine liver and brain.<sup>53</sup> Another study reported levels of 0.004-0.055  $\mu$ moles kg<sup>-1</sup> of H<sub>2</sub>S or 0.03-0.39  $\mu$ moles (kg protein)<sup>-1</sup>, corresponding to 6-80 nM in murine liver, brain, heart, muscle, oesophagus and kidney.<sup>54</sup> In agreement with these low estimates, the steady-state concentration extrapolated from measurements of H<sub>2</sub>S production and consumption rates in murine liver, kidney and brain were calculated to be 12-25 nM.<sup>55</sup> Interestingly, H<sub>2</sub>S levels in the aorta are significantly higher (~1.5  $\mu$ M).<sup>54</sup>

The steady-state concentration of H<sub>2</sub>S is the net result of its formation and decay rates. At the physiologically relevant concentration of 0.1 mM Cys, the H<sub>2</sub>S production rate is 0.484 mmol h<sup>-1</sup> (kg tissue)<sup>-1</sup> (i.e. ~ 12  $\mu$ M min<sup>-1</sup>) in murine liver and ~ 0.025 mmol h<sup>-1</sup> (kg tissue)<sup>-1</sup> (i.e. 0.6  $\mu$ M min<sup>-1</sup>) in murine brain.<sup>55</sup> The decay rates are high and as expected, they decrease dramatically under hypoxic conditions.<sup>55</sup> The apparent first order rate constant of H<sub>2</sub>S decay in murine liver under aerobic conditions was reported to be 277 min<sup>-1</sup>.<sup>55</sup> Thus, the very low steady-state tissue concentrations are primarily due to high rates of H<sub>2</sub>S oxidation.<sup>55</sup>

#### 1.1.3.1 Methylene Blue Method

The methylene blue technique of H<sub>2</sub>S measurement is based on the formation of the well-known blue phenothiazine dye, methylene blue (MB<sup>+</sup>), detectable at 670 nm. Though this method is widely used, it suffers from a number of drawbacks, including low sensitivity, lack of specificity to H<sub>2</sub>S and cross-reactivity with other sulfur species.<sup>8</sup> The starting material *N*,*N*-dimethyl-*p*-phenylenediamine (DMPD), reacts with H<sub>2</sub>S and Fe<sup>3+</sup> (e.g. ferric chloride, sodium nitroprusside) in acidic conditions. Zinc chloride is also added to trap volatile H<sub>2</sub>S (Fig. 4A).<sup>56,57</sup> The amount of MB<sup>+</sup> is usually measured spectrophotometrically, chromatographically or using mass spectrometry (MS), and H<sub>2</sub>S concentration is estimated based on the calibration curves.

#### 1.1.3.2 Lead Acetate

Lead acetate is a simpler, semi-quantitative method of  $H_2S$  measurement with low sensitivity. In the presence of  $H_2S$ , black insoluble lead sulfide is formed. This can be determined either by soaking native gels in solutions containing  $H_2S$  donors or densitometrically, on commercially available lead acetate-soaked filter paper.<sup>8</sup>

#### 1.1.3.3 Electrochemical Sensors

Polarographic sensors have been used with higher sensitivity and shorter response rates, allowing real-time monitoring of the  $H_2S$  levels/production. The sensors' limitations lie mainly in practical aspects, such as leakage and the presence of

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impurities.<sup>8</sup> This sensor is comprised of an alkaline potassium ferricyanide solution with an  $H_2S$  permeable membrane.  $H_2S$  diffuses through the membrane, reducing ferricyanide to ferrocyanide. As a result, electrons are donated to the anode, creating a measurable current proportional to the  $H_2S$  present.<sup>58</sup>

#### 1.1.3.4 Gas Chromatography

Gas chromatography (GC) methods have been used in the past with very high sensitivity for H<sub>2</sub>S; however, the drawback of this approach is that specialised and expensive (gastight) equipment is needed. One of the approaches is derivatisation of H<sub>2</sub>S to bis(pentafluorobenzyl)sulfide followed by extraction into an organic phase and analysis by GC. Some GC instruments have highly sensitive sulfur chemiluminescence detectors and could analyse the gas phase samples directly, without derivatisation.<sup>8,50</sup>

#### 1.1.3.5 Monobromobimane

Bromobimanes were originally used as fluorogenic labels for thiols (RSH)<sup>59</sup>, however monobromobimane (MBB) derivatisation was later introduced as another method of measuring H<sub>2</sub>S and for quantifying persulfides and polysulfides.<sup>50,60</sup> The mechanism of detection relies on the nucleophilic attack of H<sub>2</sub>S on MBB to form a bimane-substituted thiol, which in turn can react with another equivalent of MBB to form dibimane sulfide, a fluorogenic molecule that can then be extracted and analysed using reverse-phase HPLC coupled to an MS (Fig. 4B).



**Figure 4** – H<sub>2</sub>S detection methods. (A) Mechanism of methylene blue method. (B) Reaction of monobromobimane and H<sub>2</sub>S.

#### 1.1.3.6 Fluorescent Probes

A range of fluorescent probes have been developed over the years due to the increasing interest in understanding the importance, amounts and distribution of  $H_2S$  in cells, tissue and organs. Different probes have become available, some of which are summarised in Fig 5.<sup>61–63</sup> One approach is  $H_2S$ -mediated reduction of azide (R-N<sub>3</sub>) or nitro (R-NO<sub>2</sub>) groups, attached to a range of different fluorogenic scaffolds such as rhodamine (e.g. MeRho-Az), dansyl or naphthalimide (Fig. 5A). A further improved strategy is the use of a probe with 2 electrophilic centres, which can exploit  $H_2S'$  double nucleophilicity (Fig. 5B). A different strategy is used in copper centred probes attached to a fluorophore, where the affinity of  $H_2S$  to metal centres has been exploited (Fig. 5C).



Figure 5 - Scaffolds and structures of fluorescent H<sub>2</sub>S sensors. (A) H<sub>2</sub>S-mediated reduction of azide (R-N<sub>3</sub>) or nitro (R-NO<sub>2</sub>) groups attached to a range of fluorescent moieties. (B) Fluorescent probes with two fluorogenic centres. (C) Cu<sup>2+</sup>-based sensor.

### 1.2 Enzymatic Biosynthesis and Oxidation of H<sub>2</sub>S

#### 1.2.1 Enzymatic H<sub>2</sub>S Biosynthesis

Despite the growing interest in the biological relevance of H<sub>2</sub>S, its endogenous biosynthetic regulation and production for signalling are not yet well understood. Todate, there are three main enzymes involved in its formation. Two of the enzymes are pyridoxal 5'-phosphate (PLP)-dependent enzymes, cystathionine  $\beta$ -synthase (CBS) and cystathionine  $\gamma$ -lyase (CSE; also known as CTH), linked to the (canonical and reverse) transsulfuration pathway (TP). These enzymes are predominantly located in the cytosol, though their presence in other compartments, such as the nucleus and mitochondria, has also been reported.<sup>64-66</sup> The third enzyme is the PLP-independent, 3-mercaptopyruvate sulfurtransferase (MST; also known as MPST), which is located in the mitochondria and the cytoplasm.<sup>67</sup> The key role of the transsulfuration pathway is the synthesis of cysteine (canonical TP), which in turn results in a wide range of metabolic conversions, some of which lead to the production of H<sub>2</sub>S. It still remains unclear how the cell responds to cellular demands and switches from synthesising cysteine to catabolising cysteine and generating a controlled H<sub>2</sub>S flux. Further understanding of the regulation of H<sub>2</sub>S-producing and -oxidising enzymes may shed light on the biological relevance of  $H_2S$ .

CBS was the first H<sub>2</sub>S-producing enzyme to be identified, and is an enzyme interlinking the methionine cycle and the TP, providing sulfur for the synthesis and catabolism of cysteine and leading to H<sub>2</sub>S production. CBS alone, via a ping-pong mechanism, catalyses a spectrum of reactions through its ability to house substrates such as serine, and homocysteine (Hcy) and Cys following the elimination of H<sub>2</sub>S, shown in Fig. 6, reviewed by Filipovic *et al.*.<sup>8</sup> It has been proposed that its regulation is based on substrate affinity and concentration, such as Cys or Hcy. CBS is a cytosolic homodimer, with a subunit of ~63 KDa, and is both a PLP enzyme and a heme protein. At its N-terminal domain, it houses the PLP cofactor and a regulatory heme cofactor, which has been proposed to sensitise CBS to the binding of metal ions and oxidation.

It also binds an allosteric activator, S-adenosylmethionine (AdoMet; also known as SAM), at its C-terminal side, which causes a major conformational rearrangement activating CBS to substrate binding.<sup>68,69</sup> As such, both of its terminal domains play a role in the catalysis.<sup>8,70</sup> Moreover, the two terminal domains intercommunicate, as the binding of AdoMet makes CBS more susceptible to the binding of exogenous ligands, nitric oxide radical (NO•) and CO, which in turn have an inhibitory effect on its activity.<sup>8</sup> This inactivation by ligands has been suggested to switch the transsulfuration pathway from its canonical path of forming cysteine, to its reverse path, catabolising cysteine and generating H<sub>2</sub>S.<sup>71</sup>



Figure 6 - H<sub>2</sub>S biosynthesis and oxidation pathways. The abbreviated enzymes involved in H<sub>2</sub>S production are: CBS = cystathionine β-synthase; CSE = cystathionine γ-lyase; MST = 3 mercaptopyruvate sulfurtransferase; CAT = cysteine aminotransferase; DAO = D-amino acid oxidase.
Enzymes involved in H<sub>2</sub>S oxidation are, SQR = sulfide quinone oxidoreductase; Rho = Rhodanese;
ETHE1 = persulfide dioxygenase; SO = sulfite oxidase; TMT = thiol S-methyltransferase.

Finally, it has also been reported that the activity of CBS can be modulated by hormones (insulin and testosterone), the transcription factor cAMP and covalent SUMOylation, modifications, such S-glutathionylation as (R-SG) and phosphorylation.<sup>70</sup> For instance, SUMOylation of CBS has been reported to cause an inactivation of the enzyme and its translocation to the nucleus.<sup>64</sup> On the other hand, both glutathionylation (under oxidative stress conditions) and phosphorylation have been shown to activate it to produce more H<sub>2</sub>S.<sup>72,73</sup> CBS expression has been detected in different types of systems, such as the cardiovascular and respiratory, gastrointestinal tract, kidneys, liver, lymphocytes, uterus, plasma and pancreas islets, and has been suggested to be the determining H<sub>2</sub>S generator in the central nervous system.74

CSE is a tetramer with a 45 KDa subunit, which also houses a PLP cofactor. It catalyses an array of reactions within the transsulfuration pathway, some of which result in the production of H<sub>2</sub>S (Fig. 6). The possible regulation of its activity stems from its ability to accommodate different substrates competing for the same binding pocket, such as cystathionine, Hcy and Cys.<sup>8</sup> It has been reported that the concentration of Hcy can modulate H<sub>2</sub>S formation of CSE-catalysed reactions. In addition, CSE catalyses the synthesis of cysteine and homocysteine persulfides (Cys-SSH and Hcy-SSH) from their respective disulfide analogues.<sup>8</sup>

CSE has been proposed to be one of the major H<sub>2</sub>S-producing enzymes; however, very little is known about its regulation. One possible site of regulation is at its two CXXC motifs, with a possible redox-sensitive allosteric regulation.<sup>8</sup> CSE responds to Endoplasmic reticulum (ER) stress, with ER stressors inducing an increase in CSE expression levels (and subsequently H<sub>2</sub>S) and upregulating the activating transcription factor 4 (ATF4).<sup>75</sup> Furthermore, CSE has been suggested to be inactivated by phosphorylation,<sup>76</sup> may be activated by increased concentrations of calcium/calmodulin<sup>6</sup> and possibly modified by SUMOylation<sup>77</sup> however, the physiological relevance of these regulations is unknown. CSE is expressed in a range of different mammalian tissues, and has been reported to be the main H<sub>2</sub>S-producing enzyme in the kidneys, liver, uterus, pancreatic islet cells, and largely expressed in the cardiovascular and respiratory systems.<sup>74,78</sup>

The third H<sub>2</sub>S-producing enzyme is MST, predominantly located in the mitochondria, but also found in the cytoplasm.<sup>67</sup> In the Cys catabolism pathway, L-Cys is initially converted to 3-mercaptopyruvate (3-MP) by the PLP-dependent cysteine/alanine aminotransferase (CAT or AAT, respectively; Fig. 6).<sup>8</sup> 3-MP then serves as a substrate for MST, catalysing its conversion to pyruvate and forming a persulfidated form of MST. Persulfidated MST is then reduced in the presence of a reductant, such as a LMW thiol or thioredoxin (Trx), simultaneously eliminating H<sub>2</sub>S.<sup>79,80</sup> An alternative route for the formation of 3-MP has been reported, which includes the oxidation of D-cysteine, catalysed by D-amino acid oxidase (DAO).<sup>81</sup> The regulation of the synthesis of H<sub>2</sub>S via the CAT/MST or DAO pathways is not understood, except for the possible inhibition of CAT by calcium.<sup>8</sup> MST alone appears to be redox-regulated, as three redox-sensitive cysteines (Cys154, Cys248 and Cys263) have been reported in its structure, and the activity of MST seems to be decreased under oxidative stress.<sup>82</sup> Similar to CBS and CSE, MST is expressed in the heart, liver, lung, brain, while the kidneys seem to have a higher activity of the enzyme.<sup>74</sup>

#### 1.2.2 Enzymatic H<sub>2</sub>S Oxidation

The accumulation of H<sub>2</sub>S would be toxic to organs (through its inhibition of Complex IV in the mitochondria); therefore, there are tightly regulated oxidation processes in place for its catabolism. The most efficient known mechanism of H<sub>2</sub>S oxidation within mammalian cells takes place in the mitochondria, where H<sub>2</sub>S is oxidised to S<sub>2</sub>O<sub>3</sub><sup>2-</sup> or SO<sub>4</sub><sup>2-</sup> (Fig. 6). The first step in this oxidation pathway involves the oxidation of H<sub>2</sub>S by sulfide quinone oxidoreductase (SQR) to either an LMW persulfide, glutathione persulfide (GSSH) or S<sub>2</sub>O<sub>3</sub><sup>2-</sup>. SQR is a membrane-bound protein expressed in the mitochondrial matrix, with two important redox centres, an active site trisulfide and a flavin adenine dinucleotide (FAD) cofactor (Fig. 6), through which it can use this oxidation to funnel electrons to coenzyme Q (CoQ) and hence, the electron transport

chain (ETC).<sup>83,84</sup> The formed GSSH can be further oxidised to  $S_2O_3^{2-}$  by the sulfurtransferase, Rhodanese (Rho; also known as TST), or to  $SO_3^{2-}$  by the mitochondrial matrix protein, persulfide dioxygenase (ETHE1; also known as PDO).  $SO_3^{2-}$  can then be quickly oxidised to  $SO_4^{2-}$  by sulfite oxidase (SO) found in the mitochondrial intermembrane space (Fig. 6).<sup>8</sup> The majority of H<sub>2</sub>S is finally excreted as  $SO_4^{2-}$ , or is further metabolised through the urine.

Another mechanism for the catabolism of  $H_2S$  that remains largely understudied is its methylation, which takes place in the cytosol. This reaction is catalysed by thiol *S*-methyltransferase (TMT) and sulfur from  $H_2S$  is incorporated into organic compounds methanethiol (MeSH) and dimethylsulfide (Me<sub>2</sub>S) (Fig. 6).<sup>10,85</sup>

#### 1.2.3 Synthetic Inhibitors of H<sub>2</sub>S Biogenesis

The development of selective inhibitors of H<sub>2</sub>S-producing enzymes has been limited; however, a vast amount of more generalised H<sub>2</sub>S inhibitors displaying only partial selectivity have been reported. A common strategy for the inhibition of H<sub>2</sub>S synthesis is the use of PLP-binding site inhibitors, i.e. aminooxyacetic acid (AOAA – originally used as a CBS inhibitor, Fig. 7) and hydroxylamine.<sup>2</sup> These types of inhibitors are commonly used as more generalised H<sub>2</sub>S inhibitors, as they have demonstrated a lack of selectivity between CBS and CSE.<sup>8,86</sup> Other CBS inhibitors identified through different high-throughput screenings are tangeritin, 1,4-napthaquinone, flavinoids and benserazide.<sup>87</sup>

A commonly employed CSE inhibitor is L-propargylglycine (PG; also known as PAG, Fig. 7), originally developed for irreversible inhibition at the active site of CSE; however, PG is reported to also have off-target effects with alanine transferase.<sup>88–90</sup> Other CSE targeted inhibitors used are  $\beta$ -cyano-L-alanine (BCA, Fig. 7) and aminoethoxyvinyl glycine.<sup>86,89</sup>

Another commonly used strategy is the indirect inhibition of MST via the use of CAT/AAT inhibitors, such as aspartate or MST substrate mimics, but they display low selectivity towards MST.<sup>91</sup>



Figure 7 - Scaffolds and structures of main synthetic H<sub>2</sub>S inhibitors used. AOAA, aminooxyacetic acid; PG, L-propargylglycine; BCA,  $\beta$ -cyano-L-alanine.

# 1.3 Physiological Effects of H<sub>2</sub>S

Since the discovery that H<sub>2</sub>S controls synaptic plasticity in the brain,<sup>2</sup> the evidence relating to its role as a mediator of physiological processes has grown exponentially, with different, and at times opposing, reported effects in tissues and diseases. Given the on-going development of analytical tools to measure endogenous H<sub>2</sub>S levels and pharmacological donors/inhibitors to mimic its actions, there has been an increased interest in further understanding its physiological and pathological effects. This section briefly summarises the physiological roles of endogenously produced H<sub>2</sub>S and also addresses the pharmacological potential of H<sub>2</sub>S donors (Fig. 8).



Figure 8 - Overview of physiological and pathological effects of H<sub>2</sub>S.

#### 1.3.1 Antioxidant & Cytoprotective Capacity of H<sub>2</sub>S

Oxidative stress is the consequence of an imbalance in the reduction-oxidation (redox) capacity of cells, due to a non-physiological increase in ROS and RNS concentrations. Excessive ROS/RNS can result in molecular and cellular disruption through organelle injury, protein misfolding and DNA damage.<sup>92</sup> H<sub>2</sub>S has been found to improve disease or oxidative stress conditions in various pathological settings.<sup>8,78</sup> Different H<sub>2</sub>S donors have been described as direct scavengers of the cytotoxic oxidant, peroxynitrite (ONOO<sup>-</sup>), in dying neuronal cells.<sup>93</sup> Several studies have also found that pharmacological H<sub>2</sub>S donors display antioxidative (protective) effects in I/R injury in different organs. A relevant example of this is the capacity of H<sub>2</sub>S to scavenge other ROS, such as hydrogen peroxide ( $H_2O_2$ ) and superoxide ( $O_2^{-}$ ),<sup>15</sup> which, in the case of myocardial I/R injury, shows a significant reduction in the extent of infraction following H<sub>2</sub>S treatment.<sup>94</sup> These observed cytoprotective effects of H<sub>2</sub>S are associated with its direct antioxidant effects. Similar protective effects have been reported in kidney I/R injury where H<sub>2</sub>S reduced mortality and inflammation. However, these observations are controversial, given that conflicting evidence has emerged regarding kidney  $I/R.^{95,96}$  In the case of lung diseases, the pharmacological administration of H<sub>2</sub>S (in the form of NaSH) has shown beneficial antioxidant effects. This was investigated in rats with bleomycin-induced pulmonary fibrosis, whereby H<sub>2</sub>S treatment was shown to reduce free radical generation and lipid peroxidation in lung tissue.<sup>97</sup>

H<sub>2</sub>S has additionally been linked to aging, as experimental observations in *Caenorhabditis elegans (C. elegans)* showed that H<sub>2</sub>S treatment increases thermotolerance and longevity.<sup>98</sup> Recently, Hine *et al.* postulated that the endogenous production of H<sub>2</sub>S may be the mechanism driving the benefits behind caloric and dietary restriction (CR and DR, respectively).<sup>99</sup> However, the mechanism by which H<sub>2</sub>S relays these beneficial effects was not shown.

Although the antioxidant activity of  $H_2S$  is widely used as an explanation for the effects of  $H_2S$ ,  $H_2S$  itself is a weak antioxidant. Its rate constants with either peroxynitrite,<sup>100</sup> superoxide<sup>15</sup> or  $H_2O_2$ <sup>15</sup> are not fast enough to compete with other

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thiol pools.<sup>8</sup> Therefore, it remains unclear how  $H_2S$  exhibits all those antioxidant properties reported in the literature.

#### 1.3.2 Signalling Roles in Different Tissues

H<sub>2</sub>S was initially described as an endogenous neuromodulator, selectively increasing *N*-methyl-D-aspartate (NMDA)-mediated processes and, at high levels, inhibiting synaptic transmissions in the hippocampus.<sup>2</sup> A number of ion channels have also been reported to be modulated by H<sub>2</sub>S. In addition, there have also been multiple reports of pro- and anti-nociceptive effects of H<sub>2</sub>S donors in the nervous system.<sup>8</sup> Its mechanisms of pronociceptive effects have been suggested to proceed via transient receptor potential (TRP) channels, in contrast to its reported anti-nociceptive effects linked to ATP-dependent potassium channels (K<sub>ATP</sub>).

Numerous effects of H<sub>2</sub>S in the cardiovascular system have been published in the literature, with CSE being the predominant H<sub>2</sub>S-producing enzyme. H<sub>2</sub>S was originally labelled as a gasotransmitter that regulates blood pressure.<sup>6</sup> Cross-talk between H<sub>2</sub>S and NO signalling pathways have been described in many different settings, specifically, in the regulation of vasorelaxation and angiogenesis.<sup>8,101</sup> The role of H<sub>2</sub>S as a smooth muscle cell relaxant was first reported in the vascular system.<sup>3,6,102</sup> The pharmacological administration of H<sub>2</sub>S has also been shown to cause a decrease in blood pressure and to exert vasodilatory effects. These effects have been associated with the activation of the K<sub>ATP</sub> channel, which has been found to be persulfidated (Section 1.5.3.4), and with the activation of endothelial nitric oxide synthase (eNOS).<sup>8,103-105</sup> Furthermore, it has been reported that the activation of the vascular endothelial growth factor receptor (VEGFR) by VEGF causes an increase in CSE levels, generating H<sub>2</sub>S and resulting in the subsequent activation of eNOS.<sup>106</sup>

#### 1.3.3 Roles in Specific Diseases/Disorders

There is a growing body of literature linking  $H_2S$  to different disease states. Within the brain, neurodegenerative conditions, such as Alzheimer's disease, Huntington's

disease, Parkinson's disease (PD), spinocerebellar ataxia 3 (SCA3) and traumatic brain I/R injury exhibit positive modulation by H<sub>2</sub>S (or persulfidation).<sup>8</sup> Alzheimer's and Huntington's disease patients reported lower levels of endogenous H<sub>2</sub>S in comparison to healthy patients.<sup>107–110</sup> Following the administration of H<sub>2</sub>S (in the form of NaSH), rodent models of Alzheimer's disease showed an improvement in learning and memory.<sup>108</sup> In patients with Huntington's disease, reduced CSE expression was found to be caused by the inhibition of specificity protein 1 (Sp1, transcriptional activator of CSE) by the mutant huntingtin (Htt) protein.<sup>110</sup> Furthermore, the beneficial effects of H<sub>2</sub>S in SCA3 (polyQ repeats in ataxin 3) were tested using a *Drosophila* model overexpressing CSE, where the authors showed reversal of the disease phenotype.<sup>111</sup> The effects of H<sub>2</sub>S in both SCA3 and PD have been linked to the persulfidation of proteins by H<sub>2</sub>S.<sup>8</sup>

 $H_2S$  has also been suggested to act as a physiological mediator of inflammation; however, its precise role is controversial in different settings and organs.<sup>112</sup> Pro-inflammatory effects of  $H_2S$  have been reported in acute pancreatitis, <sup>113,114</sup> lung and neurogenic inflammation, <sup>113</sup> renal I/R injury<sup>96</sup> and sepsis.<sup>115-117</sup> Anti-inflammatory responses of  $H_2S$  have been associated with intestinal ischemic injury, inflammatory bowel diseases, intestinal I/R and different conditions of the gut.<sup>8,112</sup> The pharmacological administration of the slow-releasing  $H_2S$  donor, GYY417, showed anti-inflammatory effects through the inhibition of the transcription factor, nuclear factor κB (NF-κB), in contrast to the biphasic response caused by the addition of NaSH.<sup>118,119</sup> The exact mechanisms by which  $H_2S$  affects inflammation are still unclear.

Types 1 and 2 diabetes have been demonstrated to be affected by H<sub>2</sub>S, as well. Decreased expression levels of CSE were observed in diabetic mouse models,<sup>120</sup> and low H<sub>2</sub>S levels in patients suffering from diabetic neuropathy.<sup>121</sup> Vasoconstriction and reduced blood flow were ameliorated with the administration of NaSH.<sup>120</sup> Additionally, TST has been described as an anti-diabetic target. Its overexpression in adipocytes of mice proved to decrease the likelihood of diet-induced obesity and insulin-resistant diabetes. Moreover, TST-deficient mice showed increased incidence of developing diabetes.<sup>122</sup>

The relationship between  $H_2S$  and cancer remains controversial, with  $H_2S$  exhibiting opposing effects in cancer progression; at low concentrations  $H_2S$  is cytoprotective, but it becomes cytotoxic at high doses<sup>74</sup>.  $H_2S$  was shown to affect cancer cells by interfering with cellular bioenergetics, angiogenesis, apoptosis and intracellular signalling.<sup>74,123,124</sup>

### 1.4 Cysteine-based Redox Signalling

Life is maintained by a limited number of chemical reactions, of which sulfur-centered chemistry is particularly important. The Cys residue can undergo an extensive range of redox modifications that are exploited in multiple cellular processes and, in particular, cell signalling. Redox signalling is a biological response caused by a specific redox oxygen, nitrogen or sulfur (RSS) species. The highly reactive nature of these aforementioned species (oxidants) render them toxic if their levels were to be left unchecked, leading to cellular damage and an array of pathological conditions.<sup>125,126</sup> Over the past decades, increasing evidence has suggested that these reactive species are integrated into the physiology of non-stressed cells. As such, they have been defined as cellular secondary messengers.<sup>8,127–130</sup>

Given the reactive nature of these species, their specificity - a prerequisite for signalling - comes into question. An important mechanism by which a reactive oxidant signal is converted into a biological response is via site-specific, covalent modifications of targeted biological macromolecules. The amino acid cysteine is a commonly known target due to its ideally suited chemical reactivity, such as its sensitivity to oxidation. To that effect, most ROS/N/SS signalling proceed via posttranslational modification of specific Cys residues.<sup>126</sup>

Two general mechanisms have been proposed for the general regulation of redox signalling: a thermodynamic model and direct targetting.<sup>131</sup> The first occurs through the thermodynamic equilibrium of intracellular redox buffers, such as

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GSH/oxidised glutathione (GSSG). The second mechanism entails the direct targeting of Cys residues in proteins, which then serve as molecular switches. The important reversible thiol oxPTMs, used by cells to convey signalling, are *S*-nitrosylation (*S*-nitrosothiols, R-SNO), *S*-glutathyonylation (R-SG), disulfides (R-SS-R), *S*-sulfenylation (sulfenic acids, R-SOH) and persulfidation (persulfides, R-SSH) (Fig. 9). In parallel to these modifications, some important irreversible thiol modifications occur, such as sulfinylation (sulfinic acids, R-SO<sub>2</sub>H) and sulfonylation (sulfonic acids, R-SO<sub>3</sub>H), discussed further below.



Figure 9 - Reversible oxPTMs of Cysteine.

Initially, it is important to understand the reactivity of thiols, as only a fraction of thiols from the entire proteome become oxidised. The generalised rule for the cysteine's reactivity is that the lower the  $pK_a$  and the higher the nucleophilicity of the thiol, the more reactive it is to oxidants. However, several factors influence the thiol's  $pK_a$  and nucleophilicity, such as the local environment of the protein/the microenvironment of the residue, and steric factors for oxidant accessibility.

The pK<sub>a</sub> of free Cys is 8.3, but when placed within the protein microenvironment, this can vary dramatically, through interactions with local residues. An example of this is seen in the 23isulphide oxidoreductase, bacterial thiol 23isulphide oxidoreductase A (DsbA), which carries 2 Cys residues in the same active site with a pK<sub>a</sub> of 3.5 and 10.<sup>132</sup> Another interesting example of this complexity is mirrored in the drastically different rate constants of some active site Cys with similar
pK<sub>a</sub>. Peroxiredoxin-2 (Prx2) has an active site Cys with a pK<sub>a</sub> of 5-6 and a rate constant of 1-2 x 10<sup>7</sup> M<sup>-1</sup> s<sup>-1</sup> for the reaction with H<sub>2</sub>O<sub>2</sub>, while protein tyrosine phosphatase (PTP), PTP1B, with a Cys pK<sub>a</sub> of 5.4, reacts with H<sub>2</sub>O<sub>2</sub> with a rate constant of 10-20 M<sup>-1</sup> s<sup>-1</sup>.<sup>133,134</sup> Hence, this substantial difference in specific reactivity of each protein, coupled with the selective reactivity of the oxidants themselves (H<sub>2</sub>O<sub>2</sub>), feeds into the idea that redox signalling by reactive species is highly target-specific, as opposed to just being a result of an alteration in the redox equilibrium.<sup>131</sup>

#### 1.4.1 ROS Production & Metabolism

The group of ROS encompasses a variety of molecules, such as  $H_2O_2$ ,  $O_2^{\bullet-}$  and hydroxyl radicals (OH). These molecules differ in their reactivity and are therefore differentially explored by the cells as either damaging oxidants or signalling molecules.<sup>125,127,131</sup> The difference in reactivity is reflected by the fact that the non-radical species  $H_2O_2$  engages in 2-electron oxidation reactions, while the radical species,  $O_2^{\bullet-}$  and OH, Serve as 1-electron oxidants. Therefore, they form different products when reacting with thiols.

The species claimed to be involved in cellular signalling are  $H_2O_2$  and upon its dismutation  $O_2^{\bullet}$ . Despite the high production rate,  $O_2^{\bullet}$  is maintained intracellularly at low concentrations. Superoxide is not only intrinsically unstable (undergoes spontaneous dismutation), but it is also efficiently cleared out enzymatically by superoxide dismutases (SOD). By contrast,  $H_2O_2$  is much more stable, shows more selective reactivity and is tightly regulated at nM to low  $\mu$ M steady-state levels by detoxifying enzymes (Fig. 10).<sup>127</sup> Detoxification is achieved by enzymes such as catalase or peroxiredoxins (Prx) and glutathione peroxidases (GPx) (Fig. 10).<sup>135</sup> The latter two recycle back to their active reduced forms by nicotinamide adenine dinucleotide phosphate (NADPH)-dependent Trx/thioredoxin reductase (TrxR) or GSH/glutathione reductase (GR) systems.<sup>126</sup>

Therefore,  $H_2O_2$  has been viewed as the most likely secondary messenger in redox signalling. Unlike  $O_2^{\bullet-}$ ,  $H_2O_2$  is uncharged and can easily diffuse across membranes. However, it has been reported that  $H_2O_2$  can also exploit protein

channels called aquaporins (AQP) as transport facilitators when crossing the plasma membrane.<sup>136–138</sup> This is particularly true for the entry of  $H_2O_2$  into the cell upon the stimulation of receptor tyrosine kinases (RTK) by growth factors.<sup>139–141</sup>



Figure 10 - The fate of H<sub>2</sub>O<sub>2</sub> in cells. H<sub>2</sub>O<sub>2</sub> generated may: (i) react with protein thiols leading to their oxidation; (ii) serve as a substrate for myeloperoxidase to form an even stronger oxidant, HOCI; (iii) be involved in metal-catalysed Fenton reactions to form highly reactive OH\*; or (iv) be reduced to water (by peroxiredoxins and glutathione peroxidase) and oxygen (by catalase).

The localisation of ROS sources within a cell plays an important role in the way the cell responds to oxidants. One main source of ROS, such as  $O_2^{\bullet-}$  is the ETC in the mitochondria (Fig. 11A). More specifically, ROS formation is a consequence of electron leakage from nicotinamide adenine dinucleotide (NADH) or FADH<sub>2</sub> at complexes I and III (NADH-dehydrogenase and ubiquinone-cytochrome *c* reductase, respectively), with complex III serving as the most prominent superoxide generator in the matrix or the intermembrane space.<sup>125,142</sup> Most of the  $O_2^{\bullet-}$  formed in the mitochondria represents a significant source of cellular ROS in the form of H<sub>2</sub>O<sub>2</sub>, given its dismutation to  $O_2$  and H<sub>2</sub>O<sub>2</sub> by the manganese-SOD (MnSOD) (Fig. 11A).<sup>143,144</sup> It has been suggested that 1-2% of O<sub>2</sub> in the mitochondria is converted to  $O_2^{\bullet-}$ .<sup>143,145</sup>

In addition, the adaptor protein called p66shc is recognised as a generator of  $H_2O_2$  (Fig. 11B) and also as an apoptosis inducer, through its induction of oxidative stress.<sup>146</sup> Studies of p66shc knockout mice show lower intracellular ROS levels and oxidative damage. P66shc has also been referred to as a longevity gene, affecting lifespan and aging.<sup>147–149</sup> P66shc increases cellular ROS levels by diverting electrons from ETC at the level of cytochrome *c* (cyt *c*), and reducing  $O_2$  to  $H_2O_2$ , stimulating mitochondrial generation of ROS (Fig. 11B) or down-regulating the synthesis of antioxidant enzymes such as MnSOD or GPx1.<sup>146,150,151</sup>



Figure 11 - Cellular Sources of Reactive Oxygen Species. (A) The electron transport chain (ETC) in the mitochondria. Four protein complexes (I-IV) shuttle electrons from Nicotinamide adenine dinucleotide (NADH) and succinate of the mitochondrial matrix to oxygen reducing it to water. This establishes a proton gradient used by the complex V to generate ATP. Electron leakage at complexes I and III generate superoxide (O<sub>2</sub>••) in the matrix or intermembrane space. (B) The proapoptotic protein p66shc aids in the mitochondrial production of O<sub>2</sub>•• and H<sub>2</sub>O<sub>2</sub>. Stimuli such as, UV irradiation or growth factor deprivation cause p66shc to translocate to the mitochondria where it interacts with complex III, diverting electrons from cytochrome c (cyt c) directly to molecular oxygen. Generated H<sub>2</sub>O<sub>2</sub> can enter the cytoplasm initiating signalling or it can modulate the opening of the mitochondrial permeability transition pore (mPTP), causing mitochondrial swelling and apoptosis. (C)

NOX enzyme complexes assemble at plasma (or intracellular) membranes regulating localised production of ROS. The stimulation of receptors (such as receptor tyrosine kinases, RTK) initiate the recruitment of co-activating proteins and calcium to NOX catalytic centres. Upon enzyme activation, electrons are funnelled from cytoplasmic nicotinamide adenine dinucleotide phosphate (NADPH) to oxygen to produce either O<sub>2</sub><sup>•-</sup> (NOX1-2) or H<sub>2</sub>O<sub>2</sub> (Duox1-2). Subsequently, O<sub>2</sub><sup>•-</sup> is dismutated to H<sub>2</sub>O<sub>2</sub> and molecular oxygen, either spontaneously or by extracellular SOD. The H<sub>2</sub>O<sub>2</sub> diffuses in or is transported through an aquaporin (AQP), modulating protein activity and signalling.

Another important source of ROS is the NOX family of enzymes. NOX proteins are  $O_2^{\bullet-}$  and  $H_2O_2$  producing proteins, working in conjunction with the local SOD and associated to a more controlled and physiologically deliberate ROS release (Fig. 11C). This family of multi-unit complexes consists of membrane-bound NOX1-5 and Duox1 or Duox2, with different tissue distribution and subcellular localisation.<sup>152</sup> A range of stimuli have been identified to modulate NOX proteins and subsequently the production of ROS, such as VEGF, epidermal- and platelet-derived growth factors (EGF and PDGF, respectively), angiotensin II, transforming growth factor  $\beta$  (TGF- $\beta$ ) and cytokines.85,127,152-154 Given their multi-unit structure and depending on the isoform, these complexes require the formation of regulatory membrane (or cytosolic) co-activator subunits, the assembly of specific cofactors (such as FAD or heme), or the association of calcium ions (Ca<sup>2+</sup>).<sup>152</sup> Furthermore, the roles in cell signalling of the different NOX members are largely dependent on their compartmentalisation within the cell. Following their activation and  $O_2^{\bullet-}$  production,  $O_2^{\bullet-}$  is dismutated to  $H_2O_2$ , which subsequently diffuses (or is transported via AQP) into the cytosol (Fig. 11C). The resulting cytosolic H<sub>2</sub>O<sub>2</sub> has been reported to mediate physiological responses through a cascade of events, such as proliferation, differentiation and apoptosis.127,155,156

## 1.4.2 Cysteine SulfE/I/Onylation

Protein thiols' nucleophilicity and propensity for oxidation makes them excellent targets for redox-based modulation of proteins. The modification of protein thiols by  $H_2O_2$  (and NO), creating the starting point for a path by which cells can 'sense' intracellular alterations in the redox balance. This can lead to a cascade of cellular redox responses or to oxidative stress and cellular damage.  $H_2O_2$  has the ability to react directly with cysteine thiols in their thiolate form, via a 2-electron oxidation, forming the first oxPTM, sulfenic acid (by S-sulfenylation). As such, a portion of  $H_2O_2$ -mediated redox signalling is understood to propagate through thiol oxidation, specifically, R-SOH formation. Sulfenic acids can also undergo further irreversible

oxidation upon exposure to excess H<sub>2</sub>O<sub>2</sub>, to R-SO<sub>2</sub>H by S-sulfinylation and, under greater oxidative stress conditions, to R-SO<sub>3</sub>H, by S-sulfonylation (oxyacids) (Fig. 12). The 1<sup>st</sup> oxidation step to R-SOH has been reported to have very diverse second order rate constants spanning between 20-10<sup>7</sup> M<sup>-1</sup> s<sup>-1</sup>, depending on a multitude of properties.<sup>131</sup> The two following oxidation events to the subsequent oxyacids have been observed to have much slower rate constants (0.1-100 M<sup>-1</sup>s<sup>-1</sup>), but, again, are affected by their protein microenvironment.<sup>127</sup>



Figure 12 - Oxidative Posttranslational Modifications of Cysteine Residues Caused by the Reaction with  $H_2O_2$ .

Sulfenic acids, as mentioned above, are the first oxidation products of thiols. Due to their instability and high reactivity, they give rise to multiple important PTMs, such as disulfides, S-glutathione adducts, cyclic sulfenamides, and other oxyacids, as shown in Fig. 12. R-SOHs have been implicated in the modulation of T-cells<sup>157</sup>, as part of the catalytic cycle of important enzymes, such as Prx (Fig. 13A)<sup>158</sup> and NADPH peroxidase and Methionine Sulfoxide Reductases (Msrs, in some cases form the corresponding selenic acid modification, R-SeOH).<sup>154,156,159,160</sup> Growth factor-mediated signalling has also been shown to result in the direct sulfenylation of kinase receptors and their downstream PTPs, such as phosphatase and tensin homolog (PTEN), PTP1B and Src homology region 2 domain-containing phosphatase-2 (SHP2) (Fig. 13B).<sup>85,153</sup> Additionally, it has been shown that the endoplasmic reticulum (ER) stress sensor, IRE-1, is directly sulfenylated upon oxidative stress, causing its inactivation and initiation of the p38/SKN-1 (nuclear factor erythroid 2-related factor 2, Nrf2) antioxidant response, thereby increasing oxidative stress resistance and lifespan in both *C. elegans* and human cells.<sup>161</sup> Furthermore, the importance of sulfenylation-induced modulation of the activity of H<sub>2</sub>O<sub>2</sub>-sensing transcription factors in bacteria (organic hydroperoxide resistance transcriptional regulator, OhrR and hydrogen peroxide-inducible genes activator, OxyR) and in yeast (Prx and Yap1-Gpx3 (Oxysterol-binding protein-related protein 1, Orp1)) has also been extensively investigated and reviewed.<sup>156,160,162</sup>



Figure 13 - Some Examples of Biologically Important Protein Sulfenic Acids (Prx, EGFR, PTPs).
(A) The catalytic cycle of the homodimer 2-Cys Peroxiredoxin (Prx) reduces H<sub>2</sub>O<sub>2</sub>. The first step is the oxidation of its 'peroxidatic' (catalytic) Cys to an R-SOH. This R-SOH forms a disulfide with the resolving Cys on its other subunit that can then be reduced by Trx. R-SOH can also be further oxidised to R-SO<sub>2</sub>H, a modification that can be reversed by sulfiredoxin, Srx. (B) The activation of the Epidermal Growth Factor Receptor (EGFR) by EGF induces the generation of H<sub>2</sub>O<sub>2</sub> through NOX. The generated H<sub>2</sub>O<sub>2</sub> enters the cytoplasm by diffusion or through the aquaporin (AQP) and directly oxidises a specific Cys797 of EGFR's active site enhancing its tyrosine kinase activity. This endogenous H<sub>2</sub>O<sub>2</sub> also oxidised and inhibits protein tyrosine phosphatases (PTP), maintaining the phosphorylation of EGFR. Together this enhances activation of downstream signalling pathways. PTPs oxidised by H<sub>2</sub>O<sub>2</sub> are: PTEN, SHP2 and PTP1B.

Overall, sulfenylation of proteins has been shown to play a key role in immune responses, cell growth, proliferation and apoptosis, growth factor signalling and diseases such as cancer and neurodegeneration.<sup>127,163</sup> This wide range of responses reflects the importance of *S*-sulfenylation as an integral global signalling mechanism, analogous to phosphorylation, consequently propelling advances in the development of selective detection methods of this oxPTM.

Sulfenic acids are unstable species of transient nature, with an estimated halflife in the range of minutes, though some of them can be further stabilised by the protein microenvironment.<sup>164,165</sup> The sulfur of sulfenic acids has a formal oxidation state of 0, allowing it to exhibit both electrophilic and nucleophilic character.

One of the first indirect methods used to detect sulfenic acids was a differential alkylation approach, whereby free thiols are blocked (by *N*-ethylmaleimide, NEM or lodoacetamide, IAA), followed by a sulfenic acid reduction step by arsenite. A subsequent alkylation step is performed on the nascent thiol, with a conjugated blocking agent for detection.<sup>127</sup> However, the selectivity of arsenite as a selective reductant of sulfenic acids came into question. In addition to this, a direct labelling of R-SOH was achieved by an electrophilic blocking reagent, 4-chloro-7-nitrobenzo-2-oxa-1,3-diazole (NBD-CI), but this approach is limited to isolated proteins, given the reagent's reactivity with other nucleophilic groups in a more complex system.<sup>127,166</sup>

The probes that found widespread use are in fact nucleophiles attacking the electrophilic sulfur (Fig. 14). To date, the most common method of sulfenic acid detection is the direct labelling with probes based on a 1,3- carbonyl scaffold, mainly 5,5-dimethyl-1,3-cyclohexadione (dimedone) and its derivatives. These types of scaffolds have been found to be selective for sulfenic acids and, under physiological aqueous conditions, to not react with other Cys modifications, such as thiols and sulfinic acids and other common functional groups. Currently, a library of dimedone derivatives has been developed for different applications (Fig. 14). For example, dimedone itself has been used to label R-SOH in samples and in combination with anti-dimedone antibody (developed by the Carroll group) used for protein microarray detection and immune-blotting (Fig. 14).<sup>167,168</sup> Moreover, the biotinylated derivative

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of dimedone, DCP-Bio1, and clickable analogues, such as DYn-1 and DYn-2 (alkyne derivatives) or DAz-2 (azide derivative) have allowed for *in situ* tagging of sulfenic acids followed by enrichment for proteomic studies or in-gel detection (Fig. 14).<sup>153,169-173</sup> The Carroll group have introduced two workflow approaches for the proteomic quantification of protein sulfenylation:<sup>174,175</sup> a) isotope-coded dimedone or 2-iododimedone (ICDID); and b) isotopically light and heavy derivatives of DAz-2 or DYn-2 (Fig. 14).

This toolset was further diversified with the introduction of Benzo[c][1,2]thiazine-based molecule, BTD, Thiazolidin-4-one1,1-dioxide-based molecules (TD), pyrrolidine-2,4-dione- based molecules (PYD) and piperidine-2,4-dione- based probe (PRD) (Fig. 14). BTD was discovered to be particularly interesting, as it reacted with R-SOH several orders of magnitude faster than dimedone-based probes and proved to be a useful tool for proteomic analysis.<sup>176</sup>



Figure 14 – Methods for Sulfenic Acid Detection.

The next step in cysteine oxidation is the formation of sulfinic acids/sulfinylation (Fig. 15). Until very recently, there was a lack of tools for sulfinic acid labelling and these remained understudied. Sulfinylation has been found to have regulatory functions in a number of proteins, such as Prx<sup>177</sup>, Parkinson's disease protein 7 (DJ-1; also known as PARK7)<sup>178–180</sup>, matrilysin (MMP-7) and nitrile hydratase, alluding to it being a more controlled event, rather than simply a marker of over-oxidation, as previously considered.<sup>127,165</sup> More specifically, the best characterised family of proteins undergoing sulfinylation in their catalytic cycle are Prxs, cysteine-based peroxidases known to clear intracellular oxidants. The over-oxidation of their 'peroxidatic' Cys has been reported to cause its inactivation and, subsequently, aggregation. Moreover, and in addition, a physiological regulator of this over-oxidation has been characterised. Adenosine triphosphate (ATP)-dependent protein, sulfiredoxin (Srx), reduces Cys-SO<sub>2</sub>H of Prxs, restoring their activity; to date, it is the only known sulfinic acid reductase.<sup>181-183</sup>



Figure 15 - Sulfinic Acid Detection Approaches.

The physiological importance of sulfinic acids, as mentioned above, has remained elusive due to the lack of selective detection methods. Until recently, detection relied on the difference in a mass shift in MS; however, this approach is questionable, given that the similar mass change is found for persulfidation, as well.<sup>184</sup> In addition, antibodies recognising sulfinic acids of specific proteins have been developed, but they were not applicable for a more global understanding of protein sulfinylation.<sup>182,185</sup> Over the past few years, the Carroll group have made some advances in the development of chemical probes for the selective labelling of R-SO<sub>2</sub>H. In 2015, the group introduced the Sulfinic acid Nitroso Ligation (SNL) method using an NO-Bio probe, and showed the specificity of this approach in single proteins and whole cell lysates (Fig. 15).<sup>186</sup> More recently, the group reported electrophilic diazene probes with 'clickable' alkyne-derivatives, as well as biotinylated and fluorescent analogues, called DiaAlk, BioDiaAlk and DiaFlu, respectively (Fig. 15).<sup>187</sup> These probes were applied for in-gel, mass spectrometry-based and even proteomic identification of sulfinic acids, allowing for a chemoselective profiling of the *S*-sulfinylome.

Finally, the last step in cysteine thiol hyper-oxidation is sulfonylation, which is an irreversible modification (Fig. 12). As such, sulfonic acids are potential biomarkers of intracellular oxidative stress. Given the scarce availability of detection methods, only a handful of biomolecules have been identified, one of which is Cu,Zn-SOD, implicated in pathogenesis of amyotrophic lateral sclerosis.<sup>188</sup> Redox sensitive protein, DJ-1, undergoes oxidation to a sulfinic<sup>187</sup> and sulfonic acid,<sup>189</sup> particularly at its Cys106 (Fig. 16). Sulfonylation of DJ-1 controls its intracellular localisation and is implicated in protection against neuronal cell death.<sup>180</sup>



**Figure 16 - Mechanism of DJ-1 oxidation.** The antioxidative action of DJ-1 is modulated by its Cys106, which can be oxidised to R-SOH, R-SO<sub>2</sub>H and R-SO<sub>3</sub>H. Given the stability of its R-SO<sub>2</sub>H, it is postulated that this is its active form. Under high oxidative stress C106 hyperoxidation results in protein inactivation.

Currently, the only methods available for the detection of sulfonic acids are the use of specific antibodies for hyperoxidised (sulfonylated) proteins, with work-flows adapted to specific proteins, such as PTPs (Fig. 17B), or the use of poly-arginine-coated nanodiamonds. The latter technique is based on ionic affinity capture of R-SO<sub>3</sub>H peptides, which can then be analysed by MS.<sup>127,190</sup>



**Figure 17** - **Methods of Detection of Oxidised Thiols.** (A) Oxidised Cys residues can be detected directly by mass spectrometry (top) or using specific antibodies developed to recognize oxidised forms of targeted proteins (bottom). (B) Indirect two-step immunochemical technique for detection of Cys hyperoxidation on specific PTPs. Step 1 involves the blocking of free thiols (with NEM). The protein are subsequently digested and peptides enriched by specific antibodies that recognize hyperoxidised PTPs. Step 2 uses a second aliquot of the same original sample which is first reduced and then hyperoxidised with pervanadate to achieve absolute oxidation of all Cys. The extent of endogenous oxidation is established by comparing the ratios of the signal intensities obtained (by LC-MS/MS) in stages 1 and 2.

# 1.5 Protein Persulfidation: Biochemistry & Signalling

In order to explain the plethora of biological effects of H<sub>2</sub>S, researchers have turned their attention towards protein persulfidation.<sup>8,191</sup> Protein persulfidation (also known as *S*-sulfhydration) is an oxPTM of cysteine residue (Fig. 1) and its role has been compared to protein *S*-sulfenylation (responsible for H<sub>2</sub>O<sub>2</sub>–mediated responses) or *S*-nitrosylation (responsible for NO-mediated responses). However, biological targets affected by persulfidation, the mechanism(s) of persulfide formation and the actual physiological outcomes of this modification remain unclear, given the lack of selective tools for their detection (described further in Section 1.6 and Chapter 2).

## 1.5.1 Properties of Persulfides

Papain-, glutathione- and glutathione peroxidase 3- persulfides were among the first protein models prepared.<sup>192,193</sup> Since, protocols have also been developed for the *in situ* preparation of human and bovine serum albumin (HSA and BSA, respectively).<sup>194</sup> In addition, the synthesis of LMW persulfides have been reported, such as penicillamine-derived persulfides (*N*-Methoxycarbonyl penicillamine persulfide, nmc-PSSH),<sup>195</sup> Cys,<sup>196</sup> Hcy<sup>197</sup> and others. All model persulfides are prepared *in situ* due to their instability and susceptibility to decomposition via the disproportionation of two persulfides in aqueous solution (Eq. 3).<sup>8</sup>

$$RSSH + RSS^{-} \rightarrow RSSSR + HS^{-}$$
(3)

This inherent instability of persulfides is the reason why there is limited information on the reactivity of persulfides. Moreover, another challenge in their detection is that their chemical properties tend to overlap with other Cys-thiol modifications, such as thiols and disulfides.

The persulfide group, R-SSH, is a type of unsymmetrical disulfide, which bears two sulfur atoms of different properties (Fig. 18A). Its inner sulfur, R-<u>S</u>SH, is considered a sulfane sulfur with an oxidation state of 0, which naturally is of slightly electrophilic nature (thus a target for nucleophilic attack).<sup>198</sup> However, its outer sulfur, R-S<u>S</u>H, has

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an oxidation state of -1, making it nucleophilic (and thus it could react with electrophiles). R-SSH is ionisable and hence acidic, existing predominantly in the anionic form, R-SS<sup>-</sup>, at physiological pH 7.4 Its fully ionised nature, coupled with an alpha effect from its adjacent sulfur, makes it a much stronger nucleophile (and thus more reactive) than its corresponding thiol.8,199 The nucleophilicity of persulfides renders them reactive to 1- and 2- electron oxidants.<sup>8</sup> They are better 1-electron reductants than thiols and H<sub>2</sub>S,<sup>200</sup> and can therefore be oxidised by weaker oxidant. Persulfides have been shown to reduce ferricyanide,<sup>201</sup> ferric cyt c <sup>195</sup> and metmyoglobulin.<sup>201</sup> Additionally, as 2-electron reductants they have a 4-fold higher apparent rate constant with ONOO<sup>-</sup> than their corresponding thiols.<sup>199</sup> When oxidised by peroxynitrite, the resulting products are perthiosulfenic acid, R-SSOH, pertiosulfinic acid, R-SSO<sub>2</sub>H and perthiosulfonic acid, R-SSO<sub>3</sub>H. These resemble the oxidation products formed from thiols (Fig. 12), but with the important difference that the persulfide-derived products bear a cleavable disulfide group (R-SSOH, R-SSO<sub>2</sub>H, R-SSO<sub>3</sub>H), which can be reduced back to the original thiol (Fig. 18B).<sup>8</sup> This inherent reversibility, possibly by intracellular disulfide reductases, feeds into the idea that the persulfidation of protein thiols allows for a rescue (reversible) pathway that can prevent irreversible cysteine over-oxidation (a main topic of this thesis) (Fig. 18B).



Figure 18 - Protective Effect of Protein Persulfidation from Cysteine Over-oxidation. (A) Dual chemical nature of protein persulfides, with one sulfur atom being electrophilic and the other nucleophilic. (B) Due to their increased nucleophilicity, persulfides are better scavengers of ROS than cysteines, resulting in the formation of S-sulfonates, which could potentially be reduced by thioredoxin (Trx) (restoring back the thiolates). On the other hand, when exposed to persistent ROS, cysteine residues are irreversibly hyperoxidised leading to protein inactivation.

## 1.5.2 Persulfide Formation and Depersulfidation

The estimated percentage of persulfidated proteins varies from 5 – 25% of the entire proteome, depending on the method of detection and cellular model used.<sup>8</sup> The intracellular formation of this modification is based on two general mechanisms: (i) through non-enzymatic mechanisms achieved either by oxidation of H<sub>2</sub>S or oxidation of thiols; or (ii) enzymatically, where persulfides are formed in the catalytic cycle.

## 1.5.2.1 - Non-Enzymatic Formation of Persulfides

One common misconception is that persulfides are formed via the direct reaction of  $H_2S$  and a cysteine thiolate. However, this reaction is thermodynamically unfavourable.<sup>15,194</sup> The main mechanism by which persulfides are non-enzymatically formed are through reactions of  $H_2S$  with oxidised Cys, such as R-SS-R, R-SOH and R-SNOs, or in the reaction of Cys residues with radicals, polysulfides and other persulfides (so called transpersulfidation).

#### H<sub>2</sub>S and Sulfenic Acids

Sulfenic acids are known weak nucleophiles, soft electrophiles and typically unstable. The reaction of R-SOH with H<sub>2</sub>S generating a persulfide and water has been documented, with a 4-fold higher pH-independent rate constant when compared to the reaction with thiols.<sup>199</sup> Additionally, this reaction is the mechanism by which the persulfidated forms of proteins glyceraldehyde 3-phosphate dehydrogenase (GAPDH), BSA and HSA are prepared.<sup>194,199</sup>

A few studies have shown that upon the treatment of cells with  $H_2O_2$ , or under conditions of ER stress known to have elevated levels of ROS (specifically R-SOH), there is an increase in endogenous persulfide levels.<sup>199,202,203</sup> This suggests that the

reaction of R-SOH and H<sub>2</sub>S could be the main mechanism for persulfide formation in cells. It is important to take into account the competing reaction of R-SOH with glutathione, given the higher estimated cellular concentrations of glutathione compared to H<sub>2</sub>S.<sup>198</sup> However, H<sub>2</sub>S is freely diffusible across membranes, has a very high flux of production, and could reach deeper into the protein without any constrains.<sup>12,55</sup> The hypothesis that R-SOH serve as precursors for R-SSH formation is investigated in detail in this thesis.

#### H<sub>2</sub>S and Disulfides

GSSG, protein inter- or intra- molecular disulfides or an S-glutathionylated protein Cys residue, represent the majority of disulfides generally found in a cell.  $H_2S$  can react with disulfides, in a sulfur exchange reaction (Eq. 4), yielding an R-SSH and R-S<sup>-</sup>; however, due to the very slow rate constants and low steady-state concentrations of  $H_2S$  under physiological conditions (compared to other LMW thiols), this is an unlikely mechanism for persulfide formation.<sup>8,199</sup>

$$R-SS-R + HS^{-} \rightleftharpoons R-SS^{-} + RSH$$
(4)

The likelihood of this reaction depends on the  $pK_a$  of the thiol which forms the disulfide. Additionally, studies showed no formation of a persulfide upon the reaction of BSA or immunoglobulins (both of which contain intramolecular disulfides) with H<sub>2</sub>S at physiological conditions.<sup>15,194</sup> The slow reaction of H<sub>2</sub>S with disulfides may be more relevant for specific proteins or in some compartments, such as the ER.<sup>194</sup> This reaction can also represent a possible route for the clearance of H<sub>2</sub>S in compartments with high levels of disulfides.

#### H<sub>2</sub>S and S-Nitrosothiols

*S*-nitrosation is one of the most important posttranslational modifications of thiols through which NO conveys some of its signalling properties. The –SNO group has an unusual electronic structure, giving rise to its ambiguous reactivity. The reaction of *S*-nitrosated thiols (R-SNO) and RS<sup>-</sup> proceeds via trans-nitrosation reaction, forming RS<sup>-</sup>

and RSNO, or with RSH forming the disulfide R-SS-R and nitroxyl (HNO) - the latter being thermodynamically unfavourable.<sup>8</sup> With regards to its reactivity with H<sub>2</sub>S, R-SNO can react and form a thiol and the smallest *S*-nitrosothiol, HSNO.<sup>204</sup> HSNO represents a carrier of "NO<sup>+</sup>" (nitrosonium) moiety and can serve as a trans-nitrosation agent, as it can freely diffuse through membranes.<sup>204</sup> R-SNO and H<sub>2</sub>S can also form a persulfide and HNO (Eq. 5); however, this reaction has thermodynamic limitations and depends largely on the protein microenvironment of the –SNO bond.<sup>205</sup>

$$RSNO + HS^{-} \rightarrow RSS^{-} + HNO$$
 (5)

Overall, even though *S*-nitrosation and persulfidation may affect protein function differently, there is some evidence of a 36% proteomic overlap<sup>202</sup> of the two modifications, giving rise to the need for further deciphering of the intertwined nature of these modifications.<sup>8</sup>

#### **Radical Reactions**

Protein metal centres or strong 1-electron oxidants can contribute to the formation of persulfides through their reactivity with H<sub>2</sub>S and RSH, forming the sulfanyl (HS•) and thiyl (RS•) radicals, respectively. Following the formation of these radicals, a likely route of persulfide formation can be: (i) the radical-radical combination (Eq. 6); or (ii) reaction between HS<sup>-</sup> and RS• or RS<sup>-</sup> and HS• to form the radical anion R-SSH•- (Eq. 7-8). This radical anion can subsequently react with O<sub>2</sub>, forming RSSH (Eq. 9).<sup>8</sup> It has been reported that GAPDH, BSA and Hsp70 are persulfidated upon exposure to H<sub>2</sub>S and water-soluble heme iron.<sup>194</sup>

$$HS^{\bullet} + RS^{\bullet} \to RSSH \tag{6}$$

$$HS^{\bullet} + RS^{-} \rightarrow RSSH^{\bullet-}$$
(7)

$$RS^{\bullet} + HS^{-} \rightarrow RSSH^{\bullet-}$$
(8)

$$RSSH^{\bullet-} + O_2 \rightarrow RSSH + O_2^{\bullet-}$$
(9)

A few recent studies have demonstrated the importance of this mechanism for persulfide formation. The reaction of  $H_2S$  with cyt *c* results in the initial formation of a  $HS^{\bullet}/S^{\bullet-}$  radical. Cyt *c*-assisted protein persulfidation might represent a previously

unrecognised source of reactive sulfur species, which mediates the protective effects attributed to protein persulfidation (Fig. 19).<sup>206</sup> This was not only important for mitochondrial persulfidation, but also for apoptosis where cyt *c*-mediated persulfidation of procaspase 9 inhibited its pro-apoptotic function.



Figure 19 - Protein persulfidation catalysed by cyt c/H<sub>2</sub>S couple.

Far more intriguing was the observation that  $H_2S$  can interact with zinc finger (ZF) proteins.<sup>207</sup> ZF coordinates HS<sup>-</sup>, serving as a catalyst for the shuttling of electrons from HS<sup>-</sup> to O<sub>2</sub> forming superoxide and HS<sup>•</sup>/S<sup>•-</sup>. Subsequent ZF persulfidation disrupts the protein structure and abrogates RNA binding, resulting in higher TNF $\alpha$  mRNA levels which offers an explanation for the proinflammatory properties assigned to H<sub>2</sub>S.

#### Thiols and Polysulfides

Polysulfides ( $HS_n^{-}$ ) are products of the incomplete oxidation of  $H_2S$ , mainly caused by the presence of metal ion impurities. Smaller chain polysulfides are more stable (n = 4/5). As sulfane-sulfur-containing compounds, they are susceptible to nucleophilic attack by thiolates, resulting in the formation of persulfides.<sup>198</sup> Given their presence in contaminated solutions of  $H_2S$  or media, they can be responsible for some of the effects assigned to  $H_2S$ .<sup>8</sup> An example is the activation of TRP channels by polysulfides, a phenomenon not seen upon the treatment with clean  $H_2S$  solutions.<sup>208,209</sup> Although the intracellular presence of polysulfides is inevitable, their role as signalling molecules or their targeted role in forming persulfides remains doubtful, given their unspecific stochastic reactivity, slow diffusibility across membranes, and unregulated production.<sup>8,198</sup>

#### Thiols and Persulfides (Transpersulfidation)

As already mentioned, persulfides contain two sulfur atoms, the inner (proximal or sulfenyl) sulfur (the S atom bound to carbon) and the outer (terminal or sulfhydryl) sulfur atom, which is negatively charged (due to the low  $pK_a$ ). Therefore, the reaction with thiolates as nucleophiles predominantly proceeds via an SN2 nucleophilic attack on the inner sulfur, with formation of disulfide and release of H<sub>2</sub>S (Eq. 10). This has been documented for both LMW persulfides as well as protein persulfide models.<sup>193,195,210</sup>

$$RSSH + R'S^{-} \rightleftharpoons RSSR' + HS^{-}$$
(10)

However, transpersulfidation, a transfer of terminal sulfur atom, between thiols and persulfides, has been suggested in the literature (Eq. 11).

$$RSSH + R'S^{-} \rightleftharpoons RSH + R'SS^{-}$$
(11)

The existence of the tautomeric thiosulfoxide form (RS(H)=S) is often cited as an explanation for the transpersulfidation (Eq. 11),<sup>211–213</sup> which would then act as a perfect donor of sulfane sulfur. The SS bond in thiosulfoxides (R<sub>2</sub>S=S) may be considered as either double<sup>214</sup> or "semipolar",<sup>215</sup> depending on the electronegativity of substituents. Computational studies and bond energies, however, suggest that thiosulfoxide tautomers cannot be formed as the energy barrier for the isomerisation is >100 kJ/mol.<sup>216</sup> In case of persulfides, the isomerisation requires the protonated forms to tautomerise to RS(S)H,<sup>216</sup> but considering that at physiological pH most of the persulfides exist in deprotonated form, the tautomerisation is additionally slowed. Yet, this type of reaction has been documented in the reactions/catalytic cycles of some proteins where protein microenvironment facilitates this process (discussed in greater detail below).

#### 1.5.2.2 - Enzymatic Formation of Persulfides

Several enzymes involved in cysteine metabolism have been reported to get persulfidated in their catalytic cycle, or to form an LMW persulfide through sulfur transfer reactions. These persulfidated enzymes/LMW thiols generally have the ability to further transfer a sulfane sulfur. TST, an enzyme responsible for the detoxification of cyanide (Fig. 6) and the conversion of thiosulfate to sulfite, forms an intramolecular persulfide in its catalytic cycle (Fig. 20A).<sup>8,217</sup> The enzyme cycles between a persulfidated and a free thiol form of its active site Cys.<sup>218</sup>

The main enzymes involved in the carbon-sulfur bond cleavage are cysteine desulfurases or MSTs. Cysteine desulfurases are a family of PLP-dependent enzymes that catalyse the removal of sulfur from L-Cys, forming L-alanine and a sulfane sulfur.<sup>219</sup> The proposed mechanism proceeds via the generation of a labile protein-bound Cys-SSH intermediate. These enzymes are particularly important for the iron sulfur cluster assembly.<sup>219,220</sup>



Figure 20 - Mechanism of Persulfide Formation in Catalytic Cycles of TST, MST and SQR.

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MSTs, on the other hand, do not require a cofactor and are involved in the cysteine catabolism and the production of H<sub>2</sub>S.<sup>8</sup> As mentioned in Section 1.2 (Fig. 6), MST catalyses the conversion of 3-MP to pyruvate, proceeding via a two-step mechanism. In the first reaction, a sulfur atom is transferred from 3-MP to the Cys residue of MST, forming a Cys-SSH intermediate on MST and pyruvate (Fig. 20A). The second step involves the transfer of the nucleophilic sulfur of Cys-SSH to a nucleophilic acceptor, such as Trx, and the subsequent release of H<sub>2</sub>S from the acceptor.<sup>8,221</sup> Studies have shown that upon the treatment of cells with D-Cys (substrate of DAO), which feeds into the production of 3-MP (substrate of MST), there is an increase of persulfidation.<sup>8,42</sup>

SQR, an enzyme involved in H<sub>2</sub>S clearance in the mitochondria, is another example of an enzyme which forms an intermediate persulfide on its catalytic cysteine (Fig. 20B).<sup>222</sup> The intermediate SQR persulfide transfers its sulfane sulfur to GSH forming GSSH.<sup>223</sup> Alternatively, it can transfer it to  $SO_3^{2-}$  to form  $S_2O_3^{2-}$ .<sup>8</sup>

Finally, it is worth mentioning that both CBS and CSE could produce Cys-SSH from cystine (Cys-SS-Cys).<sup>8,196,224</sup>

#### 1.5.2.3 - Mechanism of Depersulfidation

Persulfidation is considered to be a reversible PTM and, given the increasing evidence of it having a prominent signalling role, its endogenous removal is essential. It has been postulated that the Trx/TrxR system can enzymatically reduce persulfides. Trx is a disulfide oxidoreductase and an important intracellular redox regulator. It is known to mediate the 2-electron reduction of disulfides and regenerate reduced Trx, catalysed by TrxR. Studies have suggested that the Trx/TrxR system can reduce both R-SNO<sup>225-227</sup> and possibly R-SOH<sup>165</sup>, and that it also takes part in the sulfur transfer reaction catalysed by MST.<sup>8</sup> Initial *in vitro* evidence showed that Trx has an approximately 200-fold higher efficiency at reducing Cys-SSH of PTP1B compared to the reducing agent DTT.<sup>228</sup>





Further studies compared the first order rate constants of Trx + Cys-SSH and Trx + Cys-SS-Cys, and showed that Trx was 10-fold faster at reducing Cys-SSH.<sup>42</sup> Trx has also been shown to reduce the active site Cys of GAPDH-SSH.<sup>229</sup> Using its 2 cysteine residues in its active site CXXC motif, Trx can reduce persulfide via two distinct routes, as shown in Fig. 21A.<sup>8,230</sup>

*In vivo* studies have also been performed where treatment of cell lysates with Trx or the TrxR inhibitor, Auranofin, increased H<sub>2</sub>S and persulfides levels, respectively.<sup>231</sup> Moreover, HIV-1 patients, who experience high circulatory Trx levels, show lower total plasma sulfane sulfur levels, confirming that Trx and persulfide levels are linked (inversely).<sup>42</sup> Taken together, these results prove the efficiency of Trx in reducing persulfides, which makes them excellent candidates for the key intracellular depersulfidases.

The thioredoxin related protein of 14KDa, TRP14, also a disulfide reductase, but with one redox sensitive Cys, has shown to increase persulfide levels when silenced. This suggests its potential role in depersulfidation, under oxidative stress conditions as a replacement for Trx.<sup>231</sup> The reduction of polysulfides and HSA-SSH by glutaredoxin (Grx)/ GR systems have also been shown *in vitro* (Fig. 21B).<sup>231</sup> Overall, further studies must be performed in order to reach a clear understanding of the full mechanism of depersulfidation.

# 1.5.3 Signalling via Persulfidation

As mentioned earlier within this introduction, the progress of research in the area of persulfides has led to the identification of multiple persulfidated target proteins. Below is a brief summary of the biological effects controlled by the persulfidation of specific targets.<sup>8,11</sup>

#### 1.5.3.1 – Cytoprotection and Stress Signalling

As discussed in Chapter 1.3.1 above, H<sub>2</sub>S is involved in the regulation of antioxidant response genes, a process that involves the binding of Nrf2 to the antioxidant response element (ARE). Nrf2 is considered a 'master regulator' of intracellular antioxidant response, and is involved in attenuating apoptosis and initiating mitochondrial biogenesis.<sup>232-234</sup> Under non-stress conditions, Kelch-like ECH-associated protein 1 (Keap1) binds to Neh2 domain of Nrf2 and sequesters it in the cytoplasm, where it becomes targeted for proteasomal degradation (Fig. 22). Keap1 bears reactive cysteines which can be targeted by different electrophiles.

H<sub>2</sub>S has been reported to exhibit its cardioprotective effects under ischemic stress, by persulfidating Keap1 and promoting nuclear translocation of Nrf2 and the activation of the expression of antioxidant enzymes (Fig. 22).<sup>234–236</sup> The persulfidation of Keap1 was also decreased in CSE knockout mice and mouse embryonic fibroblasts (MEF), both of which show elevated levels of oxidative stress and signs of senescence.<sup>236</sup> Moreover, upon the activation of the Keap1-Nrf2 pathway, there is an increase in the expression levels of the enzymes involved in H<sub>2</sub>S production CBS and CSE and SQR.<sup>237</sup>



Figure 22 - H<sub>2</sub>S controls cellular antioxidant defence and prevents senescence by persulfidation of Keap1. In the cytosol, Kelch-like ECH-associated protein 1 (Keap1) represses nuclear factor erythroid 2-related factor 2 (Nrf2) signalling by binding to it. Bound Nrf2 is subjected to the polyubiquitination and subsequent proteasomal degradation. Persulfidation of cysteine residues in the Keap1 protein induces the conformational change, which results in Nrf2 release. From the cytoplasm Nrf2 translocates to the nucleus where it binds to DNA and upregulates the expression of various antioxidant defence genes.

Another example of how persulfidation can assist in cellular protection against oxidative stress is through the persulfidation and subsequent inactivation of the p66shc protein (Chapter 1.4.1).<sup>238</sup> The persulfidation of p66shc attenuated its phosphorylation, preventing its translocation and generation of ROS.<sup>238</sup> These results provide an explanation of how persulfidation/H<sub>2</sub>S can inhibit mitochondrial ROS production.

The receptor of advanced glycation end products, RAGE, has been demonstrated to be persulfidated at two of its Cys.<sup>239</sup> RAGE is up-regulated in various disease conditions and contributes to the pathogenesis of diseases, such as diabetes and Alzheimer's disease. Upon H<sub>2</sub>S treatment or the overexpression of CBS, persulfidation of RAGE prevented its dimerisation, reducing the cytotoxic effects of advanced glycation end products and protecting from senescence.<sup>239</sup>

Persulfidation has also been linked to protection from ER stress, which is associated with many disease conditions.<sup>240,241</sup> During ER stress, transcription

regulator ATF4 is up-regulated, which in turn increases the expression of CSE and intracellular H<sub>2</sub>S levels.<sup>75</sup> Consequently, this causes persulfidation of many target proteins, some of which are involved in the glycolysis and tricarboxylic acid (TCA) cycle.<sup>202</sup> ER-stress induced persulfidation of targets may be aided by the elevated levels of ROS, which cause the initial sulfenylation of proteins, priming them for persulfidation.<sup>8</sup> Moreover, ER stress also induces the persulfidation of PTPs, most importantly, PTP1B, which plays a central role in ER stress signalling.<sup>153,228</sup> Persulfidation of PTP1B was shown to inhibit its activity and consequently inhibit protein translation through the activation of phosphorylated extracellular signal-related kinase (PERK).

Persulfidation-induced MEK1 activation has been associated with increased DNA damage repair.<sup>242</sup>

Finally, it has been demonstrated that H<sub>2</sub>S in the bone marrow modulates selfrenewal and osteogenic differentiation.<sup>243</sup> CBS deficiency (decreased levels of H<sub>2</sub>S), common in patients with osteoporosis, caused the reduction in persulfidation of multiple transient receptor channels, invoking a decreased Ca<sup>2+</sup> influx and downregulation of signalling pathways controlling osteogenic differentiation.<sup>243</sup>

#### 1.5.3.2 – Neuroprotection

Protein persulfidation has also been associated with many neurodegenerative diseases, one of which is Parkinson's disease (PD). Parkin is an E3 ubiquitin ligase with reactive Cys at the active site, prone to oxPTMs. Parkin is known to be regulated by oxidative/nitrosative stress via sulfenylation and nitrosylation. Persulfidation of Parkin, demonstrated even under basal conditions, leads to enzyme activation and clearance of damaged proteins (Fig. 23).<sup>244</sup> Persulfidation of Parkin has been observed *in vivo* in samples from patients with PD, which contained lower levels of persulfidated Parkin but increased levels of its nitrosylated form.<sup>244</sup>



Figure 23 - Schematic representation of the regulatory role of H<sub>2</sub>S on catalytic activity of parkin. In healthy subjects, the E3 ubiquitin ligase, Parkin, is persulfidated, which increases its enzymatic activity. This leads to ubiquitylation of diverse substrates, such as alpha-synuclein (a component of the Lewy bodies found in Parkinson's disease), and their subsequent proteasomal degradation. In patients with Parkinson's disease, parkin is S-nitrosylated with greatly decreased catalytic activity, resulting in protein aggregation, accumulation of toxic proteins and cell death.

#### 1.5.3.3 - Apoptosis

GAPDH, the first protein found to be persulfidated<sup>191</sup> and an important enzyme in glycolysis, is also involved in regulation of apoptosis and neuronal responses, such as memory deficits.<sup>11</sup> GAPDH is redox sensitive, and contains a catalytic Cys which can undergo *S*-sulfenylation and *S*-nitrosylation, both of which cause its inactivation and consequent initiation of apoptosis.<sup>245,246</sup> Persulfidation of GAPDH has been suggested to increase its enzymatic activity and, therefore, inhibits its translocation to nucleus and initiation of apoptosis.<sup>191</sup>

 $H_2S$  is also known to protect against inflammation, although the exact mechanism by which this is occurs is still under investigation. One possible mechanism is through persulfidation of the p65 subunit of NF- $\kappa$ B, the anti-apoptotic transcription factor complex known to regulate cell survival.<sup>247</sup> Persulfidation was reported to activate its binding to promoters of anti-apoptotic genes and to increase pro-survival gene expression.

#### 1.5.3.4 – Vasodilation

Multiple studies demonstrate that  $H_2S$  promotes vasorelaxation through its interaction with NO; however,  $H_2S$ -induced vasodilatory effects are probably caused by several different mechanisms.<sup>8</sup> One such mechanism is persulfidation of the ATP-dependent potassium channel ( $K_{ATP}$ ) in vascular smooth muscle cells. Persulfidation causes channel opening by increasing its binding to phosphatidylinositol-4,5-bisphosphate (PIP<sub>2</sub>), which results in potassium ion (K<sup>+</sup>) influx and consequent vasorelaxation.<sup>105</sup>

# 1.6 Methods of Persulfide Detection

Due to their greater nucleophilicity, persulfides react faster with commonly used thiolblocking electrophiles than the corresponding thiols, and they yield distinct products. Thus, alkylation of thiols yields thioethers, while disulfides are formed from persulfides. Several methods exploit these characteristics of persulfides for their detection and are briefly summarised below.

Persulfides could be detected directly by spectrophotometry via their prior derivatisation with the electrophilic blocking agent, 1-fluoro-2,4-dinitrobenzene (FDNB) (Fig. 24).<sup>248</sup> Once persulfides react with FDNB, they form a mixed disulfide, which can be subsequently treated with a reducing agent to release 2,4-dinitrobenzenethiol, a molecule with distinct spectre in visible light region. Another direct approach is MS.<sup>191</sup> However, this approach has many limitations, including that protein persulfides are quite unstable and that mass shift of 32 KDa is indistinguishable from sulfinylation. In order to avoid their instability, the initial use of a blocking reagent, such as NEM or IAA, can be installed to stabilise the proteins (Fig. 24).<sup>192,193</sup>



Figure 24 - Strategies used for the characterisation of protein persulfides. Pure protein persulfides can be analysed directly by Mass Spectrometry (MS), UV-visible, IR or NMR spectroscopy (top left). Protein persulfides can be labelled by different approaches (top right). Persulfides can either be labeled with the blocking reagent, 1-fluoro-2,4-dinitrobenzene (FDNB) and reduced with DTT to release fluorogenic adduct which absorbs at 408 nm at alkaline conditions (bottom right), or they can be blocked using blocking reagents (NEM or IAA) and analysed by MS (bottom left).

The first method for persulfidation detection that found wide application was published by Snyder *et al.*, and is known as the Modified Biotin Switch assay (Fig. 25).<sup>191</sup> This method relies on the premise that thiol blocking reagent *S*-methylmethanethiosulfonate (MMTS) would not react with persulfides but will block all other thiols, so that in the next step persulfides could be selectively labelled with *N*-(6-(biotinamido)hexyl)-3'-(2'-pyridyldithio)-propionamide (biotin-HPDP). However, it has been demonstrated that MMTS reacts readily with persulfides (if not even better than with thiols), rendering this method problematic.<sup>193</sup>



Figure 25 - Modified Biotin Switch assay for protein persulfide detection.

Snyder's group has also proposed Cy5-maleimide labelling, which has since been modified to fit specific applications (Fig. 26).<sup>247</sup> The original method involves the blocking of free thiols and persulfides using Cy5-maleimide, followed by the reduction with DTT. By comparing fluorescence loss in samples treated with DTT directly in gel, persulfidation levels could be assessed. Although very easy to perform, this method suffers from a lack of sensitivity and a decrease in the fluorescence of less abundant proteins will not be easily detectable. Another disadvantage of the use of Cy5maleimide is that it results in high background signal from the labelling of amino groups.



