



Utilisation of nanomaterials in the study of spectral markers of neurodegenerative diseases

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ÉCOLE DOCTORALE DES SCIENCES CHIMIQUES

UMR 7140, Chimie de la matière complexe

LABORATOIRE DE BIOÉLECTROCHIMIE ET SPECTROSCOPIE

THÈSE présentée par :

Krongkarn SIRINUKUNWATTANA

soutenue le : **10 Juillet 2025**

pour obtenir le grade de : **Docteur de l'université de Strasbourg**

Discipline/ Spécialité : Chimie

**Utilisation de nanomatériaux dans l'étude de marqueurs
spectraux de maladies neurodégénératives**

**Utilisation of nanomaterials in the study of spectral
markers of neurodegenerative diseases**

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*"Medicine and public health
are fundamental foundations of a country's development.
No nation can achieve sustainable and comprehensive progress
if people remain in unhealthy."*

*Royal Address of His Majesty King Bhumibol Adulyadej the Great
at the Prince Mahidol Award Presentation Ceremony for the Year 1996*

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RÉSUMÉ

1) Introduction

Cette thèse se concentre principalement sur deux maladies neurologiques : la sclérose en plaques et la maladie d'Alzheimer. La première partie de la thèse aborde la sclérose en plaques récurrente-rémittente (SEP-RR), une maladie auto-immune inflammatoire chronique du système nerveux central (SNC) caractérisée par la démyélinisation et l'inflammation. Cette condition conduit à une réduction des niveaux d'alloprégnanolone détectés dans le cerveau [1]. La démyélinisation et l'inflammation du SNC entraînent des incapacités, notamment chez les jeunes adultes. Deux molécules, l'interféron- β (IFN- β) et le XBD173, ont été utilisées pour traiter l'encéphalomyélite auto-immune expérimentale (EAE), un modèle murin de la SEP-RR. L'IFN- β est utilisée depuis plus de deux décennies pour réduire les taux de rechutes dans les formes de SEP-RR [2]. L'IFN- β agit comme un anti-inflammatoire naturel circulant dans le sang, augmentant le nombre de cellules anti-inflammatoires [2,3]. Cependant, la thérapie par IFN- β est associée à des effets secondaires graves [3]. Afin d'atténuer ces effets secondaires, le XBD173 a été exploré comme traitement alternatif. Le XBD173 est un médicament synthétique utilisé pour réduire l'anxiété sans les effets secondaires des benzodiazépines. Il présente une grande sélectivité et affinité pour le TSPO, protéine située dans la membrane externe des mitochondries. Le ligand TSPO XBD173 réduit les cytokines pro-inflammatoires, augmente l'alloprégnanolone neuroprotectrice dans la moelle épinière [4, 5] et améliore les symptômes cliniques et comportementaux chez les souris EAE induites par PLP [5].

La deuxième partie de la thèse porte sur la maladie d'Alzheimer (MA), un trouble neurodégénératif progressif. L'agrégation de peptides amyloïdes est un signe distinctif de cette maladie. Dans la MA, la voie d'agrégation convertit l'A β monomérique en fibrilles, formant ainsi des feuillets β parallèles. Ces changements impliquent la rupture/formation de liaisons H, la réorientation des brins β et l'allongement des feuillets β [6]. Les dépôts extracellulaires de feuillets β insolubles, connus sous le nom de "plaques", se composent principalement de l'isoforme A β 42, tandis que les dépôts vasculaires sont formés par A β 40 [7]. Les immunothérapies récentes contre la MA sont coûteuses, mais elles entraînent plus problématiquement des effets secondaires graves, tels que des œdèmes cérébraux et des saignements chez jusqu'à 81 % des patients. Ces effets secondaires proviennent du ciblage non spécifique des anticorps anti-A β qui ciblent d'abord les parois des vaisseaux sanguins cérébraux. Pour résoudre ce problème grave, de nouveaux peptides cycliques (PC) ciblant spécifiquement les premières assemblées de A β seront testés. Les nanoparticules d'or (GNP), en combinaison avec le PC, permettront de fournir une évaluation préclinique précoce des premières espèces de A β dans le sérum sanguin et le cerveau. Les microscopies IR et Raman seront utilisées pour la détection des PC et de leur interaction avec les oligomères de A β [8-11].

La spectroscopie infrarouge à transformée de Fourier (FTIR) est une technique très utilisée dans l'étude d'échantillons biologiques et dans des applications médicales. Le spectre IR des échantillons biologiques contient des bandes d'absorption provenant des protéines, des lipides, des acides nucléiques et des glucides, fournissant des signatures moléculaires [12]. Dans les deux

parties de la thèse, la FTIR est utilisée pour analyser le sérum et le tissu cérébral des souris. Concernant la SEP-RR, l'étude vise à sonder les effets des différents traitements (IFN- β et XBD173) sur le sérum des souris et sa relation avec les scores cliniques.

L'étude des échantillons de la MA vise à développer des outils pour le diagnostic précoce de la maladie, ainsi que le développement d'outils de prévention et de traitement de la MA en utilisant des approches de nanotechnologie.

2) Résultats et discussions

2.1 Sclérose en plaques

88 échantillons de sérum, comme indiqué dans le tableau 1, ont été reçus du Professeur A. G. Mensah-Nyagan (Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutiques, INSERM U1119). Chaque échantillon de sérum (2.5 μ L) a été analysé dans la gamme de 4000-700 cm^{-1} .

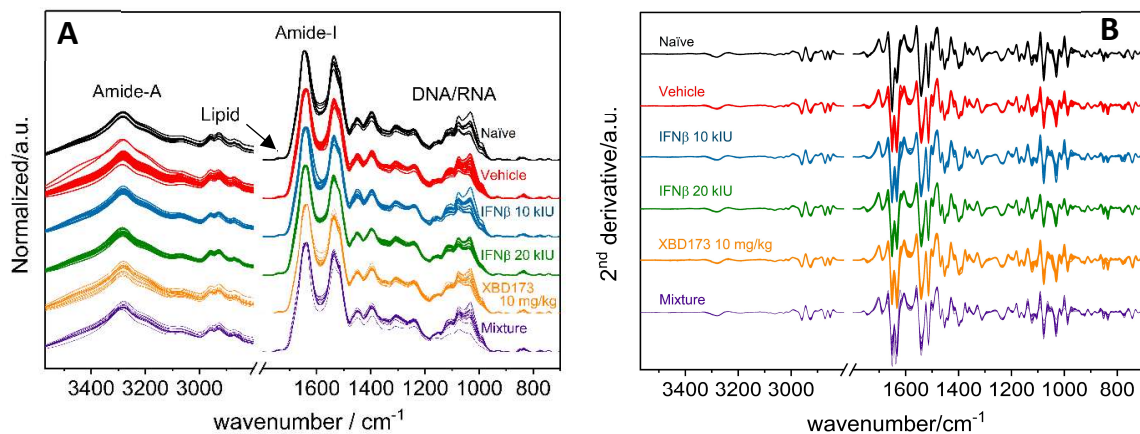


Figure 1 (A) Les spectres normalisés des échantillons de sérum dans les gammes 3400-2600 cm^{-1} et 1800-700 cm^{-1} . (B) Les dérivées secondes des spectres de sérum. Naïf (noir), Véhicule (rouge), IFN- β 10 kIU (bleu), IFN- β 20 kIU (vert), XBD173 (orange), et Mélange (violet).

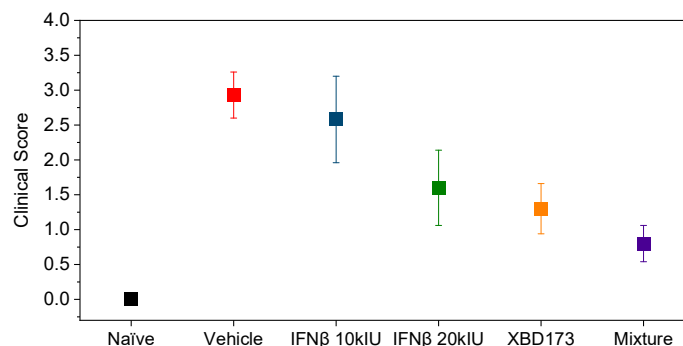


Figure 2. Les scores cliniques moyens des groupes Naïf, Véhicule et des groupes de traitement.

Tableau 1. Les cohortes de souris incluses dans cette étude ainsi que leurs scores cliniques moyens (moyenne $\pm$ SEM) au pic et au moment du sacrifice et du prélèvement des échantillons sanguins. L'échelle standard de cotation varie de 0 à 10, un score de 0 indiquant l'absence de signes cliniques. Des scores cliniques plus élevés reflètent une aggravation de la maladie, un score maximal de 10 indiquant la mort. Une valeur de $p \leq 0.05$ a été considérée comme significative (versus Naïf * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ ou versus Véhicule # $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$, #### $p \leq 0.0001$).

Cohort	Nombre total de souris	Au pic (D14-D16)		Au moment du sacrifice (D35-D37)	
		Score clinique moyens ± SEM	p	Score clinique moyens ± SEM	p
Serum					
Naïve (healthy)	7	0.00	####	0.00	####
Vehicle (untreated MS)	23	3.35 ± 0.27	****	2.93 ± 0.33	****
IFNβ 10 kIU	13	3.23 ± 0.43	****	2.58 ± 0.62	***
Treated RRMS IFNβ 20 kIU	10	2.50 ± 0.48	**	1.60 ± 0.54	*#
10 mg/kg XBD173	20	2.70 ± 0.23	***	1.30 ± 0.36	####
10 mg/kg XBD173+IFNβ 10 kIU	15	2.47 ± 0.24	**	0.80 ± 0.26	###
Brain tissue					
Naïve (healthy)	1	-	-	0	-
Vehicle (untreated MS)	1	-	-	5	-
IFNβ 20 kIU	1	-	-	2	-
Treated RRMS 10 mg/kg XBD173	1	-	-	2	-
10 mg/kg XBD173+IFNβ 10 kIU	1	-	-	1	-

Note : le score clinique varie de 0 (état sain) à 10 (décès).

Les données de scores cliniques ont été fournies par le Professeur A. G. Mensah-Nyagan. Le score clinique varie de 0 à 10, où 0 indique un état sain et 10 indique la mort. La figure 2 présente les scores cliniques des groupes Naïf, Véhicule et des différents traitements. Le groupe Naïf montre le score clinique le plus bas, tandis que le groupe Véhicule présente le plus élevé. Bien que la demi-dose d'IFN- β ait été inefficace, la dose complète a été aussi efficace que le XBD173. Fait intéressant, le traitement combiné a montré la plus grande efficacité grâce aux effets synergiques entre l'anti-inflammation et la neuroprotection.

2.1.1 La corrélation entre l'IFN β 20 kUI et le mélange de 10 mg/kg de XBD173 + IFN β 10 kUI traité dans la SEP

La corrélation entre l'IFN β 20 kUI et le mélange de 10 mg/kg de XBD173 + IFN β 10 kUI (Figure 29) montre les effets les plus associés dans la région lipidique de vibration CH (2981, 2942, 2832 et 2908 cm^{-1}) et dans la région amide A (3025 cm^{-1}). Une corrélation plus faible est observée dans la région amide A (3025, 3652, 3579, 3546, 3477, 3272, 3303, 3110, 3351, 3370, 3170 et 3201 cm^{-1}) et dans la région amide B (3060 cm^{-1}). La corrélation la plus faible est

constatée dans la région phospholipidique (1799 cm^{-1}), la région des glucides (1045 cm^{-1}) et les régions protéiques (989 et 1103 cm^{-1}). De plus, une corrélation négative entre les deux traitements entraîne une inhibition dans la région phospholipidique (1758 et 1700 cm^{-1}), la région amide I (1650 et 1614 cm^{-1}), les régions protéiques (1344 , 1319 , 1376 , 1432 et 1481 cm^{-1}) ainsi que les régions nucléiques (1207 et 836 cm^{-1}).

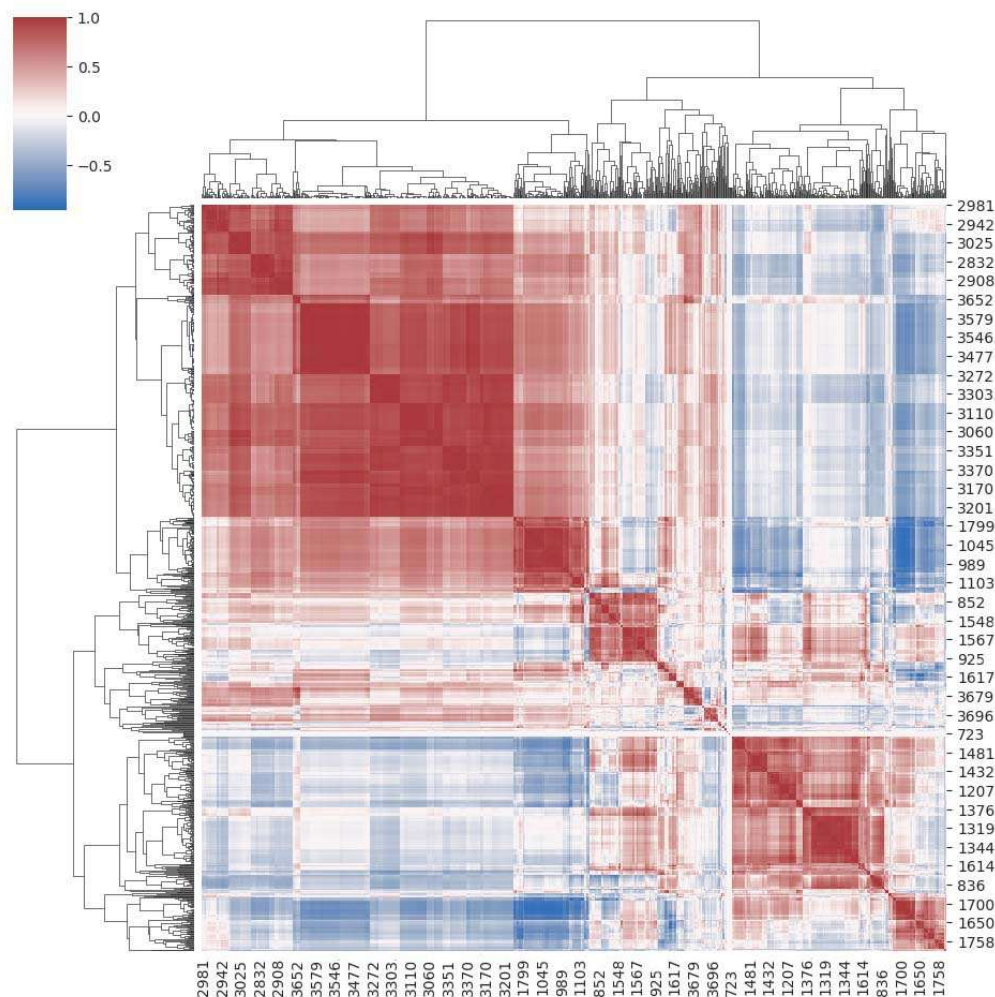


Figure 3 illustre la corrélation la plus élevée entre l'IFN β 20 kUI et le mélange de 10 mg/kg de XBD173 + IFN β 10 kUI dans la région lipidique de vibration CH. Les deux traitements amplifient le signal centré à 2959, 2927, 2872 et 2852 cm^{-1} . Notamment, le signal à 2852 cm^{-1} , correspondant à la vibration symétrique CH_2 , présente des différences significatives par rapport au véhicule.

L'influence du traitement par IFN β -1b sur les antioxydants sanguins (α -tocophérol et NADPH) dans la SEP met en évidence un effet d'épargne sur la teneur en α -tocophérol des érythrocytes. Cette molécule, principal antioxydant des membranes, protège ces structures contre la peroxydation lipidique. La diminution observée du NADPH, parallèlement à une augmentation des triglycérides plasmatiques, suggère que l'IFN β -1b stimule la synthèse des acides gras.

Le XBD173 compte parmi les ligands du TSPO (protéine translocatrice située sur la membrane externe de la mitochondrie) les plus sélectifs et à haute affinité, connus pour stimuler la neurostéroïdogénèse. La spectroscopie infrarouge biomédicale permet de quantifier les

triglycérides dans la région spectrale 2800–3200 cm^{-1} en utilisant l'ensemble de calibrations PLS. Les deux thérapies pourraient refléter l'augmentation des triglycérides sériques, qui sont des sous-produits du mécanisme antioxydant de rupture de chaîne, piégeant les radicaux peroxydes et protégeant les membranes contre les réactions peroxydatives induites par les ROS et le NO.

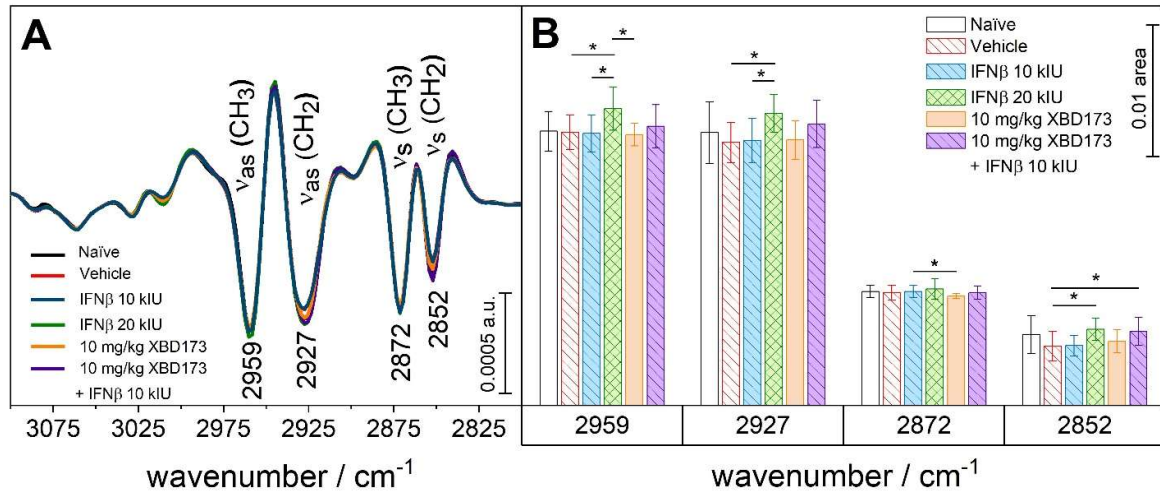


Figure 4. (A) Dérivées secondes des spectres moyens de chaque groupe de souris dans la plage spectrale 3100–2800 cm^{-1} . (B) Aires moyennes sous la courbe pour chacun des signaux centrés à 2959, 2927, 2872 et 2852 cm^{-1} . (Moyenne $\pm$ ÉT, $p < 0.05$).

2.1.2 La corrélation entre le sérum et le tissu du cervelet

La corrélation entre le sérum et le tissu du cervelet a été analysée à l'aide de la corrélation de Spearman. Le tissu du cervelet se compose de trois types distincts : la substance blanche cérébelleuse, la couche granulaire et la couche moléculaire. Pour différencier chaque type de tissu, une ACP (analyse en composantes principales) a été appliquée. La première composante principale (PCA1) représente la composante avec la plus grande variance, distinguant deux types de tissus : la substance blanche du cervelet, représentée en rouge, et les couches granulaire et moléculaire, représentées en bleu.

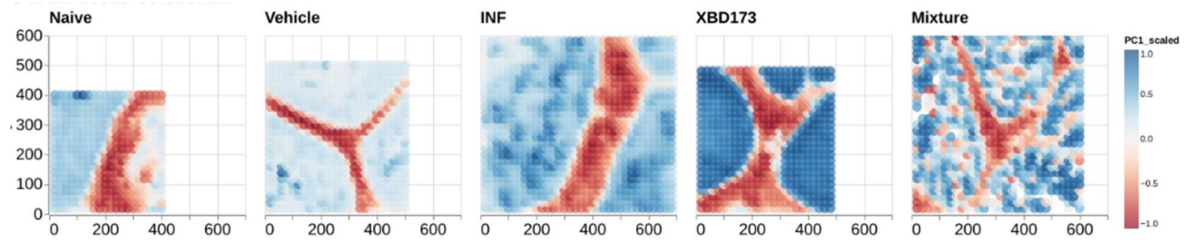


Figure 5. ACP du tissu cérébelleux, distinguant deux types de tissus : la substance blanche du cervelet (ombrage rouge) et la combinaison des couches granulaire et moléculaire (ombrage bleu). Barre d'échelle en μm .

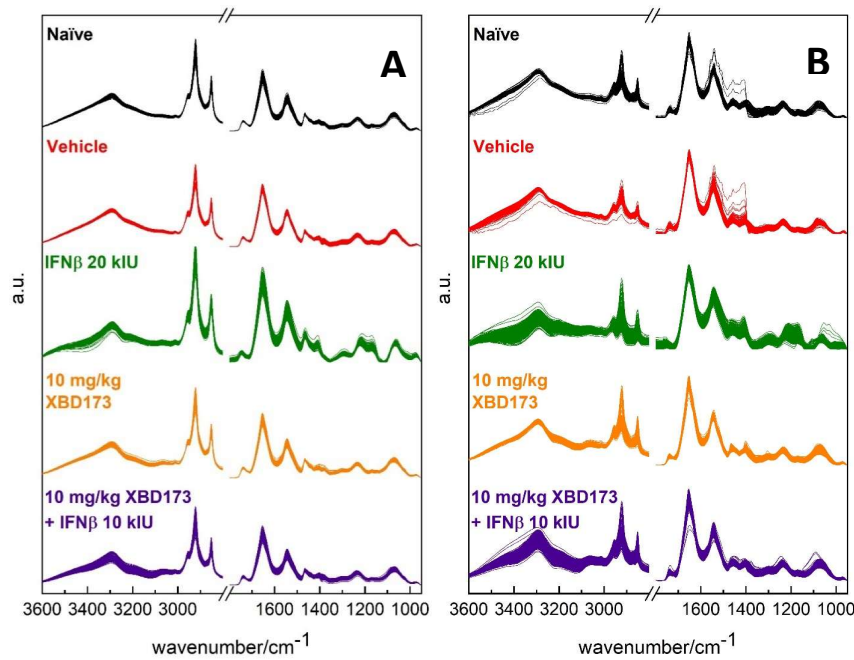


Figure 6. Les spectres des échantillons de tissu cérébelleux dans les plages de $3600\text{--}2800\text{ cm}^{-1}$ et $1800\text{--}950\text{ cm}^{-1}$, analysés par microscope FTIR sur la substance blanche du cervelet (A) et sur les couches granulaire et moléculaire (B). Les spectres naïfs sont représentés en noir, ceux du véhicule en rouge, ceux de l'IFN β 20 kIU en vert, ceux du XBD en orange, et ceux du mélange XBD+IFN β en violet.

Un total de 100 spectres ont été sélectionnés aléatoirement dans chaque région — la substance blanche du cervelet (en rouge) et les couches granulaire et moléculaire (en bleu). En raison des différences de nombres d'onde obtenus à partir de deux spectroscopies infrarouges différentes, la fonction `interpolate1d` en Python a été appliquée aux données de nombres d'onde issues de la microscopie IR. La corrélation moyenne de Spearman a été calculée pour chaque spectre sélectionné en comparaison avec chaque spectre de sérum.

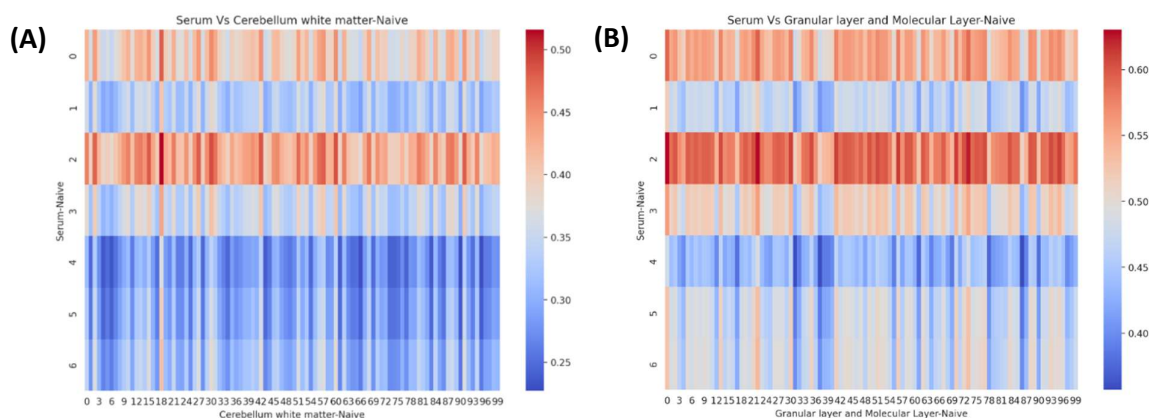


Figure 7. La corrélation moyenne de Spearman des tests par paires entre chaque échantillon de sérum et les 100 spectres obtenus à partir de la substance blanche du cervelet de l'état naïf (A), et entre chaque sérum et les 100 spectres obtenus à partir des couches granulaire et moléculaire de l'état naïf (B).