Figure 26 - Protein persulfide labelling by Cy5-maleimide.

The general chemical strategy behind the maleimide assay has been explored for proteomic analysis; this approach is often referred to as the 'biotin thiol assay'.<sup>202</sup> In this approach, both thiols and persulfides are blocked with biotin-maleimide. Following trypsinisation, immobilisation on beads, and elution using a reducing agent, only initially persulfidated proteins could be detected. Different variants of this method are reported under the names qPerS-SID<sup>249</sup> and ProPreDP (Fig. 27).<sup>231</sup> These methods have two main limitations: (i) that commonly used thiol blocking reagents react readily with sulfenylated proteins as well giving a product that cleavable by is DTT or Tris(2-carboxyethyl)phosphine hydrochloride (TCEP); and (ii) that reducing agents will release peptides originating from inter- or intra- molecular disulfides, giving rise to false positives.



Figure 27 - Different strategies using biotin-tagged alkylating agents.

A much more selective approach that has been reported for persulfide detection is the tag-switch method (Fig. 28).<sup>194,250</sup> In the first step, a thiol-blocking reagent is introduced and reacts with both thiols and persulfides. With an appropriate (aromatic) thiol-blocking reagent, methylsulfonyl benzothiazole (MSBT), an asymmetric mixed disulfide is formed in place of persulfide, with one of the sulfurs from the disulfide bond showing much enhanced reactivity to certain nucleophiles. It should be noted that thiol adducts from the first step are thioethers, which do not react with the nucleophile. In the second step, derivatives of cyanoacetic acid (reporting molecules), shown in Fig. 27, are used as nucleophiles to selectively label only persulfides.<sup>42</sup> The only limitation of the method is the poor solubility of MSBT (MSBT-A is not commercially available). This method was successfully used to study SCA3<sup>111</sup> and was recently adapted for the analysis of the persulfide proteome (persulfidome) in *Arabidopsis thaliana*.<sup>251</sup>



Figure 28 - Tag-switch method for persulfide labelling.

Finally, a more recent method has been published, known as the low pH Quantitative Thiol Reactivity Profiling (QTRP) method (Fig. 29).<sup>252</sup> This method was developed as a direct site-specific mapping technique, based on the use of an electrophilic thiol-reactive probe with an alkyne IAA-like structure called IPM and performed strictly at low pH. The method relies on the fact that persulfides have a much lower pK<sub>a</sub> compared to thiols; thus, at lower pH (pH 5), most thiols would be protonated, but persulfides will still remain fully deprotonated. As such, at pH 5 the IPM probe will label all persulfides and very few thiols. The sample is then digested and attached to a UV-cleavable biotin tag by copper(I)-catalyzed alkyne-azide cycloaddition (CuAAC) – click reaction. The biotinylated peptides were captured and photo-released, allowing for the quantitative MS analysis. However, the approach is limited to comprehensive MS analysis and cannot be used for any other detection.



Figure 29 - low-pH Quantitative thiol reactivity profiling (QTRP) method for the direct detection of persulfides.

# 1.7 Project Aims

As a third gasotransmitter, H<sub>2</sub>S has been implicated in mediating a plethora of (patho)physiological functions. However, as described above, the exact mechanism(s) of action responsible for these effects are still elusive. One of the main mechanisms by which biological effects of H<sub>2</sub>S may be explained is through persulfidation. Therefore, understanding protein persulfidation does not only have fundamental potential, for instance in unravelling new signalling pathways, but it also has pharmacological potential, particularly in fighting aging and neurodegenerative diseases. To date, the underlying mechanisms of H<sub>2</sub>S-mediated R-SSH formation remain unclear, mainly due to the lack of reliable and selective tools for persulfide labelling. The overall aim of this study was to develop a robust, chemoselective method for persulfide labelling, which can then be used for positioning this oxPTM in the global cell redox signalling scheme. The goals of this thesis were:

- <u>Development and characterisation of a selective method for persulfide labelling.</u> The chemoselectivity of the novel two-step approach for persulfide labelling, named Dimedone Switch method, was shown through kinetic and proteomic studies with LMW and protein persulfides and in different cell models.
- 2. <u>Development of the method's applicability.</u>

To establish the method's versatility, protocols were developed for the in-gel fluorescence, microscopy, anti-body microarray-like and proteomic analysis of intracellular persulfidation. Furthermore, the method was used in different model organisms such as cells, organs, *C. elegans, E. coli* and *S. cerevisiae* to demonstrate that persulfidation is evolutionarily conserved and controlled by the H<sub>2</sub>S-producing enzymes in the transsulfuration pathway and cysteine catabolism.

3. <u>Investigation of persulfide's intertwined nature with H<sub>2</sub>O<sub>2</sub> signalling and their importance for protecting cysteines from hyperoxidation.</u>

The reversibility of  $R-SH\rightarrow R-SSH\rightarrow R-SSO_3H$  cycle, catalysed by Trx, was demonstrated in kinetic and mass spectrometry studies. Moreover, the protective effect of R-SSH from cysteine over-oxidation was shown through dynamic spatiotemporal studies of R-SSH and oxPTMs, such as R-SOH and R-SO<sub>2</sub>H, revealing the presence of 'protective waves' of protein persulfidation.

4. <u>Study of the biological relevance of the protective effect of persulfidation, its</u> <u>correlation with oxidative stress resistance and aging.</u>

To determine how persulfide levels affect oxidative stress resistance and aging, stress assays were performed with different model organisms *in vitro* and *in vivo*. The ability to form persulfides was directly correlated to oxidative resistance and lifespan extension. Additionally, using *C. elegans* lifespan assays and aging models (human patients and rodents), persulfide and H<sub>2</sub>S-producing enzymes levels were shown to inversely correlate with aging.

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### **CHAPTER 2: Mini Review**

### Measurement of Protein Persulfidation: Improved Tag Switch Method

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## **Chapter 4**

### Measurement of Protein Persulfidation: Improved Tag-Switch Method

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### Abstract

Hydrogen sulfide (H<sub>2</sub>S) is an endogenously produced signaling gasotransmitter, generated by the enzymes cystathionine  $\gamma$ -lyase, cystathionine  $\beta$ -synthase, and 3-mercaptopyruvate sulfurtransferase. The involvement of H<sub>2</sub>S in numerous physiological, as well as pathophysiological conditions, was established over the past decade. However, the exact mechanism(s) of regulation of the biological functions by H<sub>2</sub>S are under active investigations. It is proposed that the oxidative posttranslational modification of protein cysteine residues, known as persulfidation, could be the main mechanism of action of H<sub>2</sub>S. Protein persulfides show similar reactivity to thiols, which represents one of the main obstacles in the development of a reliable method for detection is of utmost importance in order to fully understand the physiological and pathophysiological role of H<sub>2</sub>S. Several methods have been proposed for the detection of protein persulfidation, all of which are highlighted in this chapter. Furthermore, we provide a detailed description and protocol for the first selective persulfide labeling method, a tag-switch method, developed in our group.

**Key words** Hydrogen sulfide, Gasotransmitter, Oxidative posttranslational modification, Persulfide, Tag-switch assay, CN-BOT, CN-Cy3, MSBT

### 1 Introduction

1.1 Protein Persulfidation Over the past decade, hydrogen sulfide  $(H_2S)$  has emerged as the third gasotransmitter alongside nitric oxide (NO) and carbon monoxide (CO) [1, 2]. The production of H<sub>2</sub>S in the cell has been found to be controlled by at least three enzymes, cystathionine cystathionine β-synthase (CBS), γ-lyase (CSE), and 3-mercaptopyruvate sulfur transferase (MPST). These enzymes are expressed at different levels in different tissues and control H<sub>2</sub>S production with different efficiencies. H<sub>2</sub>S has been shown to completely or partially regulate various physiological and pathophysiological processes [3-8]. The main mechanism by which H<sub>2</sub>S has been proposed to regulate biological functions is the formation

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of persulfides on specific protein cysteine residues (referred to as *S*-sulfhydration or persulfidation), an oxidative posttranslational modification that could change the protein structure and activity [9].

Protein persulfidation can potentially explain the ample effects that  $H_2S$  has been documented to exhibit in the cell [10]. For example, parkin, a key Parkinson's disease (PD)-associated protein, is a documented case of a protein affected by oxidative/nitrosative stress [11–16]. Parkin functions as an E3 ubiquitin ligase, i.e., it catalyzes the thioester transfer of ubiquitin moieties to a variety of proteins. The loss of protein activity has been shown to be a reason for PD [17]. A recent study by Snyder's group demonstrated that parkin gets persulfidated, which consequently causes an increase of its activity [18]. This overactivation of parkin's function could rescue neurons from cell death by removing damaged proteins. The use of H<sub>2</sub>S donors may therefore help in the early treatment of PD.

Despite the growing interest for protein persulfidation, there is still limited evidence in the literature regarding the exact mechanism(s) by which proteins are persulfidated. Initial studies of protein persulfidation were based on the incorrect assumption that the deprotonated form of free thiols (thiolates) could react directly with H<sub>2</sub>S, resulting in the formation of persulfides. This reaction is, however, thermodynamically unfavorable and does not occur [19–21]. The exact mechanism by which proteins are modified by H<sub>2</sub>S is an important question, which, when clearly understood, could be a crucial linker toward the unraveling of H<sub>2</sub>S signaling. Several mechanisms have been proposed [21], but the most plausible is the reaction of H<sub>2</sub>S with oxidized thiols, more precisely, disulfides or sulfenic acids.

The proposed reaction of  $H_2S$  with disulfides could represent a route for  $H_2S$  consumption in the extracellular matrix and plasma, for example, where higher levels of disulfides are present. However, recent studies suggested that the reaction of  $H_2S$  with disulfides is too slow to be of physiological relevance [22]. Interestingly, the reaction of  $H_2S$  with sulfenic acids (RSOH) has a rate constant higher than that of the reaction of other biological thiols with RSOH [22]. The treatment of cells with hydrogen peroxide  $(H_2O_2)$  showed increased intracellular levels of protein persulfidation, a process that could be completely suppressed by inhibiting endogenous  $H_2S$  production [22].

The p $K_a$  of persulfides is lower than that of their corresponding thiols [21–23] suggesting that at physiological conditions, the majority of persulfides would be in their deprotonated form (R-S-S<sup>-</sup>), making them "super" nucleophilic. This should dramatically increase persulfides' reactivity when compared to their corresponding thiols. Indeed the rate constant of the reaction of protein persulfides with peroxynitrite (powerful oxidant formed in the diffusion controlled reaction of superoxide with nitric oxide) is found to be one order of magnitude greater than for the reaction of peroxynitrite with its corresponding thiol [22].

All of these observations led to a suggestion that protein persulfidation could serve as a protection mechanism where, via protein persulfidation, the cellular milieu gets protected from irreversible protein hyper-oxidation, induced by a high amount of reactive oxygen (ROS) and nitrogen species (RNS) [21]. Namely, thiol oxidation, which initially starts with the formation of sulfenic acids (still reversible modification) could proceed further with the irreversible formation of sulfonic acids.  $H_2S$  could react with the sulfenic acids instead, preventing this oxidation. In addition, persulfidated protein will react faster with ROS/RNS and form an adduct that could be cleaved by the action of certain enzymes restoring the free thiol.

To exert a regulatory function similar to that of phosphorylation/dephosphorylation or S-nitrosation/denitrosation, S-persulfidation levels must be enzymatically modulated [24]. Intracellular protein disulfides and S-glutathionylation levels are controlled by the thioredoxin (Trx) system [25]. The enzymatic system, consisting of Trx, thioredoxin reductase (TrxR), and NADPH, represents the main disulfide reductase system in cells. In addition to its disulfide reductase activity Trx cleaves the persulfides one order of magnitude more efficiently than it reduces corresponding disulfides. The inhibition of the Trx system leads to an increase of intracellular persulfides, confirming that this process occurs in the cells as well. Significantly lower total sulfane sulfur levels were detected in HIV patients with high viral load (and high circulatory Trx levels) compared to the treated patients, which provides evidence that Trx acts as depersulfidase additionally in vivo [26].

Recent studies have shown that proteins such as NF- $\kappa$ B, Keap1, GAPDH, KATP-channels, PTP1B, etc. undergo protein persulfidation [2, 19, 27–30]. It has been estimated that up to 25% of proteins are persulfidated [31] making this modification almost as abundant as phosphorylation, and thus being of crucial importance for cells. However, due to the lack of an accurate and selective method for persulfide detection, the total amount of persulfidated protein remains questionable.

Persulfides are made of two sulfurs with different electronegativities, which results in it demonstrating two modes of reactivity. The sulfur covalently bound to carbon (**RSSH**) is considered a sulfane sulfur, with a formal charge of 0. Subsequently, this sulfane sulfur is susceptible to nucleophilic attack [32]. The persulfide's terminal sulfur (**RSSH**), however, possesses a formal charge of -1, which makes it susceptible to reaction with electrophiles [32]. As a consequence, one of the main obstacles in the development of a reliable and selective detection method for persulfides is the similar reactivity of persulfides to other sulfur species, especially thiolates. According to literature, there has been a lot of debate on whether the detection methods used for persulfide labeling are indeed selective for protein persulfides [21]. This chapter provides an overview of the currently reported methods for protein persulfide labeling, with particular emphasis on the tag-switch method developed in our group.

**1.2 Modified Biotin-Switch Method** The first method proposed in the literature for the labeling and detection of protein persulfides was by Mustafa et al. [31]. This method was a modification of the method originally used for the detection of protein *S*-nitrosation in proteins, known as the biotin-switch assay [33]. This assay for *S*-nitrosothiols was developed as a three-step method, where free thiols were initially blocked with the electrophilic alkylating agent, *S*-methyl methanethiosulfonate (MMTS), and after removing the excess of MMTS, ascorbate was added to reduce the *S*-nitrosothiols to free thiols. The released thiols were then selectively conjugated with *N*-[6-(biotinamido) hexyl]-3'-(2-pyridyldithio) propionamide (biotin-HPDP) and captured by streptavidin beads.

Mustafa and colleagues proposed a modified biotin-switch technique (modified-BST), illustrated in Fig. 1, in which protein persulfides were postulated to remain unreacted after the blocking of thiols with MMTS. Hence after the excess MMTS is removed, the free persulfides can be labeled with the use of biotin-HPDP, as shown in Fig. 1. Using this method, Mustafa et al. claimed that up to 25% of proteins in cell lysates are modified by  $H_2S$ , under basal conditions [31].

MMTS is extensively used for the detection of protein *S*-nitrosation and used in the in vivo trapping of the thiol-disulfide state of proteins [34, 35]. However, caution must be taken when using MMTS as Karala and Ruddock documented that MMTS generates artificial intermolecular and intramolecular protein disulfide bonds, which can give rise to the misinterpretation of results [36].

The chemical foundation of the modified biotin-switch technique was the selective reactivity of MMTS with thiols. However,



**Fig. 1** Schematic overview of modified biotin-switch assay. The first step of this method was based on a chemically questionable premise that protein persulfides would not react with electrophilic thiol-blocking reagent *S*-methyl methanethiosulfonate (MMTS). In the subsequent step, persulfides are labeled with *N*-[6-(biotinamido) hexyl]-3'-(2'-pyridyldithio) propionamide (biotin-HPDP)

the potential nucleophilicity and hence reactivity of persulfides with the electrophilic MMTS was not investigated. Pan and Carroll [32] tested the reactivity of MMTS using low molecular weight (GSH persulfide) and protein persulfide models (papain persulfide and glutathione peroxidase 3 (Gpx3) persulfide). Their results demonstrated that the alkylated products were present in the product mixture following the reaction with MMTS. In the case of the low MW model, GSH persulfide, the alkylated product was obtained as a minor product, while with Gpx3 persulfide and papain persulfide, the alkylated product was obtained as the major product. Additionally, the reactivity of these persulfide models toward electrophilic and nucleophilic reagents was tested, giving a further insight into persulfide reactivity. The nucleophilicity of the terminal sulfur of tested persulfides (RSSH) was reaffirmed, showing without a doubt that they react with MMTS (and its brominated analogue BBMTS) as readily as free thiols.

1.3 Cy5-Maleimide Labeling and Further Modifications of the Method Snyder's group also [27] proposed a modified NEM (*N*-ethylmaleimide) method for the persulfide labeling of purified proteins (Fig. 2). Cy-5 labeled maleimide was used as a thiol-blocking reagent, to block both the persulfides and free thiol of tested protein sample. The product of Cy5-maleimide and persulfide is actually a disulfide that can be cleaved by dithiothreitol (DTT). The samples were then treated with DTT and the decrease of in-gel fluorescent signal monitored as readout for the persulfide levels (Fig. 2). Simplicity of this method, as well as commercial availability of the reagents, represents the two main advantages; however the analysis of complex protein mixtures becomes more difficult.



**Fig. 2** Schematic overview of Cy5-maleimide method. In this method both persulfide and free thiol would be blocked by the thiol fluorescently labeled *N*-ethyl maleimide (Cy5-conjugated maleimide). The adduct of persulfide and Cy5-maleimide is a disulfide that will be then cleaved by the DTT leading to a decrease of the in-gel fluorescence signal in the samples containing persulfides



**Fig. 3** Chemical modifications of method proposed by Sen et al. [27]. Proteins get initially labeled with alkylating agent such as biotin-maleimide, maleimide-PEG-biotin, or iodoacetyl-PEG-biotin which label both thiols and persulfides. Labeled proteins then get pulled down by streptavidin (or avidin) beads, cleaned from the rest of the mixture, and persulfidated proteins get eluted by some reducing reagent such as DTT or TCEP. Cuevasanta et al. [22], Gao et al. [37], and Longen et al. [39] trypsinized the labeled protein first and then pulled down the peptides with streptavidin beads, while Dóka et al. [38] worked with intact proteins

We described a slight modification of the method proposed by Snyder's group [22], which has since been used by several other authors with minor modifications (Fig. 3). In this methodological approach, free thiols and persulfides are initially labeled with biotinylated maleimide. Labeled proteins are then digested using trypsin and biotinylated peptides separated using streptavidin agarose beads. The alkylation of persulfides leads to the formation of a disulfide, making the elution of persulfidated peptides from streptavidin beads by DTT rather easy. After centrifugation, the eluant was analyzed by LC-MS/MS. Gao et al. [37] used a similar method to detect persulfides in cell lysates. For the blocking step, an alternative reagent was used, maleimide-PEG<sub>2</sub>-biotin (NM-biotin), which was followed by the binding of the biotin-labeled proteins on an avidin column.

Other thiol-blocking reagents could be used instead of maleimides, such as Iodoacetyl-PEG<sub>2</sub>-Biotin (IAB). Some authors tried to name this method, calling it ProPerDP [38] or qPerS-SID [39]. In the ProPerDP method, instead of digesting the alkylated proteins prior to streptavidin separation, Dóka et al. [38] separated the whole proteins on streptavidin beads, eluting the persulfidated proteins with TCEP (tris(2-carboxyethyl)phosphine). Separating the whole proteins by this approach is more prone to artifacts. In addition to inevitable elution of proteins connected by inter- and intramolecular disulfides and which do not necessarily have to contain any persulfides, the actual yield of eluted persulfidated proteins is underestimated. For example, if a protein contains two cysteine residues, of which only one is persulfidated, then the chances for the persulfidated protein to be eluted from streptavidin beads are 50%. For example, in case of another protein TRPA1 [40] whose persulfidation is postulated to contains 21 cysteine residues, those chances would be 1/21. Therefore, it is not surprising that Dóka et al. [38] reported very low protein persulfidation using the ProPerDP approach.

In the qPerS-SID method, Longen and colleagues [39] used this approach for quantitative proteomic analysis of protein persulfidation, where control cells were grown on standard cell medium, while the cells treated with H<sub>2</sub>S donors were grown on SILAC (stable isotope labeling with amino acid in culture) medium. The cells were treated with iodoacetyl-PEG2-biotin (IAB) to block thiols and persulfides, samples were mixed in a ratio of 1:1 and digested by trypsin. Peptides containing cysteine and persulfide were separated from other peptides in the mixture using streptavidin beads. Bound persulfidated peptides were separated from the bound peptides with cysteine using the reducing agent (TCEP) to cleave the disulfide bond in the persulfides. In the following step, peptides were treated with iodoacetamide (IAM) to improve their detection by LC-MS/MS analysis. However, certain limitations arise concerning this method. The critical step of this method, similar to that of the ProPerDP method [38], is its reduction step and subsequent breaking of the disulfide bonds, hence not being selective only to persulfides. As a result, the intramolecular disulfide bonds will also be reduced causing false-positive results. Furthermore, following the reduction, the use of IAM as a thiol-blocking reagent can additionally label primary amines [41] and sulfenic acids [42], as the authors suggested. Indeed, the authors did not see any significant increase of protein persulfidation in the cells treated with the most used H<sub>2</sub>S donors, NaHS or Na<sub>2</sub>S, contrary to all other published studies, which further questions this methodological approach.

To determine the persulfidation of protein tyrosine phosphatase 1B (PTP1B) in the cell lysate, Krishnan and colleagues [28] used iodoacetic acid (IAA) as a thiol-blocking reagent (Fig. 4). Free thiols and persulfides will be blocked since the persulfide reactivity is similar to that of the free thiols. However, in the second step, they used DTT to cleave the alkylated persulfides in order to form free thiols. Next, the free thiols were labeled with iodoacetamide-linked



**Fig. 4** Schematic overview of persulfide labeling approach proposed by Krishnan et al. [28]. In this method, iodoacetic acid (IAA) is used to initially block both free thiols and protein persulfides. In the subsequent steps, alkylated persulfide is cleaved with DTT and then labeled with iodoacetamide-linked biotin (IAP). Although DTT would indeed cleave this adduct, it is unclear how this method distinguishes the persulfides from intra- and intermolecular disulfides and S-nitrosothiols, which would also be reduced by DTT



**Fig. 5** Tag-switch method for persulfide labeling. (a) Schematic overview of labeling steps in tag-switch method. Methylsulfonyl benzothiazole (MSBT) is used to block thiols and persulfides in the first step, followed by the tag switch with cyanoacetate derivatives that carry a reporting molecule, in the second step. (b) Structures of reporting molecules used to label protein persulfides from the cells

biotin (IAP) and purified on streptavidin beads. The main concern with this method is the use of DTT as a reducing reagent in the second step of the protocol. Namely, DTT would cleave the alkylated persulfides, but it would also cleave all the disulfide bonds in the protein and consequently cause false-positive results.

We proposed that persulfidation can be selectively detected by the 1.4 Tag-Switch tag-switch method (i.e., using two reagents to label protein persul-Method fides in two steps) [10, 19]. In the first step, a thiol-blocking reagent should be introduced and tagging both P-SH and P-SSH to form an intermediate product (Fig. 5a). If an appropriate thiol blocking reagent is employed, the disulfide bonds in the persulfide adducts may show enhanced reactivity to certain nucleophiles than common disulfides in proteins. We screened a series of carbonbased nucleophiles as potential candidates [19]. Among these candidates, methyl cyanoacetate was particularly attractive as its ester group could allow easy installation of reporting molecules. Therefore, we could introduce a tag-switching reagent (containing both the nucleophile and a reporting molecule, such as biotin) to label only the persulfide adducts. It should be noted that thiol adducts from the first step are thioethers, which are not expected to react with the nucleophile. Moreover, in contrast to previous methods, even if the free thiol is not completely blocked, we should not expect any misidentification of persulfidated proteins because the tag-switch reagent is a nucleophile, not an electrophile.

A major challenge in this technology was whether the newly generated disulfide linkages from persulfide moieties could display a unique reactivity to a suitable nucleophile to an extent that it is differentiated from common protein disulfides. We envisioned that a reagent, which would give a mixed aromatic disulfide linkage when reacting with persulfides (-S-SH), could exert the reactivity criteria for our tag-switch technology (Fig. 5a). Indeed, by combining methylsulfonyl benzothiazole (MSBT-A) as a thiol-blocking reagent in the first step, and a biotinylated derivative of methyl cyanoacetate as a nucleophile in the second step, we could efficiently label protein persulfides (Fig. 5a) [19]. To test the selectivity of this method, we produced P-SSH on bovine serum albumin (BSA) and compared its reactivity with the tag-switch assay using glutathionylated, sulfenylated, and normal BSA (which by definition contains both intramolecular disulfides and one reactive cysteine). Only P-SSH was labeled and could be pulled down by streptavidin beads, suggesting the applicability of tag-switch assay for wide proteomic analysis [43].

The original assay used was with a biotinylated cyanoacetic acid tag, which required Western blot transfer and streptavidin or antibodies for visualization [10, 19]. To increase sensitivity, we synthesized two new cyanoacetic acid derivatives, with a fluorescent BODIPY moiety (CN-BOT) or a Cy3-dye (CN-Cy3) (Fig. 5b) [26]. Both new tags labeled persulfidated human serum albumin (HSA-SSH) resulting in the formation of fluorescent products. CN-BOT was used for the labeling of cells for microscopy (Fig. 6) and CN-Cy3 for the labeling of cell lysates. This is because the former showed low fluorescence after gel fixation, while the latter proved to be very difficult to wash out from fixed cells. This lead to the discovery that the thioredoxin/thioredoxin reductase system is essentially involved in the removal of protein persulfidation, thus acting as protein depersulfidase system. Using this improved tag-switch assay, we also demonstrated the role of protein persulfidation in a D. melanogaster disease model of spinocerebellar ataxia type 3 (SCA3) [44].

### 2 Materials

2.1 "Improved Tag-Switch" Assay for In-gel Detection

- 1. Ham's F12: DMEM (1:1) medium supplemented with 2 mM glutamine, 1% nonessential amino acids, and 10% fetal bovine serum.
- 2. PBS.
- HEN buffer: 50 mM HEPES, 0.1 mM EDTA, 1.5% SDS, 1% NP-40, 1% protease inhibitor cocktail, and 10 mM MSBT pH 7.4. See Note 1.
- 4. Methanol.



**Fig. 6** The use of the improved tag-switch method for the in situ labeling of intracellular persulfides in SH-SY5Y neuroblastoma cells. Cells were treated with 100  $\mu$ M Na<sub>2</sub>S or 2 mM p-cysteine (substrate for 3-mercaptopyruvate sulfur transferase, MPST) for 1 h, to increase the levels of intracellular persulfidation. Labeling was performed as described in the protocol, with the green fluorescence originating from CN-BOT and blue from the use of DAPI to stain the nuclei. An obvious increase of intracellular persulfidation was achieved with exogenous treatment with Na<sub>2</sub>S and even stronger effect was visible in the cells treated with 2 mM p-cysteine

- 5. Chloroform.
- 6. 50 mM HEPES with 3% SDS (pH 7.4).
- 7. Neocuproine hydrate.
- 8. 2.5 mM CN-Cy3 in acetonitrile.

2.2 "Improved Tag-Switch" Assay for In Situ Detection of Intracellular Persulfide

- 1. PBS.
- 2. Methanol.
- 3. Acetone.
- 4. 50 mM HEPES buffer (pH 7.4).
- 10 mM MSBT dissolved into 70% 50 mM HEPES (pH 7.4)/ 30% methanol.
- 6. Triton X-100.
- 7. 2.5 mM CN-BOT in acetonitrile.
- 8. DAPI.

3 Methods	
3.1 "Improved Tag-Switch" Assay	1. Grow SH-SY5Y cells in Ham's F12: DMEM medium, supple- mented with 2 mM glutamine, 1% non-essential amino acids, and 10% fetal bovine serum at 37 °C and 5% CO <sub>2</sub> in T-75 cell culture flasks.
	2. Treat the cells with respective compounds over 1 h.
	3. Wash the cells twice with warm sterile PBS.
	<ol> <li>Lyse the cells by adding 800 μL HEN buffer that contains 10 mM MSBT to the T-75 flasks.</li> </ol>
	5. Incubate the cells on ice for 10 min with occasional scrapping of the flask surface with a cell scrapper.
	6. Transfer the lysate to tubes and incubate for 1 h at 37 $^{\circ}$ C.
	<ul> <li>7. Precipitate the proteins from the lysate by chloroform/methanol precipitation. Start by adding methanol (MeOH) first (1/1, v/v) followed by vigorous vortexing and then add chloroform (final: 4/4/1, water/MeOH/CHCl<sub>3</sub>). Vortex the sample and centrifuge (20,000 × g, 20 min, 4 °C). Proteins will form a visible intermediate layer pellet between the chloroform and MeOH/water fraction. To wash the protein pellet, remove the upper fraction, and replace with MeOH, mix, and centrifuge again (20,000 × g, 20 min, 4 °C).</li> </ul>
	8. Dry the precipitated pellet and resuspend in 300 $\mu$ L 50 mM HEPES with 3% SDS.
	<ol> <li>Incubate the protein solutions with 60 μM CN-Cy3 (by adding 3 μL of a 25 mM stock solution) for 1 h at 37 °C. See Note 2.</li> </ol>
	10. Resolve by SDS-PAGE under non-reducing conditions, and record the gels on a Cy3 channel.
3.2 "Improved Tag-Switch" Assay for In Situ Detection of Intracellular Persulfide	<ol> <li>Grow cells in μ-dishes (35 mm, high) obtained from Ibidi<sup>®</sup> (Martinsried, Germany), following manufacturer's instructions.</li> </ol>
	2. Treat the cells with respective compounds over 1 h.
	3. After treatments, wash the cells twice with warm sterile PBS.
	4. Fix the cells by incubation with ice-cold methanol at $-30$ °C for 20 min. Remove methanol, and add ice-cold acetone to the cells for 5 min at $-30$ °C. Wash the dishes with PBS buffer.
	5. Incubate the cells with 0.5 mL MSBT/HEPES/methanol solution at room temperature overnight.
	<ol> <li>Wash the cells five times with PBS, and incubate with 0.5 mL of 25 μM CN-BOT (obtained by adding 5 μL of CN-BOT stock solution) in HEPES at 37 °C for 1 h.</li> </ol>

- 7. Wash the cells five times with PBS and stain with DAPI, following manufacturer's instruction.
- 8. Wash the cells again and visualize using an LSM 780 confocal laser scanning system (Carl Zeiss MicroImaging).

### 4 Notes

- 1. MSBT shows poor solubility. Phosphate buffers further decrease its solubility leading to the precipitation. Stock solutions should be prepared in methanol and added into HEN buffer which already contained 1.5% SDS, 1% NP-40, and 1% protease inhibitor cocktail.
- 2. Sulfenic acids could react with cyanoacetic acid-derived probes. Although the chances that they remain intact in the cells after the first five steps are minor (indeed we confirm that they do not interfere with the labeling [19]). An additional step could be introduced immediately after the fixation where the cells would be incubated with 1 mM dimedone in 70% 50 mM HEPES (pH 7.4)/30% methanol for 1 h at 37 °C, washed carefully, and then continue labeling as described in step 5.

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### **CHAPTER 3: Article I**

### Selective Persulfide Detection Reveals Evolutionarily Conserved Anti-Aging Effects of S-Sulfhydration

Jasmina Zivanovic, <u>Emilia Kouroussis</u>, Joshua B. Kohl, Bikash Adhikari, Biljana Bursac, Sonia Schott-Roux, Dunja Petrovic, Jan Lj. Miljkovic, Daniel Thomas-Lopez, Youngeun Jung, Marko Miler, Sarah Mitchell, Verica Milosevic, Jose Eduardo Gomes, Moran Benhar, Bruno Gonzales-Zorn, Ivana Ivanovic-Burmazovic, Roberta Torregrossa, James R. Mitchell, Matthew Whiteman, Guenter Schwarz, Solomon H. Snyder, Bindu D. Paul, Kate Carroll and Milos R. Filipovic

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### **RESOURCE ARTICLE**

### SELECTIVE PERSULFIDE DETECTION REVEALS EVOLUTIONARILY CONSERVED ANTI-AGING EFFECTS OF S-SULFHYDRATION

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### ABSTRACT

Life on Earth emerged in a hydrogen sulfide (H<sub>2</sub>S)-rich environment eons ago and with it protein persulfidation mediated by H<sub>2</sub>S evolved as a signaling mechanism. Protein persulfidation or *S*-sulfhydration is a posttranslational modification of reactive cysteine residues, which modulate protein structure and/or function. Persulfides are difficult to label and study due to their reactivity and similarity with cysteine. Here, we report a facile strategy for chemoselective persulfide bioconjugation using dimedone-based probes, to achieve highly selective, rapid, and robust persulfide labeling in biological samples with broad utility. Using this method, we show that persulfidation is an evolutionarily conserved modification and that waves of persulfidation are employed by cells to resolve sulfenylation and prevent irreversible cysteine overoxidation to preserve protein function. We report an age-associated decline in persulfidation which is conserved across evolutionary boundaries. Accordingly, dietary or pharmacological interventions to increase persulfidation associate with increased longevity and improved capacity to cope with stress stimuli.

**Keywords:** hydrogen sulfide, protein persulfidation, hydrogen peroxide, sulfenylation, sulfonylation, redox signalling, aging, calorie restriction

#### **INTRODUCTION**

Although considered a toxic gas for more than a century, hydrogen sulfide (H<sub>2</sub>S) was one of the essential ingredients required for life to emerge on Earth (Patel et al., 2015). Early anaerobic bacteria flourished in H<sub>2</sub>S-rich environments and even used H<sub>2</sub>S instead of water for the first photosynthetic process (Filipovic et al., 2018; Wang, 2012). Two decades ago H<sub>2</sub>S re-emerged as an important signaling molecule produced by cells (Filipovic et al., 2018; Paul and Snyder, 2012; Szabó, 2007; Wang, 2012). Genetic deletion of the H<sub>2</sub>S producing enzyme cystathionine  $\gamma$ -lyase (CSE) can lead to hypertension (Yang et al., 2008). Animals exposed to H<sub>2</sub>S enter a suspended animation-like state (Blackstone, 2005), while dietary restriction-induced stress resistance and lifespan extension depends on intracellular H<sub>2</sub>S production (Hine et al., 2015). Despite beneficial effects of H<sub>2</sub>S, observed in a plethora of pathological states (Filipovic et al., 2018; Paul and Snyder, 2012; Szabó, 2007; Wallace and Wang, 2015), the mechanism or mechanisms underlying these effects remain poorly characterized. However, its role in the oxidative posttranslational modification (oxPTM) of cysteine residues, known either as protein *S*-sulfhydration or persulfidation, is thought to be one of its main beneficial mechanisms of action (Paul and Snyder, 2015).

Cysteine is a rare amino acid residue that often occurs in functional sites of proteins and represents a site for redox control of protein function (Marino and Gladyshev, 2010; Paulsen and Carroll, 2013). For example, hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) signals via the oxidation of cysteine residues to sulfenic acids (P-SOH), while part of nitric oxide signaling could be explained by cysteine *S*-nitrosation (Foster et al., 2009; Paulsen and Carroll, 2013). A variety of chemical approaches have been used to label and study cysteine modifications, leading to a broad range of fundamental and applied advances (Furdui and Poole, 2014; Paulsen and Carroll, 2013). However, protein persulfides (P-SSH) and their role in cell signaling managed to remain understudied, due to the fact that P-SSH are very reactive and their reactivity is similar to that of cysteine residues (Cuevasanta et al., 2015; Filipovic et al., 2018; Pan and Carroll, 2013). Consequently, this has made it difficult to design tools for selective labeling, hampering a better understanding of the function of this specific oxPTM. Current methods rely on blocking both thiols and persulfides with electrophiles and then releasing the latter with a reducing agent, but this approach is linked to many caveats (Dóka et al., 2016; Filipovic et al., 2018; Reisz et al., 2013).

Nonetheless, due to their enhanced nucleophilicity persulfides react readily with reactive oxygen species (ROS), while H<sub>2</sub>S itself is a poor ROS scavenger (Cuevasanta et al., 2015; Filipovic

et al., 2018; Ono et al., 2014). Given the fact that ROS play an important role in signaling (D'Autréaux and Toledano, 2007; Finkel, 2011) and aging (Finkel and Holbrook, 2000), it is tempting to speculate that the general beneficial effects of  $H_2S$  are evolutionary conserved and conveyed through protein persulfidation.

To better understand persulfidation *in vivo*, we explored the phenomenon that, if first transformed to mixed aromatic disulfides, protein persulfides could be selectively labeled by certain nucleophiles. Here we report the development of a new, dimedone-based method that enables chemoselective persulfide bioconjugation of proteins from a wide range of source materials *in vitro* and *in vivo*. Using this new method, we report that persulfidation plays an integral role in hydrogen peroxide-based signal transduction. We also found that protein persulfidation decreases with age and is maintained by interventions that increase lifespan across evolutionary boundaries, and may thus play a previously unrecognized protective role against aging.

#### RESULTS

#### Development of dimedone switch method

To be able to use nucleophilic substitution to tag persulfides, P-SSH need to first be transformed into a mixed disulfide (Wedmann et al., 2016; Zhang et al., 2014) in such a way that one of the sulfurs in the S-S bond possesses a much more enhanced electrophilicity (Figure S1A). Despite their selectivity in sulfenic acid labeling (Klomsiri et al., 2010; Paulsen and Carroll, 2013; Yang et al., 2014), dimedone-based probes (Figure 1A) could be an excellent candidate in the second step, serving as the nucleophile. They are additionally attractive candidates since a plethora of those probes are commercially available with different reporting moieties and have been thoroughly tested (Furdui and Poole, 2014; Paulsen and Carroll, 2013). However, for dimedonebased probes to be used in P-SSH labeling, the initial step needs to involve a reagent which not only reacts with P-SSH and thiols but also blocks sulfenic acids. 4-chloro-7-nitrobenzofurazan (NBF-Cl) fulfils these criteria. It is used as a tool for the blocking and detection of thiols, amines and sulfenic acids (Bernal-Perez et al., 2012; Ellis and Poole, 1997) and it should form a mixed disulfide upon its reaction with P-SSH (Figure 1B). We initiated our study by monitoring the labeling of the low molecular weight persulfide, N-methoxycarbonyl penicillamine persulfide (nmc-PSSH, (Artaud and Galardon, 2014)) (Figure 1C). Nmc-PSSH reacted readily with NBF-Cl resulting in a characteristic absorbance maximum at 412 nm (Figure 1D). Next, the addition of an equimolar amount of dimedone led to a fast disappearance of the 412 nm peak, suggesting that switching did occur (**Figure 1E**). ESI-TOF MS/MS analysis of the reaction mixture confirmed that the two main products are the NBF-SH and dimedone labeled nmc-PSSH (**Figure 1C**, Figure S1B-F).

We then evaluated the selectivity of the dimedone switch method by using human serum albumin (HSA) as a model (Cuevasanta et al., 2015). HSA has 17 disulfides and 1 free thiolate and is therefore both a good control and an example of a protein with an oxPTM of cysteine. Human serum albumin (HSA-SH), its sulfenylated (HSA-SOH) and persulfidated (HSA-SSH) forms reacted with NBF-Cl to generate products with distinct absorbance maxima that fit well to those reported in the literature (Ellis and Poole, 1997) (Figure S1G, I, K). The subsequent addition of dimedone caused tag-switching only in the HSA-SSH sample (**Figure 1F,G,** Figure S1H,J) resulting in the loss of HSA-SS-NBF absorbance and formation of both HSA-S-dimedone and NBF-SH products. Separation of treated samples by electrophoresis and subsequent immunoblotting with anti-dimedone antibody (Seo and Carroll, 2009) gave a positive signal for HSA-SSH only (**Figure 2A**). To confirm the labeling of naturally occurring persulfides, we used Thiosulfate Sulfur Transferase (TST, also known as Rhodanese), an enzyme that forms a persulfide during its catalytic cycle, as a model (Filipovic et al., 2018). Both immunoblotting with anti-dimedone antibody (**Figure 2B**) and ESI-TOF-MS (**Figure 2C**) revealed TST labeled with dimedone.

With these data in hand, we envisioned that the dimedone switch method could enable the installation of various payloads onto a protein of interest that would allow specific identification/visualization. We first used DCP-Bio1, a biotinylated form of dimedone (**Figure 1A**). Persulfides of HSA and GAPDH showed positive staining (Figure S2A, B), as detected by Cy5-streptavidin. Separation of samples by streptavidin magnetic beads and subsequent in-gel detection of green fluorescence originating from amino groups labeled with NBF-Cl (**Figure 1B**), showed the selective labeling of P-SSH but not other oxPTM (Figure S2A, B).

Next, we tested if the method can be used for proteomic analysis. TST persulfide was tagswitched with DCP-Bio1 and subjected to either trypsin or chymotrypsin digestion. Combined they covered 95% of the structure (Figure S2C, Supporting Dataset S1, S2) and only C248, present as a persulfide in the active site of the enzyme, was found to be labeled (Figure S2C). Other cysteine residues and several lysine residues were labeled with NBF alone (Supporting Data S1, S2), according to the reaction scheme in **Figure 1B**.





(A) (Upper) Labeling of sulfenic acids with dimedone. (Lower) Structures of dimedone-based probes.
 (B) Proposed dimedone switch strategy for persulfide labeling. In the first step proteins react with 4-chloro-7-nitrobenzofurazan (NBF-Cl) to label persulfides, thiols, sulfenic acids, and amino groups. Reaction with amino groups gives characteristic green fluorescence. In the second step, NBF tag is switched by a dimedone-based probe, selectively labeling persulfides.

(C) Model switch reaction with 100  $\mu$ M N-methoxycarbonyl penicillamine persulfide (nmc-PSSH), 100  $\mu$ M NBF-Cl and 500  $\mu$ M dimedone. MS analysis reveals formation of 4-thio-7-nitrobenzofurazan (535 nm) and dimedone labeled nmc-penicillamine, which under MS/MS conditions decomposes along the blue or red dash line. Numbers given in the brackets represent calculated *m*/*z* for the observed ions.

We also tested the possibility of detecting P-SSH directly in gel, by installing a Cy5fluorescence moiety through Copper(I)-catalyzed Azide-Alkyne Cycloaddition (CuAAC, click chemistry). In addition to labeling cysteines, NBF-Cl also reacts with amino groups giving a characteristic fluorescence with  $\lambda_{ex}$  at 488 nm (Bernal-Perez et al., 2012). Commercially available TST (already partially present as a persulfide) was incubated with either thiosulfate or dithiothreitol (DTT) to form a fully persulfidated or reduced TST, respectively. 20 µM reduced or persulfidated TST was mixed with 50 µM NBF-Cl and the persulfide was visualized using DAz-2/Cy5 CuAAC. On the one hand, while both untreated (lane 1, Figure S2D) and thiosulfate treated (lane 3, Figure S2D) TST showed Cy5 signal, the green fluorescence signal was significantly reduced in the fully persulfidated enzyme, despite the same load. On the other hand, the green fluorescence signal was much stronger in the fully reduced enzyme (lane 2, Figure S2), suggesting that at low NBF-Cl/protein ratio, switching caused by the dimedone-based probe could affect the intensity of green fluorescence (Figure S2D), so we opted for using at least 10-fold excess of NBF-Cl (Figure S2E). This use of NBF-Cl in excess additionally offered the opportunity to use the green fluorescence as a measure of the total protein load and to therefore quantify the persulfidation levels by measuring the Cy5/488 fluorescence signal ratio. Different components of click chemistry did not show any interference with the labeling in the presence of NBF-Cl (Figure S2F). Together these data display the utility of the dimedone switch approach in biological environments.

To be able to label cell extracts, we had to ensure that the method shows sufficient selectivity. The selectivity of dimedone probes for sulfenic acids have been demonstrated previously and no cross-reactivity with disulfides, *S*-nitrosothiols, HNE-modified cysteines or any other nucelophilic centers could be observed (Charles et al., 2007; Klomsiri et al., 2010; Yang et al., 2014). In fact, a blind proteomic search for off-targets of dimedone did not identify any

<sup>(</sup>**D**) Time-resolved spectra for the reaction of 100  $\mu$ M nmc-PSSH with 100  $\mu$ M NBF-Cl (pH 7.4, 23 °C). Arrows indicated disappearance of NBF-Cl and appearance of nmc-PSS-NBF adduct at 412 nm.

<sup>(</sup>E) Time-resolved spectral changes upon addition of 200  $\mu$ M dimedone to a reaction mixture shown in (D) (pH 7.4, 23 °C). Inset: Kinetics of decay of 412 nm absorbance maximum after addition of dimedone.

<sup>(</sup>F-G) 23  $\mu$ M HAS-SSH was left to react with 100  $\mu$ M NBF-Cl over 30 min in phosphate buffer (50 mM, pH 7.4) with 1% SDS, at 37 °C and then 200  $\mu$ M dimedone was added. UV-Vis spectral changes (F) and kinetic traces (G) show the decay of the 422 nm absorbance and the appearance of a 535 nm peak.

unwanted labeling (Yang et al., 2014). However, in the dimedone-switch labeling (i) incomplete blocking of sulfenic acids by NBF-Cl and (ii) potential interference of sulfenamides could represent possible caveats (Figure S2G).

To ensure efficient and selective labeling we decided to perform the cell lysis under denaturing conditions, which will permit fast unfolding and exposure of all cysteine and amine residues to NBF-Cl. Large excess of NBF-Cl and incubation at 37 °C should provide an efficient kinetic push for fast and complete labeling and blocking. Protein unfolding will also expose cyclic sulfenamides to more water, and since they exist in the equilibrium with sulfenic acids this equilibrium will be shifted towards sulfenic acids (Gupta and Carroll, 2016). Furthermore, NBF-Cl has been demonstrated to efficiently react with cyclic sulfenamides (Figure S2G) (Gupta and Carroll, 2016). Nonetheless, we used protein tyrosine phosphatase 1B (PTP1B), which forms a stable cyclic sulfenamide, as a model system (Paulsen and Carroll, 2013). PTP1B treated with H<sub>2</sub>O<sub>2</sub> (to form a mixture of sulfenic acid and cyclic sulfenamide) was labeled with DAz-2 (that bears a biorthogonal azide group) and then subsequently coupled to Cy5-alkyne via a CuAAC reaction, as expected, but not if it first reacted with NBF-Cl (lanes 1 and 2, Figure S2H). PTP1B persulfide however, could be labeled only if it first reacted with NBF-Cl and then with DAz-2/Cy5 CuAAC (lane 3, Figure S2H). Labeling was absent when NBF-Cl step was omitted (lane 4, Figure S2H).

The method's selectivity was further confirmed in cell lysates. HeLa cells lysed with NBF-Cl and tag switched with DAz-2/Cy5 CuAAC resulted in the labeling and in-gel detection of a red fluorescence signal only when all reagents were used (**Figure 2D**, Figure S21). Barely any signal could be detected in a control sample where NBF-Cl was absent (**Figure 2D**), confirming that lysis, incubation and protein precipitation (Figure S2I) would already be sufficient to get rid of reactive sulfenic acids and cyclic sulfenamides that could have remained uncapped by NBF-Cl. We found that lysis with 5 mM NBF-Cl is already sufficient to give maximal persulfide signal (Figure S2J). Treatment of cell lysates with DTT to reduce the disulfide bond formed in the reaction of NBF-Cl and protein persulfides, abolished the detected Cy5 fluorescence (**Figure 2E**), confirming the chemical mechanism of the dimedone switch approach proposed in **Figure 1B**. Lysis with dimedone to trap all sulfenic acids (Figure S2K) and subsequent labeling with NBF-Cl and then DCP-Bio1 (Figure S2L) or DAz-2/Cy5 CuAAC (Figure S2M) to switch tag persulfides, showed that the removal of sulfenic acids prior to persulfide labeling did not affect the detected signal, further confirming that our dimedone switch approach shows no cross-reactivity with sulfenic acids and/or cyclic sulfenamides (Figures S2K-M). Additionally, treatment of HeLa cells with different sources of H<sub>2</sub>S increased the intracellular persulfidation levels several-fold (**Figure 2F, G**). 200 nM mitochondria-targeted H<sub>2</sub>S donor, AP39, induced comparable increase as 200  $\mu$ M Na<sub>2</sub>S, confirming the strong pharmacological potential of this compound (**Figure 2F, G**).

We used DCP-Bio1 labeling to identify the endogenously persulfidated proteins in human erythrocytes (**Figure 2H**, Table S1). Out of 56 identified proteins, more than half were previously identified to bear oxidized cysteine residues. These proteins were either found to be prone to cysteine oxidation in red blood cells (RBC) depleted of haemoglobin (Delobel et al., 2016) or treated with diamide (Zaccarin et al., 2014), in RBC from peroxiredoxin II deficient mice (Yang et al., 2012) or are found to be directly persulfidated (Valentine et al., 1987). More importantly, both enzymes involved in H<sub>2</sub>S production in erythrocytes were also found to be persulfidated: 3-mercaptopyruvate sulfur transferase (MPST or MST) and methanethiol oxidase. The former is known to form a persulfide during the catalytic cycle (Yadav et al., 2013) while the latter produces both H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>S (Pol et al., 2018), facilitating the oxidation of a cysteine residue to a sulfenic acid during the catalytic cycle (Wood et al., 2003), were found to be persulfidated as well. It is also worth noting that all identified peptides, even those not falling within selection criteria (at least 2 reliable peptides and -10logP>50) originated from cysteine containing proteins, indicative of the very high selectivity of this approach.



Figure 2. Protein persulfide labeling and identification.

(A-B) Selectivity of dimedone-switch method for protein persulfides. Human serum albumin (HSA, A) and TST (B) were used as models. Dimedone labeling was visualized by rabbit polyclonal anti-dimedone antibody. Ponceau S staining was used for the protein load.

(C) Deconvoluted mass spectra 20  $\mu$ M rhodanese (black), rhodanese treated with 100  $\mu$ M NBF-Cl (blue) and rhodanese treated first with 100  $\mu$ M NBF-Cl then with 500  $\mu$ M dimedone (red).

(**D**) In-gel detection of P-SSH levels in cells using the dimedone switch method. HeLa cells were lysed with or without supplementation of 10 mM NBF-Cl, and probed for persulfide labeling with or without DAz-2, followed by Cy5-alkyne using CuAAC. Gels were also stained with Coomassie Brilliant Blue. Fire pseudo-colouring was used to visually enhance the signal. Green fluorescence corresponds to the total protein load (NBF-protein adducts). (E) MEF cells lysed with or without 20 mM NBF-Cl samples and then treated with or without 20 mM DTT and labeled with DAz-2/Cy5-alkyne using CuAAC.

(**F-G**) Protein persulfidation levels in HeLa cells treated with different H<sub>2</sub>S donors: 200  $\mu$ M Na<sub>2</sub>S (H<sub>2</sub>S) for 45 min, 200  $\mu$ M GYY4137 for 2 hr, 200 nM AP39 for 2 hr and 2 mM D-cysteine (D-Cys) for 1 hr. Ratio of Cy5/488 signals is used for the quantification (**G**). Data shown as a mean ± SD. of 3 individual experiments. \*\* p<0.01 vs. control. (**H**) Schematic depiction of the protocol used for the proteomic analysis of endogenous persulfidation in RBC.

# Persulfidation is evolutionarily conserved and controlled by $H_2S$ generation from transsulfuration pathway and cysteine catabolism

Although initial studies suggested that the main source of intracellular persulfides is H<sub>2</sub>S, produced predominantly by CSE (Figure 3A) (Filipovic et al., 2018; Mustafa et al., 2009; Paul and Snyder, 2012), recent findings questioned this by claiming that persulfides are synthesized during protein translation and are not related to the transsulfuration pathway or cysteine catabolism (Figure 3A) (Akaike et al., 2017). Persulfidation levels were significantly reduced in mouse embryonic fibroblasts (MEFs) originating from CSE<sup>-/-</sup> animals (Figure 3B). Interestingly, repeated cell splitting of the same cell line leads to less pronounced differences in these levels due to compensatory overexpression of cystathionine- $\beta$ -synthase (CBS, Figure S3A, B). CSE is profoundly diminished in Huntington's Disease (HD), a neurodegenerative disease triggered by the expansion of polyglutamine repeats in the huntingtin protein (Paul et al., 2014). In striatal cellline models of HD (STHdh $Q^{7/Q7}$  and STHdh $Q^{111/Q111}$ ) harbouring 7 and 111 polyglutamine repeats, we now show that the lack of CSE results in the barely detectable P-SSH levels in the ST*Hdh*<sup>Q111/Q111</sup> cells (Figure 3C). CSE is known to be the predominant source of H<sub>2</sub>S in this cell type (Paul et al., 2014; Sbodio et al., 2016). Furthermore, the inhibition of cystine transporter, system x<sub>c</sub> with erastin also resulted in the loss of protein persulfidation (Figure 3D). Additionally, pharmacologically induced overexpression of CSE by Golgi stressor, monensin (Sbodio et al., 2018), on the other hand, resulted in an increase of intracellular persulfidation (Figure 3E).

We expanded the screening to different phyla and regna, and in all of them endogenous persulfidation was found to be controlled by  $H_2S$  produced via the transsulfuration pathway or cysteine catabolism (**Figure 3A**). We used an *E. coli* strain that is transformed with the phsABC operon (pSB74 plasmid) encoding for thiosulfate reductase, causing increased  $H_2S$  production.

Treatment of these bacteria with thiosulfate resulted in a two-fold increase of bacterial protein persulfidation when compared to control (**Figure 3F**). On the other hand, *cth-1* and *mpst-3* mutants of *C. elegans* (lacking CSE and MPST, respectively, **Figure 3G**) showed lower P-SSH levels. *Drosophila melanogaster* flies overexpressing CSE (Snijder et al., 2015) showed increased P-SSH levels (**Figure 3H**), while kidneys from CSE<sup>-/-</sup> mice showed reduced persulfidation levels (**Figure 3I**). Finally, endogenous persulfidation could be observed in human RBC, in both membrane and cytoplasm, confirming the proteomic data (**Figure 3J, Table 1**).

The dimedone switch method was also successfully applied for the visualization of intracellular persulfides by confocal microscopy (**Figure 3K**, Figure S3C). MEFs lacking CSE showed barely any detectable intracellular levels of P-SSH, while both H<sub>2</sub>S and D-cysteine treatments increased those levels several-fold. Independent of CSE, both sources of H<sub>2</sub>S increased the P-SSH levels highlighting the essential role of H<sub>2</sub>S in protein persulfide formation (**Figure 3K**, Figure S3C). Furthermore, wide-field fluorescence deconvolution microscopy provided the first high-resolution images of intracellular persulfidation (Figure S3D-F). The P-SSH signal is dispersed throughout the cell, with some of it being detected even in the nucleus. The P-SSH signal in D-cysteine treated cells seems to be predominantly localized in mitochondria, in accordance with the fact that D-cysteine is a substrate for cysteine catabolism path via MPST (Shibuya et al., 2013) (Figure S3F).

#### Broad applicability of dimedone switch method: antibody microarray

To further showcase the applicability of the dimedone switch method, we used an antibody microarray-like approach, where antibodies for specific proteins were immobilized on an NHS-activated surface (**Figure 3L**). As the samples carry both green and red fluorescence, reflecting the total load and P-SSH levels respectively, proteins of interest could be analyzed by this approach and their P-SSH levels assessed. We selected antibodies against a series of proteins (**Figure 3L**) for which persulfidation has been shown, or which form persulfides in their catalytic cycles. In general, the lack of CSE reduced P-SSH levels of target proteins while D-cysteine treatment increased it, albeit with different efficiencies. The selectivity of the method is once more demonstrated, as enzymes reported to form persulfides at their active sites during the H<sub>2</sub>S oxidation, such as sulfide:quinone oxidoreductase (SQOR or SQR) and TST, showed high endogenous P-SSH levels with minimal changes upon further D-cysteine treatment. However, a significant decrease of steady-state persulfide levels of those enzymes was observed in cells lacking CSE. Aside from the proteins whose persulfidation has already been demonstrated

(GAPDH, HSP70, Keap 1, B-actin, Parkin) (Mustafa et al., 2009; Vandiver et al., 2013; Yang et al., 2013; Zhang et al., 2014), this approach led to the observation that manganese superoxide dismutase (MnSOD) could be persulfidated as well (Figure 3L). Unlike prokaryotes, most eukaryotic MnSOD have at least one cysteine residue (Figure 3M, Figure S3G) and exhibit strong product inhibition by H<sub>2</sub>O<sub>2</sub> (Hearn et al., 2001). Our experiments with human recombinant MnSOD showed that a 15 min exposure to a 3-fold excess of H<sub>2</sub>O<sub>2</sub>, inhibited MnSOD activity  $(0.15 \pm 0.06 \text{ x } 10^3 \text{ U/mg vs. } 2.91 \pm 0.07 \text{ x } 10^3 \text{ U/mg in the control})$  while the co-treatment with 5fold excess of H<sub>2</sub>S rescued the enzymatic activity  $(1.92 \pm 0.07 \text{ x } 10^3 \text{ U/mg})$  (Figure 3N). MS/MS analysis of human recombinant MnSOD treated with H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>S and labeled by the dimedone switch method (using DCP-Bio1 as a switching agent) confirmed that C193 was indeed persulfidated (Figure S3H, I, Dataset S3, S4). Other studies have pointed out that the same cysteine residue is redox sensitive (Matsuda et al., 1990). Furthermore, persulfidated MnSOD was more resilient to tyrosine nitration by peroxynitrite (yield of nitration per subunit  $3\pm 2\%$ ) when compared to the control (15±4%/subunit), suggesting that persulfidation of MnSOD might serve as a protective mechanism against detrimental enzyme nitration found in many disease states (Szabó et al., 2007).


# Figure 3. Intracellular persulfidation is evolutionarily conserved and controlled by H<sub>2</sub>S producing enzymes.

(A) Intracellular  $H_2S$  production is catalyzed by cystathionine gamma lyase (CSE) and cystathionine beta synthase (CBS), *via* the reverse transsulfuration pathway, and by 3-mercaptopyruvate sulfur transferase (MPST) in cysteine catabolism pathway. Hcy: homocysteine; Cys: cysteine; 3MP: 3-mercaptopyruvate; CAT: cysteine aminotransferase; DAT: D-amino acid aminotransferase.

(B) P-SSH levels in MEF cells from wild type ( $CSE^{+/+}$ ) and  $CSE^{-/-}$  mice. Fire pseudo-colouring was used to visually enhance the signal. Ratio of Cy5/488 signals is used for the quantification. n = 4. \*\* p<0.01 Inset: Western blot analysis of CSE levels. n = 3.

(C) P-SSH levels in STHdh<sup>Q7/Q7</sup> and STHdh<sup>Q111/Q111</sup> cells. Ratio of Cy5/488 signals is used for the quantification. n = 4. \*\* p<0.01 Inset: Representative Western blot of CSE protein expression levels. n = 3.

(**D**) The effect of 1 and 10  $\mu$ M Erastin (18.5 hr) on P-SSH levels in CSE<sup>+/+</sup> MEF cells. Ratio of Cy5/488 signals is used for the quantification. Data shown as a mean  $\pm$  SD. of 4 individual experiments. \*\* p<0.01

(E) P-SSH levels in CSE<sup>+/+</sup> MEF cells for control, C, and treated with 1  $\mu$ M Monensin, Mone (18 hr). Ratio of Cy5/488 signals is used for the quantification. Data shown as a mean  $\pm$  SD. of 3 individual experiments. \*\* p<0.01 Inset: Representative Western blot of CSE protein expression levels. n = 3.

(F) P-SSH levels in *E. coli* without (WT) or with phsABC operon (pSB74 plasmid) that encodes thiosulfate reductase and results in H<sub>2</sub>S production. Both strains were treated with or without thiosulfate (TS, 4 hr at 37°C). Ratio of Cy5/488 signals is used for the quantification. n = 3. \* p<0.05, \*\* p<0.01

(G) P-SSH levels in wild type (N2), *cth-1* (CSE) and *mpst-3* (MPST) *C. elegans* mutants. ~ 16000 worms per sample. Ratio of Cy5/488 signals is used for the quantification. n = 3. \*\* p<0.01

(H) P-SSH levels in wild type  $(y^1w^{118})$  Drosophila melanogaster and flies with different levels of CSE overexpression. 3-4 flies per samples. Ratio of Cy5/488 signals is used for the quantification. n = 3. \* p<0.05, \*\* p<0.01

(I) P-SSH levels in kidney extracts form wild type (C57BL/6J) and CSE<sup>-/-</sup> mice. Ratio of Cy5/488 signals is used for the quantification. n = 3 animals. \*\* p<0.01

(J) Protein persulfidation in RBC membrane and cytosol from a healthy human donor.

(K) Confocal microscopy images of intracellular protein persulfide levels of  $CSE^{+/+}$  and  $CSE^{-/-}$  MEFs treated or not with 200  $\mu$ M Na<sub>2</sub>S (H<sub>2</sub>S) or 2 mM D-Cys for 1 hr. Cy5 signal corresponds to protein persulfides, 488 nm signal corresponds to NBF-adducts. Nuclei stained with DAPI. Scale bar 20  $\mu$ m.

(L) Antibody microarray-like approach to study persulfidation status of specific proteins. Schematic depiction of the method (lower part) and the actual readout (upper part) for the ten listed proteins. Cell lysates from  $CSE^{+/+}$ ,  $CSE^{-/-}$  and  $CSE^{+/+}$  MEFs treated with D-Cys (2 mM, 1 hr) were compared. Results are presented as a mean  $\pm$  SD. from 3 independent experiments.

(M) Ribbon structure of two subunits from human MnSOD (PDB: 1pl4), highlighting the cysteine residues and manganese containing active site.

(N) SOD activity of 13  $\mu$ M MnSOD, MnSOD pretreated with 3-fold excess of H<sub>2</sub>O<sub>2</sub> (15 min, 37 °C) and MnSOD pretreated with both 3-fold excess of H<sub>2</sub>O<sub>2</sub> and 5-fold excess of H<sub>2</sub>S. SOD activity was measured using cytochrome c as a reporting molecule which is reduced by the superoxide generated from the xanthine/xanthine oxide system. The increase in absorption at 550 nm recorded is proportional to the reduction of ferricytochrome c, and was measured over 2 minutes. Results are presented as a mean  $\pm$  SD. from 3 independent experiments.

# Persulfidation is intrinsically linked to $H_2O_2$

For H<sub>2</sub>S to be able to modify cysteine residues, an oxidant is required - a role that could be played by  $H_2O_2$ . Protein sulferillation, as a consequence of  $H_2O_2$  production, represents an important signaling event (Paulsen and Carroll, 2013; Poole et al., 2004). However, P-SOH formation should be controlled in order to prevent overoxidation of cysteine residues to sulfinic (P-SO<sub>2</sub>H) and sulfonic acids (P-SO<sub>3</sub>H) that results in a loss of protein function (Figure 4A). Previous studies showed that protein P-SOH react two orders of magnitude faster with  $H_2S$ , than with glutathione at pH 7.4 (Cuevasanta et al., 2015) and our proteomic analysis of persulfidated proteins in RBC showed a significant overlap with proteins known to be sulferylated (Table S1). We hypothesized that the reaction of H<sub>2</sub>S with P-SOH could represent an integral part of H<sub>2</sub>O<sub>2</sub>-induced redox signaling and the main way for resolving P-SOH back to thiols. To test this, we first exposed wild type (CSE<sup>+/+</sup> or WT) and CSE<sup>-/-</sup> MEFs to H<sub>2</sub>O<sub>2</sub>. While 100 µM H<sub>2</sub>O<sub>2</sub> induced no detectable increase in P-SOH levels in CSE<sup>+/+</sup> cells, a massive increase in sulfenylation was detected in CSE<sup>-/-</sup> that decreased as exposure time increased (Figure 4B). 500 µM H<sub>2</sub>O<sub>2</sub> was required to cause the same magnitude of P-SOH formation in  $CSE^{+/+}$  cells (Figure 4B). This effect could be completely abolished by pre-incubating the cells with 100 µM H<sub>2</sub>S donor, GYY4137 (Figure S4A). Conversely, the P-SSH levels in CSE<sup>+/+</sup> cells increased time-dependently when treated with 100 and 500 µM H<sub>2</sub>O<sub>2</sub> but remained very low in CSE<sup>-/-</sup> cells (Figure 4C). Recent development of selective probes for sulfinic acids (P-SO<sub>2</sub>H) (Akter et al., 2018) allowed us to test how sulfinylation changes in cells lacking endogenous  $H_2S$ . A strong overall increase of sulfinylation was observed in CSE<sup>-/-</sup> cells treated with 100  $\mu$ M  $H_2O_2$  for 15 min, but this P-SO<sub>2</sub>H dropped back to normal after 30 min suggesting that those cysteines either became hyperoxidized or reduced back by sulfiredoxin (Akter et al., 2018) (**Figure 4D**). 500  $\mu$ M  $H_2O_2$  caused increase of sulfinylation only on a selected group of proteins in CSE<sup>+/+</sup> cells. Sulfinylation of CSE<sup>-/-</sup> cells, caused by 500  $\mu$ M  $H_2O_2$ , seems to be somewhat lower than with 100  $\mu$ M dose, presumably due to their higher sensitivity and a stronger cysteine hyperoxidation to sulfonic acid.

To address how endogenous H<sub>2</sub>S controls H<sub>2</sub>O<sub>2</sub>-induced cysteine oxidation on a molecular level we monitored the cysteine oxidation status in a redox sensitive protein, DJ-1. C106 is known to undergo oxidation to a sulfinic (Akter et al., 2018) and even sulfonic acid (Fernandez-Caggiano et al., 2016), while our proteomic analysis identified DJ-1 as a target for persulfidation as well (Supporting Table S1). CSE<sup>+/+</sup> and CSE<sup>-/-</sup> cells were treated with 100 µM H<sub>2</sub>O<sub>2</sub> for 15 and 30 min, labeled for P-SOH (using DCP-Bio1), P-SSH (using DCP-Bio1 as a switching reagent) and P-SO<sub>2</sub>H (using BioDiaAlk) and immunoprecipitated. In parallel, using an antibody selective for DJ-1 C106 sulfonic acid, we assessed the DJ-1-SO<sub>3</sub>H levels in those samples (Figure 4E, Figure S4B). H<sub>2</sub>O<sub>2</sub> treatment of CSE<sup>+/+</sup> cells resulted in increased persulfidation of DJ-1 (in 15 and 30 min) and increased sulfenylation and sulfonylation (P-SO<sub>3</sub>H) after 30 min. However, in CSE<sup>-/-</sup> cells already low P-SSH levels continued to decrease in cells treated with H<sub>2</sub>O<sub>2</sub>. The basal levels of DJ-1-SOH, DJ-1-SO<sub>2</sub>H and DJ-1-SO<sub>3</sub>H were much higher in untreated CSE<sup>-/-</sup> cells when compared to CSE<sup>+/+</sup>. While the DJ-1-SOH decreased with H<sub>2</sub>O<sub>2</sub> treatment in CSE<sup>-/-</sup> cells, DJ-1-SO<sub>2</sub>H and DJ-1-SO<sub>3</sub>H levels continued to further increase (Figure 4E, Figure S4B) confirming that the lack of H<sub>2</sub>S to trap P-SOH results in cysteine hyperoxidation. Taken together these data confirmed that persulfidation, controlled by endogenous H<sub>2</sub>S production, is an integral part of H<sub>2</sub>O<sub>2</sub>-induced redox changes in proteins.



# Figure 4. Endogenous H<sub>2</sub>S controls cysteine oxidation caused by H<sub>2</sub>O<sub>2</sub>.

(A) The proposed mechanism for the redox switching between H<sub>2</sub>O<sub>2</sub>-induced thiol oxidation and persulfidation. (B-D) Cysteine oxPTM levels in CSE<sup>+/+</sup> (WT) and CSE<sup>-/-</sup> MEF cells treated with 100 or 500  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 15 and 30 min. (B) Protein sulfenylation (PSOH) – Proteins were labeled with DCP-Bio1 and blots were visualized with streptavidin-488 on a Typhoon FLA 9500. GAPDH was used as a loading control. Representative blots of 4 separate experiments and quantification of the streptavidin-488 signal normalized to the GAPDH levels. (C) Protein persulfidation (PSSH) - Proteins were labeled with DAz-2:Cy5 as a switching agent. Fire pseudo-colouring was used to visually enhance the PSSH signal. Green fluorescence (488 nm) corresponds to the total protein load (NBF-protein adducts). Ratio of Cy5/488 signals is used for the quantification. n = 3. (D) Protein sulfinylation (PSO<sub>2</sub>H) - Proteins were labeled with streptavidin-Cy5. GAPDH was used as a loading control. Representative blots of n = 5 and quantification of the streptavidin-Cy5 signal normalized to the GAPDH levels. PSOH and PSSH values are normalized to the levels found in untreated cells. \*p<0.01 compared to the untreated CSE<sup>+/+</sup> cells; #p<0.05 compared to the untreated CSE<sup>-/-</sup> cells.

(E) Persulfidation, sulfenylation and sulfonylation of DJ-1. WT and CSE<sup>-/-</sup> MEF cells were treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 15 or 30 min, labeled for P-SSH, P-SOH and P-SO<sub>2</sub>H using biotinylated reagents, immunoprecipitated with anti-DJ-1 antibody immobilized to agarose beads and immunoblotted with anti-biotin antibody. For sulfonylated DJ-1, antibody selective for C106 sulfonic acid of DJ-1 was used. n = 4. \*\*p<0.01 vs. untreated WT. # p<0.05, ##p<0.01 vs. untreated CSE<sup>-/-</sup> cells.

# *P-SSH waves follow P-SOH formation: implications for RTK-H<sub>2</sub>O<sub>2</sub> signaling*

The importance of P-SOH signaling is best exemplified by the receptor tyrosine kinase (RTK) activation (Finkel, 2011; Paulsen et al., 2011; Sundaresan et al., 1995), thus we looked for the temporal correlation between P-SOH and P-SSH (**Figure 5A**). HeLa cells treated with 100 ng/ml of epithelial growth factor (EGF) responded by a sharp rise in P-SOH within the first 5-15 min that dropped back to basal values by 30 min (**Figure 5B**). P-SSH levels however, followed a phase shifted curve, with the levels initially dropping at 5 min and then reaching a maximum at 30 min (**Figure 5B**). This correlated well with the increase in expression of all three H<sub>2</sub>S producing enzymes with EGF (Figure S4C).

To confirm the interplay between of P-SOH and P-SSH, we pretreated HeLa cells with GYY4137 (**Figure 5C**, Figure S4D) or with a mix of CSE and CBS inhibitors (propargylglycine and aminooxyacetic acid, **Figure 5D**, Figure S4E) for 30 min to either increase or decrease, respectively, the intracellular H<sub>2</sub>S and P-SSH levels. Pretreatment with GYY4137 indeed induced an increase of P-SSH, and upon EGF stimulation these levels continued to rise over 30 min, while P-SOH initially dropped and remained low and unchanged (**Figure 5C**, Figure S4D). In contrast, the pharmacological inhibition of endogenous H<sub>2</sub>S production resulted in a sharp rise of P-SOH levels, peaking at 5 min, and being fully resolved at 15 min, presumably due to cysteine overoxidation, as P-SSH levels remained very low and unchanged (**Figure 5D**, Figure S4E). These results strongly suggested that persulfidation represents the innate mechanism that cells use to resolve signaling by sulfenylation. At the same time, as both labeling approaches use dimedone-based probes (for PSSH using at a switching agent), these data confirmed once more that the dimedone switch method can distinguish P-SSH from P-SOH.

We then tested other RTK pathways. The treatment of human umbilical endothelial vein cells (HUVEC) with 40 ng/ml of vascular endothelial growth factor (VEGF) showed similar phase-shift curves for P-SOH and P-SSH, with P-SOH peaking at 5 min and reverting back to basal levels already after 15 min, while P-SSH levels peaked at 15 min and stayed high even after 30 min

(**Figure 5E**, Figure S4F). P-SSH increased ~ 9 times in cells exposed to VEGF for 15 min, in accordance with the very high H<sub>2</sub>S production rate that these cells possess (Filipovic et al., 2018; Lin et al., 2013). The treatment of neuroblastoma cells (SH-SY5Y) with insulin, produced again a distinct peak of P-SSH levels, with the peaking time and the intensity of change correlating directly with the dose of insulin (**Figure 5F**, Figure S4G). A sharp peak of sulfenylation was observed after 2 min of exposure to 100 ng/mL insulin and it was followed by a wave of persulfidation that peaked at 5 min (**Figure 5F**, Figure S4G). When 200 ng/mL was used, the P-SSH wave of greater amplitude was detected, with peaking time being 2 min, presumably due to a stronger and faster H<sub>2</sub>O<sub>2</sub> flux produced by higher insulin dose. The kinetics of intracellular sulfenylation preceded the P-SSH wave in a phase shifted manner (Figure S4H). Finally, we used CSE<sup>+/+</sup> and CSE<sup>-/-</sup> MEFs and treated them with 100 ng/ml EGF. The temporal profile of P-SSH in CSE<sup>+/+</sup> was quite similar to that observed for HeLa cells and was inhibited in CSE<sup>-/-</sup> cells (Figure S4I,J). On the other hand, sulfenylation was much stronger in CSE<sup>-/-</sup> cells (Figure S4I,J).

Next, we turned our attention to understanding the biological relevance of these waves of persulfidation. EGF receptor activation is regulated by sulfenylation (Paulsen et al., 2011) so we focused on understanding if and how persulfidation could control the duration of EGF signaling. We first looked for the persulfidation of EGFR using commercially available antibody microarray plates for the EGFR pathway. Pentaplicates of two different antibodies for EGFR showed a strong increase in EGFR persulfidation in HeLa cells treated with EGF for 30 min (**Figure 5G**). Persulfidation of EGFR had a functional effect on the downstream signaling. Phosphorylation of Y1068, activated by cysteine sulfenylation, was strongly impaired in GYY4137 pretreated HeLa cells (**Figure 5H**). Correspondingly, the activation of the EGF receptor monitored in live cells also revealed a stronger receptor activation in cells pretreated with CSE and CBS inhibitors (PG and AOAA), an effect that could be reduced by GYY4137 pretreatment (**Figure 5I**). Furthermore, the inhibition of H<sub>2</sub>S production to increase the half-life of EGFR sulfenylation caused a significant increase of extracellular signal-regulated kinase (ERK) phosphorylation even without EGF stimulation (Figure S4K).

Many of the cysteine containing phosphatases important for EGFR signaling have been already shown to be sulfenylated (Paulsen et al., 2011). This is particularly true for PTEN, PTP1B and SHPTP2, which we now find to be persulfidated as well (Figure S4L). In addition, using the EGFR pathway antibody microarray, we also assessed the persulfidation status of kinases downstream of EGFR (**Figure 5J**, Dataset S5). Numerous protein targets were identified with an

increased persulfidation status upon exposure to EGF. It is interesting that besides actin, kinases involved in the regulation of cytoskeleton rearrangements and cell motility are particularly affected (**Figure 5J, K**, Figures S4M-R). Taken together these data suggest that the formation of protein persulfides represents a redox switch for controlling cellular signaling initiated by  $H_2O_2$  and P-SOH formation.



Figure 5. Waves of protein persulfidation in RTK signaling.

(A) Schematic representation of the signaling events triggered by the epidermal growth factor receptor (EGFR) activation. Nox: NADPH oxidase; AQP: aquaporin.

(B) HeLa cells treated with 100 ng/mL EGF for 5, 15, 30 or 60 min were analyzed for protein sulfenylation (labeled using DCP-Bio1 and visualized with streptavidin-488, levels calculated using  $\beta$ -tubulin as a loading control) and protein persulfidation (using dimedone switch method with Cy5 as a reporting molecule, levels calculated as a ratio of Cy5/488 fluorescence readouts). (Right) In-gel fluorescence of P-SSH levels and Western blots for P-SOH levels. (Left) Temporal dynamics of P-SSH and P-SOH changes upon EGF exposure. n≥3. Values are presented as a mean ± SD. \*\* p<0.01.

(C) Quantification of P-SSH and P-SOH changes as a function of time upon EGF exposure in HeLa cells, pretreated with GYY4137 (100  $\mu$ M) for 30 min, prior the EGF treatment. n≥3. Values are presented as a mean ± SD. \*\* p<0.01. (D) Quantification of P-SSH and P-SOH changes as a function of time upon EGF exposure in HeLa cells, pretreated with 2 mM mixture of inhibitors, aminooxyacetic acid (AOAA) and propargylglycine (PG) (1:1, 30 min), prior the EGF treatment. n≥3. Values are presented as a mean ± SD. \*\* p<0.01.

(E) Quantification of P-SSH and P-SOH changes in HUVEC as a function of time upon VEGF (40 ng/mL) exposure.  $n \ge 3$ . Values are presented as a mean  $\pm$  SD. \*\* p<0.01.

(F) The effect of different insulin concentrations on P-SSH levels in neuroblastoma (SHSY5Y) cells as a function of time of insulin exposure. n $\geq$ 3. Values are presented as a mean  $\pm$  SD. \*\* p<0.01 vs. untreated, <sup>##</sup> p<0.01 100 nM vs. 200 nM.

(G) Persulfidation of EGF receptor of HeLa cells treated with 100 ng/mL EGF for 30 min, detected by two different antibodies using antibody microarray slides. Each antibody was spotted in pentaplicated. 2 technical replicates were performed. Values are presented as a mean  $\pm$  SD. \*\* p<0.01.

(H) Time-dependent phosphorylation of EGF receptor tyrosine 1068 (Y1068) as a response to EGF. HeLa cells were pretreated or not with GYY4137 (100  $\mu$ M) for 2 hr prior to exposure to EGF (100 ng/mL). n = 3. \*\* p<0.01 GYY4137 treated vs untreated.

(I) Real-time measurement of EGF receptor activation in living cells recorded with xCELLigence RTCA DP system. HeLa cells were also pretreated with GYY4137 (100  $\mu$ M, 30 min) or with 2 mM mixture of AOAA and PG (1:1, 30 min). EGF receptor activation was initiated by the addition of 150 ng/mL EGF. n = 4. Values are presented as a mean  $\pm$  SD. \*\* p<0.01.

(J) Antibody microarray analysis of persulfidation of different kinases involved in the EGF signaling. HeLa cells were treated with 100 ng/mL EGF for 30 min. Each antibody was spotted in pentaplicated. 2 technical replicates were performed.

(K) Schematic presentation of protein targets involved in actin remodeling, cytoskeleton regulation and cell motility, found to be persulfidated in cells treated with 100 ng/mL EGF for 30 min.