Les résultats sont présentés sous forme de carte thermique illustrant les scores moyens de corrélation. Le score moyen de corrélation le plus élevé entre le sérum et le tissu cérébelleux de NA_3_5VIS7 a été observé à la fois dans la substance blanche du cervelet (Figure 7A) et dans les couches granulaire et moléculaire (Figure 7B). La corrélation entre trois composantes — les spectres de sérum, le spectre moyen de la substance blanche du cervelet obtenu à partir de 100 spectres, et le spectre moyen de la couche granulaire obtenu à partir de 100 spectres — a été analysée à l'aide de la corrélation de Spearman.

La corrélation entre trois composantes — les spectres de sérum et le spectre moyen de la substance blanche du cervelet — a été analysée à l'aide du coefficient de corrélation par rang de Spearman. Les groupes Naïf (Figure 8A), IFN β 20 kIU, XBD173 à 10 mg/kg, et le mélange de XBD173 10 mg/kg + IFN β 10 kIU montrent la plus forte corrélation dans la région des vibrations CH des lipides (2800-2996 cm^{-1}). Une corrélation plus faible est observée dans les régions des phosphates (1203-1280 cm^{-1}), des glucides (1140-1183 cm^{-1}) et des acides nucléiques (994-1140 cm^{-1}). Le groupe Véhicule (Figure 8B), en revanche, montre une corrélation inverse dans ces régions. Par conséquent, l'étude a calculé la corrélation entre le sérum et le tissu du cervelet à partir du rapport entre la surface totale des régions phosphate, glucides et acides nucléiques / région des vibrations CH des lipides. La Figure 9 présente l'imagerie chimique du cerveau de souris au niveau du cervelet, illustrant le rapport entre les phosphates, glucides et acides nucléiques totaux / région des vibrations CH des lipides.

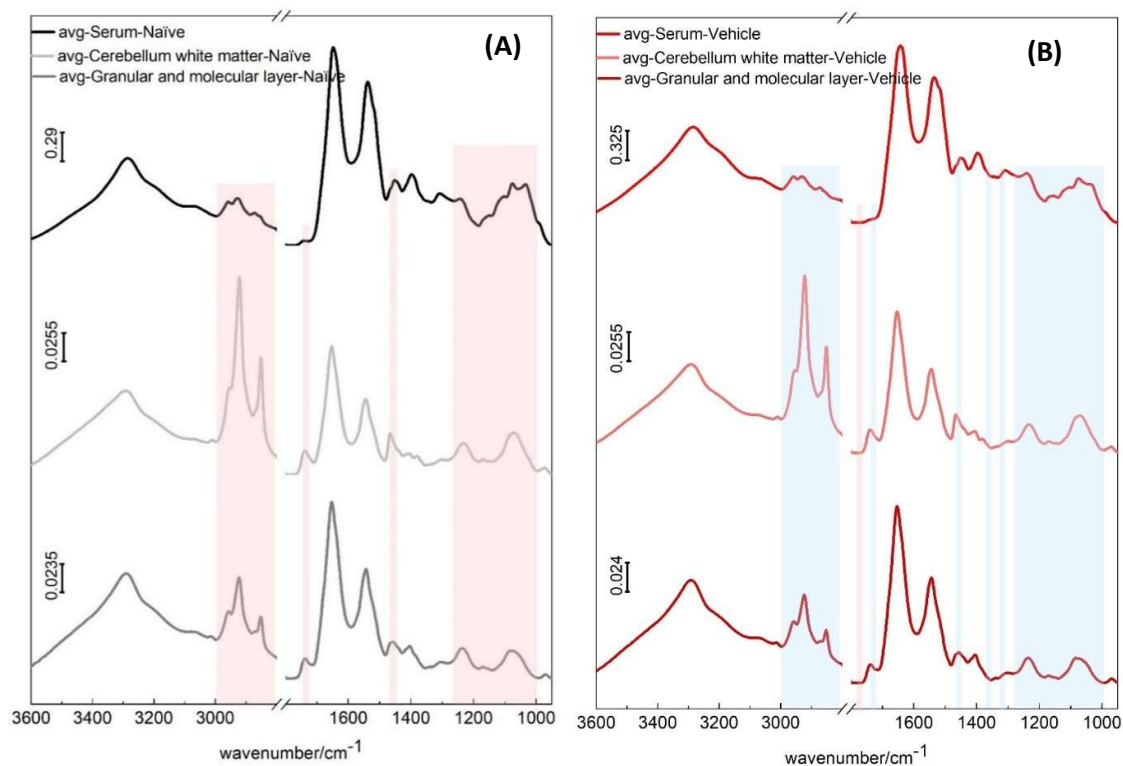


Figure 8. La corrélation entre le sérum, le spectre moyen de la substance blanche du cervelet et celui des couches granulaire et moléculaire de l'état Naïf (A) et du groupe Véhicule (B) a été analysée à l'aide du coefficient de corrélation par rang de Spearman. Un coefficient de corrélation mis en évidence en rouge représente une association forte. En revanche, s'il est mis en évidence en bleu, cela indique une association inverse.

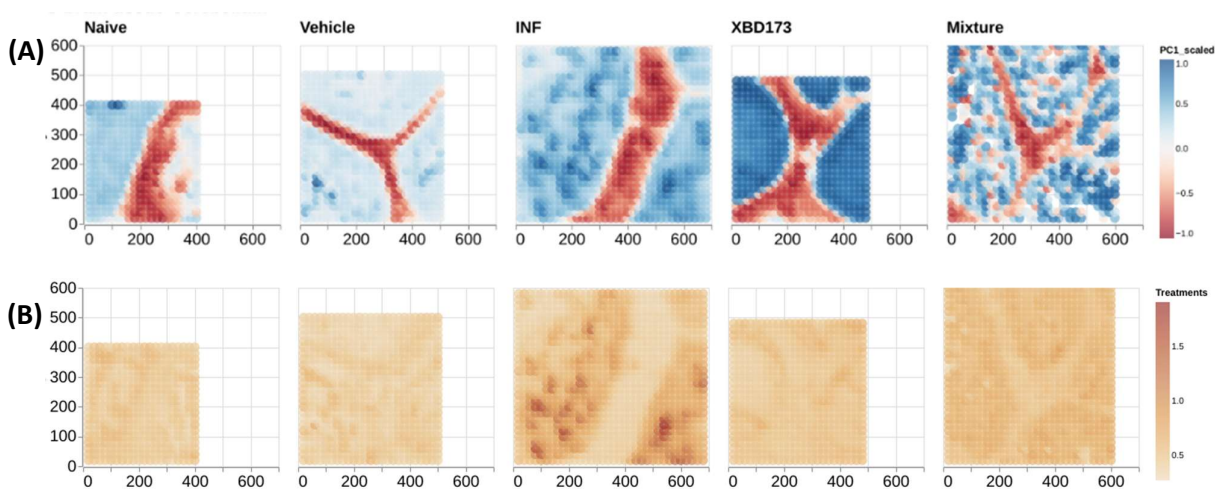


Figure 9. ACP du tissu cérébral du cervelet de souris (A). Image chimique du cervelet du cerveau de souris. La barre d'échelle située à droite de l'image chimique indique la valeur du rapport intégré des longueurs d'onde, représentée par des couleurs brune et brun clair. La couleur la plus claire correspond aux valeurs les plus faibles du rapport intégré. Le rapport total des phosphates, glucides et acides nucléiques (B) =

$$\frac{(\text{phosphates, glucides et acides nucléiques})}{\text{Région des vibrations CH des lipides}} = \frac{[994-1280] \text{ cm}^{-1}}{[2800-2996] \text{ cm}^{-1}}$$

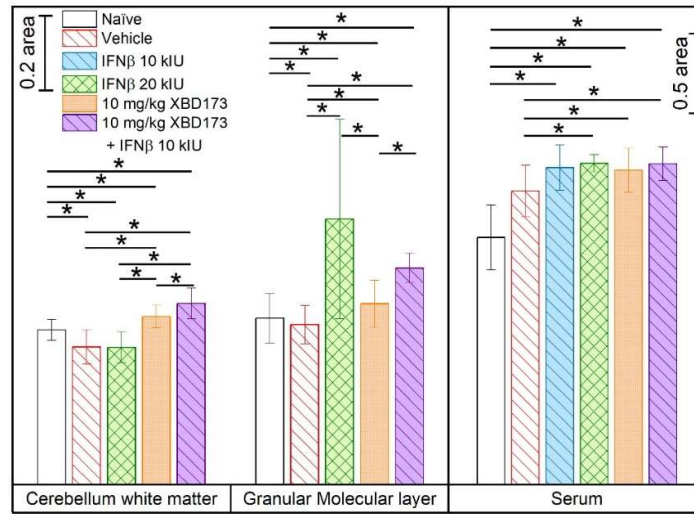


Figure 10. Le rapport de surface des phosphates, glucides et acides nucléiques dans la substance blanche du cervelet et les couches granulaire et moléculaire (à gauche) ainsi que dans le sérum (à droite). La signification statistique a été analysée pour $p < 0,05$ à l'aide du test de Kruskal-Wallis H, suivi d'une analyse post hoc avec le test de Dunn et des valeurs p ajustées selon la méthode de Holm.

La surface totale des ratios de phosphates, glucides et acides nucléiques a diminué de manière significative ($p < 0.05$) dans le tissu cérébelleux. Cet effet a été principalement observé avec le XBD173 à 10 mg/kg ainsi qu'avec la combinaison de XBD173 10 mg/kg + IFN β 10 kIU, ciblant ces zones. Par conséquent, ces traitements ont démontré une bonne efficacité pour traiter les régions affectées. De plus, le traitement par la combinaison XBD173 10 mg/kg + IFN β 10 kIU a amélioré ce ratio davantage que le XBD173 10 mg/kg seul. Le traitement par IFN β 20 kIU cible la couche granulaire moléculaire ; cependant, il ne montre pas de différence significative par rapport au véhicule dans la substance blanche du cervelet, qui est riche en gaines de myéline. Le traitement par XBD173 10 mg/kg et le mélange ciblent les deux zones du tissu cérébelleux. Ainsi, les ratios de phosphates, glucides et acides nucléiques reflètent le score clinique.

Les effets de divers traitements — Véhicule, IFN β , XBD173 à une dose de 10 mg/kg, et le mélange de XBD173 avec IFN β — sur un ratio spécifique (la surface totale des phosphates, glucides et acides nucléiques / région des vibrations CH des lipides) mesuré à la fois dans le sérum et le tissu cérébelleux. Le ratio observé a augmenté de manière significative dans tous les groupes de traitement, reflétant les tendances observées dans le tissu cérébelleux, à l'exception du groupe Véhicule. Dans ce dernier, l'augmentation du ratio est attribuée à une diminution de la surface totale de la région des vibrations CH des lipides, comme illustré à la Figure 10. Par conséquent, ce ratio pourrait constituer un biomarqueur potentiel reliant les changements du sérum aux altérations du tissu cérébelleux après traitement.

2.2 Maladie d'Alzheimer

Nous ciblerons spécifiquement les espèces précoces de A β pour le diagnostic, la prévention et le traitement. Pour ce faire, nous utilisons des nanoparticules d'or (GNP) détectables en IR et Raman, conjuguées à des peptides cycliques D,L- α pour le diagnostic. Le ciblage sélectif des oligomères d'A β dans la MA précoce sera réalisé grâce aux peptides cycliques abiotiques D,L- α récemment développés, qui sont étudiés en collaboration avec le Professeur Shai Rahimipour (Université Bar Ilan). Ces peptides cycliques (PCs) imitent les caractéristiques structurales et biochimiques des peptides A β en reproduisant la conformation des feuillets β croisés, modulant ainsi l'agrégation, inhibant la formation d'oligomères toxiques et désassemblant les fibrilles préformées. Ces peptides non toxiques et perméables aux cellules interagissent avec des protéines de diverses maladies, comme A β et tau pour la MA et l' α -synucléine pour la maladie de Parkinson. En ciblant les oligomères précoces d'A β , des analogues radiomarqués des PCs ont montré une détection précoce sans précédent d'A β à des stades présymptomatiques. Les études préliminaires ont montré que la conjugaison des peptides cycliques à des liposomes approuvés cliniquement et aux GNP améliorerait l'affinité de liaison aux espèces précoces d'A β et augmentait l'activité anti-amyloïdogénique dans les modèles de la MA de *C. elegans*. Des coupes de cerveaux d'un modèle murin sont obtenus grâce à une collaboration avec le Professeur Jens Pahnke (Université d'Oslo).

Tableau 2 : Nombre de coupes de cerveau incluses dans les groupes sains et les groupes AD non traités.

Cohort	Nombre total de coupes de cerveau
Brain tissue – coronal section	
B6 (healthy)	3
APP-Front 10 μ m (untreated AD-Frontal lobe)	4
APP-Back 10 μ m (untreated AD-Occipital lobe)	5

Dix-huit échantillons de tissus cérébraux (Tableau 2) sont inclus dans l'étude. Des échantillons de tissus cérébraux APP (MA) et B6 (control) ont été analysés des lobes frontaux et occipitaux à l'aide du microscope infrarouge LUMOS II (Bruker, Allemagne).

2.2.1 L'effet de la MA sur le tissu cérébral de souris

L'étude de l'effet de la maladie d'Alzheimer sur des échantillons de tissu cérébral s'est concentrée sur le cortex cérébral. Le tissu cérébral a été examiné dans la région somatosensorielle primaire pour les couches corticales II-III, et dans quatre régions distinctes — le cortex cingulaire antérieur, la région somatosensorielle primaire, la région somatosensorielle supplémentaire et la région piriforme — pour les couches corticales IV-V. L'ACP de la cartographie FTIR des tissus cérébraux non-AD et AD provenant de la région somatosensorielle primaire (B6-14-2, APP-Front-4 et APP-Back-7) et de la région somatosensorielle supplémentaire (B6-14-3, APP-Front-2 et APP-Back-6) est présentée dans cette partie.

Aire somatosensorielle primaire

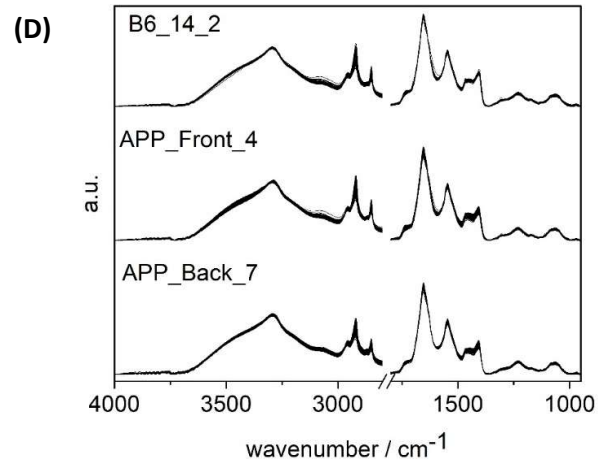
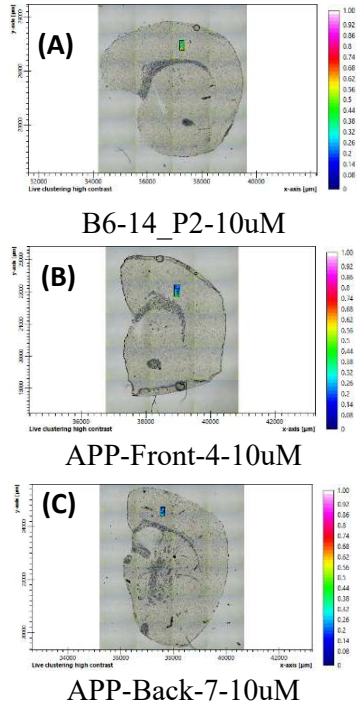


Figure 11. Les images optiques des sections cérébrales demi-coronales, mettant en évidence les zones analysées dans la région somatosensorielle primaire, sont présentées pour (A) B6-14-2, (B) APP-Front-4 et (C) APP-Back-7. (D) montre les spectres d'absorption correspondants obtenus à partir de la cartographie FTIR de B6-14-2, APP-Front-4 et APP-Back-7.

Aire somatosensorielle supplémentaire

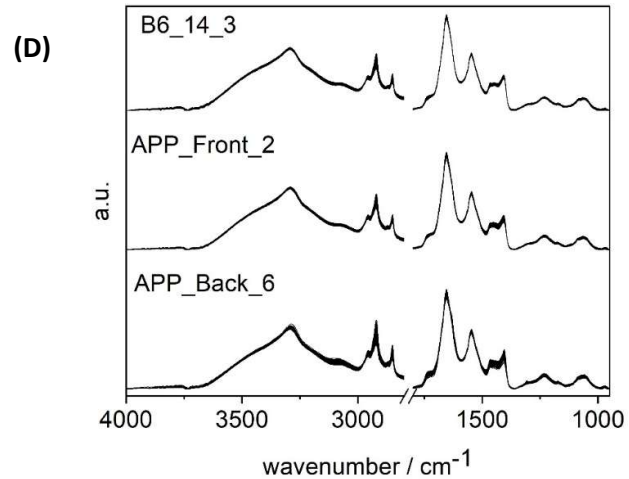
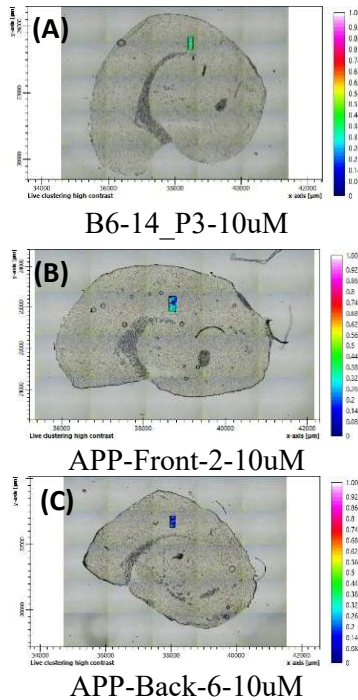


Figure 12. Les images optiques des sections cérébrales demi-coronales, mettant en évidence les zones analysées dans la région somatosensorielle primaire, sont présentées pour (A) B6-14-3, (B) APP-Front-2 et (C) APP-Back-6. (D) montre les spectres d'absorption correspondants obtenus à partir de la cartographie FTIR de B6-14-3, APP-Front-2 et APP-Back-6.

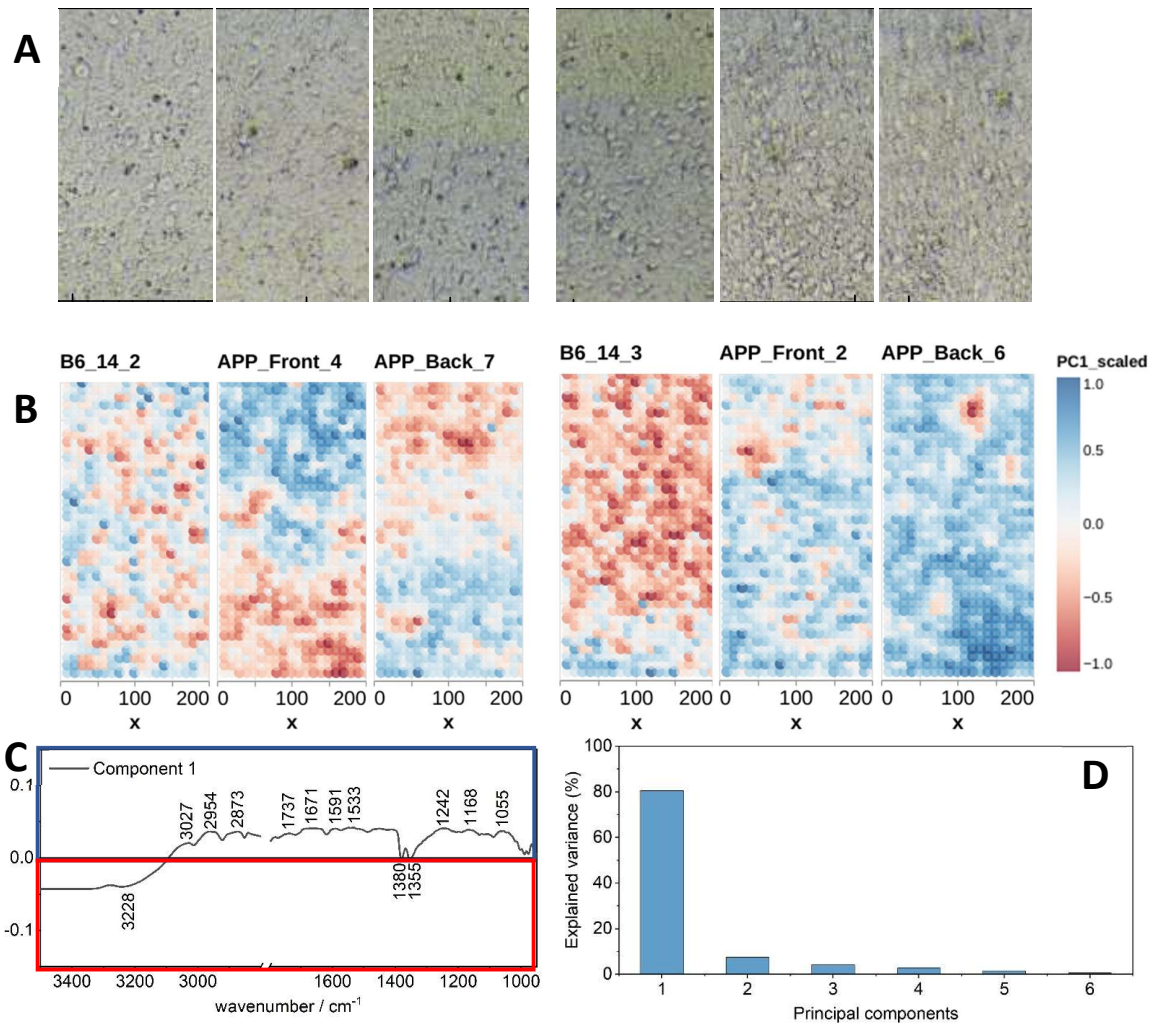


Figure 13. (A) Images optiques des zones analysées dans les régions somatosensorielle primaire et somatosensorielle supplémentaire. (B) Score du PC1 calculé à partir des spectres d'absorption de la cartographie FTIR observée sur le cortex cérébral. (C) Chargement du PC1, mettant en évidence les variables qui augmentent avec des valeurs positives (zone bleue dans la Figure 13B) et celles qui diminuent avec des valeurs négatives (zone rouge dans la Figure 13B). (D) Ratio de variance expliquée de la cartographie FTIR observée sur le cortex cérébral.

La caractérisation des tissus cérébraux de souris non-AD et AD a été analysée à l'aide de l'ACP, qui capture les variations les plus significatives des données. Les graphiques des scores représentent la relation des échantillons avec les composantes principales, aidant à identifier des regroupements, des tendances ou des valeurs aberrantes. Les chargements indiquent quelles régions spectrales contribuent le plus à chaque composante principale. Les valeurs positives et négatives dans les chargements apparaissent parce que les données sont centrées sur la moyenne, ce qui permet des variations au-dessus et en dessous de la moyenne. Les chargements positifs correspondent aux variables qui augmentent avec une composante principale, tandis que les chargements négatifs correspondent aux variables qui diminuent.

La première composante principale (PC1) a capturé la plus grande variance, reflétant le changement le plus important de l'intensité d'absorption, comme le montre la Figure 13. Les zones de variance la plus élevée sont ombrées en bleu et en rouge, indiquant des différences dans les composants chimiques. Le graphique des scores de l'ACP utilise un code de couleurs pour représenter la distribution des scores des composantes principales (PC) dans l'ensemble des échantillons. Ces scores indiquent la projection de chaque échantillon sur les composantes principales, révélant des similitudes ou des différences entre les échantillons. Les valeurs de score pour chaque PC ont été normalisées dans une plage allant de -1 à 1 (comme illustré dans la Figure 13B) à des fins de visualisation. Cela permet de représenter dans le graphique à la fois les contributions positives et négatives de chaque chargement. L'échelle située à droite du graphique des scores de l'ACP indique les valeurs de chargement à l'aide de couleurs bleu et rouge : le bleu représente les valeurs positives, tandis que le rouge indique les valeurs négatives. Le ratio de variance expliquée de PC1 est de 80.58 %, ce qui signifie que cette composante principale conserve 80.58 % de la variance totale du jeu de données.