# Protein persulfidation as a rescuing path from cysteine hyperoxidation

While protein sulfenylation (and even sulfinylation for some proteins) represents a signaling event, uncontrollable production of  $H_2O_2$  or any of the ROS would result in cysteine hyperoxidation and loss of function (**Figure 6A**). Our initial hypothesis was that due to its small size, diffusibility and high production flux (Cuevasanta et al., 2012; Filipovic et al., 2018; Vitvitsky et al., 2012),  $H_2S$  could react as the first line of cellular defence against cysteine hyperoxidation (**Figure 6A**). Formed persulfides would be much better nucleophiles than cysteines alone, and could scavenge (Cuevasanta et al., 2015) more of the damaging oxidants. Owing to their reducible S-S bond, the ensuing S-sulfocysteine should be readily reduced by disulfide reductases, such as thioredoxin (Trx) (**Figure 6A**) creating a reduction pathway which would recycle it back to its thiol form. It has been already demonstrated that Trx could reduce S-sulfocysteine formed in the active site of 3'-phosphoadenosine-5'-phosphosulfate reductase (Palde and Carroll, 2015).

To address the reversibility of persulfide oxidation, we used S-sulfocysteine (SSC) as a model system (**Figure 6B**). Incubation of human recombinant Trx with SSC at an equimolar ratio resulted in the complete oxidation of Trx, as observed by the leftward mass shift ( $\Delta m/z = -2$ ), corresponding to the formation of a disulfide bond (**Figure 6C**). When the C32S mutant, which is catalytically inactive, was incubated with the same amount of SSC, no spectral change could be observed (Figure S5A); whilst incubation with the C35S mutant, which initially reacts with the substrate but cannot complete the catalytic cycle, resulted in the characterization of the disulfide adduct of Trx with cysteine (**Figure 6D**, Figure S5B). Spectrofluorometric kinetic analysis of the reaction (**Figure 6E**, S5C) derived an estimated rate constant of 6.1 ± 0.2 x 10<sup>4</sup> M<sup>-1</sup> s<sup>-1</sup>, one order of magnitude faster than the reaction of Trx with cysteine persulfide and two orders of magnitude faster than the reaction with cysteine (Wedmann et al., 2016). Coupled with thioredoxin reductase (TrxR), the reaction was also faster than with cysteine (Figure S5D). Thioredoxin-related protein (TRP14) however, was not as efficient in cleaving SSC as human Trx (Figure S5E).

We then assessed how persulfidation levels of different living systems correlate with their ability to resist stress.  $CSE^{-/-}$  MEFs showed slower growth and were much more sensitive to  $H_2O_2$  when compared to the  $CSE^{+/+}$  cells (**Figure 6F**); *S. cerevisiae* proved to be no exception either. Despite a slightly different cysteine and  $H_2S$  metabolism (Figure S5F), the *S. cerevisiae* mutant  $\Delta cys3$  (CSE) showed growth retardation (Figure S5G) and had lower P-SSH and  $H_2S$  levels (Figure S5H,I). In parallel to this the mutant was found to be more sensitive to  $H_2O_2$  than the wild type (**Figure 6G, H**).  $\Delta cys4$  (CBS), on the other hand, exhibited somewhat higher  $H_2S$  and P-SSH levels (Figure SH,I) and was seen to be more resistant to  $H_2O_2$  (**Figure 6H**).

We also tested the sensitivity of *C. elegans* to different ROS-inducing stressors. *cth-1* (CSE) and *mpst-3 C. elegans* mutants showed enhanced sensitivity to paraquat (**Figure 6I**) with ~ 50% of *cth-1* animals dead within 4 hr of exposure (compared to ~80% alive for N2). Another ROS stressor, sodium arsenite, also proved to be more toxic to *cth-1* mutants compared to N2 (**Figure 6J**). Enhanced sensitivity of *cth-1* to paraquat and arsenite could be rescued by pretreatment with H<sub>2</sub>S donors to increase the intracellular P-SSH levels (**Figure 6K**, Figure S5J, L). Even the N2 showed better survival after exposure to paraquat or arsenite, just by a 3 hr pretreatment with either GYY4137 or AP39 (Figure S5J, K).



# Figure 6. Cytoprotective effects of protein persulfidation.

(A) The proposed mechanism for the protective effects of protein persulfidation. Trx-thioredoxin, TrxR-thioredoxin reductase.

(B) Model reaction of S-sulfocysteine (SSC) with human thioredoxin (hTrx).

(C) Deconvoluted MS spectrum of 10 μM human recombinant Trx (black) and Trx treated with 10 μM S-sulfocysteine (SSC) (red).

(**D**) Deconvolution of MS of 10  $\mu$ M C35S Trx before (black) and after (red) the reaction with 10  $\mu$ M SSC showing appearance of TrxS-S-Cys adduct in sample treated with SSC.

(E) Plot of  $k_{obs}$  vs. concentration of SSC for the reaction with human recombinant Trx. Reaction was followed fluorometrically by measuring conformational changes induced in Trx due to the cysteine oxidation. Values presented as a mean  $\pm$  SD. n = 3

(F) Toxicity of  $H_2O_2$  in CSE<sup>+/+</sup> and CSE<sup>-/-</sup> MEFs. Values presented as a mean  $\pm$  SD. n = 3.

(G-H) Flow cytometry analysis of cell death using propidium iodide (FL2A channel). Different *S. cerevisiae* strains were cultured overnight, adjusted to  $OD_{600} = 2$ , and grown for 27 hr without or with 10 mM and 20 mM H<sub>2</sub>O<sub>2</sub>. Upper left quadrant was used as a measure of dead cells. 150000 cells were analysed per measurement. n=2. \*\*p<0.01 vs. untreated cells in the same group, <sup>##</sup>p<0.01 vs. corresponding treatment of BY4742 cells.

(I-J) Survival curves of N2, *cth-1* and *mpst-3 C. elegans* strains exposed to 60 mM paraquat (I) and 5 mM sodium arsenite (J). N>80 worms. Experiments were performed in triplicate. \*\* p < 0.01.

(**K**) The effect of short-term (3 hr) pre-exposure to GYY4137 (500  $\mu$ M) or AP39 (100 nM) on survival rate of *cth-1 C. elegans* mutants treated with 60 mM paraquat. N>80 worms. Experiments were performed in triplicate. \*\* p<0.01.

# Protein persulfidation decreases with aging

With aging there is an imbalance between ROS production and removal, resulting in an increase in oxidative damage (Balaban et al., 2005; Finkel and Holbrook, 2000; Liochev, 2013; Redman et al., 2018). Moreover, two independent quantitative proteomics studies found that in *C. elegans*, CSE is decreased during aging (Aging et al., 2015; Narayan et al., 2016). Here we tested the hypothesis that protective pools of intracellular P-SSH decline with age and correlate with the lifespan of individuals within a given species. While the *cth-1 C. elegans* mutants did not display an overall significantly shorter median/maximal life span, they initially exhibited a much higher death rate (Figure S6A). Mpst-3 mutants, which had an even lower P-SSH pool (Figure 3G), lived significantly shorter (Figure S6A). Additionally, the persulfidation levels in wild type N2 worms gradually decreased from day 1 to day 7 of adulthood (Figure S6B). To confirm the evolutionary conservation of this phenomenon, we looked at Wistar rats of 1, 3, 6, 12, and 24 months of age. In brain extracts, reduced protein persulfidation levels were observed beginning at 6 months of age, with an approximately 50% lower persulfidation in 24 -month-old rats relative to 1-month-old rats (Figure 7A). The reduction of P-SSH correlated with the loss of protein expression of all three H<sub>2</sub>S producing enzymes (Figure 7B, Figure S6C). We also observed a profound decrease in protein persulfidation and H<sub>2</sub>S-generating enzyme levels (CSE, MPST) in rat hearts as a function of age (Figure 7C), while in liver P-SSH and CSE and CBS levels trended lower but were not significantly different between 12- and 24-month-old rats (Figure S6D).

Finally, as a proof-of-concept experiment we analyzed the persulfidation and sulfinylation levels in human fibroblasts originating from the same individual but collected at different ages (31 and 48 years of age). The results displayed a decrease in P-SSH levels and an increase in P-SO<sub>2</sub>H (**Figure 7D**) in accordance with our hypothesis (**Figure 6A**).

Life-span extension by dietary restriction is caused by increased protein persulfidation

Recent studies suggest that dietary restriction (DR) increases endogenous H<sub>2</sub>S production (Hine et al., 2015; Mitchell et al., 2016) and that this increase is associated with multiple benefits including extended longevity in different species. However, the mechanism by which H<sub>2</sub>S contributes to DR benefits remains poorly characterized. We tested the hypothesis that DR increases longevity in part by increasing protein persulfidation. We started with *C. elegans* by using the *eat-2* genetic model of DR which eats less and lives longer than N2 controls. An additional deletion of either *cth-1* or *mpst-3* reduced the lifespan of the *eat-2* mutant back to control levels (**Figure 7E**). Interestingly, this was in excellent concordance with the total P-SSH pool in these worms; *eat-2* mutants had approximately 3 times higher P-SSH levels than N2, while double mutants had P-SSH levels close to or even lower than N2 (**Figure 7F**). Furthermore, lifespan extension induced by 2-deoxy-D-glucose (DOG) treatment in wildtype N2 worms was completely reversed in *cth-1* and *mpst-3* mutants (**Figure 7G**).

We next looked at 7- and 20-month-old C57BL/6J mice fed *ad libitum* (AL) or subject to daily 30% calorie restricted (CR) from the age of 2 months. Liver persulfidation levels declined with age, but were higher in both CR groups compared to AL (**Figure 7H**).

Considering that glucose and insulin tolerance are affected with aging (Fink et al., 1983), and that we already observed that persulfidation is an integral part of insulin signaling (**Figure 5F**) we monitored P-SSH changes in young and old mice (2- vs 12-month-old) challenged with *i.p.* glucose injection. A lower persulfidation in old mice, as well as a decrease of the H<sub>2</sub>S producing enzymes (Figure S6E) were obvious. The peak of persulfidation was observed in the muscle tissue of young mice treated with glucose but was blunted in the 12-month-old animals (**Figure 7I**), suggesting that the loss of persulfidation capacity with aging is also affecting the insulin signaling and that the beneficial effects of CR to a glucose load (Fontana et al., 2010) could partially be due to the increased persulfidation.

Finally, we tested whether pharmacological interventions to increase persulfidation levels could extend lifespan. While many H<sub>2</sub>S donors have shown considerable pre-clinical efficacy and are currently undergoing clinical evaluation (Wallace and Wang, 2015), we opted for testing an established FDA approved therapeutic drug, sodium thiosulfate, which does not release H<sub>2</sub>S itself but exhibits beneficial effects mimicking those of H<sub>2</sub>S (Snijder et al., 2015). We observed that treatment of cell lysates with either thiosulfate, TST or both increased the persulfidation levels several fold (Figure S6F). *C. elegans* grown on medium supplemented with 1 mM thiosulfate showed higher persulfidation levels (**Figure 7J**) as such, we decided to test its effect on lifespan.

Indeed, worms grown on medium supplemented with 1 mM thiosulfate had a significant increase ( $\sim 15\%$ ) in median longevity (Figure 7K).





(A) Changes in the persulfidation levels in brain extracts of male Wistar rats 1, 3, 6, 12 and 24 months of age, calculated as a ratio of Cy5/488 signal. Values are presented as a mean  $\pm$  SD. n = 3/age group. \* p< 0.05, \*\* p< 0.01. (B) Immunohistochemical analysis of CSE, CBS and MST expression levels in the cortex of 1-, 12- and 24-monthold male Wistar rats. Images are representative of 3 animals/experimental group, magnification 20x.

(C) Protein persulfidation levels of 1-, 12- and 24-month-old hearts of male Wistar rats (top). Expression levels of CSE, CBS and MPST in hearts of 1-, 6-, 12- and 24-month-old male Wistar rats (bottom). Images are representative of 3 animals/experimental group.

(**D**) PSSH (labeled with the dimedone switch method; DAz-2:Cy5-alkyne switch agent) and PSO<sub>2</sub>H (labeled with BioDiaAlk) levels in human fibroblasts originating from the same donor but collected at the age of 31 and 48. PSSH were calculated as a ratio of Cy5/488 signal. PSO<sub>2</sub>H levels were visualized with streptavidin-488, and calculated using GAPDH as a loading control. Quantification of thiol modifications, marked on y axis as PSX, represents average  $\pm$  SD. of n = 3. \*\* p< 0.01.

(E) Survival curves for N2, *eat-2*, *eat-2*; *cth-1* and *eat-2*; *mpst-3* double mutants. n > 100 per line. N2 = 17.8±0.5 days; *eat-2* = 24.5±0.9 days; *eat-2*; *cth-1* = 20.3±0.6 days; *eat-2*; *mpst-3* = 20.2±0.7 days. For *eat-2*; *cth-1* vs. *eat-2* and *eat-2*; *mpst-3* vs. *eat-2* p<0.001.

(F) Persulfidation levels in N2, *eat-2*, *eat-2*; *cth-1* and *eat-2*; *mpst-3 C*. *elegans* mutants. For the fluorescence in-gel detection, P-SSH levels were calculated as a ratio of Cy5/488 fluorescence readouts. Values are presented as average  $\pm$  SD. Protein extracts from ~16000 worms were used for each lane. Experiments were performed in triplicates. \*\* p < 0.01 vs. N2, <sup>##</sup> p < 0.01 vs. eat-2.

(G) Survival curves for N2 and *cth-1* mutants grown in the absence or presence of 5 mM 2-deoxy-D-glucose (DOG). n = 110 per each line. N2 =  $14.2\pm0.4$  days, N2 50 mM DOG =  $17.2\pm1.0$  days; *cth-1* =  $13.3\pm0.4$  days; *cth-1* 50 mM DOG =  $13.7\pm1.0$  days. For N2 vs. N2 50 mM DOG p= 0.005; for *cth-1* vs. *cth-1* 50 mM DOG p = n.s, for N2 vs. *cth-1* p=0.0565.

(H) Age-induced PSSH changes in mice fed *ad libitum* (AL) and mice fed with calorie restriction diet (CR). PSSH levels were calculated as a ratio of Cy5/488 fluorescence readouts. Values are presented as a mean  $\pm$  SD. n=5. \*\* p < 0.01.

(I) Time-dependent PSSH changes in the muscle tissue of 2-month and 12-month old mice injected i.p. with D-glucose (2 g/kg body weight). PSSH levels were calculated as a ratio of Cy5/488 signal. Values are presented as a mean  $\pm$  SD.  $n \ge 3$ . \*\* p < 0.01 control vs. 2-month old mice, <sup>##</sup> p < 0.01 2-month vs. 12-month old mice.

(J) Persulfidation levels in N2 worms with and without treatment of 1 mM thiosulfate. PSSH levels were calculated as a ratio of Cy5/488 fluorescence readouts. Values are presented as a mean  $\pm$  SD. \*\* p < 0.01.

(K) Survival curves for N2 *C. elegans*, and N2 treated with 1 mM thiosulfate. n > 160 per group. N2 =  $18.5 \pm 0.3$  days, 1 mM thiosulfate =  $20.3 \pm 0.4$  days. p < 0.0001

# DISCUSSION

# Versatility and selectivity of dimedone switch method

By combining commercially available and well-characterized chemicals, our novel dimedone switch method is a simple, versatile and robust approach for selectively labeling protein persulfides, that enables the installation of various groups to proteins and the use of a global of detection methods. Moreover, our chemical method allows for a wide scale analysis of metabolic pathways that could be controlled by persulfidation and the identification of new redox switches. The constant green fluorescence introduced by the NBF-Cl probe provides not only the information about total load but could also be harnessed for high-throughput screening. It is easy to envision a setup where by measuring only the green fluorescence in microplates with immobilized neutravidin, total persulfidation labeled with DCP-Bio1 could be screened. The versatility of dimedone based probes also permits site-centric identification and quantitation of persulfides by MS, as done for protein sulfenylation (Yang et al., 2014). The installation of different fluorophores through CuAAC and almost undetectable unselective background in

microscopic analysis also carries a potential for further exploration of protein persulfidation in tissue sections. Finally, the use of simple in-gel detection prevents all the problems associated with column separation and Western blot transfer, commonly used in other persulfide detection methods (Dóka et al., 2016). Caution should be exercised however, to ensure that sulfenic acids and cyclic sulfenamides are completely blocked by NBF-Cl prior to the reaction with dimedone-based probes.

# Protein persulfidation is controlled by the transsulfuration pathway and cysteine catabolism

From the data presented here, we conclude that protein persulfidation is almost exclusively controlled by the three  $H_2S$  producing enzymes involved in the transsulfuration pathway and cysteine catabolism (**Figure 3A**). This is evident in all tested life forms. However, in mammalian cells and under basal substrate availability (assuming that all 3 enzymes are present in the cell), CSE is expected to be the major source of  $H_2S$  (Filipovic et al., 2018). CBS would predominantly be involved in cystathionine production while the activity of MPST would depend on thioredoxin availability and CAT activity (**Figure 3A**). MPST could nonetheless play a significant role in controlling protein persulfidation given that D-cysteine causes a dramatic increase in protein persulfidation representing an interesting venue for drug development.

# Persulfidation as integral part of $H_2O_2$ signaling or/and as evolutionary conserved route to rescue cysteines

As life emerged in an H<sub>2</sub>S-enriched environment (Olson and Straub, 2016) and under conditions of extensive radiation where ROS were formed by photolysis of water (Liang et al., 2006), protection of cysteine residues was necessitated. The formation of persulfides poses as the simplest answer. In persulfides, sulfur atoms are oxidized, however concurrently sulfur is deprotonated and highly nucleophilic, reducing ROS at least one order of magnitude faster than its corresponding thiolate (Cuevasanta et al., 2015; Filipovic et al., 2018). In other words, while a cysteine gets oxidized to form a persulfide, it forms a strong reducing agent at the same time.

 $H_2O_2$  as main ROS player is now widely accepted as a signaling molecule (D'Autréaux and Toledano, 2007; Holmström and Finkel, 2014; Yang et al., 2014). This is best exemplified in the case of growth factor receptor signaling (Paulsen and Carroll, 2013; Sundaresan et al., 1995). Approximately 1000 cysteine sites have been found to be sulfenylated, raising the question of how these sites are rescued. In our study, waves of persulfidation are observed following sulfenylation, as a response to RTK activation, confirming that persulfidation is an integral part of RTK signaling. A recent study demonstrated that a large number of proteins undergo further oxidation to sulfinic acid as well (Akter et al., 2018). Our results demonstrate that without  $H_2S$ /persulfidation proteins undergo a substantial thiol oxidation (sulfenylation, sulfinylation and sulfonylation) even with  $H_2O_2$  concentrations that cause no change in normal cells. This overlooked phenomenon questions whether and in which concentration range  $H_2O_2$  acts as a signaling molecule without  $H_2S$ .

We demonstrate that as cells age their persulfidation levels decrease due to the loss of  $H_2S$  producing enzymes. Reacting with ROS, a persulfide will form a *S*-sulfonate which we show could be readily reduced back to a thiolate by thioredoxin (**Figure 6A**). This rescue loop for preserving cysteine residues in proteins and preventing their loss of function is probably an evolutionary remnant used as a general protective mechanism in all life forms. This is best documented by the enhanced sensitivity of different life forms with low P-SSH levels to oxidant stressors and their increased resistance once the intracellular P-SSH pool is increased even by a short-term pretreatment with  $H_2S$  donors. This unifying mechanism explains the beneficial effects of DR, already known to result in  $H_2S$  overproduction (Hine et al., 2015), as well as lifespan extension caused by pharmacological increase of protein persulfidation. Thus, our results further strengthen the ROS theory of aging (Redman et al., 2018).

It is worth mentioning that beside a general protection, persulfidation of specific proteins could result in an alteration of their function (Filipovic et al., 2018; Paul and Snyder, 2015; Vandiver et al., 2013), as we observe in the case of MnSOD, whose activity is preserved after persulfidation. It is possible that DR-induced decrease of ROS (Redman et al., 2018) could also be partially related to better activity of ROS removing enzymes.

Beside aging, general protective effects of protein persulfidation could be translated to many other disease states. HD and neurodegeneration are strongly linked to CSE expression and pharmacological interventions to increase CSE levels, such as monensin treatment, show promising therapeutic effects (Paul et al., 2014; Sbodio et al., 2016, 2018). Decreased persulfidation in HD cells and increased P-SSH levels by upregulating CSE expression reported in our study could provide a general explanation for this. Taken together, our data represent a good starting point for the fine tuning of targeted therapeutic approaches to improve healthspan and lifespan.

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**Authors contribution:** JZ and EK contributed equally to this study and performed most of the experiments. MRF conceived the study. JBK, BA, SS-R, DT-L, JLjM, BB, DP, MM, BDP and MRF also performed experiments. YG, KC, RT, MW, MB, VM, II-B, GS, JEG, BGZ, BP, SHS provided tools and intellectual input, and helped with data analysis. SM and JM provided DR mouse livers and helped with data analysis. MRF wrote the manuscript with the help from all co-authors.

# **Supplementary Materials:**

Figures S1-S6

Table S1

External Databases S1-S6

KEY RESOURCES TABLE		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-β-actin, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-47778
Anti-β-tubulin, mouse monoclonal	Sigma Aldrich	Cat# T0198
Anti-GAPDH, mouse monoclonal	Sigma Aldrich	Cat# G8795
Anti-HSP70, mouse monoclonal	Abcam	Cat# Ab5439
Anti-KEAP1 P586, rabbit polyclonal	Cell Signalling	Cat# 4678
Anti-eNOS, rabbit monoclonal	Cell Signalling	Cat# 32027
Anti-Parkin, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-136989
Anti-SOD-2, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-137254
Anti-TST, rabbit polyclonal	GeneTex	Cat# GTX114858
Anti-SQRDL, rabbit polyclonal	Sigma Aldrich	Cat# HPA017079
Anti-PTEN, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-7974

#### STAR METHODS KEY RESOURCES TAB

Anti-PTP1B, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-133259			
Anti-SH-PTP2, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-7384			
Anti-EGFR, rabbit polyclonal	Santa Cruz Biotechnology	Cat# sc-03-G			
Anti-CBS, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-133154			
Anti-CBS, rabbit polyclonal	Santa Cruz Biotechnology	Cat# sc-67154			
Anti-MPST, rabbit polyclonal	Sigma Aldrich	Cat# HPA001240			
Anti-CTH, rabbit	From Prof. Snyder's lab (Johns Hopkins University School of Medicine)	(Paul et al., 2014)			
Anti-CTH, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-365382			
Anti- p-ERK, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-7383			
Anti ERK 1, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-271269			
Anti-DJ-1, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-55572			
Anti-DJ-1 AC, mouse monoclonal	Santa Cruz Biotechnology	Cat# sc-55572 AC			
Anti DJ-1 (Oxidized At C106), mouse monoclonal	BioRad	Cat# HCA024			
Anti-Biotin-Peroxidase, mouse monoclonal	Sigma Aldrich	Cat# A0185			
Anti-Dimedone, rabbit polyclonal	From Dr. Carroll's lab (The Scripps Research Institute)	(Seo and Carroll, 2009)			
Mouse IgGk BP-HRP	Santa Cruz Biotechnology	Cat# sc-516102			
Mouse anti-rabbit IgG-HRP	Santa Cruz Biotechnology	Cat# sc-2357			
Swine anti-rabbit IgG-HRP, polyclonal	Dako, Denmark	Cat# P0399			
Donkey anti-mouse IgG-HRP, polyclonal	Abcam, Cambridge, UK	Cat# ab6820			
Streptavidin Protein, DyLight 488	Thermo Fisher Scientific	Cat# 21832			
Cy®5-Streptavidin	Sigma Aldrich	Cat# GEPA45001			
ECE Pathway Phoenho Antibody Array	Full Moon Biosystems	Cat# PEG214			
EOF Failway Flospilo Antibody Allay		Bacterial and Virus Strains			
Bacterial and Virus Strains					
Bacterial and Virus Strains OP50-1	Caenorhabditis Genetics Center	N/A			
Bacterial and Virus Strains OP50-1 HB101	Caenorhabditis Genetics Center Caenorhabditis Genetics Center	N/A N/A			
Bacterial and Virus Strains OP50-1 HB101 <i>E.coli</i> MG1655 (pTrc99a)	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain	N/A N/A N/A			
Bacterial and Virus Strains OP50-1 HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74)	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain	N/A N/A N/A N/A			
Bacterial and Virus Strains OP50-1 HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74) <i>E. coli</i> BL21 DE3 Rosetta	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen	N/A N/A N/A N/A Cat# 70954-3			
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Both Fadiway Phospho Antibody Array Bacterial and Virus Strains OP50-1 HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74) <i>E. coli</i> BL21 DE3 Rosetta Biological Samples Kidneys from C57BL/6 WT and CSE <sup>-/-</sup> mice <i>D. melanogaster</i> : y <sup>1</sup> w <sup>1118</sup> <i>D. melanogaster</i> : Eip55E Chemicals, Peptides and Recombinant Proteins Chelex-100 Sodium Sulfide GYY4137 AP39	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich (Alexander et al., 2015) (Le Trionnaire et al., 2014)	N/A N/A N/A N/A Cat# 70954-3 (Markó et al., 2016) (Snijder et al., 2015) (Snijder et al., 2015) Cat# C7901 Cat# 407410 N/A N/A			
Born Failway Phospho Antibody Array         Bacterial and Virus Strains         OP50-1         HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74) <i>E.coli</i> BL21 DE3 Rosetta         Biological Samples         Kidneys from C57BL/6 WT and CSE <sup>-/-</sup> mice <i>D. melanogaster</i> : y <sup>1</sup> w <sup>1118</sup> <i>D. melanogaster</i> : Eip55E         Chemicals, Peptides and Recombinant Proteins         Chelex-100         Sodium Sulfide         GYY4137         AP39         D-Cystine	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich (Alexander et al., 2015) (Le Trionnaire et al., 2014) Sigma Aldrich	N/A N/A N/A N/A Cat# 70954-3 (Markó et al., 2016) (Snijder et al., 2015) (Snijder et al., 2015) Cat# C7901 Cat# 407410 N/A N/A N/A Cat# 30095			
Boor Framway Phospho Antroody Array         Bacterial and Virus Strains         OP50-1         HB101         E.coli MG1655 (pTrc99a)         E.coli MG1655 (pSB74)         E. coli BL21 DE3 Rosetta         Biological Samples         Kidneys from C57BL/6 WT and CSE <sup>-/-</sup> mice         D. melanogaster: y <sup>1</sup> w <sup>1118</sup> D. melanogaster: Eip55E         Chemicals, Peptides and Recombinant Proteins         Chelex-100         Sodium Sulfide         GYY4137         AP39         D-Cystine         DL-propargylglycine	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich	N/A N/A N/A N/A Cat# 70954-3 (Markó et al., 2016) (Snijder et al., 2015) (Snijder et al., 2015) Cat# C7901 Cat# 407410 N/A N/A Cat# 30095 Cat# P7888			
Bacterial and Virus Strains         OP50-1         HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74) <i>E.coli</i> BL21 DE3 Rosetta         Biological Samples         Kidneys from C57BL/6 WT and CSE <sup>-/-</sup> mice <i>D. melanogaster</i> : y <sup>1</sup> w <sup>1118</sup> <i>D. melanogaster</i> : Eip55E         Chemicals, Peptides and Recombinant Proteins         Chelex-100         Sodium Sulfide         GYY4137         AP39         DCystine         DL-propargylglycine         O-(Carboxymethyl)hydroxylamine         hemihydrochloride (AOAA)	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich	N/A         N/A         N/A         N/A         N/A         Cat# 70954-3         (Markó et al., 2016)         (Snijder et al., 2015)         (Snijder et al., 2015)         Cat# C7901         Cat# 407410         N/A         N/A         Cat# 30095         Cat# C13408			
DescriptionBacterial and Virus StrainsOP50-1HB101E. coli MG1655 (pTrc99a)E. coli MG1655 (pSB74)E. coli BL21 DE3 RosettaBiological SamplesKidneys from C57BL/6 WT and CSE-/- miceD. melanogaster: y1w1118D. melanogaster: Eip55EChemicals, Peptides and Recombinant ProteinsChelex-100Sodium SulfideGYY4137AP39D-CystineDL-propargylglycineO-(Carboxymethyl)hydroxylaminehemihydrochloride (AOAA)Hydrogen peroxide solution	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich Sigma Aldrich	N/A N/A N/A N/A Cat# 70954-3 (Markó et al., 2016) (Snijder et al., 2015) (Snijder et al., 2015) (Snijder et al., 2015) Cat# C7901 Cat# 407410 N/A N/A Cat# 30095 Cat# P7888 Cat# C13408 Cat# 216763			
Bacterial and Virus Strains         OP50-1         HB101 <i>E.coli</i> MG1655 (pTrc99a) <i>E.coli</i> MG1655 (pSB74) <i>E.coli</i> BL21 DE3 Rosetta         Biological Samples         Kidneys from C57BL/6 WT and CSE <sup>-/-</sup> mice <i>D. melanogaster</i> : y <sup>1</sup> w <sup>1118</sup> <i>D. melanogaster</i> : Eip55E         Chemicals, Peptides and Recombinant Proteins         Chelex-100         Sodium Sulfide         GYY4137         AP39         D-Cystine         DL-propargylglycine         O-(Carboxymethyl)hydroxylamine         hemihydrochloride (AOAA)         Hydrogen peroxide solution         Monensin sodium salt	Caenorhabditis Genetics Center Caenorhabditis Genetics Center From the laboratory of Gonzales- Zorn, Spain From the laboratory of Gonzales- Zorn, Spain Novagen From Professor Maik Gollasch (Charité Medical Faculty) From Professor Ody Sibon (University of Groningen) From Professor Ody Sibon (University of Groningen) Sigma Aldrich Sigma Aldrich	N/A         N/A         N/A         N/A         Cat# 70954-3         (Markó et al., 2016)         (Snijder et al., 2015)         (Snijder et al., 2015)         Cat# C7901         Cat# 407410         N/A         N/A         Cat# 30095         Cat# C13408         Cat# 216763         Cat# M5273-1G			

S-Sulfocysteine	Sigma Aldrich	Cat# C2196
Cystine	Sigma Aldrich	Cat# 30200
Imidazole	Carl Roth Gmbh	Cat# 3899.4
Isopropyl β-D-1-thiogalactopyranoside	Inalco Pharmaceuticals	Cat# 1758-1400
Dithiothreitol	GE Healthcare	Cat# 17-1318-02
4-Chloro-7-nitrobenzofurazan	Sigma Aldrich	Cat# 163260
Dimedone	Sigma Aldrich	Cat# D153303
DCP-Bio1	Kerafast	Cat# EE0028
DAz-2	Cayman Chemicals	Cat# 13382
BioDiaAlk	(Akter et al., 2018)	N/A
DiaAlk	(Akter et al., 2018)	N/A
Cyanine5 alkyne	Lumiprobe	Cat# FP-OO5590
Copper(II)-TBTA	Lumiprobe	Cat# 21050
L-Ascorbic acid	Sigma Aldrich	Cat# 795437
5-Fluoro-2'-deoxyuridine	Sigma Aldrich	#F0503
Nmc-penicillamine	(Artaud and Galardon 2014)	N/A
EGF human recombinant	PromoKine	Cat# 60170
VEGE-165 human recombinant	PromoCell	Cat# C-64422
Insulin human	Sigma Aldrich	Cat# 10908
Paraformaldehyde	Sigma Aldrich	Cat# P6148
Sodium thiosulfate	PROLABO	$C_{at#} 27,910,291$
Paraquat hydrochloride hydrate	Sigma Aldrich	Cat# 36541
Sodium (meta)arsenite	Sigma Aldrich	Cat# \$7400
2 Deoxy D glucose	Sigma Aldrich	Cat# D8375
Z-Deoxy-D-glucose Thiazolul Plue Tetrazolium Promide	Sigma Aldrich	Cat# D8375
Protesse Inhibitor	Sigma Aldrich	Cat# 193035
His Dur Ni NTA Superflow Agerose	Thormo Eisher Scientifie	Cat# 18340
Strontovidin Magnetia Danda	Sigma Aldrich	Cat# 23210
Streptavidin Magnetic Beads	Sigma Aldrich	Cat# 11 641 //8 001
NeutrAvidin Agarose Resin	Thermo Fisher Scientific	Cat# 29201
High Capacity NeutrAvidin Agarose Resin	Thermo Fisher Scientific	
Trypsin from porcine pancreas	Sigma Aldrich	Cat# 16567
Chymotrypsin Sequencing Grade	Sigma Aldrich	Cat# 000000011418467001
Human Serum Albumin (HSA)	Sigma Aldrich	Cat# A1887
GAPDH	Sigma Aldrich	Cat# G2267
Rhodanese from bovine liver (TST)	Sigma Aldrich	Cat# R1756
MnSOD	Creative BioMart	Cat# SOD2-1039H
Cytochrome c	Sigma Aldrich	Cat# 30398
Xanthine	Sigma Aldrich	Cat# X0626
PTP1B human recombinant	Abcam, Cambridge, UK	Cat# ab51277
Xanthine Oxidase	Sigma Aldrich	Cat# X1875
Thioredoxin Reductase (TrxR) from rat liver	Sigma Aldrich	Cat# T9698
TRP14 (From human fibroblast cDNA)	For this paper	N/A
hsTrx C32S	For this paper	N/A
hsTrx C35S	For this paper	N/A
hsTrx1	For this paper	N/A
NADPH	Sigma Aldrich	Cat# N5130
DAPI	Euromedex	Cat# 1050-A
MeRho-Az	From Michael D. Pluth's lab	N/A
	(Dept. of Chemistry and Biochemistry Fugene OR)	
Providium Iodide	Sigma Aldrich	Cat# P4864

Histowax®	Histolab Product AB, Sweden	Cat# 00405
Normal swine serum	Dako, Denmark	Cat# X0901
Normal donkey serum	Abcam, Cambridge, UK	Cat# X0903
3,3'-Diaminobenzidine tetrahydrochloride	Dako North America, Inc.	Cat# K3468
(DAB)	Carpinteria, CA, USA	
DPX medium	Sigma-Aldrich	Cat# 06522
Experimental Models: Cell Lines		
Human: HeLa	ECACC	Cat# 93021013
Human: HUVEC	PromoCell	Cat# C-12203
Human: SH-SY5Y	ECACC	Cat# 94030304
Human: Fibroblasts	Coriell Institute	AG08790 and AG14245
Mouse: MEF CSE <sup>+/+</sup> (WT)	From Prof. Snyder's lab (Johns Hopkins University School of Medicine)	(Sbodio et al., 2016)
Mouse: MEF CSE	From Prof. Snyder's lab (Johns Hopkins University School of Medicine)	(Sbodio et al., 2016)
Mouse: Striatal progenitor cells STHdh <sup>Q7/Q7</sup>	M. MacDonald (Massachusetts General Hospital, Boston, MA)	N/A
Mouse: Striatal progenitor cells ST <i>Hdh</i> <sup>Q111/Q111</sup>	M. MacDonald (Massachusetts General Hospital, Boston, MA)	N/A
Experimental Models: C.elegans mutants		
Wild-type Bristol N2	Caenorhabditis Genetic Center	N/A
<i>cth-1(ok3319)V</i>	Caenorhabditis Genetic Center	VC2569
mpst-3(tm4387)V	MITANI Lab, National Bio- Resource Project of the MEXT, Japan	FX04387
eat-2(tm5786)II	MITANI Lab, National Bio- Resource Project of the MEXT, Japan	FX19451
eat-2(tm5786)II;cth-1(ok3319)V	This paper	N/A
eat-2(tm5786)II;mpst-3(tm4387)V	This paper	N/A
Experimental Models: Strains/Organisms		
S. cerevisiae: BY4742 (WT)	Euroscarf	N/A
S. cerevisiae: ∆cys4	Euroscarf	ACCNO Y16696
S. cerevisiae: ∆cys3	Euroscarf	ACCNO Y16865
S. cerevisiae: ∆tum1	Euroscarf	ACCNO Y12507
Male Wistar Rat	Institute for Biological Research "Siniša Stanković", Belgrade, Serbia	N/A
Male C57BL/6J mice	NIA Aging Colony Resource at Charles River Laboratories (CRL)	N/A
C57BL/6 mice	Department of Pharmacology and Molecular Sciences, Johns Hopkins University School of Medicine, Baltimore, MD	N/A
Oligonucleotides		
5'-GAAGGAGATATACCATGGTGAAGC-3' (fwd)	Sigma Aldrich	hsTrx in pET28a
5'-CGGATCTCAGTGGTGGTG-3' (rev)	Sigma Aldrich	hsTrx in pET28a
5'-CACGTGGTCTGGGCCTTG-3' (fwd)	Sigma Aldrich	hsTrx C32S mutagenesis
5'-CAAGGCCCAGACCACGTG-3' (rev)	Sigma Aldrich	hsTrx C32S mutagenesis
5'-GTGTGGGGCCTTCCAAAATGATCAAG-3' (fwd)	Sigma Aldrich	hsTrx C35S mutagenesis

5'-CTTGATCATTTTGGAAGGCCCACAC-3' (rev)	Sigma Aldrich	hsTrx C35S mutagenesis	
5'- ACCATCACGGATCCATGGCCCGCTATGA GGAGG-3' (fwd)	Sigma Aldrich	hsTRP14 in pQE-80L	
5'- CCGGGGTACCGTTAATCTTCAGAGAACA ACATTTCCACCAG-3' (rev)	Sigma Aldrich	hsTRP14 in pQE-80L	
Recombinant DNA			
Plasmid: pET-28a(+)	Novagen	Cat# 69864-3	
Plasmid: pQE-80L	Qiagen	N/A	
Software and Algorithms			
ImageJ	NIH	https://imagej.nih.gov/ij/do wnload.html	
GraphPad Prism 5.0	GraphPad Software	https://www.graphpad.com	
PEAKS Studio	Bioinformatics Solutions Inc.	http://www.bioinfor.com/	
OriginPro 8	OriginLab	https://www.originlab.com /	
RTCA Software Version 2.0	ACEA Biosciences, Inc.	https://www.aceabio.com/	
		products/rtca-dp/	

#### CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to, and will be fulfilled by the Lead Contact, Milos Filipovic (milos.filipovic@ibgc.cnrs.fr)

# EXPERIMENTAL MODEL AND SUBJECT DETAILS

#### **Cell Lines**

HeLa cells and Neuroblastoma cells (SH-SY5Y) were both obtained from ECACC. HeLa cells were cultured in Dulbecco's modified Eagle's media (DMEM, high glucose and sodium pyruvate) supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin and 10% calf serum at 37 °C and 5% CO<sub>2</sub>.

SH-SY5Y cells were cultured in Ham's F12 : DMEM (1 : 1) media, supplemented with 2 mM glutamine, 1% penicillin-streptomycin and 10% calf serum at 37 °C and 5% CO<sub>2</sub>.

C-pooled human umbilical vein endothelial cells (HUVEC) were obtained from PromoCell, Germany and cultured in Endothelial Cell Growth Medium Kit (C-22110, PromoCell, Germany) at 37 °C and 5% CO<sub>2</sub>.

Mouse Embryonic Fibroblasts (MEF) were generated from wild type ( $CSE^{+/+}$ ) and  $CSE^{-/-}$  mice and immortalized using SV40T antigen (Sbodio et al., 2016). MEF cells were cultured in DMEM (high glucose), supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin and 10% calf serum at 37 °C and 5% CO<sub>2</sub>.

The striatal progenitor cell line ST*Hdh*<sup>Q7/Q7</sup>, expressing wild-type huntingtin, and ST*Hdh*<sup>Q111/Q111</sup>, expressing mutant huntingtin, harbouring 111 glutamine repeats (referred to as Q7 and Q111 cells, respectively), were from M. MacDonald (Massachusetts General Hospital, Boston, MA). The cells were maintained in DMEM (low glucose, no pyruvate) supplemented with 2 mM L-glutamine, 1% penicillin-streptomycin and 10% calf serum at 33 °C and 5% CO<sub>2</sub>.

Human Fibroblasts cell lines AG08790 (31 years old) and AG14245 (48 years old - a culture initiated from a biopsy taken 17 years earlier from this same donor is AG08790) were obtained from Coriell Institute for Medical Research. Fibroblasts were cultured in DMEM supplemented with 2 mM L-glutamine and 10% calf serum at 37  $^{\circ}$ C and 5% CO<sub>2</sub>.

#### C. elegans

The following strains were used in this study: Wild-type Bristol N2, *cth-1(ok3319)V*, *mpst-3(tm4387)V*, *eat-2(tm5786)II*; *eat-2(tm5786)II*; *cth-1(ok3319)V*, *eat-2(tm5786)II*; *mpst-3(tm4387)V*. The original isolate VC2569 *cth-1(ok3319)V* was provided by the Caenorhabditis Genetics Center whereas the original isolates FX04387 *mpst-3(tm4387)V* and FX19451 *eat-2(tm5786)II* were provided by the MITANI Lab through the National Bio-Resource Project of the MEXT, Japan. All mutant strains were outcrossed at least 4 times before use. Double-mutant strains were constructed by using standard techniques (Brenner, 1974; Sulston and Hodgkin, 1988) and the presence of both

mutations were checked by PCR. Worms were grown at 20 °C on standard nematode growth medium (NGM) plates, unless otherwise stated, using standard *C. elegans* techniques (Stiernagle, 2006) with sufficient food (*E. coli* OP50-1) for at least two generations prior to use.

#### S. cerevisiae

Yeast cells were grown in liquid YPD media (1% yeast extract, 1% bactopeptone, and 2% glucose), unless otherwise stated.

#### Wistar rats

Male Wistar rats used in the experiment were bred and housed at the Institute for Biological Research "Siniša Stanković", Belgrade, Serbia, under constant laboratory conditions  $(22\pm2^{\circ}C, 12-12 \text{ hr light-dark cycle})$ . Food and water were available *ad libitum*. The animals were decapitated at the ages of 1, 3, 6, 12 and 24 month(s). All animal procedures were in compliance with the EEC Directive (86/609/EEC) on the protection of animals used for experimental and other scientific purposes and were approved by the Ethical Committee for the Use of Laboratory Animals of the Institute for Biological Research "Siniša Stanković", University of Belgrade.

# Male C57BL/6J mice (AL and CR experiments)

All experiments were performed with the approval of the Harvard Medical Area Institutional Animal Care and Use Committee (IACUC). Male C57BL/6J mice were obtained from the NIA Aging Colony Resource at Charles River Laboratories (CRL) at 6 months (young) and 19 months (old) of age. Mice were allowed to acclimatize to the facility for one month to ensure weight stabilization after shipment, so at sacrifice were 7mo old (young) and 20 months (old). Initiation of caloric restriction (CR) was performed as previously described (Turturro et al., 1999) (starting at 14 weeks of age, CR is initiated in a step-down fashion, where it its increased to 25% restriction at 15 weeks, and the full 40% restriction imitated at 16 weeks where it is maintained throughout the life of the animal. Ad libitum (AL) animals were fed NIH-31 diet, while CR animals are fed NIH-31 fortified diet (Turturro et al., 1999). Other details about the husbandry conditions at CRL can be found here: https://www.nia.nih.gov/research/dab/aged-rodent-colonies-handbook/barrier-environmental-information.

Mice were single housed for the duration of their life in standard mouse cages. Upon arrival at Harvard, mice were housed in the barrier facility at HSPH in microisolator cages with corncob bedding, a nestlet and one shepherd shack. Mice receive free access to water, and either AL NIH-31, or fortified NIH-31 (1 pellet per day for the CR mice). CR mice were fed daily between the hours of 7:00-8:00am by feeding the ration of food onto the floor of the cage. Any remaining food was removed the next day prior to giving the new food ration. Mice were housed in a room on a 12/12 hr light/dark cycle (7am-7pm) with temperature between 20–23 °C with 30%–70% relative humidity. Cages were changed weekly using full sterile technique and performed in Class II laminar flow change stations with spot changes as required.

#### Male C57BL/6J mice (Glucose Tolerance Test)

Animals were housed on a 12-h light–dark schedule and received food and water ad libitum, except for the glucose tolerance experiment, where the mice were starved overnight before injection with glucose. All animals were treated in compliance with the recommendations of the National Institutes of Health and approved by the Johns Hopkins University Committee on Animal Care.

#### **METHOD DETAILS**

All chemicals were purchased from Sigma Aldrich, unless otherwise mentioned. All buffers were prepared with nanopure water and treated with Chelex-100 resins to remove traces of metal ions. Na<sub>2</sub>S solutions were prepared and handled as recommended (Wedmann et al., 2014). Nmc-penicillamine was synthesized, as previously described (Artaud and Galardon, 2014). Morpholin-4-ium 4 methoxyphenyl(morpholino) phosphinodithioate (GYY4137) and (10-oxo-10-(4-(3-thioxo-3*H*-1,2-dithiol-5-yl)phenoxy)decyl)triphenylphosphonium bromide (AP39) were synthesized in house according to (Alexander et al., 2015; Le Trionnaire et al., 2014).

# **Preparation of Specific oxPTM of Proteins**

#### Preparation of HSA-SH, HSA-SOH and HSA-SSH

Different oxPTM of HSA were prepared and their concentrations quantified as previously reported (Cuevasanta et al., 2015).

# Preparation of GAPDH persulfide

GAPDH persulfide was prepared as previously described for HSA derivatives (Cuevasanta et al., 2015).

# Preparation of TST and TST persulfide

Bovine TST (Sigma Aldrich) (already largely present as a persulfide) was used to prepare the fully persulfidated enzyme, by incubating with a 10-fold excess of sodium thiosulfate in 10 mM Tris buffer (pH 8) at 37 °C for 30 min and then cleaning with mini biospin column from Bio-Rad. The reduced enzyme was prepared by incubating with a 10-fold excess of DTT at 37 °C for 30 min and then cleaned on a mini biospin column.

#### **Dimedone Switch Method for Purified Proteins**

Protein was incubated with 5 or 10 mM NBF-Cl in 50 mM PBS (40mM Na<sub>2</sub>HPO<sub>4</sub>, 10 mM NaH<sub>2</sub>PO<sub>4</sub> and 135 mM NaCl, pH 7.4) supplemented with SDS (final conc. 2%) for 30 min at 37 °C. The solution was then precipitated by methanol/chloroform precipitation; Sample/MeOH/CHCl<sub>3</sub>, 4/4/1 (v/v/v) was added and centrifuged (14000 x g, 15 mins, 4 °C). The protein pellet obtained will be between the organic and aqueous layers, both layers was aspirated and H<sub>2</sub>O/MeOH/CHCl<sub>3</sub>, 4/4/1 (v/v/v) was added to the protein pellet and centrifuged. Supernatant was aspirated again and the pellet was subsequently washed with MeOH 2-3 times. Pellet was resuspended in 50 mM PBS containing 2% SDS, incubated with either 50 µM dimedone or DCP-Bio1 for 1 hr at 37 °C, precipitated with methanol/chloroform as previously mentioned, and re-suspended in PBS containing 2% SDS. 1 equivalent of Laemmli (4X) buffer (BioRad) supplemented with 10% β-mercaptoethanol, was then added to 3 equivalents of sample for SDS-PAGE and boiled at 95 °C for 5 min protected from light.

For some experiments, the DCP-Bio1-labeled samples were redissolved in 50 mM PBS only, incubated with neutravidin beads (Thermo Fischer Scientific) for 2 hr at room temperature (RT) with continuous mixing. Beads were then washed with 10 volumes of PBS supplemented with 0.01% Tween-20 and bound proteins were eluted by boiling the beads in a minimum volume of Laemmli buffer supplemented with 10%  $\beta$ -mercaptoethanol (1X with PBS) buffer for SDS-PAGE for 5 min. Samples were resolved by SDS-PAGE and gels were fixed in fixation buffer for 30 min, protected from light. The gel was recorded, at 635 nm for the Cy5 signal and 473 nm for NBF-Cl signal, on the Typhoon FLA 9500 (GE Healthcare).

#### ESI-TOF MS of the dimedone switch reaction with LMW persulfides

Mass spectrometry was performed on maXis 5G, Bruker Daltonic (Bremen, Germany), an ESI-TOF MS capable of resolution of at least 40,000 FWHM. Detection was in positive-ion mode. 100  $\mu$ M nmc-penicillamine persulfide was mixed with 100  $\mu$ M NBF-Cl in ammonium bicarbonate buffer, (pH 7.4, 23 °C) and the reaction monitored for 15 min. 500  $\mu$ M dimedone was then added and the reaction monitored for an additional 15 min.

#### MS of Protein Persulfide Models Labeled with DCP-Bio1

Protein persulfides switch labeled with DCP-Bio1 as previously described and resolved by SDS-PAGE, and protein bands excised and digested with either trypsin or chymotrypsin following previously described protocol (Crouzet et al., 2017). Digested peptides were analyzed by LC-MS/MS on a Thermo Scientific Q Exactive Orbitrap mass spectrometer in conjunction with a Proxeon Easy-nLC II HPLC (Thermo Fisher Scientific) and Proxeon nanospray source at Bordeaux Proteomic Platform. The digested peptides were loaded a 100 micron x 25 mm Magic C<sub>18</sub> 100Å 5U reverse phase trap where they were desalted online before being separated with a 75 micron x 150 mm Magic C18 200Å 3U reverse phase column. Peptides were eluted using a 120 min gradient with a flow rate of 300 nL/min. An MS survey scan was obtained for the m/z range of 350-1600; MS/MS spectra were acquired using a top 12 method, where the top 12 ions in the MS spectra were subjected to High Energy Collisional Dissociation (HCD). An isolation mass window of 2 m/z was used for the precursor ion selection, and normalized collision energy of 27% was used for fragmentation. Five second duration was used for the dynamic exclusion. Peptide identification was performed using PEAKS Studio (BSI, Canada) (Zhang et al., 2012). The search settings were: precursor  $\Delta$ m tolerance = 10 ppm, fragment  $\Delta$ m tolerance = 0.2 Da, missed cleavages = 2, modifications of lysine: NBF (163.0012), modifications of cysteine: NBF (163.0012), DCP-Bio1 (394.1557), or hydrolyzed DCP-Bio1 (168.0786).

#### Proteomic Analysis of Persulfidated Proteins in Red Blood Cell Lysates

9 mL of peripheral whole blood from participants of this study, who provided informed consent in accordance with the Declaration of Helsinki, was collected in citrate and processed immediately following previously described protocol (Pasini et al., 2006) with the modification that the lysis buffer contained 5 mM NBF-Cl. After 30 min of incubation with lysis buffer at 4 °C, additional NBF-Cl was added (to final concentration of 15 mM NBF-Cl) with SDS (to final concentration of 2%) and incubated for 30 min. Methanol/chloroform precipitation was performed as previously described, and the protein pellet obtained was redissolved in 50 mM PBS supplemented with 0.1% SDS. Endogenously biotinylated proteins were precleared by incubating with Pierce<sup>TM</sup> NeutrAvidin<sup>TM</sup> Agarose (Thermo Fisher Scientific) at RT for 2 hr with agitation. The resins were subsequently removed on a Pierce<sup>TM</sup> Disposable Column (Thermo Fisher Scientific) and the solution obtained was precipitated by methanol/chloroform. The resulting protein pellet was redissolved in 50 mM PBS supplemented with 50 µM DCP-Bio1

at 37 °C for 1.5 hr. Solution was precipitated with methanol/chloroform and redissolved in 50 mM PBS supplemented with 0.1% SDS. The protein solution was incubated with Pierce<sup>™</sup> High Capacity NeutrAvidin<sup>™</sup> Agarose (Thermo Fisher Scientific) at 4 °C overnight with agitation. Samples were then brought to RT and loaded on a column. The resins were washed with 8 column volumes of 50 mM PBS supplemented with 0.001% Tween-20, 2 column volumes of 50 mM PBS and finally with 1 column volume of H<sub>2</sub>O. After washing, the resins were collected from the column and incubated with 2.25 M ammonium hydroxide at RT, overnight with agitation. The sample was then neutralized with formic acid and protein concentration was determined. 1 equivalent of enzyme digestion buffer was added (for trypsin digestion: 100 mM ammonium bicarbonate buffer; for chymotrypsin digestion: 100 mM Tris and 10 mM CaCl<sub>2</sub>, pH 7.8). Digestion was performed at an enzyme-to-substrate ratio of 1:50 (wt:wt) and incubated at 37 °C overnight with agitation. Enzyme was added again to solution at an enzyme-to-substrate ratio of 1:20 (wt:wt) and reincubated at 37 °C for 5 hr with agitation. The resulting peptide sample was then quenched by bringing to pH 3, with conc. HCl, and analyzed at Bordeaux Proteomic Platform. Trypsin digested peptides were analyzed by LC-MS/MS on a Thermo Scientific Q Exactive Orbitrap mass spectrometer in conjunction with a Proxeon Easy-nLC II HPLC (Thermo Fisher Scientific) and Proxeon nanospray source. Samples were separated on 300-µm ID x 5-mm C18 PepMapTM precolumn and 75 µm ID x 25 cm nanoViper C18, 2 µm, 100 Å – Acclaim® PepMap RSLC column using 4-40% gradient of B (A: H<sub>2</sub>O/MeCN/HCOOH, 95/05/0.1, B: H<sub>2</sub>O/MeCN/HCOOH, 20/80/0.1). Peptides were eluted using a 120 min gradient with a flow rate of 300 nL/min. An MS survey scan was obtained for the m/z range of 350-1600; MS/MS spectra were acquired using a top 12 method, where the top 12 ions in the MS spectra were subjected to High Energy Collisional Dissociation (HCD). An isolation mass window of 2 m/z was used for the precursor ion selection, and normalized collision energy of 27% was used for fragmentation. Five second duration was used for the dynamic exclusion. Peptide identification was performed using PEAKS Studio (BSI, Canada). The search settings were: precursor  $\Delta m$  tolerance = 10 ppm, fragment  $\Delta m$  tolerance = 0.2 Da, missed cleavages = 2, -10logP>50, modifications of lysine: NBF (163.0012), modifications of cysteine: NBF (163.0012), DCP-Bio1 (394.1557), or hydrolyzed DCP-Bio1 (168.0786).

#### In Gel Detection of Persulfidation (Dimedone Switch Method)

#### Preparation of DAz-2:Cy-5 Click Mix

Final concentrations of 1 mM DAz-2 (Cayman Chemical), 1 mM Cyanine5 alkyne (Lumiprobe), 2 mM copper(II)-TBTA complex (Lumiprobe) and 4 mM ascorbic acid made *in situ*, were added sequentially in 15 mM PBS buffer mixed with 30% (vol/vol) acetonitrile. The solution was mixed at RT overnight and then quenched with 20 mM ethylenediaminetetraacetic acid (EDTA) and mixed at RT for 2 hr.

# Persulfidation of PTP1B

PTP1B, human recombinant, was purchased from Abcam at 1 mg/mL. Protein was desalted on biospin columns (BioRad) and concentration adjusted to 10  $\mu$ M. Samples were either left untreated, exposed to 50  $\mu$ M H<sub>2</sub>O<sub>2</sub>, or combination of 50  $\mu$ M H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>S for 15 min at 37 °C. Samples were then desalted and treated with or without 1 mM NBF-Cl in HEPES buffer supplemented with 2 % SDS for 1 h at 37 °C. Following a desalting step on biospin column, the same samples were treated with 20  $\mu$ M DAz-2:Cy-5 Click Mix for 30 min at at 37 °C. Unreacted reagent was removed by desalting on biospin columns and samples mixed with Laemmli buffer supplemented with 10%  $\beta$ -mercaptoethanol.

#### Persulfide Detection in Cell Lysates

Cells were grown to 80-90% confluency in a 10 cm Petri dish, and following respective treatments, were gently washed twice with cold 15 mM PBS. 1 ml of cold HEN lysis buffer (50 mM Hepes, 1 mM EDTA, 0.1 mM Neocuproine, 1% IGEPAL and 2% SDS, adjusted to pH 7.4, 1% protease inhibitor) supplemented with 5 mM 4-chloro-7-nitrobenzofurazan (NBF-CI) was then added onto the cells. Cells were gently scrapped, lysates were collected, homogenized with a syringe and needle and immediately placed for incubation at 37 °C for 30 min, protected from light. A methanol/chloroform precipitation was performed, as previously described, protected from light. Protein pellets obtained were then redissolved in 50 mM Hepes (Euromedex, adjusted pH 7.4) supplemented with SDS (2% final conc.). Once fully dissolved, protein concentration was determined using a DC Assay (BioRad) and adjusted to approx. 3 mg/ml. DAz-2:Cy-5 click mix (final. conc. 25  $\mu$ M), prepared as described above, was added to the adjusted samples and incubated at 37 °C for 30 min, protected from light. The sample was then precipitated by methanol/chloroform and the protein pellet obtained was redissolved in Hepes with SDS (final conc. 2%). The protein concentration was adjusted to 2-2.5 mg/ml and sample was prepared for SDS-PAGE and recorded, as previously described. A step-by-step protocol is provided as External Database S6.

For H<sub>2</sub>S donor treatments were performed with 200  $\mu$ M Na<sub>2</sub>S for 45 min, 200  $\mu$ M GYY4137 for 2 hr, 200 nM AP39 for 2 hr and 2 mM D-Cysteine for 1 hr. Erastin treatments were performed by treating cells with 1  $\mu$ M and 10  $\mu$ M erastin for 18.5 hr. Monensin treatments were performed with 1  $\mu$ M monensin for 18 hr.

For P-SSH labeling in thiosulfate and TST treated HeLa lysates, cells were lysed with RIPA lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 2 mM EDTA, 1% IGEPAL and 2% SDS, adjusted to pH 7.4) supplemented with 1% protease inhibitor, as previously described. Lysates were precipitated by TCA precipitation; 100% TCA stock solution was added to sample to obtain a final mixture of 20% TCA. The mixture was incubated on ice for 10 min followed by centrifugation (30000 x g, 10 min, 4 °C) and supernatant was aspirated. Protein pellet was then washed with cold acetone and centrifuged (30000 x g, 10 min, 4 °C) twice, and left to dry. Protein pellet was redissolved in 50 mM Hepes and treated with or without 500  $\mu$ M thiosulfate, 5 $\mu$ M thiosulfate sulfur transferase (TST) or a co-treatment of both, for 1 hr at 37 °C. Next, 10 mM NBF-Cl was added to the mixtures, incubated for 1 hr at 37 °C and cleaned by methanol/chloroform precipitation. Samples were then switch labeled with DAz-2:Cy5 preclick mix and processed for SDS PAGE as previously described.

#### Persulfide Detection in Escherichia coli Lysates

The laboratory strain *E. coli* MG1655 was transformed with the pSB74 plasmid that contains the *phsABC* operon of *S. Enterica* serovar Typhimurium for H<sub>2</sub>S production. As a negative control, *E. coli* was transformed with an empty vector (pTrc99a). Both strains were streaked on TSA plates supplemented with ampicillin 50 mg/ml, and incubated overnight at 37 °C. The next day, one colony of each strain was inoculated into TSB medium supplemented with 50 mg/ml of ampicillin and grown at 37 °C with agitation (100 rpm), as a starter inoculum. 10 µl of each of these overnight-grown cultures were transferred into 10 ml of fresh TSB medium supplemented with the appropriate antibiotic. Both strains were treated with or without 20 mM sodium thiosulfate for 4 hr at 37 °C with agitation (120 rpm). After the incubation time, samples were harvested by centrifugation at 5000 rpm for 4 min, washed with icecold PBS and resuspended in HEN lysis buffer supplemented with 1% protease inhibitor and 25 mM NBF-Cl. Cells were disrupted on ice by sonication, 20 seconds at 190 MHz 2 times with a 2 min pause, and incubated at 37 °C for 1 hr, protected from light. Samples were then precipitated and protein pellets were processed as previously described for persulfide labeling, with 50 µM DAz-2:Cy5 preclick mix, for 1 hr at 37 °C protected from light.

#### Persulfide Detection in Saccharomyces cerevisiae Lysates

200  $\mu$ L of yeast cell strains WT,  $\Delta cys3$ ,  $\Delta cys4$  and  $\Delta tum1$  were mixed with 1 mL HEN lysis buffer supplemented with 1% protease inhibitor for yeast and 20 mM NBF-Cl. Mixture was added dropwise into liquid nitrogen and grinded using a mortar and pestle together with glass beads, reaching a fine powder consistency. Samples were transferred in 2 ml tubes and centrifuged (1500 x g, 15 min at 4 °C). Supernatants were collected and incubated at 37 °C for 1 hr, protected from light. After precipitation, protein pellets were processed as previously described with 50  $\mu$ M DAz-2:Cy5 preclick mix for 45 min at 37 °C.

#### Persulfide Detection in Caenorhabditis elegans Lysates

Synchronous populations of embryos were obtained by lysing gravid hermaphrodites in alkaline bleach as previously described (Emmons et al., 1979). Once washed free of the alkaline bleach by centrifugation, the embryos were inoculated on standard NGM agar plates seeded with E. coli OP50-1, ~4000 embryos/plate. At Day-1 adult stage worms of different strains (N2, cth-1, mpst-3, eat-2, eat-2; cth-1, eat-2; mpst-3) were collected from the NGM plates, 4 plates/strain, into 15 ml falcons using M9 buffer and washed three times. Worm pellets were frozen in liquid nitrogen and 500 µl of glass beads was added in every tube. Samples were put in the bead beater (FastPrep-24, MP Biomedicals, California, USA) for 35 seconds at speed 6.5 m/s, followed by an additional cycle at the same speed for 20 seconds. HEN lysis buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl was added to each tube, and centrifuged for 15 min at 13000 rpm at 4°C. Supernatants were collected and incubated at 37 °C for 45 min. Samples were then precipitated and protein pellets were switch labeled for persulfides and processed as previously described. Synchronous populations of embryos were obtained as described above and inoculated on standard NGM agar or NGM agar plates supplemented with 1 mM sodium thiosulfate seeded with E. coli OP50-1. The plates were then incubated at 20 °C until worms reached Day-1 adult stage. Worms were collected from the plates and processed for persulfide labeling, as previously described. For the detection of persulfide levels in aging N2 worms (Day-1, Day-3 and Day-7) synchronous populations of embryos were placed on standard NGM-agar plates seeded with E. coli OP50-1. L4 staged worms were transferred to NGM agar plates containing 25 µM 5-Fluoro-2'-deoxyuridine (FUdR). One day after transfer, worms were collected with M9 buffer and filtered (Cell Strainer 40 µm, ClearLine) in order to remove the eggs. Worms were either directly lysed and processed for persulfide labeling as previously described (for Day-1 of adulthood) or placed back onto FUdR containing plates and grown for respective times (Day-3 and -7 of adulthood), whilst filtering and transferring to fresh FUdR plates every 2-3 days.

# Persulfide Detection in Drosophila melanogaster Lysates

As wild-type control, the  $y^l w^{118}$  Drosophila line was used. Eip55E (Drosophila CSE)-overexpressing lines were a kind gift from Professor Ody Sibon (University of Groningen). 3-4 whole flies were grinded in dounce homogenizer

with 50  $\mu$ L of HEN lysis buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl on ice. Homogenates were centrifuged at 30000 x g for 20 min and the supernatant was incubated for 30 min at 37 °C, protected from light. Samples were then precipitated and protein pellets were switch labeled for persulfides and processed as previously described.

#### Persulfide Detection in Mouse Kidney Lysates

CSE <sup>+/+</sup> and CSE <sup>-/-</sup> C57BL/6 mice were generated and previously characterized (Markó et al., 2016). CSE<sup>+/-</sup> males and females were bred to obtain CSE<sup>+/+</sup> and CSE<sup>-/-</sup> littermates. Mice were allowed free access to standard chow and water. The mice were kept in a 12:12-hr light-dark cycle. The kidneys from these mice were kind gift from Professor Maik Gollasch (Charité Medical Faculty). 5-10 mg of kidney tissue was homogenized with 500  $\mu$ L of HEN lysis buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl on ice in dounce homogenizer. Homogenates were centrifuged at 30 000 x g for 20 min and the supernatant was incubated for 30 min at 37 °C, protected from light. Samples were then precipitated and protein pellets were switch labeled for persulfides and processed as previously described.

#### Persulfide Detection in Rat Tissue Lysates

Immediately after decapitation, the brain, heart and liver were quickly removed and snap frozen in liquid nitrogen. The organs were shredded and homogenized in HEN lysis buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl using a dispersion system (Ultra-Turax T25, Janke & Kunkel, IKA-Labortechnik, Germany) at 8000 rpm on ice. After 1 hr incubation at 37 °C protected from light, samples were precipitated, processed for persulfidation labeling (with 50 µM DAz-2:Cy5 preclick mix) as previously described.

#### Persulfide Detection in Liver of Aging Mice following dietary restriction (AL and CR)

On the day of the experiment, mice were brought to the procedure room (6.30 am) and placed in clean cages. Food for AL mice was transferred to the hopper, and CR mice were fed per usual at 7am with one pellet per mouse on the floor of the cage. They were allowed to eat for 2 hr and then starting at 9am, mice were anesthetized with isoflurane (2-5% in oxygen) and a cardiac puncture was performed to withdraw blood. Cervical dislocation was performed to ensure euthanasia and tissues were excised and snap frozen in liquid nitrogen. From the time of cervical dislocation to excision and snap freezing of the liver, this period did not exceed 30 seconds (mice were not fasted for this experiment). 20 mg of liver was cut into small pieces with a scalpel placed in 1 ml of HEN buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl, which were then homogenized using a dispersion system on ice and switch labeled with 50  $\mu$ M DAz-2:Cy5 preclick mix and processed as previously above.

# Persulfide Detection in Skeletal Muscle of Mice following a Glucose Tolerance Test

A glucose tolerance test (GTT) was performed on 2-month old and 12-month-old mice which were fasted for 16 hr prior to being injected with D-glucose (i.p. 2 g/kg body weight). Blood glucose level was recorded by tail vein bleeding immediately before and at indicated time points (15 min and 60 min) after injection using an Ascensia Contour blood glucose meter and test strips. At the indicated time points, the mice were euthanized by cervical decapitation and the skeletal muscle was isolated and snap frozen in liquid nitrogen. 20 mg of frozen muscle was cut in to small pieces with a scalpel and placed in 2 ml of HEN buffer supplemented with 1% protease inhibitor and 20 mM NBF-Cl. The suspension was then homogenized using a dispersion system on ice and processed for persulfidation labeling (50  $\mu$ M DAz-2:Cy5 preclick mix), as previously described.

#### Persulfide Detection in Human Erythrocyte Lysates

Erythrocyte lysates were prepared in two ways, to obtain membranes and cytosol. Packed erythrocytes were lysed with 5 volumes of 10 mM phosphate buffer containing 5 mM NBF-Cl for 30 min at 4 °C with frequent vortexing. After 20 min centrifugation (30,000 x g) supernatant was separated from cell membrane pellet. SDS (for a final conc. of 2%) and 10 mM NBF-Cl (final conc.) were added to the supernatant and incubated for an additional 60 min at 4 °C. Membrane pellets were washed 3 times with PBS containing 5 mM NBF-Cl, and then resuspended in PBS containing 5 mM NBF-Cl and SDS (final conc. 2%) and incubated for 30 min at 37 °C. Both membrane and cytosol proteins were precipitated and processed for persulfidation labeling (50  $\mu$ M DAz-2:Cy5 preclick mix) as previously described.

#### Persulfidation Detection by Confocal Microscopy and Epifluorescence Deconvolution Microscopy

 $CSE^{+/+}$  and  $CSE^{-/-}$  MEF cells were grown in  $\mu$ -Dish (35 mm, high Glass Bottom, 81158) obtained from Ibidi® (Martinsried, Germany) following manufacturer's instructions. The treatments with 2 mM D-cysteine and 200  $\mu$ M Na<sub>2</sub>S (H<sub>2</sub>S) were performed over 1 hr at 37 °C. After treatments, the cells were washed twice with warm sterile PBS,

and incubated with 1 mM NBF-Cl in PBS for 30 min at 37 °C. Fixation was carried out by incubation with ice-cold methanol at -20 °C for 20 min and subsequent permeabilization with ice-cold acetone at -20 °C for 5 min. The dishes were washed with PBS and incubated with an additional 1 mM (final conc.) NBF-Cl in 2 ml PBS for 1 hr at 37 °C. Cells were washed with PBS overnight with agitation, and incubated with 10 µM (final conc.) DAz-2:Cy-5 click mix in 2 ml PBS for 1 hr at 37 °C. For the negative control cells were incubated with 10 µM DAz-2:Cy-5 click mix prepared without DAz-2. After overnight washing with PBS, cells were washed with methanol 3 x 10 min, followed by an additional washing with PBS. DAPI staining was performed by incubating cells with 300 nM DAPI (final conc.) in 2 ml 15 mM PBS, for 5 min with agitation, protected from light, and then cells were washed 5 times gently with 15 mM PBS. Images were obtained using a Confocal Leica TCS SP5 microscope equipped with an Argon laser (458, 476, 488, 514 nm), a diode laser (405 nm) and Helium-Neon laser (633 nm). A x40 oil objective lens was used. For examination of co-localization of immunofluorescence, single optical sections at the same focus plane were taken separately and the 3 corresponding channels (405 nm (DAPI), 488 nm (NBF-adducts) and 633 nm (Cy5 for PSSH)) were merged into a 8-bit RGB tiff-file using ImageJ. Z-stack images were taken on Olympus IX81 inverted fluorescence microscope using x 100 oil objective lens, used for image deconvolution.

#### Antibody Array-like Approach for Detection of Persulfidation

Each antibody was added into the appropriate wells of a 96 well plate with 3D-NHS Surface (PolyAn, Berlin) at a final volume of 50  $\mu$ l in PBS buffer (150 mM Na<sub>2</sub>HPO<sub>4</sub> / NaHPO<sub>4</sub> and 50 mM NaCl, pH 8.5). Additionally, in the negative control wells 50  $\mu$ l of 5% BSA in TBST (137 mM NaCl and 20 mM Trizma base, pH 7.4, supplemented with 0.1% Tween) and 0.002% NaN<sub>3</sub> was added. The plate was covered and incubated at 4 °C overnight with agitation. The solutions were discarded and the wells were washed 5 times with 15 mM PBS buffer supplement with 0.01% Tween, using a multi-channel and inverting the clean plate against paper towels for complete removal of liquid. All wells were then blocked with 50 mM ethanolamine in 100 mM Tris at pH 9 for 2 hr at RT with agitation. The blocking solution was discarded and wells were re-blocked with 5% BSA in TBS with 0.01% Tween-20 for a further 1 hr at RT with agitation. Wells were washed again as described above. Following complete removal of liquid, 100  $\mu$ l of treated samples were added to appropriate wells and incubated at 4 °C, overnight with agitation. After washing, the plate was recorded on Typhoon FL9500 at 473 nm and 635 nm.

For experiments where CSE<sup>+/+</sup>, CSE<sup>-/-</sup> and CSE<sup>+/+</sup> treated with D-cysteine MEF cells, lysates were obtained and labeled for persulfides, as previously described and redissolved in 50 mM Hepes at a concentration of 0.3 mg/ml. The following antibodies were used: 1. β-actin (0.04 mg/ml, sc-47778, Santa Cruz Biotechnology); 2. β-tubulin (0.04 mg/ml, T0198, Sigma Aldrich); 3. GAPDH (0.04 mg/ml, G8795, Sigma Aldrich); 4. HSP70 (0.04 mg/ml, ab5439, Abcam); 5. KEAP1 P586 (0.0062 mg/ml, 4678, Cell Signalling); 6. eNOS (0.0025 mg/ml, 32027, Cell Signalling); 7. Parkin (0.04 mg/ml, sc-136989, Santa Cruz Biotechnology); 8. SOD-2 (0.04 mg/ml, sc-137254, Santa Cruz Biotechnology); 9. Anti-TST (0.04 mg/ml, GTX114858, GeneTex); 10. Anti-SQR (0.04 mg/ml, HPA017079, Sigma Aldrich).

For experiments with lysates of HeLa cells treated in a time-dependent manner with EGF and labeled as described above. The following antibodies were used: PTEN (1:1000, sc-7974, Santa Cruz Biotechnology,); 2. PTP1B (1:1000, sc-133259, Santa Cruz Biotechnology); 3. SH-PTP2 (0.04 mg/ml, sc-7384, Santa Cruz Biotechnology); 4. EGFR (0.04 mg/ml, sc-03-G, Santa Cruz Biotechnology).

#### **MnSOD** Persulfidation and Activity Experiments

Human recombinant MnSOD was purchased from Creative BioMart. SOD activity was measured using cytochrome c assay, as described previously (Liu et al., 2007). Peroxynitrite was prepared following well established protocol (Filipovic et al., 2012) and tyrosine nitration assessed using characteristic spectral properties: an increase in the absorbance at 430 nm was attributed to nitrotyrosine formation due to the characteristic shift (from 430 to 357 nm) observed with decreasing pH (Filipovic et al., 2012).

#### **Detection of Protein Sulfenylation**

Following respective treatments, cells were lysed in cold HEN lysis buffer supplemented with 1% protease inhibitor and 100  $\mu$ M DCP-Bio1. Cells were gently scrapped, lysate was collected, homogenized with a syringe and needle and immediately placed for incubation at 37 °C for 1 hr. A methanol/chloroform precipitation was performed as previously described, and protein pellet obtained was redissolved in 50 mM Hepes with SDS (final conc. 2%). Protein pellets were prepared for SDS-PAGE, as previously explained. After resolving samples using SDS-PAGE, protein transfer was performed on nitrocellulose membrane, followed by blocking in 5% dry-milk in PBS supplemented with 0.1% Tween (PBST) and incubated with Streptavidin Protein DyLight 488 in 50 mM PBS (1:10000, 21832, Thermo Fisher Scientific) for 1 hr protected from light. Nitrocellulose membrane was recorded at 473 nm, using a Typhoon FLA 9500 (GE Healthcare). Membrane was then striped using 0.04M NaOH, incubated with agitation for 5 mins twice, and then washed with PBST (5 times, 5 min), blocked as previously described and incubated with GAPDH or  $\beta$ - tubulin as a loading control. Membranes were washed and incubated with respective secondary horseradishconjugated antibodies for 2 hr at RT. Membranes were then washed and visualized using Clarity<sup>TM</sup> Western ECL Substrate (BioRad) on a G:Box Chemi-XT4 (Syngene).

#### **Detection of Protein Sulfinylation**

Following respective treatments, cells were lysed in cold HEN lysis buffer supplemented with 1% protease inhibitor and 5 mM NBF-Cl or 20 mM NEM. Cells were gently scrapped, lysate was collected, homogenized with a syringe and needle and immediately placed for incubation at 37 °C for 30 min or 1 hr respectively. A methanol/chloroform precipitation was performed and protein pellet obtained was redissolved and protein concentration adjusted as previously described. Samples were treated with 1 mM BioDiaAlk at RT for 1 hr, protected from light. Reaction was quenched by the addition of 1 mM DTT incubated overnight at 4 °C or by a methanol/chloroform precipitation. 3 equivalents of sample was then mixed with 1 equivalent of Laemmli buffer (4X) supplemented with 10%  $\beta$ mercaptoethanol and incubated for 20 min at 55 °C. After resolving samples using SDS-PAGE, protein transfer was performed, followed by blocking in 5% dry-milk in PBST and incubated with Cy5-Streptavidin (1:4000, 21832, Thermo Fisher Scientific) for 1 hr protected from light. Nitrocellulose membrane was recorded at 635 nm, using a Typhoon FLA 9500 (GE Healthcare). GAPDH was used as a loading control as described above.

#### **Receptor Tyrosine Kinase Activation of Cells**

#### Activation Conditions

EGF (PromoKine) treatments in HeLa and MEF cells, and insulin treatments in SH-SY5Y cells, were performed with cells cultured in 100 mm cell culture dishes at a 80-90% confluency. VEGF (PromoCell) treatments were performed with HUVECs cultured in T25 flasks (Greiner) at a 70-80% confluency. For pretreatments, media was replaced with complete media supplemented with 100  $\mu$ M GYY4137 or inhibitor mix (PG and AOAA, 1 mM of each), incubated at 37 °C and 5% CO<sub>2</sub> for 30 min and then washed with warm sterile PBS. Subsequently, media was replaced with complete media (as described above) for control and media supplemented with respective treatments (100 or 200 ng/ml EGF; 100 nM or 200 nM insulin; 40 ng/ml VEGF). Following treatments, cells were washed with cold PBS twice and lysed for persulfide or sulfenic acid labeling, as previously explained.

#### Real-time activity of EGFR in HeLa cells

Cells were seeded at  $1 \times 10^4$  cell/well in an equilibrated E-Plate VIEW 16 PET (ACEA Biosciences, San Diego, USA) and grown overnight at 37 °C with 5% CO<sub>2</sub>. Next, cells were incubated in serum-free complete medium for 4 hr prior to experiments and pretreated with 100  $\mu$ M of GYY4137 or inhibitor mix (PG and AOAA, 1 mM of each), as indicated. Basal receptor tyrosine kinase activity in cells was recorded for 20 min and upon the addition of 150 ng/ml EGF, cells were further recorded in 2 hr with the integration time of 1 minute using xCELLigence RTCA DP system (ACEA Biosciences, San Diego, USA).

#### Antibody Microarray Detection of Persulfidation of EGFR Pathway kinases

The persulfidation of the proteins associated to the EGFR pathways were assessed using an EGF pathway phospho antibody array comprising of 214 antibodies related to the EGF pathway (Full Moon Biosystems, CA), performed in duplicates. The glass strips (barcode: 4000026018, 4000026019, 4000026022 and 4000026023) were equilibrated to RT for 1 hr and dried for 45 min. They were then blocked with 5% BSA in TBST by rocking at 55 rpm at RT for 45 mins. The slides were then washed with TBST (twice), TBS (twice) and then with H<sub>2</sub>O (5 times). Lysates of HeLa cells treated with or without 100 ng of EGF for 30 mins and labeled for persulfides, as previously explained. Samples were then dissolved in 50 mM Hepes with SDS (final conc. 2%) and further diluted 60 times with 1% BSA in TBS up to 6 ml. The glass strips were then submerged in the samples and incubated by rocking at 55 rpm for 2 hr. The slides were washed as done previously, left to dry and recorded at 473 nm and 635 nm, using a Typhoon FLA 9500 (GE Healthcare). Persulfide levels of each dot for each type of antibody (in pentaplicates on glass strip) were averaged. The intensity of their cy5 (635 nm) signal was adjusted to the 473 nm signal for the internal standards, GAPDH and  $\beta$ -actin.

#### Immunoblotting

Untreated or specifically treated cells were washed twice with cold PBS and harvested in RIPA lysis buffer supplemented with 1% protease inhibitor. Cells were scrapped, lysates were collected, homogenized with syringe and needle, and centrifuged at 30,000 x g for 5 min at 4 °C. Clear lysate was transferred to a new tube. Protein concentration of lysate was determined using the DC assay (BioRad). Protein samples were resolved by SDS-PAGE and transferred to a nitrocellulose membrane (GE Healthcare).

Primary antibodies used: CBS (1:1000, sc-133154, Santa Cruz Biotechnology), MPST (1:4000, HPA001240, Sigma Aldrich), CTH (MEF cells, 1:4000), CTH (HeLa cells, 1:1000, sc-374249, Santa Cruz Biotechnology), p-ERK
(1:1000, sc-7383, Santa Cruz Biotechnology), total-ERK (1:1000, sc-271269, Santa Cruz Biotechnology), DJ-1 (1:250, sc-55572, Santa Cruz Biotechnology), DJ-1 Oxidized At C106 (1:1000, HCA024, BioRad) and  $\beta$ -tubulin (1:5000, T0198, Sigma Aldrich). Species-specific horseradish-conjugated secondary antibodies (1:5000, Santa Cruz Biotechnology) were used for antigen detection and visualized using Clarity<sup>TM</sup> Western ECL Substrate (BioRad) on a G:Box Chemi-XT4 (Syngene).

## Immunoprecipitation and detection of DJ-1 Persulfidation, Sulfinylation and Sulfenylation Following H<sub>2</sub>O<sub>2</sub> Treatment

MEF cells (CSE<sup>+/+</sup> and CSE<sup>+/-</sup>) were treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 15 or 30 min. Samples were labeled for oxPTMs as previously described; for the detection of persulfides NBF-Cl was used and switched with DCP-Bio1; for sulfenic acid labeling DCP-Bio1 was used; for sulfinic acid labeling NEM was used and then labeled with BioDiaAlk. Proteins were then precipitated and resuspended in 50 mM Hepes with SDS (final conc. 0.01%) at 1 mg/ml. Samples were incubated overnight at 4 °C with anti-DJ-1 agarose coupled antibody (Santa Cruz Biotechnology, sc-55572 AC). After incubation agarose resins were collected by centrifugation (2,000 x g, 10 min) and washed with 10 mM TBS supplemented with 0.001% Tween (3 times) and with 10 mM TBS (twice). DJ-1 protein was eluted from the resins for 10 min at 95 °C in 10 mM TBS with 3.5% SDS. This was then mixed with Laemmli buffer (4X) supplemented with 10%  $\beta$ -mercaptoethanol (3 eq. sample : 1 eq. buffer) and incubated overnight at 55 °C. Elution fractions were collected by centrifugation (30,000 x g, 20 min) and supernatants were resolved by SDS-PAGE. Protein transfer was performed, followed by blocking in 1% BSA in TBST. Monoclonal anti-Biotin-Peroxidase-conjugated antibody (1:1000, Sigma Aldrich) was used for detection and visualized using Clarity<sup>TM</sup> Western ECL Substrate (BioRad) on a G:Box Chemi-XT4 (Syngene).

### Trx-catalyzed reduction of S-sulfocysteine (SSC)

### Cloning and Mutagenesis of Human TRP14 and Trx1

Genetic sequence of human TRP14 was obtained by generating cDNA from mRNA isolated from human fibroblasts. The target sequence amplified by PCR forward primer 5'was using ACCATCACGGATCCATGGCCCGCTATGAGGAGG-3' containing a BamHI restriction site (underlined) and a codon (boldface combination with reverse primer 5'start type), in CCGGGGTACCGTTAATCTTCAGAGAACAACATTTCCACCAG-3' 3'containing a KpnI restriction site (underlined) and a stop codon (boldface type). The sequence of the target band was verified by commercial sequencing (GATC Biotech). The PCR product was ligated into a pQE80-L expression vector (Qiagen). Human Thioredoxin 1 (Trx1) was expressed in E. coli using pET28a expression vector. Mutagenesis of the C32S & C35S variants were conducted using PCR-mediated site-directed mutagenesis, and the Trx1-pET28a expression plasmid was used as a scaffold.

### **Expression and Purification of Human TRP14 and Trx1**

E. coli BL21 (DE3) Rosetta cells were transformed with the respective expression plasmid and cultured at 37 °C, 140 rpm in LB medium supplemented with the necessary antibiotics and grown until  $OD_{600} = 0.5$ . Protein expression was induced using isopropyl-β-D-thiogalactopyranosid (IPTG) at a final concentration of 250 μM. The expression temperature and rotation was changed to 30 °C, 120 rpm for TRP14, and 18 °C, 120 rpm for Trx1 variants; expression was conducted for ~16 hr. Cells were harvested and lysed in lysis buffer (50 mM potassium phosphate, pH 7.4, 250 mM NaCl, 10 mM imidazole) supplemented with complete EDTA-free protease inhibitor (Roche). Cells were lysed using egg white lysozyme (VWR) and homogenized in an Emulsiflex. Residual nucleic acids were disrupted using a sonication rod at 25% amplitude, 10 seconds. The cell lysate was centrifuged at 48000 x g for 45 min, and His-tagged proteins were isolated using His-Pur Ni-NTA Superflow Agarose (Thermo Fisher Scientific) in accordance with the manufacturer's protocol. Elution was performed using lysis buffer supplemented with 250 mM imidazole. Elution fractions were analyzed using SDS-PAGE and subsequent Coomassie Brilliant Blue staining, and fractions containing increased amounts of target protein were further purified using anion exchange chromatography (Source 15Q, GE Healthcare; low salt buffer: 50 mM sodium phosphate, pH 7.4; high salt buffer: 50 mM sodium phosphate, pH 7.4, 1 M NaCl). Elution was performed in a gradient over 5 column volumes. Elution fractions were again analyzed using SDS-PAGE and Coomassie Brilliant Blue staining. Relevant fractions were buffer exchanged into storage buffer (50 mM sodium phosphate, pH 7.4, 150 mM NaCl, 100 µM DTT), flash-frozen in liquid nitrogen and stored at -80 °C. Proteins were again buffer exchanged after thawing into the relevant experimental buffer.

### Coupled Assay of Trx1 / TRP14 and TrxR with Cystine / SSC

Activities of hTrx and TRP14 with cystine and SSC were recorded measuring NADPH oxidation on a Tecan Infinite M200 plate reader, set to record absorption at 340 nm. 3  $\mu$ M hTrx1 or TRP14, 30 nM TrxR from rat liver (Sigma Aldrich) and 250  $\mu$ M NADPH were used in all measurements. The concentration of cystine or SSC were set at 1 mM. Absorption at 340 nm were recorded in 10 seconds intervals over 900 seconds. The initial rate of A<sub>340</sub> decrease was fitted linearly, and consumption of NAPDH over time was determined using an NADPH extinction coefficient  $\epsilon_{340}$  of 6020 M<sup>-1</sup> cm<sup>-1</sup>.

### Kinetics of Direct Reaction of Human Trx with SSC

Kinetics of the reaction of hTrx with cysteine persulfide was monitored on a FP-8200 spectrofluorometer (Carry Eclipse, Agilent) using an excitation wavelength of 280 nm and a maximal emission of 345 nm. Concentration of the enzyme was kept at 4  $\mu$ M while substrate concentrations ranged between 25  $\mu$ M and 100  $\mu$ M. Given pseudo first-order conditions, observed rate constants  $k_{obs}$  were obtained by fitting the decrease in emission at 345 nm at a given SSC concentration using a first order exponential decay fit in Origin® analysis software.

#### Ultra High Resolution ESI-TOF Mass Spectrometry of Reaction Between Trx and SSC

10  $\mu$ M of human recombinant Trx, Trx C35S or Trx C32S were incubated with 10  $\mu$ M SSC in 20 mM ammonium carbonate buffer pH 7.8 for 5 min and recorded on maXis 5G (Bruker Daltonics) ESI-TOF MS capable of resolution of at least 40,000 FWHM, following previously described protocol (Wedmann et al., 2016).

### **MEF cells Stress Assays**

MEF cells (CSE<sup>+/+</sup> and CSE<sup>-/-</sup>) were plated in 96-well plates at  $5 \times 10^4$  cells/well. Cells were treated with H<sub>2</sub>O<sub>2</sub>, as indicated, for 24 hr and the cell survival was assessed by an MTT assay as previously described (Liu et al., 2015).

### C. elegans Stress Assays

Worms were initially synchronised by picking approximately 100 young adult worms per strain onto NGM-plates, and allowed to lay eggs over 4 hr at 20 °C. The young adults were then removed, and the remaining embryos were washed off and collected from the NGM-plates using M9 buffer, and centrifuged (850 x g, 1 min) and washed with M9 buffer 3 times. The resulting pellet of embryos was re-suspended in 12 ml of M9 buffer, and incubated with agitation (100 rpm) for 24 hr at 20 °C. The synchronised L1 worms were collected, centrifuged (850 x g, 1 min) and re-suspended in S-basal buffer. The number and synchronicity of L1 worms was determined and the worms were transferred to an Erlenmeyer, diluted in the respective amount of HB101 in S-Basal buffer for a final conc. of 60 worms/10 mg of HB101/ml of S-basal buffer. They were then incubated with agitation (115 rpm) for 48 hr at 20 °C. The worms were then collected, centrifuged (400 rpm, 1 min), washed with M9 buffer 3 times and re-suspended in M9 buffer.

For experiments with the pretreatment of worms, synchronised young adults (as described above) were transferred in an Erlenmeyer with 12 ml of M9 buffer in which GYY4137 (final conc. 500  $\mu$ M) or AP39 (final conc. 100 nM) was added. Worms were then incubated for 3 hr at 20 °C with agitation (115 rpm). Worms were centrifuged (400 rpm, 1 min) and re-suspended in M9 buffer. To assess the effect of CTH on stress resistance, paraquat dichloride hydrate (final conc. 60 mM) or sodium (meta) arsenite (final conc. 5 mM) was added to the synchronised young adult worm suspension and plated on a 96 well plate with approximately 5 - 10 worms per well. Viability was monitored by counting dead worms over 5 - 6 hr, whilst incubating the plate at 20 °C, with agitation (110 rpm).

### Yeast stress Assays and H<sub>2</sub>S production

Spot assays were carried out by spotting 5  $\mu$ l of early exponential phase cultures (OD<sub>600</sub>=0.5) of different strains of *S. cerevisiae*, sequentially diluted (approximately 3.5 x 10<sup>4</sup> to 3.5 cells) on plates with YPD media supplemented with different concentrations of H<sub>2</sub>O<sub>2</sub> (0, 1 mM, 2 mM and 5 mM). Growth was recorded after incubation of 24 and 48 hr, at 30 °C. Survival assays were done by preparing overnight cultures in YPD media (cell in stationary phase), from which the experimental cultures were set, by diluting to OD<sub>600</sub>=2 in a 5 ml final volume, with or without respective concentrations of H<sub>2</sub>O<sub>2</sub> (0, 10 mM and 20 mM). The cultures were subsequently grown in culture tubes for 27 hr at 30 °C with agitation (180 rpm). Yeast cells were then washed once in PBS and collected by centrifugation (5000 x *g*, 3 min). Cells were then resuspended in PBS supplemented with 2  $\mu$ M propidium iodide, at 1×10<sup>6</sup> cells/ml and incubated for 5 min in the dark. Analysis was performed by flow cytometry using 150,000 cells per condition, on a BD Accuri<sup>TM</sup> C6 (BD Biosciences) and results were analysed using the CFlow Plus Software. For the quantification of H<sub>2</sub>S levels, overnight cultures were washed with PBS and diluted as described for survival assays. Cell suspensions were incubated with 20  $\mu$ M MeRho-Az sensor for 45 mins at 30 °C and analysed by flow cytometry, on a BD Accuri<sup>TM</sup> C6 (BD Biosciences).

### C. elegans Lifespans

For the lifespan experiments a synchronous population of worms was obtained by transferring 5-6 young adults on medium plates and allowing them to lay eggs over 3 hr at 20 °C. Lifespan measurements were conducted at 20 °C, worms were transferred daily during the reproductive period. Death was scored by failure of the animal to move in response to gentle prodding with a platinum wire. For lifespan analysis with 1 mM sodium thiosulfate treatment or 5 mM 2-deoxy-D-glucose (DOG), new plates containing treatments in NGM agar were prepared every second day. Lifespan measurements were repeated at least twice unless otherwise stated.

### Immunohistochemistry on Rat Brains

Brains were fixed in 4% paraformaldehyde for 24 hr, dehydrated in a series of increasing concentrations of ethanol (30%–100%), enlightened in xylene and embedded in Histowax® (Histolab Product AB, Göteborg, Sweden). Sagittal plane of each brain was sectioned at 5 µm thickness on a rotary microtome (RM 2125RT Leica Microsystems, Wetzlar, Germany). Sections were placed on Superfrost Ultra Plus® manufactured slides and used for immunohistochemical staining. After tissue deparaffinization, brain sections were exposed to heat-induced antigen retrieval to demask target antigens. Slides were placed in a container and covered with 0.01 mol/l sodium citrate buffer pH 6.0, and then heated at 750 W in microwave oven for 3 x 7 min. Next, sections were incubated with 0.3% H<sub>2</sub>O<sub>2</sub> in MeOH for 15 min to block endogenous peroxidase. Slides were washed in PBS (pH 7.4) and reduction of non-specific background staining was achieved by incubation with normal swine (1:10, X0901, Dako) and donkey serum (1:10, X0903, Abcam) for 45 min at RT. Next, sections were incubated with primary antibodies; CBS (1:200, sc-67154, Santa Cruz Biotechnology), MPST (1:500, HPA001240, Sigma Aldrich), or with CTH/CSE antibody (1:200, sc-365382, Santa Cruz Biotechnology), overnight at 4 °C. For the negative control, the primary antibody was substituted with PBS. After washing for 5 min in PBS, brain tissue sections were incubated with swine-anti-rabbit (1:100, P0399, Dako) and donkey-anti-mouse IgG-HRP (1:100, ab6820, Abcam) for 1 hr at RT. Slides were washed in PBS and visualization was performed using Dako liquid 3,3'-diaminobenzidine tetrahydrochloride (DAB) substrate chromogen system (Dako) at concentrations suggested by the manufacturer. Hematoxylin was used as counterstain and slides were mounted in DPX medium (Sigma Aldrich).

### QUANTIFICATION AND STATISTICAL ANALYSIS

The experiments were performed in at least in triplicates by at least 2 different researchers. Key methodological experiments were verified by at least three different researchers in three different labs. Protein expression levels, persulfidation, sulfenylation and sulfinylation levels were compared with an unpaired t test with \*p < 0.05 and \*\*p < 0.01. Lifespan data were analyzed using Kaplan-Meier survival analysis to detect statistical differences. Plotting of the data were performed using GraphPad Prism 5.0, Origin 8 and Microsoft Excel.



# Figure S1. Testing dimedone switch method on low molecular weight and protein persulfides. Related to Figure 1.

(A) Original Improved tag switch strategy for persulfide labeling.

(B) ESI-TOF-MS spectrum of the reaction mixture containing 100  $\mu$ M nmc-penicillamine persulfide, 100  $\mu$ M NBF-Cl, and 500  $\mu$ M dimedone (ammonium carbonate buffer, pH 7.4, 23 °C). 100  $\mu$ M nmc-penicillamine persulfide and 100  $\mu$ M NBF-Cl were mixed for 10 min and after the completion of the reaction dimedone was added and the reaction

was monitored by ESI-TOF-MS. (C-E) Speciation of the observed (red) peaks and simulation of the isotopic distribution for each species (black).

(F) MS/MS spectrum of m/z 344 peak. Asterisk marks the position of m/z 344 peak that decomposed to fragments which correspond to species shown in Figure 1C.

(G) UV-vis spectral changes upon addition of 100  $\mu$ M NBF-Cl to 23  $\mu$ M HSA-SH (50 mM phosphate buffer, pH 7.4 with 1% SDS, at 37 °C).

(H) UV-vis spectral changes caused by subsequent addition of 100  $\mu$ M dimedone to the reaction mixture from (G). Inset: Kinetic trace at 420 nm.

(I) UV-vis spectral changes upon addition of 100  $\mu$ M NBF-Cl to 23  $\mu$ M HSA-SOH (50 mM phosphate buffer, pH 7.4 with 1% SDS, at 37 °C).

(J) UV-vis spectral changes caused by subsequent addition of 100  $\mu$ M dimedone to the reaction mixture from (I). Inset: Kinetic trace at 420 nm.

(K) UV-vis spectral changes upon addition of 100  $\mu$ M NBF-Cl to 23  $\mu$ M HSA-SSH (50 mM phosphate buffer, pH 7.4 with 1% SDS, at 37 °C).



Figure S2. Probing the selectivity and conditions for persulfide labeling by the Dimedone switch method. Related to Figure 2.

(A) Human serum albumin (HSA), sulfenylated HSA and HSA persulfide were treated with 5 mM NBF-Cl (in 50 mM phosphate buffer, pH 7.4 with 1% SDS, at 37 °C), precipitated and cleaned from NBF-Cl, and then incubated with

100  $\mu$ M DCP-Bio1 for 30 min at 37 °C. Following precipitation, proteins were resuspended in 50 mM phosphate buffer, pH 7.4 and spotted on a nitrocellulose membrane. Detection of dimedone-labeled adduct was done with streptavidin-Cy5. In addition, the same samples were incubated with streptavidin magnetic beads and after careful washing, the bound protein was eluted by boiling with Laemmli buffer for 5 min at 95 °C. Eluted proteins were separated by electrophoresis and in-gel fluorescence of NBF-protein adduct detected by Typhoon FLA 9500.

(B) Detection of GAPDH and GAPDH-persulfide switch labeled with DCP-Bio1. Proteins were spotted on a nitrocellulose membrane and detection of dimedone-labeled (PSSH) adduct was done with streptavidin-Cy5. Green fluorescence (NBF-protein adduct) served as a measure of protein load.

(C) MS/MS of peptide fragment obtained by trypsin digestion of bovine rhodanese (TST) shows labeling of C248 with hydrolyzed DCP-Bio1. Full MS data are given in Data S1-2.

(**D**) Commercially available TST was either incubated with thiosulfate (TS) or DTT to form, the fully persulfidated or reduced form, respectively. 20  $\mu$ M enzyme was mixed with 50  $\mu$ M NBF-Cl and switch tagged for persulfide detection with DAz-2:Cy5 CuAAC. While both untreated and thiosulfate treated showed a Cy5 signal, the green fluorescence signal was significantly reduced in the fully persulfidated enzyme, despite having the same load. On the other hand, the green fluorescence signal was much stronger in the fully reduced enzyme, suggesting that at low NBF-Cl/protein ratio, switching caused by the dimedone-based probe could affect the intensity of green fluorescence. Top image: Coomassie Brilliant Blue (CBB, protein load); Middle image: 488 signal (NBF adduct); Bottom image: Cy5 image (persulfides).

(E) 1 mM NBF-Cl (excess) was used to initially react with 20  $\mu$ M of TST, in an experiment similar to that shown in (D). The Cy5 signal was reduced when DTT treated TST was used, whilst the green fluorescence (488 nm) signal remained stable, suggesting that the green fluorescence can be used as a measure of the total protein load when excess NBF-Cl is used. It is worth mentioning that it is known that DTT is unable to fully reduce TST persulfide (Tandon and Horowitz, 1989). Top image: Coomassie Brilliant Blue (CBB, protein load); Middle image: green fluorescence signal (488, NBF adduct – protein load); Bottom image: Cy5 image (persulfides)

(F) The reaction of HSA-SH with CuAAC reagents in all possible combinations (left) show no nonselective labeling. The same samples run 2 weeks after being kept at -20 °C show some small unselective labeling in the sample treated with Cy5-alkyne, Cu(II)-TBTA and ascorbate (due to the side reaction of alkynes with thiols – as a result of no NBF-Cl used), but the signal was still negligible when compared to the signal obtained for fully labeled HSA-SSH. Top row: Cy5 signal; Middle row: 488 nm signal (NBF-adducts); Bottom row: Coomassie Brilliant Blue (CBB).

(G) Cyclic sulfenamides exist in equilibrium with sulfenic acids and react with NBF-Cl (Gupta and Carroll, 2016).

(H) Dimedone switch method efficiently distinguishes between protein sulfenic acid (and cyclic sulfenamide) and protein persulfides. PTP1B was used as a model system. Top row: Cy5 signal; Middle row: 488 nm signal (NBF-adducts); Bottom row: Coomassie Brilliant Blue.

(I) Depiction of experimental design used to test method's selectivity, shown in Figure 2D.

(J) In-gel detection of protein persulfidation levels (left; fire image) in HeLa cells, labeled with DAz-2/Cy5-alkyne CuAAC, with different concentrations of NBF-Cl. Green fluorescence (right) corresponds to NBF-protein adducts.

(K-L) Depiction of experimental design used to test method's cross reactivity with intracellular sulfenic acids and sulfenamides (K) and obtained Westernblot analysis visualized by streptavidine Cy5 (L). Blotting for vinculin was used to visualized the load.

(M) Effect of dimedone pretreatment on persulfidation signal in MEF cells. Following steps depicted in (K), MEF cell lysates were labelled for protein persulfidation using DAz-2:Cy5 preclick mix. Bands were visualized using Typhoon 9500. Green signal corresponds to the protein load, and pseudocoloring with fire was used for PSSH signal.



### Figure S3. Broad applicability of dimedone switch method. Related to Figure 3.

(A-B) Protein persulfidation levels (A) and corresponding CSE and CBS expression levels (B) in CSE<sup>+/+</sup> and CSE<sup>-/-</sup> cells of passage (P) 2 and 10. Green fluorescence (NBF adducts) used as a loading control for gels and GAPDH as a loading control for blots for normalization of signal.

(C) Confocal microscopy of *in situ* labeling of intracellular protein persulfidation. Negative control cells were incubated with DAz-2:Cy5 preclick mix prepared without DAz-2 (switching agent).  $CSE^{-/-}$  MEF cells treated with 200  $\mu$ M Na<sub>2</sub>S (H<sub>2</sub>S) or 2 mM D-Cys for 1 hr. Cy5 signal corresponds to protein persulfides, 488 nm signal corresponds to NBF-adducts. Nuclei stained with DAPI. Scale bar 20  $\mu$ m. Related to the **Figure 3K**.

(**D-F**) High-resolution images of protein persulfidation in  $CSE^{+/+}$  (**D**),  $CSE^{-/-}$  (**E**) and  $CSE^{+/+}$  MEF cells treated with D-Cys (2 mM, 1 hr) (**F**), obtained by wide-field fluorescence deconvolution.

(G) Protein evolution phylogenic tree showing the common origin of cysteine containing MnSOD.

(H-I) MS/MS spectra of peptide obtained by chymotrypsin (H) and trypsin (I) digestion of persulfide labeled by Dimedone switch method (switch agent DCP-Bio1) MnSOD containing C193 labeled with hydrolyzed DCP-Bio1.



### Figure S4. Protein persulfidation is an integral part of the cellular response to H<sub>2</sub>O<sub>2</sub> and RTK activation. Related to Figures 4 and 5.

(A) Representative blot showing the effect of GYY4137 (100  $\mu$ M, 30 min) on time-dependent H<sub>2</sub>O<sub>2</sub> (500  $\mu$ M)-induced sulfenylation in CSE<sup>+/+</sup> and CSE<sup>-/-</sup> MEF cells. Sulfenylation was labeled with DCP-Bio1 and visualized by streptavidin-488 and normalized to GAPDH. n=3.

**(B)** Persulfidation, sulfenylation and sulfonylation of DJ-1. WT and CSE<sup>-/-</sup> MEF cells were treated with 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> for 15 or 30 min, labeled for P-SSH, P-SOH and P-SO<sub>2</sub>H using biotinylated reagents, immunoprecipitated with anti-DJ-1 antibody immobilized to agarose beads and immunoblotted with anti-biotin antibody. For sulfonylated DJ-1, antibody selective for C106 sulfonic acid of DJ-1 was used. Related to **Figure 4E**.

(C) Expression levels of H<sub>2</sub>S producing enzymes, MST, CBS and CSE in HeLa cells after treatment with 100 ng/ml EGF for the indicated amount of time. Densitometric analysis of data from 3 independent experiments was normalized to  $\beta$ -tubulin. Values represent mean  $\pm$  SD. \* p< 0.05, \*\* p<0.01.

(D-E) Representative images showing protein persulfidation and sulfenylation, used for Figure 5C and 5D respectively. HeLa cells were pretreated with 100  $\mu$ M GYY4137 (30 min, D) or with 2 mM mixture of AOAA and PG (1:1, 30 min, E) and then exposed to 100 ng/ml EGF for the indicated amount of time. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. Sulfenylation was visualized by streptavidin-488 and normalized to  $\beta$ -tubulin. n=3.

(F) Representative images showing protein persulfidation and sulfenylation, used for Figure 5E. HUVEC were treated with 40 ng/ml VEGF for the indicated amount of time. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. Sulfenylation was visualized by streptavidin-488 and normalized to  $\beta$ -tubulin. n=3.

(G) Representative images showing protein persulfidation used for Figure 5F. SH-SY5Y cells were treated with either 100 nM or 200 nM insulin for indicated time points. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. n=3.

(H) Sulfenylation changes in SH-SY5Y cells treated with either 100 nM or 200 nM insulin for indicated time points. Sulfenylation was visualized by streptavidin-488 and normalized to GAPDH. n = 3.

(I-J) Changes in persulfidation and sulfenylation dynamics in WT ( $CSE^{+/+}$ ) and  $CSE^{-/-}$  MEF cells after treatment with 100 ng/ml of EGF for indicated time points. Representative images (I) and quantification of the change (J). Values are given as a mean  $\pm$  SD. from n = 3. \* p<0.05, \*\* p<0.01.

(K) Inhibition of H<sub>2</sub>S production by the pretreatment of HeLa cells with 2 mM mixture of AOAA and PG (1:1, 30 min) and subsequent treatment with 100 ng/ml EGF for indicated time points causes changes in phosphorylation levels of pERK. Expression levels of pERK were normalized against total ERK expression in the same immunoblot, while total ERK was normalized to  $\beta$ -tubulin. Densitometric analysis of the data from 3 independent experiments is shown as a mean  $\pm$  SD. \* p<0.05, \*\* p<0.01.

(L) Antibody microarray-like approach was used to address persulfidation status of EGFR, PTEN, PTP1B and SHPTP2 from HeLa cells lysates treated with 100 ng/mL EGF for the indicated amount of time. Negative control represents wells were samples were added, but 5% BSA was added instead of an antibody. Original readouts obtained by Typhoon FLA 9500 are pseudo-coloured in ImageJ to visually enhance the changes in the signal (right). Quantification of the data from two independent experiments (left). \* p < 0.05, \*\* p < 0.01.

(M-R) Persulfidation levels of  $\beta$ -catenin (M), actin (N), E-cadherin (O), Dok-2 (P), VAV1 (Q), RhoA (R) in HeLa cells lysates treated with or without 100 ng/ml EGF for 30 min and labeled for persulfides (switching agent DAz-2:Cy5), measured using EGFR pathway microarray glass slips. Related to Figures 5J, K. Each antibody was spotted in pentaplicates. Values are given as a mean ± SD. from two independent experiments. \* p< 0.05, \*\* p<0.01.



Figure S5. Trx-catalyzed reduction of S-sulfocysteine (SSC) and protection against ROS-induced death. Related to Figure 6

(A) Deconvoluted mass spectra of 10  $\mu$ M C32S Trx before (black) and after (red) the reaction with 10  $\mu$ M SSC showing no difference. Related to Figure 6C.

(B) ESI-TOF-mass spectra of 10  $\mu$ M C35S Trx before (black) and after the reaction with 10  $\mu$ M SSC (red). The arrow indicates the TrxS-S-Cys peak that is absent in the control spectrum. Related to Figure 6D.

(C) Kinetics of human Trx (4  $\mu$ M) oxidation with 25  $\mu$ M (blue line) and 50  $\mu$ M SSC (red line), followed by tryptophan fluorescence ( $\lambda_{ex}$  280 nm) changes. Spontaneous oxidation of Trx alone (Control) is shown in black.

(**D-E**) Activities of human Trx (**D**) with cystine (Cys<sub>2</sub>, black) or SSC (red) and, Trx1 (black) or TRP14 with SSC (**E**) where NADPH oxidation was recorded on a Tecan Infinite M200 plate reader, set to record absorption at 340 nm. 3  $\mu$ M human Trx1 or TRP14, 30 nM TrxR from rat liver and 250  $\mu$ M NADPH were used in all measurements. Concentration of cystine or SSC were set at 1 mM. The initial rate of A<sub>340</sub> decrease was fitted linearly, and consumption of NAPDH over time was determined using an NADPH extinction coefficient  $\varepsilon_{340}$  of 6020 M<sup>-1</sup> cm<sup>-1</sup>. (**F**) Metabolic pathways for H<sub>2</sub>S biosynthesis in *S. cerevisiae*.

(G) Growth curves for wild type,  $\Delta cys3$ ,  $\Delta cys4$  and  $\Delta tum1$  mutants of S. cerevisiae. \*\* p<0.01.

(H) Persulfidation level in wild type,  $\Delta cys3$ ,  $\Delta cys4$  and  $\Delta tum1$  mutants of *S. cerevisiae*. Green fluorescence (488, NBF-adducts) was used as a loading control. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. Quantification of persulfidation levels in different mutants (right). Values represent mean  $\pm$  SD. \*\* p<0.01.

(I) Flow cytometric analysis of H<sub>2</sub>S levels (green fluorescence, FL1A) in BY4247 (WT) and the  $\Delta cys3$  mutant of S. *cerevisiae*. Cells were incubated with 20  $\mu$ M MeRho-Az sensor (45 min, 30 °C). \*\* p<0.01.

(J) The effect of short-term (3 hr) pre-exposure to GYY4137 (500  $\mu$ M) or AP39 (100 nM) on the percentage of dead *C. elegans* after 5 hr of 60 mM paraquat. N>50 worms. \*\* p<0.01.

(**K**) The effect of short-term (3 hr) pre-exposure to GYY4137 (500  $\mu$ M) on the survival rate of N2 and *cth-1* mutant *C. elegans* mutants exposed to 5 mM sodium arsenite over 5 hr. N>50 worms. \*\* p<0.01.



### Figure S6. P-SSH levels correlate with aging. Related to Figure 7.

(A) Lifespan analysis of N2, *cth-1* and *mpst-3* mutant strains. n > 110 per line. N2 = 17.8±0.5 days, *mpst-3* = 15.7±0.4 days, p=0.0003.

(B) Persulfidation levels in N2 C. *elegans* at different days of adulthood. Intensity of persulfidation is expressed as a Cy5/488 signal ratio. Values represent mean  $\pm$  SD. from n=3. \*\* p<0.01.

(C) Expression levels of CSE, CBS and MPST in brain extracts from 1-, 6-, 12- and 24-month-old Wistar rats. GAPDH is used as a loading control. Representative blots from n=3.

(**D**) Persulfidation levels (left) and expression levels of CSE, CBS and MPST in liver extracts of 1-, 6-, 12- and 24month-old Wistar rats. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. GAPDH was used as a loading control for the expression levels. Representative images from n=3.

(E) Expression levels of CSE, CBS and MPST in muscle extracts of 2- and 12-month-old mice. GAPDH is used as a loading control. Densitometric analysis (right) of the data from 3 independent experiments is shown as a mean  $\pm$  SD. \*\* p<0.01. Related to Figure 7I.

(F) Protein persulfidation levels in HeLa cell lysates, treated with or without thiosulfate (500  $\mu$ M) or thiosulfate sulfur transferase (TST, 5  $\mu$ M) or both, for 1 hr, 37 °C. Persulfidation was detected in-gel by measuring Cy5/488 signal ratio. Values represent mean  $\pm$  SD. \*\* p<0.01.

Protein name Coverage Nº **UniProtKB** -10logP **Peptides** MW (Da) Modification/Ref.\*\* (UniProtKB database) % PSX/(Delobel et al., 1 Carbonic anhydrase 1 P00915 308.61 54 73 28,870 2016) Spectrin alpha chain, PSX/(Zaccarin et 2 P02549 101 43 280,014 266.65 erythrocytic 1 al., 2014) PSX/(Zaccarin Spectrin beta chain, et 3 P11277 248.03 61 30 246,468 erythrocytic al., 2014) PSX/(Yang et al., 2012; Zaccarin et 4 Ankyrin 1 P16157 218.79 32 21 206,265 al., 2014) PSX/(Delobel et al., 5 Catalase P04040 218.65 35 45 59,756 2016; Yang et al., 2012) PSX/(Delobel et al., 64 6 Flavin reductase P30043 197.55 17 22,119 2016) Band 3 anion transport PSX/(Zaccarin et 7 P02730 197.26 23 26 101.792 protein al., 2014) PSX/(Delobel et al., 65 8 Carbonic anhydrase 2 P00918 192.22 25 29,246 2016) PSX/(Delobel et al., 9 Peroxiredoxin 2 P32119 187.35 59 21,892 2016; Zaccarin et 26 al., 2014) Bisphosphoglycerate PSX/(Delobel et al., 10 P07738 135.16 14 51 30.005 mutase 2016) PSX,PSSH/ Glyceraldehyde-3-(Valentine et al., 11 phosphate P04406 9 39 36,053 131.26 1987; Zaccarin et dehydrogenase al., 2014) Protein/nucleic PSX/(Delobel et al., acid 099497 50 12 129.49 8 19,891 deglycase DJ-1 2016) PSX/(Delobel et al., 13 10 Peroxiredoxin 1 Q06830 123.63 45 22,110 2016) Protein DDI1 homolog Q8WTU0 6 22 14 121.85 44,124 Fructose-bisphosphate PSOH/(Valentine et 9 15 P04075 116.71 20 39,420 aldolase A al., 1987) nucleoside PSX/(Delobel et al., Purine 16 P00491 114.54 7 30 32,118 2016) phosphorylase 7 17 17 Methanethiol oxidase Q13228 112.56 52,391 PSX/(Yang et al., 18 7 10 2012; Zaccarin et Protein 4.1 P11171 112.26 97,017 al., 2014) Transitional PSX/(Yang et al., 19 endoplasmic reticulum P55072 164.3 16 21 89,322 2012) ATPase PSX/(Delobel et al., 52 20 Peroxiredoxin 6 P30041 153 11 25,035 2016) Stress-induced-PSX/(Delobel et al., 21 P31948 140.13 11 19 62,639 phosphoprotein 1 2016) Triosephosphate PSX/(Delobel et al., 22 30,791 P60174 125.54 6 31 isomerase 2016) Phosphoglycerate PSSH/(Valentine et 19 23 P00558 120.79 8 44.615 kinase 1 al., 1987)

Table S1. List of proteins found to be endogenously persulfidated in human red blood cells.\*Related to Figure 2D.

24	Heat shock cognate 71 kDa protein	P11142	119.28	10	20	70,898	PSX/(Delobel et al., 2016; Yang et al., 2012)
25	Tropomyosin alpha-3 chain	P06753	112.7	6	25	32,950	
26	L-lactate dehydrogenase B chain	P07195	111.71	10	25	36,638	PSX/(Delobel et al., 2016)
27	Alpha enolase	P06733	107.69	7	21	47,169	PSX, PSSH/ (Delobel et al., 2016; Valentine et al., 1987; Yang et al., 2012)
28	T-complex protein 1 subunit theta	P50990	106.44	7	16	59,621	
29	Low molecular weight phosphotyrosine protein phosphatase	P24666	103.76	5	38	18,042	
30	Alpha adducin	P35611	99.94	7	11	80,955	PSX/(Yang et al., 2012)
31	Erythrocyte band 7 integral membrane protein	P27105	98.78	7	29	31,731	PSX/(Zaccarin et al., 2014)
32	Eukaryotic translation initiation factor 5 alpha	P55010	95.91	4	31	49,223	
33	Heat shock protein HSP 90-alpha	P07900	94.83	7	13	84,660	
34	Peroxiredoxin 4	Q13162	93.27	6	11	30,540	
35	Adenylate kinase isoenzyme 1	P00568	91.30	5	23	21,635	PSSH/(Valentine et al., 1987)
36	Ubiquitin-like modifier- activating enzyme 1	Q5JRR6	89.97	4	5	56,852	PSX/(Yang et al., 2012)
37	Ubiquitin carboxyl- terminal hydrolase 14	P54578	87.61	4	13	56,069	PSX/(Yang et al., 2012)
38	β adducin	P35612	87.23	3	5	80,854	
39	Hsc70 interacting protein	P50502	85.19	4	12	41,332	
40	Proteasome subunit alpha type-5	P28066	82.21	3	18	26,411	
41	Thioredoxin	P10599	79.82	4	31	11,737	
42	Rab GDP dissociation inhibitor $\beta$	P50395	79.5	4	10	50,663	PSX/(Delobel et al., 2016)
43	Glutathione S- transferase A1	P08263	78.34	3	13	25,631	PSX/(Delobel et al., 2016)
44	Erythrocyte membrane protein band 4.2	P16452	75.97	4	8	77,009	PSX/(Zaccarin et al., 2014)
45	Rho GDP dissociation inhibitor 1	P52565	75.91	3	16	23,207	
46	Dematin	Q08495	75.59	4	12	45,514	
47	Ankyrin 3	Q12955	75.45	5	1	480,410	PSX/(Zaccarin et al., 2014)
48	3-mercaptopyruvate sulfur transferase	P25325	68.09	4	9	33,178	
49	Carbonic anhydrase 3	P07451	69.76	3	14	29,557	PSX/(Delobel et al., 2016)
50	Ubiquitin conjugating enzyme E2	P51668	72.33	3	30	16,602	

51	Serine/threonine protein kinase OSR1	095747	68.46	4	8	58,022	
52	Copper chaperone for superoxide dismutase	O14618	67.22	3	10	29,041	
53	Transaldolase	P37837	66.97	4	9	37,540	PSX/(Delobel et al., 2016)
54	Protein S100-A6	P06703	80.68	5	43	10,180	
55	Malate dehydrogenase	P40925	60.29	3	13	36,426	PSX/(Delobel et al., 2016)
56	Glutamatecysteine ligase catalytic subunit	P48506	59.76	2	10	72,766	
57	Proteasome subunit alpha type-1	P25786	59.18	2	10	29,556	
58	14-3-3 protein beta/alpha	P31946	50.89	2	8	28,082	

\* The table consists of proteins identified by at least 2 reliable peptides and -10logP>50, obtained by trypsin and/or chymotrypsin digestion.

\*\*PSX denotes any kind of DTT-reducible cysteine oxidation (PSSH, PSOH, PSSP). PSSH denotes protein persulfidation specifically.

## **CHAPTER 4: Article II**

# Interdependency of host and pathogen protein persulfidation governs disease severity in experimental and human aspergilloses

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Interdependency of host and pathogen protein persulfidation governs disease severity in experimental and human aspergilloses

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### 22 INTRODUCTORY PARAGRAPH

23 The ability to adapt to the harsh conditions imposed by a host is fundamental for pathogens' 24 infective capacity and, concomitantly, the host cellular response must be finely tuned to mount an 25 efficient response against the pathogen and clear infection. Post-translational modifications (PTMs) 26 are important for adaptation to stress and therefore are expectedly crucial for both pathogen and 27 host. Here we investigated the relevance of the PTM persulfidation for virulence of the human 28 fungal pathogen Aspergillus fumigatus and for antifungal host defence in mammalian lung cells. We 29 show that a weakly persulfidating A. fumigatus mutant is more susceptible to host-mediated killing 30 and displays reduced virulence in a murine model of infection. Besides, we found that a single 31 nucleotide polymorphism (SNP) in the human gene encoding cystathionine- $\gamma$ -lyase, the main enzyme 32 responsible for protein persulfidation in the lungs, predisposes to invasive pulmonary aspergillosis in 33 hematopoietic stem cell transplant recipients, and we further show that correct levels of 34 persulfidation are required for optimal antifungal activity of lung-resident host cells. Interestingly, 35 levels of host protein persulfidation determine the levels of fungal persulfidation, reflecting a direct 36 host-pathogen cross-talk.

37

Post-translational modifications (PTMs) constitute a rapid acting response mechanism that permits fast adaptation to short-lasting and varying stresses. Therefore, appropriately timed and executed PTM modifications are likely crucial for the survival of pathogens inside their hosts [1] as well as for optimal host responses [2]. Hydrogen sulphide (H<sub>2</sub>S) is a gaseous signalling molecule or gasotransmitter, which is produced in mammalian tissues by at least three enzymes —cystathionine  $\beta$ -synthase (CBS), cystathionine  $\gamma$ -lyase (CSE) and 3-mercaptopyruvate-sulphurtransferase (MST)— [3, 4]. It has been postulated that H<sub>2</sub>S exerts its signalling via protein persulfidation [5], a post-

46 translational modification that consists of the conversion of a thiol (-SH) into a persulfide (–SSH) 47 group in cysteine residues of target proteins [6]. The exact mechanism by which  $H_2S$  becomes 48 activated to modify specifically target cysteine residues remains unclear [7]. H<sub>2</sub>S is. Persulfidation 49 can increase or decrease function or activity of a given protein, which translates into a prominent 50 regulatory role for various physiological functions [8], including inflammation and counteracting 51 endoplasmic reticulum stress [5]. Furthermore, the number of proteins discovered to undergo 52 persulfidation is steadily increasing [8]. Nevertheless, despite evidence of its importance, little is 53 known about the role and relevance of protein persulfidation for mammalian immunity.

In contrast to extensive research undertaken on H<sub>2</sub>S signalling and persulfidation in mammalian cells, insights about their relevance in microbes are limited. H<sub>2</sub>S production has been shown to be important for antibiotic susceptibility of several bacteria [9] and their defence against the host immune response [10] and inflammation [11]. The relevance of persulfidation specifically, has only been studied so far in *Staphylococcus aureus*, where it could be linked to resistance against antibiotics and cellular redox stress and to the global regulation of the production of virulence factors [12].

Here we address the relevance of persulfidation for adaptation of the human pathogenic fungus 61 62 Aspergillus fumigatus to its mammalian host, and for host defence against pathogen challenge. In 63 the wild, A. fumigatus produces millions of airborne spores that, due to their small size, can penetrate the human respiratory tract. Inhalation of A. fumigatus spores rarely has any adverse 64 65 effects in immunocompetent individuals, since the spores are efficiently eliminated by host innate 66 immunity. However, immune disorders may lead to a spectrum of diseases collectively named 67 aspergilloses [13, 14]. In Europe, the number of clinical conditions caused by A. fumigatus exceeds 68 two millions cases per year, including around 50,000 cases of lethal invasive pulmonary aspergillosis 69 (IPA) [15].

70 Here we reveal that disruption of the cystathionine-y-lyase encoding gene in both A. fumigatus and 71 human alveolar epithelial cells diminishes their protein persulfidation levels. Reduced persulfidation 72 of A. fumigatus proteins is correlated with decreased virulence, as we have shown that this PTM 73 modulates the activity of at least two proteins relevant for fungal pathogenicity, a peroxiredoxin 74 (Aspf3) and an alcohol dehydrogenase (AlcA). We further show that correct host persulfidation levels 75 are required for maximum antifungal potency of alveolar macrophages and epithelial cells, which 76 correlates with the observed higher incidence of IPA in hematopoietic stem cell transplant recipients 77 carrying a single nucleotide polymorphism (SNP) in the gene coding for cystathionine- $\gamma$ -lyase. Finally, 78 we show that the extent of host protein persulfidation, which directly correlates with its capacity to 79 defend against A. fumigatus infection, determines the level of A. fumigatus persulfidation, which 80 correlates with its capacity to adapt to the conditions imposed by the host in the course of infection.

81

### 82 RESULTS

### 83 Persulfidation is an essential PTM in Aspergillus fumigatus

84 Making use of the BLAST tool at the NCBI server (http://www.ncbi.nlm.nih.gov/Blast.cgi) and using 85 the three human proteins described to be involved in  $H_2S$  production as query, we identified the A. 86 fumigatus orthologue genes encoding cystathionine-y-lyase (mecB, AFUA\_8G04340), cystathionine-87 (mecA, AFUA\_2G07620) and 3-mercaptopyruvate sulphurtransferase (mstA, β-synthase 88 AFUA 8G01800). MecB and MecA are highly similar to their human counterparts (MecB 53% 89 identity, 69% similarity; MecA 54% identity, 69% similarity) strongly suggesting a conserved activity, 90 whilst MstA has a lower similarity rate (37% identity, 51% similarity), which might still indicate an 91 analogous function.

To gain insight into the intrinsic control of persulfidation in *A. fumigatus*, we constructed deletion
strains for the three identified genes by homologous gene replacement in the wild-type ATCC46645

94 strain, employing a self-excising recyclable marker [16]. Persulfidation levels were monitored by 95 both in-gel detection (Fig. 1a) and fluorescence microscopy (Fig. 1c), via the dimedone switch 96 method [17]. Relative to wild-type, quantification of persulfidation levels in whole protein extracts 97 revealed a significantly decreased persulfidation in  $\Delta mecA$  (32.4% P=0.005) and  $\Delta mecB$  (41.6% 98 reduction, P=0.001) deletion mutants, which was more pronounced in the  $\Delta mecB$  strain (Fig. 1b). 99 Microscopy mediated quantification of persulfidation levels in hyphae growing in minimal medium 100 (MM) and in DMEM revealed a reduction, relative to wild-type, in the  $\Delta mecA$  and  $\Delta mecB$  mutants, 101 which was statistically significant for the latter (P=0.0013 in MM and P=0.046 in DMEM) (Fig. 1d). 102 Aiming to reduce the levels of persulfidation further, we attempted to construct a double  $\Delta mecA$ 103 AmecB mutant by targeting each gene for deletion the corresponding single mutant strain but 104 repeatedly failed. We therefore tested if loss of function in both genes could have a synthetic lethal 105 phenotype by using the heterokaryon rescue technique, a method designed for identification of 106 essential genes in Aspergillus species [18]. We indeed observed that conidia from primary 107 transformants could be propagated in non-selective media but not in selective medium, which 108 means that the double transformant nuclei can only be maintained in heterokaryosis and therefore 109 that the loss of function of both gene products has synthetic lethal outcome. Hence, we postulate 110 that persulfidation is an essential PTM for A. fumigatus viability and cannot be completely disrupted.

111 We evaluated the sensitivity of all three single mutants to a variety of common stressors, and found 112 that in most conditions they displayed identical growth rate to the wild-type (Fig. S1). Remarkably, 113 all mutants were more sensitive than the wild-type to  $H_2O_2$  (Fig. S1a) and the glutathione-disturbing 114 antifungal Fludioxonil (Fig. S1b); in addition *AmecB* was more sensitive to the thiol-oxidizing drug 115 diamide (Fig. S1c) and slightly to menadione (Fig. S1d). Besides, the *AmecA* mutant was sensitive to 116 the cell wall stressor SDS (Fig. S1e). None of the mutants was sensitive to high temperature (48°C), hypoxia (1% O<sub>2</sub>), osmotic stress (NaCl or KCl) or other cell wall disturbing agents (Congo Red, 117 Calcofluor White or Caffeine) (Fig. S1e). Persulfidation is known to be very important for cellular 118 119 redox processes (for a review see [19]), due to its role in preventing cysteine hyperoxidation [17]. 120 Therefore, it is not surprising that reduced levels of persulfidation cause sensitivity to oxidative 121 stressors in *A. fumigatus*, as has been described in other organisms [17]. Besides, the  $\Delta mecB$  mutant 122 showed the same sensitivity profile to the antifungals Amphotericin B, Voriconazole and 123 Anidulafungin, respectively representing polyenes, azoles and echinocandins, as the wild-type (table 124 S1), suggesting that persulfidation is not important for antifungal resistance.

125 Therefore, to investigate if persulfidation dependent responses are relevant for pathogen 126 adaptation to host conditions, we selected the low-level persulfidation mutant  $\Delta mecB$  for 127 subsequent analyses. It is important to note that all phenotypes displayed by the  $\Delta mecB$  mutant are 128 due to a ~45% reduction of persulfidation, rather than absolute absence of this PTM.

129

### 130 Persulfidation affects the activity of proteins known to be relevant for A. fumigatus pathogenicity

131 Seeking A. fumigatus proteins which are highly persulfidated, we utilised two independent 132 methodologies, the Biotin Thiol Assay [20] and the improved switch tag technique [21], and we 133 observed that a number of A. fumigatus proteins are persulfidated (Fig. 2a). By mass-spectrometry 134 of cut-off bands we identified highly persulfidated proteins, and among them we found proteins described to be important for A. fumigatus pathogenic potential. Remarkably, we identified the 135 136 peroxiredoxin Aspf3 (AFUA\_6G02280), which is strictly required for A. fumigatus pathogenicity [22], 137 and the alcohol dehydrogenase AlcA (AFUA\_7G01010). The latter is of potential relevance given that 138 at least one of the three alcohol dehydrogenases encoded by the A. fumigatus' genome has been implicated in virulence, AlcC (AFUA\_5G06240) [23]. To confirm that the level of persulfidated Aspf3 139 is indeed diminished by ~20% in the  $\Delta mecB$  isolate compared to the wild-type progenitor, we 140 enriched the persulfidation fraction of full protein lysates and specially detected Asp3 by Western-141 blot (Fig 2b and S2a). In order to investigate whether reduced persulfidation levels affect the activity 142 143 of this protein, extracellular peroxiredoxin activity of fungal mycelia was measured by the

144 degradation rate of tert-butyl hydroperoxide [24] (Fig. S2b). A. fumigatus  $\Delta mecB$  showed a 145 significant decrease  $\sim$ 35% in Aspf3 activity (P=0.0082) relative to the wild-type (Fig. 2c). This 146 demonstrated that reduced persulfidation levels impact peroxiredoxin activity, probably due to a 147 lower capacity to prevent hyperoxidation of the enzyme's catalytic site [17, 25]. We also measured 148 alcohol dehydrogenase activity from crude protein extracts and found that the activity was 149 significantly increased by ~25% (P=0.025) in the low persulfidation  $\Delta mecB$  mutant, compared to the 150 wild-type strain, whilst the activity of the  $\Delta alcC$  strain was strongly diminished, as expected (Fig 2d 151 and S3c).

### 152 Correct persulfidation levels are relevant for A. fumigatus pathogenic potential

153 Given the importance of Aspf3 for oxidative stress resistance during infection [22] and the observed 154 higher susceptibility of  $\Delta mecB$  to oxidative stressors (Fig. S1), we speculated that the lower 155 peroxiredoxin activity in the  $\Delta mecB$  mutant would translate into a higher susceptibility to killing by 156 immune effector cells. Therefore, we performed spore killing assays using murine (Raw624.7) and 157 human (THP-1) macrophage cell lines. In both cases the percentage of killed  $\Delta mecB$  conidia was 158 significantly higher than that of the wild-type (28.3% VS 16.7% for Raw624.7, P<0.0001, and 14.3 VS 159 10.8 for THP-1, P= 0.0111) (Fig. 3a). This suggests that altered activity of relevant fungal proteins in 160 the  $\Delta mecB$  mutant results in a higher susceptibility to killing by effector immune cells. We then 161 investigated the  $\Delta mecB$  pathogenic potential in a leukopenic murine model of invasive pulmonary 162 aspergillosis (IPA) (Fig. 3b). We observed that the  $\Delta mecB$  mutant indeed showed a significant (P= 163 0.0265) reduction in virulence compared to the wild-type strain. Mice infected with  $\Delta mecB$  survived 164 slightly longer and at day 7 after infection four out of eleven mice were alive and had recovered 165 from infection. In addition, fungal burden at day +3 post-infection was significantly lower in mice 166 infected with  $\Delta mecB$  than in mice infected the wild-type strain (P=0.0229) (Fig. 3c).

In summary, these results demonstrate that a moderate reduction in *A. fumigatus* persulfidation of
 ~45% correlates with a significant decreased in its virulence.

A SNP in the human cystathionine-γ-lyase encoding gene predisposes hematopoietic stem cell
 transplant recipients to invasive pulmonary aspergillosis.

171 Cystathionine- $\gamma$ -lyase has been implicated in various hyperinflammatory conditions [26, 27]. 172 Therefore, we speculated that balanced persulfidation in host cells based on correct cystathionine-y-173 lyase activity must be required to properly defend against invading pathogens. To evaluate this 174 hypothesis we investigated the relationship between genetic variability of the cystathionine-y-lyase 175 encoding gene NG 00804 (mainly responsible for persulfidation in lung tissues [28]) and the 176 incidence of IPA after hematopoietic stem cell transplantation. We observed that the presence of 177 the mutant homozygous (TT) genotype of the non-synonymous SNP S403I (rs1021737) in transplant recipients resulted in a 10% increase in cumulative incidence of IPA after transplant relative to the 178 179 other genotypes (32 % for TT vs. 22% GG+GT, P=0.22) (Fig. 4a). Actually, the SNP is more frequent in 180 IPA patients than in controls (11.3% in IPA vs 7.1% in controls, P=0.14) (Table 1), suggesting an 181 association with disease. These increments were not statistically significant, probably due to the low 182 number of patients (n=36) that carry this SNP in homozygosis. However the genotype-dependent 183 trend towards increased incidence of IPA in recipients prompted us to analyse the cytokine 184 environment in the bronchoalveolar lavage (BAL) of patients with IPA, according to \$403I genotypes. 185 Since only one BAL sample from a homozygous patient was available, we also included samples from 186 IPA patients with the SNP in heterozygosis. In general, the presence of the SNP was associated with 187 an increase in concentration of several cytokines (IFN- $\gamma$ , IL-18 and TNF- $\alpha$ ) and chemokines (eotaxin, 188 IP10, RANTES and MCP-1) (Fig. S3a and b), which tended to be more pronounced in the TT 189 homozygous sample. This could suggest that the heightened risk of fungal infection derives from a 190 prevalent hyper-inflammatory environment that is permissive to fungal infection. A remarkable 191 exception was IL-8, which was strongly diminished in patients with the SNP (P=0.02) (Fig. 4b). IL-8 is 192 known to be important for the defence against A. fumigatus [29, 30] and often reflects a more 193 specific contribution of epithelial cells [31], which is in agreement with an increased risk of infection 194 when the SNP is on the recipient side. Interestingly, in contrast to the general tendency of increased cytokine presence in patients' BAL samples, macrophages derived from donors' PBMCs carrying the SNP in homozygosis showed a decreased production of pro-inflammatory cytokines upon stimulation with *A. fumigatus* conidia compared to macrophages derived from non-carriers of the SNP (Fig. S4). Together, these results suggest that the SNP causes a general imbalance in cytokine production with likely consequences to the antifungal immune response.

200 Low persulfidation levels correlate with a decrease in the antifungal potency of alveolar 201 macrophages and epithelial cells

202 The fact that an increase in IPA incidence occurs in when the SNP is in the recipient but not in the 203 donor suggests that the effect of persulfidation is more relevant in non-hematopoietic cells (e.g. 204 epithelial cells) and immune cells that are resistant to conditioning treatments (e.g. alveolar 205 macrophages, AMs [32]). To further investigate this line of reasoning, we made use of the cystathionine-y-lyase knock-out C57BL/6<sup>CSE-/-</sup> [33] mouse line, which was previously demonstrated to 206 207 have decreased levels of persulfidation in the lung tissues [34]. We isolated bone marrow neutrophils (representative of donor derived cells in a transplant recipient) and AMs (representative 208 of host recipient cells) from C57BL/6 and C57BL/6<sup>CSE-/-</sup> and challenged them with A. fumigatus wild-209 210 type and  $\Delta mecB$  conidia ex vivo. Both immune effector cells killed  $\Delta mecB$  spores to a ~10% higher degree (Fig. 5a), further supporting the relevance of persulfidation in the pathogen. Interestingly, 211 C57BL/6<sup>CSE-/-</sup> AMs, but not neutrophils, showed a defect in conidial killing (~11% for wild-type 212 P<0.0001, and ~9% for  $\Delta mecB P$ =0.0007), which supports the notion that correct persulfidation is 213 214 required in host resident cells to sufficiently control A. fumigatus infection.

Aiming to investigate the contribution of non-hematopoietic cells in more detail, we disrupted the cystathionine- $\gamma$ -lyase encoding gene in the human alveolar epithelial cell line A549 using a commercial CRISPR/Cas9 strategy, which inserts a puromycin resistance gene in three locations within the targeted ORF. The resulting  $CSE^{-/-}$  cell line (Fig S5a) showed a significant reduction in persulfidation levels (Fig. S5b). As in *A. fumigatus*, persulfidation was not completely abrogated in 220 this cell line and, hence the effects described below are correlated with the significant reduction of ~55% in the persulfidation levels. We challenged the A549 and  $CSE^{-/-}$  cell lines with A. fumigatus 221 222 wild-type and  $\Delta mecB$  strains and measured epithelial cell detachment to evaluate the degree of host damage incurred by the pathogen (Fig. 5b). Interestingly, we observed that the A. fumigatus  $\Delta mecB$ 223 strain induced slightly less detachment than the wild-type in both A549 and CSE<sup>-/-</sup> cell layers (5.5% 224 and 9.7% less damage respectively). In addition, the  $CSE^{-/-}$  cell monolayer suffered a higher 225 226 detachment rate during incubation with both wild-type (113.2%, P=0.0306) and  $\Delta mecB$  (109%, not 227 significant) spores. This experiment suggests that persulfidation is relevant for both the fungal 228 potential to cause damage as well as the host capacity to withstand assault. In order to investigate 229 the killing capability of the epithelial cells, we calculated the percentage of dead conidia after 6 230 hours of co-incubation (Fig. 5c) and observed that 1) the A. fumigatus AmecB conidia were killed to a higher extent than wild-type (31.1% VS 21.1% conidia killed by A549 P<0.001 and 26.8% VS 16.7% by 231  $CSE^{-/-}$  P<0.001) and 2) the  $CSE^{-/-}$  cells were less efficient in killing fungal conidia compared with the 232 233 progenitor A549 cells (16.7% VS 21.1% killed wild-type conidia P=0.0727 and 26.8% VS 31.1% killed 234  $\Delta mecB P=0.0549$ ). Therefore, correct persulfidation levels are important for fungal survival and also for the capacity of epithelial cells to kill A. fumigatus. Surprisingly, we found that CSE<sup>-/-</sup> cells had 235 internalised significantly more spores than A549 cells 4 hours after challenge (Fig. 5d and S6), 236 237 suggesting that this process is more efficient with low persulfidation. Hence, low persulfidation more 238 likely causes a defect in pathogen killing rather than in phagocytosis. However, since the exact 239 mechanism that epithelial cells utilise to kill A. fumigatus spores has not been elucidated yet [35] we 240 could not determine the underlying reason of this defect. We then measured IL-8 production in challenged and unchallenged cells and detected that  $CSE^{-/-}$  cells produce significantly more IL-8 than 241 242 A549 cells (Fig. 5e). This result might seem to be in conflict with the lower level of IL-8 detected in patients' BAL; however, the total cytokine levels in patients' BAL are derived from several cell 243 populations [36, 37], and the reduced persulfidation level may affect other IL-8 producing cells 244 245 differently. Altogether, our observations suggest that deficiency in cysthathionine- $\gamma$ -lyase, and

consequently low persulfidation levels, favour the development of a pro-inflammatory environment
in the airways, which is consistent with other studies reporting the relevance of this enzyme for
hyperinflammatory conditions [26, 27].

### 249 Host persulfidation determines the level of persulfidation in A. fumigatus

250 Our observations suggest that the level of persulfidation in host cells correlates with their capacity to 251 kill A. fumigatus and, inversely, the level of persulfidation in the fungus correlates with its capacity to 252 survive in the host. We thus hypothesised that correct persulfidation levels in the host (reflecting 253 high killing capacity) may directly increase the persulfidation levels in the fungal pathogen in order 254 to better survive the more potent host antifungal activity. To test that hypothesis, we measured the 255 level of persulfidated Aspf3 (a fungal protein serving as reporter of persulfidation specifically in A. fumigatus), in A. fumigatus wild-type and  $\Delta mecB$  strains infecting A549 or  $CSE^{-/-}$  cell lines (Fig. 6a and 256 257 S7). As expected, the level of persulfidated Aspf3 was always higher in wild-type than in  $\Delta mecB$ . 258 Interestingly, the amount of persulfidated Aspf3 was higher when infecting A549 than  $CSE^{/2}$ , which 259 suggests that a higher level of host persulfidation triggers a higher level of the same modification in 260 the pathogen. The increase in persulfidation was significant in A. fumigatus wild-type (162%, P=0.0017%) but not in in  $\Delta mecB$  (139%, P=0.107), which points to MecB as an important fungal 261 protein involved in the specific response to host challenge. We also measured total levels of 262 263 persulfidation in hyphae challenged with cell lines, using the persulfidation fluorescence imaging 264 protocol [17] and an automated image processing and analysis macro created to mask the fungus 265 from the human cells based on fungal-specific Calcofluor White staining (Fig. S8). As expected, the 266 absolute mean persulfidation fluorescence signal in A. fumigatus wild-type hyphae significantly 267 exceeded the overall level in  $\Delta mecB$  mutant in all conditions. In agreement with the previous result, 268 the level of persulfidation was higher when A. fumigatus was in contact with A549 than with CSE<sup>7-</sup> 269 cells and the increment was significant in wild-type (124%, P<0.0001) but not in AmecB (106%, 270 P=0.89) hyphae (Fig. 6b and S8). Therefore, the level of host persulfidation influences the level of A.

271 fumigatus persulfidation. We hypothesised that this interaction may occur through two alternative 272 mechanisms, either by host-produced H<sub>2</sub>S diffusing to fungal cells and directly impacting the level of 273 persulfidation, or by an active response in A. fumigatus to the stress caused by the host cells. To gain 274 some further insight, we tested A. fumigatus persulfidation levels in vitro in the presence of a 275 sulphide donor or an oxidative stressor. Addition of the H<sub>2</sub>S donor GYY4137 did not affect A. 276 fumigatus persulfidation levels, whilst incubation of mycelia in the presence of peroxide significantly 277 increased the persulfidation levels of the wild-type strain by ~1.4 fold (P=0.0138), and of the  $\Delta mecB$ 278 by ~1.3 fold (P=0.052,) (Fig 6c). This suggests that the increase in persulfidation of A. fumigatus in 279 the presence of epithelial cells is due to an active response to stress caused by the effector cells, 280 such as oxidative stress, which is partly dependent on the activity of MecB.

281 DISCUSSION

282 Adaptation is paramount in host-pathogen interactions. Pathogens must be able to adapt to the 283 harsh and varying conditions encountered inside a host. Concomitantly, host cells must respond 284 properly to the challenge to kill the pathogen and mount a proper immune response. Persulfidation 285 is a post-translational modification known to be important for a variety of physiological processes 286 [8]. In pathogens, it has only been studied in *Staphylococcus aureus*, in which it has been related to the production of virulence factors and cytotoxicity [12, 38]. However, the impact of low 287 288 persulfidation for S. aureus virulence in the context of infection was not well defined. In a mouse 289 model of infection a low persulfidation mutant was reported to cause reduced bacterial burden, but 290 the consequences of low persulfidation for bacterial fitness and/or resistance to host killing was not 291 investigated. Interestingly, in S. aureus a double mutant of the cystathionine-β-synthase (CBS) and 292 cystathionine- $\gamma$ -lyase (CSE) encoding genes ( $\Delta CBS\Delta CSE$ ) had decreased persulfidation, produced 293 supernatants with higher cytotoxic potential, but did not have reduced virulence [12]. We have 294 shown that a partial reduction of persulfidation levels in A. fumigatus affects the activity of at least 295 two proteins known to be important for virulence, increases susceptibility to host-mediated killing

296 and accordingly decreases its pathogenic potential. Furthermore, our results suggest that 297 persulfidation cannot be completely abolished in A. fumigatus and therefore appears to be an 298 essential process for viability. This could seem to be in disagreement with S. aureus, where a 299  $\Delta CBS\Delta CSE$  mutant is viable [12]. However, the level of persulfidation in this double mutant was 300 approximately 45% of the wild-type, which is similar to the reduction we observed in our single A. 301 fumigatus AmecB mutant (CSE). Therefore, it is likely that other enzymes (as MST) or mechanisms 302 are more relevant for persulfidation in this bacterial pathogen than in A. fumigatus. Moreover, 303 persulfidation seems to be an essential process in mammalian cells as well, as to or knowledge there 304 is no report of a double  $\Delta CBS \Delta CSE$  cell line constructed. Therefore, we presume that persulfidation 305 might be an essential PTM for all organisms.

306 The presence of the SNP S403I (rs1021737) in the human cystathionine-y-lyase encoding gene 307 appears to partially contribute to an increased incidence of invasive pulmonary aspergillosis (IPA) in 308 hematopoietic stem cell transplant recipients. This SNP has been proposed to affect phosphorylation 309 sites and decrease protein-substrate affinity, therefore it is believed to reduce the activity of the 310 encoded enzyme [39]. In addition it seems to decrease expression of the gene [40]. Actually, it has 311 been associated with cystathioninuria and higher homocysteine plasma concentration [41], which could be a direct result of a reduction in enzyme amount and activity. We demonstrated that lack of 312 313 cystathionine-y-lyase enzymatic activity in alveolar epithelial cells reduces the level of persulfidation, 314 which decreases their antifungal potency and de-regulates cytokine production. A previous study 315 found increased pro-inflammatory cytokine production upon inhibition of CSE expression in human 316 macrophages [42] and cystathionine- $\gamma$ -lyase is known to play a role in various chronic pro-317 inflammatory airway diseases [27]. The underlying mechanism could be related to the persulfidation of NF-KB, which has been described to affect its activity [34]. Therefore, we propose that 318 319 hematopoietic stem cell transplant recipients carrying the SNP S403I in homozygosis are more prone 320 to develop IPA because reduced enzymatic activity and thus persulfidation levels lead to the 321 development of a hyperinflammatory environment that is permissive to fungal infection, which cannot be properly controlled as their alveolar epithelial cells and alveolar macrophages have reduced antifungal potency. Optimal activity of CSE has been shown to be important for the defence against respiratory syncytial virus *in vitro* [43] and in a murine model [44], which supports the notion that the action of this enzyme is important for the defence against pathogens.

326 Interestingly, we have shown that host persulfidation influences the level of persulfidation in A. 327 *fumigatus*. We explored the possibility that a higher production of  $H_2S$  in the host could directly 328 diffuse to fungal cells and affect their persulfidation level. However, in contrast to other organisms 329 [12, 17, 20, 45], the sulphide donor GYY4137 alone did not significantly alter persulfidation levels in 330 A. fumigatus. We speculate this could be due to a lower diffusion of  $H_2S$  through the A. fumigatus cell wall and/or a low capacity to oxidize non-enzymatically derived H<sub>2</sub>S [46, 47]. Alternatively, we 331 332 hypothesised that the elevated host antifungal potency in competent persulfidating cells may induce 333 more oxidative stress to A. fumigatus, which could then be sensed by protein sulfenylation [48] and 334 in turn increase persulfidation in the fungal cell [17]. In agreement, we have shown that  $H_2O_2$  alone 335 could trigger an increase in persulfidation levels. Therefore, we propose that the persulfidation 336 levels in A. fumigatus are modulated as a direct response to host cell defence mechanisms, which are balanced by its own persulfidation levels. 337

In summary, we show that correct protein persulfidation is important for both *A. fumigatus* pathogenic potential and host antifungal defence and also that host persulfidation determine the level of persulfidation in the fungal pathogen. Furthermore, we propose that persulfidation is an essential cellular process. Therefore, persulfidation must be considered as a relevant posttranslational modification for infection, where its modulation may be a promising and novel strategy to target both pathogens and immune responses.

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362

### 363 Author Contribution

MSO performed the majority of experiments and wrote the manuscript; SG constructed the  $CSE^{-/-}$  KO epithelial cell line and assayed their ROS production, phagocytosis and cytokine production; JS helped with various experiments and performed the murine model of infection; YY and MS performed the killing assay with  $CSE^{-/-}$  AMs and neutrophils; CC and AC analysed the SNP and measured cytokines in patients' BAL and in monocyte-derived macrophages; EM and JZ contributed

in acquisition of data and measurements of persulfidation levels; DT contributed in to the acquisition 369 of single cell fluorescence images, conceptualised and wrote the automated image 370 processing/analysis macro and revised the manuscript; PB participated in the conception of cellular 371 analyses and revised the manuscript; AB participated in the design of the murine experiments and 372 373 revised the manuscript; AC designed the human analyses and revised the manuscript; MRF 374 participated in the conception of the persulfidation experiments and revised the manuscript; EB participated in the conception of the project and revised the manuscript; JA designed the project 375 and wrote the manuscript. 376

### 377 Data availability statement

- 378 The raw data that support the findings of this study are available from the corresponding author
- 379 upon reasonable request.

### 380 Code availability statement

- 381 Code for Fiji for automated analysis of microscopy data is available as Supplementary Files. The in-
- house Fiji batch image processing and counting algorithm is available upon reasonable request to
- the corresponding author and with permission of Prof Elaine Bignell.
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  - 495

496
### **Table 1. Association of SNP with disease**

SNP rs#	Alleles: status	Genotype, n (%)		P value
number		G/G + G/T	Т/Т	_
Recipient				
rs1021737	IPA	102 (88.7)	13 (11.3)	0.14
	Controls	301 (92.9)	23 (7.1)	

Fig. 1



## Fig. 1. A. fumigatus persulfidation levels decrease with the deletion of genes involved in $H_2S$ production.

a) Representative images of in-gel detection of persulfidation levels in whole protein extracts of *A. fumigatus* wild-type and mutants  $\Delta mecB$  (cystathionine- $\gamma$ -lyase),  $\Delta mecA$  (cystathionine- $\beta$ -synthase) and  $\Delta mstA$  (3-mercaptopyruvate sulphurtransferase. 4-chloro-7-nitrobenzofurazan (NBF-Cl) labels persulfides, thiols, sulfenic acids, and amino groups; reaction with amino groups produces the green signal, therefore it reflects the whole protein context and is used to normalise the persulfidation levels. The red signal is produced by the dimedone-Cy5 labelled probe, which selectively switches NBF-Cl in persulfide groups [17]. b) Quantification of persulfidation levels, measured as the ratio of red signal normalised to the green signal, revealed a significant decrease in persulfidation level of  $\Delta mecB$  relative to wild-type (n=3). c) Representative images (scale bar=15 µm) and d) quantification of persulfidation levels measured by microscopy showed a significant decrease in  $\Delta mecB$ . The intensity of fluorescence is represented as 32 bit arbitrary units. (n=3, >10 photos per sample). All data in panels b and d are depicted as mean+/-SD and were analysed using one-way ANOVA with Dunnett's multiple comparisons.



## Fig. 2. Decreased persulfidation affects the activity of the virulence associated proteins Aspf3 and AlcC

a) Persulfidated proteins were detected by two different methods, the biotin-thiol assay (persulfidated proteins are eluted in the presence of DTT) and the Tag-switch. The three more intense bands were used to identify proteins by mass-spectrometry. b) The ratio of persulfidated Aspf3 (normalised to total Aspf3 levels, Fig. S2a) was significantly decreased in the  $\Delta mecB$  mutant relative to wild-type, as analysed using an unpaired *t*-test with Welch's correction. (n=3) c) In a peroxiredoxin enzymatic assay  $\Delta mecB$  and  $\Delta aspf3$  showed a significant decrease of activity compared to wild-type (n=4, 3 technical replicates per biological repetition). Data was analysed using a one-way ANOVA with Dunnett's multiple comparisons. d) In an alcohol dehydrogenase enzymatic assay  $\Delta alcC$  had a significantly decreased and  $\Delta mecB$  increased activity in comparison with wild-type. n=3, 3 technical replicates per biological repetition). All data are depicted as mean+/-SD and were analysed using a one-way ANOVA with Dunnett's multiple comparisons.

Fig. 3



# Fig. 3. Reduced persulfidation renders A. *fumigatus* more susceptible to killing by host effector cells and reduced its virulence in a murine model of infection.

a)  $\Delta mecB$  was more sensitive to killing by murine Raw 624.7 macrophages (unpaired *t*-test) and human THP-1 macrophages (Mann-Whitney test). (n=3 with 3 technical replicates and 6 photos per well). b) The  $\Delta mecB$  strain showed a significant reduction in virulence in a leukopenic murine model of invasive pulmonary aspergillosis (*P*=0.0265 Log-rank test) (n=11 animals per group). c) Lungs of mice infected with the  $\Delta mecB$  mutant showed a decreased fungal burden compared to wild-type infected (n=5, 3 technical replicates per qPCR). Data is depicted as mean+/-SD and was compared using an unpaired *t*-test. Fig. 4



## Fig. 4. A SNP in the human cystathionine-γ-lyase is associated with higher incidence of invasive pulmonary aspergillosis in hematopoietic stem cell transplant recipients.

a) Cumulative incidence of invasive pulmonary aspergillosis (IPA) in donors and recipients with and without the non-synonymous SNP S403I (rs1021737) after hematopoietic stem cell transplantation. b) Levels of IL-8 were reduced in bronchoalveolar lavages (BAL) of IPA patients carrying the SNP (n=8) compared with control patients (n=13). Data for the SNP in heterozygosis was analysed using a Mann-Whitney test. Only one BAL for the SNP in homozygosis was available, so no statistical analysis is possible.



Fig. 5. Defect in cystathionine-y-lyase reduces the antifungal potency of alveolar macrophages and alveolar epithelial cells

a) Alveolar macrophages isolated from  $CSE^{-/-}$  knock-out mice showed a lower capability of killing conidia compared with AMs derived from wild-type mice, whereas bone marrow neutrophils killed conidia at the same level. The *A. fumigatus*  $\Delta mecB$  mutant was more sensitive to killing by both immune cell populations. (n=3 mice; 5 photos per well) b) The  $CSE^{-/-}$  alveolar epithelial cell line suffered a higher percentage of detachment than A549 after challenging with  $\Delta mecB$  and wild-type spores. (n=4; with 3 technical replicates) c) The  $CSE^{-/-}$  alveolar epithelial killed *A. fumigatus* conidia slightly less efficiently compared to its A549 parental line. *A. fumigatus*  $\Delta mecB$  was killed significantly more than the wild-type strain (n=4; with 3 technical replicates and 6 photos per condition). Data from a), b) and c) were analysed with two-way ANOVA. d) After 4 hours challenge, the  $CSE^{-/-}$  cell line internalised significantly more spores than A549 (n=4 with 4 technical replicates unpaired *t*-test). e) The  $CSE^{-/-}$  alveolar epithelial cell line showed a dysregulated increment of IL-8 production (n=3 with 2 technical replicates, two-way ANOVA). All data in the figure are depicted as mean+/-SD.





# Fig. 6. The level of persulfidation in wild-type *A. fumigatus* is influenced by contact with the host and by oxidative stress.

a) The ratio of persulfidated Aspf3 in *A. fumigatus* wild-type is higher when the fungus is in contact with A549 than with *CSE*<sup>-/-</sup> cells. The ratio of persulfidated Aspf3 is always lower in the *A. fumigatus*  $\Delta mecB$  mutant. (n=3) b) Measurement of total persulfidation by fluorescence microscopy reflected that *A. fumigatus* wild-type has higher persulfidation level when it is in contact with A549 than with *CSE*<sup>-/-</sup> cells and that the level of persulfidation is lower in the *A. fumigatus*  $\Delta mecB$  mutant. (n=4 with 2 technical replicates and 5 photos per well) c) The total level of persulfidation did not increase in the presence of a H<sub>2</sub>S donor. In contrast, incubation with the oxidative stressor H<sub>2</sub>O<sub>2</sub> triggered a significant increment of persulfidation levels in the wild-type strain, and only a slight increase in the  $\Delta mecB$  mutant (n=5). All data are depicted as mean+/-SD and were analysed using one way ANOVA tests.

### Fig. S1





### Fig. S1. Decreased persulfidation levels affect *A. fumigatus* sensitivity to oxidative stress.

A. fumigatus mutants were not sensitive to high temperature (48 °C), hypoxia, or osmotic stress (300 mM NaCl and KOH). The  $\Delta mecA$  mutant was slightly more sensitive to the cell wall stressor SDS (0.01%), but not Congo Red (30 µg/ml), CalcofluorWhite (200 µg/ml) and Caffeine (5 mM). All mutants were more sensitive to H<sub>2</sub>O<sub>2</sub> and Fludioxonil and the  $\Delta mecB$  mutant was also more sensitive to Diamide and slightly to Menadione.



## Fig. S2. Persulfidation affects the activity of the peroxirredoxin Aspf3.

a) Representative Western blot of full lysates and persulfidated enriched fraction of wild-type and  $\Delta mecB$  protein extracts using an Aspf3 antiserum. b) Degradation rate of tert-Butyl hydroperoxide reflects peroxiredoxin activity. The  $\Delta aspf3$  mutant showed a strong reduction in degradation rate. The  $\Delta mecB$  mutant also had a lower degradation rate. The curve shows one representative experiment with 3 technical replicates. c) The ratio of NADP+ reduction to NADPH reflects alcohol dehydrogenase enzymatic activity and is measured by NADPH absorbance at 340 nm. The  $\Delta alcC$  mutant showed a strong reduction in NADPH production. The  $\Delta mecB$  mutant had a slightly increased speed in NADPH production. The curve shows one representative experiment with 3 technical replicates. Fig. S3



# Fig. S3. Pro-inflammatory cytokine environment in the BAL of IPA patients with a SNP in the cystathionine- $\gamma$ -lyase encoding gene.

The level of several pro-inflammatory cytokines a) and chemokines b) was elevated in the BAL of IPA patients with the SNP S403I (rs1021737) in the cystathionine- $\gamma$ -lyase encoding gene in heterozygosis (n=8) compared with the control patients (n=13). Data were compared using Mann-Whitney tests. Only one BAL from a patient with the SNP in homozygosis was available, so no statistical analysis could be applied. This patient seemed to have similar or even stronger tendencies than heterozygous patients.