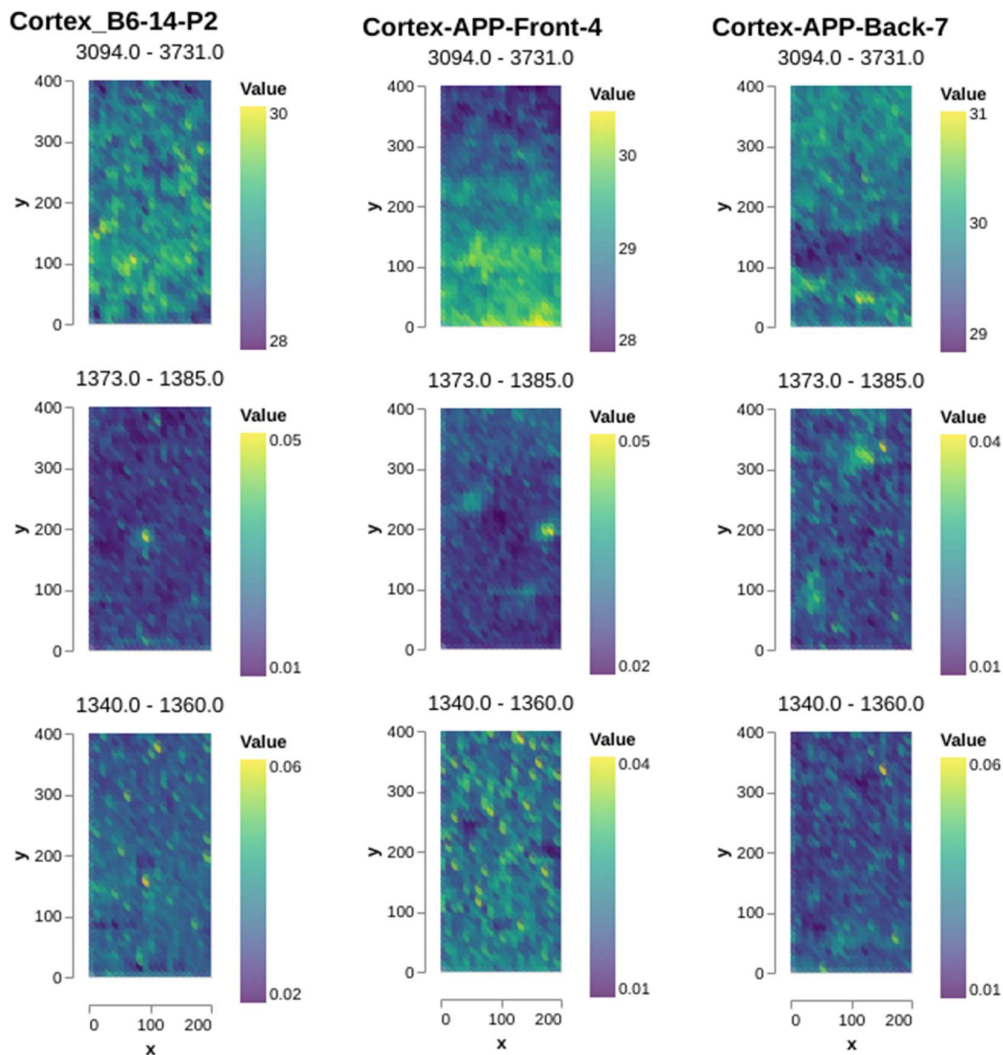


Figure 14. Images chimiques analysées sur le cortex cérébral dans la région somatosensorielle primaire en utilisant la plage de nombres d'onde correspondant aux valeurs négatives du

chargement de la PC1. Barre d'échelle = 100 μm . Barre de couleur : le jaune indique une intensité élevée ; le violet indique une intensité faible.

Les zones de plus grande variance ont été ombrées en bleu et en rouge, comme le montre la Figure 13B, indiquant des différences dans les composants chimiques. La Figure 14 et la Figure 15 présentent les modifications de composition chimique identifiées à l'aide de la plage de nombres d'onde correspondant aux valeurs négatives de la PC1 (Figure 13C), qui correspondent à la zone ombrée en rouge observée dans la Figure 13B. Cet ombrage rouge représente les plus grands changements d'absorption associés aux étirements O-H et N-H ($3094\text{-}3731\text{ cm}^{-1}$), au pliage symétrique du CH_3 ($1373\text{-}1385\text{ cm}^{-1}$), et au pliage du CH ($1340\text{-}1360\text{ cm}^{-1}$) au niveau des plaques. Les images chimiques révèlent une accumulation de signaux à $1373\text{-}1385\text{ cm}^{-1}$ et une diminution d'intensité à $1340\text{-}1360\text{ cm}^{-1}$ dans les mêmes zones, indiquant à la fois un gain et une perte de composants. Cela pourrait être lié au mécanisme d'extraction des lipides de la bicouche lipidique, dans lequel le peptide toxique se lie aux lipides puis les extrait de la membrane.

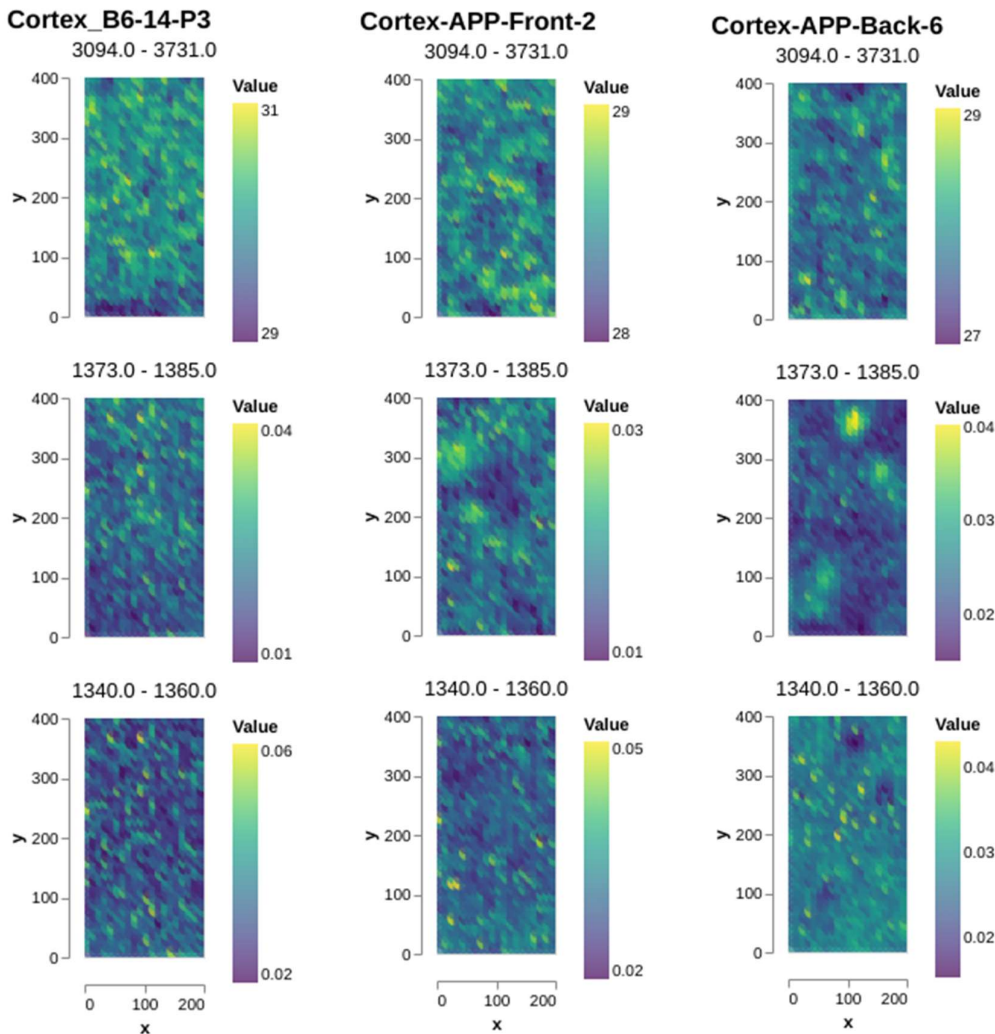


Figure 15. Images chimiques analysées sur le cortex cérébral dans la région somatosensorielle supplémentaire en utilisant la plage de nombres d'onde correspondant aux valeurs négatives du

chargement de la PC1. Barre d'échelle = 100 μm . Barre de couleur : le jaune indique une intensité élevée ; le violet indique une intensité faible.

2.2.2 Agrégation des fibrilles d'A β 40

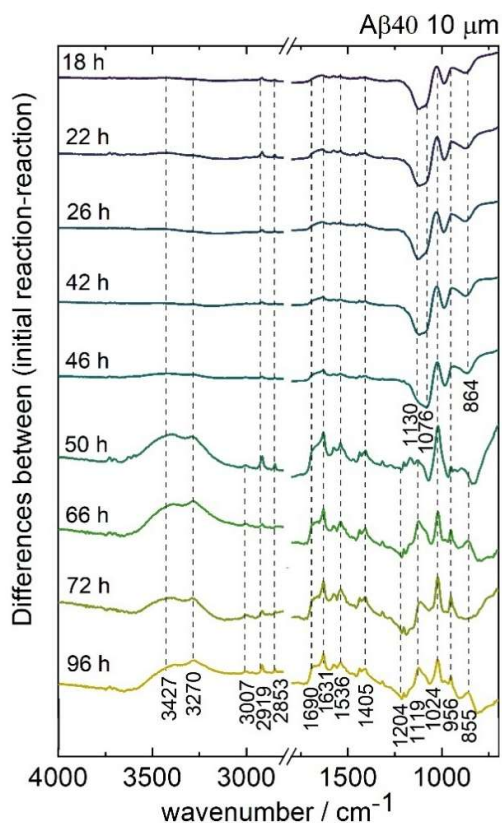


Figure 16. Spectres FTIR différentiels entre l'état de réaction et l'état initial (réaction à 1 h moins xh), enregistrés à partir de films secs lors de l'agrégation de l'A β 40 monomérique à une concentration de 10 μM .

Pour analyser la cinétique du processus d'agrégation, une spectroscopie différentielle a été appliquée en soustrayant le spectre FTIR enregistré à 1 h (état initial) des spectres obtenus à chaque point temporel suivant (réaction à 1 h - point temporel de la réaction). L'interprétation des spectres différentiels est la suivante : une valeur positive indique que l'intensité d'absorption de la réaction à 1 h est supérieure à celle de la réaction au point temporel considéré. Inversement, une valeur négative indique que l'intensité d'absorption à ce point temporel est supérieure à celle de la réaction initiale à 1 h.

La spectroscopie différentielle présentée dans la Figure 16 révèle les plus grands changements dans les régions de 1180-1060 cm^{-1} , 1041-1014 cm^{-1} , 956-900 cm^{-1} et 856-698 cm^{-1} , respectivement. Ces régions correspondent aux vibrations d'élongation C-O, aux liaisons latérales C-O-C, aux vibrations C-C et C-O de la chaîne principale, et aux déformations hors du plan des cycles aromatiques. Les changements les plus marqués ont été observés aux temps de 18 h, 22 h, 26 h, 42 h et 46 h.

Une diminution a été observée dans les régions 3646-3146 cm^{-1} , 3007 cm^{-1} , 2919 cm^{-1} , 2853 cm^{-1} ainsi que 1702-1371 cm^{-1} à partir de 18 heures. Cette diminution concerne les régions d'étirement N-H et C-H, ainsi que les bandes amide I (étirement C=O) et amide II (déformation N-H et étirement C-N). À mesure que le temps de réaction dépasse 50 heures, une augmentation est observée dans la région 1364-1143 cm^{-1} , correspondant aux vibrations d'étirement C-N et de déformation C-H. Ainsi, cette région 1364-1143 cm^{-1} soutient l'hypothèse d'une réorganisation des chaînes latérales lors de l'agrégation fibrillaire de l'A β 40. La diminution d'intensité dans la région 1180-1060 cm^{-1} pendant l'agrégation de l'A β 40 monomérique en fibrilles est attribuée à une réduction de la mobilité et de l'exposition des groupes fonctionnels C-O, liée à la formation de structures fibrillaires riches en feuillets β , ce qui entraîne une baisse de l'activité vibrationnelle détectable par FTIR.

3) Conclusion générale

La spectroscopie FTIR a permis de détecter avec succès de petites modifications dans la composition biomoléculaire du sérum. Des différences significatives ont été observées entre les groupes Naïf et Véhicule (MS). Ces changements distincts nous ont permis de différencier le Véhicule MS du Naïf, avec des effets du Véhicule sur la signature infrarouge des protéines et des lipides dans le sérum. L'IFN- β et le XBD173 ont tous deux pu restaurer le ratio feuillets β dans le sérum. Seul l'IFN- β à 20 kIU a amélioré efficacement le ratio feuillets β /hélice α dans la région amide I. La combinaison du XBD173 avec une faible dose d'IFN- β a donné des effets comparables à ceux d'un traitement avec une dose complète d'IFN- β .

En comparant les composants moléculaires des plaques dans le tissu cortical atteint de la maladie d'Alzheimer avec ceux de l'A β 40 fibrillaire à une concentration de 10 μM , une similitude notable est observée dans leurs caractéristiques spectrales. Les deux présentent une perte d'intensité dans les régions d'étirement O-H et N-H, reflétant une réduction de l'hydratation. En revanche, les deux systèmes montrent un gain dans les régions d'étirement C-N et de déformation C-H : spécifiquement, 1364-1143 cm^{-1} pour l'A β 40 fibrillaire, et 1366-1395 cm^{-1} ainsi que 1087-1113 cm^{-1} pour le tissu cérébral atteint de la maladie d'Alzheimer.

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LISTE DES PUBLICATION

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Notations

μ is the mean value

σ is the standard deviation

$\text{var}(x)$ is the variance

$\text{cov}(x, y)$ is the covariance of x and y

x_i, y_i are the values of x and y

$\bar{x}, \bar{y}$ are the mean values of x and y

A is a square matrix of covariance

v is Eigen vector

λ is Eigen value

ev is eigenvector

B is a matrix of regression coefficients

E is a matrix of residuals

$F1$ and $F3$ are outer fences

$Q1$ is the first quartile

$Q3$ is the third quartile

$q1$ is the first quartile

$q2$ is the second quartile

$q3$ is the third quartile

k_n is the kurtosis factor

$Z_{\alpha/2}$ is the alpha level's z-score for a two tailed test

X_{Ai} is the sample in group A which rank 1 has the lowest value

W_A is the rank sum value

r_s is Spearman's rank correlation coefficient

d_i is the difference in ranks for x and y

n represents the number of observations

$\bar{X}$ is the sample mean

$t_{\alpha/2, n-1}$ is the t-multiplier

$\frac{8}{\sqrt{n}}$ is the standard error

Abbreviations

FTIR	Fourier Transform Infrared spectroscopy
FPA	Focal plane array detectors
NMOSD	Neuromyelitis optica spectrum disorder
MS	Multiple sclerosis
ALS	Amyotrophic lateral sclerosis
CNS	Central nervous system
BBB	Blood-brain barrier
CSF	Cerebrospinal fluid
capCAA	Capillary cerebral amyloid angiopathy
Ag	Antigen
APC	Antigen-presenting cell
IL	Interleukin
MMP	Matrix metalloproteinase
TH	T helper cell
AD	Alzheimer's disease
PD	Parkinson's disease
A β	Amyloid- β
MRI	Magnetic resonance imaging
BECs	Brain endothelial cells
ABC	ATP-binding cassette
ROS	Reactive oxygen species
TJ	Tight junction
APCs	Antigen-presenting cells

ECs	Endothelial cells
EAE	Experimental autoimmune encephalomyelitis
MRI	Magnetic resonance imaging
IFN β	Interferon-beta
AQP-4	anti-aquaporin-4 antibodies
PLP	Proteolipid protein
NK	Natural killer
MBP	Myelin basic protein
TSPO	Translocator protein
RRMS	Relapsing-remitting multiple sclerosis
VDAC	Voltage-dependent anion channel
OMM	Outer mitochondrial membrane
IMM	Inner mitochondrial membrane
ANT	Adenine nucleotide translocator
APP	Amyloid precursor protein
PCA	Principal Component Analysis
PLS	Partial least squares regression
ROC	Receiver Operating Characteristic
SEM	Standard error of mean
IQR	Interquartile range
SIQR	Semi-interquartile range
ANOVA	Analysis of variance
FDR	False discovery rate
DDW	Double distilled water
PBS	Phosphate buffer saline
NGF	Nerve growth factor

Chapter I

Introduction

FTIR spectroscopy is a technique used to study the interaction between molecules and electromagnetic radiation, producing a characteristic spectrum. This technique provides a spectral fingerprint that serves as a unique signature, revealing the specific characteristics of the studied sample. Because it is nondestructive, FTIR can be applied to the analysis of biomolecules, cells and tissues. In medical applications, IR spectroscopy in the mid-IR range (4000 cm^{-1} to 400 cm^{-1}) is commonly used. When IR radiation is absorbed by molecules, it induces their rotational and vibrational motions. The IR spectrum of biological samples contains specific absorption bands corresponding to proteins, lipids, nucleic acids, and carbohydrates⁴⁻⁶ as shown in Figure 1.

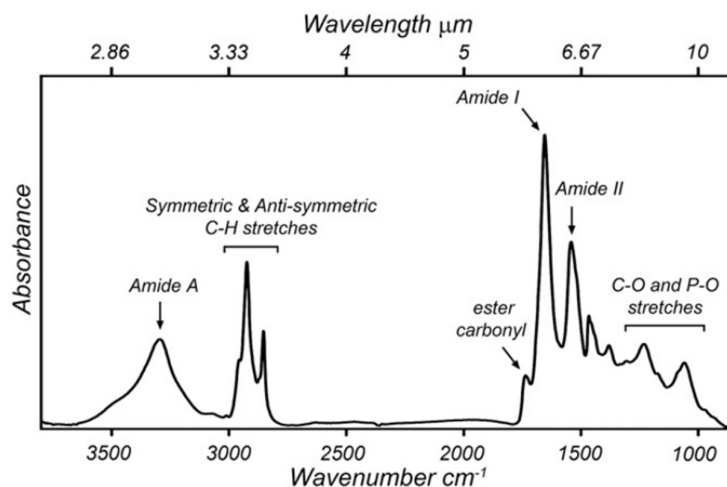


Figure 1. Infrared spectrum from a biological sample highlighting major macromolecular functional group⁴.

A particular advantage of FTIR spectroscopy is its ability to investigate protein structure and monitor structural changes under conditions that are not directly or noninvasively accessible by other spectroscopic techniques^{4,7}. FTIR can be applied across diverse environments, including aqueous solutions, crystalline states, organic solvents, and even complex biological matrices containing other biomolecules.

These assignment rules were derived from extensive studies of polypeptides and proteins with well-defined secondary structures. As such, FTIR has been widely used in biomedical applications, including the diagnosis and characterization of amyloid-related diseases such as AD,

where conformational changes in protein structures are a hallmark. Beyond structural analysis, FTIR is also employed in infrared clinical chemistry (e.g., quantitative analysis of blood or other biofluids), infrared pathology (e.g., tissue biopsy assessment), and in vivo diagnostics for noninvasive disease monitoring⁵.

Using infrared microscopy, spatially resolved biochemical information can be obtained with a resolution of a few micrometers. When combined with FPA detectors, it becomes possible to rapidly analyze the biochemical composition of each pixel within a tissue slice—often within minutes. Advanced statistical methods, such as hierarchical cluster analysis, allow for the generation of false-color images that visually differentiate tissue regions based on their spectral features. These annotated images can then be used by pathologists to train neural networks, enabling the development of automated classification models for various tissue types. FTIR spectroscopy has been successfully applied, for instance, in the diagnosis of skin cancer. It enables the clear distinction between the spectral signatures of normal epidermal regions, hair follicles, dermis, and basal cell carcinoma tissue⁴. Moreover, FTIR spectroscopy is now a well-established tool for monitoring biochemical changes in tissues and body fluids, allowing for reagent-free and noninvasive diagnostics⁴.

FTIR spectroscopy enables the simultaneous, reagent-free quantification of at least six serum analytes—albumin, cholesterol, glucose, total protein, triglycerides, and urea—within clinically accepted standard error margins. It has also been successfully applied in the rapid quantitative assessment of collagen levels following myocardial infarction, the diagnosis of arthritis using human synovial fluid samples, and the real-time monitoring of tissue oxygenation (e.g., after plastic surgery)⁸. Label-free FTIR spectroscopy offers improved accuracy and reproducibility in the diagnosis of cancer and autoimmune inflammatory diseases, while eliminating the need for complex and time-consuming clinical processing of tissue samples, as currently required in computerized histopathological analysis⁵.

ATR-FTIR spectroscopy coupled with random forest classification machine learning algorithm were utilized to differentiate NMOSD and MS which both are autoimmune inflammatory and demyelinating diseases of the central nervous system between healthy and disease to determine any differences specifically related to disease progression based on the protein, lipid and nucleic acids infrared contributions⁹. With this method helps differentiation between MS and ALS relies on the clinical assessment of the symptoms along with magnetic resonance imaging and sampling CSF for the search of biomarkers for either disease. This approach shows that infrared spectroscopy is powerful in discriminating between the two diseases and healthy controls by offering high specificity for MS (100%) and ALS (98%)¹⁰.

Therefore, FTIR spectroscopy has been applied to research on brain diseases, including MS and AD.

1. The first project, focusing on MS, presents spectroscopic studies of two treatment strategies in mice: anti-inflammatory and neuroprotective as well as a combination of both.
2. The second project, focusing on AD, aims to understand the interaction between CP-2 and A β ₄₀, as well as the effects of AD in a mouse model as revealed by spectroscopic analysis.

1. Multiple sclerosis

1.1 MS pathology

Approximately 2.8 million people worldwide are afflicted with MS, a chronic inflammatory and neurodegenerative disease of the CNS that leads to significant physical disability, particularly in young adults, with a higher incidence among women¹¹⁻¹². MS is characterized by a wide range of symptoms, including sensory and visual disturbances, motor impairments, fatigue, pain, and cognitive deficits¹³. A hallmark of the disease is the formation of lesions in the CNS, resulting from the infiltration of immune cells across the BBB. This immune response triggers inflammation, demyelination, gliosis, and neuroaxonal degeneration, ultimately disrupting neuronal signaling and contributing to disease progression¹⁴⁻¹⁶.

In MS pathology, disruption of the BBB represents an early stage in lesion development, preceding both the massive infiltration of leukocytes—primarily T-lymphocytes and monocyte-derived macrophages—and the subsequent destruction of nervous tissue¹⁷. The BBB is a specialized structure that protects the CNS from the systemic circulation by restricting the entry of unwanted molecules and immune cells into the brain. It also actively removes cytotoxic compounds and supplies the brain with essential nutrients and oxygen via specific transport mechanisms¹⁷⁻¹⁸. Impaired BBB function has been implicated in several neuroinflammatory and neurodegenerative diseases, including MS, capCAA, AD, epilepsy, and PD¹⁹. In MS in particular, BBB disruption is a critical and early marker of disease pathophysiology, preceding immune cell infiltration and tissue damage^{17, 20}. Figure 2 illustrates neuroinflammatory changes at the BBB during neuroinflammation in MS.

The BBB is composed of highly specialized BECs and functions to restrict both transcellular and paracellular passage of cells and molecules from the systemic circulation into the CNS²¹. The transcellular transport of hydrophilic molecules is particularly limited due to several factors: a low rate of transcytotic vesicle formation, minimal pinocytotic activity, the expression of active efflux transporters—such as P-glycoprotein—belonging to the ABC family, and high metabolic activity involving cytosolic enzymes and transporters²².

Axonal damage plays a critical role in the pathology of MS. Early signs of axonal injury are often observed in regions of active demyelination and inflammation²³⁻²⁴. Numerous studies have demonstrated that axonal loss is a major contributor to the irreversible neurological disability seen in MS patients²⁵. Given the irreversible nature of axonal degeneration and its strong association with inflammatory processes, it is essential that anti-inflammatory therapies are administered early in the disease course. Furthermore, incorporating neuroprotective strategies may enhance therapeutic outcomes by helping to preserve axonal integrity and prevent long-term neurological decline^{17, 26}.