Macrophages derived from PBMCs of healthy donors with the SNP S403I (rs1021737) in homozygosis showed a decrease pro-inflammatory cytokine production upon stimulation with *A. fumigatus* conidia. (n=43 GG, 29 GT and 7 TT for IL-1b, TNF $\alpha$  and IL-6 and n=39 GG, 21 GT and 2 TT for IL-8) Data are depicted as mean+/-SD and were analysed using Kruskal-Wallis tests with Dunn's multiple comparisons.

Fig. S5

C









Fig S5. Deletion of the CSE gene from A549 cells decreases the levels of protein persulfidation.

a) Representative Western Blot of the *CSE* protein in the A549 and its derivative *CSE*<sup>-/-</sup> epithelial cell lines. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as loading control. b) Representative image of in-gel detection of persulfidation levels in A549 and *CSE*<sup>-/-</sup> cell lines. 4-chloro-7-nitrobenzofurazan (NBF-CI) labels persulfides, thiols, sulfenic acids, and amino groups; reaction with amino groups produces the green signal, therefore it reflects the whole protein context and is used to normalise the persulfidation levels. The red signal is produced by the dimedone-Cy5 labelled probe, which selectively switches NBF-CI in persulfide groups [17]. b) Quantification of persulfidation levels, measured as the level of red signal normalised to the green signal, showed a significant decrease in persulfidation level of *CSE*<sup>-/-</sup> relative to A549 (n=4). Data was analysed using a *t*-test with Welch's correction. C) Representative images (scale bar=15 µm) of persulfidation levels detected by microscopy.



All channels



TdTomato and CW overlay



### Fig. S6. Phagocytosis Microscopy

Representative images of the quantification of phagocytosis. Red colour displays the TdTomato signal, which constitutes the whole conidia population. Green colour displays the Calcofluor White signal, which constitutes the extracellular (i.e. non-phagocytosed) conidia population. Scale bar =30  $\mu$ m.



Fig. S7. Western-blot of persulfidated ratio of Aspf3.

Representative Western-blot of full protein lysate and persulfidated enriched fractions using an Aspf3 antiserum.



## Fig. S8. Measurement of fungal persulfidation in infection experiments by epifluorescence microscopy.

Representative images of in-gel detection of persulfidation levels in protein extracts of A. fumigatus wild-type and mutants  $\Delta mecB$  (cystathionine-y-lyase), ∆mecA (cystathionine-β-synthase) and ∆mstA (3-mercaptopyruvate sulphurtransferase. The green signal reflects all proteins and is used to normalise the red signal, which specifically labels persulfidated proteins (as described in materials and methods) b) Quantification of persulfidation levels, measured as the level of red signal normalised to green signal, showed a significant decrease in persulfidation level of  $\Delta mecB$  relative to wild-type (n=3). Representative images of the measurement of persulfidation levels in hyphae infecting an epithelial cell monolayer 4-chloro-7-nitrobenzofurazan (NBF-Cl) labels persulfides, thiols, sulfenic acids, and amino groups; reaction with amino groups produces the green signal, therefore it reflects the whole protein context and is used to normalise the persulfidation levels. The red signal is produced by the dimedone-Cy5 labelled probe, which selectively switches NBF-Cl in persulfide groups [17]. Calcofluor White dye specifically stains chitin in the fungal cell wall. This blue channel can be used to automatically segment hyphae (mask) and hence permits to measure the green and red signals exclusively from fungal cells in images containing mixed fungal and human cells. Scale bar=  $30 \mu m$ .

## **CHAPTER 5: Article III**

# Beneficial Effects of Metformin are CSE/H<sub>2</sub>S/PSSH dependent

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Manuscript in preparation

## INTRODUCTION

Currently approved for the treatment of diabetes, metformin is a widely-used drug whose benefits on a cellular level extend far beyond insulin and glucose regulation.<sup>1–3</sup> Metformin has been shown to inhibit mTOR,<sup>4</sup> inhibit mitochondrial complex 1 in the electron transport chain and reduce production of reactive oxygen species (ROS),<sup>5,6</sup> activate AMP-activated kinase (AMPK),<sup>7,8</sup> and reduce DNA damage,<sup>9</sup> among other effects.

Overall, metformin is considered to favourably influence metabolic and cellular processes closely associated with age-related conditions.<sup>3</sup> This goes some way to explain the reasons why metformin treatment has been shown to be capable of extending the lifespan of different animals.<sup>10,11</sup> To date, there is no evidence to suggest similar effects in humans, while it is currently not clear whether metformin has multiple effects on multiple pathways, or whether effects reflect downstream consequences of a primary action on a single mechanism of aging.

A recent study by the Gladyshev group showed that one of the general features of interventions resulting in lifespan extension is the overexpression of CSE.<sup>12</sup> According to the results of our study, CSE-controlled H<sub>2</sub>S production and subsequent protein persulfidation (R-SSH) represents an evolutionarily conserved path to protect proteins from inactivation caused by cysteine hyperoxidation.<sup>13</sup> Furthermore, we also observed that CSE-controlled persulfidation is downregulated in a Huntington's disease model.

The earlier work of the Paul and Snyder lab has identified the important role of CSE in Huntington's disease.<sup>14–16</sup> CSE was found to be downregulated in brain samples of CSE knockout mice and humans, and CSE knockout animals showed Huntington's disease-like features. The latter is caused by the disordered amino acid homeostasis, which results in dysfunctions of the transcription factor ATF4, a master regulator of amino acid disposition. The regulation of CSE by ATF4 is particularly interesting and could unravel new venues for pharmacological manipulation.

In this study, we tested the hypothesis that the master pathway for the beneficial effects of metformin is ATF4-CSE-H<sub>2</sub>S-RSSH, and that metformin could be used as a pharmacological tool to increase CSE expression levels (and persulfidation levels) in Huntington's disease.

### **RESULTS AND DISCUSSION**

Metformin Up-regulates CSE and Increases Persulfide Levels in Mouse Embryonic Fibroblasts. CSE is part of the reverse transsulfuration pathway and is involved in the biosynthesis of cysteine, leading to the production of H<sub>2</sub>S. A range of stress stimuli have been reported to induce the expression of CSE; thus, we looked at the effect of metformin on CSE expression. Mouse embryonic fibroblasts treated in a dose-dependent manner with metformin for 24 hours responded with an increase in CSE levels, peaking at 5 µM of metformin (Fig. 1A). As we have previously shown, CSE is a major source of H<sub>2</sub>S and controller of endogenous protein persulfidation, integrated in redox signalling and a global protective modification from oxidative stress.13 Thus, questioned whether we the beneficial protective effects of metformin are due, in part, to the protein persulfidation. As with metformin in a dose-dependent manner and, such, we treated MEFs using our Dimedone-switch method,<sup>13</sup> found that R-SSH levels were indeed directly correlated to metformin, mirroring its induction of CSE expression (Fig. 1B).



#### Figure 1 - Metformin triggers CSE up-regulation and protein persulfides in MEFs.

(A) CSE expression levels in MEF cells treated with Metformin (Sigma, PHR1084) for 24 h. Visualized using rabbit polyclonal anti-CTH antibody and Clarity<sup>™</sup> Western ECL Substrate (BioRad) on a G:Box Chemi-XT4 (Syngene).
 (B) In-gel detection of R-SSH levels in metformin treated cells, using the Dimedone-switch method. Proteins were switch labelled with DAz-2:Cy5 and visualized on a Typhoon FLA 9500. Fire pseudo-colouring was used to visually enhance the R-SSH signal. Green fluorescence corresponds to the total protein load (NBF-protein adducts). Results are presented as a mean ± SD. from 3 independent experiments.

**Metformin Rescues CSE and Protein Persulfide Depletion in HD Cell Lines.** Huntington's Disease (HD) is a neurodegenerative disorder triggered by gluatamate repeats (111) in the huntingtin protein (Htt), and has been associated with having increased oxidative stress and mitochondrial dysfunction.<sup>17</sup> Paul et al. showed that the HD model cell line, ST*H*dh<sup>Q111/Q111</sup> (Q111), has severely depleted CSE levels and, consequently, is more sensitive to oxidative stress stress compared to the control cells, ST*H*dh<sup>Q7/Q7</sup> (Q7).<sup>14</sup>

More recently, we have additionally shown that this lack of CSE in the HD cell line causes a significant drop in persulfide levels of the disease model (Q111) (Fig. 2A).<sup>13</sup> This substantial decrease in R-SSH levels in the cells of the HD model provides an explanation for its increased oxidative stress levels, which can also be seen by their increased endogenous levels of cysteine sulfenic acids (R-SOH) (Fig. 2A).

Subsequently, we moved to look at whether metformin can alleviate the endogenous stress of HD by rescuing its CSE expression and persulfide levels. We first treated both striatal cell lines, Q7 and Q111, for 24 hours with metformin in a dose-dependent manner, and observed a correlative increase in the CSE levels of both lines, with a rescue of CSE in Q111 cells (Fig. 2B). We then went on to show that the treatment of HD cells with metformin caused an immediate increase in persulfide levels in the same dose-dependent manner when treated for 24 hours (Fig. 2C). This CSE-controlled increase in persulfides was also seen in the control of Q7 cells upon treatment with metformin for an even shorter period of 9 hours (Fig. 2C). Furthermore, we tested the oxidation status of these cells with metformin and observed that they responded by decreasing their R-SOH levels, with the lowest being with 5 mM metformin (Fig. 2D). This exemplified the hypothesis that metformin can relay its beneficial effects by increasing CSE and subsequently R-SSH levels to protect against overoxidation.



Figure 2 – Beneficial effects of metformin through the induction of CSE and protective persulfide levels. (A) Endogenous levels of R-SSH, R-SOH and CSE in striatal cell line models STHdh<sup>Q7/Q7</sup> (Q7) and STHdh<sup>Q111/Q111</sup> (Q111). R-SSH levels (left) where detected in-gel using the Dimedone-switch method, with Fire pseudo-colouring used for R-SSH and green fluorescence as a loading control. R-SOH levels (middle) were detecting by labeling with DCP-Bio1, immunoblotting and visualized with Streptavidin Protein DyLight 488 (Sigma, 21832) on a Typhoon FLA 9500. Anti-GAPDH (Sigma, G2267) was used as an internal standard. CSE levels (right) were detected using the rabbit polyclonal anti-CTH antibody and GAPDH as a loading standard. (B) CSE Levels in Q7 and Q111 cell lines upon treatment with 0, 1, 2.5 and 5 mM Metformin for 24 h; visualized the rabbit polyclonal anti-CTH antibody and GAPDH as a loading standard. (C) In-gel R-SSH levels in Q7 cells as a control and Q111 cell line upon treatment with metformin for 24 h and in Q7 with metformin treatment for 9 h (right); fire pseudo-colouring used for R-SSH and green fluorescence as a loading control. (D) R-SOH levels in Q7 cells upon treatment with metformin for 24h. Proteins labeled with DCP-Bio1 and visualized with Streptavidin-488. Quantification of the streptavidin-488 signal normalized to the GAPDH levels, presented as a mean ± SD. from 3 independent experiments.

Lifespan Extension by Metformin Linked to CSE and Persulfide Levels. Metformin has shown age-related effects, which are believed to diverge from its effect on glucose metabolism.<sup>3</sup> The exact cellular mechanism through which its documented lifespan extension occurs is still under investigation. To explore whether this involves CSE, and the way ways in which the deletion of CSE would impact meformin's effect, we performed survival curves with N2 (wild-type) and *cth-1* (CSE) mutant *C. elegans* on synchronous population of worms (grown on NGM plates supplemented with 50 mM Metformin from Day 2 of adulthood; Fig. 3). Indeed, we observed that metformin extended the median lifespan of N2 worms by 17.6 %; however, this extension was abolished in *cth-1* mutants exposed to metformin, with no significant increase in their median lifespan compared to the control *cth-1* group (Fig. 3). As such, we show that metformin requires CSE to mediate lifespan extension effects. Previously, we have also shown that *cth-1* mutant worms have lower R-SSH levels compared to N2.<sup>13</sup>

Together, these results clearly correlate with our observation that when CSEcontrolled persulfide levels are increased, they exhibit protective effects against overoxidation and aging.<sup>13</sup>



Figure 3 - Life-span extension by metformin is abolished in cth-1 mutant C. elegans. Survival curves of N2 and cth-1 (CSE) mutant C. elegans and N2 and cth-1 exposed to 50 mM Metformin (Met, Sigma, D150959) from Day 1 of adulthood. n > 90 per group. N2 = 14.99 ± 0.65 days, N2 + 50 mM Met = 17.64 days ± 0.73 days, cth-1 = 14.29 ± 0.64 days and cth-1+ 50 mM Met = 15.63 days ± 0.99 days. p < 0.0001. Data were analyzed using Kaplan-Meier survival analysis to detect statistical differences and plot graph.</li>

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# CHAPTER 6: Conclusions and Future Perspectives

The past decade has witnessed a burgeoning of literature on H<sub>2</sub>S signalling, with a plethora of physiological and pathophysiological functions claimed to be controlled by this gaseous molecule. This is perhaps best exemplified by the reports in which H<sub>2</sub>S is claimed to be the main reason for the caloric restriction–induced lifespan extension. Understanding molecular mechanisms by which such a small molecule can provide almost a universal protection remains, however, a long-sought goal.

One of the main ways through which H<sub>2</sub>S relays its signalling properties is by modification of cysteine residues – a process known as S-sulfhydration or persulfidation. Yet, H<sub>2</sub>S cannot directly modify cysteine residues, meaning that the mechanism for persulfide formation remains unclear. In order to gain a deeper understanding of their role in signalling, one must selectively label protein persulfides, which represents a major obstacle due to their instability and similarity to cysteine residues.

In the present thesis, we report a new and selective method which combines the availability and familiarity of the toolset widely used for the detection of sulfenic acids (dimedone-based probes) with a unique chemical 'switch', making this approach easy to use and widely applicable. The method is robust and is adapted for highthroughput screening, as well as for proteomic analysis or analysis by confocal microscopy. There are many possible applications of this method, all of which suggest that persulfidation is an evolutionarily conserved modification controlled by the transsulfuration pathway and cysteine catabolism in all tested life forms (from bacteria to humans).

The main way of persulfide formation is the reaction of sulfenic acids with  $H_2S$ . Waves of protein persulfidation always follow waves of sulfenylation, suggesting that they are an integral part of  $H_2O_2$ /sulfenic acid signalling (like in the case of receptor tyrosine kinases) – which has not been considered before. In fact, it seems that in the absence of  $H_2S$  to resolve sulfenylation,  $H_2O_2$  leads to cysteine hyperoxidation, even at signalling concentrations.

We also provide evidence that persulfidation levels decrease with aging in *C. elegans*, mice and rats, and that dietary restriction results in a strong increase of protein persulfidation. The reduction of protein persulfidation by genetic manipulation abolishes beneficial effects of dietary restriction on lifespan, while the pharmacological increase of persulfidation levels results in longer lifespan and improved ability to cope with oxidative stress. This further strengthens the ROS theory of aging.

A general mechanism is proposed (which we believe is an evolutionary remnant of the times when life emerged and flourished in a  $H_2S$  environment) in which persulfidation represents a rescue loop from cysteine hyper-oxidation and subsequent loss of protein function (expected to accumulate with aging). In other words, the higher the persulfidation, the longer the life.

Protective effects of protein persulfidation are used by pathogens such as *Aspergilus fumigatus* to survive cells' defence mechanism, which mainly consists of ROS/RNS. Higher levels of pathogens' persulfidation suggest higher virulence, as well.

Finally, metformin's beneficial effects may largely be mediated by its ability to stimulate the ATF4-CSE expression pathway, resulting in higher persulfidation levels and lower levels of cysteine oxidation. CSE is essential for metformin's lifespan extension effects.

Dimedone switch method opens doors to a new chapter of redox biochemistry, by allowing us to visualise, quantify and understand the role of persulfidation. The results presented within this thesis appear to be only the tip of the proverbial iceberg. Further studies should clarify to what extent persulfidation is only protective, and to what extent it is regulatory. Quantitative, site-centric proteomic analysis of persulfidated cysteines, combined with metabolomic analysis and individual structural analysis of modified targets, would provide further insights about the functional impact of this posttranslational modification.

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The intertwined nature of  $H_2O_2$  and  $H_2S$  signalling described in our work warrants further investigation. We show this to be the case for receptor tyrosine kinase signalling, though  $H_2O_2$  signalling has been implicated in many other physiological processes. The role of  $H_2S$  (i.e. persulfidation) in these processes remains to be further investigated, in order to position this PSOH/PSSH switch globally in the signalling scheme.

Finally, the ways in which and reasons why  $H_2S$ -producing enzymes decrease with aging remains to be understood, as well as the exact mechanism by which increased persulfidation correlates with lifespan extension.

## **APPENDIX 1: Buffers and Media**

The following buffers and media were used in this study

- NBF-Cl stock solution (500 mM) Dissolve 49.9 mg of 4-Chloro-7-nitrobenzofurazan (MW. 199.55) in 500 μl DMSO. Critical. Prepare right before use and keep light protected.
- DAz-2 stock solution (50 mM) Dissolve 5 mg DAz-2 (MW. 195.2) in 502.3 μl of argonated DMSO. At -20 °C it can be aliquoted and stored long term.
- Ascorbate solution (100 mM) Dissolve 1.76 mg of L-Ascorbic acid (MW. 176.12) in 200 μl dH<sub>2</sub>O. Critical. Prepare solution directly before addition.
- Tris buffer (1 M, pH 9) Dissolve 24.2 mg in 200  $\mu$ l of dH<sub>2</sub>O and adjust pH to 9.
- EDTA stock solution (300 mM EDTA in Tris buffer) Dissolve 55.8 mg EDTA (MW. 372.24) in 500 μl Tris buffer (1M). Stored in 4 °C.
- DAz-2:Cy5 preclick mix (1 mM DAz-2, 1 mM Cy5-alkyne, 2 mM Copper(II)-TBTA, 4 mM Ascorbate solution in PBS and 30% v/v acetonitrile) Add 43 μl acetonitrile to 60.5 μl PBS, and add 3 μl of 50 mM DAz-2, 7.5 μl of 20 mM Cy5-alkyne and 6 μl Ascorbate solution, consecutively whilst vortexing after each reagent addition. Solution should be mixed at RT overnight. Mixture is quenched with 10 μl of EDTA stock solution for 2 hr with agitation.
- HEN buffer (50 mM Hepes, 1 mM EDTA, 0.1 mM Neocuproine, 1% IGEPAL and 2% SDS, pH 7.4) Dissolve 5.96 g of Hepes, 186.12 mg of EDTA, 10.4 mg Neocuproine, 5 ml IGEPAL and 50 ml of 20% SDS in 500 ml H<sub>2</sub>O, and adjust pH to 7.4. Critical. Stir with Chelex-100 overnight before use.
- RIPA buffer (50 mM Trizma base, 150 mM NaCl, 2 mM EDTA, 1% IGEPAL, 0.5% Sodium deoxycholate and 2% SDS, pH 7.4) Dissolve 1.51 g Trizma base, 2.19 g NaCl, 0.18 g EDTA, 2.5 ml IGEPAL, 1.25 ml sodium deoxycholate and 5 g SDS in 250 ml dH<sub>2</sub>O and adjust to pH 7.4. Critical. Stir with Chelex-100 overnight before use.
- HEPES buffer (50 mM, pH 7.4) Dissolve 5.96 g Hepes (MW. 238.30) in 500 ml dH<sub>2</sub>O and adjust pH to 7.4. Critical. Stir with Chelex-100 overnight before use.
- Laemmli buffer (4X Laemmli and 10% v/v β-Mercaptoethanol) Add 100 μl of β Mercaptoethanol to 900 of 4X Laemmli.

- 10X Running Buffer (250 mM Tris Base, 2 M Glycine and 35 mM SDS) Dissolve 30.3 g Tris base, 144.0 g glycine and 10 g SDS in 1000 ml dH<sub>2</sub>O.
- Fixation Buffer (83.5% v/v H<sub>2</sub>O, 12.5% v/v methanol and 4% v/v acetic acid) Dilute
  125 ml methanol in 835 ml of water and add 40 ml acetic acid.
- 10X Transfer Solution (250 mM Tris base and 2 M Glycine) Dissolve 30.3 g Tris base and 144.0 g glycine in 1000 ml dH<sub>2</sub>O.
- Transfer Buffer (10% v/v 10X Transfer solution and 20% v/v EtOH) Dissolve 100 ml
  10X Transfer solution into 200 ml MeOH in 700 ml dH<sub>2</sub>O.
- 5X PBS buffer (500 mM) (200mM Na<sub>2</sub>HPO<sub>4</sub>, 50 mM NaH<sub>2</sub>PO<sub>4</sub> and 680 mM NaCl, pH 7.4) Dissolve 35.6 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O, 39.7 g NaCl and 7.8 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O in 1000 ml dH<sub>2</sub>O.
- 5X TBS buffer (500 mM) (137 mM NaCl, 20 mM Trizma base, pH 7.4) Dissolve 40 g
  NaCl and 12.1 g of Trizma Base in 1000 ml dH<sub>2</sub>O and adjust to pH 7.4.
- **PBST or TBST** Add 0.1% Tween and 1X PBS or TBS, respectively.
- 10X Phosphate Buffer (500 mM) Dissolve 68.9 g of Na<sub>2</sub>HPO<sub>4</sub>·2H<sub>2</sub>O and 17.61 g NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O in 1000 ml dH<sub>2</sub>O and adjust to pH 7.4.
- Chymotrypsin Buffer (100 mM Tris HCl and 10 mM CaCl<sub>2</sub>) Dissolve 15.76 g Tris HCl and 1.11 g CaCl<sub>2</sub> in 1000 ml dH<sub>2</sub>O and adjust to pH 7.8. Critical. Stir with Chelex-100 overnight before use.
- Ponceau S (1.5 mM Ponceau S and 5% v/v acetic acid) Dissolve 250 mg Ponceau S (MW. 672.63) and 12.5 ml acetic acid and added dH<sub>2</sub>O up to 250 ml.
- Coomassie Brilliant Blue (1.2 mM CBB, 10% v/v acetic acid and 40% v/v MeOH)
  Dissolve 0.5 g Coomassie Brilliant Blue R250 (825.97), 50 ml acetic acid, 200 ml
  MeOH in 250 ml dH<sub>2</sub>O.
- S-Basal (102.7 mM NaCl, 6.9 mM K<sub>2</sub>HPO<sub>4</sub> and 44 mM KH<sub>2</sub>PO<sub>4</sub>) Dissolve 3 g NaCl,
  0.6 g K<sub>2</sub>HPO<sub>4</sub> and 3 g KH<sub>2</sub>PO<sub>4</sub> in 500 ml dH<sub>2</sub>O. Autoclave at 120 °C for 30 min.
  Before use, add 0.5 ml cholesterol (50 mg ml<sup>-1</sup> in ethanol).
- M9 Buffer (85.6 mM NaCl, 42.3 mM Na<sub>2</sub>HPO<sub>4</sub> and 22 mM KH<sub>2</sub>PO<sub>4</sub>) Dissolve 5 g NaCl, 3 g KH<sub>2</sub>PO<sub>4</sub>, and 6 g Na<sub>2</sub>HPO<sub>4</sub> in 1000 ml dH<sub>2</sub>O. Autoclave at 120 °C for 30 min. Before use, add 1 ml 1M MgSO<sub>4</sub>.

- Potassium phosphate (1 M, pH 6) Dissolve 136.1 g KH<sub>2</sub>PO<sub>4</sub> in 800 ml dH<sub>2</sub>O and adjust to pH 6 with KOH<sub>(s)</sub>. Autoclave at 120 °C for 20 min.
- NGM Agar (1 L) Add 3 g NaCl, 17 g bactoagar and 2.5 g bactopeptone (10 g bactopeptone for Enriched NGM Agar) in 975 ml dH<sub>2</sub>O. Autoclave at 110 °C for 30 min. Supplement media before use after melting 1 ml cholesterol (50 mg ml<sup>-1</sup> in ethanol), 1 ml CaCl<sub>2</sub> (1 M), 1 ml MgSO<sub>4</sub> (1 M) and 1 ml potassium phosphate (1 M).
- YPD Media (500 ml) Add 5 g Yeast extract, 5 g bactopeptone (12 g agar if YPD Agar) and 25 ml glucose (40 %) in 500 ml dH<sub>2</sub>O. Autoclave at 110 °C for 30 min.
- LB Broth Dissolve 10 g LB Broth (Lennox) (add 15 g bactoagar for solid media) in 500 ml dH<sub>2</sub>O. Autoclave 121 °C for 15 min.
- Complete Media for MEF and HeLa (2mM Glutamine, 1% Penicillin-Streptomycin, DMEM and 10% FCS) 500 μl L-Glutamine 100 X, 500 μl Penicillin-Streptomycin, 44 ml DMEM and 5 ml FCS.

## **APPENDIX 2: Protocols**

The following protocols of the Dimedone Switch Method on different model systems, were used in this study.

### Selective labelling of protein persulfidation in cells.

1. Grow cells in 100 mm cell culture dishes in respective medium to 80-90% confluency.

*Critical*. Cells should be cultured carefully (i.e., regular schedule for splitting with no overgrowing, no more than 15 passages, mycoplasma testing) to avoid cell stress.

- 2. (Optional) For cell treatments, aspirate medium from cells and replace it with medium supplemented with treatment and incubate for respective time.
- Aspirate medium, wash cells twice, gently but quickly, with 7 ml of cold PBS and add 1 ml of cold lysis buffer supplemented with 5 mM 4-chloro-7nitrobenzofurazan onto cells.

*Critical*. 4-Chloro-7-nitrobenzofurazan (NBF-CI) should be supplemented right before step 3 if  $\leq$  5 mM is used. If > 5mM is used (typically for larger cell culture flasks; 10 mM NBF-CI in 3 ml lysis buffer for T75 flaks) it should be added during the 2<sup>nd</sup> wash with PBS before aspiration, and lysis buffer should be RT. From this point on sample should always be protected from light.

4. Using a cell scraper, gently scrape cells off dish and collect them into two 1.5 ml Eppendorf tubes (600 µl cell suspension per tube). Homogenize cells with a 1 ml syringe and a 23G needle 7 times, and place tubes immediately for incubation at 37 °C for 30 min.

*Critical*. Avoid formation of excess bubbles during homogenization. Steps 3 and 4 need to be done consecutively, 1 dish at a time within 2-3 min.

 Precipitate proteins by methanol/chloroform precipitation (lysate:methanol:chloroform = 4:4:1, v/v/v) and centrifuge samples at 14,000g for 15 min at 4 °C.

*Critical*. Methanol used for precipitation is typically stored at -20 °C. After the addition of each solvent during precipitation, suspension should be vortexed.

6. Remove aqueous and organic layers, keeping protein pellet. Repeat precipitation by adding  $H_2O$ :methanol:chloroform (4:4:1, v/v/v) to protein pellet obtained and

centrifuge samples at 14,000*g* for 15 min at 4 °C. Remove aqueous and organic layers and keep protein pellet. Wash pellet 2 times with ice cold methanol and leave to dry for 5-10 min.

Pause Point. The protein pellet can be stored at -20 °C for several weeks.

- 7. Re-dissolve each protein pellet by first adding 10 μl of 20% SDS onto pellet, and then 90 μl Hepes buffer (for a 2% final conc. of SDS). Vortex sample and then place for sonication to ensure pellet is completely dissolved. Combine the 2 protein solutions from the same dish.
- Use a DC Assay to determine the protein concentration. Adjust protein concentration to approx. 3 mg ml<sup>-1</sup> by diluting sample with Hepes buffer supplemented with SDS (final conc. 2%). The final volume prepared is typically 100 µl of protein suspension.
- Add a final concentration of 25 μM of DAz-2:Cy5 preclick mix to adjusted sample and incubate at 37 °C for 30 min.

*Critical.* For samples of protein concentrations > 3 mg/ml, 50  $\mu$ M of DAz-2:Cy5 preclick mix should be used and incubated at 37 °C for 1hr.

Alternative. For other applications such as persulfide detection using ECL, proteomics or immunoprecipitation, the alternative switch label 100  $\mu$ M DCP-Bio1 can be added instead at this point to adjusted sample, and incubated at 37 °C for 1 hr.

10. Repeat Precipitation Steps 5, 6 and 7.

*Pause Point.* The protein pellet can be stored at -20 °C for a week.

11.Use a DC Assay to determine the protein concentration. Dilute the protein solution with Hepes buffer supplemented with SDS (final conc. 2%), to a protein concentration of approx. 2-2.5 mg ml<sup>-1</sup>.

*Alternative*. For other applications, protein pellet obtained should be redissolved in appropriate buffer (without SDS). Protocol can be stopped here and samples are fully labeled and ready for appropriate use. *Limitations*. It should be noted that for applications where no SDS should be present, complete re-dissolving of a fully labelled protein pellet may pose a problem. For

immunoprecipitation studies, it is possible that NBF labelling may interfere with some antibody epitope attachment, thus antibody of interest should be previously tested with fully labeled sample. *Troubleshooting*. If  $\leq$  0.1% SDS can be used, dissolve pellet in a minimal volume of buffer with 1 % SDS as a final concentration and once fully dissolved, dilute with only buffer  $\leq$  10 times. If no SDS is possible, add 0.1 M urea as a final concentration to dissolve the proteins.

- 12.Add Laemmli buffer:sample, 1:3, v/v, boil mixture at 95 °C for 5 min and allow to cool to RT.
- 13. Resolve sample by SDS PAGE. Typically load 20 μl of sample into well (approx.40-50 μg of protein).

Alternative. For samples switch labeled with DCP-Bio1, after resolving they can at this point be transferred on a nitrocellulose membrane, followed by blocking in 1 % BSA in TBST supplemented with 0.1% Tween (PBST) for 30 min, washed with TBST and incubated with anti-Biotin-Peroxidase (1:1000 in 1% BSA in TBST) at 4 °C overnight. Membrane should then be washed and visualized using Clarity<sup>™</sup> Western ECL Substrate (BioRad) on a G:Box Chemi-XT4 (Syngene).

- 14. Wash the gel once in dH<sub>2</sub>O and replace with fixation buffer. Fix gel for 30 min with agitation.
- 15.Record the gel at 635 nm for the Cy5 signal and 473 nm for NBF-Cl signal, on a Typhoon FLA 9500 (GE Healthcare).

## Selective labelling of protein persulfidation in C. elegans

1. Collect and transfer worms from NGM plate with M9 buffer, using a Pasteur pipette, into 15 ml falcons.

Troubleshooting. Need approximately 15,000 worms / condition.

- 2. Centrifuge worm suspension at 400 rpm for 1 min.
- Remove supernatant and add 8 ml of M9 buffer to worms.
  Critical. Do not let worms dry, leave some buffer above worm pellet.
- 4. Repeat steps 2 and 3 two times.
- Using forceps, fill one 2 ml fast prep tube with liquid nitrogen and then quickly drop the concentrated worm suspension into the fast prep tube.
   Troubleshooting. Approximately 8,000 worms / fast prep tube.
- 6. Fill half the fast prep tube containing the frozen worms with glass beads, and keep on ice.
- 7. Cool FastPrep 24 machine with dry ice, by spinning at 4 m/s for 10 s with a few pieces of dry ice.
- Break samples in the FastPrep24 machine by spinning at 6.5 m/s for 35 s and 6.5 m/s for 30 s.
- Add 1 ml of lysis buffer supplemented with 20 mM NBF-Cl into tubes with beads and broken worms.
- 10. Incubate on ice for 10 min.
- 11. Centrifuge at 13,000 rpm for 15 min, at 4 °C.
- 12. Collect supernatant, above beads, into two 1.5 Eppendorf tubes.
- 13. Incubate samples at 37 °C for 1 h.
- Follow Steps 5-15 of protocol for 'Selective labelling of protein persulfidation in cells'.

## Selective labelling of protein persulfidation in S. cerevisiae

 Add 200 μl of yeast cell pellet into 2 ml cold lysis buffer, supplemented with 20 mM 4-chloro-7-nitrobenzofurazan (NBF-Cl).

*Critical.* NBF-Cl should be supplemented right before use. As > 10 mM NBF-Cl is only partially soluble in aqueous buffers, the lysis buffer should not be too cold and needs to be resuspended a few times in lysis buffer, with a pipette. Any precipitate formed will dissolve during incubation step From this point on sample should always be protected from light.

 Add this yeast cell suspension dropwise into a mortar containing liquid nitrogen. Add glass beads and grind frozen cells with a pestle until a fine powder consistency is reached.

*Troubleshooting.* Add liquid nitrogen whilst grinding when necessary to keep sample frozen.

- Collect ground sample into a two tubes and centrifuge at 1500 x g for 15 min at 4 °C.
- 4. Collect supernatant and incubate for 1 hr at 37 °C.
- 5. Split sample into four 1.5 ml Eppendorf tubes, and follow Steps 5-15 of protocol for 'Selective labelling of protein persulfidation in cells'.

## Selective labelling of protein persulfidation in organs

- 1. Weigh out 15-20 mg of snap frozen organ sample on a balance, and then place on a petri dish, on ice.
- Mince organ with a scalpel and transfer directly into a 5ml tube containing 1.5 ml of cold lysis buffer, supplemented with 20 mM 4-chloro-7-nitrobenzofurazan.
  Critical. NBF-Cl should be supplemented right after step 1 as it is not very soluble.
  Always mix lysis buffer suspension by pipetting up and down twice before use.
  From this point on sample should always be protected from light.
- 3. Homogenize organ with a 1 ml syringe and a 23G needle 10 times, and a homogenizer for 5 s.

*Troubleshooting*. For lysing of liver samples or muscles, organs need to be minced more thoroughly with the scalpel on ice, and the homogenizer needs to be used for longer.

- 4. Place tube for incubation at 37 °C for 1 hr.
- 5. Transfer lysate into a 2 ml tube and centrifuge sample at 30,000g for 10 min at 4 °C.
- 6. Collect supernatant into two 1.5 ml Eppendorf tubes.
- Follow Steps 5-15 of protocol for 'Selective labelling of protein persulfidation in cells'.
## **APPENDIX 3: Supplementary Dataset S1**

MS/MS data of the persulfidated TST digested with trypsin Related to CHAPTER 3 – Article I (Figure 2)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Scan	Source File	Accession	PTM	AScore
GSVNMPFMN FLTEDGFEKS PEELR	122,14	2773,2727	24	2,1	925,4335	40,76	12931	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
GSVNMPFMN FLTEDGFEKS PEELR	87,76	2773,2727	24	4,2	925,4354	41,28	13116	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
GSVNMPFMN FLTEDGFEKS PEELR	19,63	2773,2727	24	0	925,4315	41,79	13290	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	113,52	2023,9962	19	3,1	1013,0085	22,25	6034	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	109,96	2023,9962	19	4,5	675,6757	23,81	6619	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	109,21	2023,9962	19	3,6	675,6751	22,25	6033	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	108,41	2023,9962	19	3,4	675,675	23,3	6428	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	105	2023,9962	19	3,3	675,6749	22,77	6228	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	80,48	2023,9962	19	2,5	675,6744	45,19	14464	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	74,64	2023,9962	19	3,1	675,6747	46,22	14816	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	73,58	2023,9962	19	3,2	675,6749	38,49	12114	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	73,42	2023,9962	19	3,9	675,6753	46,74	14995	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	71,2	2023,9962	19	1,6	675,6738	33,88	10403	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	71,02	2023,9962	19	4	675,6754	43,63	13928	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	69,26	2023,9962	19	4	675,6754	41,07	13039	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	68,82	2023,9962	19	2,3	675,6743	52,9	17110	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	61,55	2023,9962	19	2,3	675,6743	40,56	12858	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	60,34	2023,9962	19	10,1	675,6795	20,54	5423	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGLDSGHIR	48,92	2023,9962	19	71,8	675,7211	40,41	12804	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SRPEPAIFK	110,77	1976,9955	18	4,4	989,5093	16,07	3787	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SRPEPAIFK	101,51	1976,9955	18	0,1	660,0059	16,48	3938	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SRPEPAIFK	94,69	1976,9955	18	3,6	660,0081	15,97	3749	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SRPEPAIFK	93,29	1976,9955	18	2,4	660,0073	16,98	4118	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		

EGHPVTSEP SRPEPAIFK	77,34	1976,9955	18	1,6	660,0068	17,49	4294	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
EGHPVTSEP SRPEPAIFK	74,42	1976,9955	18	3,3	660,0079	18,51	4672	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	72,47	1976,9955	18	4,4	660,0087	18	4479	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	63,91	1976,9955	18	-10,9	659,9986	19,85	5176	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
EGHPVTSEP SRPEPAIFK	61,54	1976,9955	18	0,9	989,5059	15,55	3593	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	38,07	1976,9955	18	1	989,506	16,64	3997	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	31,03	1976,9955	18	3,3	660,0079	29,71	8822	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	27,61	1976,9955	18	0,8	660,0063	24,86	7016	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
EGHPVTSEP SRPEPAIFK	22,66	1976,9955	18	3,7	660,0082	44,74	14311	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	98,34	1607,8154	13	2,4	804,9169	25,48	7250	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	97,71	1607,8154	13	2,7	536,9472	26,48	7623	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	95,03	1607,8154	13	4,2	536,948	25,95	7425	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	91,49	1607,8154	13	3,2	804,9176	25,98	7435	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	91,27	1607,8154	13	4,2	536,948	25,44	7236	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	89,8	1607,8154	13	3,7	536,9478	26,99	7808	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	89,22	1607,8154	13	4,4	536,9481	29,97	8922	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	88,96	1607,8154	13	0,7	536,9461	27,49	7988	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	88,93	1607,8154	13	3,7	536,9478	28,52	8369	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	88,34	1607,8154	13	2	804,9166	24,96	7055	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	87,01	1607,8154	13	2,5	536,9471	29,46	8725	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	85,13	1607,8154	13	3,6	536,9477	32,01	9693	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	82,3	1607,8154	13	-0,9	536,9453	28	8175	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	81,65	1607,8154	13	1,1	536,9464	31	9313	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	81,14	1607,8154	13	2,1	536,9469	30,48	9112	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN

TYEQVLENLE SKR	80,83	1607,8154	13	2,6	804,9171	26,59	7663	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	79,09	1607,8154	13	2,3	536,947	31,51	9502	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	78,99	1607,8154	13	4,2	536,948	34,7	10716	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	77,88	1607,8154	13	4,4	804,9185	27,13	7862	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	77,5	1607,8154	13	2,1	536,9469	33,15	10120	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
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TYEQVLENLE SKR	76,33	1607,8154	13	2,8	536,9473	35,21	10904	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	76,11	1607,8154	13	6	536,949	39,41	12443	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
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TYEQVLENLE SKR	72,52	1607,8154	13	3,6	536,9477	35,75	11110	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	72,41	1607,8154	13	3,7	536,9478	46,68	14973	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	72,28	1607,8154	13	5,8	536,9489	34,18	10517	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	72,25	1607,8154	13	7,4	536,9497	42,5	13534	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	71,91	1607,8154	13	2,4	536,947	37,31	11689	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	70,2	1607,8154	13	-1,3	536,9451	36,27	11300	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TYEQVLENLE SKR	68,93	1607,8154	13	7,4	536,9497	43,52	13891	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
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TYEQVLENLE SKR	67,78	1607,8154	13	7,5	536,9498	41,99	13358	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	67,38	1607,8154	13	3,2	536,9474	40,44	12815	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	66,43	1607,8154	13	3,7	536,9478	40,97	13000	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
TYEQVLENLE SKR	65,46	1607,8154	13	17,6	536,9552	45,07	14423	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN

TYEQVLENLE SKR	64,75	1607,8154	13	3,8	536,9478	43,02	13709	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SKR	64	1607,8154	13	0,3	536,9459	47,36	15213	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	61,16	1607,8154	13	16,5	536,9546	44,03	14071	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	61,02	1607,8154	13	2,4	536,947	36,8	11494	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	60,34	1607,8154	13	3,5	536,9476	51,58	16649	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SKR	59,81	1607,8154	13	5,9	536,9489	48,4	15573	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	59,47	1607,8154	13	4,2	536,948	47,89	15397	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
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TYEQVLENLE SKR	58,43	1607,8154	13	17,3	536,955	45,59	14606	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	56	1607,8154	13	6,6	536,9493	49,43	15937	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
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TYEQVLENLE SKR	53,26	1607,8154	13	6,7	536,9493	49,99	16121	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	50,86	1607,8154	13	-3,1	804,9125	27,64	8043	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	46,49	1607,8154	13	0,1	536,9458	53,38	17280	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	42,58	1607,8154	13	-3,1	804,9125	29,08	8582	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	24,72	1607,8154	13	-4,6	804,9113	34,04	10464	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SKR	23,07	1607,8154	13	0,9	804,9157	31,37	9449	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	94,35	1451,7144	12	3,5	726,867	29,7	8816	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	92,81	1451,7144	12	4,5	726,8677	29,17	8614	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	89,49	1451,7144	12	2,3	726,8661	30,22	9015	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	88,39	1451,7144	12	3,9	726,8673	30,73	9206	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	86,61	1451,7144	12	4	726,8674	32,3	9796	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	85,89	1451,7144	12	0,7	726,8649	31,25	9403	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	

TYEQVLENLE SK	83,84	1451,7144	12	5,5	726,8685	31,79	9606	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	82,07	1451,7144	12	0	484,9121	28,81	8474	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	75,04	1451,7144	12	1,3	726,8654	33,33	10191	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	70,3	1451,7144	12	1,7	726,8657	32,81	9990	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	59,88	1451,7144	12	4,9	726,868	34,21	10528	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	58,01	1451,7144	12	0,9	726,8651	35,02	10831	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	57,66	1451,7144	12	3,7	726,8671	37,61	11798	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	54,55	1451,7144	12	5,8	726,8687	40,16	12713	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	54,22	1451,7144	12	7,2	726,8697	39,65	12528	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	52,42	1451,7144	12	2,6	726,8663	38,63	12165	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	46,4	1451,7144	12	8,9	726,8709	42,82	13644	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	45,03	1451,7144	12	2,9	726,8666	39,14	12346	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	42,5	1451,7144	12	2,3	726,8661	35,52	11025	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
TYEQVLENLE SK	40,18	1451,7144	12	3,8	726,8672	43,92	14033	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	39,09	1451,7144	12	4,6	726,8678	45,47	14562	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	39,07	1451,7144	12	1,2	726,8653	41,8	13291	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	38,64	1451,7144	12	3,8	726,8672	38,12	11980	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	37,63	1451,7144	12	4,3	726,8676	48,57	15635	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	36,46	1451,7144	12	0,3	726,8647	40,68	12901	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	35,35	1451,7144	12	6,6	726,8693	26,36	7577	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	35,02	1451,7144	12	6,1	726,8689	43,34	13829	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	34,41	1451,7144	12	2,2	726,866	45,97	14732	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	32,73	1451,7144	12	4,8	726,8679	41,29	13119	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
TYEQVLENLE SK	29,98	1451,7144	12	-28,5	726,8438	42,37	13491	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	

TYEQVLENLE SK	28,82	1451,7144	12	1,4	726,8655	49,09	15822	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	28,22	1451,7144	12	3	726,8666	47,52	15270	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	26,1	1451,7144	12	2	726,8659	44,96	14386	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	24,51	1451,7144	12	8,8	726,8708	50,04	16135	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
TYEQVLENLE SK	22,52	1451,7144	12	4,8	726,8679	42,31	13473	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	17,58	1451,7144	12	5,5	726,8685	50,55	16307	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	17,44	1451,7144	12	-1,8	726,8632	54	17498	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
TYEQVLENLE SK	16,51	1451,7144	12	5,8	726,8687	52,12	16837	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
NWLKEGHPV TSEPSRPEPA IFK	92,11	2518,2969	22	1,5	840,4409	20,46	5402	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
NWLKEGHPV TSEPSRPEPA IFK	44,87	2518,2969	22	-7,1	840,4336	23,65	6561	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
GSVNMPFMN FLTEDGFEK	90,94	2061,9175	18	3,4	1031,9695	44,51	14236	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
GSVNMPFMN FLTEDGFEK	48,75	2061,9175	18	1,7	688,3143	44,09	14091	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
GSVNMPFMN FLTEDGFEK	47,2	2061,9175	18	4,1	1031,9702	44	14059	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
HVPGASFFDI EEC(+163.00) RDK	89,41	2011,8477	16	4,5	671,6262	28,37	8313	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	88,04	2011,8477	16	0,6	671,6235	27,86	8124	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	76,92	2011,8477	16	1,7	671,6243	28,99	8547	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	24,74	2011,8477	16	4,6	671,6262	34,6	10678	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	21,73	2011,8477	16	5,7	671,627	34,06	10471	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	19,95	2011,8477	16	5,4	671,6268	32,59	9905	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C13:NBF- C:1000.00
HVPGASFFDI EEC(+163.00) RDK	17,4	2011,8477	16	5,1	671,6266	32,02	9697	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C13:NBF- C:1000.00
APPETWVSQ GK	87,52	1198,5981	11	3,7	600,3086	18,29	4592	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ GK	86,35	1198,5981	11	1,4	600,3072	17,26	4215	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ GK	84,87	1198,5981	11	1,8	600,3074	17,78	4402	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ GK	83,22	1198,5981	11	2,1	600,3076	16,76	4039	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		

APPETWVSQ GK	82,06	1198,5981	11	1,6	600,3073	16,25	3860	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
APPETWVSQ GK	73,93	1198,5981	11	4	600,3087	18,8	4777	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	63,99	1198,5981	11	3,4	600,3084	20,15	5290	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
APPETWVSQ GK	59,33	1198,5981	11	4,1	600,3088	19,64	5097	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	56,26	1198,5981	11	0,8	600,3068	21,53	5761	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	51,37	1198,5981	11	-1,1	600,3057	20,94	5563	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	51,17	1198,5981	11	1,8	600,3074	31,23	9395	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	49,71	1198,5981	11	3,3	600,3083	30,46	9107	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	47,41	1198,5981	11	1,1	600,307	24,88	7023	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	44,27	1198,5981	11	0,7	600,3068	32,67	9937	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	43,15	1198,5981	11	6,7	600,3104	26,46	7613	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	41,3	1198,5981	11	1	600,3069	33,57	10286	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	41,23	1198,5981	11	5,3	600,3095	38,33	12055	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	36,59	1198,5981	11	4,2	600,3088	25,57	7283	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	35,17	1198,5981	11	3,4	600,3084	32,07	9716	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
APPETWVSQ GK	33,2	1198,5981	11	2,4	600,3078	37,22	11651	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
APPETWVSQ GK	32,06	1198,5981	11	2,6	600,3079	44,06	14080	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	
APPETWVSQ GK	30,36	1198,5981	11	3,6	600,3085	50,81	16391	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	30,08	1198,5981	11	7,2	600,3107	45,8	14672	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	29,69	1198,5981	11	4,1	600,3088	44,67	14287	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	29,66	1198,5981	11	6,3	600,3101	43,55	13900	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	27,97	1198,5981	11	9,2	600,3119	41,5	13189	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	25,73	1198,5981	11	5,1	600,3094	40,97	13001	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	
APPETWVSQ GK	24,64	1198,5981	11	2,5	600,3079	48,42	15581	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	

APPETWVSQ GK	24,56	1198,5981	11	3,3	600,3083	35,12	10869	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ GK	23,4	1198,5981	11	0,3	600,3065	53,42	17294	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	23,32	1198,5981	11	10,1	600,3124	49,72	16035	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	22,45	1198,5981	11	5	600,3093	49,01	15789	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	21,73	1198,5981	11	6,2	600,3101	47,92	15406	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	21,15	1198,5981	11	5,4	600,3096	51,61	16661	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	19,33	1198,5981	11	6,9	600,3105	50,27	16210	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK	18,27	1198,5981	11	6,3	600,3101	42,85	13649		protein1 sp  P00586 TH		
APPETWVSQ GK	16,41	1198,5981	11	4,2	600,3088	52,18	16857	- mf18031 3 02.raw	protein1 sp  P00586 TH		
DTHVVVYDG DDLGSFYAP	87,18	2124,9751	19	2,8	709,3343	30,46	9105	- mf18031 3 02.raw	protein1 sp  P00586 TH		
GISNDTHVVV YDGDDLGSF	85,34	2496,1558	23	4,2	833,0627	30,11	8972	- mf18031 3 02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	84,97	1350,6567	12	2,8	676,3375	24,83	7003	- mf18031 3 02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	82,66	1350,6567	12	3,4	676,338	25,85	7385		protein1 sp  P00586 TH		
VLDASWYSP GTR	78,24	1350,6567	12	4,2	676,3385	26,37	7580	 mf18031 3_02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	78,08	1350,6567	12	2,9	676,3376	25,34	7202	mf18031 3_02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	73,92	1350,6567	12	4,7	676,3388	26,96	7799	mf18031 3_02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	69,13	1350,6567	12	1,1	451,2267	24,43	6851	mf18031 3_02.raw	protein1 sp  P00586 TH		
VLDASWYSP GTR	26,57	1350,6567	12	105,2	451,2735	22,3	6054	mf18031 3_02.raw	protein1 sp  P00586 TH		
ALVSTKWLA ESVR	80,1	1458,8195	13	1,2	730,4179	24,78	6982	mf18031 3_02.raw	protein1 sp  P00586 TH		
ALVSTKWLA ESVR	38,54	1458,8195	13	-17	487,2722	31,89	9646	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GK(+163.00)G	74,83	1603,7373	14	4	802,8792	28,38	8316	mf18031 3_02.raw	protein1 sp  P00586 TH	NBF-N	K11:NBF- N:13.38
APPETWVSQ GK(+163.00)G	67,87	1603,7373	14	2,9	802,8782	28,89	8505	mf18031 3_02.raw	protein1 sp  P00586 TH	NBF-N	K11:NBF- N:29.32
VYDGDDLGS FYAPR	72,55	1573,7048	14	-1,1	787,8588	28,04	8194	mf18031 3_02.raw	protein1 sp  P00586 TH		
APPETWVSQ GKG	68,6	1255,6196	12	2,1	628,8184	16,75	4037	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		

APPETWVSQ GKG	61,2	1255,6196	12	-0,2	628,817	16,23	3854	mf18031 3_02.raw	protein1 sp  P00586 TH		
MNFLTEDGF EKSPEELR	65,2	2040,9462	17	1,3	681,3235	28,03	8189	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	63,43	1048,5665	10	1,7	525,2914	21,12	5626	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	63,17	1048,5665	10	2,4	525,2918	21,63	5800	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	52,08	1048,5665	10	-4,9	525,288	20,62	5449	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	51,47	1048,5665	10	3,9	525,2926	20,04	5249	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	46,86	1048,5665	10	-0,4	525,2903	29,34	8677	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	43,37	1048,5665	10	0,8	525,291	29,85	8873	mf18031 3_02.raw	protein1 sp  P00586 TH		
TVSVLNGGF R	29,98	1048,5665	10	13,8	525,2978	31,92	9655	mf18031 3_02.raw	protein1 sp  P00586 TH		
KVDLTKPLIAT	62,27	1197,7333	11	3,2	599,8759	20,95	5568	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	60,75	1019,5512	8	3	510,7844	14,8	3320	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	58,91	1019,5512	8	2,3	510,784	15,32	3512	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	47,12	1019,5512	8	0,1	510,7829	15,86	3708	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	38,3	1019,5512	8	-1	510,7823	16,42	3917	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	20,47	1019,5512	8	5,4	510,7856	17,92	4452	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
RFQLVDSR	17,91	1019,5512	8	5,7	510,7858	42,15	13415	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
AGKVGPGLR	60,14	853,5134	9	-2,4	427,7629	10,94	1969	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
PVTSEPSRPE PAIFK	57,31	1653,8726	15	2,8	552,2997	17,38	4254	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
SPEELR	55,52	729,3657	6	-0,5	365,6899	11,03	1996	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
SPEELR	51,06	729,3657	6	1,7	365,6907	11,55	2184	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
GDDLGSFYA PR	55,38	1196,5461	11	5,9	599,2839	23,84	6630	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
AMFEAKK	53,85	823,4261	7	1,1	412,7208	11,2	2050	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
FLTEDGFEK	53,74	1084,5077	9	1,2	543,2618	21,07	5609	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
AGK(+163.00) VGPGLR	53,52	1016,5146	9	6,1	509,2677	23,77	6604	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K3:NBF- N:1000.00

PETWVSQGK	53,03	1030,5083	9	3,1	516,263	13,85	2990	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
FLTEDGFEKS PEELR	52,49	1795,8628	15	1,1	898,9397	23,22	6398	mf18031 3_02.raw	protein1 sp  P00586 TH
FLTEDGFEKS PEELR	51,18	1795,8628	15	0,3	599,6284	23,12	6359	mf18031 3_02.raw	protein1 sp  P00586 TH
FLTEDGFEKS PEELR	31,61	1795,8628	15	3,8	599,6305	23,58	6535	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	52,34	863,4501	7	6,1	432,735	17,7	4370	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	52,13	863,4501	7	4,2	432,7341	18,21	4560	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	50,09	863,4501	7	3,6	432,7339	18,73	4750	mf18031 3 02.raw	protein1 sp  P00586 TH
FQLVDSR	42,35	863,4501	7	4,4	432,7342	34,26	10550	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	41,05	863,4501	7	-0,7	432,732	36,42	11355	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	39,53	863,4501	7	1,9	432,7331	32,73	9959	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	38,27	863,4501	7	5	432,7345	37,82	11875	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	38,27	863,4501	7	2,1	432,7332	32,22	9766	mf18031 3_02.raw	protein1 sp  P00586 TH
FQLVDSR	37,97	863,4501	7	1,5	432,733	33,24	10155	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
FQLVDSR	37,88	863,4501	7	8,2	432,7359	39,68	12538	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
FQLVDSR	37,71	863,4501	7	3	432,7336	30,46	9106	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
FQLVDSR	36,92	863,4501	7	5,6	432,7347	45,71	14643	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
FQLVDSR	35,84	863,4501	7	3,3	432,7337	34,78	10748	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
FQLVDSR	34,63	863,4501	7	0,7	432,7326	36,93	11543	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TVSVLNGGF RNWLK	52	1589,8678	14	0,2	530,9633	28,94	8526	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TVSVLNGGF RNWLK	46,89	1589,8678	14	2,7	530,9647	29,46	8726	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
TVSVLNGGF RNWLK	15,17	1589,8678	14	-8,9	530,9585	34,8	10755	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
VWWMFR	50,37	923,4476	6	4,6	462,7332	35,29	10934	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
VWWMFR	50,14	923,4476	6	3	462,7325	34,78	10749	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
VWWMFR	49,67	923,4476	6	2,4	462,7322	35,81	11133	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN

VWWMFR	47,97	923,4476	6	-0,2	462,731	36,32	11317	mf18031 3 02.raw	protein1 sp  P00586 TH	
	47.24	023 4476	6	47	462 7222	37.97	11202	- mf18031	IR_BOVIN protein1 sp	
	47,24	923,4470	0	4,7	402,7555	57,07	11093	3_02.raw	TR_BOVIN	
VWWMFR	46,7	923,4476	6	2,9	462,7324	38,39	12078	mf18031	protein1 sp  P00586 TH	
								5_02.1aw	TR_BOVIN	
VWWMFR	46,38	923,4476	6	2,3	462,7321	37,34	11701	mf18031 3_02.raw	P00586 TH	
								mf18031	protein1 sp	
VWWMFR	46,37	923,4476	6	4,5	462,7332	38,91	12265	3_02.raw	P00586 TH TR BOVIN	
	46.00	023 4476	6	0	462 7311	36.83	11504	mf18031	protein1 sp	
	40,09	923,4470	0	0	402,7311	30,02	11504	3_02.raw	TR_BOVIN	
VWWMFR	45 98	923 4476	6	49	462 7333	42 52	13541	mf18031	protein1 sp  P00586ITH	
	.0,00	0_0,0	Ĵ	.,0	,	,0_		3_02.raw	TR_BOVIN	
VWWMFR	45 66	923 4476	6	31	462 7325	40 47	12823	mf18031	protein1 sp  P00586ITH	
	10,00	020,1110	Ű	0,1	102,1020	10,11	12020	3_02.raw	TR_BOVIN	
	15 23	023 1176	6	2.8	462 7324	13 04	13720	mf18031	protein1 sp	
	45,25	923,4470	0	2,0	402,7324	43,04	13720	3_02.raw	TR_BOVIN	
	44.00	000 4470	0	5.0	400 7000	44.40	40400	mf18031	protein1 sp	
VVVVVIFR	44,92	923,4476	6	5,9	462,7338	41,49	13186	3_02.raw	TR BOVIN	
								mf18031	protein1 sp	
VWWMFR	44,89	923,4476	6	4,3	462,7331	42,01	13367	3_02.raw	P00586 TH TR BOVIN	
								mf18031	protein1 sp	
VWWMFR	44,66	923,4476	6	3,6	462,7328	39,42	12448	3_02.raw	P00586 TH	
								mf18031	protein1 sp	
VWWMFR	43,6	923,4476	6	3,9	462,7329	51,42	16597	3_02.raw	P00586 TH	
									protein1 sp	
VWWMFR	43,39	923,4476	6	2,5	462,7322	40,98	13007	3_02.raw	P00586 TH	
								-	protein1 sp	
VWWMFR	43,18	923,4476	6	2,9	462,7325	39,95	12637	mf18031 3 02.raw	P00586 TH	
									TR_BOVIN protein1lspl	<u> </u>
VWWMFR	42,32	923,4476	6	2	462,732	44,08	14089	mf18031 3 02 raw	P00586 TH	
								0_02.101	TR_BOVIN protein1lspl	
VWWMFR	41,6	923,4476	6	4,6	462,7332	44,59	14260	mf18031	P00586 TH	
								5_02.1aw	TR_BOVIN	
VWWMFR	41,5	923,4476	6	4,8	462,7333	45,62	14614	mf18031	P00586 TH	
								3_02.1aw	TR_BOVIN	_
VWWMFR	41,32	923,4476	6	2,9	462,7324	51,95	16778	mf18031	P00586 TH	
				-				3_02.raw	TR_BOVIN	
VWWMFR	41.09	923,4476	6	1.2	462,7317	48.88	15746	mf18031	P00586ITH	
	,			-,_	,	,		3_02.raw	TR_BOVIN	
VWWMFR	41 07	923 4476	6	3.8	462 7328	50.91	16428	mf18031	protein1 sp  P00586ITH	
	,07		Ű	0,0				3_02.raw	TR_BOVIN	<u> </u>
VWWMFR	40 22	923 4476	6	32	462 7326	43 57	13906	mf18031	protein1 sp  P00586ITH	
	10,22	020,4470	Ŭ	0,2	102,7020	10,01		3_02.raw	TR_BOVIN	
	39.86	923 4476	6	14	462 7317	54 07	17524	mf18031	protein1 sp  P00586ITH	
	00,00	020,7770		1,-#		UT,UI	11024	3_02.raw	TR_BOVIN	
	20.64	022 4476	6	2.2	462 7226	52.40	16062	mf18031	protein1 sp	
	59,01	323,4470	0	5,5	402,1320	52,48	10903	3_02.raw	TR_BOVIN	

VWWMFR	39,58	923,4476	6	1,6	462,7318	47,35	15211	mf18031 3_02.raw	protein1 sp  P00586 TH
VWWMFR	37,75	923,4476	6	1,7	462,7319	53,02	17149	mf18031 3 02.raw	protein1 sp  P00586 TH
VWWMFR	35,96	923,4476	6	3,2	462,7326	45,11	14436	mf18031	TR_BOVIN protein1 sp  P00586 TH
VWWMFR	35,62	923,4476	6	4,1	462,733	49,89	16091	mf18031	TR_BOVIN protein1 sp  P00586 TH
	35 35	923 4476	6	2.8	462 7324	40 30	15924	3_02.raw mf18031	TR_BOVIN protein1 sp  P00586ITH
	05,00	000 4470	°	2,0	402,7024	40,00	15524	3_02.raw mf18031	TR_BOVIN protein1 sp
VWWMFR	35,03	923,4476	6	3,7	462,7328	48,37	15563	3_02.raw	TR_BOVIN
VWWMFR	32,2	923,4476	6	2,7	462,7323	46,13	14788	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
VWWMFR	31,84	923,4476	6	4,1	462,733	50,4	16257	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
VWWMFR	31,71	923,4476	6	5,5	462,7336	46,64	14960	mf18031 3_02.raw	protein1 sp  P00586 TH
VWWMFR	31,3	923,4476	6	3,2	462,7326	47,86	15387	mf18031 3_02 raw	protein1 sp  P00586 TH
VWWMFR	30,55	923,4476	6	1	462,7315	53,54	17338	mf18031	TR_BOVIN protein1 sp  P00586 TH
HOVLYR	50 21	814 4449	6	16	408 2304	11 28	2083	3_02.raw mf18031	TR_BOVIN protein1 sp  P00586ITH
	00,21	011,1110	Ŭ	1,0	100,2001	11,20	2000	3_02.raw	TR_BOVIN protein1 sp
KEYLER	50,17	836,4392	6	10,3	419,2312	10,83	1925	mf18031 3_02.raw	P00586 TH TR_BOVIN
KEYLER	49,73	836,4392	6	1,1	419,2273	11,34	2104	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
KEYLER	43,74	836,4392	6	10,3	419,2312	11,85	2281	mf18031 3_02.raw	protein1 sp  P00586 TH
KEYLER	35,56	836,4392	6	1,4	419,2274	12,37	2457	mf18031 3 02 raw	protein1 sp  P00586 TH
KEYLER	34,29	836,4392	6	25,7	419,2376	15,22	3473	mf18031	TR BOVIN protein1 sp  P00586 TH
KEYLER	30.6	836.4392	6	-0.9	419.2265	13.31	2794	mf18031	TR_BOVIN protein1 sp  P00586ITH
	, -	,	-	- , -	-,	- , -		3_02.raw	TR_BOVIN protein1 sp
EYLER	48,72	708,3442	5	0,4	355,1795	12,09	2368	mf18031 3_02.raw	P00586 TH TR_BOVIN
KVDLTKPL	48,02	912,5644	8	4	457,2913	16,03	3774	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN
AVGLDSGHIR	47,89	1023,5461	10	2,2	512,7815	13,13	2729	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN
APPETWVSQ GKGGK	47,73	1440,7361	14	-13,9	481,246	18,17	4543	mf18031 3_02.raw	protein1 sp  P00586 TH TP_BOV(N
APPETWVSQ GKGGK	16,47	1440,7361	14	68,5	481,2856	18,05	4497	mf18031 3 02.raw	protein1 sp  P00586 TH
WLAESVR	47,13	859,4552	7	6,4	430,7376	17,54	4314	 mf18031 3_02.raw	protein1 sp  P00586 TH
			I		I			1	

WLAESVR	40,84	859,4552	7	2,1	430,7358	20,72	5484	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
WLAESVR	40,02	859,4552	7	5,3	430,7372	20,21	5316	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
ATLNRSLLK	45,76	1014,6185	9	1,6	508,3174	13,68	2928	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
K(+163.00)EY LER	41,32	999,4404	6	4,7	500,7298	22,44	6105	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-N	K1:NBF- N:1000.00
FQLVDSRAQ GR	41,01	1275,6683	11	3	426,2313	14,82	3328	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
FQLVDSRAQ GR	19,02	1275,6683	11	1,5	638,8424	14,85	3338	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
DASWYSPGT R	39,38	1138,5043	10	3,6	570,2615	19,52	5052	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN		
DASWYSPGT R	16,97	1138,5043	10	-0,6	570,2591	24,59	6912	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
VHQVLYR	37,52	913,5134	7	2,4	457,7651	12,38	2463	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
VHQVLYR	36,69	913,5134	7	0,6	457,7643	11,87	2290	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
AMFEAK(+163 .00)K	36,3	986,4274	7	1,9	494,2219	26,23	7523	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K6:NBF- N:24.44
ALVSTK(+163. 00)WLAESVR	35,74	1621,8207	13	1,7	811,919	39,2	12367	mf18031 3_02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-N	K6:NBF- N:1000.00
PEPDAVGLD SGHIR	34,67	1461,7212	14	-3,4	731,8654	21,8	5864	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ	32,88	1013,4818	9	4,4	507,7504	23,16	6374	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
APPETWVSQ	26,89	1013,4818	9	5,9	507,7512	23,67	6566	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SR	32,32	1194,5629	11	7,4	598,2932	9,94	1596	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
NWLKEGHPV TSEPSRPEPA	30,58	2130,0493	19	3,4	711,0261	16,09	3795	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD	29,11	1018,4607	9	1,7	510,2385	17,52	4305	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
PSRPEPAIFK	28,46	1140,629	10	-1,4	571,321	15,69	3643	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
GSWFEWFH R	26,67	1250,5621	9	-18,2	626,277	35,36	10963	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
QPEPDAVGL DSGHIR	24,58	1589,7798	15	1,1	795,898	22,02	5952	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
KVDLTKPLIAT C(+163.00)R	20,56	1619,8447	13	1	810,9304	24,04	6704	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C12:NBF- C:1000.00
KPLIATC(+16 8.08)RK	19,91	1196,6951	9	7,4	399,9245	19,25	4948	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	Dio- cleaved	C7:Dio- cleaved:1000. 00
QVLENLESK	19,83	1058,5608	9	-8,9	530,283	15,32	3510	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		

TYEQVLENLE SK(+163.00)R	17,99	1770,8167	13	9,6	886,4241	41,72	13263	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K12:NBF- N:1000.00
EGHPVTSEP SRPEPAIFKA TLNR	17,7	2532,3083	23	-6,3	845,1047	22,49	6126	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
EGHPVTSEP SRPEPAIFKA TLNR	16,53	2532,3083	23	-10,4	845,1013	21,71	5831	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
KVDLTK(+163 .00)PLIATC(+1 68.08)R	17,57	1787,9235	13	-39	596,9585	24,29	6796	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N; Dio- cleaved	K6:NBF- N:5.81;C12:Di o- cleaved:1000. 00
EPDAVGLDS GHIR	15,93	1364,6685	13	23,1	683,3573	13,11	2724	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
YLGTQPEPD AVGL	15,65	1358,6718	13	1,7	680,3443	31,43	9473	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		
GHPVTSEPS RPEPAIFK	15,02	1847,9529	17	-3,1	616,9897	15,2	3463	mf18031 3_02.raw	protein1 sp  P00586 TH TR_BOVIN		

## **APPENDIX 4: Supplementary Dataset S2**

MS/MS data of the persulfidated TST digested with chymotrypsin Related to CHAPTER 3 – Article I (Figure 2)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Scan	Source File	Accession	PTM	AScore
LTEDGFEKSP EEL	82,53	1492,6932	13	7,4	747,3594	24,46	5819	mf18031 3_Chymo	protein1 sp  P00586 TH		
								_02.1aw mf18031	protein1lspl		
LTEDGFEKSP	36,21	1492,6932	13	2,4	747,3557	24,97	5971	3_Chymo	P00586 TH		
EEL								_02.raw	TR_BOVIN		
								mf18031	protein1 sp		
FHRAPPETW	66,07	1139,5511	9	4,4	570,7853	17,38	3712	3_Chymo	P00586 TH		
								02.raw	Protein ISP		
FHRAPPETW	65,54	1139,5511	9	3,1	570,7846	16,88	3571	3_Chymo	P00586 TH		
								02 row mf18031	protein1lspl		
FHRAPPETW	57,59	1139,5511	9	3,2	570,7847	16,36	3410	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		
	- 4 0-	4400 5544	0		000 0507	10.00	0.570	mf18031	protein1 sp		
FHRAPPEIW	54,65	1139,5511	9	2,8	380,8587	16,88	3573	3_Chymo			
								_02.1aw mf18031	protein1lspl		
FHRAPPETW	51,61	1139,5511	9	3,5	570,7849	17,89	3886	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		
								mf18031	protein1 sp		
FHRAPPETW	51	1139,5511	9	5,9	570,7862	18,41	4049	3_Chymo	P00586 TH		
								_02.raw mf18031	nrotein1lsnl		[
FHRAPPETW	48.33	1139.5511	9	4.4	380.8593	17.4	3719	3 Chymo	P00586ITH		
	- ,	,	_	,	,	,		_02.raw	TR_BOVIN		
								mf18031	protein1 sp		
FHRAPPETW	47,59	1139,5511	9	3,2	380,8589	16,37	3411	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		
FHRAPPETW	33 48	1139 5511	9	51	570 7858	18 91	4198	3 Chymo	P00586ITH		
	00,40	1100,0011	Ũ	0,1	070,7000	10,01	4100	02.raw	TR BOVIN		
								mf18031	protein1 sp		
FHRAPPETW	30,98	1139,5511	9	6,1	570,7863	19,43	4343	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		
	28.06	1130 5511	0	7.6	570 7872	10.05	4515	mf18031	protein1 sp		
	20,00	1139,3311	9	7,0	510,1012	19,95	4010	02 raw	TR BOVIN		
								mf18031	protein1 sp		
FHRAPPETW	18,66	1139,5511	9	4,8	570,7856	21,5	4955	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		L
EAKKVDLTKP	61 22	1240 730	11	3.4	621 3780	15 / 1	3114	mf18031	protein1 sp		
L	01,25	1240,733		5,4	021,5703	13,41	5114	02 raw	TR BOVIN		
								mf18031	protein1 sp		
	52,37	1240,739	11	0,7	621,3772	14,9	2969	3_Chymo	P00586 TH		
L								_02.raw	TR_BOVIN		
EAKKVDLTKP	10.46	1240 720	11	2	111 5000	15 90	2262	mf18031	protein1 sp		
L	49,40	1240,739	11	3	414,0002	15,69	3203	02 raw	TR BOVIN		
								mf18031	protein1 sp		
	47,41	1240,739	11	5,2	414,5891	15,38	3106	3_Chymo	P00586 TH		
L								_02.raw	TR_BOVIN		<u> </u>
EAKKVDLTKP	25.64	1240 720	11	0.4	111 5071	11 07	2050	mf18031	protein1 sp		
L	23,04	1240,739	11	0,4	414,3071	14,07	2959	02 raw	TR BOVIN		
								mf18031	protein1 sp		
	59,09	1258,6458	11	3,2	420,5572	24,09	5697	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		
LERHVPGAS	55.04	4050 0450			000 00 45	00.0	<b>FF</b> 4 <b>F</b>	mf18031	protein1 sp		
FF	55,31	1258,0458	11	6,9	030,3345	23,0	5545	3_Cnymo			
									protein1lspl		
	52,05	1258,6458	11	7	420,5588	23,57	5538	3_Chymo	P00586 TH		
								_02.raw	TR_BOVIN		L
LERHVPGAS	40.50	4050 0450			000 0007	04.40	F740	mf18031	protein1 sp		
FF	43,53	1208,6458	11	0,9	03U,33U/	24,12	5710	3_Cnymo			
LOTODEDE								mf18031	protein1lspl		
	58,95	1195,6084	12	7	598,8157	29,73	7306	3_Chymo	P00586 TH		
VGL								_02.raw	TR_BOVIN		1

LGTQPEPDA VGI	57,76	1195,6084	12	4,7	598,8143	30,24	7440	mf18031 3_Chymo	protein1 sp  P00586 TH	
								_02.raw	TR_BOVIN	
LGTQPEPDA	49.42	1195.6084	12	3.9	598.8138	30.74	7593	3 Chymo	P00586ITH	
VGL	,	,		-,-	,	,-		_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
VGL	37,65	1195,6084	12	2,6	598,813	28,71	7016	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
LGTQPEPDA	34 92	1195 6084	12	73	598 8159	33 29	8230	3 Chymo	P00586ITH	
VGL	0.,01		.=	.,.	000,0100	00,20	0200	_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
VGL	31,79	1195,6084	12	8,8	598,8167	31,25	7737	3_Chymo	P00586 TH	
_								_02.raw	TR_BOVIN	
LGTQPEPDA	28 54	1195 6084	12	45	598 8141	31 76	7855	3 Chymo	P00586ITH	
VGL	20,04	1100,0004	12	4,0	000,0141	01,70	1000	02.raw	TR BOVIN	
								mf18031	protein1 sp	
	27,82	1195,6084	12	8,2	598,8164	32,27	7986	3_Chymo	P00586 TH	
VOL								_02.raw	TR_BOVIN	
LGTQPEPDA	26.2	1105 6084	10	6.0	500 0150	20.22	7166	mf18031	protein1 sp	
VGL	20,2	1195,0004	12	0,2	590,015Z	29,22	/ 100	02 raw	TR BOVIN	
								mf18031	protein1 spl	
LGTQPEPDA	21,07	1195,6084	12	7,4	598,8159	33,8	8345	3_Chymo	P00586 TH	
VGL								_02.raw	TR_BOVIN	
LGTQPEPDA	10.01		10	_				mf18031	protein1 sp	
VGL	19,81	1195,6084	12	5	598,8145	34,82	8610	3_Chymo	P00586 TH	
								_02.raw	nrotein1Ispl	
LAESVRAGK	58.88	1352.7776	14	1.5	677.3971	17.65	3803	3 Chymo	P00586ITH	
VGPGL	,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		.,-	,	,		_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
VGPGI	52,36	1352,7776	14	1,1	451,9336	17,63	3797	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
LAESVRAGK	32 42	1352 7776	14	1.8	451 934	18 14	3060	3 Chymo	P00586ITH	
VGPGL	02,42	1002,1110	1-7	1,0	-01,00-	10,14	0000	02.raw	TR BOVIN	
								mf18031	protein1 sp	
	55,76	2090,0796	19	5,5	697,7043	20,29	4627	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	55 59	002 4828	Q	0.8	407 2401	14 55	2862	MT18031	protein 1  sp	
	55,50	992,4020	0	0,0	497,2491	14,55	2002	02 raw	TR BOVIN	
								mf18031	protein1 sp	
HRAPPETW	44,51	992,4828	8	0,3	497,2488	14,04	2699	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	20.0	000 4000	0	0.7	407.05	45.07	2017	mf18031	protein1 sp	
HRAPPEIW	29,8	992,4828	8	2,7	497,25	15,07	3017	3_Cnymo	PUU586 TH	
								mf18031	protein1 sp	
HRAPPETW	21,04	992,4828	8	-1,7	497,2478	15,86	3253	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
LGTQPEPDA	FF 0.4	1000.000	40	4.0	004 0040	00.00	4700	mf18031	protein1 sp	
VGLDSGHIR	55,04	1860,933	18	4,8	621,3212	20,92	4788	3_Chymo		
									protein1lspl	
SPGTREARK	53,24	1292,6472	11	-0,1	647,3308	10,71	1648	3 Chymo	P00586 TH	
EY								_02.raw	TR_BOVIN	
SPGTREARK	_ /							mf18031	protein1 sp	
EY	51,79	1292,6472	11	0,1	431,889 <i>1</i>	10,73	1653	3_Chymo	P00586 TH	
								_02.raw	nrotein1len	
SPGTREARK	41,78	1292,6472	11	13,7	431,8956	10,21	1501	3 Chvmo	P00586ITH	
EY	, -	, <u>-</u>			,			_02.raw	TR_BOVIN	 
SPGTRFARK								mf18031	protein1 sp	 
EY	41,63	1292,6472	11	0,8	431,89	11,23	1795	3_Chymo	P00586 TH	
								_02.raw	IK_BUVIN	<b> </b>
SPGTREARK	16.09	1292.6472	11	0.7	431.89	12.11	2079	3 Chvmo	P00586ITH	
EY	.,	,=			. ,	,		_02.raw	TR_BOVIN	 

SPGTREARK	45.00	4000 0470		40.7	404 0050	10.00	00.40	mf18031	protein1 sp	
EY	15,86	1292,6472	11	13,7	431,8956	12,93	2340	3_Chymo		
									protein1 sp	
AGKVGPGLR	52,77	1065,6658	11	4,3	356,2307	18,54	4085	3_Chymo	P00586 TH	
VL								_02.raw	TR_BOVIN	
AGKVGPGLR								mf18031	protein1 sp	
VL	36,31	1065,6658	11	6	533,8434	18,48	4069	3_Chymo	P00586 1H	
								_02.raw	nrotein1lsnl	
NGGFRNW	52.61	849.3882	7	3.3	425,7028	17.44	3732	3 Chymo	P00586ITH	
	,- :			-,-	,	,		_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
NGGFRNW	47,31	849,3882	7	1,9	425,7022	21,75	5024	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
NGGERNW	46 52	849 3882	7	4.6	425 7033	19.32	4316	3 Chymo	P00586ITH	
	40,02	040,0002	,	4,0	420,7000	10,02	4010	02.raw	TR BOVIN	
								mf18031	protein1 sp	
NGGFRNW	44,8	849,3882	7	5,9	425,7039	18,78	4161	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	44.25	040 2002	7	170.0	105 775	17 75	2025	mf18031	protein1 sp	
NGGFRNW	44,35	849,3882	/	173,3	425,775	17,75	3835	3_Chymo		
									protein1lspl	
NGGFRNW	42,77	849,3882	7	2,9	425,7026	19,85	4482	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
NGGFRNW	41,31	849,3882	7	175,4	425,7759	18,27	4007	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
NGGERNW	40 11	849 3882	7	8	425 7048	22 26	5172	3 Chymo	P00586ITH	
	,				,	,		_02.raw	TR_BOVIN	
								mf18031	protein1 sp	
NGGFRNW	36,54	849,3882	7	7	425,7043	23,57	5536	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
NGGERNW	36 30	840 3882	7	6	425 7039	20.36	4645	3 Chymo	P00586ITH	
NOOLINIW	50,55	043,3002	'	0	425,7055	20,50	4043	02 raw	TR BOVIN	
								mf18031	protein1 sp	
NGGFRNW	33,03	849,3882	7	1,8	425,7021	24,91	5949	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	24.67	040 2002	7	4.4	405 7000	20.96	4770	mf18031	protein1 sp	
NGGERNW	24,07	049,3002	/	4,4	425,7052	20,00	4770	3_Chymlo 02 raw		
								mf18031	protein1 spl	
NGGFRNW	16,56	849,3882	7	7,8	425,7047	27,68	6730	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
			_			<u> </u>		mf18031	protein1 sp	
NGGFRNW	15,79	849,3882	1	5,7	425,7038	25,74	6222	3_Chymo		
									protein1lspl	
QLVDSRAQG	52,61	1291,6632	11	0,7	646,8394	13,77	2613	3 Chymo	P00586 TH	
Rĭ								_02.raw	TR_BOVIN	
QLVDSRAQG								mf18031	protein1 sp	
RY	47,35	1291,6632	11	0,7	431,562	13,77	2612	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
QLVDSRAQG	45.54	1291.6632	11	1.1	431.5622	14.79	2936	3 Chymo	P00586ITH	
RY	,	,		.,.	,	,		02.raw	TR BOVIN	
								mf18031	protein1 sp	
RY	44,73	1291,6632	11	1,6	431,5624	14,29	2779	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	<b> </b>
QLVDSRAQG	41 32	1291 6632	11	٨٩	646 8395	13 26	2450	3 Chymo	POOSSAITH	1
RY	11,02	1201,0002		0,0	0.0,0000	.0,20	2-100	02.raw	TR BOVIN	1
								mf18031	protein1 sp	
RY	41,2	1291,6632	11	0,4	431,5619	13,26	2449	3_Chymo	P00586 TH	1
								_02.raw	TR_BOVIN	<b> </b>
QLVDSRAQG	10.61	1201 6622	11	2	131 562	15.2	2002	mt18031	PO05861TL	1
RY	40,01	1231,0032		5	431,303	10,0	3083	02 raw		1
					1					L

QLVDSRAQG	25.26	1001 6620	11	4.4	424 5626	15.00	2244	mf18031	protein1 sp	
RY	35,30	1291,0032	11	4,4	431,0030	19,02	3241	3_Chymo 02.raw	TR BOVIN	
								mf18031	protein1 sp	
RY	21,31	1291,6632	11	1,3	646,8397	14,29	2780	3_Chymo	P00586 TH	
								_02.raw mf18031	protein1lspl	
	52,43	1490,7728	12	6,6	497,9348	23,52	5521	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
EQVLENLESK	20.2	1400 7728	12	3.0	407 0335	24.03	5678	mf18031	protein1 sp	
RF	29,2	1490,7720	12	5,9	497,9555	24,03	5078	02.raw	TR BOVIN	
EOVI ENI ESK								mf18031	protein1 sp	
RF	25,04	1490,7728	12	6,5	746,3985	23,52	5520	3_Chymo	P00586 TH	
								_02.raw mf18031	Drotein1lspl	
TSEPSRPEPA	51,6	1329,6564	12	7,2	665,8403	23,16	5420	3_Chymo	P00586 TH	
IF								_02.raw	TR_BOVIN	
TSEPSRPEPA	10.69	1320 6564	12	0.4	665 9352	22.66	5260	mf18031	protein1 sp	
IF	49,00	1329,0304	12	-0,4	005,0552	22,00	5209	02.raw	TR BOVIN	
								mf18031	protein1 sp	
GGF	50	1242,6469	12	3,6	415,2244	21,22	4871	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
YSPGTREAR	49,56	1455,7106	12	-0,1	486,2441	11,76	1967	3 Chymo	P00586 TH	
KEY								_02.raw	TR_BOVIN	
LAESVRAGK	40.00	4704 0044	47	1.0	574 005	00.70	5000	mf18031	protein1 sp	
VGPGLRVL	49,22	1721,0311	17	1,2	574,085	22,73	5288	3_Chymo 02 raw	TR BOVIN	
								mf18031	protein1 sp	
VGPGI RVI	17,15	1721,0311	17	9,1	574,6895	22,22	5159	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
GHRTVSVL	49.03	867,4926	8	1.4	434,7542	14.28	2778	3 Chymo	P00586ITH	
	- ,	,	-	,	- , -	, -	_	02.raw	TR_BOVIN	
	10.01	007 (000			404 7507	40 70	0015	mf18031	protein1 sp	
GHRIVSVL	48,04	867,4926	8	0,2	434,7537	13,78	2615	3_Chymo	P00586 TH	
								mf18031	protein1 sp	
GHRTVSVL	36,74	867,4926	8	61,5	434,7803	14,77	2928	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
GHRTVSVL	31.5	867,4926	8	1.6	434,7543	15.18	3051	3 Chymo	P00586ITH	
	- ,-	,	_	.,-	,	,		_02.raw	TR_BOVIN	
						10 -0		mf18031	protein1 sp	
KATLNRSLL	47,86	1014,6185	9	1,6	508,3174	16,52	3460	3_Chymo		
								mf18031	protein1 sp	
KATLNRSLL	39	1014,6185	9	1,8	508,3175	16,01	3302	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
KATI NRSI I	32 48	1014 6185	9	43	508 3187	17 03	3615	3 Chymo	P00586ITH	
	,	,	_	.,-	,	,		_02.raw	TR_BOVIN	
	o			1.0				mf18031	protein1 sp	
KATLNRSLL	24,7	1014,6185	9	1,2	508,3172	17,56	3776	3_Chymo	P00586 TH	
								mf18031	protein1 sp	
KATLNRSLL	20,14	1014,6185	9	1,3	508,3172	19,19	4274	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
KATI NRSI I	16 93	1014 6185	9	37	508 3184	18 65	4123	3 Chymo	P00586ITH	
	,	,	Ŭ	0,1		,		_02.raw	TR_BOVIN	
DALLYSTIC	4		_	- /	100	4- 4 -		mf18031	protein1 sp	
RALVSTKW	47,79	959,5552	8	5,1	480,7873	17,11	3639	3_Chymo	P00586 TH	
								mf18031	protein1lspl	
RALVSTKW	43,28	959,5552	8	5	480,7873	17,1	3637	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
RAIVSTKW	37 32	959 5552	8	14	480 7856	16 50	3480	mt18031	P00586ITH	
	01,02	000,0002		1,4	-00,7000	10,00	0-00	_02.raw	TR_BOVIN	

RALVSTKW         28.61         99.552         8         3.3         480.786         18.12         3961         3.0 mm         Processol in the processol in t	RALVSTKW	35,98	959,5552	8	1	480,7854	17,61	3791	mf18031 3_Chymo	protein1 sp  P00586 TH		
RALVSTKW         29.91         99.95552         8         3.3         480,7865         18.12         391         3.Chyme         PROWN         PROWN           RALVSTKW         17.93         99.95552         8         1.9         480,7855         19.73         4443         5.Chyme         PORSBOTH									_02.raw	TR_BOVIN		
Name         Disk         Disk <thdisk< th="">         Disk         Disk         <thd< td=""><td>RAI VSTKW</td><td>29.61</td><td>959 5552</td><td>8</td><td>33</td><td>480 7865</td><td>18 12</td><td>3961</td><td>3 Chymo</td><td>P00586ITH</td><td></td><td></td></thd<></thdisk<>	RAI VSTKW	29.61	959 5552	8	33	480 7865	18 12	3961	3 Chymo	P00586ITH		
RALVSTKW         17.33         959.552         8         1.9         480.785         19.73         4443         5.07mo         POSSBT14         Optimal TR BOVIN           VGSLGISNOT         47.46         1098,5305         11         3         550.2742         16.34         440.7858         1.77         4443         5.07mo         POSSBT14         Ozraw         R BOVIN           REKASPY         46.89         1472.5984         11         8.8         737.3129         16.67         3473         2.07mo         R BOVIN         NBF-C         C4/NBF-C         C100.00           NRSLLKTY         44.8         993.5607         8         0.7         497.788         14.74         2919         5.07mo         POSSBT14         Ozraw         R BOVIN           NRSLLKTY         44.8         993.5607         8         0.07         497.788         14.74         2919         5.07mo         POSSBT14         Ozraw         R BOVIN         Ozraw         R BOVIN         C4/NBF-C         C4/NBF-C         C6/NMP         POSSBT14         Ozraw         R BOVIN         Ozraw         R BOVIN </td <td>1012001100</td> <td>20,01</td> <td>000,0002</td> <td>Ũ</td> <td>0,0</td> <td>400,7000</td> <td>10,12</td> <td>0001</td> <td>02.raw</td> <td>TR BOVIN</td> <td></td> <td></td>	1012001100	20,01	000,0002	Ũ	0,0	400,7000	10,12	0001	02.raw	TR BOVIN		
RALVSTKW         17.33         959.5552         8         1.9         490.7563         19.73         4443         3_Chymo P005861TH 10017m TR_BOVIN           VGSLGINDT H         47.46         1098.5305         11         3         550.2742         16.34         4405         5_Chymo P005861TH 0.0 raw         CMIND 100131         CMIND 1001111111         C									mf18031	protein1 sp		
VGSLGISNO H         VGSLGISNO H <thvgslgisno H         <thvgslgisno H</thvgslgisno </thvgslgisno 	RALVSTKW	17,93	959,5552	8	1,9	480,7858	19,73	4443	3_Chymo	P00586 TH		
VGSLGISNDT H         47.46         1098.5305         11         3         550.2742         16.34         3405         3C/Mm         PO0288[H] D.2.7w1         NEP-C         C4.NBF- C.1000.00           RDKASPY         46.89         1472.5984         11         8.8         737,312         16.57         3473         3.07m1         PO0588[H] D.2.7w1         NEB-C         C4.NBF- C.1000.00           NRSLLKTY         44.8         993.5607         8         0.7         497.788         14.74         2919         3.07m0         P00588[H] D.2.7w1         NEB-CU         C4.NBF- C.1000.00           NRSLLKTY         43.4         993.5607         8         -100.4         497.738         14.98         2962         3.07m0         P00588[H] D.2.7w1         NEBOVIN           YAPRVW         42.28         790.4128         6         6.2         396.216         20.5         48813         3.07m0         P0058[H] P0058[H]         2.7w1         R.BOVIN									_02.raw	TR_BOVIN		
H         47.46         1098.5305         11         3         50.27/2         16.34         3405         3.Chymo         PODB8[H]           LEEC(+63.00) RDKASPY         46.89         1472.5864         11         8.8         737.3129         16.57         3473         3.Chymo         PODB8[H]         NBF-C         C4:NBF- C: 1000.00           NRSLLKTY         44.8         993.5607         8         0.7         497.788         14.74         2919         3.Chymo         POD688[H]         2.2         C1:00.00         76:00.00           NRSLLKTY         43.4         993.5607         8         -100.4         497.7378         14.98         2992         3.Chymo         POD688[H]         2.2         78:00H1         2.2         78:0058[H]         2.2         7	VGSLGISNDT								mf18031	protein1 sp		
LEC(+163.00 RDKASPY         48,89         1472.5984         11         8.8         737.3129         16.57         3473         3.0000         Tober 1801 Protein 1801 Protein 1801 Protein 1801         NBF-C         C4.NBF- C.1000.00           NRSLLKTY         44,8         993.5607         8         0.7         497.788         14,74         2919         3.Chymo         PD0588[TH D27aw         TR. BOVIN         2           NRSLLKTY         43.4         993.5607         8         -100.4         497.7378         14,98         2992         3.Chymo         PD0588[TH D27aw         TR. BOVIN           YAPRVW         42.28         790.4126         6         6.2         396.216         20.5         4681         3.Chymo         PD0588[TH D27aw         TR. BOVIN           YAPRVW         30.25         790.4126         6         6.2         396.216         20.5         4681         3.Chymo         PD0588[TH D27aw         TR. BOVIN           YAPRVW         30.65         790.4126         6         3.1         386.2148         21.03         4824         3.Chymo         PD0588[TH D27aw         TR. BOVIN           YAPRVW         36.8         110.2739         14         602.3075         21.11         4844         3.Chymo         PD0588[TH	н	47,46	1098,5305	11	3	550,2742	16,34	3405	3_Chymo	P00586 1H		
LEEC(+163.00) RDKASPY         46,89         1472,5984         11         8,8         737,3129         16,57         3473         3Comme         Propression (1000)         NBF-C         C4:NBF- C: 1000.00           NRSLLKTY         44,8         993,5607         8         0.7         497,788         14.74         2919         3Chyme         Propression (11001)         NBF-C         C4:NBF- C: 1000.00           NRSLLKTY         43.4         993,5607         8         -100.4         497,7738         14.98         2992         3Chyme         Propossinth									_02.raw	TR_BOVIN		
RDKASEY         Note         Note         Note         Note         Note         Note         Condect         Note         Condect         Note         Condect         Note         Condect         Condect         Note         Condect         Condect         Note         Condect         Condect <thcondet< th="">         Condect         <thcondet< t<="" td=""><td>IEEC(+163.00)</td><td>46 89</td><td>1472 5984</td><td>11</td><td>8.8</td><td>737 3129</td><td>16.57</td><td>3473</td><td>3 Chymo</td><td>P00586ITH</td><td>NBE-C</td><td>C4:NBF-</td></thcondet<></thcondet<>	IEEC(+163.00)	46 89	1472 5984	11	8.8	737 3129	16.57	3473	3 Chymo	P00586ITH	NBE-C	C4:NBF-
NRSLLKTY         44.8         993,5607         8         0.7         497,786         14.74         2919         0.718031         potent ispl 0.2raw         potent ispl mf8031           NRSLLKTY         43.4         993,5607         8         -100.4         497,7378         14.98         2992         3. Chymo P005861TH 0.2raw         TR BOVIN           YAPRVW         42.28         790.4126         6         4.7         396.216         19.99         4527         3.Chymo P005861TH 0.2raw         TR BOVIN           YAPRVW         30.25         790.4126         6         6.2         396.216         20.5         4681         3.Chymo P005861TH 0.2raw         TR BOVIN           YAPRVW         16.66         790.4126         6         3.1         396.214         21.03         4824         3.Chymo P005861TH 0.2raw         TR BOVIN           YAPRVW         16.66         790.4126         6         3.1         396.214         21.03         4824         3.Chymo P005861TH 0.2raw         TR BOVIN           YAPRVW         39.8         1202.5931         11         6.1         602,3075         21.11         4842         3.Chymo P005861TH 0.2raw         TR BOVIN           GISNDTHVW         39.88         813.4286         6	RDKASPY	10,00	1112,0001		0,0	101,0120	10,01	0110	02.raw	TR BOVIN		C:1000.00
NRSLLKTY         44.8         993.5607         8         0.7         497.788         14.74         2919         3.Chymo P00580TH D.2 raw TR BOVIN           NRSLLKTY         43.4         993.5607         8         -100.4         497.7378         14.98         2992         3.Chymo P00580TH D.2 raw TR BOVIN           YAPRVW         42.28         790.4126         6         4.7         396.216         19.99         4527         3.Chymo P00580TH D.2 raw TR BOVIN           YAPRVW         30.25         790.4126         6         6.2         396.216         20.5         4681         7163031 proteinTispi D.2 raw TR BOVIN           YAPRVW         16.66         790.4126         6         3.1         396.216         20.5         4681         717807H         78.00TH           GISNDTHVVV V         16.66         790.4128         6         3.1         396.216         21.91         4842         3.Chymo P00580TH           GISNDTHVVV V         38.8         1202.5031         11         6.1         602.3075         21.11         4844         3.Chymo P00580TH           GISNDTHVVV V         15.54         1202.5031         11         6.1         602.3075         21.11         4844         3.Chymo P00580TH         27.80 Chymo P00580TH									mf18031	protein1 sp		
NRSLLKTY         43.4         993.5607         8         -100.4         497.7378         14.98         2992         3.Ctymo P00580[TH D02 raw         TR BOVIN           YAPRVW         42.28         790.4126         6         4.77         396.215         19.99         4527         3.Ctymo P00580[TH D02 raw         TR BOVIN           YAPRVW         30.25         790.4126         6         6.2.         396.216         20.5         4661         3.Ctymo P00580[TH D02 raw         TR BOVIN           YAPRVW         16.66         790.4126         6         6.2.         396.216         21.03         4824         3.Ctymo P00580[TH D02 raw         TR BOVIN           YAPRVW         16.66         790.4126         6         3.1         396.2148         21.03         4824         3.Ctymo P00580[TH D2 raw         TR BOVIN           LERHVPGAS F         41.71         1111.5774         10         2.5         556.7974         15.97         3288         3.Ctymo P00580[TH D02 raw         TR BOVIN           GISNDTHVVV Y         39.8         813.4286         6         9.6         407.7233         26.87         6550         3.Ctymo P00580[TH D2 raw         TR BOVIN           APRVWW         32.83         813.4286         6         9.6	NRSLLKTY	44,8	993,5607	8	0,7	497,788	14,74	2919	3_Chymo	P00586 TH		
NRSLLKTY         43.4         993.5607         8         -100.4         497.7378         14.98         299         mf18031 protein fispl 3Chymo P00580TH 0.02 raw         P00580TH TR.BOVIN           YAPRVW         30.25         790.4126         6         4.7         396.2154         19.99         4527         3Chymo P00580TH 0.02 raw         TR.BOVIN           YAPRVW         30.25         790.4126         6         6.2         396.216         20.5         4881         mf18031 protein fispl 0.02 raw         TR.BOVIN           YAPRVW         10.66         790.4126         6         31         396.2148         21.03         482         3Chymo P00580TH 0.02 raw         TR.BOVIN           LERHVPGAS         41.71         1111.5774         10         2.5         556.7974         15.97         3288         3Chymo P00580TH 0.02 raw         TR.BOVIN           V         39.8         1202.5931         11         6.1         602.3065         21.11         4844         3Chymo P00580TH 0.02 raw         7BOVIN           V         15.54         1202.5931         11         6.1         602.3075         21.11         4844         3Chymo P00580TH 0.02 raw         7BOVIN           APRVWW         39.8         813.4286 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>_02.raw</td><td>TR_BOVIN</td><td></td><td></td></t<>									_02.raw	TR_BOVIN		
NRSLLKY         43,4         993,5807         8         -100,4         497,738         14,98         2992         3_Chymo         POORSITH           YAPRVW         42,28         790,4126         6         4,7         396,2164         19,99         427         3_Chymo         POORSITH         02,raw         TR_BOVIN           YAPRVW         30,25         790,4126         6         6,2         396,216         20,5         4681         3_Chymo         POORSITH         02,raw         TR_BOVIN           YAPRVW         16,66         790,4126         6         3,1         396,2148         21,03         4861         3_Chymo         POORSITH         02,raw         TR_BOVIN           YAPRVW         16,66         790,4126         6         3,1         396,2148         21,03         4823         3_Chymo         POORSITH         02,raw         TR_BOVIN           GISNDTHVVV         39,8         1202,5931         11         4         602,3063         21,61         4984         3_Chymo         POORSITH         02,raw         TR_BOVIN           GISNDTHVVV         39,58         13,4286         6         4,3         407,723         26,87         6503         3_Chymo         POORSITH         02,raw <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td>mf18031</td> <td>protein1 sp </td> <td></td> <td></td>									mf18031	protein1 sp		
YAPRVW         42,28         790,4126         6         4.7         396,215         19.99         4527         711,000         PODERITISP PODERITISP           YAPRVW         30,25         790,4126         6         6.2         396,216         19.99         4527         716,033         protein1sp           YAPRVW         30,25         790,4126         6         6.2         396,216         20.5         4681         3_Chymo         PODERS[TH 02_zwr         TR_BOVIN           YAPRVW         16,66         790,4126         6         3.1         396,2148         21.03         4824         3_Chymo         PODERS[TH 02_zwr         TR_BOVIN           YAPRVW         16,66         790,4126         6         3.1         396,2148         21.03         4824         3_Chymo         PODERS[TH 02_zwr         TR_BOVIN           LERHVPGAS         41.71         1111,5774         10         2.5         566,7974         15.97         3288         3_Chymo         PODERS[TH 02_zwr         TR_BOVIN           GISNDTHVVV Y         39,8         1202,5931         111         6,1         602,3075         21,11         4844         3_Chymo         PODESS[TH 02_zwr         TR_BOVIN           APRVWW         39,88         813,	NRSLLKTY	43,4	993,5607	8	-100,4	497,7378	14,98	2992	3_Chymo	P00586 TH		
YAPRVW         42,28         790,4128         6         4,7         396,2154         19,99         4527         3_C,1ym         P00536[TH]           YAPRVW         30,25         790,4128         6         6,2         36,218         20,5         4681         3_C,1ym         P00586[TH]           YAPRVW         16,66         790,4128         6         3,1         396,2148         21,03         4624         3_C,1ym         P00586[TH]           YAPRVW         16,66         790,4128         6         3,1         396,2148         21,03         4624         3_C,1ym         P00586[TH]           YAPRVW         16,66         790,4128         11         4         602,3063         21,81         4986         3_C,1ym         P00586[TH]         0_Z,aw         TR BOVIN           GISNDTHVVV Y         39,8         1202,5931         111         6,1         602,3075         21,81         4986         3_C,1ym         P00586[TH]         0_Z,aw         TR BOVIN           GISNDTHVVV Y         39,58         813,4286         6         4,07,723         26,87         6533         3_C,1ym         P00586[TH]         0_Z,aw         TR BOVIN           APRVWW         32,83         813,4286         6									_02.raw	TR_BOVIN		
MINUN         Rue         Josh Ro         Sol Ro <td></td> <td>42.28</td> <td>790 4126</td> <td>6</td> <td>47</td> <td>306 2154</td> <td>10 00</td> <td>4527</td> <td>3 Chymo</td> <td>PO0586ITH</td> <td></td> <td></td>		42.28	790 4126	6	47	306 2154	10 00	4527	3 Chymo	PO0586ITH		
YAPRVW         30,25         790,4126         6         6,2         396,216         20,5         4681         3_Crymo         P00586[TH]           YAPRVW         16,66         790,4126         6         3,1         396,216         20,5         4681         3_Crymo         P00586[TH]           VAPRVW         16,66         790,4126         6         3,1         396,2148         21,03         4824         3_Crymo         P00586[TH]           LERHVPGAS         41,71         1111.5774         10         2,5         556,7974         15.97         32.88         3_Crymo         P00586[TH]           GISNDTHVVV         39,8         1202,5931         11         4         602,3075         21,11         4844         3_Crymo         P00586[TH]           Q2 raw         TR BOVIN         mf18031         protein1spi         02 raw         TR BOVIN           MIREX         1202,5931         11         6,1         602,3075         21,11         4844         3_Crymo         P00586[TH]           Q2 raw         TR BOVIN         mf18031         protein1spi         02 raw         TR BOVIN           MRVWW         39,58         813,4286         6         3,5         407,7255         27,38         <		72,20	750,4120	0	ч, г	000,2104	10,00	4521	02 raw	TR BOVIN		
YAPRVW         30,25         790,4126         6         6.2         396,216         20.5         4681         3_C Hymo         Protein Tispi protein Tispi S_C Hymo         Protein Tispi Protein Tispi S_C Hymo         Protein Tispi Protein Tispi TR_BOVIN           LERHVPGAS F         41.71         1111.5774         10         2.5         556,7974         15.97         3288         5_C Hymo         P005861TH 									mf18031	protein1 sp		
VAPRVW         16,6         790,4126         6         3,1         396,2148         21,03         4824         3,Chyme         Podem11spl poten1spl 3,Chyme         Method 1         proten1spl poten1spl 3,Chyme         Method 1         Podem1spl 3,Chyme         Method 1	YAPRVW	30,25	790,4126	6	6,2	396,216	20,5	4681	3 Chymo	P00586 TH		
YAPRVW         16.66         790,4126         66         3,1         396,2148         21,03         4824         mf18031 (30,0)         rotein1isp (30,0)         rotein1isp (30,0) <throtein1isp (30,0)         rotein1isp (30,0)</throtein1isp 									_02.raw	TR_BOVIN		
YAPRVW         16.66         790.4126         6         3.1         396.2148         21.03         4824         3.Chym Po0586[TH D2:raw TR BOVIN           LERHVPGAS F         41.71         1111.5774         10         2.5         556.7974         15.97         3288         3.Chym Po0586[TH D2:raw TR BOVIN           GISNDTHVVV Y         39.8         1202.5931         11         4         602.3063         21.61         4986         3.Chym Po0586[TH D2:raw TR BOVIN           GISNDTHVVV Y         15.54         1202.5931         11         6.1         602.3075         21.11         4844         3.Chym Po0586[TH D2:raw TR BOVIN           APRVWW         39.58         813.4286         6         4.3         407.7233         26.87         6503         3.Chym Po0586[TH D2:raw TR BOVIN           APRVWW         33.88         813.4286         6         9.6         407.723         26.87         6503         3.Chym Po0586[TH D2:raw TR BOVIN           APRVWW         33.88         813.4286         6         9.6         407.723         26.86         6300         3.Chym Po0586[TH D2:raw TR BOVIN           APRVWW         32.83         813.4286         6         3.5         407.723         26.36         6380         3.Chym Po0586[TH D2:raw TR BOVIN									mf18031	protein1 sp		
LERHVPGAS F         41,71         1111,5774         10         2,5         556,7974         15,97         32.88         3,C1ymo 0,2 raw TR BOVIN         TR BOVIN           GISNDTHVVV Y         39.8         1202,5931         111         4         602,3063         21,61         4986         3,Chymo 0,2 raw TR BOVIN         Polos66[TH 02 raw TR BOVIN           GISNDTHVVV Y         15,54         1202,5931         111         6,1         602,3075         21,11         4444         mf18031 motein1ispl 02 raw TR BOVIN           APRVWW         39,88         813,4286         6         4,3         407,7253         27,38         6658         3,Chymo 900566[TH         7         7           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3,Chymo 900566[TH         7         7         7         26,36         6300         3,Chymo 900566[TH         7         7         7         26,36         6300         3,Chymo 900566[TH         7         7         1         8,931         9743         3,Chymo 900566[TH         7         7         1         8,931         9743         3,Chymo 900566[TH         7         7         1         8,931         9743         3,Chymo 900566[TH         7 <td>YAPRVW</td> <td>16,66</td> <td>790,4126</td> <td>6</td> <td>3,1</td> <td>396,2148</td> <td>21,03</td> <td>4824</td> <td>3_Chymo</td> <td>P00586 TH</td> <td></td> <td></td>	YAPRVW	16,66	790,4126	6	3,1	396,2148	21,03	4824	3_Chymo	P00586 TH		
LERHVPGAS F         41.71         1111.5774         10         2.5         556,7974         15.97         3288         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           GISNDTHVVV Y         39,8         1202,5931         11         4         602,3063         21.61         4986         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           GISNDTHVVV Y         15.54         1202,5931         11         6.1         602,3075         21.11         4844         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           APRVWW         39,58         813,4286         6         4.3         407,7233         26.87         6530         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           APRVWW         33,88         813,4286         6         9.6         407,7235         27.38         66530         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           APRVWW         32.83         813,4286         6         3.5         407,723         26.87         6530         3_Chymo         PO0586[TH U.2, rw         TR BOVIN           EVMLPSEAG FADY         39.4         1427,6278         13         5,7         714,8253         39,31         9743         3_Chymo         P00586[TH U.2, rw         TR BOVIN									_02.raw	TR_BOVIN		
F         41,71         111,374         10         2,3         336,7374         15,97         3268         3_2,7100         TR_BOVIN           GISNDTHVVV Y         39,8         1202,5931         11         4         602,3063         21,61         4986         3_2,7100         7R_BOVIN           GISNDTHVVV Y         15,54         1202,5931         11         6,1         602,3063         21,61         4986         3_2,7100         7C,7000         P005861TH           GISNDTHVVV Y         15,54         1202,5931         11         6,1         602,3075         21,11         4844         3_2,7100         7C,7000         P005861TH           APRVWW         39,58         813,4286         6         4,3         407,7255         27,38         66530         3_2,7000         P005861TH           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3_2,7000         P005861TH           APRVWW         32,83         813,4286         6         3,5         714,823         39,31         9743         3_2,7000         P005861TH           EVMLPSEAG         39,4         1427,6278         13         5,7         714,823         39,33         974	LERHVPGAS	41 71	1111 5774	10	25	EEC 7074	15.07	2200	mf18031	protein1 sp		
GISNDTHVW Y         39,8         1202,5931         11         4         602,3063         21,61         4986         3_Chymo P00586[TH D2_raw         TR_BOVIN           GISNDTHVW Y         15,54         1202,5931         11         6,1         602,3075         21,11         4844         3_Chymo P00586[TH D2_raw         TR_BOVIN           APRVWW         39,58         813,4286         6         4,3         407,7233         26,87         6530         3_Chymo P00586[TH D2_raw         TR_BOVIN           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3_Chymo P00586[TH D2_raw         TR_BOVIN           APRVWW         32,83         813,4286         6         3,5         407,725         26,36         6380         3_Chymo P00586[TH D2_raw         TR_BOVIN           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8253         39,31         9743         3_Chymo P00586[TH D2_raw         TR_BOVIN           BVMLPSEAG FADY         30,61         1415,6616         13         1,5         714,8253         39,31         9743         3_Chymo P00586[TH D2_raw         7R_BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13	F	41,71	1111,5774	10	2,5	550,7974	15,97	3200	02 raw			
GISNDTHVVV Y         39.8         1202,5931         11         4         602,3063         21.61         4986         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           GISNDTHVVV Y         15.54         1202,5931         11         6.1         602,3075         21.11         4844         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           APRVWW         39,58         813,4286         6         4.3         407,7233         26.87         6530         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           APRVWW         33,88         813,4286         6         9.6         407,7255         27,38         6658         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           APRVWW         32.83         813,4286         6         9.6         407,725         27,38         6658         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           EVMLPSEAG         39.4         1427,6278         13         5,7         714,8253         39,31         9743         3_Chymo         P00586[Th 0.2 raw         TR         BOVIN           EVMLPSEAG FADY         30,61         1415,6616         13         19,3         472,9036         23,35 <t< td=""><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td>protein1lspl</td><td></td><td></td></t<>										protein1lspl		
Y         IAB	GISNDTHVVV	39.8	1202.5931	11	4	602.3063	21.61	4986	3 Chymo	P00586ITH		
GISNDTHVVV Y         15,54         1202,5931         11         6,1         602,3075         21,11         4844         3, Chymo 3, Chymo 02,raw         TR BOVIN           APRVWW         39,58         813,4286         6         4,3         407,7233         26,87         6530         3, Chymo 02,raw         TR BOVIN           APRVWW         33,88         813,4286         6         9,6         407,7235         27,38         6658         3, Chymo 02,raw         TR BOVIN           APRVWW         32,88         813,4286         6         9,6         407,7255         27,38         6658         3, Chymo 02,raw         TR BOVIN           APRVWW         32,83         813,4286         6         9,6         407,723         26,36         6380         3, Chymo 02,raw         TR BOVIN           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3, Chymo 02,raw         TR BOVIN           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8223         39,83         9890         3, Chymo 02,raw         TR BOVIN           DSGHIRGSV NMFF         38,78         1415,6616         13         19,3         472,9036         23,35	Y	, -	- ,			,	, -		02.raw	TR BOVIN		
SISINITIV Y         15,54         1202,5931         11         6,1         602,3075         21,11         4844         3, Chymo         P00586[TH O2:raw         TR         BOVIN           APRVWW         39,58         813,4286         6         4,3         407,7233         26,87         6530         3, Chymo         P00586[TH O2:raw         TR         BOVIN           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3, Chymo         P00586[TH O2:raw         TR         BOVIN           APRVWW         32,83         813,4286         6         9,6         407,723         26,36         6380         3, Chymo         P00586[TH O2:raw         TR         BOVIN           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3, Chymo         P00586[TH O2:raw         TR         BOVIN           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8253         39,31         9743         3, Chymo         P00586[TH O2:raw         TR         BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         1,5         714,8253         39,83         9									mf18031	protein1 sp		
APRVWW         39,58         813,4286         6         4,3         407,7233         26,87         6530         3_Chymo         P00586[TH           APRVWW         33,88         813,4286         6         9,6         407,725         27,38         6658         3_Chymo         P00586[TH         0.2,raw         TR         BOVIN           APRVWW         33,88         813,4286         6         9,6         407,725         27,38         6658         3_Chymo         P00586[TH         0.2,raw         TR         BOVIN	V	15,54	1202,5931	11	6,1	602,3075	21,11	4844	3_Chymo	P00586 TH		
APRVWW         39,58         813,4286         6         4,3         407,7233         26,87         6530         3_CNm0         Potent [sp]           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3_CNm0         Potent [sp]           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3_CNm0         Potent [sp]         mf18031         proteint [sp]         mf18031         moteint [sp]         m	•								_02.raw	TR_BOVIN		
APRVWW         39,38         813,4268         6         4,3         407,7233         26,87         6530         3_C1rw         TR <bovin< th="">           APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3_C1rw         TR<bovin< td="">           APRVWW         32,83         813,4286         6         9,6         407,7255         27,38         6658         3_C1rym         P00586[TH           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6658         3_C1rym         P00586[TH           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3_C1rym         P00586[TH           Common April 1427,6278         13         5,7         714,8253         39,31         9743         3_Chrym         P00586[TH           Common April 1427,6278         13         1,5         714,8223         39,83         9890         3_Chrym         P00586[TH           DSGHIRGSV         36,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chrym         P00586[TH           DSGHIRGSV         30,61         &lt;</bovin<></bovin<>		20 50	040 4000	<u> </u>	4.0	407 7000	00.07	0500	mf18031	protein1 sp		
APRVWW         33,88         813,4286         6         9,6         407,725         27,38         6658         3_Chymo         P00586[TH D02,raw         TR         BOVIN           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3_Chymo         P00586[TH D02,raw         TR         BOVIN           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3_Chymo         P00586[TH D02,raw         TR         BOVIN           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,823         39,31         9743         3_Chymo         P00586[TH D2,raw         TR         BOVIN           EVMLPSEAG FADY         37,9         1427,6278         13         1,5         714,8223         39,83         9890         3_Chymo         P00586[TH D2,raw         TR         BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo         P00586[TH D2,raw         TR         BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13         4,7         472,8967         23,88	APRVVVV	39,58	813,4280	0	4,3	407,7233	20,87	6530	3_Chymo			
APRVWW         33,88         813,4286         6         9,6         407,7255         27,38         6658         3.Chymo         P00586[TH]           APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3.Chymo         P00586[TH]           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8253         39,31         9743         3.Chymo         P00586[TH]           EVMLPSEAG FADY         37,9         1427,6278         13         5,7         714,8223         39,83         9890         3.Chymo         P00586[TH]           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3.Chymo         P00586[TH]           DSGHIRGSV NMPF         38,61         1415,6616         13         4,7         472,8967         23,88         5628         5.Chymo         P00586[TH]           DSGHIRGSV NMPF         36,78         801,4596         7         0,2         401,7372         11,72         35,53         8767         3.Chymo P00586[TH]         0.2raw         TR BOVIN           EAKKVDL         36,78         801,4596         7         0,2         401,7372									_02.1aw mf18031	protein1lspl		
APRVWW         32.83         813.4286         6         3.5         407.723         26,36         6380         3.Chymo         PO0586[TH         PO0586[TH           EVMLPSEAG FADY         39,4         1427,6278         13         5.7         714,8253         39,31         9743         3.Chymo         P00586[TH         02.raw         TR BOVIN         P00586[TH         P0058	APRVWW	33.88	813.4286	6	9.6	407.7255	27.38	6658	3 Chymo	P00586ITH		
APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         mf18031 3_Chymo         protein1 spl P00586 TH           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8253         39,31         9743         3_Chymo         P00586 TH           EVMLPSEAG FADY         37,9         1427,6278         13         5,7         714,8223         39,83         9890         3_Chymo         P00586 TH         02.raw         TR BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         1,5         714,8223         39,83         9890         3_Chymo         P00586 TH         02.raw         TR BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo         P00586 TH         02.raw         TR BOVIN           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo         P00586 TH         02.raw         TR BOVIN           EAKKVDL         36,78         801,4596         7         0,2         401,7372         11,72         1951         3_Chymo         P00586 TH </td <td></td> <td>,</td> <td>,</td> <td>-</td> <td>- , -</td> <td>- ,</td> <td>,</td> <td></td> <td>02.raw</td> <td>TR BOVIN</td> <td></td> <td></td>		,	,	-	- , -	- ,	,		02.raw	TR BOVIN		
APRVWW         32,83         813,4286         6         3,5         407,723         26,36         6380         3_Chymo         P00586 TH           EVMLPSEAG FADY         39,4         1427,6278         13         5,7         714,8253         39,31         9743         3_Chymo         P00586 TH           EVMLPSEAG FADY         37,9         1427,6278         13         5,7         714,8223         39,83         9890         3_Chymo         P00586 TH         02.raw         TR BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         1,5         714,8223         39,83         9890         3_Chymo         P00586 TH         02.raw         TR BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo         P00586 TH         02.raw         TR BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13         4,7         472,8967         23,88         5628         3_Chymo         P00586 TH         02.raw         TR BOVIN           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo         P00586 TH									mf18031	protein1 sp		
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	APRVWW	32,83	813,4286	6	3,5	407,723	26,36	6380	3_Chymo	P00586 TH		
EVMLPSEAG FADY       39,4       1427,6278       13       5,7       714,8253       39,31       9743       3_Chymo 02_raw       P00586[TH 02_raw									_02.raw	TR_BOVIN		
FADY       39,4       1427,6278       13       5,7       714,6233       39,31       9743       3_Chymb Pubseling       Pubseling         EVMLPSEAG FADY       37,9       1427,6278       13       1,5       714,8223       39,83       9890       3_Chymb Pubseling       716,0131       protein1 spl 02.raw       TR BOVIN         DSGHIRGSV NMPF       38,78       1415,6616       13       19,3       472,9036       23,35       5474       3_Chymb Pubseling       718,031       protein1 spl 02.raw       7R BOVIN         DSGHIRGSV NMPF       30,61       1415,6616       13       4,7       472,8967       23,88       5628       3_Chymb Pubselin1 spl 02.raw       7R BOVIN         MNFLTEDGF EKSPEEL       38,17       1884,8451       16       6,2       943,4357       35,53       8767       3_Chymb Pubselin1 spl 02.raw       7R BOVIN         MNFLTEDGF EKSPEEL       38,17       1884,8451       16       6,2       943,4357       35,53       8767       3_Chymb Pubselin1 spl 02.raw       7R BOVIN         EAKKVDL       36,78       801,4596       7       0,2       401,7372       11,72       1951       3_Chymb Pubselin1 spl 02.raw       7R BOVIN         RSLLKTY       36,04       879,5178       7       1,9<	EVMLPSEAG	20.4	1407 6079	10	F 7	714 0050	20.24	0742	mf18031	protein1 sp		
EVMLPSEAG FADY         37,9         1427,6278         13         1,5         714,8223         39,83         9890         3_Chymo 02.raw         P00586[TH TR BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo 02.raw         P00586[TH TR BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo 02.raw         P00586[TH TR BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13         4,7         472,8967         23,88         5628         3_Chymo 02.raw         P00586[TH TR BOVIN           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo 02.raw         P00586[TH TR BOVIN           EAKKVDL         36,78         801,4596         7         0,2         401,7372         11,72         1951         3_Chymo 02.raw         TR BOVIN           RSLLKTY         36,04         879,5178         7         1,9         440,767         14,53         2856         3_Chymo 0.2.raw         TR BOVIN           DGDDLGSFY         35,88         987,3821         9	FADY	39,4	1427,0270	15	Э, <i>1</i>	/ 14,0200	39,31	9743	3_Chymo			
EVMLPSEAG FADY         37,9         1427,6278         13         1,5         714,8223         39,83         9890         3_Chymo P00586[TH O2.raw         P00586[TH R BOVIN           DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo 3_Chymo P00586[TH O2.raw         P00586[TH R BOVIN           DSGHIRGSV NMPF         30,61         1415,6616         13         4,7         472,8967         23,88         5628         3_Chymo P00586[TH O2.raw         TR BOVIN           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo P00586[TH O2.raw         TR BOVIN           EAKKVDL         36,78         801,4596         7         0,2         401,7372         11,72         1951         3_Chymo P00586[TH O2.raw         TR_BOVIN           RSLLKTY         36,04         879,5178         7         0,2         401,7372         11,72         1951         3_Chymo P00586[TH O2.raw         P00586[TH P									mf18031	protein1lspl		
FADY         Image: Constraint of the second se	EVMLPSEAG	37,9	1427,6278	13	1,5	714,8223	39,83	9890	3 Chvmo	P00586ITH		
DSGHIRGSV NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         mf18031 3_Chymo         protein1 sp  P00586 TH 02.raw         mf18031	FADY								_02.raw	TR_BOVIN		
NMPF         38,78         1415,6616         13         19,3         472,9036         23,35         5474         3_Chymo         P00586 TH           DSGHIRGSV NMPF         30,61         1415,6616         13         4,7         472,8967         23,88         5628         3_Chymo         P00586 TH           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo         P00586 TH           MNFLTEDGF EKSPEEL         36,78         801,4596         7         0,2         401,7372         35,53         8767         3_Chymo         P00586 TH           22.raw         TR_BOVIN         11884,8451         16         6,2         943,4357         35,53         8767         3_Chymo         P00586 TH           02.raw         TR_BOVIN         11,72         11,72         1951         3_Chymo         P00586 TH         02.raw         TR_BOVIN           EAKKVDL         36,04         879,5178         7         0,2         401,7372         11,72         1951         3_Chymo         P00586 TH         02.raw         TR_BOVIN           RSLLKTY         36,04         879,5178         7         1,9         440,767         14,53	DSGHIRGSV								mf18031	protein1 sp		
$ \begin{array}{c ccccccccccccccccccccccccccccccccccc$	NMPF	38,78	1415,6616	13	19,3	472,9036	23,35	5474	3_Chymo	P00586 TH		
DSGHIRGSV NMPF       30,61       1415,6616       13       4,7       472,8967       23,88       5628       3_Chymo       P00586 TH         MNFLTEDGF EKSPEEL       38,17       1884,8451       16       6,2       943,4357       35,53       8767       3_Chymo       P00586 TH       10         EAKKVDL       36,78       801,4596       7       0,2       401,7372       11,72       1951       3_Chymo       P00586 TH       10									_02.raw	TR_BOVIN		
NMPF         30,01         1413,0010         13         4,7         412,0007         23,60         30,20         3_010         P00380[1H]           MNFLTEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo         P00586[TH]           MNFLTEDGF EKSPEEL         36,78         801,4596         7         0,2         401,7372         11,72         1951         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7         0,2         401,7372         11,72         1951         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7         0,2         401,7372         11,72         1951         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7         0,2         401,7372         11,72         1951         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7         1,9         440,767         14,53         2856         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7         1,9         440,767         14,53         2856         3_Chymo         P00586[TH]           202.raw         TR_BOVIN         7	DSGHIRGSV	30 61	1/15 6616	12	47	172 2067	22.00	5620	11118031			
MNFLTEDGF EKSPEEL       38,17       1884,8451       16       6,2       943,4357       35,53       8767       3_Chymo 3_Chymo -02_raw       TR b0VIN       MR b01 sp  Protein1 sp  _02_raw       mr b0586 TH _BOVIN         EAKKVDL       36,78       801,4596       7       0,2       401,7372       11,72       1951       3_Chymo _02_raw       TR BOVIN       16         RSLLKTY       36,04       879,5178       7       1,9       440,767       14,53       2856       3_Chymo _02_raw       P00586 TH _02_raw       1900586 TH _02_raw       11         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo _02_raw       P00586 TH _02_raw       18031       protein1 sp  _02_raw       18031       protein1 sp  _02_raw       18031       18	NMPF	30,01	1415,0010	15	4,7	472,0907	23,00	5020	02 raw			
MINFLIEDGF EKSPEEL         38,17         1884,8451         16         6,2         943,4357         35,53         8767         3_Chymo 3_Chymo         P00586[TH P00586[TH _02.raw         TR_BOVIN           EAKKVDL         36,78         801,4596         7         0,2         401,7372         11,72         1951         3_Chymo _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.raw         P00586[TH _02.									mf18031	protein1lspl		
EKSPEEL       Image: Constraint of the image: Constraint	MNFLTEDGF	38,17	1884,8451	16	6,2	943,4357	35,53	8767	3 Chymo	P00586 TH		
EAKKVDL       36,78       801,4596       7       0,2       401,7372       11,72       1951       mf18031 3_Chymo D2.raw       protein1 sp  P00586 TH _02.raw       protein1 sp  TR_BOVIN         RSLLKTY       36,04       879,5178       7       1,9       440,767       14,53       2856       3_Chymo D2.raw       protein1 sp  TR_BOVIN         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo D2.raw       protein1 sp  TR_BOVIN	ENSPEEL								_02.raw	TR_BOVIN		
EAKKVDL       36,78       801,4596       7       0,2       401,7372       11,72       1951       3_Chymo       P00586 TH         RSLLKTY       36,04       879,5178       7       1,9       440,767       14,53       2856       3_Chymo       P00586 TH         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo       P00586 TH         02.raw       TR_BOVIN       14,53       2856       3_Chymo       P00586 TH       14,53         0.00000000000000000000000000000000000									mf18031	protein1 sp		
RSLLKTY       36,04       879,5178       7       1,9       440,767       14,53       2856       mf18031       protein1 sp          DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo       P00586 TH         02.raw       TR_BOVIN       14,53       2856       3_Chymo       P00586 TH       02.raw       TR_BOVIN	EAKKVDL	36,78	801,4596	7	0,2	401,7372	11,72	1951	3_Chymo	P00586 TH		
RSLLKTY       36,04       879,5178       7       1,9       440,767       14,53       2856       3_Chymo       P00586 TH         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo       P00586 TH         02.raw       TR_BOVIN       mf18031       protein1 sp        mf18031       protein1 sp          02.raw       TR_BOVIN       mf18031       protein1 sp        mf18031       protein1 sp          02.raw       TR_BOVIN       mf18031       protein1 sp        mf18031       protein1 sp									_02.raw	TR_BOVIN		
NOLLINIT       30,04       079,5178       7       1,9       440,707       14,93       2050       3_Chymio       P00586[1H]         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo       P00586[TH]         DGDDLGSFY       35,88       987,3821       9       2,8       494,6997       37,01       9140       3_Chymo       P00586[TH]         02.raw       TR_BOVIN       02.raw       TR_BOVIN       14,957       <	DOLLATY	36.04	870 5170	7	10	110 767	11 52	2856	mt18031	protein1 sp		
DGDDLGSFY         35,88         987,3821         9         2,8         494,6997         37,01         9140         3_Chymo         P00586 TH	ROLLNII	30,04	019,3110	/	1,9	440,707	14,00	2000	02 raw			
DGDDLGSFY         35,88         987,3821         9         2,8         494,6997         37,01         9140         3_Chymo         P00586 TH									mf18031	protein1lsnl		
_02.raw TR_BOVIN	DGDDLGSFY	35,88	987,3821	9	2,8	494,6997	37,01	9140	3_Chymo	P00586ITH		
		<u> </u>			, 		·		_02.raw	TR_BOVIN		

	33 93	987 3821	q	17	494 6992	37 52	9282	mf18031	protein1 sp	
DODDEGGI	00,00	307,3021	5	1,7	404,000Z	57,52	5202	02.raw	TR BOVIN	
								mf18031	protein1 sp	
MFRVF	35,73	698,3574	5	3	350,187	26,73	6490	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
MERVE	33 27	698 3574	5	8 1	350 1888	27 24	6625	3 Chymo	P00586ITH	
	00,21	000,0014	Ũ	0,1	000,1000	21,24	0020	02.raw	TR BOVIN	
								mf18031	protein1 sp	
MFRVF	16,58	698,3574	5	9,2	350,1892	27,76	6752	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	25.20	1050 5206	0	1 0	251 1004	10.44	1560	mf18031	protein1 sp	
VDSRAQGRT	35,39	1050,5200	9	-1,2	331,1004	10,44	1509	02 raw	TR BOVIN	
								mf18031	protein1 sp	
VDSRAQGRY	21,14	1050,5206	9	0	526,2676	13,33	2474	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	45 40	4050 5000	0	4.0	500.007	10.14	4504	mf18031	protein1 sp	
VDSRAQGRY	15,42	1050,5206	9	-1,2	526,267	10,41	1561	3_Cnymo		
								_02.1aw	protein1lspl	
LKEGHPVTSE	34,9	1829,9271	17	0,2	610,9831	12,54	2217	3 Chymo	P00586 TH	
PSRPEPA	-	-						_02.raw	TR_BOVIN	
KEGHPVTSE								mf18031	protein1 sp	
PSRPEPAIF	34,64	1976,9955	18	5,9	660,0096	19,11	4251	3_Chymo	P00586 TH	
								_02.raw	IR_BOVIN	
QLVDSRAQG	34 47	1404 7473	12	13	469 257	17 65	3804	3 Chymo	P00586ITH	
RYL	01,11	1101,1110		1,0	100,201	11,00	0001	02.raw	TR BOVIN	
								mf18031	protein1 sp	
EQVLENLESK	33,61	1187,6034	10	4,4	594,8116	19,83	4476	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	22.20	004 5045	0	0.4	454 7747	10.40	0470	mf18031	protein1 sp	
KATLINKSL	33,30	901,5345	8	0,4	451,7747	12,42	2173	3_Chymo		
								mf18031	protein1 spl	
RVLDASWY	33,33	1008,5029	8	2,3	505,2599	26,32	6367	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
GTOPEPDAV								mf18031	protein1 sp	
GL	32,54	1082,5244	11	7,4	542,2735	25,82	6239	3_Chymo	P00586 TH	
								_02.raw 	IR_BOVIN	
GHRTVSV	32.47	754,4086	7	-0.4	378.2114	11.21	1788	3 Chymo	P00586ITH	
	- ,	. ,		- ,	,	,		02.raw	TR BOVIN	
								mf18031	protein1 sp	
GHRTVSV	22,36	754,4086	7	-37,9	378,1973	11,2	1783	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	32 10	778 4337	7	-0.8	300 2238	10.67	1634	3 Chymo		
	52,15	110,4001	'	-0,0	000,2200	10,07	1004	02 raw	TR BOVIN	
								mf18031	protein1 sp	
ENLESKRF	32,15	1021,5192	8	1,3	511,7675	13,1	2396	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
	04 70	000 5004	0	0	405 770	10.07	1005	mf18031	protein1 sp	
LAESVRAGK	31,79	929,5294	9	0	405,772	10,07	1035	3_Chymo		
								mf18031	protein1 sp	
VGPGLRVL	31,37	809,5123	8	4,6	405,7653	22,89	5334	3_Chymo	P00586 TH	
								_02.raw	TR_BOVIN	
QLVDSRAQG		1100.0	40		077 0000	10.00	4704	mf18031	protein1 sp	
R	30,9	1128,6	10	-1	377,2069	10,92	1704	3_Chymo		
								_02.1aw mf18031	protein1lspl	
QLVDSRAQG	22,11	1128,6	10	0,1	565,3073	10,92	1703	3_Chymo	P00586ITH	
к								_02.raw	TR_BOVIN	
						4- 6 -		mf18031	protein1 sp	
VHQVLY	30,84	/57,4122	6	2,2	379,7142	15,81	3239	3_Chymo	P00586 TH	
								_02.raw	IK_BOVIN	
LAESVRAGK	30.8	1239.6935	13	-1.6	414.2378	13.51	2522	3 Chvmo	P00586ITH	
VGPG	,-		-	,-	,	- ,		_02.raw	TR_BOVIN	

LAESVRAGK VGPG	29,31	1239,6935	13	0,6	620,8544	13,49	2518	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN		
YAPRVWW	27,66	976,4919	7	0,3	489,2534	29,56	7259	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
EKSPEEL	26,19	830,4021	7	-1,4	416,2077	13,05	2379	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN		
LAESVRAG	26,07	801,4344	8	-0,9	401,7242	12,02	2053		protein1 sp  P00586 TH TR BOVIN		
QLVDSRAQ	25,49	915,4774	8	-0,9	458,7455	11,69	1943	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
SEPSRPEPAI F	25	1228,6088	11	-1,2	615,3109	22,73	5289	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
SLLKTY	24,87	723,4167	6	2,8	362,7166	17,44	3730	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
SPGTREARK EYL	23,96	1405,7313	12	2,9	469,5858	13,29	2460	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
VSQGK(+163. 00)GGK	23,87	922,4251	8	56,3	462,2458	14,81	2941	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K5:NBF- N:28.36
RVLDASW	23,77	845,4395	7	3,8	423,7286	22,06	5109	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
GGFRNW	21,71	735,3452	6	2,5	368,6808	17,47	3746	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
VSQGKGGK(+ 163.00)	21,18	922,4251	8	1,6	462,2206	15,1	3026	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K8:NBF- N:0.00
DIEEC(+163.0 0)RDKASPY	20,58	1587,6254	12	7,7	530,2198	22,23	5162	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-C	C5:NBF- C:1000.00
DIEEC(+163.0 0)RDKASPY	16,23	1587,6254	12	1,1	794,8208	21,74	5022	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN	NBF-C	C5:NBF- C:1000.00
VGSLGISNDT HVVVY	18,32	1558,7991	15	3,7	780,4097	28,38	6926	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN		
DGDDLGSFY APR	18,12	1311,5731	12	2,5	656,7955	28,41	6935	mf18031 3_Chymo 02.raw	protein1 sp  P00586 TH TR BOVIN		
GHRTVSVLN	17,96	981,5356	9	0,7	491,7754	13,11	2398	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
ERHVPGASF F	17,84	1145,5618	10	17,1	573,798	53,39	13453	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
ERHVPGASF F	17,79	1145,5618	10	19,6	573,7994	53,9	13615	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
K(+163.00)AT LNR	17,32	864,4196	6	4,1	433,2188	19,54	4378	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-N	K1:NBF- N:1000.00
EVMLPSEAG F	16,44	1078,5005	10	8,6	540,2621	36,23	8942	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
ALVSTKW	16,27	803,4541	7	7,4	402,7373	20,65	4717	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN		
LC(+163.00)G KPDVAIY	15,98	1240,5541	10	12,6	621,2921	29,81	7328	mf18031 3_Chymo _02.raw	protein1 sp  P00586 TH TR_BOVIN	NBF-C	C2:NBF- C:1000.00

## **APPENDIX 5: Supplementary Dataset S3**

MS/MS data of the persulfidated MnSOD digested with trypsin Related to CHAPTER 3 – Article I (Figure 3)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Scan	Source File	Accession	PTM	AScore
YMAC(+168.0 8)K(+163.00)	21,63	945,3355	5	18,2	473,6837	17	4122	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	Dio- cleaved; NBF-N	C4:Dio- cleaved:1000. 00;K5:NBF- N:1000.00
YMAC(+168.0 8)K(+163.00)	19,41	945,3355	5	12,6	473,681	13,6	2873	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	Dio- cleaved; NBF-N	C4:Dio- cleaved:1000. 00;K5:NBF- N:1000.00
YMAC(+168.0 8)K(+163.00)	18,09	945,3355	5	19,2	473,6841	17,51	4294	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	Dio- cleaved; NBF-N	C4:Dio- cleaved:1000. 00;K5:NBF- N:1000.00
HHAAYVNNL NVTEEK(+163 .00)YQEALAK	103,24	2704,2625	22	7,7	902,4351	30,12	8832	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:44.72
HHAAYVNNL NVTEEK(+163 .00)YQEALAK	89,35	2704,2625	22	-2	902,4263	29,58	8630	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:28.95
HHAAYVNNL NVTEEK(+163 .00)YQEALAK	79,6	2704,2625	22	-3,5	902,4249	32,87	9860	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:27.66
HHAAYVNNL NVTEEK(+163 .00)YQEALAK	73,45	2704,2625	22	0	902,4281	21,42	5694	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:14.15
NVTEEK(+163 .00)YQEALAK	61,03	2704,2625	22	1,8	902,4297	26,41	7489	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:21.15
NVTEEK(+163 .00)YQEALAK	54,74	2704,2625	22	2,3	1353,1416	29,73	8689	mf18031 3_03.raw	P04179 SO DM HUMAN	NBF-N	K15:NBF- N:6.08
NVTEEK(+163 .00)YQEALAK	40,5	2704,2625	22	4,5	902,4321	24,51	6807	mf18031 3_03.raw	P04179 SO DM HUMAN	NBF-N	K15:NBF- N:47.00
NVTEEK(+163 .00)YQEALAK	20,5	2704,2625	22	18,6	902,4448	20,9	5522	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K15:NBF- N:21.15
DFGSFDKFK( +163.00)EK	89,53	1509,6519	11	4,8	755,8368	34,54	10505	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K9:NBF- N:33.18
DFGSFDKFK( +163.00)EK	70,18	1509,6519	11	3,7	504,2264	34,47	10478	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K9:NBF- N:15.73
DFGSFDKFK( +163.00)EK	68,8	1509,6519	11	2,2	504,2257	38,64	12095	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K9:NBF- N:0.00
DFGSFDKFK( +163.00)EK	54,62	1509,6519	11	2,8	504,226	40,18	12713	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K9:NBF- N:0.00
GSGWGWLG FNK(+163.00) ER	87,37	2482,1772	22	3,9	828,403	45,3	14732	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ER	72,19	2482,1772	22	5	1242,1021	45,54	14828	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ER	44,54	2482,1772	22	4	828,403	53,83	18003	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ER	23,02	2482,1772	22	0,3	828,4	50,31	16664	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ER	20,73	2482,1772	22	2	1242,0984	53,88	18024	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00

LTAASVGVQ GSGWGWLG FNK(+163.00) ER	18,58	2482,1772	22	5,2	828,404	51,58	17143	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ER	17,51	2482,1772	22	4,8	828,4037	49,69	16428	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K20:NBF- N:1000.00
DFGSFDKFK EK(+163.00)	77,4	1509,6519	11	5,5	755,8373	34,01	10299	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K11:NBF- N:26.31
DFGSFDKFK EK(+163.00)	69,04	1509,6519	11	3,4	504,2263	33,96	10279	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K11:NBF- N:13.67
DFGSFDK(+1 63.00)FKEK	75,86	1509,6519	11	4,5	755,8366	35,57	10901	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:22.85
DFGSFDK(+1 63.00)FKEK	75,81	1509,6519	11	3,1	504,2261	35,6	10911	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:32.28
DFGSFDK(+1 63.00)FKEK	56,45	1509,6519	11	3,5	755,8359	36,12	11118	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:18.53
DFGSFDK(+1 63.00)FKEK	40,72	1509,6519	11	4,8	755,8368	40,22	12729	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:11.10
DFGSFDK(+1 63.00)FK	71,66	1252,5143	9	4,1	627,267	44,4	14383	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:22.85
DFGSFDK(+1 63.00)FK	71,01	1252,5143	9	4,1	627,267	43,88	14180	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:18.53
DFGSFDK(+1 63.00)FK	69,71	1252,5143	9	4,1	627,267	41,66	13304	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:18.53
DFGSFDK(+1 63.00)FK	69,7	1252,5143	9	3,7	627,2667	44,92	14584	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:20.41
DFGSFDK(+1 63.00)FK	58,84	1252,5143	9	3,9	627,2668	45,43	14784	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:14.63
DFGSFDK(+1 63.00)FK	44,26	1252,5143	9	5,4	627,2678	49,41	16321	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:12.81
DFGSFDK(+1 63.00)FK	42,42	1252,5143	9	3,5	627,2666	42,82	13765	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:12.81
DFGSFDK(+1 63.00)FK	40,13	1252,5143	9	1,2	627,2652	48,9	16123	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:14.63
DFGSFDK(+1 63.00)FK	25,99	1252,5143	9	2,4	627,2659	45,95	14985	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:5.99
GDVTAQIALQ PALK(+163.00 )	66,29	1586,8047	14	6,4	794,4147	47,05	15423	mf18031 3_03.raw	P04179 SO DM_HUMAN	NBF-N	K14:NBF- N:1000.00
RDFGSFDK(+ 163.00)FK	59,78	1408,6154	10	-0,1	470,5457	32,55	9737	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K8:NBF- N:18.53
NVRPDYLK(+ 163.00)	57,92	1166,5463	8	4	584,2827	28,15	8122	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K8:NBF- N:1000.00
GELLEAIK(+1 63.00)R	57,8	1190,6038	9	4,4	596,3118	36,83	11404	mf18031 3_03.raw	PO4179 SO DM_HUMAN	NBF-N	K8:NBF- N:1000.00
GELLEAIK(+1 63.00)R	56,71	1190,6038	9	4,6	596,3119	37,36	11609	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K8:NBF- N:1000.00
DFGSFDKFK( +163.00)	56,6	1252,5143	9	2,7	627,2661	43,37	13978	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K9:NBF- N:15.73

DFGSFDK(+1 63.00)FK(+163 .00)EK	54,17	1672,6531	11	4	837,3372	52,16	17370	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	NBF-N	K7:NBF- N:11.01;K9:NB F-N:6.59
YMAC(+163.0 0)K	42,34	777,2569	5	3,4	389,6371	15,36	3526	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	NBF-C	C4:NBF- C:1000.00
GGGEPK(+16 3.00)GELLEAI KR	39,27	1715,8585	15	4,3	572,9626	33	9910	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K6:NBF- N:47.82
LTAASVGVQ GSGWGWLG FNKERGHLQI AAC(+163.00) PNQDPLQ	39,26	4067,9443	37	32	1357,0321	38,26	11963	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C	C30:NBF- C:1000.00
YMAC(+163.0 0)KK	38,33	905,3518	6	3,6	453,6848	12,23	2382	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C	C4:NBF- C:1000.00
YMAC(+163.0 0)KK	33,83	905,3518	6	2,9	453,6845	12,74	2564	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C	C4:NBF- C:1000.00
YMAC(+163.0 0)KK	32,4	905,3518	6	0,2	453,6833	14,1	3048	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C	C4:NBF- C:1000.00
YMAC(+163.0 0)KK	28,83	905,3518	6	1,9	453,6841	13,41	2799	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C	C4:NBF- C:1000.00
NVRPDYLK(+ 163.00)AIWNV INWENVTER	35,68	2891,4097	22	4,1	964,8145	50,61	16776	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K8:NBF- N:1000.00
LTAASVGVQ GSGWGWLG FNK(+163.00) ERGHLQIAAC (+163.00)PNQ	28,74	3777,7231	33	51,3	1260,3129	38,7	12121	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N; NBF-C	K20:NBF- N:1000.00;C30 :NBF- C:1000.00
HINHSIFWTN LSPNGGGEP KGELLEAIK(+ 163.00)RDFG SFDK	27,74	4173,0088	36	-7,1	1392,0004	18,51	4671	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K28:NBF- N:0.00
YMAC(+163.0 0)K(+163.00)K	27,15	1068,353	6	3,9	535,1859	27,5	7879	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-C; NBF-N	C4:NBF- C:1000.00;K5: NBF-N:7.65
NGGGEPK(+1 63.00)GELLE AIKR	20,58	1829,9014	16	8,5	915,9658	42,15	13500	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K7:NBF- N:49.35
DFGSFDKFK EK(+163.00)L TAASVGVQG	18,77	2393,1282	21	25,3	798,7368	17,83	4411	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	NBF-N	K11:NBF- N:11.10
VINWENVTE RYMAC(+394. 16)KK	27,79	2377,1262	16	1,5	793,3839	24,05	6647	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	DCP- Bio1	C14:DCP- Bio1:1000.00
VINWENVTE RYMAC(+394. 16)KK	21,19	2377,1262	16	7,9	793,3889	22,5	6083	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	DCP- Bio1	C14:DCP- Bio1:1000.00
VINWENVTE RYMAC(+394. 16)KK	20,96	2377,1262	16	-6,5	793,3776	24,66	6861	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	DCP- Bio1	C14:DCP- Bio1:1000.00
VINWENVTE RYMAC(+394. 16)KK	18,3	2377,1262	16	3,1	1189,5741	23,23	6345	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	DCP- Bio1	C14:DCP- Bio1:1000.00
YMAC(+394.1 6)K	26,68	1008,4113	5	3,7	505,2148	26,34	7465	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	DCP- Bio1	C4:DCP- Bio1:1000.00
HSLPDLPYDY GALEPHINAQ IMQLHHSK	158,74	3223,5872	28	4,2	1075,5409	29,83	8728	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN		
HSLPDLPYDY GALEPHINAQ IMQLHHSK	146,76	3223,5872	28	2,1	1075,5386	29,3	8533	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN		
HSLPDLPYDY GALEPHINAQ IMQLHHSK	142,21	3223,5872	28	1,4	1075,5378	28,79	8351	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN		

HSLPDLPYDY								mf18031	protein1 sp	
GALEPHINAQ	130,3	3223,5872	28	4,7	1075,5414	30,33	8914	3 03.raw	P04179 SO	
IMQLHHSK									DM_HUMAN	
HSLPDLPYDY	74 70	2002 5070	20		1075 5107	22.05	10005	mf18031	protein1 sp	
	/1,/0	3223,3072	20	-15,5	1075,5197	<i>აა</i> ,00	10235	3_03.raw		
									protein1lspl	
GAI FPHINAQ	53 94	3223 5872	28	4 5	1075 5411	36	11069	mf18031	P04179ISO	
IMQLHHSK	00,01	0220,0072	20	1,0	1070,0111	00	11000	3_03.raw	DM HUMAN	
HSLPDLPYDY								(10001	protein1 sp	
GALEPHINAQ	18,05	3223,5872	28	7,3	1075,5442	43,25	13934	mf18031	P04179 SO	
IMQLHHSK		-						3_03.raw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	147,24	2541,2612	22	2,3	848,0963	18,55	4685	3 03 raw	P04179 SO	
ALAK								5_05.1aw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	140,12	2541,2612	22	2,4	848,0964	19,08	4867	3 03.raw	P04179 SO	
ALAK									DM_HUMAN	
	100.05	0544 0040	22	0	040.0004	01.00	5704	mf18031	protein1 sp	
NVIEEKIQE	136,05	2541,2012	22	2	848,0961	21,03	5764	3_03.raw	P04179 50	
								_	DIVI_HUIVIAN	
	131 43	2541 2612	22	4	848 0978	20.1	5241	mf18031	P04179IS0	
	101,40	2041,2012	22	-	040,0070	20,1	5241	3_03.raw	DM HUMAN	
HHAAYVNNI									protein1lspl	
NVTEEKYQE	110.27	2541.2612	22	3	848.0969	26.78	7623	mf18031	P04179ISO	
ALAK	,			-		,		3_03.raw	DM HUMAN	
HHAAYVNNL									protein1 sp	
NVTEEKYQE	105,63	2541,2612	22	-0,3	1271,6375	19,8	5131	11118031	P04179 SO	
ALAK								3_03.raw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	99,78	2541,2612	22	-1,9	848,0927	29,46	8592	3 03 raw	P04179 SO	
ALAK								0_00.1uw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	68,54	2541,2612	22	-0,1	848,0942	38,18	11928	3 03.raw	P04179 SO	
									DM_HUMAN	
	64.40	2544 2642	22	2.2	949 007	20.00	10000	mf18031	protein 1  sp	
NVIEEKIQE	04,42	2041,2012	22	3,2	040,097	30,99	12230	3_03.raw	P04179 30	
									protein1 spl	
NVTEEKYOE	62 39	2541 2612	22	12	848 0954	43 48	14021	mf18031	P04179ISO	
ALAK	02,00	2011,2012		.,_	010,0001	10,10		3_03.raw	DM HUMAN	
HHAAYVNNL									protein1 sp	
NVTEEKYQE	61,17	2541,2612	22	5,4	848,0989	40,68	12913	mf18031	P04179 SO	
ALAK								3_03.raw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	59,03	2541,2612	22	1,8	848,0959	39,54	12457	3 03 raw	P04179 SO	
ALAK								0_00.1aw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	52,39	2541,2612	22	0,4	848,0947	47,86	15728	3 03.raw	P04179 SO	
								-	DM_HUMAN	
	33 43	2541 2612	22	2.2	848 0062	515	17115	mf18031		
	33,42	2041,2012	22	2,2	040,0902	51,5	17115	3_03.raw		
									protein1lspl	
NVTEEKYOE	30.26	2541 2612	22	33	848 0972	46 74	15297	mf18031	P04179ISO	
ALAK	00,20	2011,2012		0,0	010,0012	10,11	10201	3_03.raw	DM HUMAN	
HHAAYVNNL									protein1 sp	
NVTEEKYQE	30,03	2541,2612	22	-0,9	848,0936	52,54	17508	mf18031	P04179 SO	
ALAK	,			,	,			3_03.raw	DM HUMAN	
HHAAYVNNL								mf10021	protein1 sp	
NVTEEKYQE	28,78	2541,2612	22	2	848,096	48,88	16117	3 03 row	P04179 SO	
ALAK								3_03.1aw	DM_HUMAN	
HHAAYVNNL								mf18031	protein1 sp	
NVTEEKYQE	25,54	2541,2612	22	6,1	848,0995	49,93	16519	3 03 raw	P04179 SO	
ALAK									DM_HUMAN	
HHAAYVNNL	00.0	0544 0040		<u>.</u>	040.004	50.04	475.40	mf18031	protein1 sp	
INVIEEKYQE	23,8	2541,2612	22	0,4	848,0947	52,64	17548	3_03.raw		
	16 11	2541 2612	22	31	848 0072	15.07	2/12	mf18031		
	10,11	2071,2012	~~	5,4	0-10,0372	10,07	5415	3_03.raw		

LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	139,46	2034,0322	20	2,7	1018,0261	47,37	15541	3 03 raw	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	134,14	2034,0322	20	3,1	1018,0266	47,89	15740	3 03.raw	P04179 SO	
								_	DM_HUMAN	
	124.05	2024 0222	20	15	1019 025	10 10	15044	mf18031	protein Isp	
	134,05	2034,0322	20	1,5	1016,025	40,42	15944	3_03.raw		
ITAASVGVO									protein1lsnl	
GSGWGWLG	133 67	2034 0322	20	3	1018 0264	50.97	16910	mf18031	P04179ISO	
FNK	100,07	2004,0022	20	Ū	1010,0204	00,07	10010	3_03.raw	DM HUMAN	
LTAASVGVQ								(10001	protein1 sp	
GSGWGWLG	133,15	2034,0322	20	2,6	1018,026	46,35	15139	mf18031	P04179 SO	
FNK	-					-		3_03.raw	DM_HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	131,35	2034,0322	20	2,6	1018,0261	49,43	16328	3 03 raw	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	131,28	2034,0322	20	2,7	1018,0261	48,92	16132	3 03 raw	P04179 SO	
FNK								0_00.1uw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	131,12	2034,0322	20	4,8	1018,0283	44,31	14349	3 03.raw	P04179 SO	
FNK									DM_HUMAN	
	100.00	2024 0222	20	<u> </u>	1010 0050	40.00	45044	mf18031	proteiniispi	
GSGWGWLG	130,28	2034,0322	20	2,3	1018,0258	40,80	15344	3_03.raw		
								_	DIVI_HUIVIAN	
CSCWCWLC	130.25	2034 0322	20	3	1018 0264	15.93	1/037	mf18031		
	130,25	2034,0322	20	3	1010,0204	40,00	14937	3_03.raw		
									protein1lsnl	
GSGWGWLG	129.2	2034 0322	20	23	1018 0257	50 45	16715	mf18031	P04179ISO	
FNK	120,2	2004,0022	20	2,0	1010,0207	00,40	107 10	3_03.raw	DM HUMAN	
LTAASVGVQ									protein1lspl	
GSGWGWLG	129.05	2034.0322	20	2	1018.0255	49.94	16525	mf18031	P04179ISO	
FNK	,			_	,	,		3_03.raw	DM HUMAN	
LTAASVGVQ								(1000.1	protein1 sp	
GSGWGWLG	128,93	2034,0322	20	5,1	1018,0286	44,82	14544	mf18031	P04179 SO	
FNK								3_03.raw	DM_HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	127,54	2034,0322	20	3,5	1018,027	42,78	13750	3 03 row	P04179 SO	
FNK								3_03.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	127,12	2034,0322	20	4,5	1018,028	45,32	14741	3 03 raw	P04179 SO	
FNK								•_•••	DM_HUMAN	
LIAASVGVQ	100.00	0004 0000	00	0.4	1010 0050	40.00	40050	mf18031	protein1 sp	
GSGWGWLG	126,99	2034,0322	20	2,1	1018,0256	43,29	13950	3_03.raw	P04179 SO	
								_	DM_HUMAN	
CSCWCWLC	125.0	2034 0322	20	2	1018 0254	52.5	17/03	mf18031		
GSGWGWLG	125,5	2004,0022	20	2	1010,0234	52,5	17435	3_03.raw		
LTAASVGVO									protein1lspl	
GSGWGWLG	125.81	2034.0322	20	2.9	1018.0264	42.27	13545	mf18031	P04179ISO	
FNK	,			_,-	,	,		3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	125,64	2034,0322	20	2	1018,0255	54,54	18283	mf18031	P04179 SO	
FNK								3_03.raw	DM_HUMAN	
LTAASVGVQ								mf19024	protein1 sp	
GSGWGWLG	125,36	2034,0322	20	2,1	1018,0256	51,48	17104	3 03 row	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	125,21	2034,0322	20	2,6	1018,026	51,98	17300	3 03 raw	P04179 SO	
FNK								•_•••	DM_HUMAN	
LTAASVGVQ	404.00	0004 0005			1010 0010	<b>F</b> 1 0 0	40000	mf18031	protein1 sp	
GSGWGWLG	124,39	2034,0322	20	1,4	1018,0248	54,03	18083	3 03.raw	P04179 SO	
FNK										
LIAASVGVQ	124.22	2034 0222	20	2.2	1010 0050	F2 01	17600	mf18031		
GSGWGWLG	124,33	2034,0322	20	2,3	1010,0208	55,01	1/090	3_03.raw		
GSGWGWU	10/	2034 0222	20	15	1018 028	41 21	12125	mf18031		
FNIK	124	2007,0022	20	ч,5	1010,020	<b>,∠</b> 1	10120	3_03.raw		
1 1 1 1 1										

LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	123,49	2034,0322	20	4,4	1018,0278	40,7	12921	3 03.raw	P04179 SO	
FNK								0_00a.i	DM_HUMAN	
LIAASVGVQ	400.00	0004 0000	00		1010 0070	10.40	40740	mf18031	protein1 sp	
	123,30	2034,0322	20	4,4	1018,0278	40,18	12/12	3_03.raw		
									protein1lspl	
GSGWGWLG	122 78	2034 0322	20	0.5	1018 0239	53 52	17888	mf18031	P04179ISO	
FNK	122,10	2004,0022	20	0,0	1010,0200	00,02	17000	3_03.raw	DM HUMAN	
LTAASVGVQ								(1000.1	protein1 sp	
GSGWGWLG	121,1	2034,0322	20	3,6	1018,0271	43,81	14154	mf18031	P04179 SO	
FNK	,	,	_	- , -	,-	- , -	_	3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	121,03	2034,0322	20	3,5	1018,027	41,73	13335	3 03 row	P04179 SO	
FNK								3_03.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	120,15	2034,0322	20	2	1018,0254	38,14	11914	3 03.raw	P04179 SO	
FNK									DM_HUMAN	
	100.00	0004 0000	20	2	1010 0005	20.47	10005	mf18031	proteiniispi	
GSGWGWLG	120,06	2034,0322	20	3	1018,0265	39,17	12305	3_03.raw	P04179 SO	
								-	DIVI_HUIVIAN	
CSCWCWLC	116 39	2034 0322	20	3	1018 0264	30.67	12500	mf18031		
FNK	110,50	2004,0022	20	5	1010,0204	55,07	12000	3_03.raw		
LTAASVGVO									protein1lspl	
GSGWGWLG	115 45	2034 0322	20	23	679 0196	50.28	16649	mf18031	P04179ISO	
FNK	110,10	2001,0022	20	2,0	010,0100	00,20	10010	3_03.raw	DM HUMAN	
LTAASVGVQ									protein1lspl	
GSGWGWLG	115.38	2034.0322	20	3.1	1018.0266	37.63	11718	mf18031	P04179ISO	
FNK	,			-,.	,	.,		3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	114,99	2034,0322	20	2,6	679,0198	47,19	15472	mf18031	P04179 SO	
FNK	· · ·	,		,				3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	113,96	2034,0322	20	2,4	679,0197	47,72	15675	11118031	P04179 SO	
FNK								3_03.raw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	113,51	2034,0322	20	2,6	679,0198	48,73	16063	3 03 raw	P04179 SO	
FNK								0_00.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	113,34	2034,0322	20	2,7	679,0198	46,15	15065	3 03.raw	P04179 SO	
FNK									DM_HUMAN	
	440.07	0004 0000	20	0.7	070 0405	40.00	45070	mf18031	proteiniispi	
GSGWGWLG	113,27	2034,0322	20	0,7	679,0185	48,23	15870	3_03.raw		
									Divi_HUIVIAN	
GSGWGWLG	112 70	2034 0322	20	24	679 0197	46 68	15271	mf18031	P04179ISO	
FNK	112,75	2004,0022	20	∠,⊤	070,0107	40,00	10271	3_03.raw		
LTAASVGVQ									protein1lspl	
GSGWGWLG	110.5	2034.0322	20	3.5	679.0204	45.63	14860	mf18031	P04179ISO	
FNK	<i>,</i>	,		,				3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	109,62	2034,0322	20	4,8	679,0212	44,6	14462	3 02	P04179 SO	
FNK								3_03.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	109,37	2034,0322	20	1,9	679,0193	51,82	17239	3 03 raw	P04179 SO	
FNK								0_00.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	108,54	2034,0322	20	1,5	679,019	53,9	18033	3 03.raw	P04179 SO	
FNK									DM_HUMAN	
LIAASVGVQ	100 54	2024 0000		<u> </u>	670 0407	E0 70	10044	mf18031		
GSGWGWLG	108,51	2034,0322	20	2,5	0/9,019/	50,79	10844	3_03.raw		
FNK								-	DM_HUMAN	
CSCWCWLC	108.00	2034 0322	20	31	670 0201	13 57	14050	mf18031		
	100,09	2004,0322	20	J, I	013,0201	-3,37	14009	3_03.raw		
I TAASVGVO									protein1lsnl	
GSGWGWLG	107 73	2034 0322	20	12	679 0189	49 75	16452	mf18031	P04179ISO	
FNK	,	_007,0022	20	•,-	5. 5,6 100	.0,10	10402	3_03.raw	DM HUMAN	
LTAASVGVO									protein1lspl	
GSGWGWLG	107.39	2034.0322	20	2.5	679,0197	49.25	16257	mt18031	P04179 SO	
FNK	,	· ·			, -	, -	-	3_03.raw	DM_HUMAN	

LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	106,96	2034,0322	20	4,7	679,0212	41,51	13244	3_03.raw	P04179 SO	
								-	DM_HUMAN	
GSGWGWLG	106.9	2034 0322	20	39	679 0206	39.22	12324	mf18031	P04179ISO	
FNK	100,0	2001,0022	20	0,0	010,0200	00,22	12021	3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	105,96	2034,0322	20	1,9	679,0193	38,2	11936	111110031 3 03 raw	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	105,76	2034,0322	20	2,6	679,0198	51,32	17041	3 03.raw	P04179 SO	
									DM_HUMAN	
GSGWGWLG	104.9	2034 0322	20	2.5	679 0197	42 54	13654	mf18031		
FNK	104,0	2004,0022	20	2,0	010,0101	42,04	10004	3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	104,53	2034,0322	20	2,1	679,0195	52,85	17630	111110031 3 03 raw	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	103,69	2034,0322	20	2,2	679,0195	52,33	17432	3_03.raw	P041/9 SO	
								_	DIVI_HUIVIAN	
GSGWGWLG	103 57	2034 0322	20	2.5	679 0197	37 69	11739	mf18031	P04179ISO	
FNK	,			_,•	010,0101	0.,00		3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	103,1	2034,0322	20	4,8	679,0212	39,73	12534	3 03 raw	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ	100.04	0004 0000		4.0	070 0044	40.05	40054	mf18031	protein1 sp	
GSGWGWLG	103,01	2034,0322	20	4,9	679,0214	43,05	13854	3_03.raw		
								_	protein1 spl	
GSGWGWLG	102 28	2034 0322	20	1.1	679 0187	53 37	17832	mf18031	P04179ISO	
FNK	102,20	2001,0022	20	.,.	010,0101	00,07	11002	3_03.raw	DM HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	102,08	2034,0322	20	3,3	679,0203	42,04	13453	111110031 3 03 row	P04179 SO	
FNK								5_05.1aw	DM_HUMAN	
LTAASVGVQ	400.04	0004 0000		4.0	070 0040	4- 44	4 4 9 5 9	mf18031	protein1 sp	
GSGWGWLG	102,04	2034,0322	20	4,8	679,0212	45,11	14658	3_03.raw	P041/9 SO	
									protein1lspl	
GSGWGWLG	101.9	2034.0322	20	3.4	679.0203	41	13040	mf18031	P04179ISO	
FNK	,.			-, -	,			3_03.raw	DM HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	100,88	2034,0322	20	3	679,02	44,09	14260	3 03 raw	P04179 SO	
FNK								0_00.14W	DM_HUMAN	
	100 72	2024 0222	20	10	670 0212	40.49	10000	mf18031	protein'i  sp	
GSGWGWLG	100,75	2034,0322	20	4,0	079,0213	40,40	12033	3_03.raw	P04179 30	
LTAASVGVQ									protein1lspl	
GSGWGWLG	99,46	2034,0322	20	1,8	679,0192	54,93	18398	mf18031	P04179 SO	
FNK					-			3_03.raw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	97,07	2034,0322	20	1	679,0187	54,42	18237	3 03.raw	P04179 SO	
FNK									DM_HUMAN	
CSCWCWLC	96.34	2034 0333	20	2.1	670 0105	38 7	12110	mf18031		
GSGWGWLG FNK	90,34	2034,0322	20	۷,۱	079,0195	50,7	12119	3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	32,78	2034,0322	20	4,7	1018,0281	36,02	11075	mt18031	P04179 SO	
FNK								3_03.raw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	136,42	2291,1699	22	3,3	764,7331	40,61	12886	3 03.raw	P04179 SO	
GENK								-	DM_HUMAN	
OGSCWCW	136 21	2201 1600	22	3.2	764 733	30 58	12474	mf18031		
GENK	100,21	2201,1000	~~	5,2	107,100	55,50	127/4	3_03.raw	DM HUMAN	
EKLTAASVGV									protein1 sp	
QGSGWGWL	136,05	2291,1699	22	2,7	764,7327	39,08	12272	mt18031	P04179 SO	
GFNK								3_03.raw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	135,33	2291,1699	22	4,8	764,7343	37,55	11686	3_03.raw	P04179 SO	
GENK							1		DM_HUMAN	

EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	134,83	2291,1699	22	3	764,7329	41,11	13086	3 03 raw	P04179 SO	
GFNK								5_05.1aw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	132,48	2291,1699	22	2,5	764,7325	40,1	12683	3 03.raw	P04179 SO	
								_	DM_HUMAN	
EKLIAASVGV	122.02	2201 1600	22	2.6	764 7222	41.60	12200	mf18031		
GENK	132,03	2291,1099	22	3,0	704,7333	41,02	13209	3_03.raw	P0417930	
EKI TAASVGV									protein1lspl	
OGSGWGWI	129 82	2291 1699	22	26	764 7326	33 44	10074	mf18031	P04179ISO	
GENK	120,02	2201,1000	22	2,0	104,1020	00,44	10014	3_03.raw	DM HUMAN	
EKLTAASVGV								(10001	protein1 sp	
QGSGWGWL	128,96	2291,1699	22	3	764,7329	36,01	11073	mf18031	P04179 SO	
GFNK					-			3_03.raw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	128,51	2291,1699	22	3,6	764,7333	34,47	10477	3 03 raw	P04179 SO	
GFNK								5_05.1aw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	125,92	2291,1699	22	2,2	764,7322	34,98	10672	3 03 raw	P04179 SO	
GFNK								0_00.10W	DM_HUMAN	<u> </u>
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	125,33	2291,1699	22	2,7	764,7327	42,13	13490	3 03.raw	P04179 SO	
GENK									DM_HUMAN	
EKLIAASVGV	104 47	2204 4000	22	0.7	704 7007	40.40	10000	mf18031	protein1 sp	
	124,47	2291,1699	22	2,7	104,1321	43,10	13898	3_03.raw	P04179 50	
								_	DIVI_HUMAN	
OGSGWGWI	122.26	2201 1600	22	3.0	764 7335	35.5	10972	mf18031		
GENK	123,20	2291,1099	22	3,0	704,7335	35,5	10072	3_03.raw		
									protein1lspl	
OGSGWGWI	123.1	2291 1699	22	34	764 7332	43 66	14095	mf18031	P04179ISO	
GENK	120,1	2201,1000		0,4	104,1002	40,00	14000	3_03.raw	DM HUMAN	
EKLTAASVGV									protein1 spl	
QGSGWGWL	122.67	2291,1699	22	3.8	764,7335	33.95	10276	mf18031	P04179ISO	
GFNK	, -	-,		- ) -	. ,	,		3_03.raw	DM HUMAN	
EKLTAASVGV								me£10001	protein1 sp	
QGSGWGWL	122,5	2291,1699	22	3,2	764,733	42,65	13698	mf18031	P04179 SO	
GFNK								3_03.raw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	121,89	2291,1699	22	-1,4	764,7295	38,57	12070	3 03 raw	P04179 SO	
GFNK								0_00.1aw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	120,39	2291,1699	22	4,3	764,7339	37,03	11482	3 03.raw	P04179 SO	
GFNK									DM_HUMAN	
EKLIAASVGV	110 70	2201 1600	22	0.5	764 724	E2 E7	17010	mf18031	protein I spj	
	119,72	2291,1099	22	0,5	704,731	55,57	17910	3_03.raw	P04179 50	
									DIVI_HUIVIAIN	
OGSGWGWI	119 11	2291 1699	22	3.6	764 7333	36 52	11279	mf18031	P04179ISO	
GENK	110,11	2201,1000	22	0,0	104,1000	00,02	11210	3_03.raw	DM HUMAN	
EKLTAASVGV									protein1lspl	
QGSGWGWL	118,47	2291,1699	22	1.6	764,7318	54,09	18109	mt18031	P04179ISO	1
GFNK	· · ·	,		,		<i>.</i>		3_03.raw	DM HUMAN	
EKLTAASVGV								me£10001	protein1 sp	
QGSGWGWL	116,22	2291,1699	22	2,2	764,7323	49,92	16517	11118031	P04179 SO	
GFNK								3_03.1aw	DM_HUMAN	
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	114,28	2291,1699	22	2,6	764,7326	46,25	15102	3 03 raw	P04179 SO	
GFNK								0_00.14W	DM_HUMAN	ļ
EKLTAASVGV	400.00	0004 4005		_	704 7000	40.00	10110	mf18031	protein1 sp	l
QGSGWGWL	108,68	2291,1699	22	3	/64,/329	48,86	16110	3_03.raw	P04179 SO	l
GENK								-	DM_HUMAN	
ENLIAASVGV	106 44	2201 1000	22	10	761 7015	50 F0	17500	mf18031	protein 1 sp	l
	100,44	2291,1699	22	1,2	104,1315	52,52	1/502	3_03.raw		l
OGSGWGW	101 33	2201 1600	22	24	764 7324	52 01	17313	mf18031	PO4170190	l
	101,33	2231,1099	22	∠,+	104,1324	JZ,U I	1/515	3_03.raw		l
FKI TAASVOV			-						protein1lsnl	
QGSGWGWI	62 23	2291 1699	22	58	1146 5989	39 32	12364	mf18031	P04179ISO	l
GFNK	52,20	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		0,0		55,0L		3_03.raw	DM HUMAN	l

EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	41,99	2291,1699	22	-11,3	1146,5793	40,75	12942	3 03 raw	P04179 SO	
GFNK								0_00.1aw	DM_HUMAN	 
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	41,04	2291,1699	22	0,3	1146,5925	53,61	17925	3 03 raw	P04179 SO	
GFNK								0_00.14W	DM_HUMAN	 
EKLTAASVGV								mf18031	protein1 sp	
QGSGWGWL	39,24	2291,1699	22	-4,8	1146,5868	41,34	13173	3 03 raw	P04179 SO	
GFNK								0_00.1uw	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	131,68	2319,176	22	-0,2	774,0658	38,4	12014	3 03 raw	P04179 SO	
FNKER								5_05.1aw	DM_HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	130,95	2319,176	22	0,8	774,0666	39,44	12416	111110031	P04179 SO	
FNKER								3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	129,96	2319,176	22	4,5	774,0695	37,39	11621	111110031	P04179 SO	
FNKER	-			-	-			3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 sp	
GSGWGWLG	128,7	2319,176	22	2,9	774,0682	36,86	11416	mf18031	P04179 SO	
FNKER				-	-			3_03.raw	DM HUMAN	
LTAASVGVQ								(10001	protein1 sp	
GSGWGWLG	128.21	2319.176	22	3.2	774.0684	36.34	11201	mf18031	P04179ISO	
FNKER	· · ·	,						3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 spl	
GSGWGWLG	128.05	2319,176	22	1.1	774.0668	42.57	13667	mf18031	P04179ISO	
FNKFR	,			-,-	,	,		3_03.raw	DM HUMAN	
ITAASVGVQ									protein1lspl	
GSGWGWLG	127 97	2319 176	22	3	774 0682	34 25	10389	mf18031	P04179ISO	
ENKER		2010,110		Ũ	111,0002	01,20	10000	3_03.raw	DM HUMAN	
LTAASVGVO									protein1lspl	 
GSGWGWLG	127 52	2310 176	22	21	774 0676	38.02	12208	mf18031		
ENIKER	127,52	2010,170	22	2,1	114,0010	00,02	12200	3_03.raw		
									protein1 snl	 
GSGWGWLG	127 36	2310 176	22	0.7	774 0665	37 80	11810	mf18031		
	127,30	2319,170	22	0,7	774,0005	57,09	11019	3_03.raw		
									Divi_HOlviAN	 
	102.00	2210 176	22	1 4	774.067	42.6	14070	mf18031		
GSGWGWLG	123,99	2319,170	22	1,4	//4,00/	43,0	14070	3_03.raw	P04179 50	
									DIVI_HUIVIAN	 
	100.07	0040 470	22	0.7	774 0004	25.00	40700	mf18031	proteinnispi	
GSGWGWLG	122,97	2319,170	22	2,7	774,0001	JJ,∠0	10765	3_03.raw	P04179 50	
									DIVI_HUIVIAN	 
CECINICIAI	100.10	2210 176	22	1 4	774 067	45 14	14674	mf18031		
GSGWGWLG	122,13	2319,176	22	1,4	774,067	45,14	14071	3 03.raw	P04179 50	
FNKER								-	DM_HUMAN	 
LIAASVGVQ	404 74	0040 470	00		774 0005	04 77	40504	mf18031	protein I spj	
GSGWGWLG	121,74	2319,176	22	3,3	774,0685	34,77	10591	3 03.raw	P04179 SO	
FNKER								-	DM_HUMAN	 
LIAASVGVQ	404.00	0040 470	00		774 0004	00.40	0000	mf18031	protein I spj	
GSGWGWLG	121,39	2319,176	22	3,2	774,0684	32,19	9602	3 03.raw	P04179 SO	
FNKER								-	DM_HUMAN	 
LIAASVGVQ	101.00							mf18031	protein1 sp	
GSGWGWLG	121,36	2319,176	22	2,8	774,0681	35,8	10990	3 03.raw	P04179 SO	
FNKER									DM_HUMAN	 
LIAASVGVQ	10/ 05	0040 170					4.40-0	mf18031	protein1 sp	
GSGWGWLG	121,22	2319,176	22	1,9	//4,06/4	44,12	14273	3 03.raw	P04179 SO	
FNKER									DM_HUMAN	 
LIAASVGVQ	404.55	00/0 /==	~~			<u></u>	40.000	mf18031	protein1 sp	
GSGWGWLG	121,03	2319,176	22	1,3	774,067	33,73	10190	3 03 raw	P04179 SO	
FNKER									DM_HUMAN	 ļ
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	120,96	2319,176	22	0,6	774,0664	53,4	17842	3 03 raw	P04179 SO	
FNKER									DM_HUMAN	 ļ
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	120,41	2319,176	22	4,5	774,0695	33,21	9991	3 03 raw	P04179 SO	
FNKER								5_55.1UW	DM_HUMAN	 ļ
LTAASVGVQ				_				mf18031	protein1 sp	
GSGWGWLG	119,74	2319,176	22	2,1	774,0676	43,08	13865	3 03 raw	P04179 SO	
FNKER								5_50.1aw	DM_HUMAN	 ļ
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	119,09	2319,176	22	3,7	774,0688	32,7	9800	3 03 raw	P04179 SO	
FNKER								5_50.1aw	DM_HUMAN	

LTAASVGVQ									protein1 sp	
GSGWGWLG	118,03	2319,176	22	2,5	774,0679	44,63	14474	mf18031	P04179 SO	
FNKER	· · ·	,		,				3_03.raw	DM HUMAN	
I TAASVGVQ									protein1 spl	
GSGWGWLG	117 81	2310 176	22	07	774 0665	46 17	15072	mf18031	P04179ISO	
ENKED	117,01	2010,170		0,7	114,0000	40,17	10072	3_03.raw		
									Divi_TIOIVIAN	
LIAASVGVQ	447.40	0040 470			774 0050	45.05	4 4 9 9 7	mf18031	proteinnispi	
GSGWGWLG	117,12	2319,176	22	-0,9	774,0652	45,65	14867	3 03 raw	P04179 SO	
FNKER								0_00.1an	DM_HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	108,89	2319,176	22	1,5	774,0671	48,74	16065	11110031	P04179 SO	
FNKER				-	-			3_03.raw	DM HUMAN	
I TAASVGVO									protein1lsnl	-
GSGWGWLG	Q/ Q7	2210 176	22	11	1160 5925	53 77	17094	mf18031		
GSGWGWLG	04,07	2319,170	22	-11	1100,3023	55,77	17904	3_03.raw		
FINKER								_		
LIAASVGVQ								mf18031	proteiniispi	
GSGWGWLG	83,44	2319,176	22	-8,7	1160,5852	39,44	12417	3 03 raw	P04179 SO	
FNKER								0_00.14W	DM_HUMAN	
LTAASVGVQ								mf10021	protein1 sp	
GSGWGWLG	80,22	2319,176	22	-8,5	1160,5854	40,51	12847	11116031	P04179 SO	
FNKFR	,	, -		- , -		- , -	-	3_03.raw	DM HUMAN	
I TAASVGVO									protein1 snl	
	76.60	2210 176	22	0.1	1100 5047	40.66	10701	mf18031		
GSGWGWLG	70,03	2319,170	22	-9,1	1100,5647	42,00	13701	3 03.raw	F04179 30	
FNKER								-	DM_HUMAN	
LTAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	71,46	2319,176	22	-10,1	1160,5836	42,07	13465	2.02 1000	P04179 SO	
FNKER								3_03.raw	DM HUMAN	
LTAASVGVQ									protein1 spl	
GSGWGWLG	69.87	2319 176	22	-6.9	1160 5873	37 17	11539	mf18031	P04179ISO	
ENKED	00,07	2010,170	~~	0,0	1100,0070	07,17	11000	3_03.raw		
LIAASVGVQ								mf18031	proteiniispi	
GSGWGWLG	64,78	2319,176	22	-8,3	1160,5857	36,64	11326	3 03 raw	P04179 SO	
FNKER								0_00.14	DM_HUMAN	
LTAASVGVQ								me£10001	protein1 sp	
GSGWGWLG	61.63	2319.176	22	-8.2	1160.5858	35.4	10835	1118031	P04179ISO	
ENKER	- ,	, -		- ,	,	/		3_03.raw	DM HUMAN	
I TAASVGVO									protein1lsnl	-
	60 55	2210 176	22	7.0	1160 5962	127	14111	mf18031		
GSGWGWLG	00,55	2319,170	22	-7,9	1100,5002	43,7	14111	3 03.raw	F04179 30	
FINKER								_	DM_HUMAN	
LIAASVGVQ								mf18031	protein1 sp	
GSGWGWLG	59,96	2319,176	22	-5	1160,5895	44,74	14511	3 03 raw	P04179 SO	
FNKER								5_05.1aw	DM_HUMAN	
LTAASVGVQ								me£10021	protein1 sp	
GSGWGWLG	48	2319.176	22	-4.9	1160.5896	37.69	11741	mf18031	P04179ISO	
ENKER	_	, -		, -	,	- ,		3 03.raw		
I TAASVGVO									DM HUMAN	
	27.02								DM_HUMAN protein1lspl	
GSGWGWLG		2210 176	22	1	1160 5064	40.99	16502	mf18031	DM_HUMAN protein1 sp	
	37,02	2319,176	22	1	1160,5964	49,88	16502	mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
FNKER	37,02	2319,176	22	1	1160,5964	49,88	16502	mf18031 3_03.raw	DM_HUMAN protein1 sp  P04179 SO DM_HUMAN	
FNKER LTAASVGVQ	37,02	2319,176	22	1	1160,5964	49,88	16502	mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp	
FNKER LTAASVGVQ GSGWGWLG	31,59	2319,176 2319,176	22 22	-7,4	1160,5964 1160,5867	49,88 45,26	16502 14716	mf18031 3_03.raw mf18031	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER	31,59	2319,176 2319,176	22 22	1 -7,4	1160,5964 1160,5867	49,88 45,26	16502 14716	mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH	31,59	2319,176 2319,176	22 22	1 -7,4	1160,5964 1160,5867	49,88 45,26	16502 14716	mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN	31,59	2319,176 2319,176 2636,252	22 22 25	1 -7,4 2.1	1160,5964 1160,5867 1319,136	49,88 45,26 23.95	16502 14716 6610	mf18031 3_03.raw mf18031 3_03.raw mf18031	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05	2319,176 2319,176 2636,252	22 22 25	1 -7,4 2,1	1160,5964 1160,5867 1319,136	49,88 45,26 23,95	16502 14716 6610	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK ENGGGHINH	31,59 129,05	2319,176 2319,176 2636,252	22 22 25	1 -7,4 2,1	1160,5964 1160,5867 1319,136	49,88 45,26 23,95	16502 14716 6610	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH	31,59 129,05	2319,176 2319,176 2636,252	22 22 25	1 -7,4 2,1	1160,5964 1160,5867 1319,136	49,88 45,26 23,95	16502 14716 6610	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN	31,59 129,05 124,74	2319,176 2319,176 2636,252 2636,252	22 22 25 25	1 -7,4 2,1 3,5	1160,5964 1160,5867 1319,136 879,761	49,88 45,26 23,95 23,76	16502 14716 6610 6540	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74	2319,176 2319,176 2636,252 2636,252	22 22 25 25	1 -7,4 2,1 3,5	1160,5964 1160,5867 1319,136 879,761	49,88 45,26 23,95 23,76	16502 14716 6610 6540	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH	31,59 129,05 124,74	2319,176 2319,176 2636,252 2636,252	22 22 25 25	1 -7,4 2,1 3,5	1160,5964 1160,5867 1319,136 879,761	49,88 45,26 23,95 23,76	16502 14716 6610 6540	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN SIFWTNLSPN	31,59 129,05 124,74 124,39	2319,176 2319,176 2636,252 2636,252 2636,252	22 22 25 25 25 25	1 -7,4 2,1 3,5 4,3	1160,5964 1160,5867 1319,136 879,761 879,7617	49,88 45,26 23,95 23,76 24,28	16502 14716 6610 6540 6724	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39	2319,176 2319,176 2636,252 2636,252 2636,252	22 22 25 25 25 25	1 -7,4 2,1 3,5 4,3	1160,5964 1160,5867 1319,136 879,761 879,7617	49,88 45,26 23,95 23,76 24,28	16502 14716 6610 6540 6724	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH	31,59 129,05 124,74 124,39	2319,176 2319,176 2636,252 2636,252 2636,252	22 22 25 25 25 25	1 -7,4 2,1 3,5 4,3	1160,5964 1160,5867 1319,136 879,761 879,7617	49,88 45,26 23,95 23,76 24,28	16502 14716 6610 6540 6724	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp]	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN	31,59 129,05 124,74 124,39 69,33	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374	49,88 45,26 23,95 23,76 24,28 39,98	16502 14716 6610 6540 6724 12638	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031	DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO DM HUMAN protein1[sp] P04179[SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39 69,33	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374	<ul> <li>49,88</li> <li>45,26</li> <li>23,95</li> <li>23,76</li> <li>24,28</li> <li>39,98</li> </ul>	16502 14716 6610 6540 6724 12638	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM HUMAN protein1 sp  P04179 SO DM HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39 69,33	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374	<ul> <li>49,88</li> <li>45,26</li> <li>23,95</li> <li>23,76</li> <li>24,28</li> <li>39,98</li> </ul>	16502 14716 6610 6540 6724 12638	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39 69,33	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374	49,88 45,26 23,95 23,76 24,28 39,98	16502 14716 6610 6540 6724 12638	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1 sp          P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN	31,59 129,05 124,74 124,39 69,33 51,94	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736	49,88 45,26 23,95 23,76 24,28 39,98 39,47	16502 14716 6610 6540 6724 12638 12426	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1 sp          P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         HUMAN           protein1 sp            P04179 SO           DM         PUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39 69,33 51,94	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736	49,88 45,26 23,95 23,76 24,28 39,98 39,47	16502 14716 6610 6540 6724 12638 12426	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM_HUMAN         Protein1[sp]           P04179 SO         DM_HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH	31,59 129,05 124,74 124,39 69,33 51,94	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736	49,88 45,26 23,95 23,76 24,28 39,98 39,47	16502 14716 6610 6540 6724 12638 12426	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN	31,59 129,05 124,74 124,39 69,33 51,94 49,41	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9 -20,8	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736 879,7396	<ul> <li>49,88</li> <li>45,26</li> <li>23,95</li> <li>23,76</li> <li>24,28</li> <li>39,98</li> <li>39,47</li> <li>42,58</li> </ul>	16502 14716 6610 6540 6724 12638 12426 13671	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	31,59 129,05 124,74 124,39 69,33 51,94 49,41	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9 -20,8	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736 879,7396	<ul> <li>49,88</li> <li>45,26</li> <li>23,95</li> <li>23,76</li> <li>24,28</li> <li>39,98</li> <li>39,47</li> <li>42,58</li> </ul>	16502 14716 6610 6540 6724 12638 12426 13671	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH	31,59 129,05 124,74 124,39 69,33 51,94 49,41	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9 -20,8	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,736 879,7396	49,88 45,26 23,95 23,76 24,28 39,98 39,47 42,58	16502 14716 6610 6540 6724 12638 12426 13671	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN	31,59 129,05 124,74 124,39 69,33 51,94 49,41 46,85	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9 -20,8	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,7374 879,736 879,7396	<ul> <li>49,88</li> <li>45,26</li> <li>23,95</li> <li>23,76</li> <li>24,28</li> <li>39,98</li> <li>39,47</li> <li>42,58</li> <li>41,54</li> </ul>	16502 14716 6610 6540 6724 12638 12426 13671 13256	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1[sp]         P04179 SO           DM         HUMAN	
FNKER LTAASVGVQ GSGWGWLG FNKER FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK FNGGGHINH SIFWTNLSPN GGGEPK	37,02 31,59 129,05 124,74 124,39 69,33 51,94 49,41 46,85	2319,176 2319,176 2636,252 2636,252 2636,252 2636,252 2636,252 2636,252	22 22 25 25 25 25 25 25 25 25 25	1 -7,4 2,1 3,5 4,3 -23,4 -24,9 -20,8 -22,8	1160,5964 1160,5867 1319,136 879,761 879,7617 879,7374 879,7374 879,7396 879,7396	49,88 45,26 23,95 23,76 24,28 39,98 39,47 42,58 41,54	16502         14716         6610         6540         6724         12638         12426         13671         13256	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	DM         HUMAN           protein1 sp          P04179 SO           DM         HUMAN	

FNGGGHINH SIFWTNLSPN	34,93	2636,252	25	-17,1	1319,1107	36,42	11237	mf18031	protein1 sp  P04179 SO
GGGEPK								5_03.1aw	DM_HUMAN
FNGGGHINH	25 72	2626.252	25	0.1	1210 1226	25.20	10701	mf18031	protein1 sp
GGGEPK	25,72	2030,252	20	-0, 1	1319,1220	35,29	10791	3_03.raw	DM HUMAN
FNGGGHINH								mf10021	protein1 sp
SIFWTNLSPN	20,6	2636,252	25	-12,9	1319,1162	35,9	11027	3 03 raw	P04179 SO
GGGEPK								0_00.14W	DM_HUMAN
EKLTAASVGV	125 37	2576 3135	24	24	850 7805	20.60	8674	mf18031	protein1 sp
GENKER	120,07	2070,0100	24	2,4	003,7000	23,03	0074	3_03.raw	DM HUMAN
EKLTAASVGV								mf10021	protein1 sp
QGSGWGWL	121,48	2576,3135	24	3,3	859,7812	29,16	8482	3 03 raw	P04179 SO
GFNKER								0_00.10W	DM_HUMAN
EKLTAASVGV	106.01	2576 3135	24	7 1	1280 1732	20.25	9514	mf18031	protein1 sp
GENKER	100,01	2070,3130	24	7,1	1209,1732	29,25	0014	3_03.raw	DM HUMAN
EKLTAASVGV								me£10001	protein1 sp
QGSGWGWL	100,83	2576,3135	24	-7,6	859,7719	34,94	10656	mf18031	P04179 SO
GFNKER								3_03.1aw	DM_HUMAN
EKLTAASVGV	54.50	0570 0405	04	40.0	050 744	00.00	0700	mf18031	protein1 sp
	54,53	25/0,3135	24	-43,0	859,741	32,69	9796	3_03.raw	
EKLTAASVGV								610001	protein1lspl
QGSGWGWL	25,69	2576,3135	24	-11	859,769	39,26	12341	mf18031	P04179 SO
GFNKER								3_03.raw	DM_HUMAN
EKLTAASVGV								mf18031	protein1 sp
QGSGWGWL	23,86	2576,3135	24	-13,4	859,7669	40,99	13036	3_03.raw	P04179 SO
								_	DM_HUMAN
QGSGWGWL	20.33	2576.3135	24	-2.7	859,7761	53.54	17897	mf18031	P04179ISO
GFNKER	,			_,.	,	,		3_03.raw	DM_HUMAN
ΗΗΔΔΥΥΝΝΙ								mf18031	protein1 sp
NVTEEK	123,56	1737,8434	15	1,7	869,9304	13,81	2946	3 03.raw	P04179 SO
									DM_HUMAN
HHAAYVNNL	102 46	1737 8434	15	-27	580 2869	17 11	4157	mf18031	P04179ISO
NVTEEK	102,10		10	<b>_</b> ,.	000,2000	,	1101	3_03.raw	DM HUMAN
								mf18031	protein1 sp
NVTEEK	98,79	1737,8434	15	3,4	580,2903	20,06	5227	3 03 raw	P04179 SO
								0_00.1.a.i	DM_HUMAN
HHAAYVNNL	98 44	1737 8434	15	87	580 2035	13 79	2038	mf18031	
NVTEEK	50,44	1707,0404	10	0,7	500,2505	10,70	2000	3_03.raw	DM HUMAN
									protein1 sp
	92,79	1737,8434	15	4,2	580,2908	24,49	6800	3 03 raw	P04179 SO
								5_00.1aw	DM_HUMAN
HHAAYVNNL	80.80	1737 8/3/	15	37	580 2005	10.55	5034	mf18031	protein1 sp
NVTEEK	09,09	1757,0454	15	3,7	560,2905	19,55	5054	3_03.raw	DM HUMAN
									protein1 sp
	89,75	1737,8434	15	2,3	580,2897	26,2	7418	mf18031	P04179 SO
INVIEER								3_03.1aw	DM_HUMAN
HHAAYVNNL				<b>F</b> 4	500.0044	10.01		mf18031	protein1 sp
NVTEEK	00 74	4707 0404	4 -				4470	111110031	D04470100
	89,71	1737,8434	15	5,1	580,2914	10,01	4476	3_03.raw	P04179 SO
	89,71	1737,8434	15	5,1	580,2914	10,01	4476	3_03.raw	P04179 SO DM HUMAN protein1 sp
HHAAYVNNL	89,71 85,08	1737,8434 1737,8434	15 15	0,8	580,2914	22,13	4476 5951	3_03.raw	P04179 SO DM HUMAN protein1 sp  P04179 SO
HHAAYVNNL NVTEEK	89,71 85,08	1737,8434 1737,8434	15 15	5,1 0,8	580,2914 580,2889	22,13	4476 5951	3_03.raw mf18031 3_03.raw	P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN
HHAAYVNNL NVTEEK HHAAYVNNL	89,71	1737,8434 1737,8434	15	0,8	580,2914	22,13	4476 5951	3_03.raw mf18031 3_03.raw mf18031	P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  D04112020
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK	89,71 85,08 82,73	1737,8434 1737,8434 1737,8434	15 15 15	0,8 1,5	580,2914 580,2889 580,2892	22,13 25,29	4476 5951 7088	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO           DM HUMAN           protein1 sp            P04179 SO           DM HUMAN           protein1 sp            P04179 SO           DM HUMAN           protein1 sp            P04179 SO           DM HUMAN
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK	89,71 85,08 82,73	1737,8434 1737,8434 1737,8434	15 15 15	5,1 0,8 1,5	580,2914 580,2889 580,2892	22,13 25,29	4476 5951 7088	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO DM HUMAN protein1 sp  P04179 SO DM HUMAN protein1 sp  P04179 SO DM_HUMAN protein1 sp
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL	89,71 85,08 82,73 74,62	1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15	5,1 0,8 1,5 -0,5	580,2914 580,2889 580,2892 580,2881	22,13 25,29 23,77	4476 5951 7088 6544	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM_HUMAN         protein1 sp          P04179 SO         DM_HUMAN         protein1 sp          P04179 SO         DM_HUMAN         Protein1 sp          P04179 SO
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK	89,71 85,08 82,73 74,62	1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15	5,1 0,8 1,5 -0,5	580,2914 580,2889 580,2892 580,2881	22,13 25,29 23,77	4476 5951 7088 6544	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM_HUMAN
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNI	89,71 85,08 82,73 74,62	1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15	5,1 0,8 1,5 -0,5	580,2914 580,2889 580,2892 580,2881	22,13 25,29 23,77	4476 5951 7088 6544	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM_HUMAN         protein1 sp          P04179 SO
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK	89,71 85,08 82,73 74,62 72,58	1737,8434 1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15 15	5,1 0,8 1,5 -0,5 -1,1	580,2914 580,2889 580,2892 580,2881 580,2878	22,13 25,29 23,77 26,77	4476 5951 7088 6544 7621	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM_HUMAN         Protein1 sp          P04179 SO         PM_HUMAN
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK	89,71 85,08 82,73 74,62 72,58	1737,8434 1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15 15	5,1 0,8 1,5 -0,5 -1,1	580,2914 580,2889 580,2892 580,2881 580,2878	22,13 25,29 23,77 26,77	4476 5951 7088 6544 7621	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO           DM HUMAN           protein1 sp            P04179 SO           DM HUMAN           protein1 sp            P04179 SO           DM_HUMAN
HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL NVTEEK HHAAYVNNL	<ul> <li>89,71</li> <li>85,08</li> <li>82,73</li> <li>74,62</li> <li>72,58</li> <li>62,56</li> </ul>	1737,8434 1737,8434 1737,8434 1737,8434 1737,8434	15 15 15 15 15 15	5,1 0,8 1,5 -0,5 -1,1 3,1	580,2914 580,2889 580,2892 580,2881 580,2878 580,2902	22,13 25,29 23,77 26,77 37,1	4476 5951 7088 6544 7621 11510	mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw mf18031 3_03.raw	P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM HUMAN         protein1 sp          P04179 SO         DM_HUMAN         protein1 sp          P04179 SO

HHAAYVNNL NVTEEK	58,02	1737,8434	15	2,4	580,2898	49,74	16446	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEPHINAQ IMQLHH	114,88	3008,4602	26	-0,1	1003,8273	32,27	9632	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	112,37	1423,8035	14	3,2	712,9113	37,95	11840	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	111,69	1423,8035	14	2,3	712,9106	35,14	10733	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	111,38	1423,8035	14	3,3	712,9114	36,4	11227	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	110,73	1423,8035	14	3,8	712,9117	34,12	10339	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	110,16	1423,8035	14	5,1	712,9127	36,92	11440	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	109,76	1423,8035	14	4,4	712,9122	34,62	10535	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	109,21	1423,8035	14	5,8	712,9131	37,44	11643	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	109,04	1423,8035	14	3,7	712,9116	30,04	8805	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	108,65	1423,8035	14	1,8	712,9103	39,59	12476	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	107,97	1423,8035	14	3,5	712,9115	32,09	9566	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	106,74	1423,8035	14	2,7	712,9109	39,08	12273	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	106,4	1423,8035	14	2,7	712,9109	40,61	12887	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,92	1423,8035	14	2,3	712,9106	29,53	8615	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,47	1423,8035	14	3,8	712,9117	31,05	9174	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,42	1423,8035	14	2,1	712,9105	33,61	10144	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,17	1423,8035	14	1,7	712,9102	30,54	8989	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,13	1423,8035	14	3,9	712,9118	41,12	13088	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	105,02	1423,8035	14	3,6	712,9116	43,67	14097	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,72	1423,8035	14	2,5	712,9108	52,42	17465	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,57	1423,8035	14	3,3	712,9114	32,6	9757	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,56	1423,8035	14	0,8	712,9095	28,51	8254	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,48	1423,8035	14	2,6	712,9109	52,93	17658	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,38	1423,8035	14	2,5	712,9108	51,4	17077	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
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GDVTAQIALQ PALK	104,35	1423,8035	14	2	712,9105	29,03	8435	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	104,32	1423,8035	14	2,6	712,9109	28	8069	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	104,24	1423,8035	14	3,8	712,9117	26,47	7511	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	104,16	1423,8035	14	3,3	712,9114	42,66	13700	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	104,14	1423,8035	14	3,3	712,9114	45,74	14902	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,14	1423,8035	14	2,9	712,9111	40,1	12684	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	104,1	1423,8035	14	0,3	712,9092	53,43	17855	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	103,91	1423,8035	14	1	712,9097	49,86	16492	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	103,75	1423,8035	14	3	712,9111	41,63	13290	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	103,37	1423,8035	14	2,8	712,911	51,91	17271	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	103,36	1423,8035	14	2,6	712,9108	42,15	13498	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,74	1423,8035	14	5	712,9125	38,55	12065	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,73	1423,8035	14	3,1	712,9112	31,57	9371	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,41	1423,8035	14	0,7	712,9095	53,96	18057	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,4	1423,8035	14	3,5	712,9115	48,85	16104	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,37	1423,8035	14	3,3	712,9114	50,88	16878	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	102,01	1423,8035	14	0,8	712,9096	26,98	7698	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,78	1423,8035	14	3	712,9111	50,37	16685	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,57	1423,8035	14	0,6	712,9094	48,34	15914	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,44	1423,8035	14	3,4	712,9114	33,1	9948	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,39	1423,8035	14	2,8	712,911	49,35	16296	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,33	1423,8035	14	3,4	712,9114	45,22	14699	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	101,24	1423,8035	14	6,5	712,9136	25,95	7320	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GDVTAQIALQ PALK	101,24	1423,8035	14	3,5	712,9115	44,18	14297	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	100,11	1423,8035	14	2,8	712,911	47,81	15710	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	99,57	1423,8035	14	3	712,9111	47,28	15509	mf18031 3_03.raw	protein1 sp  P04179 SO	
GDVTAQIALQ PALK	99,54	1423,8035	14	2,9	712,9111	46,24	15100	mf18031 3_03.raw	protein1 sp  P04179 SO	
GDVTAQIALQ PALK	99,42	1423,8035	14	2	712,9104	46,77	15310	mf18031 3_03.raw	protein1 sp  P04179 SO	
GDVTAQIALQ PALK	99,17	1423,8035	14	2,9	712,9111	43,16	13899	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	98,97	1423,8035	14	1,8	712,9103	27,49	7877	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	98,67	1423,8035	14	4,2	712,912	44,69	14497	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	93,37	1423,8035	14	4,3	712,912	25,44	7144	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	91,33	1423,8035	14	3,2	475,61	26,52	7529	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	91,32	1423,8035	14	2,4	475,6096	26	7340	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	87,13	1423,8035	14	2,2	475,6095	25,49	7162	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	84,55	1423,8035	14	5,3	475,6109	28,8	8355	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	83,4	1423,8035	14	1,8	712,9103	54,87	18384	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	81,09	1423,8035	14	3	475,6098	27,71	7956	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	79,39	1423,8035	14	3	475,6098	33,63	10150	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	78,15	1423,8035	14	4,9	475,6107	35,79	10985	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	76,49	1423,8035	14	3,9	475,6103	32,05	9548	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	73,68	1423,8035	14	4	475,6104	35,25	10773	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	73,55	1423,8035	14	2,9	475,6098	34,13	10346	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	72,79	1423,8035	14	3,3	475,61	44,47	14410	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	72,37	1423,8035	14	4,5	475,6106	34,65	10546	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	71,62	1423,8035	14	3	475,6099	30,24	8880	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	69,12	1423,8035	14	2,5	475,6096	32,57	9747	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GDVTAQIALQ PALK	68,23	1423,8035	14	2,1	475,6094	42,3	13560	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	67,58	1423,8035	14	3,7	475,6102	41,74	13338	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	67,03	1423,8035	14	3,1	475,6099	31,53	9354	mf18031 3_03.raw	protein1 sp  P04179 SO	
GDVTAQIALQ PALK	66,82	1423,8035	14	4,3	475,6105	37,42	11634	mf18031 3_03.raw	protein1 sp  P04179 SO	
GDVTAQIALQ PALK	66,63	1423,8035	14	3,3	475,61	43,44	14005	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	65,98	1423,8035	14	3,9	475,6103	30,77	9074	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	65,75	1423,8035	14	2,4	475,6096	48,17	15849	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	64,4	1423,8035	14	3	475,6099	45,08	14647	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	64,21	1423,8035	14	1,3	475,609	47,14	15458	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	63,94	1423,8035	14	3,1	475,6099	50,8	16846	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	63,75	1423,8035	14	2,8	475,6098	39,46	12424	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	62,49	1423,8035	14	2,4	475,6096	40,68	12914	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	62,11	1423,8035	14	3,2	475,61	36,31	11189	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	60,77	1423,8035	14	1,5	475,6091	52,34	17434	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	60,63	1423,8035	14	3,1	475,6099	46,62	15246	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	59,28	1423,8035	14	2,8	475,6097	51,32	17042	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	58,14	1423,8035	14	1,7	475,6093	51,83	17241	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	57,16	1423,8035	14	1,1	475,6089	38,21	11942	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	55,66	1423,8035	14	3,9	475,6103	50,28	16650	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	55,52	1423,8035	14	3	475,6098	47,66	15652	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	55,23	1423,8035	14	0,9	475,6089	38,94	12218	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	53,86	1423,8035	14	0,2	475,6085	54	18073	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	53,58	1423,8035	14	2,8	475,6098	46,1	15045	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GDVTAQIALQ PALK	52,69	1423,8035	14	4,4	475,6105	52,96	17671	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GDVTAQIALQ PALK	52,46	1423,8035	14	3,1	475,6099	43,95	14207	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PALK	47,99	1423,8035	14	3,5	475,6101	36,9	11431	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AAYVNNLNVT EEKYQEALAK	112,22	2267,1433	20	2,2	756,7234	23,84	6568	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPYDY GALEPHINAQ	110,07	2249,0752	20	3,8	750,7018	31,79	9452	mf18031 3_03.raw	protein1 sp  P04179 SO	
HSLPDLPYDY GALEPHINAQ	87,09	2249,0752	20	3,4	750,7015	32,35	9662	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPYDY GALEPHINAQ	39,06	2249,0752	20	26,4	750,7188	33,68	10171	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	104,5	1742,874	14	2,3	872,4463	46,19	15079	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	101,98	1742,874	14	2,6	872,4466	47,74	15685	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	101,27	1742,874	14	0	872,4443	48,25	15880	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	100,73	1742,874	14	4	872,4478	44,14	14281	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	100,66	1742,874	14	3,9	872,4477	45,68	14880	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	100,19	1742,874	14	3,8	872,4476	45,17	14683	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	100,01	1742,874	14	2,2	872,4462	46,71	15284	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	99,25	1742,874	14	3,8	872,4476	43,62	14076	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	98,57	1742,874	14	3,8	872,4476	43,1	13875	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	98,55	1742,874	14	2,5	872,4465	50,3	16658	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	98,08	1742,874	14	4,4	872,4481	44,65	14481	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	95,6	1742,874	14	2,6	872,4465	49,27	16264	mf18031 3_03.raw	protein1 sp  P04179 SO <u>DM_HUMAN</u>	
AIWNVINWEN VTER	95,58	1742,874	14	0,6	872,4448	49,78	16461	mf18031 3_03.raw	protein1 sp  P04179 SO <u>DM_HUMAN</u>	
AIWNVINWEN VTER	95,17	1742,874	14	2,7	872,4467	47,22	15486	mf18031 3_03.raw	protein1 sp  P04179 SO <u>DM_HUMAN</u>	
AIWNVINWEN VTER	93,97	1742,874	14	2,5	581,9667	46,4	15158	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	93,79	1742,874	14	4,2	872,4479	41,52	13245	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	93,69	1742,874	14	2,8	872,4467	48,76	16072	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	93,51	1742,874	14	3,1	581,9671	45,89	14960	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

AIWNVINWEN VTER	92,69	1742,874	14	1,9	872,4459	51,35	17054	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	91,85	1742,874	14	3,9	581,9675	44,87	14562	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	91,83	1742,874	14	3,1	872,447	50,82	16854	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	91,53	1742,874	14	3,5	872,4473	39,42	12406	mf18031 3_03.raw	protein1 sp  P04179 SO	
AIWNVINWEN VTER	91,09	1742,874	14	3,8	872,4476	52,88	17641	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	91,06	1742,874	14	2,5	872,4465	51,85	17250	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	90,77	1742,874	14	2,2	872,4462	52,37	17446	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	90,49	1742,874	14	3,3	872,4472	38,89	12198	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	89,12	1742,874	14	3,1	872,447	38,37	12006	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	88,79	1742,874	14	2,2	581,9666	48,15	15843	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	88,32	1742,874	14	3,7	581,9674	44,34	14361	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	88,29	1742,874	14	3,3	581,9672	42,78	13751	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	87,96	1742,874	14	-1	872,4434	53,92	18042	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	87,23	1742,874	14	4	581,9676	40,72	12929	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	87,12	1742,874	14	4,2	581,9677	45,38	14765	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	87,03	1742,874	14	3,6	581,9673	41,23	13132	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	86,24	1742,874	14	2,5	581,9667	49,17	16227	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	83,09	1742,874	14	2,9	581,967	38,66	12102	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	82,89	1742,874	14	3,3	581,9672	51,21	17003	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	81,61	1742,874	14	1,1	581,9659	53,77	17982	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	78,99	1742,874	14	1,6	581,9662	53,26	17789	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	78,68	1742,874	14	3,1	581,9671	39,16	12303	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	78,32	1742,874	14	2,2	581,9666	52,76	17593	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	77,72	1742,874	14	1,1	581,9659	49,69	16427	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

AIWNVINWEN VTER	76,5	1742,874	14	3	581,967	52,24	17399	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	75,5	1742,874	14	3,3	581,9672	50,19	16617	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	74,19	1742,874	14	1,6	581,9662	54,28	18181	mf18031 3_03.raw	protein1 sp  P04179 SO	
AIWNVINWEN VTER	73,86	1742,874	14	5,3	872,4489	37,87	11810	mf18031 3_03.raw	protein1 sp  P04179 SO	
AIWNVINWEN VTER	72,88	1742,874	14	2,7	581,9669	50,7	16811	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTER	71,67	1742,874	14	1,6	581,9662	51,73	17204	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	61,6	1742,874	14	-9,2	872,4363	54,92	18395	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AIWNVINWEN VTER	23,71	1742,874	14	-0,4	581,965	26,81	7634	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
LNVTEEKYQE ALAK	103,62	1634,8516	14	-0,2	818,4329	17,28	4210	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAKGD VTAQIALQPA LK	102,65	2227,2212	21	2	743,4158	26,49	7520	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAKGD VTAQIALQPA LK	38,6	2227,2212	21	0,7	743,4149	53,52	17890	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YVNNLNVTEE KYQEALAK	102,42	2125,0691	18	2,9	709,3657	21,94	5878	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YVNNLNVTEE KYQEALAK	47,85	2125,0691	18	3,3	709,366	22,45	6068	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLKAI WNVINWENV TER	101,44	2728,4084	22	-5	910,4722	43,99	14221	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLKAI WNVINWENV TER	92,06	2728,4084	22	-7,3	910,4701	47,12	15449	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLKAI WNVINWENV TER	36,69	2728,4084	22	-9,3	910,4683	48,77	16078	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VGVQGSGW GWLGFNK	98,46	1590,7943	15	4,1	796,4077	35,37	10820	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GGGHINHSIF WTNLSPNGG GEPK	96,91	2375,1406	23	0,7	792,7214	21,45	5704	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPYDY GALEPH	95,57	1822,8525	16	2,4	912,4357	29,37	8556	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPYDY GALEPH	93,96	1822,8525	16	1,8	608,6259	29,34	8548	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VTEEKYQEAL AK	95,49	1407,7245	12	1	704,8702	13,17	2705	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VTEEKYQEAL AK	74,81	1407,7245	12	2,1	470,2498	13,2	2715	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VTEEKYQEAL AK	55,28	1407,7245	12	13,5	704,879	18,66	4719	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VTEEKYQEAL AK	52,22	1407,7245	12	-7,4	704,8643	18,4	4630	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

VTEEKYQEAL AK	37,71	1407,7245	12	-0,6	704,8691	19,17	4903	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
VTEEKYQEAL AK	16,03	1407,7245	12	-0,6	704,8691	20,27	5304	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GHINHSIFWT NLSPNGGGE PK	94,5	2261,0977	21	1	754,7073	21,75	5809	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
RDFGSFDKF K	93,44	1245,6141	10	3,9	416,2136	16,14	3826	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
RDFGSFDKF K	55,32	1245,6141	10	2,8	623,8161	16,14	3828	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HAAYVNNLN VTEEK	92,82	1600,7845	14	6,3	801,4046	14,97	3374	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HAAYVNNLN VTEEK	73,41	1600,7845	14	6,2	534,6054	15	3383	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	91,45	1346,6506	11	1,4	674,3336	19,47	5005	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	90,14	1346,6506	11	2,4	674,3342	17,93	4446	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	88,93	1346,6506	11	-0,7	674,3322	18,45	4648	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	87,57	1346,6506	11	-0,1	674,3325	18,96	4823	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	84,85	1346,6506	11	2,1	674,334	19,98	5200	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	83,87	1346,6506	11	7,6	674,3377	17,42	4260	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	75,97	1346,6506	11	0,1	449,8909	18,44	4647	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	75,83	1346,6506	11	3,2	449,8923	19,97	5194	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	75,1	1346,6506	11	2,7	449,892	20,48	5375	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	75,03	1346,6506	11	1,5	449,8915	18,96	4822	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	74,85	1346,6506	11	2	449,8917	45,91	14971	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	74,27	1346,6506	11	2,1	449,8918	19,46	5003	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	74,01	1346,6506	11	1,9	449,8917	42,51	13640	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	73,8	1346,6506	11	2,8	449,8921	47,45	15571	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	73,67	1346,6506	11	9,3	449,895	17,92	4443	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	73,53	1346,6506	11	4	674,3353	15,96	3755	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	73,08	1346,6506	11	3,5	449,8924	43,82	14156	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDKFK EK	72,84	1346,6506	11	3,8	449,8925	44,87	14563	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	72,77	1346,6506	11	2	449,8917	22,03	5910	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	71,93	1346,6506	11	3,8	449,8925	15,95	3752	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	71,68	1346,6506	11	3,6	449,8925	45,38	14766	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK EK	71,25	1346,6506	11	2,7	449,892	51,56	17135	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	70,89	1346,6506	11	3,1	449,8922	40,94	13018	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	70,84	1346,6506	11	3,3	449,8923	40,42	12812	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	70,02	1346,6506	11	2,9	449,8921	50,02	16556	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	69,95	1346,6506	11	2,6	449,892	21,52	5727	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	69,72	1346,6506	11	2,6	449,892	41,99	13433	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	69,55	1346,6506	11	5,1	449,8931	37,52	11675	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	69,53	1346,6506	11	2,5	449,8919	52,06	17331	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK EK	69,51	1346,6506	11	1,5	449,8915	52,57	17519	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	69,51	1346,6506	11	3,3	449,8923	47,97	15771	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	69,44	1346,6506	11	0,7	449,8911	21	5556	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	69,34	1346,6506	11	-2	449,8899	38,03	11869	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,8	1346,6506	11	2,9	449,8921	51,05	16941	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,79	1346,6506	11	2,4	449,8919	50,53	16745	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,74	1346,6506	11	2,3	449,8918	48,48	15969	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,73	1346,6506	11	0,9	449,8912	31,29	9269	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,5	1346,6506	11	2,6	449,892	35,93	11042	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,45	1346,6506	11	3,6	449,8924	44,35	14362	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,16	1346,6506	11	2,8	449,8921	49	16163	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK EK	68,07	1346,6506	11	2,2	449,8918	46,43	15171	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDKFK EK	67,94	1346,6506	11	3,5	449,8924	29,75	8697	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	67,79	1346,6506	11	3,8	449,8925	33,87	10242	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	67,71	1346,6506	11	3,7	449,8925	33,35	10042	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	67,38	1346,6506	11	3,8	449,8925	41,46	13222	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	67,38	1346,6506	11	2,5	449,8919	53,08	17717	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	67,35	1346,6506	11	4,2	674,3354	21,53	5728	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	67,24	1346,6506	11	2,9	449,8921	46,95	15380	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	66,86	1346,6506	11	3	449,8922	30,25	8886	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	66,7	1346,6506	11	4,3	449,8927	31,81	9459	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	66,55	1346,6506	11	2,2	449,8918	39,36	12383	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	66,54	1346,6506	11	1,5	449,8915	34,4	10448	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	66,32	1346,6506	11	4	449,8926	30,76	9071	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	65,9	1346,6506	11	2,6	449,892	49,52	16360	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	65,55	1346,6506	11	1,3	449,8914	43,3	13952	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,99	1346,6506	11	3,7	449,8925	34,92	10646	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,89	1346,6506	11	3	449,8922	32,32	9651	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,66	1346,6506	11	3,2	449,8922	39,9	12601	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,57	1346,6506	11	3,8	449,8925	26,43	7495	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,2	1346,6506	11	2,6	449,892	37	11471	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	64,01	1346,6506	11	1,3	449,8914	26,96	7687	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	63,91	1346,6506	11	3,2	449,8922	23,3	6367	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	63,7	1346,6506	11	3,2	674,3348	22,36	6038	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	62,32	1346,6506	11	3	449,8922	29,22	8504	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	59,32	1346,6506	11	0,3	449,891	53,59	17919	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN

DFGSFDKFK EK	59,29	1346,6506	11	4,4	449,8928	22,79	6188	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	58,62	1346,6506	11	2,5	449,8919	27,47	7869	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	57,98	1346,6506	11	3,4	449,8923	35,42	10840	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK EK	56,37	1346,6506	11	3,9	449,8926	27,99	8065	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	55,59	1346,6506	11	0,4	449,891	25,9	7305	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	50,76	1346,6506	11	1,3	449,8914	54,11	18116	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	47,64	1346,6506	11	0,8	674,3331	15,15	3443	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	20,97	1346,6506	11	-1	449,8904	16,45	3939	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK EK	18,25	1346,6506	11	4,9	449,893	14,76	3301	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSIFWTNLSP NGGGEPK	91,04	1839,8904	17	4,7	614,3069	24,33	6744	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSIFWTNLSP NGGGEPK	54,01	1839,8904	17	3,4	614,3062	24,91	6949	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
HSIFWTNLSP NGGGEPK	32,95	1839,8904	17	8,1	920,96	33,11	9951	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSIFWTNLSP NGGGEPK	22,16	1839,8904	17	14,4	920,9658	29,86	8742	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSIFWTNLSP NGGGEPK	19,33	1839,8904	17	6	920,9579	30,53	8985	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
HSIFWTNLSP NGGGEPK	15,91	1839,8904	17	-10	920,9433	27,89	8025	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
NLNVTEEKY QEALAK	89,56	1748,8944	15	1,6	583,973	18,34	4600	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
NLNVTEEKY QEALAK	79,24	1748,8944	15	1,9	583,9732	18,84	4781	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
NLNVTEEKY QEALAK	74,36	1748,8944	15	-1,7	875,453	18,85	4783	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
NLNVTEEKY QEALAK	19,87	1748,8944	15	-1,8	875,4529	20,39	5343	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
KAIWNVINWE NVTER	87,35	1870,969	15	2,7	624,6653	32,07	9555	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
SIFWTNLSPN GGGEPK	87,23	1702,8314	16	5	852,4272	30,07	8815	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
SIFWTNLSPN GGGEPK	20,1	1702,8314	16	-14,6	852,4106	31,87	9484	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
LGIDVWEHA YYLQYK	86,96	1896,941	15	1,4	633,3218	33,47	10089	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
LGIDVWEHA YYLQYK	85,9	1896,941	15	3,7	633,3233	33,98	10289	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN

VTAQIALQPA LK	86,63	1251,755	12	4,4	626,8875	22,63	6131	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VTAQIALQPA LK	54,01	1251,755	12	-0,5	626,8845	25,62	7209	mf18031 3_03.raw	protein1 sp  P04179 SO	
HSLPDLPYDY GALEPHINAQ	84,95	2493,1997	22	4,4	832,0775	36,22	11153	mf18031 3_03.raw	protein1 sp  P04179 SO	
HSLPDLPYDY GALEPHINAQ	56,19	2493,1997	22	4,8	1247,6132	36,32	11193	mf18031 3_03.raw	protein1 sp  P04179 SO	
GGHINHSIFW TNLSPNGGG	84,7	2318,1191	22	4,5	773,7172	21,55	5736	mf18031 3_03.raw	protein1 sp  P04179 SO	
AYVNNLNVTE EKYQEALAK	84,63	2196,1062	19	2,4	733,0444	23,23	6344	mf18031 3_03.raw	protein1 sp  P04179 SO	
AYVNNLNVTE EKYQEALAK	49,2	2196,1062	19	2	1099,0626	23,31	6372	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
ALEPHINAQI MQLHHSK	84,5	1966,0206	17	4,4	656,3503	17,44	4266	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
RDFGSFDKF KEK	84,04	1502,7517	12	4,5	501,9268	14,45	3175	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
RDFGSFDKF KEK	58,21	1502,7517	12	3,7	752,3859	14,45	3176	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	83,96	1089,5131	9	2,3	545,7651	24,56	6825	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	83,76	1089,5131	9	4	545,766	23,03	6275	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	83,3	1089,5131	9	3,6	545,7657	22	5901	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,99	1089,5131	9	4	545,766	45,47	14798	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,48	1089,5131	9	2,4	545,7651	52,26	17404	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,36	1089,5131	9	2,6	545,7652	41,82	13367	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,25	1089,5131	9	3,6	545,7657	39,76	12547	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,12	1089,5131	9	2,1	545,765	42,35	13578	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	82,08	1089,5131	9	4,5	545,7662	22,52	6092	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	81,79	1089,5131	9	3,5	545,7657	43,9	14190	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	81,79	1089,5131	9	3,8	545,7659	24,05	6646	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	81,73	1089,5131	9	3,3	545,7656	45,97	14997	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	81,64	1089,5131	9	1,4	545,7646	23,55	6458	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	81,61	1089,5131	9	-0,9	545,7633	38,17	11925	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDKFK	81,29	1089,5131	9	5,1	545,7666	38,73	12134	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	81,15	1089,5131	9	1,3	545,7645	51,75	17211	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	80,96	1089,5131	9	5,9	545,767	31,9	9490	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	80,88	1089,5131	9	2,7	545,7653	35,6	10912	mf18031 3_03.raw	protein1 sp  P04179 SO
DFGSFDKFK	80,14	1089,5131	9	3,3	545,7656	42,88	13789	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	80,01	1089,5131	9	3,3	545,7656	29,49	8601	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	80	1089,5131	9	3,3	545,7656	32,99	9906	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,92	1089,5131	9	3	545,7654	48,16	15844	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,74	1089,5131	9	2,4	545,7651	33,51	10102	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,7	1089,5131	9	3	545,7654	34,03	10308	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,63	1089,5131	9	3,1	545,7655	44,95	14596	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,6	1089,5131	9	3,3	545,7656	43,39	13987	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	79,45	1089,5131	9	3,3	545,7656	46,51	15206	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	79,15	1089,5131	9	4,6	545,7663	32,42	9688	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	79,13	1089,5131	9	4,6	545,7663	30,81	9088	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	78,96	1089,5131	9	1,4	545,7646	53,32	17808	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,8	1089,5131	9	2,7	545,7653	51,23	17011	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	78,77	1089,5131	9	3,7	545,7658	36,11	11114	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,77	1089,5131	9	2,4	545,7651	40,27	12750	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,68	1089,5131	9	1,2	545,7645	20,41	5349	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,24	1089,5131	9	3,3	545,7656	37,66	11728	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,04	1089,5131	9	3,8	545,7659	21,49	5717	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78,02	1089,5131	9	2,2	545,765	50,73	16822	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	78	1089,5131	9	1,5	545,7646	27,94	8045	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN

DFGSFDKFK	77,92	1089,5131	9	2,3	545,7651	49,2	16239	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	77,9	1089,5131	9	3,7	545,7658	41,31	13164	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	77,77	1089,5131	9	2,6	545,7652	47,64	15643	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,97	1089,5131	9	1,4	545,7646	49,71	16435	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,86	1089,5131	9	2,6	545,7652	48,68	16042	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,76	1089,5131	9	3,2	545,7656	36,63	11323	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,69	1089,5131	9	3,9	545,7659	37,14	11527	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,61	1089,5131	9	1,9	545,7648	25,08	7010	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,58	1089,5131	9	2	545,7649	35,07	10707	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	76,54	1089,5131	9	2	545,7649	52,79	17606	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	75,82	1089,5131	9	3	545,7654	47,11	15447	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	75,06	1089,5131	9	3,9	545,7659	34,54	10506	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	74,79	1089,5131	9	5,8	545,767	40,79	12959	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	74,78	1089,5131	9	5	545,7665	44,43	14394	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	74,68	1089,5131	9	2,6	545,7652	50,23	16630	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	74,54	1089,5131	9	-0,7	545,7634	25,64	7216	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	73,47	1089,5131	9	2,6	545,7652	30,3	8901	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	73,28	1089,5131	9	2,2	545,765	26,9	7666	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	71,22	1089,5131	9	1,2	545,7645	54,36	18211	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	68,8	1089,5131	9	-0,2	545,7637	53,85	18011	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	67,12	1089,5131	9	7,6	364,1811	22,15	5955	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	66,27	1089,5131	9	2,2	545,765	20,96	5542	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	66,04	1089,5131	9	3,1	364,1794	21,63	5766	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	65,97	1089,5131	9	4,9	364,1801	22,66	6142	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDKFK	64,81	1089,5131	9	2	364,179	24,73	6885	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	63,31	1089,5131	9	3,3	364,1795	24,2	6694	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	62,75	1089,5131	9	0,9	364,1786	25,24	7071	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	61,88	1089,5131	9	2,5	364,1792	23,18	6326	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	59,9	1089,5131	9	1,1	364,1787	23,69	6512	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	58	1089,5131	9	2,3	364,1791	48,76	16074	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	57,98	1089,5131	9	3,4	364,1795	41,05	13059	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	56,64	1089,5131	9	1,1	364,1787	53,2	17765	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	55,98	1089,5131	9	2,6	364,1792	50,3	16660	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	55,7	1089,5131	9	1,8	364,179	49,27	16266	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	54,73	1089,5131	9	1,7	364,1789	51,68	17182	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	54,11	1089,5131	9	1,6	364,1789	38,03	11868	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	54,1	1089,5131	9	1	364,1786	20,46	5365	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	54,1	1089,5131	9	2,2	364,1791	35,75	10967	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	53,95	1089,5131	9	3,4	364,1795	45,63	14861	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	53,89	1089,5131	9	1,7	364,1789	29,53	8616	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DFGSFDKFK	53,78	1089,5131	9	2,2	364,1791	34,2	10369	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	53,46	1089,5131	9	2,5	364,1792	36,28	11178	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	53,17	1089,5131	9	0,8	364,1786	49,78	16462	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	53,16	1089,5131	9	1,8	364,179	32,15	9587	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	52,91	1089,5131	9	2,6	364,1792	34,71	10569	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	52,88	1089,5131	9	1,6	364,1789	52,19	17378	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	52,86	1089,5131	9	1,4	364,1788	28,96	8408	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	52,78	1089,5131	9	2,2	364,1791	46,15	15066	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN

DFGSFDKFK	52,78	1089,5131	9	2,4	364,1792	43,7	14110	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	52,51	1089,5131	9	3,1	364,1794	44,21	14308	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	52,42	1089,5131	9	2,3	364,1791	50,82	16856	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	51,59	1089,5131	9	2,3	364,1791	35,22	10762	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	51,25	1089,5131	9	2,6	364,1792	33,18	9978	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDKFK	51,25	1089,5131	9	1,9	364,179	43,18	13906	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	51,23	1089,5131	9	1,1	364,1787	52,7	17570	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	51,16	1089,5131	9	0,4	364,1784	21,09	5583	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	51,09	1089,5131	9	1,9	364,179	47,75	15687	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	51,04	1089,5131	9	2,5	364,1792	42,66	13702	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	50,98	1089,5131	9	0,7	364,1786	39,37	12385	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	50,84	1089,5131	9	4,1	364,1798	37,42	11635	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	50,82	1089,5131	9	3,7	364,1796	32,66	9781	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	50,36	1089,5131	9	1	364,1786	33,69	10173	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDKFK	49,89	1089,5131	9	2,9	364,1794	40,48	12837	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	49,24	1089,5131	9	2,3	364,1791	39,95	12625	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	49,14	1089,5131	9	2,9	364,1794	42,09	13473	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	48,36	1089,5131	9	1,9	364,179	38,82	12168	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	48,17	1089,5131	9	1,9	364,179	46,68	15273	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	48,01	1089,5131	9	3,4	364,1795	47,19	15474	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	47,62	1089,5131	Ø	-0,5	364,1781	48,25	15881	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	45,52	1089,5131	9	4,3	364,1798	45,12	14660	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	45,21	1089,5131	9	2,2	364,1791	41,56	13264	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKFK	43,24	1089,5131	9	3,7	364,1797	30,18	8857	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDKFK	42,87	1089,5131	9	2,8	364,1793	30,85	9103	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	42,53	1089,5131	9	0,2	364,1784	53,71	17961	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
DFGSFDKFK	42,5	1089,5131	9	1,8	364,179	54,24	18168	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
WNVINWENV TER	83,47	1558,7528	12	0,1	780,3837	38,36	12000	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
SGWGWLGF NK	83,28	1150,5559	10	1,5	576,2861	33,58	10132	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
GDVTAQIALQ PALKF	82,84	1570,8718	15	4,4	786,4467	34,6	10528	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
IDVWEHAYYL QYK	81,89	1726,8354	13	3,7	576,6212	28,26	8166	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
YVNNLNVTEE K	81,08	1321,6514	11	5,4	661,8365	15,81	3693	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
SKHHAAYVN NLNVTEEK	80,66	1952,9705	17	1,1	651,9982	13,16	2701	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
GGGEPKGEL LEAIKR	80,48	1552,8572	15	-0,9	518,6259	16,18	3842	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
GSGWGWLG FNK	80,31	1207,5774	11	4	604,7984	33,22	9993	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
GSGWGWLG FNK	35,95	1207,5774	11	-17,4	604,7855	43,92	14196	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSLPDLPYDY GALEPHIN	80,07	2049,9795	18	-1,5	1025,9955	31,58	9373	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSLPDLPYDY GALEPHIN	74,25	2049,9795	18	5	684,3372	30,97	9149	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
HSLPDLPYDY GALEPHIN	61,98	2049,9795	18	-0,2	684,3336	32,64	9776	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
NVTEEKYQE ALAK	79,87	1521,7675	13	3,4	761,8936	14,28	3113	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
NVTEEKYQE ALAK	69,06	1521,7675	13	3,3	508,2648	14,33	3131	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
NVTEEKYQE ALAK	32,85	1521,7675	13	3	761,8932	19,34	4961	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
NVTEEKYQE ALAK	25,41	1521,7675	13	4	761,894	18,82	4775	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
DVTAQIALQP ALK	79,81	1366,782	13	1,2	684,3991	27,98	8061	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN
KHHAAYVNN LNVTEEKYQE ALAK	79,6	2669,3562	23	2,4	890,7948	17,89	4430	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
AQIMQLHHSK	78,41	1191,6183	10	1,3	596,8172	11,34	2069	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
AQIMQLHHSK	64,46	1191,6183	10	2,9	398,2145	11,29	2050	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN
INAQIMQLHH SK	78,2	1418,7452	12	5,6	710,3839	13,88	2968	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN

INAQIMQLHH SK	72,78	1418,7452	12	5,2	473,9248	13,86	2963	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVINWENVT ER	77,44	1372,6735	11	1	687,3447	24,63	6849	mf18031 3_03.raw	protein1 sp  P04179 SO	
KHHAAYVNN LNVTEEK	76,84	1865,9384	16	1,7	622,9878	13,28	2750	mf18031 3_03.raw	protein1 sp  P04179 SO	
YGALEPHINA QIMQLHHSK	75,52	2186,1055	19	4,5	729,7124	19,57	5044	mf18031 3_03.raw	protein1 sp  P04179 SO	
EHAYYLQYK	75,4	1213,5768	9	2,6	607,7972	15,33	3516	mf18031 3_03.raw	protein1 sp  P04179 SO	
EHAYYLQYK	67,04	1213,5768	9	2,3	405,5338	15,4	3542	mf18031 3_03.raw	protein1 sp  P04179 SO	
HINHSIFWTN LSPNGGGEP	74,47	2204,0762	20	4,2	735,7024	22,31	6017	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	73,95	814,3497	7	2,7	408,1832	20,13	5253	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	72,73	814,3497	7	1,5	408,1827	18,76	4753	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	72,39	814,3497	7	3,9	408,1837	21,97	5889	 mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	70,84	814,3497	7	2,6	408,1832	21,47	5709	 mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	70,15	814,3497	7	2,9	408,1833	20,64	5433	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	68,95	814,3497	7	2,1	408,183	19,28	4941	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	66,7	814,3497	7	3,1	408,1834	18,24	4563	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	60,97	814,3497	7	1,8	408,1828	46,43	15172	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	59,92	814,3497	7	0,6	408,1823	51,77	17220	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	59,55	814,3497	7	1,8	408,1828	48	15783	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	58,69	814,3497	7	2,4	408,1831	46,95	15381	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	57,61	814,3497	7	3	408,1833	24,51	6806	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDK	57,29	814,3497	7	2,7	408,1832	44,35	14363	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	56,62	814,3497	7	1,9	408,1829	49,01	16168	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	56,28	814,3497	7	3	408,1833	45,39	14767	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	56,02	814,3497	7	2,8	408,1833	49,53	16362	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDK	55,96	814,3497	7	2,3	408,183	47,48	15584	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDK	55,19	814,3497	7	2,7	408,1832	51,26	17023	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	55,18	814,3497	7	3,4	408,1835	43,84	14165	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	54,38	814,3497	7	2,7	408,1832	50,03	16558	 mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	53,85	814,3497	7	2,2	408,183	48,51	15978	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	53,5	814,3497	7	0,1	408,1822	53,5	17882	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	52,89	814,3497	7	3,6	408,1836	44,87	14564	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	52,21	814,3497	7	2,7	408,1832	42,75	13740	- mf18031 3_03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	51,96	814,3497	7	2,4	408,1831	52,99	17682	mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	51,7	814,3497	7	2,9	408,1833	32,14	9582	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK	51,27	814,3497	7	2,1	408,183	42,23	13532	- mf18031 3 03.raw	protein1 sp  P04179 SO	
DFGSFDK	50,93	814,3497	7	1,7	408,1828	54,06	18097	 mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	50,35	814,3497	7	2,3	408,183	41,19	13115	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	49,54	814,3497	7	2,7	408,1832	41,71	13323	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	49,29	814,3497	7	2,7	408,1832	31,53	9353	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	48,31	814,3497	7	3,6	408,1836	28,13	8115	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	48,29	814,3497	7	3	408,1833	40,08	12673	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	47,42	814,3497	7	2,7	408,1832	40,65	12901	mf18031 3_03.raw	protein1 sp  P04179 SO	
DFGSFDK	46,75	814,3497	7	2,3	408,183	50,74	16824	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDK	46,31	814,3497	7	2,2	408,183	45,91	14972	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	45,14	814,3497	7	2,7	408,1832	39,53	12452	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	43,87	814,3497	7	3,5	408,1835	30,24	8882	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DFGSFDK	43,39	814,3497	7	3,6	408,1836	30,91	9126	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDK	42,24	814,3497	7	1,5	408,1827	33,18	9979	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDK	42,03	814,3497	7	5,1	408,1842	14,52	3207	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