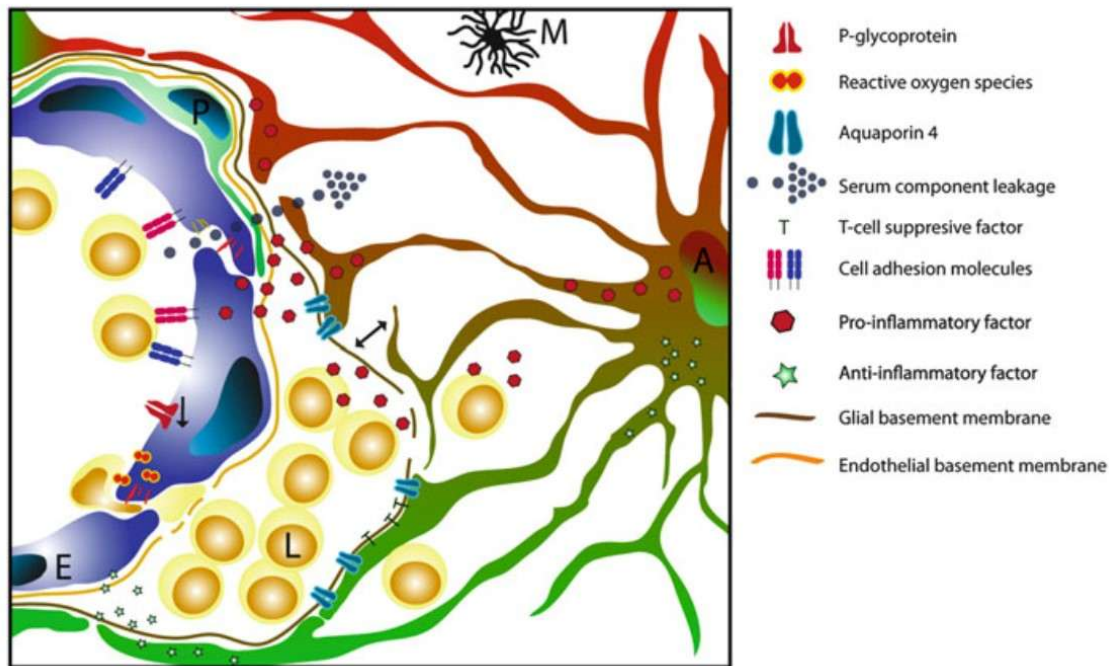


Figure 2. Neuroinflammatory changes at the BBB. During neuroinflammation in MS, the BBB exhibits a loss of structural integrity, resulting in the leakage of serum components into the CNS. Brain endothelial cells (E) upregulate cell adhesion molecules, facilitating the adhesion and migration of activated leukocytes (L) across the barrier into the CNS. These leukocytes locally release ROS, which disrupt TJ complexes and further compromise barrier function. Additionally, the expression of the efflux transporter P-glycoprotein is downregulated on endothelial cells, impairing the barrier's protective capabilities. Reactive astrocytes (A) and activated microglia (M) contribute to the neuroinflammatory environment by secreting pro-inflammatory chemokines and cytokines. Abnormal expression of aquaporin-4 in astrocytic end feet is believed to exacerbate BBB disruption. However, reactive astrocytes also play a protective role through the expression of T-cell suppressive molecules and the release of anti-inflammatory factors such as sonic hedgehog. The role of pericytes (P) in inflammatory BBB breakdown remains unclear, although pericyte loss is strongly associated with BBB dysfunction¹⁷.

Inflammation-related tissue damage in the CNS of MS patients is driven by both autoreactive, antigen-specific CD4⁺ and CD8⁺ T cells²⁷. Among these, IL-17-producing memory CD4⁺ T cells, known as Th17 cells, have been identified within active MS lesions and are implicated in promoting inflammation and tissue damage²⁸. Among APCs, infiltrating monocyte-derived macrophages are thought to play a central role in demyelination and axonal injury^{17, 29}.

Before entering the CNS, leukocytes must transmigrate across the endothelial cells (ECs) of the BBB. Monocytes, which are key effector cells within MS lesions, are recruited in large numbers to the perivascular space³⁰. During monocyte trafficking across the BBB, ROS have been shown to play a crucial role. ROS are generated by monocytes upon firm adhesion to ECs, enhancing both their adhesion and transmigration³¹. In animal models of EAE, treatment with antioxidants has been shown to reduce disease severity by limiting the infiltration of inflammatory cells into the CNS. Furthermore, superoxide, a predominant ROS, has been shown to disrupt BBB

integrity by inducing TJ rearrangements and cytoskeletal alterations, thereby facilitating leukocyte migration^{17, 32}.

The diagnosis of MS is primarily based on clinical evaluation, which includes neurological exams and a thorough clinical history³³. If a diagnosis based on clinical presentation alone is not possible, radiological and laboratory assessments such as MRI and CSF analysis become essential for confirming the diagnosis of MS. The McDonald criteria are widely used to guide this diagnostic process, incorporating clinical, imaging, and laboratory findings to establish the diagnosis with greater accuracy. MRI is particularly useful in detecting MS lesions in both the brain and spinal cord, providing evidence of lesion dissemination in time and space—key elements emphasized in the McDonald criteria³⁴. CSF analysis can also provide supportive evidence, particularly through the detection of CNS-derived antibodies, such as oligoclonal bands, which are commonly present in MS patients^{17, 35}.

An ideal biomarker for a certain disease is characterized by high sensitivity and specificity, along with a simple, cost-effective, reproducible, and non-invasive detection method. Currently, the diagnosis and prognosis of MS, as well as the monitoring of treatment response and the assessment of the risk of side effects, can be supported by several established biomarkers. These include oligoclonal bands and the IgG index, AQP-4 antibodies, neutralizing antibodies IFN β and natalizumab, as well as anti-JCV and anti-VZV antibodies³⁶⁻³⁷.

1.2 Spectroscopic analysis of MS brain tissue and body fluids

The different stages of demyelination in MS brain tissue are depicted in Figure 3. The total lipids (Figure 3E-H) and amide I (Figure 3I-L) showed reduced levels of lipids and amides in MS tissue, with lesions being the most affected compared to controls. Inactive plaques in MS white matter exhibited the most significant changes when compared to control white matter. Similar changes were detected in active lesions, though to a lesser extent. In contrast, remyelinated plaques were less affected than inactive plaques, possibly reflecting structural recovery within shadow plaques. A significant decrease in total lipids was observed in all MS lesions when compared to controls. The degree of lipid unsaturation was assessed by examining the olefinic region, which showed a significant decrease only in MS inactive plaques when compared to control white matter³⁸.

Demyelination in MS leads to significant alterations in the FTIR spectrum of affected areas, reflecting the degradation of the myelin sheath and the accumulation of breakdown products. FTIR has been successfully used to characterize white and grey matter as well as MS plaques from human CNS tissue, identifying a pronounced loss of lipids in MS plaques through alterations in lipid bands. The impact of free radical accumulation on MS pathogenesis was studied using FTIR spectroscopy on demyelinated MS lesions. The oxidation of proteins and lipids within the MS lesions was identified, supporting the co-accumulation of free radicals in the pathogenesis of MS³⁹.

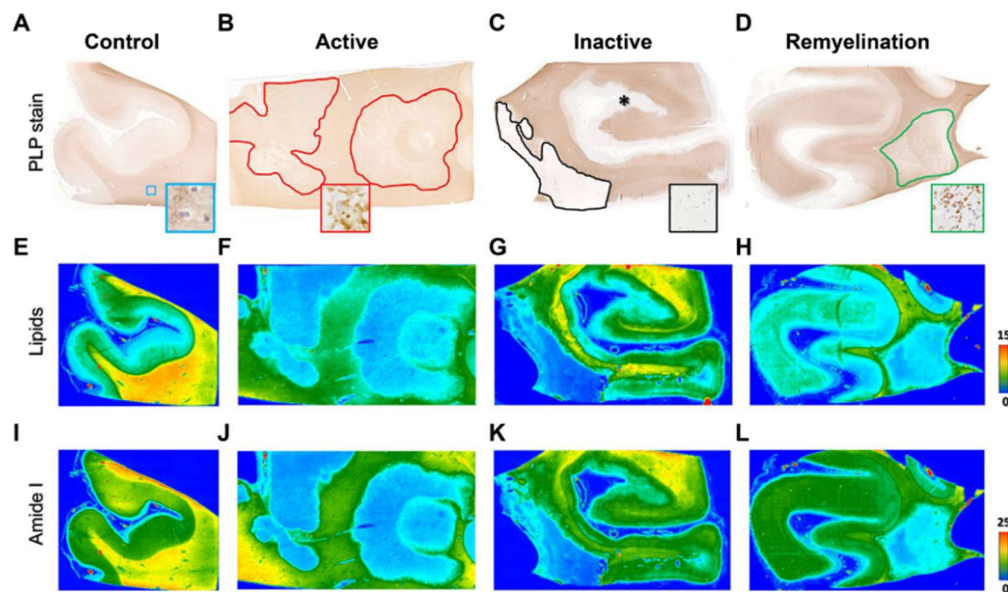


Figure 3. Hyperspectral imaging detects regions of demyelinating activity in MS brain tissue. Panels A-D show immunohistochemical staining of PLP. Active demyelination, inactive demyelinated regions, and remyelinated regions in MS brain tissue are outlined in red, black, and green, respectively. Hyperspectral images in panels E-H represent total lipids, while panels I-L show amide I. These images were generated using absorbance profiles from 3000-2800 cm^{-1} for total lipids and 1700-1600 cm^{-1} for amide I³⁸.

Spectroscopic analysis of body fluids is an ideal approach for diagnosing neurodegenerative diseases. CSF is a promising body fluid for the identification and characterization of neurodegenerative diseases. Since the CSF compartment is in close anatomical contact with brain interstitial fluid, it may directly reflect the biochemical changes associated with neurodegenerative diseases, including MS³⁹. Clinically relevant and promising biomarkers from both blood serum and CSF are presented, which are useful for MS diagnosis, prognosis, and the assessment of therapy response and effectiveness, as shown in Table 1.

Table 1. Overview of main findings of previous spectroscopic studies of MS disease markers in body fluids and brain tissue.

CSF ³⁹	Serum ⁴⁰	Brain ^{38, 41}
-Increase in carbonyl band intensity -Decrease in intensity of amide I/amide II signals ratio -Decrease in intensity lipid/protein signals ratio	-The band at 1265 cm⁻¹ is blue-shifted 19 cm⁻¹ occurs in MS indicated conformational change in amide III. -The intensity ratio of β-sheet to α-helix secondary structure increased. -The intensity of RNA at 1125 cm⁻¹ decreases significantly for MS compared to control. -The increased intensity of total lipid in serum of MS patients.	-Reductions of the signal of lipids, proteins, and phosphate in white matter lesions. -Changes in unsaturated lipids and lipid chain length. -Cortical lesions had increased olefinic lipid content and abnormal membrane structure in normal appearing MS cortex compared to controls. -A dramatic loss of intensity in lipid ester carbonyl (~1735 cm⁻¹), which co-localised with an increase in nucleic acids (965 cm⁻¹). This finding is consistent with both the loss of lipid rich myelin in the demyelinating lesions and the infiltration of nucleated cells such as T cells, astrocytes, macrophages, and microglia.

1.3 MS treatments

The loss of BBB integrity during neuroinflammation is intriguing, as the absence of various pro-inflammatory mediators, such as TNF- α or IFN- γ , in EAE animals alters the composition and quantity of infiltrating immune cells in spinal cord lesions, but does not increase BBB permeability¹⁷. IFN β was the first disease-modifying therapy approved for the treatment of MS, offering patients a treatment that led to reduced relapse rates and delays in the onset of disability^{37, 42}.

1.3.1 Interferon beta as a treatment of RRMS

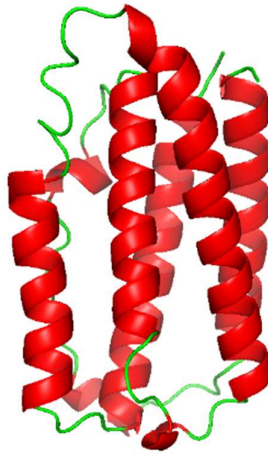


Figure 4. Structure of human Interferon beta® (IFNβ). The structure is taken from Protein Data Bank entry 1AU1.

The structure of IFNβ as shown in Figure 4 is a cytokine with a wide range of anti-inflammatory properties and is used to treat relapsing-remitting MS. Its mechanism of action is complex (Figure 5), involving the stimulation of anti-inflammatory cytokines such as IL-10, while inhibiting proinflammatory cytokines like IL-17 and osteopontin. IFNβ limits leukocyte migration across BBB and supports tissue repair through the production of trophic factors. Additionally, IFNβ can increase CD56^{bright} natural killer (NK) cells, which produce anti-inflammatory cytokines, and modulate immune responses. IFNβ helps stabilize CNS inflammation by shifting the immune response from a proinflammatory Th1/Th17 profile to an anti-inflammatory Th2 response, thereby reducing neuronal damage. It also reduces T-cell activation, adhesion, and penetration into the CNS, and disrupts antigen presentation in B cells and antigen-presenting cells⁴³⁻⁴⁴.

There are several side effects associated with MS medications, such as flu-like symptoms, chills, muscle and joint pain, liver dysfunction, and mood disorders^{42, 45}. Consequently, patients undergoing MS therapies must cope with various constraints, including frequent blood tests, MRI scans, and medical consultations to ensure the effectiveness of treatments and to assess the severity of side effects, which may require discontinuing or changing the ongoing therapy. Therefore, the characterization of effective and safe MS drugs with minimal or no side effects remains a significant challenge for biomedical researchers. In particular, the identification of novel compounds that can efficiently protect against the destruction of MBP — a crucial factor in axonal myelination⁴⁵ — may help prevent MS-induced axonal damage, which causes severe disability and presents major health concerns with substantial socio-economic consequences⁴⁵.

There are several proposed mechanisms of action for IFN β in MS

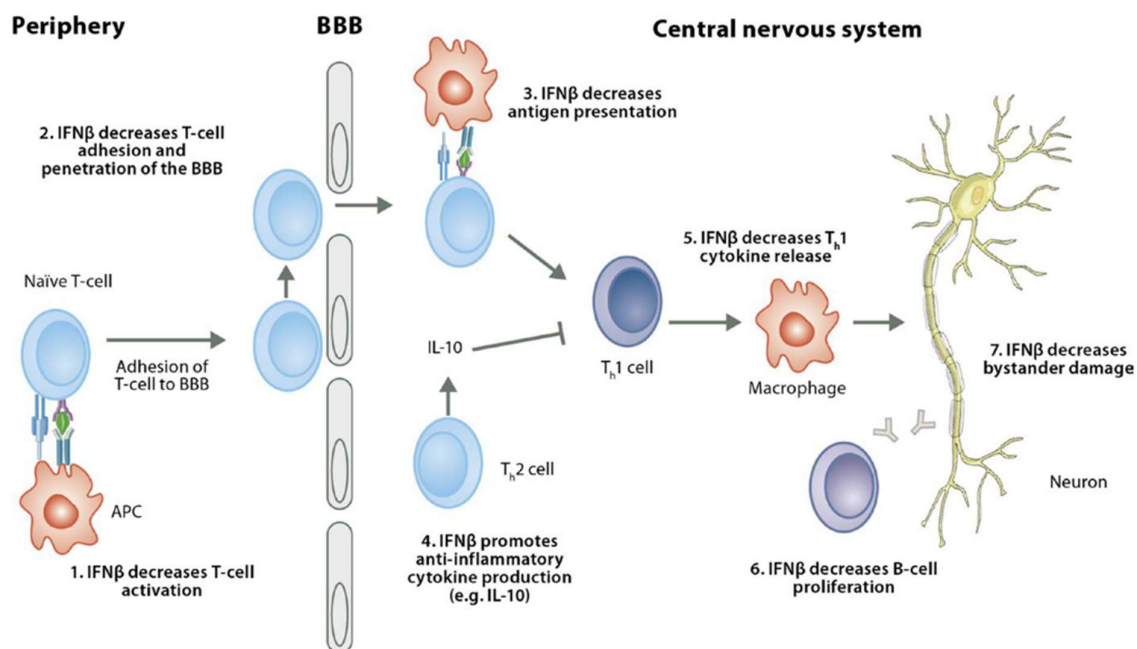


Figure 5. Mechanism of action for IFN β in MS. IFN β exerts its biological effects by binding to specific receptors on the surface of human cells. This binding initiates a complex cascade of intracellular events that leads to the expression of numerous interferon-induced gene products and markers⁴⁶.

1.3.2 XBD173 experimental treatment

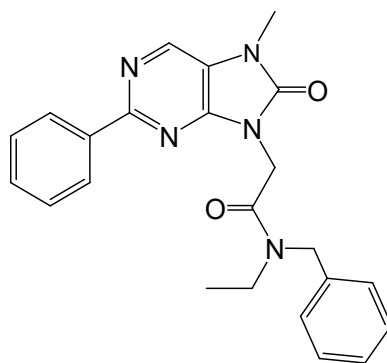


Figure 6. Chemical structure of Phenylpurine XBD173.

XBD173 (Emapunil®, Figure 6) is a selective and high-affinity ligand that targets the translocator protein (TSPO), which is found on the outer membrane of mitochondria. TSPO as shown in Figure 7 plays an important role in controlling cholesterol movement, steroid synthesis, and the production of neurosteroids in brain and immune cells. These neurosteroids have anti-

inflammatory, anxiolytic (anxiety-reducing), and neuroprotective effects, making TSPO a key player in immune modulation, especially in neurodegenerative diseases^{45, 47-48}.

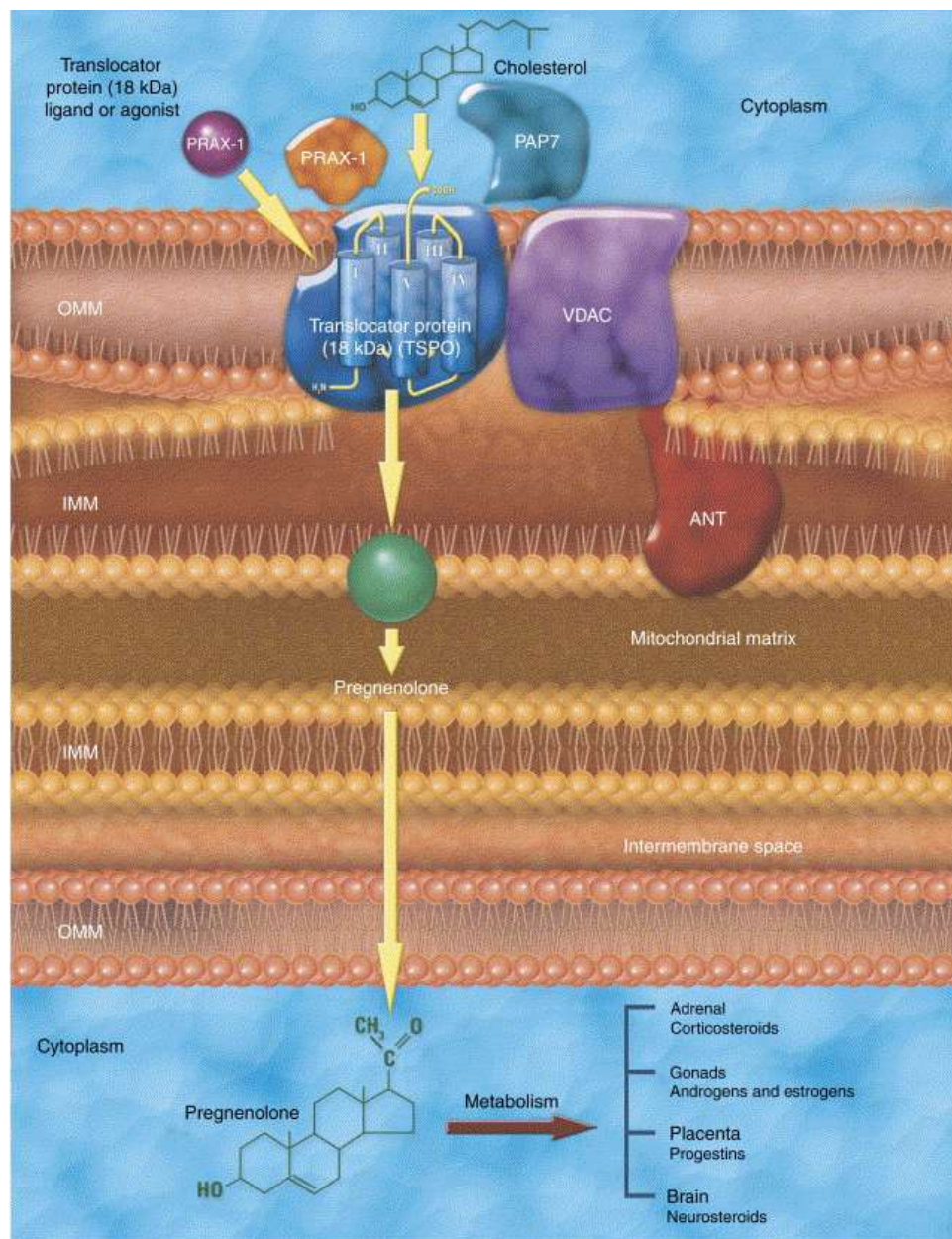


Figure 7. TSPO plays a crucial role in the synthesis of steroids by mediating the transport of cholesterol into mitochondria. A model of TSPO-mediated cholesterol transport is shown, where TSPO (also known as PBR) interacts with the voltage-dependent anion channel (VDAC) — a protein found at the outer mitochondrial membrane (OMM)–inner mitochondrial membrane (IMM) contact sites — and the IMM adenine nucleotide translocator (ANT). This interaction forms a complex that is preferentially located in the OMM–IMM contact sites. Associated proteins such as PRAX-1 and PAP7 may facilitate the formation of this complex or help target cholesterol to TSPO. Cholesterol transport across the OMM by TSPO is activated by specific ligand binding to the protein. Once cholesterol reaches the IMM, it is delivered to P450scc, where it is cleaved

to produce pregnenolone, which is further metabolized into tissue-specific steroids by enzymes in the endoplasmic reticulum⁴⁷.

Research has shown that activating TSPO with ligands like etifoxine can decrease immune cell infiltration in the spinal cord and promote regeneration of oligodendrocytes (cells responsible for myelination) after inflammation. This makes TSPO ligand-based therapies a promising option for treating neurodegenerative diseases, including MS^{45, 47-48}.

The compound XBD173 has been shown to stimulate neurosteroid production, which helps in reducing inflammation and improving neuroprotection. In experimental models of RRMS, XBD173 has demonstrated significant therapeutic effects^{45, 47-48}, such as:

- Reducing the production of pro-inflammatory cytokines like IL-17, IL-16, and TNF- α .
- Increasing the production of allopregnanolone (a neurosteroid) in the spinal cord and brain.
- Counteracting demyelination by reversing the repression of the MBP gene.
- Delaying the onset of disease symptoms.
- Reducing the severity of clinical and behavioral symptoms in animal models.

In mice, XBD173 has been shown to be effective at a low, well-tolerated dose (10 mg/kg)⁴⁵, and its effects are both safe and beneficial in improving MS symptoms without the negative side effects commonly seen with other treatments, such as benzodiazepines. XBD173 represents a promising treatment strategy for MS and other neurodegenerative diseases, offering a novel approach to managing inflammation and promoting recovery⁴⁵.

Therefore, we made the hypothesis that the concomitant injection of low doses of IFN β and XBD173 may boost their beneficial actions against MS symptoms and additionally decrease IFN β -related side effects. To check our hypothesis, we performed the present pilot study to determine the effects of separate and concomitant injections of selected doses of IFN β and XBD173 on the clinical scores of controls and RRMS mice. We combined in vivo scoring of RRMS symptoms with FTIR spectroscopy of sera samples to compare the therapeutic potential of (i) a well-tolerated dosage of the neuroprotective compound XBD173 alone, (ii) IFN β therapy alone and (iii) a concomitant treatment with both XBD173 and IFN β . The data analysis particularly focused on the spectroscopic signature of lipids and proteins, the two main components of myelin sheaths to determine the efficacy of three therapeutic strategies aiming to treat RRMS clinical symptoms.

2. Alzheimer's disease

AD is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, behavioral changes, and ultimately death. It is the most common cause of dementia and is projected to affect approximately 153 million individuals worldwide by 2050⁴⁹. Globally, AD ranks as the seventh leading cause of death⁵⁰. Neuropathologically, the disease is marked by the accumulation of abnormal proteins, leading to the formation of extracellular amyloid plaques and

intracellular neurofibrillary tangles (NFTs). Amyloid plaques primarily consist of neurotoxic A β peptides, while NFTs are composed of abnormally hyperphosphorylated tau protein aggregates⁵¹⁻⁵³. Currently, no effective therapeutic treatments are available to halt or significantly slow disease progression. Therefore, gaining a deeper understanding of AD pathogenesis is essential for the development of effective therapeutic strategies⁵²⁻⁵⁴.

In AD, neuropathological changes can begin up to thirty years before clinical symptoms appear. The earliest pathological event is the deposition of A β peptides, which initially occurs in cerebral regions with high metabolic and bioenergetic activity, such as the association cortices. This pathology progresses in a stereotypical pattern, spreading from the neocortex to the allocortex, then to the brainstem, and eventually reaching the cerebellum (Figure 8) ^{52-53, 55}.

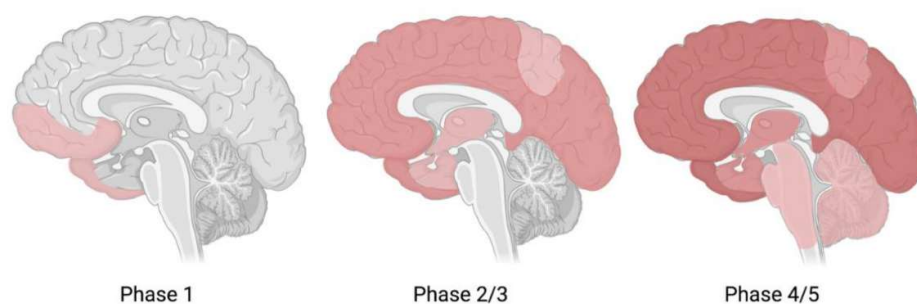


Figure 8. The traditional neuropathological phases of A β deposition in AD dementia follow a stereotypical spatial pattern. In phase 1, A β initially accumulates in the prefrontal cortex during the early preclinical stage, represented by red shading. Continued deposition in the same area is illustrated by progressively darker shading in subsequent phases, indicating ongoing accumulation. In each phase, newly affected areas are marked in red. During phase 1, A β deposition begins in neocortical regions, particularly in the association cortices. In phases 2 and 3, additional accumulation occurs in allocortical regions and the midbrain. In the later clinical stages (phases 4 and 5), A β deposits are also found in the brainstem and cerebellum. The change in shading intensity reflects the continuous progression of A β pathology over time⁵²⁻⁵³.

In AD, the oligomerization of A β into soluble oligomers and their accumulation in the brain are considered primary pathogenic events leading to synaptic loss and selective neuronal cell death. A β is a 39–42 amino acid-long peptide produced through the proteolytic cleavage of the transmembrane APP by β -secretase and γ -secretase. In its native form, A β exhibits neuroprotective properties and supports brain development. However, A β misfolding, overproduction, and an imbalance in the A β_{40} -to-A β_{42} ratio contribute to pathological conditions⁵⁶.

2.1 Mechanism of A β aggregation

A β misfolding and overproduction lead to its aggregation, a critical event in AD pathogenesis. The aggregation process (Figure 9) follows a nucleation-dependent mechanism, where A β Ms undergo conformational changes to form β -sheet-rich structures. These monomers can aggregate via the "on-pathway mechanism," leading to the formation of spherical A β Os, elongated protofibrils, and mature A β Fs, which are characterized by a cross- β structure with individual strands oriented perpendicular to the fibril axis. Alternatively, A β Ms can aggregate through an "off-pathway mechanism," forming amorphous, non-fibrillar aggregates. These off-

pathway species may dissociate into smaller aggregates that re-enter the on-pathway process. Additionally, on-pathway aggregates can undergo fragmentation, further contributing to the propagation of aggregation⁵⁶⁻⁵⁷.

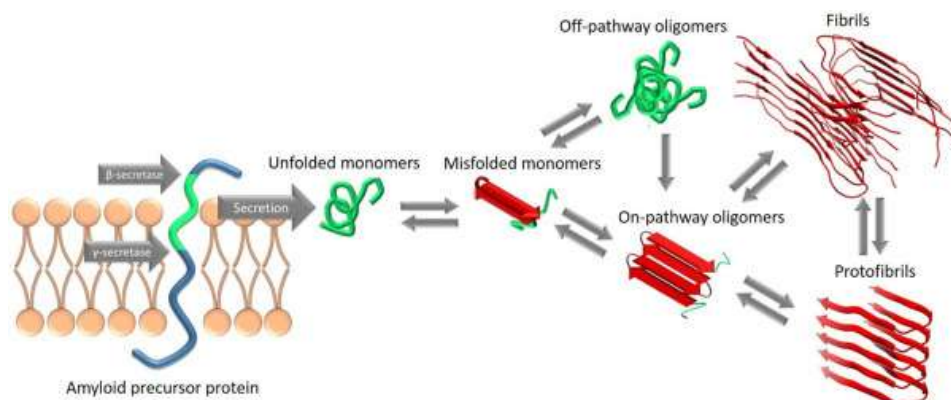


Figure 9. A β is produced through the sequential proteolytic cleavage of the APP, with β -secretase initiating the cleavage, followed by γ -secretase. The APP segment involved in this process is typically highlighted in green in structural representations. Once the unfolded A β Ms are secreted into the extracellular environment, they can misfold and begin to aggregate. These A β Ms can adopt various conformational states and follow distinct aggregation pathways, contributing to amyloid pathology in AD⁵⁶.

Cheng *et al.*⁵⁸ demonstrated that the interaction of A β_{40} with lipid vesicles is independent of lipid composition but is significantly influenced by the peptide-to-lipid (P:L) molar ratio. At a high P:L ratio, A β_{40} undergoes a rapid conformational transition from an α -helix to a β -sheet structure within 4 hours, forming β -sheet-rich oligomers (A β_{40} Os) without progressing to protofibrils or fibrils. These oligomers are capable of inducing membrane content leakage. Conversely, at a low P:L ratio, A β_{40} transitions from an α -helix to a random coil structure, leading to the formation of off-pathway A β_{40} Os. These off-pathway oligomers promote lipid mixing between neighboring vesicles, resulting in vesicle fusion. These findings underscore the critical role of the P:L ratio in determining the structural and functional outcomes of A β_{40} interactions with lipid membranes^{56, 58}.

2.2 Mechanism of A β O interaction with cell membrane

While A β Ms and A β Fs are often considered relatively non-toxic, growing evidence supports the A β Oligomer Hypothesis, which identifies A β Os as the most toxic and pathogenic form of A β . A β Os have been shown to impair learning and cognition, disrupt synaptic function, trigger cell death through lysosomal enzyme leakage, inhibit mitochondrial activity, increase the production of ROS, and promote neuroinflammation. Additionally, A β Os can permeabilize cell membranes via multiple mechanisms, including the formation of pore-like structures or ion channels, and lipid extraction. These diverse pathways contribute to the broad and potent toxicity exhibited by A β Os⁵⁶.

2.2.1 Mechanism of A β O interaction with cell membrane by channel-like structures

Recent studies have demonstrated that A β peptides can form ion channel-like structures in lipid bilayers, contributing to cellular toxicity observed in AD. These channels are heterogeneous, ranging from tetrameric to hexameric and higher-order assemblies, and their formation correlates with the oligomerization state of A β peptides upon membrane insertion. The structural diversity of these channels is influenced by the specific A β isoforms and their conformational dynamics. Notably, A β oligomers can form pores that facilitate calcium influx, disrupting ionic homeostasis—a key factor in early pathophysiological changes in neurons⁵⁹⁻⁶⁰.

These amyloid ion channels provide a direct mechanism for A β -induced toxicity. When soluble A β is no longer adequately cleared from the CSF, its levels rise and promote aggregation and membrane insertion. This results in abnormal ion flux, particularly calcium dysregulation, which contributes to synaptic dysfunction and neurodegeneration⁶¹.

Under pathological conditions, the accumulation of calcium-permeable amyloid channels over time can lead to toxic intracellular calcium concentrations. This cascade ultimately contributes to neuronal damage and degeneration, highlighting amyloid channel formation as a critical target in therapeutic strategies against AD⁵⁹⁻⁶¹.

2.2.2 Mechanism of A β oligomer interaction with cell membrane by detergent-like effect

Recent studies have demonstrated that A β O_s exert a detergent-like effect on lipid membranes, leading to significant membrane disruption. Atomic force microscopy and electron microscopy have revealed that while A β monomers have minimal impact on membrane integrity, and fibrils tend to embed into the bilayer without causing substantial disruption, A β oligomers uniquely induce lipid extraction from the membrane. This lipid removal results in membrane curvature, discontinuities, and the formation of lipid deposits, phenomena not observed with monomeric or fibrillar forms of A β as shown in Figure 10. These findings suggest that A β oligomers play a critical role in compromising membrane integrity, contributing to the pathogenesis of AD⁶².

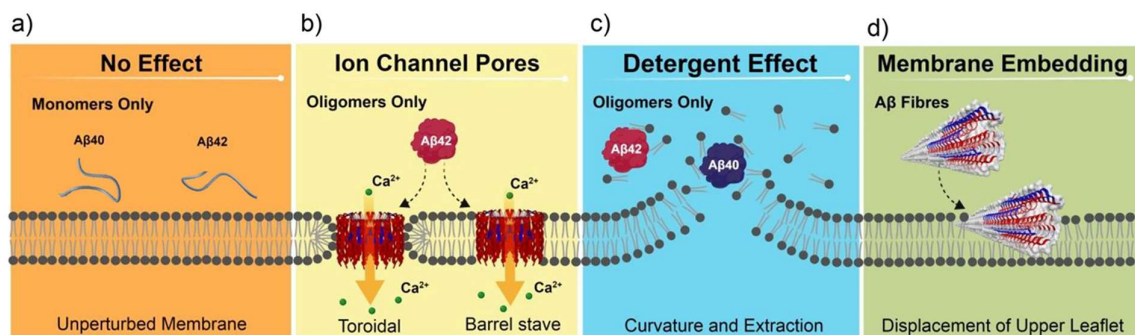


Figure 10. The mechanism of A β -mediated membrane disruption is highly dependent on the structural form of A β . Monomers, oligomers, and fibrils each have distinct effects on membrane integrity. (a) A β Ms exhibit minimal interaction with lipid membranes and have little to no impact on membrane structure. (b) In contrast, only oligomeric A β has the capacity to form ion channel-like pores within cellular membranes, thereby altering ionic homeostasis. (c) Oligomers of both

A β ₄₀ and A β ₄₂ also exert a potent detergent-like effect, resulting in lipid extraction, bilayer curvature, and membrane rupture. (d) This disruptive potential is lost when A β assembles into fibrils; instead, amyloid fibers can laterally embed into the membrane, displacing the upper leaflet of the bilayer without significantly compromising overall membrane integrity⁶².

2.2.3 Mechanism of A β oligomer interaction with cell membrane by lipid extraction

Recent studies have demonstrated that the interaction of A β Os with lipid membranes is size-dependent (Figure). Atomic force microscopy (AFM) imaging revealed that large-size A β Os, corresponding to high-molecular-weight species, interact with brain total lipid extract (BTLE) membranes by accelerating A β fibrillogenesis on the membrane surface without compromising membrane integrity. In contrast, small-size A β Os, corresponding to low-molecular-weight species, induce pore formation and subsequent disintegration of the BTLE membrane. These findings suggest that while both forms of A β Os are neurotoxic, their mechanisms of membrane disruption differ significantly¹.

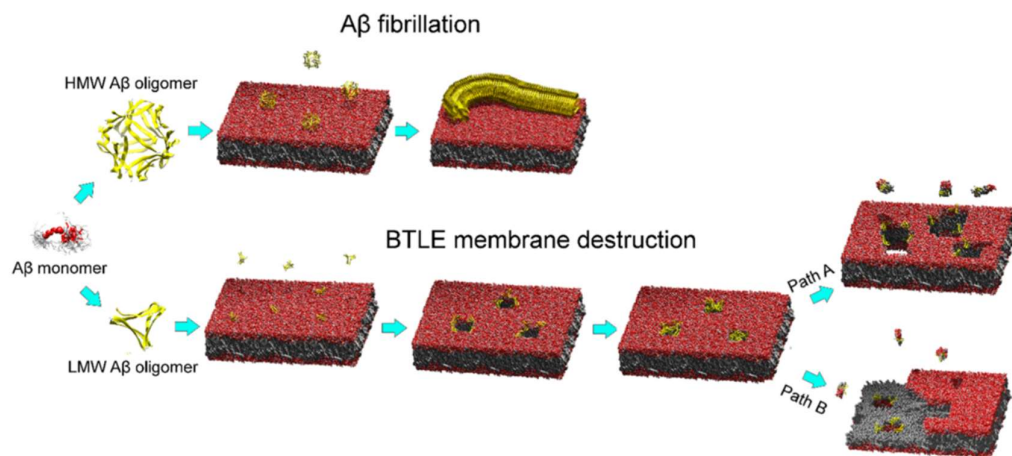


Figure 11. Illustration of the proposed mechanism of oligomer interaction with the bilayer¹. The high-molecular-weight species accelerating amyloid fibril interacts with brain total lipid extract membranes without membrane integrity. The low-molecular-weight species induces pore formation and subsequent disintegration of the brain lipid membrane.

2.3 AD treatment

Recent studies of our collaborations^{2, 63-65} have highlighted the potential of cyclic D,L- α -peptides as therapeutic agents against A β aggregation in AD. Specifically, a cyclic peptide (CP-1) and its optimized derivative (CP-2) have demonstrated the ability to bind A β , inhibit its aggregation, and reduce its toxicity in neuronal-like PC-12 cells. The chemical structures of CP-1 and CP-2 are shown in Figure 12. These cyclic peptides mimic structural features of amyloidogenic proteins, allowing them to interact with A β and prevent its pathological assembly. Notably, CP-2 not only inhibits A β aggregation but also disassembles preformed fibrils without generating additional toxic oligomers. NMR studies suggest that the anti-amyloidogenic activity

of these peptides is mediated through π - π and hydrophobic interactions, stabilizing less toxic forms of A β and altering its aggregation pathway².

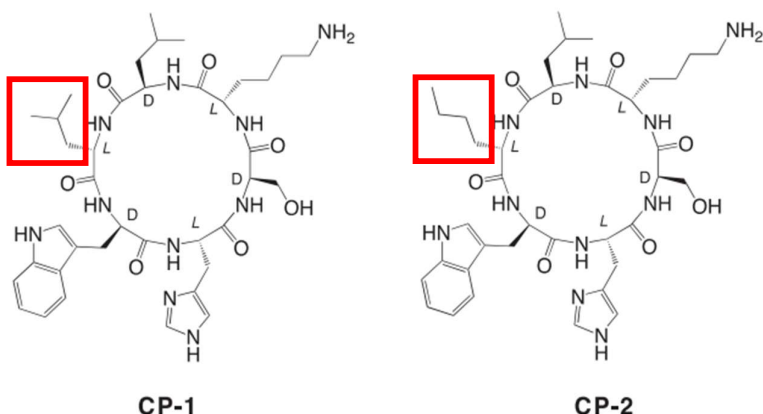


Figure 12. Chemical structure of CP-1 and CP-2² highlighted the difference by red rectangle.

While FDA-approved monoclonal antibodies like Aducanumab® and Lecanemab® have shown promise in targeting A β , concerns regarding side effects such as micro-hemorrhages, brain swelling, high treatment costs, and limited blood-brain barrier penetration underscore the need for alternative therapies. Cyclic D,L- α -peptides, with their unique structural properties and ability to modulate A β aggregation, represent a promising avenue for the development of more effective and safer treatments for AD⁶³.

The biological activity of most bioactive cyclic D,L- α -peptides is sensitive to minor amino acid modifications. Due to the vast potential sequence space within cyclic D,L- α -peptides that can interact with A β , a 'one-bead-one-compound' combinatorial approach was employed to select peptides capable of cross-reacting with A β and inhibiting its aggregation and toxicity. To investigate the general topological requirements for antiamyloidogenic activity, representative amino acids with diverse functional side chains—including positively charged (Lys), negatively charged (Glu), neutral hydrophilic (Ser), neutral hydrophobic (Leu), and aromatic (Trp, His) residues—were introduced at five positions of the cyclic D,L- α -peptide hexamer. Position one was fixed with Lys to facilitate attachment to a solid support via the free amine side chain and enable head-to-tail cyclization⁶⁶.

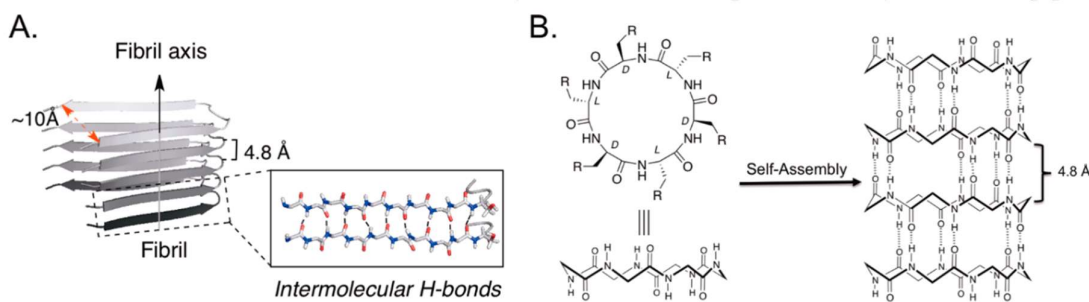


Figure 13. Structural similarity between (A) A β peptide and (B) cyclic D,L- α -hexapeptide⁶⁶.

Prefibrillar assemblies of different proteins have been shown to interact similarly with synthetic phospholipid bilayers and cell membranes, leading to membrane destabilization. In most cases, membrane interaction occurs through a two-step mechanism: first, electrostatic interactions between positively charged residues and negatively charged or polar lipid headgroups; second, insertion of hydrophobic regions into the membrane. This mechanism resembles that of membrane-active antimicrobial peptides, which form pore-like assemblies on membranes. Additionally, amyloid toxicity may involve increased production of ROS and indirect activation of microglia and immune cells by A β species⁶⁶⁻⁶⁷.

Remarkably, the structural and biochemical characteristics of amyloids closely resemble those of cyclic D,L- α peptide nanotubes. These supramolecular structures result from the self-assembly of simple cyclic peptides comprising an even number of alternating D- and L- α amino acids, forming flat, ring-shaped conformations. Under conditions favoring hydrogen bond formation, these cyclic peptides stack to form hollow, β -sheet-like tubular structures that can act as ion channels. The hydrogen bonds in such structures align peptides approximately 4.8 Å apart—an intermolecular distance similar to that found in A β -strands (Figure 13). Like amyloids, the structure of cyclic D,L- α peptide nanotubes is primarily dictated by backbone hydrogen bonding, stemming from the alternating D- and L-configuration. These peptides can exhibit various biological activities, including antibacterial and antiviral effects. Interestingly, their mode of action involves membrane interaction and disruption through nonspecific ion channel or pore formation, resulting in membrane integrity loss and cytotoxicity—again resembling amyloid behavior⁶⁶⁻⁶⁷.

Given these strong structural similarities, it has been hypothesized that appropriately designed cyclic D,L- α peptides could cross-react with A β through complementary hydrogen-bond donor and acceptor sequences, thereby modulating A β aggregation and toxicity⁶⁶⁻⁶⁷.

2.4 The studies of antiamyloidogenic self-assembled cyclic D,L- α peptide

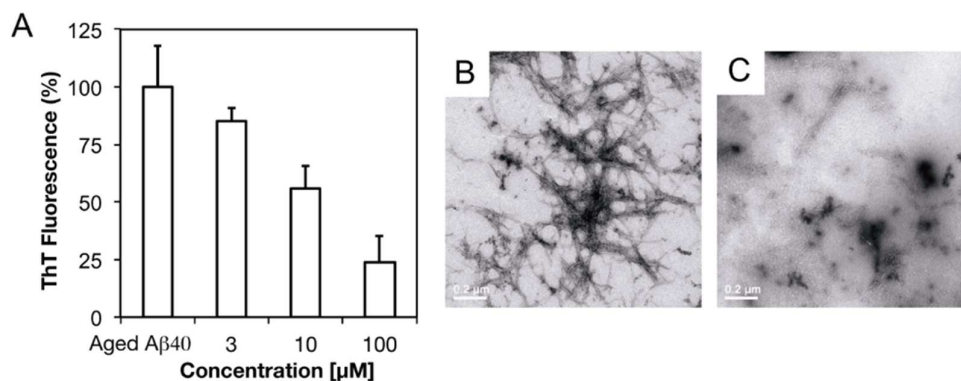


Figure 14. (A) A β ₄₀ (10 μM) was aged for 4 days to afford maximal ThT fluorescence. The aged samples were then incubated with freshly prepared cyclic peptide CP-2 (3, 10, or 100 μM) for further 72 h and then examined by the ThT assay. The disaggregation of (B) A β fibrils to (C) amorphous aggregates was examined using TEM⁶⁶.

To examine whether the cyclic D,L- α peptide CP-2 can disassemble aggregated or fibrillar A β , A β_{40} was incubated at 37 °C for four days to allow fibril formation and then treated with increasing concentrations of CP-2. The peptide was found to reduce the aggregation signal in a dose-dependent manner (Figure14A). The disaggregation of fibrillar A β by CP-2 was further confirmed by transmission electron microscopy (TEM), which showed a transformation from a well-defined fibrillar network (Figure14B) to amorphous-like structures upon treatment (Figure14C)⁶⁶.

In this study, we built upon previous findings regarding cyclic D,L- α peptides, particularly CP-2, known for their potent antiamyloidogenic activity. The experimental design was divided into three main parts to investigate the interaction between CP-2 and A β_{40} . First, we examined the aggregation of monomeric A β_{40} . Second, we studied the disaggregation of preformed A β_{40} fibrils in the presence of gold nanoparticles (GNPs) functionalized with CP-2. Third, we assessed the aggregation behavior of monomeric A β_{40} when exposed to CP-2-functionalized GNPs. Additionally, to gain deeper insight into the pathological features of AD, FTIR microscopy was employed to characterize the prefrontal and occipital cortex of AD model mouse brain tissue. FTIR is used to study the A β structures because this technique provides the detection of the secondary structure (amide I band $\sim 1600\text{--}1700\text{ cm}^{-1}$) arising mainly from C=O stretching vibrations in the peptide backbone. Moreover, in order to monitor the aggregation kinetics over time by observing changes in the Amide I region as the amyloid forms and matures as well as to observe the disaggregation kinetics of A β Fs in the presence of GNPs functionalized with CP-2.

Chapter II

Materials and methods

1. Spectroscopic studies of MS of mice sera

1.1 Sample collections

1.1.1 Animals

As received 88 EAE-PLP serum samples and EAE-PLP brain tissue samples of mice sample as shown in Table 2 from Prof. A. G. Mensah-Nyagan (Biopathologie de la Myéline, Neuroprotection et Stratégies Biopathologie de la Myéline, Neuroprotection et Stratégies Thérapeutique UMR S_1119, Fédération de Médecine Translationnelle de Strasbourg (FMTS)).

Nine-eleven-week-old SJL/jRj female mice were used in the present study. The mice were obtained from a commercial source (Janvier, Le Genest-St-Isle, France) and housed under standard laboratory conditions in a 12 h light/dark cycle with food and water ad libitum. Before starting the experiments, the animals were allowed a one-week acclimatization period after delivery in our animal house. Animal care and manipulations were conducted according to the European Community Council Directives (2010/63/UE) and under the supervision of authorized investigators. All experiments performed minimized the number of animals used and their suffering in accordance with the Alsace Department of Veterinary Public Health Guide for the Care and Use of Laboratory Animals. A national project authorization was delivered by the French Ministry of Higher Education and Research and by CREMEAS a local ethical committee (Project authorization number 9374–201,605,111,128,746-v2). The experiments also followed the International Association for the Study of Pain ethical guidelines.

1.1.2 Production of RRMS (EAE-PLP) and control mice

EAE was induced in nine-eleven-week-old mice by subcutaneous injection of an emulsion containing 150 µg proteolipid protein (PLP139-151) peptide (Eurogentec, Liège, Belgium) emulsified 1:1 in Complete Freund's Adjuvant (CFA) to each hind flank (100 µL by flank). The CFA is prepared from Incomplete Freund's Adjuvant (IFA) (Santa Cruz Biotechnology, INC, Heidelberg, Germany) with *mycobacterium tuberculosis* (Difco Laboratories, Franklin Lakes, NJ) at 5 mg/mL. Then, the mice received 200 ng pertussis toxin (Enzo life sciences, Lausen, Swiss) in 200 µL sterile phosphate-buffered saline (PBS) intraperitoneally (i.p.) on day 0 and day

2 post-immunization (D0 and D2). Control mice were immunized without PLP139-151 according to a similar schedule. Clinical evaluation of symptoms was performed daily according to the standard EAE (RRMS) grading scale.

1.1.3 Drug treatment

XBD173 purchased from Sigma-Aldrich (Saint- Quentin-Fallavier, France) was dissolved in hydroxypropyl cellulose (HPC) 0.3 % (Alfa Aesar, Haverhill, Massachusetts, USA) before i.p. injection (200 μ L) to mice at 10 mg/kg. IFN β (TebuBio, France) was diluted in PBS before i.p. injection (100 μ L) at 10 000 International Units (10 kIU) or 20 kIU.

The total cohort of 88 mice used for the present study was divided into 6 groups organized as follows: naive or healthy control mice (n = 7); RRMS (EAE-PLP) mice treated with IFN β (10kIU) alone (n = 13); RRMS mice treated with IFN β (20kIU) alone (n = 10); RRMS mice treated with XBD173 (10 mg/kg) alone (n = 20); RRMS mice concomitantly treated with XBD173 (10 mg/kg) + IFN β (10kIU)(n = 15); RRMS mice concomitantly treated with HPC 0.3 % (vehicle for XBD173) + PBS (vehicle for IFN β)(n = 23). Mice were treated every two days from D4 post immunization until sacrifice.

1.1.4 Sampling of serum, preparation conservation

The animals were sacrificed during the late remitting phase. They were deeply anesthetized with ketamine (200 mg/kg)/Rompun (20 mg/kg) (Centravet, France). Blood was removed, centrifuged at 1500 g at 4 °C for 30 min and the serum was stored at - 80 °C until the beginning of FTIR spectroscopy experiments.

1.2 ATR-FTIR spectroscopy

Frozen serum samples were stored at -80°C. The samples were thawed in an ice box at -4°C before measurement. The serum spectrum was recorded immediately after a background measurement to control atmospheric conditions, using a 64 scans per spectrum and 20 kHz rate in the wavenumber range of 4000-700 cm^{-1} by ATR FTIR spectroscopy (Vertex 70, Bruker, Germany). A volume of 2.5 μ L of serum was placed on the diamond crystal of the ATR, and 50 spectra were taken with a resolution of 4 cm^{-1} at room temperature. The last 10 spectra were averaged, baseline-corrected, and normalized using the min-max method. The second derivative spectra were smooth nine points using the Savitzky-Golay algorithm. Data recording and manipulation were performed with OPUS software version 7.2.