DFGSFDK   40.6   814,3497   7   1.8   408,828   33,69   10174   9,03 nw 9,03 nw DH   10140AN 10140AN     DFGSFDK   36,4   814,3497   7   2,1   408,183   36,34   11205   10174   3,03 nw DH   10140AN     DFGSFDK   35,4   814,3497   7   3,7   408,183   37,42   11833   mf8031   potentilspi potentilspi 3,03 nw   DFIGSFDK   32,81   814,3497   7   5,1   408,1833   35,37   10823   g.03 nw   DH HUMAN     DFGSFDK   31.26   814,3497   7   3   408,1833   35,37   10823   g.03 nw   DH HUMAN     DFGSFDK   31.26   814,3497   7   3   408,1833   35,37   10823   potentilspi potentilspi 3,03 nw   DH HUMAN     GELLEAIKR   69,78   1027,6025   9   3,9   514,8105   18,2   4548   3,03 nw   DH HUMAN     GELLEAIKR   66,79   1027,6025   9   1,4   514,8105   17,7   4931	DFGSFDK	41,84	814,3497	7	2,3	408,183	32,66	9782	mf18031 3 03.raw	protein1 sp  P04179 SO	
DEGSFDK   36.4   B14.3497   7   2,1   408,183   36,34   11205   mf8031   POEINISING DM HUMAN     DFGSFDK   35.4   814.3497   7   3,7   408,183   36,34   11205   mf8031   POEINISING DM HUMAN     DFGSFDK   32.61   814.3497   7   5,1   408,183   36,37   10822   mf8031   POEINISING DM HUMAN     DFGSFDK   31.26   814.3497   7   5,1   408,1833   36,37   10822   mf8031   POEINISING DM HUMAN     GELLEAIKR   72,31   1027,6025   9   2   514,8096   16,67   4014   mf8031   POEINISING DM HUMAN     GELLEAIKR   69,78   1027,6025   9   3,9   514,8105   18.2   4548   mf8031   POEINISING DM HUMAN     GELLEAIKR   66,79   1027,6025   9   1,4   514,8105   17,7   4361   3,03 rw   MHUMAN   MHUMAN     GELLEAIKR   66,73   1027,6025   9   1,4   514,8105   17,7	DFGSFDK	40,6	814,3497	7	1,8	408,1828	33,69	10174	- mf18031	DM_HUMAN protein1 sp  P04179 SO	
DFGSFDK   35.4   814.3497   7   3.7   408.1836   37.42   11633   mftmans mftmans   potentilspi potentilspi g.03.rw     DFGSFDK   32.61   814.3497   7   5.1   408.1836   37.42   11633   mftmans   potentilspi g.03.rw   DM HUMAN     DFGSFDK   31.28   814.3497   7   3.1   408.1833   35.37   10822   mftmans   potentilspi g.03.rw   DM HUMAN     GELLEAIKR   81.2   814.3497   7   3   408.1833   35.37   10822   mftmans   potentilspi g.03.rw   DM HUMAN     GELLEAIKR   9,78   1027,6025   9   2   514,8105   18.2   4548   mftmans   potentilspi g.03.rw   DM HUMAN     GELLEAIKR   66,79   1027,6025   9   1,4   514,8105   18,2   4548   3.03.rw   DM HUMAN     GELLEAIKR   66,63   1027,6025   9   3,1   514,8105   2,76   6177   mftmans   potentilspi g.03.rw   DM HUMAN     GELLEAIKR	DFGSFDK	36,4	814,3497	7	2,1	408,183	36,34	11205	mf18031	DM_HUMAN protein1 sp  P04179 SO	
Di Gol Bit   O.H. 1010   D. 1   O.H. 1010   D.H. 1010   D.H. 1010     DFGSFDK   32,61   814,3497   7   5.1   408,1842   36,89   11420   mf1803   PP04179800     DFGSFDK   31,2e   814,3497   7   3   408,1833   35,37   10822   mf1803   PP04179800     GELLEANKR   72,31   1027,8025   9   2   514,8086   16,67   4014   mf1803   PP04179800     GELLEANKR   68,76   1027,8025   9   6,2   514,8105   18,2   4548   mf1803   PP04179800     GELLEANKR   68,76   1027,8025   9   6,2   514,8107   17,18   4176   mf1803   PP0417980     GELLEANKR   66,63   1027,8025   9   1,4   514,808   19,73   5102   mf1803   PP0417980     GELLEANKR   66,63   1027,8025   9   3,1   514,8105   19,22   6177   mf1803   PP0417980     GELLEANKR   65,72	DEGSEDK	35.4	81/ 3/07	7	3.7	408 1836	37 / 2	11633	3_03.raw mf18031	DM_HUMAN protein1 sp	
DFGSFDK   32,61   814,3497   7   5.1   408,1842   36,89   11429   mf18031 m1429   Post mission pot m	DI GGI DR	33,4	014,3497	/	5,7	400,1030	57,42	11033	3_03.raw	DM_HUMAN	
DFGSFDK   31,28   814,3497   7   3   408,1833   35,37   10822   n18031 n18031   protein 1801 n01 HUMAN     GELLEANKR   72,31   1027,6025   9   2   514,8066   16,67   4014   mf18031 n01 HUMAN   protein 1801 potein 1801 n01 HUMAN     GELLEANKR   68,78   1027,6025   9   6,22   514,8105   18,2   4544   mf18031 n01 HUMAN   protein 1801 n01 HUMAN     GELLEANKR   66,78   1027,6025   9   6,22   514,8117   17,1   4365   .03,789 3,03,789   PO4779ISO DM HUMAN     GELLEANKR   66,79   1027,6025   9   1,4   514,8105   17,7   4366   .017037   mf18031 3,03,789   PO4779ISO DM HUMAN     GELLEANKR   66,61   1027,6025   9   3,1   514,8105   22,76   6177   mf18031 904779ISO 3,03,789   PO4779ISO DM HUMAN     GELLEANKR   65,72   1027,6025   9   3,1   514,8104   49,09   16195   m178031 904779ISO 3,03,789   PO4779ISO 3,03,789   PO4779ISO 3,03,789   PO477	DFGSFDK	32,61	814,3497	7	5,1	408,1842	36,89	11429	mf18031 3_03.raw	P04179 SO DM_HUMAN	
GELLEAIKR   72,31   1027,6025   9   2   514,8096   16,67   4014   mf18031 3_0.3.mb   pdf179180 p4179180     GELLEAIKR   69,78   1027,6025   9   3,9   514,8105   18,2   4548   mf18031 3_0.3.mb   pdf1911801 p41791805     GELLEAIKR   68,76   1027,6025   9   6,2   514,8117   17,18   4178   mf18031 3_0.3.mb   pdf1911801 p41791805     GELLEAIKR   66,79   1027,6025   9   1.4   514,8103   17,7   4381   mf18031 3_0.3.mb   pdf1911801 p41791805     GELLEAIKR   66,63   1027,6025   9   4.4   514,8105   22,76   6177   mf18031   pdf1911801 p41791805     GELLEAIKR   65,8   1027,6025   9   3,1   514,8105   22,76   6177   mf18031   pdf18031	DFGSFDK	31,26	814,3497	7	3	408,1833	35,37	10822	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR   69,78   1027,6025   9   3,9   514,8105   18,2   4548   mf18031 pot 17980 3_0.3.rm   mf18031 pot 17980 DM   mf18031 pot 17980 DM <thmf18031< td=""><td>GELLEAIKR</td><td>72,31</td><td>1027,6025</td><td>9</td><td>2</td><td>514,8096</td><td>16,67</td><td>4014</td><td>mf18031 3_03.raw</td><td>protein1 sp  P04179 SO DM_HUMAN</td><td></td></thmf18031<>	GELLEAIKR	72,31	1027,6025	9	2	514,8096	16,67	4014	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR   68,76   1027,6025   9   6.2   514,8117   17,18   4178   mf18031 3_0.3 raw DM   protein rispl PO4179[SO     GELLEAIKR   66,79   1027,6025   9   1.4   514,803   17,7   4361   mf18031 3_0.3 raw DM   PO4179[SO     GELLEAIKR   66,63   1027,6025   9   4.4   514,8105   22,76   6177   mf18031 mf18031   PO4179[SO     GELLEAIKR   65,8   1027,6025   9   3.8   514,8105   22,76   6177   mf18031 mf18031   PO4179[SO     GELLEAIKR   65,72   1027,6025   9   3.1   514,8105   22,76   6177   mf18031 mf18031   PO4179[SO     GELLEAIKR   64,62   1027,6025   9   3.1   514,8095   21,73   5800   mf18031   PO4179[SO     GELLEAIKR   63,8   1027,6025   9   5   514,8095   21,73   5800   mf18031   PO4179[SO     GELLEAIKR   62,49   1027,6025   9   5   514,8099   50,62	GELLEAIKR	69,78	1027,6025	9	3,9	514,8105	18,2	4548	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR   66,79   1027,6025   9   1.4   514,8093   17.7   4361   mf18031 mf18031   protein1ispl po	GELLEAIKR	68,76	1027,6025	9	6,2	514,8117	17,18	4178	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR   66,63   1027,6025   9   4.4   514,8108   19,73   5102   mf18031 g-03,rav   protein1[sp] P04179[SO] 3_03,rav   p	GELLEAIKR	66,79	1027,6025	9	1,4	514,8093	17,7	4361	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR   65.8   1027,6025   9   3.8   514,8105   22.76   6177   mf18031   P04179]SO     GELLEAIKR   65.72   1027,6025   9   3.1   514,8105   22.76   6177   mf18031   P04179]SO     GELLEAIKR   65.72   1027,6025   9   2.4   514,8098   19.22   4919   mf18031   P04179]SO     GELLEAIKR   64.62   1027,6025   9   2.4   514,8098   19.22   4919   mf18031   P04179]SO     GELLEAIKR   63.8   1027,6025   9   1,9   514,8095   21,73   5800   mf18031   P04179]SO     GELLEAIKR   62.99   1027,6025   9   5   514,8111   37,39   11623   mf18031   P04179]SO     GELLEAIKR   62.49   1027,6025   9   2,7   514,8099   50.62   16781   mf18031   P04179]SO     GELLEAIKR   61.87   1027,6025   9   3,1   514,8105   48.06   15805   mf18031 <t< td=""><td>GELLEAIKR</td><td>66,63</td><td>1027,6025</td><td>9</td><td>4,4</td><td>514,8108</td><td>19,73</td><td>5102</td><td>- mf18031 3_03.raw</td><td>protein1 sp  P04179 SO</td><td></td></t<>	GELLEAIKR	66,63	1027,6025	9	4,4	514,8108	19,73	5102	- mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR   65.72   1027,6025   9   3.1   514,8101   49.09   16195   mf18031 protein1[sp] 0.03.raw DM HUMAN   mf18031 protein1[sp] 0.04.79[SO     GELLEAIKR   64,62   1027,6025   9   2.4   514,8098   19.22   4919   mf18031 protein1[sp] P04179[SO   protein1[sp] P04179[SO     GELLEAIKR   63,8   1027,6025   9   1.9   514,8095   21.73   5800   mf18031 3_0.3.raw DM HUMAN     GELLEAIKR   62,99   1027,6025   9   5   514,8111   37.39   11623   mf18031 3_0.3.raw DM HUMAN     GELLEAIKR   62,99   1027,6025   9   2.7   514,8105   48.06   15805   mf18031 3_0.3.raw DM HUMAN     GELLEAIKR   62,06   1027,6025   9   3.8   514,8105   48.06   15805   mf18031 3_0.3.raw DM HUMAN     GELLEAIKR   61,87   1027,6025   9   3.1   514,8106   22,25   5995   mf18031 3_0.3.raw DM HUMAN     GELLEAIKR   61,72   1027,6025   9   3.1   514,8101   47,03	GELLEAIKR	65,8	1027,6025	9	3,8	514,8105	22,76	6177	mf18031	DM_HUMAN protein1 sp  P04179 SO	
GELLEAIKR   64,62   1027,6025   9   2,4   514,8098   19,22   4919   mf18031 3_0.3raw _0.03.raw   protein1[sp] P04179[SO _0.03.raw     GELLEAIKR   63,8   1027,6025   9   1,9   514,8095   21,73   5800   mf18031 _0.03.raw   protein1[sp] P04179[SO _0.03.raw   protein1[sp] P04179[SO _04179[SO]     GELLEAIKR   62,99   1027,6025   9   5   514,8111   37,39   11623   mf18031 _0.03.raw   protein1[sp] P04179[SO _04179[SO]     GELLEAIKR   62,49   1027,6025   9   2,7   514,8099   50,62   16781   mf18031 _0.03.raw   protein1[sp] P04179[SO _04179[SO]     GELLEAIKR   62,66   1027,6025   9   3,8   514,8105   48,06   15805   mf18031 _0.03.raw   protein1[sp] P04179[SO]     GELLEAIKR   61,87   1027,6025   9   3,1   514,8106   22,25   5995   mf18031 _0.03.raw   protein1[sp] P04179[SO]     GELLEAIKR   61,61   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 _0.03.raw   pro	GELLEAIKR	65,72	1027,6025	9	3,1	514,8101	49,09	16195	mf18031	DM_HUMAN protein1 sp  P04179 SO	
GELLEAIKR   63.8   1027,6025   9   1,9   514,8095   21,73   5800   mf18031 mf18031 3_03,raw DM   protein1[sp] Pod179[SO 3_03,raw DM   protein1[sp] Pod179[SO 3_03,raw     GELLEAIKR   62,99   1027,6025   9   5   514,8111   37,39   11623   mf18031 mf18031 3_03,raw   protein1[sp] Pod179[SO 3_03,raw     GELLEAIKR   62,49   1027,6025   9   2,7   514,8099   50,62   16781   mf18031 3_03,raw   protein1[sp] Pod179[SO 3_03,raw     GELLEAIKR   62,06   1027,6025   9   3,8   514,8105   48,06   15805   mf18031 3_03,raw   protein1[sp] Pod179[SO 3_03,raw     GELLEAIKR   61,87   1027,6025   9   3,1   514,8106   22,25   5995   mf18031 3_03,raw   protein1[sp] Pod179[SO 3_0,3raw     GELLEAIKR   61,72   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 3_03,raw   protein1[sp] Po4179[SO 3_0,3raw     GELLEAIKR   61,69   1027,6025   9   1,8   514,8094   51,75   17213   mf18031 3_03,raw	GELLEAIKR	64,62	1027,6025	9	2,4	514,8098	19,22	4919	mf18031	DM_HUMAN protein1 sp  P04179 SO	
GELLEAIKR   62,99   1027,6025   9   5   514,8111   37,39   11623   mf18031 3_03.raw DM HUMAN   protein1[sp] P04179[SO DM HUMAN     GELLEAIKR   62,49   1027,6025   9   2,7   514,8099   50,62   16781   mf18031 3_03.raw DM HUMAN   protein1[sp] P04179[SO DM HUMAN     GELLEAIKR   62,06   1027,6025   9   3,8   514,8105   48,06   15805   mf18031 3_03.raw DM HUMAN   protein1[sp] P04179[SO DM HUMAN     GELLEAIKR   61,87   1027,6025   9   3,1   514,8106   22,25   5995   mf18031 3_03.raw DM HUMAN     GELLEAIKR   61,87   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 3_03.raw DM HUMAN     GELLEAIKR   61,69   1027,6025   9   1,8   514,8094   51,75   17213   mf18031 3_03.raw DM HUMAN     GELLEAIKR   61,33   1027,6025   9   3,5   514,8107   44,3   14342   mf18031 3_03.raw DM HUMAN     GELLEAIKR   61,31   1027,6025   9   3,5   514,81	GELLEAIKR	63,8	1027,6025	9	1,9	514,8095	21,73	5800	mf18031	DM_HUMAN protein1 sp  P04179 SO	
GELLEAIKR   62,99   1027,6025   9   5   514,8111   37,39   11623   m118031 3_03.raw protein1[sp]   po4179[SO DM HUMAN     GELLEAIKR   62,49   1027,6025   9   2,7   514,8099   50,62   16781   mf18031 mf18031   protein1[sp] po4179[SO 3_03.raw   mf18031   protein1[sp] po4179[SO 3_03.raw   mf18031   protein1[sp] po4179[SO 3_03.raw   mf18031   protein1[sp] po4179[SO 3_03.raw   mf18031   protein1[sp] po4179[SO     GELLEAIKR   61,87   1027,6025   9   3,1   514,8106   22,25   5995   mf18031 3_03.raw   protein1[sp] Po4179[SO     GELLEAIKR   61,72   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 mf18031   protein1[sp] Po4179[SO 3_03.raw   protein1[sp] Po4179[SO     GELLEAIKR   61,69   1027,6025   9   1,8   514,8094   51,75   17213   mf18031 3_03.raw   protein1[sp] Po4179[SO     GELLEAIKR   61,33   1027,6025   9   3,5   514,8107   44,3   14342   mf18031 3_03.raw   protein1[sp] Po4179[SO			,		,	,	,		3_03.raw	DM_HUMAN protein1 sp	
GELLEAIKR   62,49   1027,6025   9   2,7   514,8099   50,62   16781   mf18031 3_03.raw protein1[sp]   protein1[sp] P04179]SO DM   protein1[sp] P04179]SO     GELLEAIKR   62,06   1027,6025   9   3,8   514,8105   48,06   15805   mf18031 3_03.raw   protein1[sp] P04179]SO DM   P04179]SO     GELLEAIKR   61,87   1027,6025   9   4   514,8106   22,25   5995   mf18031 3_03.raw   protein1[sp] P04179]SO     GELLEAIKR   61,72   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 3_03.raw   protein1[sp] P04179]SO     GELLEAIKR   61,69   1027,6025   9   3,1   514,8101   47,03   15413   mf18031 3_03.raw   protein1[sp] P04179]SO     GELLEAIKR   61,69   1027,6025   9   1,8   514,8107   44,3   14342   mf18031 3_03.raw   protein1[sp] P04179]SO     GELLEAIKR   61,31   1027,6025   9   3,5   514,8104   49,6   16392   mf18031 3_03.raw   protein1[sp] P04179]SO DM </td <td>GELLEAIKR</td> <td>62,99</td> <td>1027,6025</td> <td>9</td> <td>5</td> <td>514,8111</td> <td>37,39</td> <td>11623</td> <td>mf18031 3_03.raw</td> <td>P04179 SO DM_HUMAN</td> <td></td>	GELLEAIKR	62,99	1027,6025	9	5	514,8111	37,39	11623	mf18031 3_03.raw	P04179 SO DM_HUMAN	
GELLEAIKR 62,06 1027,6025 9 3,8 514,8105 48,06 15805 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,87 1027,6025 9 4 514,8106 22,25 5995 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,72 1027,6025 9 3,1 514,8101 47,03 15413 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,69 1027,6025 9 1,8 514,8094 51,75 17213 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,33 1027,6025 9 1,8 514,8094 51,75 17213 mf18031 3_0.3.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,33 1027,6025 9 4,1 514,8107 44,3 14342 mf18031 3_0.3.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_0.3.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103	GELLEAIKR	62,49	1027,6025	9	2,7	514,8099	50,62	16781	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR 61,87 1027,6025 9 4 514,8106 22,25 5995 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,72 1027,6025 9 3,1 514,8101 47,03 15413 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,69 1027,6025 9 1,8 514,8094 51,75 17213 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,33 1027,6025 9 4,1 514,8107 44,3 14342 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8107 44,3 14342 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN protein1[sp] P04179[SO DM_HUMAN	GELLEAIKR	62,06	1027,6025	9	3,8	514,8105	48,06	15805	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR 61,72 1027,6025 9 3,1 514,8101 47,03 15413 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,69 1027,6025 9 1,8 514,8094 51,75 17213 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,33 1027,6025 9 4,1 514,8107 44,3 14342 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8107 44,3 14342 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN	GELLEAIKR	61,87	1027,6025	9	4	514,8106	22,25	5995	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR 61,69 1027,6025 9 1,8 514,8094 51,75 17213 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,33 1027,6025 9 4,1 514,8107 44,3 14342 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN	GELLEAIKR	61,72	1027,6025	9	3,1	514,8101	47,03	15413	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR 61,33 1027,6025 9 4,1 514,8107 44,3 14342 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN	GELLEAIKR	61,69	1027,6025	9	1,8	514,8094	51,75	17213	mf18031 3_03.raw	P04179 SO	
GELLEAIKR 61,31 1027,6025 9 3,5 514,8104 49,6 16392 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN   GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1 sp  P04179 SO DM_HUMAN	GELLEAIKR	61,33	1027,6025	9	4,1	514,8107	44,3	14342	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR 61,24 1027,6025 9 3,4 514,8103 45,98 14999 mf18031 3_03.raw protein1[sp] P04179[SO DM_HUMAN	GELLEAIKR	61,31	1027,6025	9	3,5	514,8104	49,6	16392	mf18031 3_03.raw	protein1 sp  P04179 SO	
	GELLEAIKR	61,24	1027,6025	9	3,4	514,8103	45,98	14999	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	

GELLEAIKR	61,19	1027,6025	9	2,2	514,8097	20,7	5454	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	60,81	1027,6025	9	3,2	514,8102	48,57	16000	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR	60,67	1027,6025	9	1,4	514,8093	27,39	7838	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIKR	60,59	1027,6025	9	6,2	514,8117	31,71	9421	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	60,46	1027,6025	9	2,7	514,8099	52,26	17406	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	60,17	1027,6025	9	4	514,8106	41,71	13324	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	60,16	1027,6025	9	2,6	514,8099	23,37	6394	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	60	1027,6025	9	2,7	514,8099	52,79	17604	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	59,89	1027,6025	9	1,5	514,8093	53,31	17807	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	59,51	1027,6025	9	1,9	514,8095	21,22	5626	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	59,44	1027,6025	9	3,2	514,8102	50,11	16589	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	59,33	1027,6025	9	4,5	514,8109	44,82	14545	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	59,03	1027,6025	9	2,8	514,81	36,34	11204	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	58,93	1027,6025	9	3,9	514,8105	43,78	14143	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,84	1027,6025	9	2	514,8096	26,01	7342	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,83	1027,6025	9	3,9	514,8105	34,81	10605	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,81	1027,6025	9	3,9	514,8105	35,82	10997	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,3	1027,6025	9	4,8	514,811	30,17	8854	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,27	1027,6025	9	3,3	514,8102	51,13	16969	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,27	1027,6025	9	4	514,8106	45,36	14755	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	58,03	1027,6025	9	2,2	514,8097	27,9	8028	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	57,89	1027,6025	9	4,7	514,811	42,75	13739	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	57,87	1027,6025	9	2,5	514,8098	47,54	15604	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	57,5	1027,6025	9	3,4	514,8103	46,51	15205	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GELLEAIKR	57,49	1027,6025	9	3,7	514,8104	32,22	9615	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	57,29	1027,6025	9	2,2	514,8097	42,24	13534	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	57,01	1027,6025	9	0,6	514,8088	31,19	9231	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	56,98	1027,6025	9	3,7	514,8104	41,19	13117	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	56,9	1027,6025	9	3,8	514,8105	23,9	6591	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	56,51	1027,6025	9	4	514,8106	30,68	9037	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	56,12	1027,6025	9	3,9	514,8105	33,76	10200	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIKR	56,01	1027,6025	9	3,8	514,8105	36,86	11417	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,88	1027,6025	9	1,6	514,8094	53,85	18010	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,71	1027,6025	9	3,9	514,8105	34,29	10405	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,54	1027,6025	9	3,2	514,8102	35,31	10797	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,42	1027,6025	9	1,9	514,8095	38,62	12088	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,35	1027,6025	9	1,8	514,8094	43,26	13936	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	55,34	1027,6025	9	3,3	514,8102	29,62	8645	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	54,25	1027,6025	9	4,4	514,8108	26,52	7530	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	53,88	1027,6025	9	3,3	514,8102	18,71	4735	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	53,14	1027,6025	9	4	514,8106	33,24	10003	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	51,37	1027,6025	9	5,9	514,8116	32,73	9809	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIKR	50,17	1027,6025	9	6,2	514,8117	25,49	7163	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
FNGGGHINH SIFWTNLSPN	71,32	2110,9973	19	0,8	704,6736	27,29	7802	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VNNLNVTEEK	71,2	1158,588	10	-0,3	580,3011	13,27	2743	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VINWENVTE R	71	1258,6306	10	4,6	630,3255	23,01	6267	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VINWENVTE R	17,29	1258,6306	10	7,4	630,3273	35,78	10978	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LTAASVGVQ GSGW	70,86	1231,6196	13	3,3	616,8192	28,83	8364	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

PYDYGALEP HINAQIMQLH HSK	70,5	2561,2485	22	-3,4	854,7538	28,42	8222	mf18031 3_03.raw	protein1 sp  P04179 SO	
PYDYGALEP HINAQIMQLH HSK	41,67	2561,2485	22	1,5	854,7581	28,93	8399	mf18031 3_03.raw	protein1 sp  P04179 SO	
WTNLSPNGG GEPK	69,71	1355,647	13	4,2	678,8336	15,89	3725	mf18031 3_03.raw	protein1 sp  P04179 SO	
AAYVNNLNVT EEK	69,66	1463,7256	13	2,7	732,872	18,14	4523	mf18031 3_03.raw	protein1 sp  P04179 SO	
INHSIFWTNL SPNGGGEPK	69,58	2067,0173	19	-0,7	690,0126	25,51	7171	mf18031 3_03.raw	protein1 sp  P04179 SO	
KGDVTAQIAL QPALK	69,54	1551,8984	15	1,1	776,9573	20,37	5334	mf18031 3_03.raw	protein1 sp  P04179 SO	
KGDVTAQIAL QPALK	67,8	1551,8984	15	1	518,3073	20,43	5357	mf18031 3_03.raw	protein1 sp  P04179 SO	
IWNVINWENV TER	68,95	1671,8369	13	2,5	558,2877	35,68	10944	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
IWNVINWENV TER	27,58	1671,8369	13	-7,2	558,2822	30,3	8903	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AKGDVTAQIA LQPALK	68,14	1622,9355	16	2,8	541,9873	20,59	5416	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEPHINAQ IMQI	67,29	2734,3425	24	8	912,4621	39,39	12395	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEPHINAQ IMQL	41,99	2734,3425	24	-0,5	912,4543	41,16	13106	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FNGGGHINH SIFWTN	67,14	1699,7855	15	3,7	567,6046	23,04	6277	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FNGGGHINH SIFWTN	49,2	1699,7855	15	5	567,6053	22,23	5989	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DYGALEPHIN AQIMQLHHSK	66,62	2301,1323	20	1,8	768,0527	28,41	8217	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DYGALEPHIN AQIMQLHHSK	29,88	2301,1323	20	8,8	768,0581	31,46	9328	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DYGALEPHIN AQIMQLHHSK	28,33	2301,1323	20	4,5	768,0549	22,75	6175	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
DYGALEPHIN AQIMQLHHSK	22,5	2301,1323	20	3,4	768,054	33,98	10290	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DYGALEPHIN AQIMQLHHSK	15,5	2301,1323	20	5,2	768,0554	36,87	11418	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
WEHAYYLQY K	65,83	1399,656	10	1,4	700,8362	19,4	4983	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
WEHAYYLQY K	64,29	1399,656	10	2,1	467,5602	19,36	4971	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAK	65,4	821,4283	7	2	411,7222	11,51	2130	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAK	64,69	821,4283	7	0,3	411,7215	13,56	2857	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAK	64,55	821,4283	7	0,7	411,7217	13,05	2660	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	

YQEALAK	63,87	821,4283	7	2,4	411,7224	14,09	3044	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	63,74	821,4283	7	2,3	411,7224	12,04	2308	mf18031 3 03.raw	protein1 sp  P04179 SO	
YQEALAK	63,68	821,4283	7	0,3	411,7216	12,55	2501	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	56,51	821,4283	7	1,9	411,7222	19,14	4889	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	54,95	821,4283	7	4,3	411,7232	15,37	3528	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	50,5	821,4283	7	3,2	411,7227	45,41	14775	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	47,62	821,4283	7	3,2	411,7227	49,45	16332	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	47,62	821,4283	7	2,9	411,7226	46,98	15392	mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	47,48	821,4283	7	2,1	411,7223	51,88	17260	- mf18031 3 03.raw	DM_HUMAN protein1 sp  P04179 SO	
YQEALAK	47,33	821,4283	7	2,3	411,7224	49,96	16530	- mf18031 3 03.raw	protein1 sp  P04179 SO	
YQEALAK	45,9	821,4283	7	5,2	411,7235	14,8	3315	 mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	45	821,4283	7	-0,3	411,7213	48,26	15882	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	44,98	821,4283	7	2,8	411,7226	39,42	12408	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	44,76	821,4283	7	2,1	411,7223	50,83	16857	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	44,15	821,4283	7	1,8	411,7222	33,88	10246	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	44,07	821,4283	7	2,3	411,7224	47,72	15677	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	44,04	821,4283	7	4,1	411,7231	44,9	14577	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	42,77	821,4283	7	2,6	411,7225	40,11	12689	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	42,29	821,4283	7	2	411,7222	46,45	15180	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	42,15	821,4283	7	2,2	411,7223	35,83	11002	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	41,25	821,4283	7	3,7	411,7229	45,93	14978	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	40,66	821,4283	7	2,9	411,7226	43,86	14172	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	39,69	821,4283	7	1,6	411,7221	43,34	13967	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAK	39,63	821,4283	7	3,2	411,7227	35,2	10758	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

YQEALAK	39,03	821,4283	7	3,6	411,7229	37,48	11659	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	38,61	821,4283	7	1,2	411,7219	34,55	10510	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	38,59	821,4283	7	2	411,7222	51,36	17059	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YQEALAK	36,69	821,4283	7	2,9	411,7226	44,37	14372	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	36,65	821,4283	7	3,4	411,7228	32,78	9829	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	36,56	821,4283	7	1,8	411,7222	42,7	13720	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	36,33	821,4283	7	3,3	411,7228	41,59	13278	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	34,73	821,4283	7	2,5	411,7224	36,94	11448	mf18031 3_03.raw	protein1 sp  P04179 SO	
YQEALAK	34,15	821,4283	7	2,4	411,7224	40,64	12898	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
YQEALAK	31,82	821,4283	7	1,2	411,7219	54,12	18118	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
TAQIALQPAL K	64,75	1152,6866	11	0,7	577,351	25,57	7191	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
TAQIALQPAL K	46,98	1152,6866	11	0,5	577,3509	26,1	7378	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFKEK	64,17	1231,6237	10	2,7	616,8207	14,54	3214	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFKEK	52,77	1231,6237	10	2,7	411,5496	14,6	3236	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFKEK	47,38	1231,6237	10	1,4	616,8199	17,68	4353	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FWTNLSPNG GGEPK	63,02	1502,7153	14	6,2	752,3696	22,97	6252	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	62,92	1003,545	8	1,1	502,7803	12,38	2437	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	59,68	1003,545	8	4,2	502,7819	15,95	3750	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	59,25	1003,545	8	6	502,7828	14,96	3371	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	58,92	1003,545	8	2,5	502,781	16,46	3940	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	57,71	1003,545	8	3,2	502,7814	13,92	2981	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	56,6	1003,545	8	4,1	502,7819	13,41	2798	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	56,52	1003,545	8	4,4	502,782	16,98	4117	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	55,23	1003,545	8	3,9	502,7818	19,68	5086	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

NVRPDYLK	54	1003,545	8	5	502,7823	19,17	4900	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	53,39	1003,545	8	4,1	502,7819	17,49	4286	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	53,38	1003,545	8	6,3	502,783	12,9	2611	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	53,16	1003,545	8	3,7	502,7816	14,44	3173	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	50,04	1003,545	8	1,3	502,7804	18,64	4714	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	49,91	1003,545	8	3	502,7813	23,47	6430	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	49,71	1003,545	8	3	502,7813	47,33	15528	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	49,16	1003,545	8	2,8	502,7812	28,16	8125	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	48,69	1003,545	8	4,6	502,7821	44,65	14482	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	48,55	1003,545	8	3,7	502,7817	22,3	6015	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	48,41	1003,545	8	2,3	502,781	18,01	4475	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	48,03	1003,545	8	3,9	502,7817	43,11	13877	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	47,53	1003,545	8	3,1	502,7814	43,62	14078	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	47,12	1003,545	8	0,2	502,7799	25,16	7039	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	47,09	1003,545	8	2,3	502,7809	41	13043	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	47,05	1003,545	8	2	502,7808	49,92	16516	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	46,8	1003,545	8	3,2	502,7814	51,06	16943	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	46,55	1003,545	8	3,3	502,7815	39,95	12624	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	46,32	1003,545	8	4,4	502,782	22,96	6251	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	46,15	1003,545	8	3,9	502,7817	41,52	13247	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	45,97	1003,545	8	3,3	502,7814	39,42	12407	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	45,68	1003,545	8	0,3	502,7799	21,78	5819	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	45,26	1003,545	8	2,7	502,7811	45,89	14961	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	45,17	1003,545	8	3,1	502,7814	30	8792	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

NVRPDYLK	45,14	1003,545	8	3,8	502,7817	40,48	12836	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	44,8	1003,545	8	2,6	502,7811	29,01	8431	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	44,6	1003,545	8	1,9	502,7807	53,18	17755	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	44,42	1003,545	8	2,8	502,7812	52,16	17368	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	44,38	1003,545	8	1,2	502,7804	42,04	13456	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	44,36	1003,545	8	1,1	502,7804	26,05	7359	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	44,18	1003,545	8	1,9	502,7807	37,77	11771	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	44,16	1003,545	8	1,2	502,7804	48,36	15923	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	43,34	1003,545	8	3	502,7813	37,25	11567	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NVRPDYLK	43,03	1003,545	8	1,7	502,7806	38,9	12200	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	43,01	1003,545	8	3,6	502,7816	49,39	16311	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	42,95	1003,545	8	3,6	502,7816	36,19	11142	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	42,76	1003,545	8	1,3	502,7805	27,53	7892	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	42,62	1003,545	8	1,9	502,7808	52,66	17558	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	42,1	1003,545	8	2,9	502,7812	47,84	15721	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	42,05	1003,545	8	1,7	502,7807	26,66	7579	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	41,32	1003,545	8	3,3	502,7815	48,87	16113	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	41,28	1003,545	8	0,7	502,7802	33,48	10092	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	41,23	1003,545	8	2,3	502,781	35,17	10743	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	41,06	1003,545	8	3,1	502,7814	46,4	15159	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	40,8	1003,545	8	3,5	502,7816	45,36	14756	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	40,48	1003,545	8	3	502,7813	24,6	6840	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	40,42	1003,545	8	3,3	502,7815	51,13	16971	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	40,28	1003,545	8	2,8	502,7812	42,58	13668	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

NVRPDYLK	39,36	1003,545	8	3,7	502,7816	44,14	14282	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	38,48	1003,545	8	3,7	502,7817	50,45	16716	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	38,06	1003,545	8	3,6	502,7816	36,71	11357	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	37,28	1003,545	8	1,1	502,7803	51,64	17168	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	36,99	1003,545	8	-0,9	502,7793	53,68	17950	mf18031 3_03.raw	protein1 sp  P04179 SO	
NVRPDYLK	36,56	1003,545	8	3,4	502,7815	32,98	9901	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NVRPDYLK	15,87	1003,545	8	72,5	502,8162	44,77	14523	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
RDFGSFDK	62,22	970,4508	8	3,6	486,2344	13,42	2800	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
RDFGSFDK	56,69	970,4508	8	4,4	486,2348	13,92	2983	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
PKGELLEAIK R	62,07	1252,7502	11	6,1	418,5932	15	3386	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
PKGELLEAIK R	24,39	1252,7502	11	4,4	627,3851	15,06	3409	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GWGWLGFN K	59,96	1063,5239	9	4,7	532,7717	33,38	10053	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GWGWLGFN K	40,36	1063,5239	9	2,2	532,7704	33,89	10250	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GELLEAIK	59,68	871,5015	8	-1,2	436,7575	21,1	5586	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	56,21	871,5015	8	2,9	436,7593	22,15	5957	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	54,52	871,5015	8	2,5	436,7591	21,61	5759	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	53,87	871,5015	8	2,9	436,7593	20,59	5415	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	51,19	871,5015	8	1,2	436,7585	48,29	15897	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	49,38	871,5015	8	2,3	436,759	48,82	16094	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	49,36	871,5015	8	3,3	436,7595	45,11	14659	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	48,73	871,5015	8	3,6	436,7596	50,39	16694	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	48,05	871,5015	8	2,5	436,7591	43,45	14010	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	47,9	871,5015	8	4,2	436,7599	52,54	17510	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	47,87	871,5015	8	2,2	436,759	52,02	17314	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GELLEAIK	47,45	871,5015	8	2,1	436,7589	47,56	15614	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GELLEAIK	45,33	871,5015	8	2,3	436,759	42,67	13707	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	44,63	871,5015	8	5,4	436,7604	26,66	7580	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	44,6	871,5015	8	3,7	436,7596	37,58	11697	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	44,59	871,5015	8	2,6	436,7591	28,16	8124	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	44,21	871,5015	8	2,3	436,759	47,03	15415	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	43,15	871,5015	8	0	436,758	49,84	16485	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	41,43	871,5015	8	4,1	436,7598	51,48	17107	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	32,37	871,5015	8	44,7	436,7775	42,7	13719	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	26,24	871,5015	8	40,7	436,7757	42,06	13464	mf18031 3_03.raw	protein1 sp  P04179 SO	
GELLEAIK	25,61	871,5015	8	41,4	436,7761	41,55	13259	mf18031 3_03.raw	protein1 sp  P04179 SO	
LTAASVGVQ GSGWGWLG	57,93	1791,8944	18	3,2	896,9573	51,18	16991	mf18031 3_03.raw	protein1 sp  P04179 SO	
LTAASVGVQ GSGWGWLG	36,27	1791,8944	18	2,6	896,9568	51,7	17192	mf18031 3_03.raw	protein1 sp  P04179 SO	
LTAASVGVQ GSGWGWLG F	22,79	1791,8944	18	5,4	896,9593	50,68	16802	mf18031 3_03.raw	protein1 sp  P04179 SO	
LTAASVGVQ GSGWGWLG F	16,72	1791,8944	18	3,3	896,9574	52,22	17389	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HHAAYVNNL NVT	57,01	1351,6633	12	3,3	676,8412	15,08	3418	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HHAAYVNNL NVT	42,44	1351,6633	12	16,7	676,8502	14,57	3228	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
IMQLHHSK	56,16	992,5226	8	-0,4	497,2684	10,44	1736	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
PHINAQIMQL HHSK	56,09	1652,8569	14	5,8	551,9628	14,01	3014	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HAAYVNNLN VTEEKYQEAL AK	55,6	2404,2021	21	1,1	1203,1096	20,83	5501	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
QIALQPALK	55,44	980,6018	9	3	491,3097	19,06	4859	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPYDY GALEPHINAQ IMQ	55,16	2621,2583	23	-14	874,7478	35,59	10910	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
IALQPALK	54,29	852,5433	8	4,2	427,2807	18,27	4574	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
KGELLEAIKR	53,02	1155,6975	10	4,4	386,2415	14,76	3299	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

GSFDKFK	52,79	827,4177	7	2,2	414,717	21,68	5784	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GSFDKFK	46,21	827,4177	7	1,5	414,7168	13,23	2728	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GSFDKFK	19,31	827,4177	7	3,8	414,7177	22,2	5977	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AQIALQPALK	52,27	1051,6389	10	3,6	526,8286	19,66	5075	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AQIALQPALK	29,63	1051,6389	10	1,5	526,8275	25,61	7206	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GDVTAQIALQ PA	49,98	1182,6244	12	2	592,3207	27,86	8011	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFK	49,66	974,4861	8	0,9	488,2507	16,72	4032	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFK	43,54	974,4861	8	4,2	488,2524	22,4	6052	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FGSFDKFK	18,88	974,4861	8	2,8	488,2517	21,66	5777	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GSFDKFKEK	49,13	1084,5553	9	1,3	362,5262	11,81	2226	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GSFDKFKEK	17,51	1084,5553	9	2,9	543,2865	18,12	4517	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GSFDKFKEK	16,75	1084,5553	9	3,9	543,287	17,61	4323	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
SFDKFKEK	48,91	1027,5338	8	2,4	514,7754	11,17	2010	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
LTAASVGVQ GS	48,48	988,5189	11	2,3	495,2679	18,04	4488	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NWENVTER	48,14	1046,4781	8	-1,7	524,2455	38,42	12023	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NWENVTER	37,23	1046,4781	8	-26,7	524,2324	38,29	11971	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GVQGSGWG WLGFNK	47,7	1491,7258	14	2,9	746,8724	38,04	11874	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GVQGSGWG WLGFNK	40,27	1491,7258	14	-2,3	746,8685	38,69	12117	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GVQGSGWG WLGFNK	19,42	1491,7258	14	3,9	746,8731	34,12	10341	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GVQGSGWG WLGFNK	15,91	1491,7258	14	5,3	746,8741	40,41	12808	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
GGEPKGELL EAIKR	46,04	1495,8358	14	1,9	499,6201	16,19	3844	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
INWENVTER	45,23	1159,5621	9	5,5	580,7915	21,25	5638	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEPHINAQ IMQLHHSKHH AAYVNNLNVT EEKYQEALAK GDVTAQIALQ PALK	45,16	7152,6309	64	1,1	1193,1138	39,56	12463	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

LNVTEEK	45,06	831,4338	7	2	416,725	11,57	2150	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
NLNVTEEK	45	945,4767	8	2,9	473,747	12,19	2366	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
LTAASVGVQ GSG	44,39	1045,5404	12	1,4	523,7782	17,75	4382	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
AYYLQYK	43,43	947,4752	7	-0,1	474,7448	17,81	4404	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEP	43,27	1685,7937	15	12,5	843,9147	33,18	9980	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HSLPDLPYDY GALEP	23,56	1685,7937	15	3,5	843,907	38,29	11974	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NLSPNGGGE PK	42,33	1068,5199	11	1,1	535,2678	11,09	1980	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HHAAYVNNL NVTEEKYQE ALAKGDVTA QIALQPALKF NGGGHINHSI FWTNLSPNG GGEPK	41,88	6565,2954	61	1,3	1095,2246	39,52	12449	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
WENVTER	39,97	932,4352	7	1,5	467,2256	13,63	2884	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
DFGSFDKF	39,69	961,4181	8	2,6	481,7176	30,32	8909	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
SGWGWLGF NKER	39,67	1435,6997	12	3,5	479,5755	26,75	7614	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HHAAYVNNL NVTEEKYQE ALAKGDVTA QIALQPALK	38,83	3947,054	36	-5,9	1316,6842	33,8	10215	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VNNLNVTEEK YQEALAK	38,64	1962,0057	17	-17,3	981,9932	29,67	8666	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LSPNGGGEP K	38,59	954,4771	10	1,6	478,2466	10,54	1775	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GTTGLIPLLGI DVWEHAYYL QYK	38,35	2649,3843	23	2,5	884,1376	46,27	15111	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LLEAIKR	37,41	841,5385	7	-1,1	421,7761	13,57	2861	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LLEAIKR	21,2	841,5385	7	1,2	421,777	17,25	4200	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LLEAIKR	16,74	841,5385	7	3,1	421,7778	32,64	9773	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
FDKFKEK	36,94	940,5018	7	-1,5	471,2574	17,71	4364	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
FNGGGHINH SIFWT	36,72	1585,7426	14	2,8	529,5896	23,45	6423	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
EKLTAASVGV Q	36,52	1101,6029	11	3,9	551,8109	15,93	3745	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
ELLEAIKR	36,41	970,5811	8	2	486,2988	16,7	4025	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

ELLEAIKR	33,46	970,5811	8	2,5	486,299	17,21	4185	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
ELLEAIKR	33,24	970,5811	8	3,6	486,2996	17,72	4371	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
ELLEAIKR	24,38	970,5811	8	4,6	486,3	18,31	4590	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GHLQIAA	35,88	708,3918	7	3,8	355,2045	14,42	3165	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
FNGGGHINH SIFWTNLSPN GGGEPKG	35,55	2693,2734	26	-42,8	898,7267	25,08	7011	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
EKLTAASVGV QGS	35,52	1245,6565	13	4,8	623,8385	15,84	3708	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
PDYLKAIWNV INWENVTER	35,37	2359,196	19	-24,5	1180,5764	36,48	11260	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
KNVRPDYLK	34,81	1131,64	9	1,7	378,2213	11,3	2055	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GWLGFNK	34,47	820,4232	7	1,8	411,2196	23,53	6451	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
FKEKLTAASV GVQGSGWG WLGFNK	34,15	2566,3333	24	-10,7	856,4426	35,9	11031	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
HSLPDLPY	33,61	940,4654	8	2,6	471,2412	24,04	6642	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YYLQYK	33,38	876,4381	6	1,2	439,2269	16,78	4057	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN VTE	30,79	1586,7729	13	2,6	794,3958	46,74	15299	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LTAASVGVQ GSGWGWLG FN	29,57	1905,9373	19	4	953,9797	49,25	16259	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VRPDYLK	29,54	889,5021	7	1,8	445,7592	12,14	2346	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VRPDYLK	23,91	889,5021	7	0,1	445,7584	12,65	2533	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
VRPDYLK	20,57	889,5021	7	1	445,7588	13,16	2699	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LEAIKR	28,34	728,4545	6	-3,8	365,2331	16,67	4015	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
LEAIKR	25,85	728,4545	6	-3,8	365,2331	17,18	4179	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINWEN	28,16	1257,6143	10	3,9	629,8168	43,6	14071	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YLQYK	28	713,3748	5	0,5	357,6949	13,02	2650	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
YLQYK	24,02	713,3748	5	0,5	357,6949	13,55	2853	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
TAASVGVQG SGWGWLGF NK	26,88	1920,9482	19	2,8	641,3251	36	11068	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GWLGFNKER	26,68	1105,5669	9	3,5	553,7927	18,08	4503	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	

AIWNVIN	26,36	828,4494	7	2,2	415,2329	33,96	10281	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
SIFWTNLSPN GGGEPKGEL LEAIK	26,24	2556,3223	24	-34,7	853,0851	30,3	8905	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
PYDYGALEP HINAQIMQLH HSKHHAAYV	26,03	3239,5723	28	2,1	1080,8669	31,92	9498	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GWGWLGFN KER	25,95	1348,6676	11	-16,2	675,3301	36,12	11117	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
GSRQKHSLP DLPYDYGALE PHINAQIMQL HHSK	24,37	3779,8953	33	-15,6	1260,9528	26,94	7682	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
NNLNVTEEKY QEALAK	24,24	1862,9374	16	-0,4	932,4756	18,86	4788	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
EPHINAQIMQ LHHSK	24,1	1781,8995	15	7	594,978	28,45	8231	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
EPHINAQIMQ LHHSK	16,18	1781,8995	15	-33,5	594,9539	28,39	8213	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
EKLTAASVGV QGSG	23,68	1302,6779	14	-4,6	652,3432	15,69	3647	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
RPDYLK	19,4	790,4337	6	0,4	396,2243	12,65	2535	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
RPDYLK	18,94	790,4337	6	0,6	396,2244	13,15	2698	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
RPDYLK	18,92	790,4337	6	1	396,2245	12,14	2349	mf18031 3_03.raw	protein1 sp  P04179 SO DM_HUMAN	
AIWNVINW	18,02	1014,5287	8	3	508,2732	45,98	15000	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
SFDKFK	16,29	770,3962	6	3	386,2065	21,73	5802	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HHAAYVNNL NVTEE	16,07	1609,7484	14	4,7	805,8853	15,99	3768	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	
HAYYLQYKN VRPDYLKAIW NVINWENVT	15,2	3794,9321	30	-3,1	1265,9807	38,59	12079	mf18031 3_03.raw	protein1 sp  P04179 SO DM HUMAN	

## **APPENDIX 6: Supplementary Dataset S4**

MS/MS data of the persulfidated MnSOD digested with chymotrypsin Related to CHAPTER 3 – Article I (Figure 3)

Peptide	-10lgP	Mass	Length	ppm	m/z	RT	Scan	Source File	Accession	PTM	AScore
K(+163.00)HS LPDLPY	17,07	1231,5616	9	2,6	616,7897	36,74	10508	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	NBF-N	K1:NBF- N:1000.00
K(+163.00)NV RPDYL	28,38	1166,5463	8	3,3	584,2823	30,31	8507	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	NBF-N	K1:NBF- N:1000.00
MAC(+163.00) KK	19,35	742,2885	5	3,3	372,1528	13,11	2561	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	NBF-C	C3:NBF- C:1000.00
MAC(+163.00) KK	18,23	742,2885	5	1,3	372,152	11,48	1952	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	NBF-C	C3:NBF- C:1000.00
TNLSPNGGG EPK(+163.00) GELL	17,45	1744,801	16	37,5	873,4405	25,08	6659	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	NBF-N	K12:NBF- N:1000.00
MAC(+394.16) KK	33,99	973,443	5	4,2	487,7308	18,68	4451	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	DCP- Bio1	C3:DCP- Bio1:1000.00
AASVGVQGS GW	37,46	1017,4879	11	3,2	509,7528	24,92	6603	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
AKGDVTAQIA LQPALKF	45,06	1770,0039	17	-0,8	591,0081	30,55	8588	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
ALEPHIN	15,55	792,413	7	4,5	397,2155	15,42	3361	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
ALEPHINAQI M	34,16	1235,6332	11	8,1	618,8289	26,21	7061	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
AQIALQPALK F	53,54	1198,7074	11	5,4	600,3642	31,37	8858	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
AQIALQPALK F	34,03	1198,7074	11	9,3	600,3666	31,88	9017	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
AQIMQLHHSK HHAAY	33,59	1770,8737	15	2,7	591,3001	14,01	2880	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
AQIMQLHHSK HHAAY	23,56	1770,8737	15	2,8	591,3002	14,52	3064	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
ASVGVQGSG W	17,11	946,4508	10	0,2	474,2328	24,56	6476	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
DFGSFDKF	18,36	961,4181	8	6,4	481,7194	32,55	9234	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		
DKFKEKL	44,1	906,5174	7	1,3	454,2665	12,12	2203	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N		

DVTAQIALQP ALKF	22,25	1513,8503	14	3	757,9348	39,05	11210	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPH	35,44	900,3977	8	4,5	451,2082	19,47	4728	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPH	28,29	900,3977	8	1,3	451,2067	18,96	4550	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN	55,96	1127,5247	10	5,7	564,7728	25,82	6925	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN	52,65	1127,5247	10	5,3	564,7726	26,33	7105	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN	38,37	1127,5247	10	5,5	564,7727	25,31	6740	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN	36,99	1127,5247	10	1,7	564,7706	26,86	7292	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN	19,4	1127,5247	10	3,3	564,7715	27,39	7477	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN A	34,31	1198,5618	11	-0,4	600,2879	26,8	7274	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQ	36,68	1326,6204	12	5	664,3208	25,52	6818	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIM	68,16	1570,745	14	6,4	786,3848	35,3	10096	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIM	46,5	1570,745	14	4,7	786,3835	35,82	10235	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIM	40,08	1570,745	14	5,1	786,3838	34,79	9933	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIM	19,59	1570,745	14	-1,5	786,3786	36,83	10540	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIM	15,94	1570,745	14	9,5	786,3872	36,33	10392	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIMQL	45,9	1811,8876	16	8,1	906,9584	40,6	11648	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIMQL	25,25	1811,8876	16	5,2	906,9558	41,11	11787	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
DYGALEPHIN AQIMQLH	25,4	1948,9465	17	6,4	650,6603	33,58	9556	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

DYGALEPHIN AQIMQLH	18,21	1948,9465	17	3,7	650,6585	34,08	9721	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFG	28,76	934,4872	8	2,8	468,2522	12,65	2403	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF	64,66	1168,5876	10	4,8	585,3039	19,66	4793	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF	16,21	1168,5876	10	2,7	390,5375	19,32	4678	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF D	17,15	1283,6145	11	4,9	642,8177	19,12	4607	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF DKF	71,46	1558,778	13	0,9	780,397	22,77	5839	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF DKF	60,88	1558,778	13	1,9	520,6009	22,74	5831	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EAIKRDFGSF DKF	31,8	1558,778	13	5,6	520,6028	23,54	6107	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
EHAYYLQY	48,08	1085,4818	8	4,8	543,7508	23,22	5988	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	45,09	909,4192	7	1,7	455,7177	13,44	2680	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	44,75	909,4192	7	3,2	455,7183	13,95	2860	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	41,84	909,4192	7	2,8	455,7181	12,93	2498	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	36,74	909,4192	7	3,7	455,7186	14,47	3043	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	22,26	909,4192	7	193,9	455,805	20,64	5121	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	20,52	909,4192	7	156	455,7878	19,63	4781	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	20,47	909,4192	7	154,4	455,7871	19,05	4584	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERY	20,03	909,4192	7	5,5	455,7194	18	4214	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
ENVTERYM	30,64	1040,4597	8	5,7	521,2401	17,18	3939	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHIN	18,1	849,4344	8	5,1	425,7267	17,15	3929	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
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GALEPHINAQ	17,4	1048,5302	10	2,4	525,2736	16,69	3778	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ I	19,63	1161,6141	11	3	581,8161	24,24	6355	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IM	51,66	1292,6547	12	5,1	647,3379	27,4	7484	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IM	46,36	1292,6547	12	-1	647,334	26,9	7307	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IM	41,87	1292,6547	12	4,5	647,3375	27,91	7665	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IM	28,85	1292,6547	12	5	647,3378	28,42	7851	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IM	25,9	1292,6547	12	5,4	1293,6689	27,16	7398	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IMQL	54,18	1533,7974	14	6,4	767,9109	33,62	9571	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GALEPHINAQ IMQL	18,74	1533,7974	14	-13,1	767,8959	30,1	8438	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
galephinaq Imqlh	41,01	1670,8562	15	4,4	557,9618	27,69	7592	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
galephinaq Imqlh	26,01	1670,8562	15	3,4	836,4382	27,2	7412	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GDVTAQIALQ PALKF	37,48	1570,8718	15	6,4	786,4482	36,41	10417	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GFNKERGHL	47,68	1056,5464	9	1,6	529,2813	11,48	1953	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GFNKERGHL	37,77	1056,5464	9	0,9	353,1897	11,48	1954	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GFNKERGHL Q	36,72	1184,605	10	-0,2	593,3096	11,03	1773	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GFNKERGHL Q	23,07	1184,605	10	-13,7	395,8702	11,05	1784	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA <u>N</u> N	
GGGEPKGEL L	28,73	955,4974	10	5,1	478,7584	17,1	3910	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

GGGHINHSIF W	49,77	1223,5836	11	1,9	612,8002	22,7	5818	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GGGHINHSIF W	47,81	1223,5836	11	5,7	408,8708	22,91	5883	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GGGHINHSIF W	25,14	1223,5836	11	2,3	612,8005	22,19	5649	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GGHINHSIF	40,05	980,4828	9	3,4	491,2503	15,68	3451	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GGHINHSIFW	19,06	1166,5621	10	0,4	584,2886	22,36	5712	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GHINHSIF	22,51	923,4613	8	2,9	462,7393	15,91	3531	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GSFDKF	41,9	699,3228	6	3,2	350,6698	17,9	4179	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
GSRQKHSLP DLPYDY	36,95	1774,8638	15	-31,3	592,61	21,41	5381	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
HHSKHHAAY	54,43	1086,5107	9	1	544,2632	10,33	1551	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
HHSKHHAAY	44,26	1086,5107	9	-8,1	363,1746	10,18	1493	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
HSKHHAAY	27,9	949,4518	8	2,2	475,7342	10,16	1485	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
HSLPDLPY	35,07	940,4654	8	5,6	471,2426	25,83	6928	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
HSLPDLPYDY	35,27	1218,5557	10	4,4	610,2878	29,85	8346	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	36,94	999,6116	9	5,7	500,8159	31,37	8857	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	35,62	999,6116	9	3,2	500,8147	30,87	8692	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	33,58	999,6116	9	7,9	500,817	31,88	9016	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	28,36	999,6116	9	5,9	500,816	32,9	9342	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	27,34	999,6116	9	4,9	500,8156	32,39	9183	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

IALQPALKF	26,41	999,6116	9	4,2	500,8152	30,22	8479	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IALQPALKF	16,1	999,6116	9	0,5	500,8133	34,92	9973	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
IMQLHHSKH	22,95	1129,5815	9	2,5	565,7994	10,73	1665	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
INAQIMQL	28,17	929,5004	8	3,3	465,759	33,1	9405	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
INAQIMQLH	18,53	1066,5593	9	6	534,2902	23,52	6098	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
INHSIFW	23	915,4603	7	2,9	458,7387	28,43	7854	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KAIWNVI	23,92	842,5014	7	5,6	422,2603	29,47	8222	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KAIWNVINW	35,95	1142,6237	9	9,1	572,3243	37,68	10791	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG	26,72	1002,5709	10	2	502,2937	12,06	2180	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQ	33,38	1229,6979	12	4,2	615,8588	14,21	2948	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQ	26,9	1229,6979	12	-54,1	615,8229	14,2	2946	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	78,93	1616,8522	16	5,6	539,961	21,28	5337	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	75,3	1616,8522	16	5,5	809,4378	21,32	5351	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	53,27	1616,8522	16	2	809,435	20,79	5168	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	46,37	1616,8522	16	4,7	539,9605	21,79	5514	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	29,32	1616,8522	16	4,1	809,4367	21,84	5531	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KEKLTAASVG VQGSGW	28,85	1616,8522	16	-13,9	809,4221	27,07	7366	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KGDVTAQIAL QPALKF	20,54	1698,9668	16	2,9	567,3312	30,82	8678	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

KHSLPDLPY	45,41	1068,5603	9	6,7	535,291	21,05	5256	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	43,58	1068,5603	9	5,3	535,2903	21,56	5429	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	40,16	1068,5603	9	4,8	357,1957	21,07	5265	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	31,94	1068,5603	9	4	535,2896	20,54	5083	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	29,85	1068,5603	9	3,6	535,2894	22,06	5605	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	25,22	1068,5603	9	5,9	535,2906	23,36	6042	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPY	18,37	1068,5603	9	4,4	535,2898	24,14	6321	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	66,17	1346,6506	11	3,2	674,3347	24,86	6582	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	58,69	1346,6506	11	5,9	674,3365	25,37	6761	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	56,49	1346,6506	11	-1,6	449,8901	25,39	6770	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	53,65	1346,6506	11	5,9	674,3365	25,89	6953	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	51,11	1346,6506	11	-1,6	449,8901	24,37	6401	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	47,49	1346,6506	11	-1,6	449,8901	24,88	6590	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	40,18	1346,6506	11	-1,6	449,8901	25,92	6966	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	34,88	1346,6506	11	4,6	674,3357	26,4	7130	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	20,32	1346,6506	11	-2,4	674,331	26,91	7310	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	17,7	1346,6506	11	-1,6	449,8901	27,31	7453	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD Y	16,51	1346,6506	11	-56,3	449,8656	27,01	7345	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

KHSLPDLPYD Y	15,43	1346,6506	11	-1,6	449,8901	26,73	7247	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YG	18,62	1403,6721	12	2,2	702,8449	24,01	6272	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YGAL	29,48	1587,7932	14	5,4	530,2745	30,09	8434	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YGALEPH	55,12	1950,9475	17	4,3	651,3259	27,94	7677	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YGALEPHIN	63,81	2178,0745	19	0,4	727,0324	30,46	8558	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YGALEPHIN	57,49	2178,0745	19	3,5	727,0347	30,97	8727	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KHSLPDLPYD YGALEPHINA Q	46,31	2377,1702	21	6,7	793,4026	30,03	8414	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	48,38	1003,545	8	2,3	502,7809	14,68	3122	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	47,99	1003,545	8	3,8	502,7817	14,18	2936	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	44,34	1003,545	8	1,9	502,7807	15,75	3478	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	44,15	1003,545	8	3,5	502,7816	15,24	3303	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	42,78	1003,545	8	3,6	502,7816	16,8	3815	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	34,18	1003,545	8	6,1	502,7828	17,32	3985	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	34,1	1003,545	8	2,5	502,781	16,26	3639	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	30,49	1003,545	8	4,8	502,7822	19,09	4596	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	24,12	1003,545	8	-31	502,7643	19,2	4636	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	19,45	1003,545	8	4,6	502,7821	19,71	4810	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYL	16,05	1003,545	8	5,6	502,7826	18,07	4241	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

KNVRPDYLK	32,16	1131,64	9	1,4	566,8281	11,9	2116	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK	27,4	1131,64	9	1,3	378,2211	12,03	2166	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK A	26,89	1202,6771	10	5,6	602,3492	12,99	2519	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK A	15,08	1202,6771	10	2,5	401,9007	12,95	2506	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	53,04	1501,8405	12	3,6	501,6226	25,57	6836	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	51,95	1501,8405	12	0,7	751,928	24,59	6485	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	50,52	1501,8405	12	2,7	501,6221	24,56	6473	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	49,52	1501,8405	12	2,9	501,6222	25,06	6651	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	47,38	1501,8405	12	3,2	751,9299	25,11	6668	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	44,28	1501,8405	12	2,8	751,9296	25,63	6858	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	43,02	1501,8405	12	7,2	501,6244	26,09	7026	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	34,61	1501,8405	12	0,5	501,621	26,6	7205	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	29,92	1501,8405	12	0,8	501,6212	27,11	7380	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	28,84	1501,8405	12	5,4	501,6235	28,67	7940	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	22,17	1501,8405	12	4,1	501,6228	29,17	8120	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
KNVRPDYLK AIW	19,69	1501,8405	12	4,6	501,6231	29,79	8327	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LGFNKERGH LQ	38,33	1297,6891	11	5,1	433,5725	13,36	2649	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LGFNKERGH LQ	20,21	1297,6891	11	4,2	649,8546	13,33	2638	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

LGIDVWEHA Y	55,35	1201,5768	10	6,2	601,7994	34,61	9881	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LGIDVWEHA Y	42,61	1201,5768	10	1,6	601,7966	35,11	10037	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LGIDVWEHA YY	47,53	1364,64	11	1,2	683,3281	36,82	10534	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LGIDVWEHA YY	30,84	1364,64	11	5,9	683,3313	37,33	10683	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LQPALKF	19,15	815,4905	7	3,8	408,7541	24,14	6319	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LQYKNVRPD YL	32	1407,751	11	6,9	704,8876	20,1	4940	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
LSPNGGGEP KGELL	26,37	1366,7092	14	3	684,364	20,6	5104	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI	41,74	1004,4788	10	2,6	503,248	11,31	1887	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	66,28	1151,5472	11	3,5	576,7829	16,1	3589	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	56	1151,5472	11	5,5	576,7841	15,58	3414	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	50,15	1151,5472	11	2,4	576,7823	16,6	3751	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	23,15	1151,5472	11	4,9	384,8582	15,61	3422	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	21,98	1151,5472	11	6	576,7844	17,19	3941	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI F	19,97	1151,5472	11	7,3	576,7851	19,98	4897	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	65,85	1337,6265	12	3,8	669,8231	23,13	5958	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	65,77	1337,6265	12	0,7	669,821	22,63	5800	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	63,74	1337,6265	12	4,6	669,8236	23,65	6148	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	59,69	1337,6265	12	3,1	669,8226	24,17	6330	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

NGGGHINHSI FW	57,37	1337,6265	12	4,6	669,8236	24,67	6515	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	54,78	1337,6265	12	6,8	669,8251	26,04	7011	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	54,14	1337,6265	12	2,4	669,8221	22,12	5622	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	52,93	1337,6265	12	3,1	669,8226	27,08	7372	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	45,2	1337,6265	12	4,1	446,8846	23,68	6158	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	44,59	1337,6265	12	1,3	446,8833	22,64	5804	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	44,26	1337,6265	12	5,2	669,824	27,58	7553	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	41,03	1337,6265	12	5,4	446,8852	24,7	6523	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	40,36	1337,6265	12	13,5	446,8888	22,13	5628	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	39,45	1337,6265	12	2,6	446,8839	24,19	6338	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	32,95	1337,6265	12	6,8	669,8251	30,91	8708	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	31,98	1337,6265	12	4,5	446,8848	23,15	5965	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	28,42	1337,6265	12	79,7	669,8738	26,57	7194	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	28,36	1337,6265	12	5	669,8239	29,12	8101	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	27,8	1337,6265	12	4,1	446,8846	25,2	6702	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	25,34	1337,6265	12	7	669,8252	32,95	9360	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	22,61	1337,6265	12	8,7	446,8867	31,93	9029	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	20,51	1337,6265	12	2,9	669,8224	32,32	9160	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

NGGGHINHSI FW	19,09	1337,6265	12	7,8	669,8257	31,44	8880	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	18,84	1337,6265	12	0,9	446,8832	30,66	8624	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	17,3	1337,6265	12	3,3	669,8227	35,88	10256	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	15,77	1337,6265	12	4,7	446,8849	28,66	7938	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NGGGHINHSI FW	15,77	1337,6265	12	2,5	669,8222	30,4	8534	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NKERGHLQI	25,23	1093,5992	9	2,3	365,5412	12,18	2225	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NKERGHLQIA A	28,09	1235,6735	11	2,7	412,8995	12,37	2296	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NKERGHLQIA A	16,46	1235,6735	11	1,5	618,8449	12,37	2297	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NLNVTEEKY	16,51	1108,54	9	5,9	555,2806	17,33	3990	mf18031 3_Chymo _03.raw	P04179 SO DM_HUMA N	
NLNVTEEKY QEAL	25,23	1549,7623	13	4,4	775,8918	24,24	6356	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NLSPNGGGE PKGELL	40,67	1480,7521	15	1	741,384	21,97	5577	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NLSPNGGGE PKGELL	30,58	1480,7521	15	1	741,384	22,49	5761	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ER	51,12	1372,6735	11	5,1	687,3475	26,43	7142	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	68,45	1535,7368	12	6,3	768,8805	31,48	8894	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	68,39	1535,7368	12	3,5	768,8784	30,95	8722	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	66,83	1535,7368	12	1	768,8765	30,45	8551	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	64,56	1535,7368	12	7	768,881	32,5	9217	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	64,48	1535,7368	12	8	768,8818	31,99	9051	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

NVINWENVT ERY	62,39	1535,7368	12	5,6	768,88	33,52	9539	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	61,68	1535,7368	12	5,2	768,8797	33,01	9376	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	61,06	1535,7368	12	6,9	768,881	35,63	10183	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	60,55	1535,7368	12	1,9	768,8771	35,12	10039	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	56,54	1535,7368	12	5,2	768,8796	34,62	9883	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	55,2	1535,7368	12	5,3	768,8798	36,14	10335	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	54,2	1535,7368	12	5,3	768,8798	34,11	9731	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	52,16	1535,7368	12	1,1	768,8765	38,17	10943	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	51,65	1535,7368	12	5,2	768,8796	40,22	11541	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	51,43	1535,7368	12	8,3	768,8821	41,24	11821	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	50,94	1535,7368	12	2	768,8772	37,67	10786	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	50,38	1535,7368	12	5,8	768,8801	40,73	11685	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	48,11	1535,7368	12	3,2	768,8781	36,65	10482	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	47,42	1535,7368	12	3,9	768,8787	48,17	13676	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	47,17	1535,7368	12	2,6	768,8777	51,76	14755	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	47,04	1535,7368	12	2,1	768,8773	38,69	11103	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	46,97	1535,7368	12	1,4	768,8768	53,29	15219	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	45,39	1535,7368	12	5,9	768,8802	39,2	11257	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

NVINWENVT ERY	44,11	1535,7368	12	2,8	768,8778	50,22	14285	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	42,82	1535,7368	12	4,3	768,879	46,64	13270	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	41,82	1535,7368	12	-0,2	768,8755	41,75	11965	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	40,81	1535,7368	12	7	768,881	47,15	13399	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	39,92	1535,7368	12	4,8	768,8793	39,71	11395	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	39,36	1535,7368	12	2,2	768,8774	47,67	13539	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	39,28	1535,7368	12	-0,6	768,8752	52,78	15062	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	39,06	1535,7368	12	2,1	768,8773	52,26	14910	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	38,85	1535,7368	12	2,8	768,8778	48,69	13831	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	37,23	1535,7368	12	2,1	768,8773	51,24	14595	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	36,45	1535,7368	12	2,1	768,8773	49,19	13984	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	35,12	1535,7368	12	0,3	768,8759	54,32	15560	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	32,44	1535,7368	12	4,3	768,879	46,12	13133	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	32,03	1535,7368	12	3,2	768,8781	53,8	15390	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	31,42	1535,7368	12	0,9	768,8763	50,73	14439	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVINWENVT ERY	25,76	1535,7368	12	5,7	768,8801	49,71	14140	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NVRPDYL	25,11	875,4501	7	3,5	438,7339	17,86	4168	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
NWENVTERY	33,05	1209,5414	9	3,5	605,7801	21,97	5575	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

PLQGTTGLIP L	16,33	1108,6492	11	10	555,3374	39,51	11343	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QGTTGLIPL	29,43	898,5124	9	-26,3	450,2516	18,35	4335	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QGTTGLIPLL	23,16	1011,5964	10	-7,1	506,8019	17,9	4180	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QIALQPALKF	24,17	1127,6703	10	3,9	564,8446	31,04	8750	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QLHHSKHHA AY	53,55	1327,6533	11	1	664,8346	11,21	1849	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QLHHSKHHA AY	53,14	1327,6533	11	-0,9	664,8333	10,71	1658	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QLHHSKHHA AY	51,72	1327,6533	11	0,7	664,8344	10,2	1500	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QLHHSKHHA AY	33,32	1327,6533	11	0,5	443,5586	10,73	1667	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QLHHSKHHA AY	29,44	1327,6533	11	1	443,5588	10,22	1512	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QPALKF	40,76	702,4064	6	4	352,2119	19,17	4625	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
QYKNVRPDY L	27,8	1294,667	10	4,2	648,3435	16,49	3716	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
RQKHSLPDL PYDY	28,21	1630,8103	13	-36	544,5911	22,95	5897	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SLPDLPY	34,8	803,4065	7	5,4	402,7127	34,2	9758	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SLPDLPYDY	42,6	1081,4967	9	4,5	541,7581	37,15	10631	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SLPDLPYDY	15,12	1081,4967	9	2,3	541,7569	37,67	10787	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SLPDLPYDYG ALEPHIN	52,11	1912,9207	17	1,8	957,4693	38,92	11172	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SPNGGGEPK GELL	62,46	1253,6251	13	3,2	627,8218	17,81	4151	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SPNGGGEPK GELL	61,5	1253,6251	13	5,7	627,8234	17,3	3979	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

SPNGGGEPK GELL	28,65	1253,6251	13	2,9	627,8217	18,32	4325	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
SPNGGGEPK GELL	24,05	1253,6251	13	7,1	627,8243	18,86	4510	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TAASVGVQG SGW	61,76	1118,5356	12	7,5	560,2793	25,97	6985	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TAASVGVQG SGW	47,87	1118,5356	12	4,1	560,2774	25,47	6797	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TAASVGVQG SGW	22,08	1118,5356	12	-4,1	560,2728	26,48	7160	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGEL	23,16	1468,7157	15	3,8	735,3679	17,34	3993	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	69,01	1581,7998	16	1,7	791,9085	22,33	5701	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	68,19	1581,7998	16	3,8	791,9102	23,86	6218	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	67,01	1581,7998	16	5,2	791,9113	23,34	6033	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	64,19	1581,7998	16	2,3	791,909	22,84	5861	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	59,82	1581,7998	16	5	791,9111	27,98	7689	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	56,66	1581,7998	16	5,5	791,9115	27,46	7505	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	53,07	1581,7998	16	3	791,9095	29,03	8069	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	52,45	1581,7998	16	3,9	791,9103	31,14	8782	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	50,44	1581,7998	16	8,9	791,9142	31,65	8951	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	49,78	1581,7998	16	4,4	791,9106	46,09	13122	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	49,35	1581,7998	16	5,4	791,9114	43,04	12344	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	49,28	1581,7998	16	-0,8	791,9066	30,63	8616	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

TNLSPNGGG EPKGELL	49,12	1581,7998	16	7,5	791,9131	34,45	9834	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	48,65	1581,7998	16	3,9	791,9103	30,11	8443	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	47,85	1581,7998	16	6,2	791,912	29,56	8254	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	46,84	1581,7998	16	2,5	791,9092	34,96	9988	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	45,1	1581,7998	16	5	791,9111	41,49	11891	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	44,91	1581,7998	16	7,3	791,913	33,6	9562	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	44,45	1581,7998	16	1,7	791,9085	39,02	11200	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	44,33	1581,7998	16	3,6	791,91	38,51	11048	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	43,99	1581,7998	16	4,2	791,9105	40,03	11490	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	43,93	1581,7998	16	3,7	791,9101	33,09	9403	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	43,09	1581,7998	16	10,8	791,9157	35,47	10145	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	42,41	1581,7998	16	2,3	791,909	36,99	10584	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	41,48	1581,7998	16	1,3	791,9082	47,61	13522	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	40,22	1581,7998	16	7,5	791,9131	44,04	12596	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	39,9	1581,7998	16	1,6	528,2747	22,44	5745	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	39,27	1581,7998	16	1,6	791,9084	48,64	13818	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	39,09	1581,7998	16	5,3	791,9114	45,58	12984	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	38,18	1581,7998	16	6,1	791,912	43,54	12478	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

TNLSPNGGG EPKGELL	38,07	1581,7998	16	4,4	791,9106	37,5	10734	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	37,88	1581,7998	16	4,8	791,9109	46,59	13255	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	37,59	1581,7998	16	1,7	791,9085	38	10891	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	37,39	1581,7998	16	0,4	791,9075	50,17	14269	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	37,14	1581,7998	16	6,2	791,9121	36,49	10437	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	36,63	1581,7998	16	7,5	791,9131	39,53	11347	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	35,93	1581,7998	16	10,8	791,9157	45,07	12851	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	35,76	1581,7998	16	8,2	791,9137	47,1	13389	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	35,2	1581,7998	16	2,7	791,9093	35,97	10285	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	34,71	1581,7998	16	4,3	791,9106	48,12	13664	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	34,41	1581,7998	16	3	791,9095	53,77	15381	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	34,38	1581,7998	16	3,3	791,9098	51,71	14739	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	34,38	1581,7998	16	2,4	791,9091	52,23	14897	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	33,32	1581,7998	16	3,3	791,9098	50,69	14423	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	32,9	1581,7998	16	2,8	791,9094	52,74	15051	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	31,94	1581,7998	16	1,8	791,9086	53,26	15207	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	31,91	1581,7998	16	4,9	791,9111	40,99	11756	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	31,37	1581,7998	16	3,1	791,9096	51,19	14581	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

TNLSPNGGG EPKGELL	31,01	1581,7998	16	-33,9	791,8804	44,79	12788	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	29,55	1581,7998	16	4,6	791,9108	44,56	12728	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	28,06	1581,7998	16	2,2	791,9089	49,15	13971	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	27,76	1581,7998	16	3,1	791,9097	42	12039	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	27,54	1581,7998	16	-0,1	791,9071	54,29	15548	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELL	26,27	1581,7998	16	3,9	791,9103	49,66	14127	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELLEA	16,5	1781,8795	18	3,3	891,95	23,06	5933	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
TNLSPNGGG EPKGELLEAI K	24,66	2023,0585	20	7,6	675,3652	23,88	6224	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VINWENVTE RY	28,22	1421,6938	11	3,6	711,8568	29,18	8123	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VINWENVTE RY	26,1	1421,6938	11	2	711,8556	29,7	8299	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK	39,61	1158,588	10	5,7	580,3046	14,22	2952	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK Y	61,74	1321,6514	11	2,5	661,8346	18,94	4541	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK Y	61,72	1321,6514	11	2,7	661,8347	18,41	4354	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK Y	33,4	1321,6514	11	2,8	661,8348	19,45	4720	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEAL	62,26	1762,8737	15	5,3	882,4488	25,82	6926	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEAL	37,19	1762,8737	15	7,1	588,636	25,37	6762	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEAL	24,65	1762,8737	15	5,8	882,4492	26,33	7106	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEALA	19,77	1833,9108	16	3	917,9654	25,61	6850	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

VNNLNVTEEK YQEALAK	55,69	1962,0057	17	5,1	655,0125	21,51	5413	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEALAKGD VTAQ	71,64	2533,2659	23	0,8	845,4299	26,89	7305	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEALAKGD VTAQ	21,18	2533,2659	23	3,5	845,4322	27,92	7670	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VNNLNVTEEK YQEALAKGD VTAQIA	23,87	2717,3872	25	0,6	906,8036	30,51	8577	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VRPDYL	15,4	761,4072	6	5,7	381,713	17,01	3880	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VRPDYLKAIW	15,79	1259,7026	10	4,4	630,8614	29,24	8143	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
VTAQIALQPA LKF	24,49	1398,8235	13	4,8	700,4224	33,55	9548	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WENVTERY	20,96	1095,4985	8	4,1	548,7588	19,24	4651	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	70,65	1767,8792	17	6,6	884,9527	28,5	7876	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	64,73	1767,8792	17	3,2	884,9497	29,02	8067	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	53,14	1767,8792	17	5,3	884,9515	27,99	7692	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	46,56	1767,8792	17	6,2	884,9523	29,53	8243	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	44,09	1767,8792	17	-0,2	884,9467	30,56	8593	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	40,68	1767,8792	17	3,9	884,9503	33,33	9481	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	38,18	1767,8792	17	2,5	884,9491	32,09	9085	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	18,65	1767,8792	17	6,1	884,9523	32,82	9314	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
WTNLSPNGG GEPKGELL	15,91	1767,8792	17	4,2	884,9506	34,38	9813	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	
YLKAIW	20,31	792,4534	6	5,4	397,2361	25,82	6927	mf18031 3_Chymo _03.raw	protein1 sp  P04179 SO DM_HUMA N	

## **APPENDIX 7: Supplementary Dataset S5**

Antibody microarray analysis of protein persulfidation Related to CHAPTER 3 – Article I (Figure 5) PDK1 (Phospho-Ser241) Raf1 (Phospho-Ser259) MEK2 (Phospho-Thr394) STAT1 (Phospho-Tyr701) STAT3 (Phospho-Tyr705) STAT3 (Phospho-Ser727) AKT (Phospho-Ser473) AKT (Phospho-Thr308) FAK (Phospho-Tyr861) HER2 (Phospho-Tyr877) HER2 (Phospho-Tyr1221/Tyr1222) HER2 (Phospho-Tyr1248) EGFR (Phospho-Ser1070) EGFR (Phospho-Tyr1092) Paxillin (Phospho-Tyr118) Caveolin-1 (Phospho-Tyr14) Src (Phospho-Tyr418) Catenin beta (Phospho-Thr41/Ser45) FAK (Phospho-Tyr925) IKK-alpha (Phospho-Thr23) VAV1 (Phospho-Tyr174) MKK3 (Phospho-Ser189) JAK1 (Phospho-Tyr1022) JAK2 (Phospho-Tyr221) JAK2 (Phospho-Tyr1007) Src (Phospho-Tyr529) MEK1 (Phospho-Ser221) STAT1 (Phospho-Ser727) SEK1/MKK4 (Phospho-Thr261) SEK1/MKK4 (Phospho-Ser80) Paxillin (Phospho-Tyr31) Rac1/cdc42 (Phospho-Ser71) Raf1 (Phospho-Ser338) MEK1 (Phospho-Ser217) Catenin beta (Phospho-Ser33) Catenin beta (Phospho-Ser37) EGFR (Phospho-Tyr1172) EGFR (Phospho-Tyr1197) EGFR (Phospho-Tyr869) p44/42 MAP Kinase (Phospho-Thr202) p44/42 MAP Kinase (Phospho-Tyr204) SAPK/JNK (Phospho-Thr183) SAPK/JNK (Phospho-Tyr185) EGFR (Phospho-Tyr1110) Dok-2 (Phospho-Tyr299)



Gab1 (Phospho-Tyr627) MEK1 (Phospho-Thr291) EGFR (Phospho-Thr678) Shc (Phospho-Tyr349) Shc (Phospho-Tyr427) PDK1 (Ab-241) Raf1 (Ab-259) MEK2 (Ab-394) STAT1 (Ab-701) STAT3 (Ab-705) STAT3 (Ab-727) AKT (Ab-473) AKT (Ab-308) HER2 (Ab-877) HER2 (Ab-1221/1222) HER2 (Ab-1248) EGFR (Ab-1070) EGFR (Ab-1092) FAK (Ab-861) Catenin beta (Ab-41/45) Paxillin (Ab-118) Caveolin-1 (Ab-14) Src (Ab-418) MKK3 (Ab-189) JAK1 (Ab-1022) JAK2 (Ab-221) JAK2 (Ab-1007) SEK1/MKK4 (Ab-261) FAK (Ab-925) IKK-alpha (Ab-23) VAV1 (Ab-174) Src (Ab-529) MEK1 (Ab-221) STAT1 (Ab-727) Paxillin (Ab-31) Rac1/cdc42 (Ab-71) Raf1 (Ab-338) MEK1 (Ab-217) FAK (Ab-397) Catenin beta (Ab-37) EGFR (Ab-1172) EGFR (Ab-1197) EGFR (Ab-869) p44/42 MAP Kinase (Ab-202) p44/42 MAP Kinase (Ab-204)



SAPK/JNK (Ab-183) SAPK/JNK (Ab-185) EGFR (Ab-1110) Dok-2 (Ab-299) Gab1 (Ab-627) MEK1 (Ab-291) EGFR (Ab-678) EGFR (Ab-693) Shc (Ab-349) MKK6 (Phospho-Ser207) FAK (Phospho-Tyr397) EGFR (Phospho-Thr693) FAK (Phospho-Tyr576) IKK-alpha/beta (Phospho-Ser180/181) IKK-beta (Phospho-Tyr188) IKK-gamma (Phospho-Ser31) EGFR (Phospho-Tyr1016) FAK (Phospho-Tyr407) IKK-beta (Phospho-Tyr199) JNK1/2/3 (Phospho-Thr183/Tyr185) PAK1 (Phospho-Thr212) PAK1/2/3 (Phospho-Thr423/402/421) Raf1 (Phospho-Tyr341) Raf1(Phospho-Ser621) c-Raf (Phospho-Ser296) MEK1 (Phospho-Ser298) MEK1 (Phospho-Thr286) Ras-GRF1 (Phospho-Ser916) Gab1 (Phospho-Tyr659) PKC pan activation site (Phospho) AKT (Phospho-Tyr326) Catenin beta (Phospho-Tyr654) c-Raf (Phospho-Ser43) EGFR (Phospho-Tyr1069) ERK3 (Phospho-Ser189) ERK8 (Phospho-Thr175/Tyr177) FAK (Phospho-Ser910) PAK1/2 (Phospho-Ser199) IKK gamma (Phospho-Ser85) PI3-kinase p85-subunit alpha/gamma (Phospho-Tyr467/Tyr199) PI3-kinase p85-alpha (Phospho-Tyr607) Rho/Rac guanine nucleotide exchange factor 2 (Phospho-Ser885) VAV2 (Phospho-Tyr142) Catenin beta (Phospho-Tyr489)

MAP3K1/MEKK1 (Phospho-Thr1381)



MKK3/MAP2K3 (Phospho-Thr222) MKK7/MAP2K7 (Phospho-Ser271) MKK7/MAP2K7 (Phospho-Thr275) PAK1 (Phospho-Ser204) Src (Phospho-Ser75) Src (Phospho-Tyr216) GAB2 (Phospho-Ser159) PLCG1 (Phospho-Tyr771) PLCG1 (Phospho-Tyr783) PLCG2 (Phospho-Tyr753) CBL (Phospho-Tyr774) CBL (Phospho-Tyr700) PLCG1 (Phospho-Tyr1253) PLCG2 (Phospho-Tyr1217) SEK1/MKK4/JNKK1 (Phospho-Ser257) MKK6 (Ab-207) Catenin beta (Ab-33) Shc (Ab-427) FAK (Ab-576) IKK-alpha/beta (Ab-180/181) IKK-beta (Ab-188) IKK-gamma (Ab-31) EGFR (Ab-1016) FAK (Ab-407) IKK-beta (Ab-199) JNK1/2/3 (Ab-183/185) PAK1 (Ab-212) PAK1/2/3 (Ab-423/402/421) Raf1 (Ab-341) Raf1 (Ab-621) RhoA (Ab-188) c-Raf (Ab-296) MEK1 (Ab-298) MEK1 (Ab286) Ras-GRF1 (Ab-916) Gab1 (Ab-659) PKC pan activation site AKT (Ab-326) Catenin beta (Ab-654) c-Raf (Ab-43) EGFR (Ab-1069) ERK3 (Ab-189) FAK (Ab-910) PAK1/2 (Ab-199) IKK gamma (Ab-85)



PI3-kinase p85-subunit alpha/gamma (Ab-467/199) Rho/Rac guanine nucleotide exchange factor 2 (Ab-885) VAV2 (Ab-142) Catenin beta (Ab-489) MKK3/MAP2K3 (Ab-222) MKK7/MAP2K7 (Ab-271) PAK1 (Ab-204) Raf1 (Ab-289) Src (Ab-75) GAB2 (Ab-159) SEK1/MKK4 (Ab-80) PLCG1 (Ab-771) PLCG1 (Ab-783) PLCG2 (Ab-753) PLCG2 (Ab-1217) SEK1/MKK4/JNKK1 (Ab-257) E-cadherin (N-term) ERK1/2 (N-term) NCK2 (C-term) RAS(p21 H and K) (inter) MEKKK 4 (inter) IP3KA (C-term) JNKK (MKK4) (inter) MEKKK 1 (inter) IP3KC (inter) RASE (inter) RASF4 (inter) NCKX6 (inter) EGF-Like Module-Containing Mucin-Like Receptor 1 (EMR1) (inter) EGF-Like Module-Containing Mucin-Like Receptor 2 (EMR2) (inter) EGF-Like Module-Containing Mucin-Like Receptor 3 (EMR3) (inter) IP6K3 (inter) IP6K2 (inter) PIP5K (inter) Actin GAPDH