1.3 FTIR microscope

EAE-PLP brain tissue samples were analyzed on the cerebellum as shown in Figure 15 by LUMOS infrared microscope from Bruker. The spectra were recorded with a diaphragm of $20 \times 20 \mu\text{m}$ and spectral resolution of 4 cm^{-1} . All spectra were baseline corrected prior to normalization with vector normalization using OPUS software version 8.7.41.

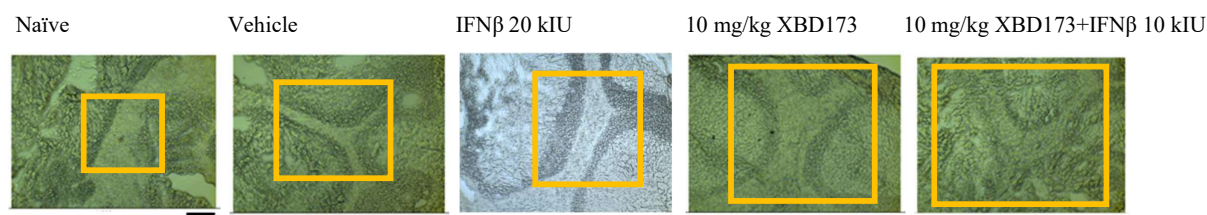


Figure 15. The analyzed cerebellum region highlighted with yellow rectangle. Scale bar = 100 μ m.

Table 2. Cohorts of mice included in this study along with their average clinical scores (mean $\pm$ SEM) at the peak and at the moment of sacrifice and blood samples collection³. The standard grading scale ranges from 0 to 10 where a score of 0 indicates the absence of clinical signs. Higher clinical scores reflect disease worsening with a maximal score of 10 indicating death. A p-value ≤ 0.05 was considered significant (versus naïve * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$ or versus vehicle # $p \leq 0.05$, ## $p \leq 0.01$, ### $p \leq 0.001$, #### $p \leq 0.0001$).

Cohort		Total number of mice	At peak (D14-D16)		At the moment of sacrifice (D35-D37)	
			Average clinical score ± SEM	p	Average clinical score ± SEM	p
Serum						
Naïve (healthy)		7	0.00	####	0.00	####
Vehicle (untreated MS)		23	3.35 ± 0.27	****	2.93 ± 0.33	****
Treated RRMS	IFNβ 10 kIU	13	3.23 ± 0.43	****	2.58 ± 0.62	***
	IFNβ 20 kIU	10	2.50 ± 0.48	**	1.60 ± 0.54	*#
	10 mg/kg XBD173	20	2.70 ± 0.23	***	1.30 ± 0.36	*####
	10 mg/kg XBD173+IFNβ 10 kIU	15	2.47 ± 0.24	**	0.80 ± 0.26	###
Brain tissue						
Naïve (healthy)		1	-	-	0	-
Vehicle (untreated MS)		1	-	-	5	-
Treated RRMS	IFNβ 20 kIU	1	-	-	2	-
	10 mg/kg XBD173	1	-	-	2	-
	10 mg/kg XBD173+IFNβ 10 kIU	1	-	-	1	-

1.4 Multivariate analysis Methods

The unsupervised multivariate analysis method is PCA commonly used chemometrics technique. The supervised multivariate analysis method is Random Forest applied for classification of healthy control, untreated MS and the various treatments. PLS, a supervised classification technique, was used to study the correlated wavenumbers between IFN β 10 kIU and IFN β 20 kIU revealing inflammation sensitive spectral range and the amount of IFN β in the mixture of 10 mg/kg XBD173 + IFN β 10kIU.

1.4.1 Unsupervised multivariate analysis

PCA is the method to reduce the dimension in the meantime preserve the variability of the data⁶⁸. PCA captures the highest variance in data that represents the overall structure and downsizes the dimensions/features/variables to the essential key factors. PCA helps to project absorption spectra onto a lower-dimensional plane which clarifies the interpretation and visualization. PCA was conducted over the range of 3500-700 cm⁻¹ to select the key wavenumbers. PCA simplifies this data by transforming it into a set of principal components (PCs) that capture the most significant variations. PCA results are presented as score and loading plots. Scores show how each sample relates to the PCs, helping to identify grouping, trends, or outliers. Loadings reveal which wavenumbers (spectral regions) contribute most to each PC, aiding interpretation of biochemical differences.

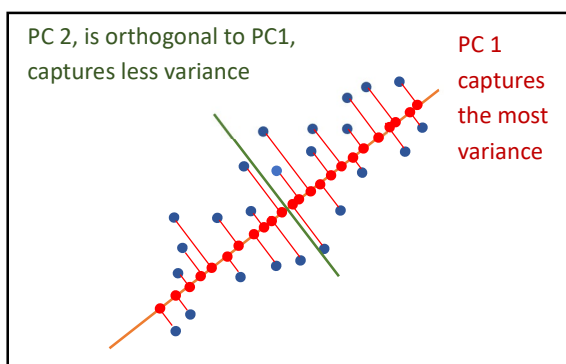


Figure 16. The projection of high-dimensional data onto a lower-dimensional space for PC1 and PC2.

Figure 16 shows how to transform two-dimensional data to one dimensional data by projection on one-dimensional straight red line. That red line preserves the highest variance (PC1). The second axis (PC2) is orthogonal to PC1 to capture less variance. PC3 will be orthogonal to both the previous two axes.

The procedure for PCA calculation includes the following steps

1. Standardization of data

$$z = \frac{x - \mu}{\sigma} \quad (\text{Eq. 1})$$

where μ is the mean value

σ is the standard deviation

z is the standardized value

x is the individual data point

2. Calculate the covariance matrix⁶⁹ from the data.

$$\text{var}(x) = \frac{\sum(x_i - \bar{x})^2}{N} \quad \text{cov}(x, y) = \frac{\sum(x_i - \bar{x})(y_i - \bar{y})}{N} \quad (\text{Eq. 2})$$

where $\text{var}(x)$ is the variance

$\text{cov}(x, y)$ is the covariance of x and y

x_i, y_i are the values of x and y

$\bar{x}, \bar{y}$ are the mean values of x and y

N is the number of observations

This matrix is defined as

$$\text{Covariance matrix} = \begin{bmatrix} \text{var}_a & \text{cov}_{a,b} & \text{cov}_{a,c} \\ \text{cov}_{a,b} & \text{var}_b & \text{cov}_{b,c} \\ \text{cov}_{a,c} & \text{cov}_{b,c} & \text{var}_c \end{bmatrix}$$

3. Computing eigenvalues and eigenvectors from eigenequation⁷⁰

$$Av = \lambda v \quad (\text{Eq. 3})$$

where A is a square matrix of covariance

v is eigenvector

λ is eigenvalue

These two values, are eigenvectors and eigenvalues, help in the interpretation of the chemometric analysis. The eigenvalues, is scalar, gives the information about the variation in each PCA. The eigenvectors or loading vectors give the information about the weighting function for PCA calculation⁷¹. These eigenvectors are filtered from the related highest eigenvalue to the lowest.

4. Calculation of the feature vector⁷²

$$\text{Feature vector} = (ev_1 \ ev_2 \dots ev_n) \quad (\text{Eq. 4})$$

where ev is eigenvector

5. Orient data from the original axes to new principal component axes⁷²

$$PC = \text{Feature vector}^T \times \text{Standardized original data}^T \quad (\text{Eq. 5})$$

1.4.2 Supervised multivariate analysis

Random Forests analysis, developed by Leo Breiman in 1996⁷³, is a supervised algorithm that uses out-of-bag estimation. Each tree in the forest is provided with different spectra to work with through bootstrapping, which is sampling with replacement. The spectra that are not chosen become the test set, called out-of-bag samples. This allows us to obtain multiple training groups from the original dataset. For each tree, a random subset of features is selected at each split, preventing the trees from becoming too similar and encouraging diversity among them. For classification as shown in Figure 17, each tree in the forest votes for a class, and the majority vote determines the final prediction. Random Forests, combined with boosting and bagging algorithms, have the ability to reduce bias (resulting in high accuracy) and variance (resulting in high precision)⁷⁴.

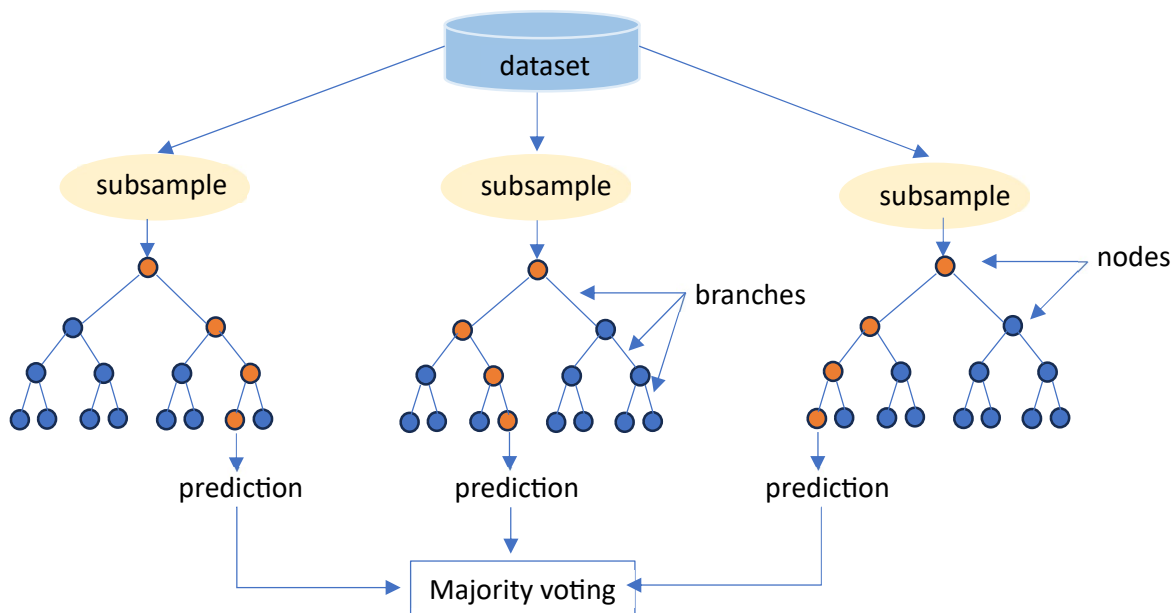


Figure 17. Random Forest algorithm is a supervised machine learning classifier that utilizes an ensemble of decision trees. Each tree consists of nodes and branches which nodes represent features, while branches guide the split based on those features. Each tree gives an individual prediction, and then a majority vote is taken to yield a more reliable and accurate result.

88 second derivative spectra have been analyzed using the Weka 3.9.6 program (<https://www.cs.waikato.ac.nz/ml/weka/>), which is free and open source, developed at the University of Waikato, Hamilton, New Zealand. The second derivative spectra have been classified using two-fold cross-validation with the Random Forest machine learning algorithm. The spectra are randomly split into two sets for each pathology: a training set and a test set, repeated for 10 different iterations. The evaluation of the model is presented as a confusion matrix, showing the percentages of ROC (Receiver Operating Characteristic) sensitivity, specificity, and precision.

1.4.3 Statistics

The nonparametric statistical test was used to evaluate the data whether normal or non-normal distribution. The comparable non-parametric statistical test with parametric statistical test used in this experiment are shown in Table 3. The statistically significant was $p = 0.05$ for all comparisons of Vehicle and treatments. The clinical score was expressed as mean $\pm$ standard error of mean (SEM). The signature of serum change after MS disease and the treatments was analyzed by fence boxplot prior to Kruskal Wallis test. The dominant effect of XBD173 and the Mixture treated MS analyzed by Wilcoxon Rank sum test. The correlation between IFN β 20 kIU-treated and Mixture-treated MS was evaluated using Spearman's rank correlation.

Table 3 The non-parametric statistical test applied whether normal or non-normal distribution is equivalent to parametric test for comparing two groups.

Parametric test	Non-Parametric equivalent
One way Analysis of variance (ANOVA)	Kruskal Wallis test
Paired t-test	Wilcoxon Rank sum test
Pearson correlation	Spearman's rank correlation

1.4.4 The effect of MS disease and all treatments

The simple way to identify the effect of all MS treatments is the outlier of the boxplot. The use of “inner fences” and “outer fences” has been suggested by “Tukey”(1997)⁷⁵. The fences procedure calculated from IQR, which is the difference between Q1 and Q3, according to following equations⁷⁶:

$$f_1 = Q1 - 1.5IQR \text{ and } f_3 = Q3 + 1.5IQR \quad (\text{Eq. 6})$$

$$F_1 = Q1 - 3.0IQR \text{ and } F_3 = Q3 + 3.0IQR \quad (\text{Eq. 7})$$

where f_1 and f_3 are inner fences

F_1 and F_3 are outer fences

Q1 is the first quartile

Q3 is the third quartile

$$IQR = Q3 - Q1$$

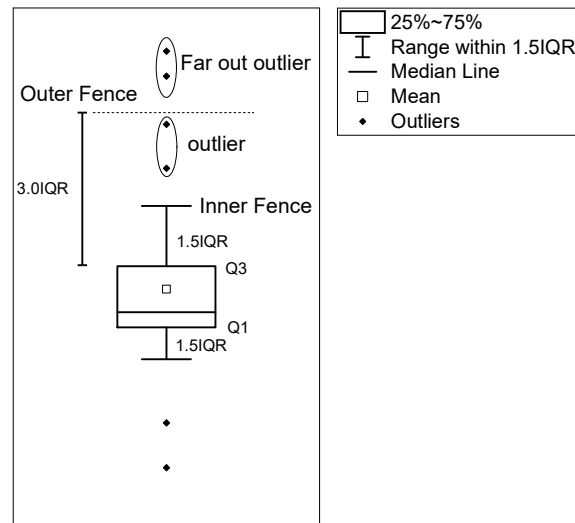


Figure 18. The components of a boxplot: median displays as a line inside the box dividing the data into two sets, quartiles cover from Q1(25 percentiles) to Q3(75 percentiles) which captures

the middle 50% of data points, fences extend from the edge of the plot 1.5 times the IQR and outliers are the data points outside fences.

The distribution fall between the inner and outer fences in each direction “outside” outliers, while those that fall below the outer fence F1 or above the outer fence F3 are “far out” outliers (Figure 18). This “inner fences” and “outer fences” is sensitive to both a normal population and very large sample. To establish fences for the asymmetrical distributions and small samples, Carling’s⁷⁷ considered based on probabilities and thin-tailed asymmetric distribution using SIQR instead of IQR. The lower and upper fences are calculated according to the following equation⁷⁶:

$$q2 - \frac{2(q2-q1)}{k_n} Z_{\alpha/2} \quad \text{and} \quad q2 + \frac{2(q3-q2)}{k_n} Z_{\alpha/2} \quad (\text{Eq. 8})$$

where $q1$ is the first quartile

$q2$ is the second quartile

$q3$ is the third quartile

k_n is the kurtosis factor ($k_n=1.34322^{76}$ for $n=52$, number of the input wavenumber)

$Z_{\alpha/2}$ is the alpha level’s z-score for a two tailed test (95% confidence level: $Z_{\alpha/2} = 1.96$)

The fence outlier method was applied to interpret the effect of each treatment. The area under the curve of the preprocessed second derivatives was calculated by integrating from the baseline mode using the Quasar 1.7.0 program⁷⁸⁻⁷⁹. The average second derivative area of the MS treatment was subtracted from that of the vehicle. The absolute difference was ranked using a boxplot. The changes obtained from the fence outlier analysis were statistically analyzed using Python 3.9 at $\alpha < 0.05$. The analysis of variance (ANOVA) was performed using the Kruskal-Wallis H test with false discovery rate (FDR) correction for p-value adjustment, followed by post hoc analysis using Dunn’s test with p-values adjusted by the Holm method⁸⁰.

1.4.5 The dominant effect of 10mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU

The dominant effect of XBD173 and the Mixture treated MS analyzed by Wilcoxon rank sum test⁸¹. This statistical test used to compare mean or median of the distribution between two groups. Moreover, it can be applied with non-normal distribution⁸² and can be used with the small samples and different sizes⁸³. The Wilcoxon rank sum test aims to test the separation between two groups using magnitude-based ranking calculated according to the following equation.

$$W_A = \sum_{i=1}^{n_A} \text{rank}(X_{Ai}) \quad (\text{Eq. 9})$$

where X_{Ai} is the sample in group A which rank 1 has the lowest value

W_A is the rank sum value

If the obtained interval-valued p-value < 0.05 , the null hypothesis (the mean of two groups is the same) will be rejected. The distribution of the absorption spectra plot in Figure 19 shows

that both the mean and median values (Table 4) for the Vehicle group are lower than those of the Naïve group. Treatment with 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU effectively restores these mean and median values to levels comparable to the Naïve group. To assess the significance of these observations, the Wilcoxon rank sum test will be employed. This non-parametric test is suitable for comparing two independent samples and helps clarify the dominant effects of 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU on the observed values.

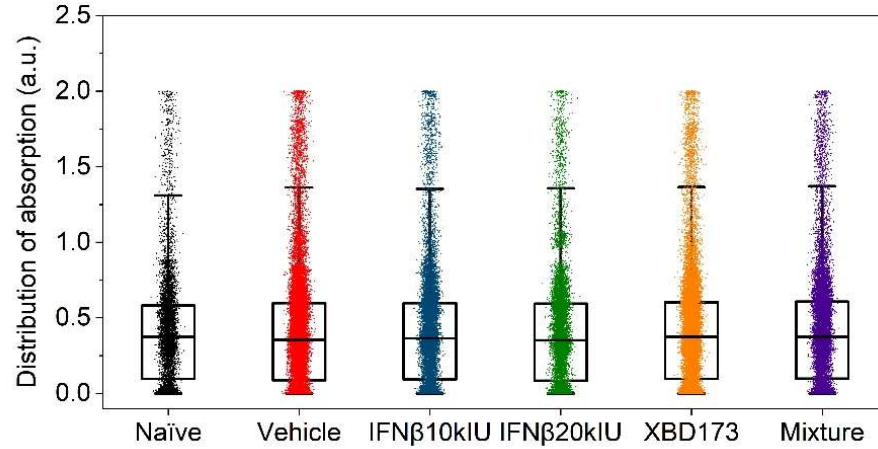


Figure 19. The distribution of absorption spectra plot of Naïve, Vehicle and all treatments.

Table 4. Mean and median calculated from the distribution of absorption spectra

Cohort	Mean	Median
Naïve	0.435	0.37
Vehicle	0.424	0.36
IFN β 10kIU	0.428	0.36
IFN β 20kIU	0.423	0.35
10 mg/kg XBD173	0.430	0.37
10 mg/kg XBD173+IFN β 10 kIU	0.431	0.37

1.4.6 The correlation between IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10kIU

The correlation between the data from IFN β 20 kIU and the data from the mixture of 10 mg/kg XBD173 + IFN β 10kIU was analyzed using Spearman's rank correlation coefficient, a non-parametric metric that studies the strength of the relationship between variables, regardless of whether they are continuous or discrete⁸⁴. For two variables, x and y, the formula for calculating the sample Spearman's correlation coefficient is:

$$r_s = 1 - \frac{6 \sum_{i=1}^n d_i^2}{n(n^2-1)} \quad (\text{Eq. 10})$$

where r_s is Spearman's rank correlation coefficient

d_i is the difference in ranks for x and y

n represents the number of observations

If the coefficient is positive, the variables tend to increase together (as shown in Figure 20A). Conversely, if the coefficient is negative, as one variable increases, the other tends to decrease (Figure 20B). A correlation coefficient of zero (Figure 20C) indicates no relationship exists between the two variables. The closer the correlation coefficient is to ± 1 , the stronger the correlation.

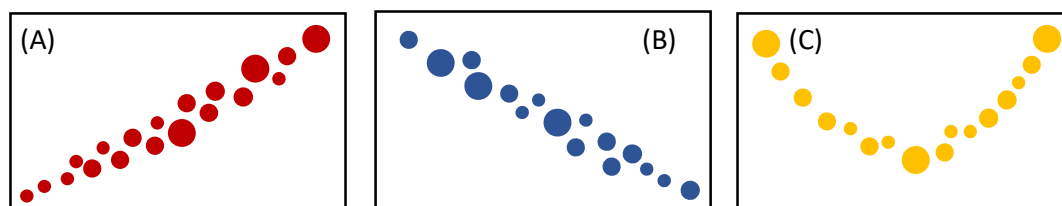


Figure 20. Spearman's rank correlation coefficient illustrates different types of monotonic relationships: (A) a perfect positive correlation (+1), where ranks increase together; (B) a perfect negative correlation (-1), where one rank increases as the other decreases; and (C) no correlation (0), indicating no consistent monotonic relationship between the variables.

The absorption spectra of IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10kIU were analyzed using Spearman's rank correlation coefficient calculated using Python 3.9. Since there are 10 samples of IFN β 20 kIU and 15 samples of the mixture, a weighting of 1.5 times were applied to IFN β 20 kIU.

1.4.7 The correlation between serum and cerebellum tissue

The correlation between serum and cerebellum tissue was analyzed using Spearman correlation. The cerebellum tissue consists of three distinct types: cerebellum white matter, the granular layer, and the molecular layer. To differentiate each tissue type, PCA was applied. PCA1 (Figure 21) represents the component with the highest variance, distinguishing two tissue types: cerebellum white matter, shaded in red, and the granular and molecular layers, shaded in blue⁸⁵

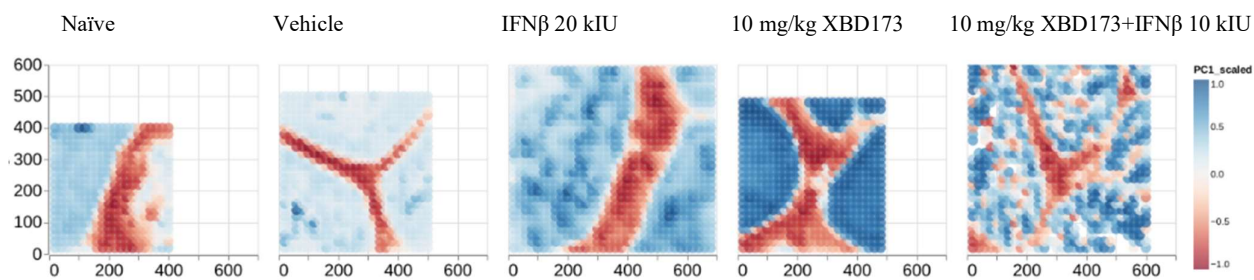


Figure 21. PCA of cerebellum tissue, distinguishing two tissue types: cerebellum white matter (red shading) and the combined granular and molecular layers (blue shading) scale bar in μm .

A total of 100 spectra were randomly selected from each region—cerebellum white matter (red) and the granular and molecular layers (blue). Due to differences in wavenumbers obtained from two different IR spectroscopies, the interpolate1d function in Python was applied to the wavenumber data from IR microscopy. The average Spearman correlation was calculated for each

selected spectrum in comparison to each serum spectrum. The 95% confidence interval (CI)⁸⁶ was determined using the following formula:

$$\bar{X} \pm t_{\alpha/2, n-1} \left(\frac{8}{\sqrt{n}} \right) \quad (\text{Eq. 11})$$

where:

$\bar{X}$ is the sample mean,

$t_{\alpha/2, n-1}$ is the t-multiplier, which depends on sample size (through $n-1$, the degrees of freedom) and the confidence level $(1 - \alpha) \times 100$

$\frac{8}{\sqrt{n}}$ is the standard error.

At the 95% $(1 - \alpha) \times 100$ confidence level, the higher and lower confidence limits correspond to 0.975 $(1 - \alpha)$ and 0.025 $(\frac{\alpha}{2})$, respectively.

The results are shown in Supplementary Tables S11-S15, which describe the average correlation scores in a heatmap plot. The highest average correlation score between serum and cerebellum tissue was observed as follows: NA_3_5V1S7 for Naïve, VE_19_2S3VT3 for Vehicle, IF_19_4S3RT1 for IFN, XB_3_2RT2S8 for 10 mg/kg XBD173, and IX_14_4V8S7 for the mixture of 10 mg/kg XBD173 + IFN β 10kIU. The correlation between three components—serum spectra, the average spectrum of cerebellum white matter obtained from 100 spectra, and the average spectrum of the granular layer obtained from 100 spectra—was analyzed using Spearman correlation.

2. Spectroscopic studies of Cyclic D,L- α -Peptide and AD mice tissue

2.1 Characterization of the AD's model mouse brain tissue

2.1.1 Sample collections

As received 12 corona section of mice brain tissue with thickness of 10 μm as shown in Table 5 from Prof. Jens PAHNKE (Medical Faculty, University of Oslo, Norway).

Table 5. Number of brain-tissue per group.

Cohort	Total number of slides
Brain tissue – coronal section	
B6 (healthy)	3
APP-Front 10 μm (untreated AD-Frontal lobe)	4
APP-Back 10 μm (untreated AD-Occipital lobe)	5

2.1.2 FTIR microscopy study of the AD brain tissue

AD brain tissue samples were analyzed in the cerebral cortex (Tables s16 and s23) using a LUMOS II infrared microscope (Bruker). The cortical structure of the cerebral cortex comprises six cellular layers⁸⁷. The cerebral brain tissue was examined in the primary somatosensory area for cortical layers II–III, as shown in Figure 16s, and in four distinct regions—anterior cingulate, primary somatosensory, supplemental somatosensory, and piriform areas—for cortical layers IV–V, as illustrated in Figure 23s. Each mapping measurement covered an area of 80,000 μm^2 using a diaphragm size of 10 $\mu\text{m} \times 10 \mu\text{m}$, with a spectral resolution of 4 cm^{-1} . Background spectra were recorded from adjacent, non-tissue areas on each CaF_2 window. All spectral maps underwent atmospheric compensation, baseline correction, and vector normalization using OPUS software version 8.7.41 prior to concatenation along the wavenumber axis. Statistical evaluation of the spectral data was performed using PCA to assess disease-related changes. All absorbance spectra were scaled within each wavenumber for PCA calculations using Python. Finally, chemical images were generated based on the wavenumber ranges corresponding to eigenvalue loadings.

2.2 The interaction between Cyclic D,L- α -Peptide (CP-2) and $\text{A}\beta_{40}$

2.2.1 GNP-functionalized with CP-2

As received four tubes of 200 μM /245 μL monomeric $\text{A}\beta_{40}$ and 980 μM of GNP-functionalized with CP-2 (Figure 22) from Prof. Shai Rahimipour (Department of chemistry, Bar-Ilan University, Israel). The average particle size and zeta potential received which detailed in Table s44.

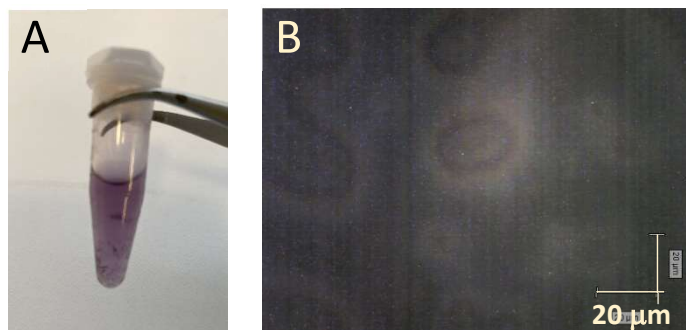


Figure 22. (A) as-receive 980 μM of GNP-functionalized with CP-2 (B) optical image of 2 μL of GNP-functionalized with CP-2 in PBS 10 μM in solution using 50x objective lens.

2.2.2 Storage and Handling of $\text{A}\beta$

$\text{A}\beta_{40}$ was purchased from Hanhong Scientific (Shanghai, China), purified to homogeneity (>95%) by RP-HPLC, and stored as a lyophilized powder at -20°C until used. The monomeric state of the peptide was ensured by evaporation from high-grade 1,1,1,3,3,3-hexafluoroisopropanol (HFIP) three times using a stream of nitrogen. The sample was dried under high vacuum for 1 h. The resulting film was dissolved in 10 mM NaOH to a concentration that was determined by UV absorption at 280 nm using the extinction coefficient of tyrosine (1280

$\text{M}^{-1} \text{cm}^{-1}$). The solution was divided into equal portions, freeze-dried, and stored at -80°C . Desired amounts of A β were freshly dissolved in DDW/PBS mixture (1:1; 50 mM, pH 7.4) and sonicated to homogeneity for ~ 20 s before each experiment⁶⁵.

2.2.3 The aggregation of monomeric A β_{40}

1. As-received A β_{40} was dissolved in 245 μL of double distilled water (DDW), this concentration of A β_{40} was afford a stock solution of 200 μM . Sonicate this solution for 75 sec to obtain monomeric form of A β .

2. Phosphate buffer saline (PBS) was freshly prepared by 106.87 mg of K_2HPO_4 , 52.60 mg of KH_2PO_4 and 151.94 mg of NaCl to gain concentration of 100 mM by 260 mM NaCl, pH 7.4. PBS was filtered sterilization with 0.2 μm PES filter.

3. The stock solution of 200 μM was divided into 6 Eppendorf tubes containing 30 μL of each. The last Eppendorf tube contained 20 μL . The rest of the stock solution was lyophilized and stored in -20°C for the next use.

4. Diluted 30 μL of the stock solution obtained from 1.1 (200 μM of monomeric A β_{40}) with 285 μL DDW and homogenized using vortex. Add to this solution 285 μL of freshly prepared PBS obtained from 1.2 and well mixed. This dilution was resulted in 600 μL of A β_{40} in PBS (~ 50 mM, 130 mM NaCl) at concentration of 10 μM .

5. 600 μL of A β_{40} 10 μM will be divided into 2 Eppendorf tubes containing 300 μL each tube.

6. Six tubes of monomeric A β_{40} obtained from 1.5 was incubated at 37°C with shaking 720 rpm for 2 min every hour, 96 h in total. Three tubes were sampling 5 μL for FTIR and 10 μL for Raman spectroscopy at 1, 18, 22, 26, 42, 46, 50, 66, 72 and 96 hours. The other three tubes were incubated 96 hours in order to get A β_{40} fibrils.

2.2.4 Disaggregation of A β_{40} fibrils in presence of GNP-functionalized with CP-2

Three tubes obtained from 1.6 had been fibrillated for 96 hours. These 96hr A β_{40} fibrils were mixed 980 μM GNP functionalized with CP-2 for a final concentration of 10 μM , to obtain a 1:1 ratio. For that ~ 3 μL of GNP functionalized with CP-2 was added to each tube. This disaggregation reaction was undertaken under the same conditions as protocol 6 of the aggregation of monomeric A β_{40} .

2.2.5 Inhibition of A β_{40} monomeric in presence of GNP-functionalized with CP-2

For aggregation experiment, three tubes monomeric A β_{40} obtained from 1.5 were mixed ~ 3 μL of 980 μM GNP functionalized with CP-2. This inhibition reaction was conducted following the 6 of the aggregation of monomeric A β_{40} .

2.2.6 Difference spectroscopy

The aggregation of monomeric A β_{40} , the disaggregation of A β_{40} fibrils in the presence of GNPs functionalized with CP-2, and the inhibition of A β_{40} monomers by GNPs functionalized with CP-2 at a concentration of 10 μM were investigated in the dried state using FTIR absorption

spectroscopy. All samples were stored frozen at $-20\text{ }^{\circ}\text{C}$. The experimental conditions for these reactions were consistent with those used in the previous serum experiments.

In Figure 23, the background absorptions of the unchanged part of the protein were removed remaining the reacting group. The background absorption of the protein is 10^3 - 10^4 times greater than the absorption of those reacting groups. Thus, the measurement conditions were controlled exactly the same⁸.

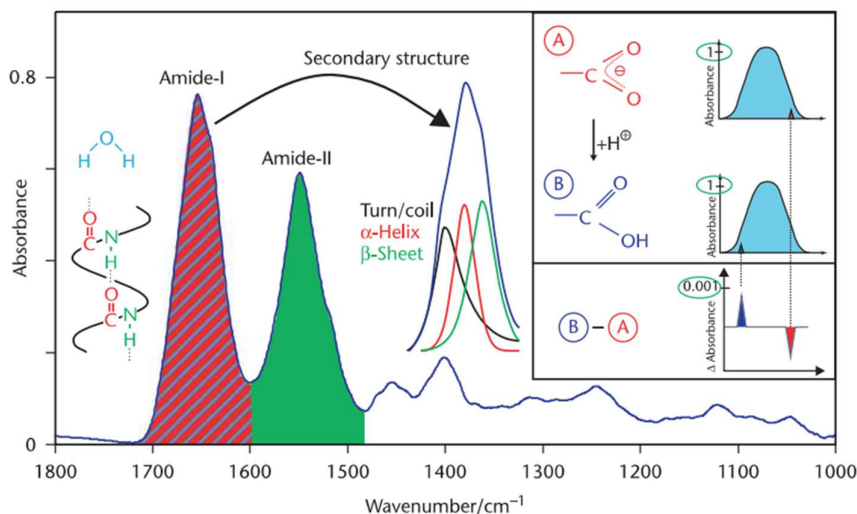


Figure 23. The absorbance spectrum of a protein typically features distinct regions corresponding to specific vibrational modes: the red-shaded area represents the C=O stretching vibration associated with the amide I band; the blue-shaded region corresponds to the bending vibration of water; and the green-shaded area indicates the combination of N–H bending and C–N stretching vibrations characteristic of the amide II band. The inset of Figure 23 displays two protein absorption spectra that differ solely in the protonation state of a carboxyl group. The lower portion of the figure presents the resulting difference spectrum, highlighting the spectral changes between the two protonation states⁸.

The amide I band is particularly sensitive to the protein's secondary structure. Based on empirical correlations, bands in the 1660 – 1650 cm^{-1} range are generally assigned to α -helices, those in the 1640 – 1620 cm^{-1} region to β -sheets, the 1695 – 1660 cm^{-1} range to β -sheets and β -turns, and those between 1650 – 1620 cm^{-1} to unordered structures⁸⁸.

Chapter III

Results and discussion

1. Spectroscopic studies of MS of mice sera

1.1 Random Forest supervised multivariate analysis

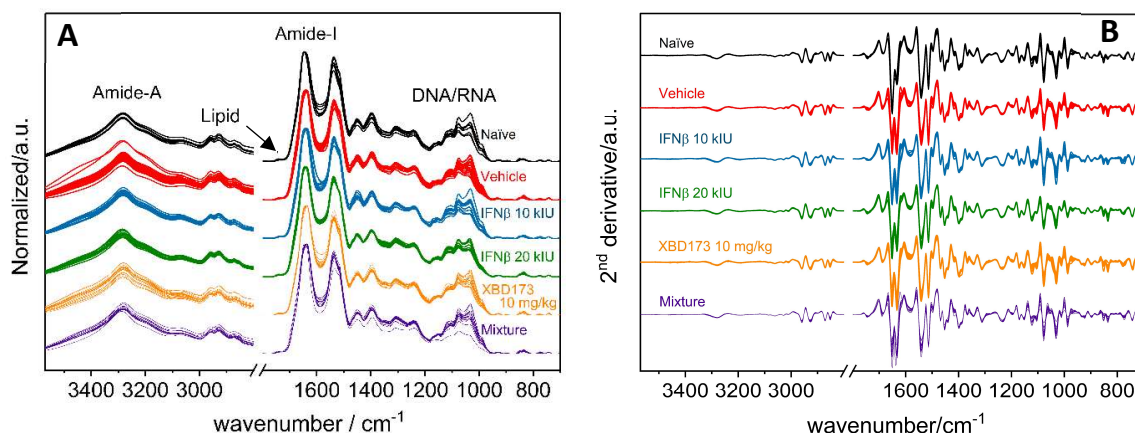


Figure 24. (A) The normalized spectra of serum samples in 3750-2800 cm⁻¹ and 1800-700 cm⁻¹ (B) The second derivatives of serum spectra. Naïve spectra are shown in black, Vehicle's in red, IFN β 10 kIU's in blue, IFN β 20 kIU's in green, XBD's in orange and the mixture of XBD+IFN β 's in violet.

Two-fold Random Forest cross-validation was performed on 88 second-derivative spectra (Figure 24). The confusion matrix is shown in Table 6, along with the classification performance for each group. There is minimal risk of misdiagnosing Vehicle, IFN β , XBD173, and Mixture as Naïve. Naïve shows the highest sensitivity (71.4%) because only two samples were misclassified as IFN β 20 kIU and XBD173. However, 6 out of 13 IFN β 20 kIU samples were misclassified as Vehicle, and 2 as Mixture, resulting in the lowest sensitivity (38.5%). Similarly, XBD173 samples also exhibit low sensitivity (40.0%) because the majority were misclassified as IFN β 10 kIU. These results suggest that tracking the progress of treated patients using serum label-free FTIR by Random Forest algorithms is challenging.

Table 6 summarizes the confusion matrix of the second derivative spectra classified by two-fold Random Forest cross-validation algorithm. The average value with standard deviation results from 10 iterations.

	Pathology	Classified as						ROC (%)	Sensitivity (%)	Specificity (%)	Precision (%)
		Naïve	Vehicle	IFN β 10 kIU	IFN β 20 kIU	XBD 173	Mixture				
2-fold validation set (10 iterations)	Naïve	5	0	0	1	1	0	99.47 (99.3 $\pm$ 0.8)	71.4 (93.9 $\pm$ 2.3)	100.0 (38.2 $\pm$ 2.9)	100.0 (95.0 $\pm$ 2.4)
	Vehicle	0	12	5	3	0	3	69.13 (66.6 $\pm$ 4.5)	52.2 (70.2 $\pm$ 3.7)	72.3 (29.4 $\pm$ 4.1)	40.0 (60.1 $\pm$ 5.8)
	IFN β 10 kIU	0	3	2	1	4	0	81.03 (69.6 $\pm$ 9.1)	55.0 (77.0 $\pm$ 3.0)	86.8 (30.4 $\pm$ 6.9)	55.0 (72.5 $\pm$ 7.8)
	IFN β 20 kIU	0	6	0	5	0	2	73.28 (77.0 $\pm$ 6.1)	38.5 (85.0 $\pm$ 1.5)	92.0 (16.8 $\pm$ 4.7)	45.5 (81.5 $\pm$ 6.6)
	XBD173	0	4	11	0	1	4	89.29 (84.8 $\pm$ 6.1)	40.0 (88.6 $\pm$ 2.0)	97.4 (15.7 $\pm$ 9.8)	66.7 (85.3 $\pm$ 7.5)
	Mixture	0	5	2	1	0	7	73.47 (71.0 $\pm$ 6.7)	50.0 (84.2 $\pm$ 2.5)	87.7 (26.3 $\pm$ 9.3)	43.8 (82.6 $\pm$ 8.0)

Note : ROC is the trade-off between sensitivity and false-positive rate, Sensitivity is the fraction of positive cases predicted positive, Specificity is the fraction of negative cases predicted as negative, and Precision is positive predictive value⁸⁹.

1.2 The effect of MS disease and all treatments

The effects of MS, as well as the target effects of each treatment, are calculated from the absolute difference between Vehicle and Naïve, along with all treatments showing outliers as indicated by the boxplot method in Figure 25. The effects of the MS disease are observed in amide I at 1650 cm^{-1} (α -helix structure), amide II at 1543 cm^{-1} , amide I at 1630 cm^{-1} (β -sheet structure), nucleic acids at 1396 cm^{-1} and amide II at 1581 cm^{-1} , phospholipids and carbohydrates at 1079 cm^{-1} , and RNA at 988 cm^{-1} .

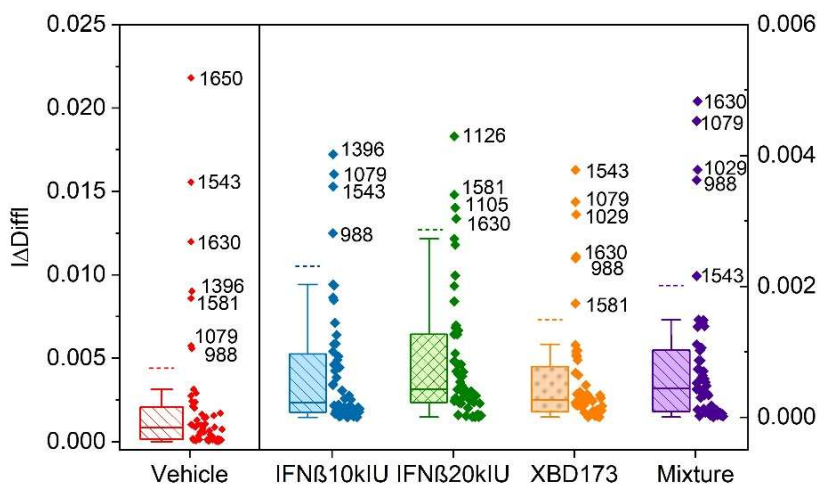


Figure 25 illustrates the boxplot method, indicating outliers using an upper fence (dashed line).

The target effects of a half dose of IFN β (10 kIU) are observed at 1396 cm⁻¹, 1079 cm⁻¹, 1543 cm⁻¹, and 988 cm⁻¹. A full dose of IFN β (20 kIU) targets 1126 cm⁻¹ (carbohydrates, polysaccharides, nucleic acids), 1581 cm⁻¹, 1105 cm⁻¹ (proteins), and 1630 cm⁻¹. 10 mg/kg XBD173 targets 1543, 1079, 1029, 1630, 988, and 1581 cm⁻¹. The mixture of 10 mg/kg XBD173+ IFN β 10kIU treatment targets 1630, 1079, 1029, 988, and 1543 cm⁻¹. All treatments have major target in proteins (1720–1480 cm⁻¹ and 1301–1229 cm⁻¹)⁹⁰ and carbohydrates and nucleic acids (1200–900 cm⁻¹)⁹⁰.

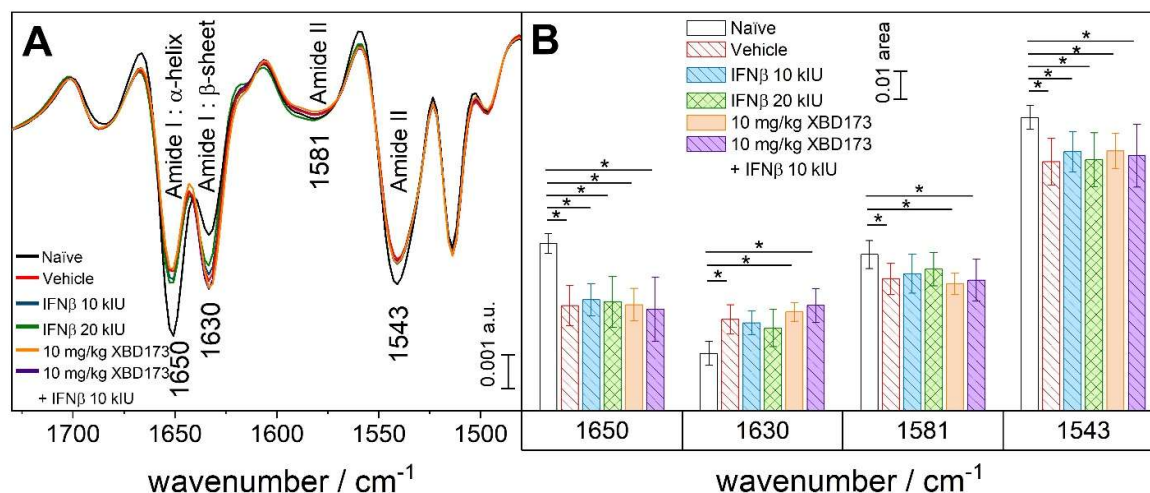


Figure 26. (A) Second derivatives of the average spectra of each group of mice in the 1700-1500 cm⁻¹ spectral range. (B) Average areas under curve for each of the signals centered at 1650, 1630, 1581 and 1543 cm⁻¹. (Mean $\pm$ SD, $p < 0.05$)

The effects of MS on the protein region were quantified using the area under the curve of the second derivative spectra, as shown in Figure 26. The amount of α -helix structure in amide I decreased significantly in the Vehicle and all therapy groups compared to Naïve. MS disease caused a significant increase in the β -sheet structure of amide I at 1630 cm⁻¹. While IFN β therapy helped reduce this increase, treatments with 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173+ IFN β 10kIU did not show recovery in this region. The Vehicle, 10 mg/kg XBD173, and the mixture of 10 mg/kg XBD173+ IFN β 10kIU groups also showed a significant reduction from Naïve at 1581 cm⁻¹. IFN β therapy did not differ significantly from Naïve in the regions at 1630 and 1581 cm⁻¹, suggesting these regions are associated with inflammatory agents³. The amount of amide II at 1543 cm⁻¹ in the Vehicle and all therapy groups was significantly lower than in Naïve.

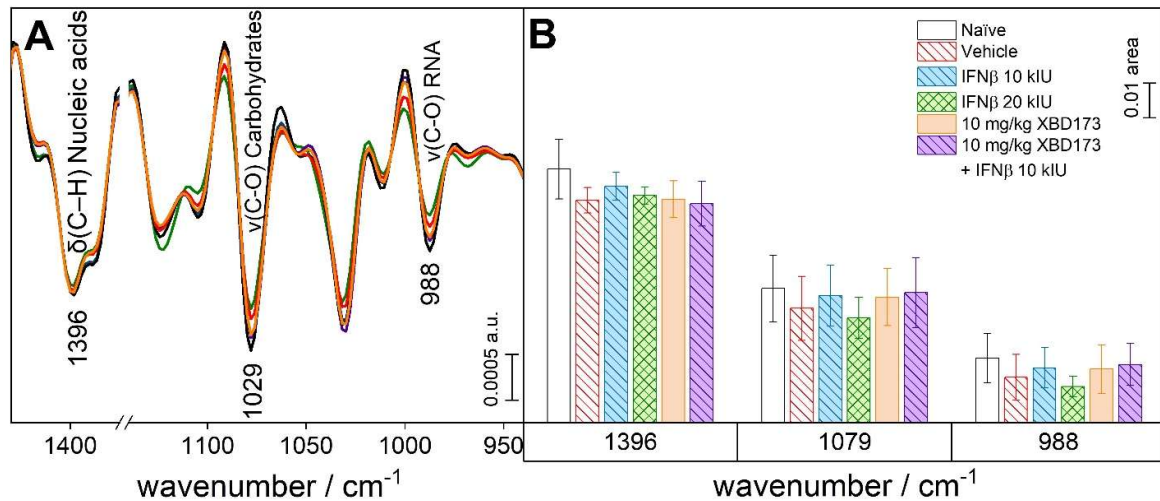


Figure 27. (A) Second derivatives of the average spectra of each group of mice in the 1500-950 cm⁻¹ spectral range. (B) Average areas under curve for each of the signals centered at 1396, 1079 and 988 cm⁻¹. (Mean ± SD, $p < 0.05$)

The effects of MS in the regions 1396, 1079, and 988 cm⁻¹ were quantified using the area under the curve of the second derivative spectra, as shown in Figure 27. There were no significant differences among the Naïve, Vehicle, and all therapy groups in these regions. However, treatment with IFNβ 10kIU showed a tendency to restore levels of nucleic acids at 1396 cm⁻¹, nucleic acids, phospholipids, and carbohydrates at 1079 cm⁻¹, and RNA at 988 cm⁻¹, demonstrating a better effect than IFNβ 20kIU therapy. Treatments with 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFNβ 10kIU showed similar behavior to the lower dose of IFNβ at 1079 cm⁻¹ and 988 cm⁻¹.

The major impact of MS in the Vehicle group's sera was a reduction in amide I (α -helix structure), amide II, nucleic acids, and RNA levels, except for amide I (β -sheet structure). This effect is reflected in the box plot of the absorption spectra in Figure 25, illustrating that the Vehicle group's mean and median values (Table 3) were lower than any other groups. In MS, an enhanced synthesis of IgG immunoglobulins has been observed, with average IgG content in MS patients' sera about 195 times higher than in their CSF⁹¹⁻⁹². IgGs with β -sheet structures⁹³ in MS sera are active in hydrolyzing DNA and MBP⁹⁴. DNase antibodies (Abzs) are intrinsic to IgGs, IgMs, and IgAs in MS sera⁹⁵ and cause nuclear DNA fragmentation, leading to apoptosis (induce cell death)⁹⁶.

1.3 The dominant effect of 10mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU

The dominant effect of 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU was analyzed using the Wilcoxon rank-sum test ($p < 0.05$). To compare differences between the two groups, the first group included Naïve, Vehicle, IFN β 10kIU, and IFN β 20kIU, while the candidate group included 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU.

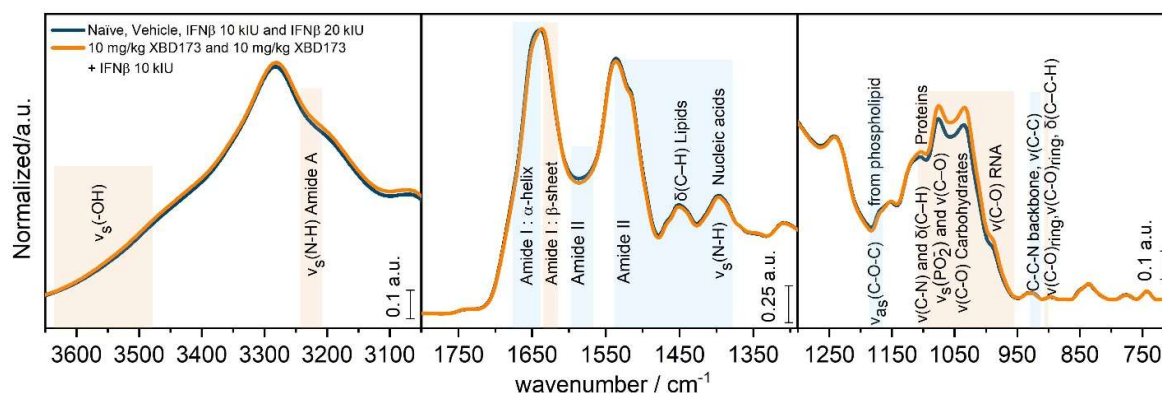


Figure 28. The average absorption spectra of two groups are shown. The first group, represented by a blue line, includes Naïve, Vehicle, IFN β 10kIU, and IFN β 20kIU. The candidate group, represented by an orange line, includes 10 mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU. Significant differences, analyzed using the Wilcoxon rank-sum test ($p < 0.05$), are highlighted in orange for the superior effect and in blue for the inferior effect of the candidate.

The superior and inferior effects of the candidate are shown in Figure 28. The candidate group consisted of 10 mg/kg XBD173 and a mixture of 10 mg/kg XBD173 + IFN β 10 kIU. The superior effects of XBD173 therapies showed significant dominance in the OH region (3635–3484 cm^{-1}), the amide-A region (3635–3210 cm^{-1}), protein (1104 cm^{-1}), carbohydrates (1075, 1033, 904 cm^{-1}), and RNA (988 cm^{-1}). In contrast, significant inferior effects were observed in amide I (both α -helix and β -sheet structures), amide II, lipids (1425 cm^{-1}), nucleic acids (1397 cm^{-1}), phospholipids (1172 cm^{-1}), and DNA (925 cm^{-1}). However, the candidate demonstrated dominance in the β -sheet structure of amide-A (1635–1616 cm^{-1}), which is related to the T-cell immune system⁹¹⁻⁹².

Among the candidates, different effects were observed in two regions: the lipid and fatty acid region (1801–1776 cm^{-1}) and the amide I region of the β -sheet structure (1626–1608 cm^{-1}), as shown in Figure 29. Treatment with the mixture containing an additional 10 kIU of IFN β reduces the β -sheet structure but increases the amount of phospholipids compared to treatment with XBD173 alone.

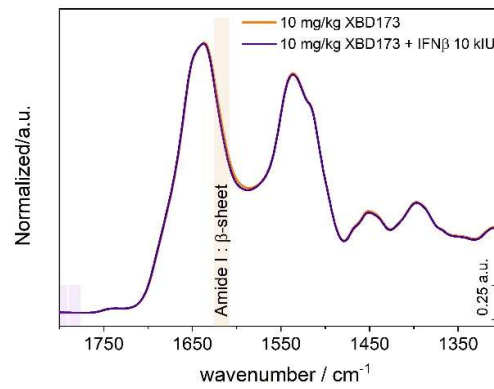


Figure 29. The average absorption spectra of 10mg/kg XBD173 represented by an orange line and the mixture of 10 mg/kg XBD173 + IFN β 10kIU represented by a purple line. Significant differences, analyzed using the Wilcoxon rank-sum test ($p < 0.05$), are highlighted in purple color for the higher and orange color for the lower intensity of the mixture.

The XBD173 therapy, at a dosage of 10 mg/kg in PLP-induced EAE mice, decreases pro-inflammatory cytokine production (IL-17, IL-6, and TNF- α) detected in serum, enhances allopregnanolone synthesis in the spinal cord and brain, and counteracts MBP gene expression-evoked demyelination⁸. Therefore, the superior effect observed with XBD173 treatment is associated with the stimulation of MBP gene expression and increased MBP mRNA levels.

1.4 The dominance effect of IFN β 20kIU and IFN β 10kIU

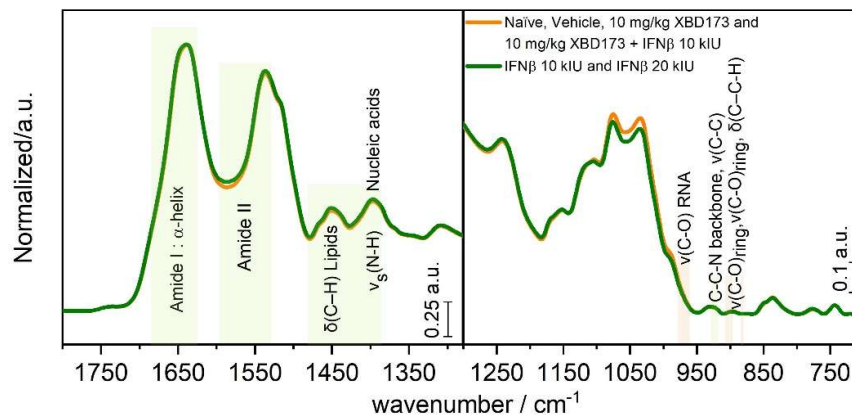


Figure 30. The average absorption spectra of two groups are shown. The first group, represented by an orange line, includes Naïve, Vehicle, 10mg/kg XBD173 and the mixture of 10 mg/kg XBD173 + IFN β 10kIU. The clinical use, represented by a green line, includes IFN β 20kIU and IFN β 10kIU. Significant differences, analyzed using the Wilcoxon rank-sum test ($p < 0.05$), are highlighted in green color for the advantage in and orange color for the inferior of the clinical use.

The superior and inferior effects of clinical use are shown in Figure 30. Full-dose (IFN β 20 kIU) and half-dose (IFN β 10 kIU) IFN β treatments were categorized as a group of clinical use. The superior effects of IFN β therapies were observed significantly in the amide I region (1685–1629 cm^{-1} , α -helix structure), amide II region (1598–1550 cm^{-1}), lipids (1451 cm^{-1}), nucleic acids

(1398 cm^{-1}), and left-handed DNA (Z-form, 926 cm^{-1}). In contrast, inferior effects were significantly observed in RNA (966 cm^{-1}) and carbohydrates (896 cm^{-1}).

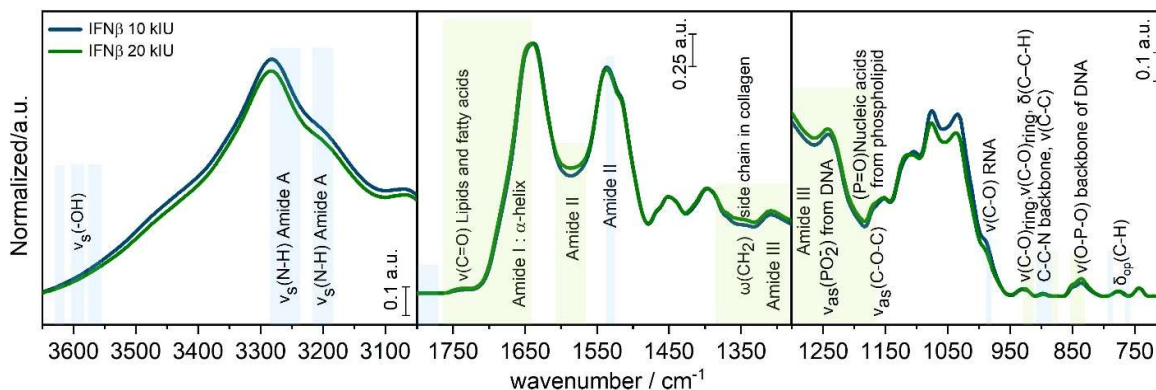


Figure 31. The average absorption spectra of IFN β 10kIU represented by a blue line and IFN β 20kIU represented by a green line. Significant differences, analyzed using the Wilcoxon rank-sum test ($p < 0.05$), are highlighted in blue color for the superior effect of IFN β 10kIU and green color for the superior of IFN β 20kIU.

Figure 31 illustrates the differential effects between the full dose and half dose of IFN β . The superior effect of the IFN β 10 kIU dose was significantly dominant in the OH region (3625–3619, 3604–3592, 3585, and 3575–3559 cm^{-1}), amide-A region (3280–3235 and 3216–3187 cm^{-1}), amide II (1531 cm^{-1}), RNA (984 cm^{-1}), and carbohydrate (896 cm^{-1}). Conversely, the superior effect of the full dose (20 kIU) was observed in lipids and fatty acids (1743 cm^{-1}), amide I (α -helix structure), amide II (1581 cm^{-1}), collagen (1345 cm^{-1}), amide III (1307 cm^{-1}), DNA (1242 cm^{-1}), nucleic acids (1193 cm^{-1}), phospholipids (1169 cm^{-1}), carbohydrate (928 cm^{-1}), and DNA (835 cm^{-1}).

IFN β increases the expression and concentration of the anti-inflammatory cytokine IL-10, reduces the production of pro-inflammatory IL-17 and osteopontin, and impairs leukocyte trafficking across the BBB by modulating MMP activity. It also promotes the secretion of factors such as NGF to support repair after neurological insults and enhance neuronal survival. Additionally, IFN β increases the presence of CD56^{bright} natural killer cells in peripheral blood. Thus, the superior effect of the 20 kIU dose is attributed to higher levels of IL-10, NGF, and CD56^{bright} natural killer cells.

1.5 The correlation between IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10kIU treated MS

The correlation between IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10 kIU (Figure 32) shows the highest associated effects in the CH-vibration lipid region (2981, 2942, 2832, and 2908 cm^{-1}) and the amide-A region (3025 cm^{-1}). A lower correlation is observed in the amide-A region (3025, 3652, 3579, 3546, 3477, 3272, 3303, 3110, 3351, 3370, 3170, and 3201 cm^{-1}) and the amide-B region (3060 cm^{-1}). The lowest correlation occurs in the phospholipid region (1799 cm^{-1}), carbohydrate region (1045 cm^{-1}), and protein regions (989 and 1103 cm^{-1}).

Additionally, a negative correlation between both treatments results in inhibition in the phospholipid region (1758 and 1700 cm^{-1}), amide-I region (1650 and 1614 cm^{-1}), protein regions (1344, 1319, 1376, 1432, and 1481 cm^{-1}), and nucleic regions (1207 and 836 cm^{-1}).

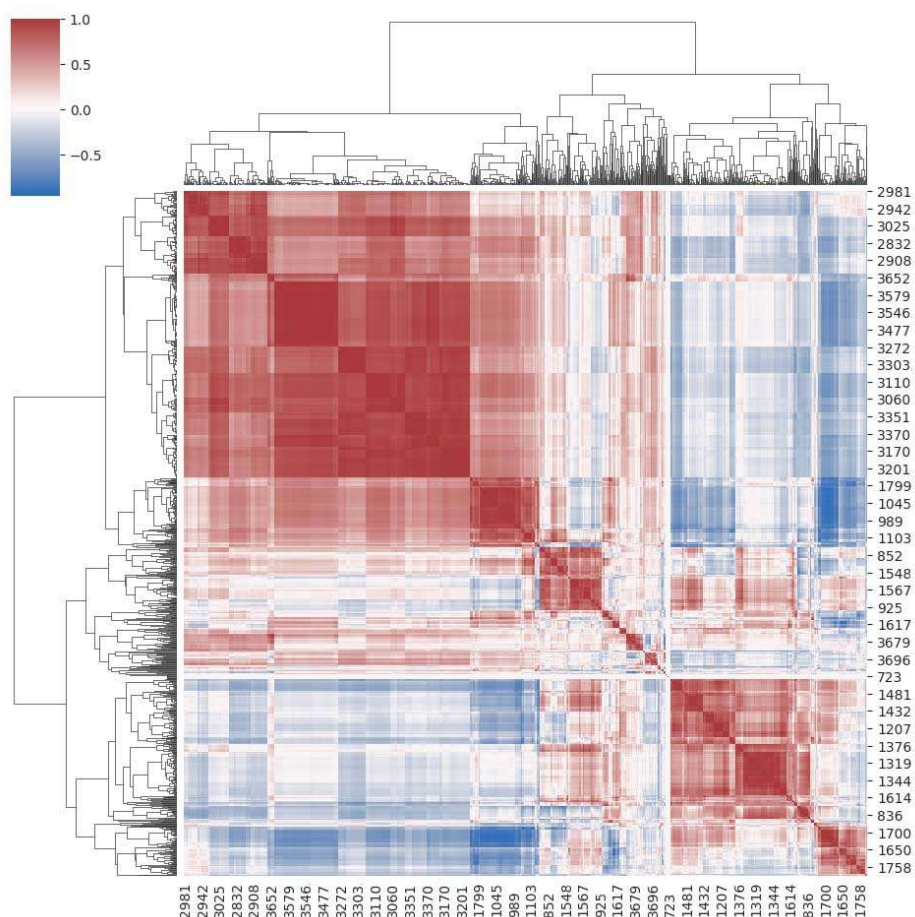


Figure 32. Heatmap plot of the correlation between IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10 kIU analyzed by Spearman's rank correlation coefficient. The scale bar on the left side of the heatmap indicates the Spearman correlation coefficients. The correlation coefficient closer to +1 indicates the higher association (red). On the other hand, if close to -1, the association is inverse (blue).

Figure 33 illustrates the highest correlation between IFN β 20 kIU and the mixture of 10 mg/kg XBD173 + IFN β 10 kIU in the CH-vibration lipid region. Both treatments enhance the signal centered at 2959, 2927, 2872, and 2852 cm^{-1} . Notably, the signal at 2852 cm^{-1} , corresponding to CH₂ symmetric vibration, exhibits significant differences compared to the vehicle.

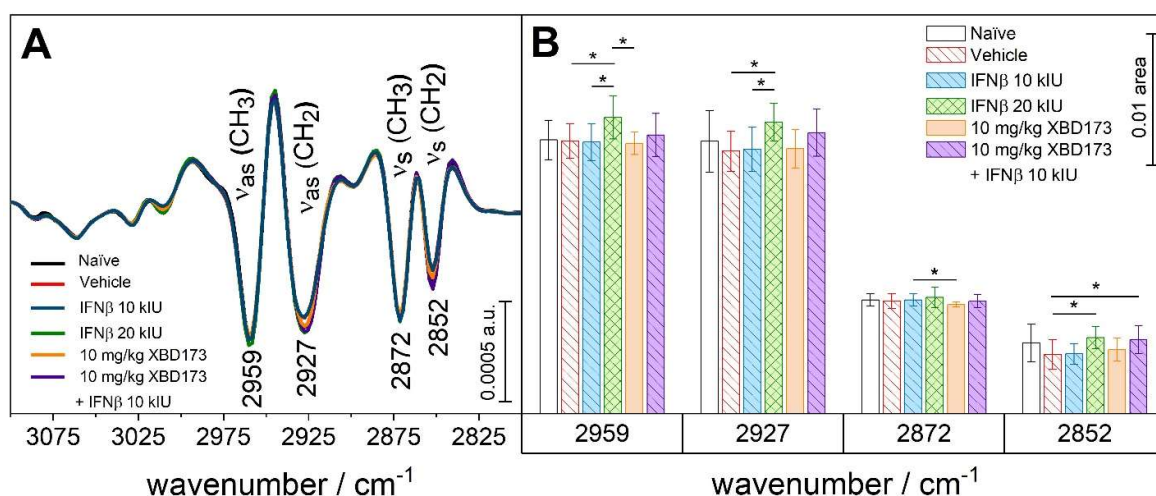


Figure 33. (A) Second derivatives of the average spectra of each group of mice in the 3100-2800 cm⁻¹ spectral range. (B) Average areas under curve for each of the signals centered at 2959, 2927, 2872 and 2852 cm⁻¹. (Mean ± SD, *p* < 0.05)

The influence of IFNβ-1b therapy on blood antioxidants (α-tocopherol and NADPH) in MS demonstrates a sparing effect on erythrocyte α-tocopherol content⁹⁷. This molecule, the primary antioxidant in membranes, protects these structures against lipid peroxidation. The observed decrease in NADPH, alongside an increase in plasma triglycerides, suggests that IFNβ-1b stimulates fatty acid synthesis⁹⁷.

XBD173 is among the most selective and high-affinity TSPO (translocator protein on the outer mitochondrial membrane) ligands, known to stimulate neurosteroidogenesis⁴⁵. Biomedical IR spectroscopy quantifies triglycerides within the spectral region of 2800–3200 cm⁻¹ using the set of PLS calibration⁹⁸. Both therapies may reflect the increase triglycerides in serum, which are by-products of the chain-breaking antioxidant mechanism that scavenges peroxide radicals and protects membranes against ROS- and NO-induced peroxidative reactions⁹⁷.

1.6 The correlation between serum and cerebellum tissue

The inflammatory state in MS of Vehicle affects brain lipids, leading to an increase in the olefinic ratio (simplified as the vibration of C=CH stretching, indicating higher unsaturated lipid content), a decrease in lipid chain length (-CH₂ groups from long hydrocarbon chains in lipids), a reduction in methylated content (-CH₃ groups from hydrocarbon chains in lipids), and an increase in the carbonyl ratio (concentration of ester C=O stretching in lipids). These changes are observed in both the cerebellum's white matter and the granular and molecular layers, showing significant differences (*p* < 0.05) compared to naïve, except for lipid chain length in the granular and molecular layers, where no significant difference is found. Details of the chemical imaging can be found in Supplementary Table S3. The area ratios of olefinic ratio, lipid chain length, methylated content, and carbonyl ratio are presented in Supplementary Table S4. These findings align with previous studies that have reported alterations in lipid composition within MS brain tissue. The utilizing FTIR spectroscopy has identified significant changes in lipid profiles, including increased unsaturated lipids and decreased lipid chain lengths, suggesting oxidative damage in MS brain tissue^{38-39, 99}.

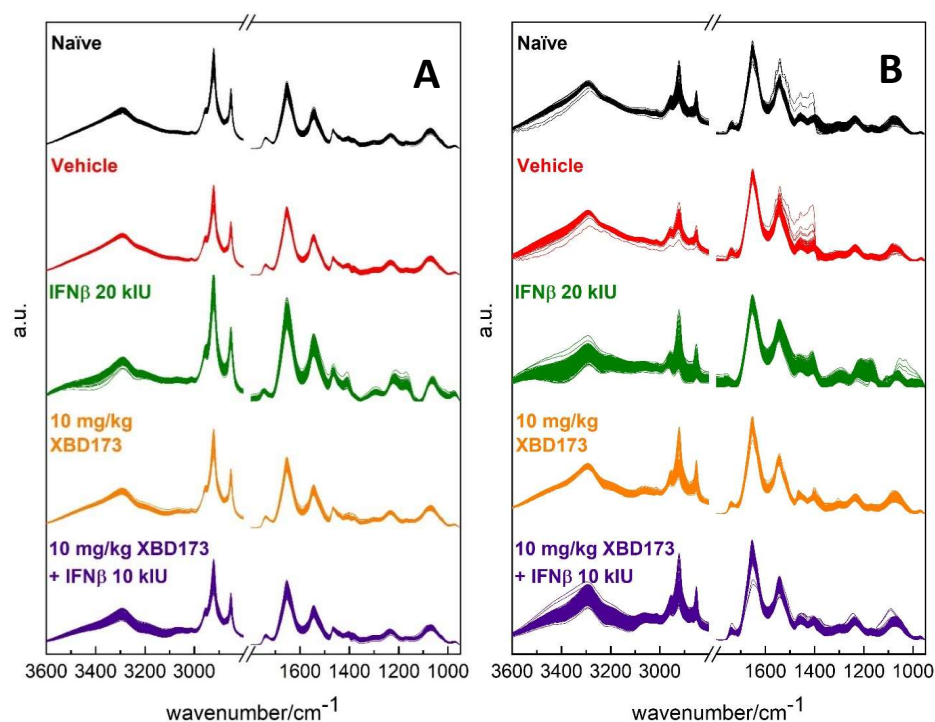


Figure 34. The spectra of cerebellum brain tissue samples in 3600-2800 cm^{-1} and 1800-950 cm^{-1} analyzed by FTIR microscope on cerebellum white matter (A) and granular layer and molecular layer (B). Naïve spectra are shown in black, Vehicle's in red, IFN β 20 kIU's in green, XBD's in orange and the mixture of XBD+IFN β 's in violet.

The ratios of beta sheet to alpha helix structures, phosphate, carbohydrates, and nucleic acids as presented in Supplementary Table S4. In the case of the vehicle group, these ratios significantly decreased in both the cerebellum white matter and the granular and molecular layers. However, the beta sheet to alpha helix ratio did not show a significant difference in the cerebellum white matter.

The correlation between three components—serum spectra, the average spectrum of cerebellum white matter—was analyzed using Spearman's rank correlation coefficient. The results are shown in Supplementary Tables S10- S14. Naïve, IFN β 20kIU, 10mg/kg XBD173, and the mixture of 10 mg/kg XBD173 + IFN β 10kIU show the highest correlation in the CH-vibration lipid region (2800-2996 cm^{-1}). A lower correlation is observed in phosphate (1203-1280 cm^{-1}), carbohydrate (1140-1183 cm^{-1}), and nucleic acid (994-1140 cm^{-1}). Vehicle, however, shows an inverse correlation for those regions. Therefore, the study calculated the correlation between serum and cerebellum tissue from the ratio between the total area of phosphate, carbohydrate, and nucleic acids/CH-vibration lipid region.

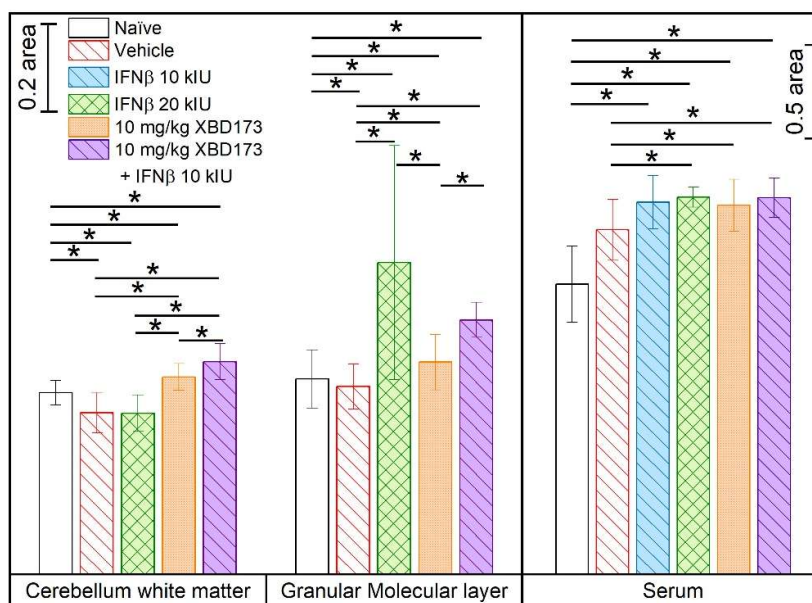


Figure 35. The area ratio of phosphate, carbohydrate and nucleic acids ratio of cerebellum white matter and granular and molecular layer (left) and serum (right). The significance was analyzed at $p < 0.05$ using the Kruskal-Wallis H test, followed by post hoc analysis using Dunn's test with p -values adjusted by the Holm method⁸⁰.

The total area of phosphate, carbohydrate, and nucleic acid ratios decreased significantly ($p < 0.05$) in cerebellum tissue. This effect was predominantly observed with 10 mg/kg XBD173 and the combination of 10 mg/kg XBD173 + IFN β 10 kIU (Figure 28), targeting these areas. Therefore, these treatments demonstrated good efficacy in addressing the affected regions. Moreover, treatment with the combination of 10 mg/kg XBD173 + IFN β 10 kIU enhanced this ratio more than 10 mg/kg XBD173 alone. This was due to the dominant effect of IFN β 10 kIU (Figure 31) on phosphate, carbohydrate, and nucleic acids, similar to 10 mg/kg XBD173 alone. Additionally, two-fold Random Forest cross-validation algorithm (Table 6) found that 40% of IFN β 10 kIU was classified as XBD173, and 55% of XBD173 was classified as IFN β 10 kIU, indicating the similarity between XBD173 and IFN β 10 kIU.

The effects of various treatments—Vehicle, IFN β , XBD173 at a dosage of 10 mg/kg, and the mixture of XBD173 with IFN β —on a specific ratio (the total area of phosphate, carbohydrate, and nucleic acids/CH-vibration lipid region) measured in both serum and cerebellum tissue. The observed ratio increased significantly across all treatment groups, mirroring trends seen in cerebellum tissue, with the exception of the Vehicle group. In the Vehicle group, the increase in the ratio is attributed to a decrease in the total CH-vibration lipid region, as illustrated in Figure 35. Consequently, this ratio may serve as a potential biomarker linking serum changes to cerebellar tissue alterations post-treatment.

2. Spectroscopic studies of Cyclic D,L- α -Peptide and AD mice brain tissue

2.1 The effect of AD on mice brain tissue

The study of the effect of AD on brain tissue samples focused on the cerebral cortex. The cerebral brain tissue was examined in the primary somatosensory area for cortical layers II-III, as shown in Figure S16, and in four distinct regions—anterior cingulate, primary somatosensory, supplemental somatosensory, and piriform areas—for cortical layers IV-V (Supplementary Table S23). PCA of FTIR mapping of non-AD and AD brain tissues from the primary somatosensory area (B6-14-2, APP-Front-4, and APP-Back-7) and the supplemental somatosensory area (B6-14-3, APP-Front-2, and APP-Back-6) are presented in this chapter.

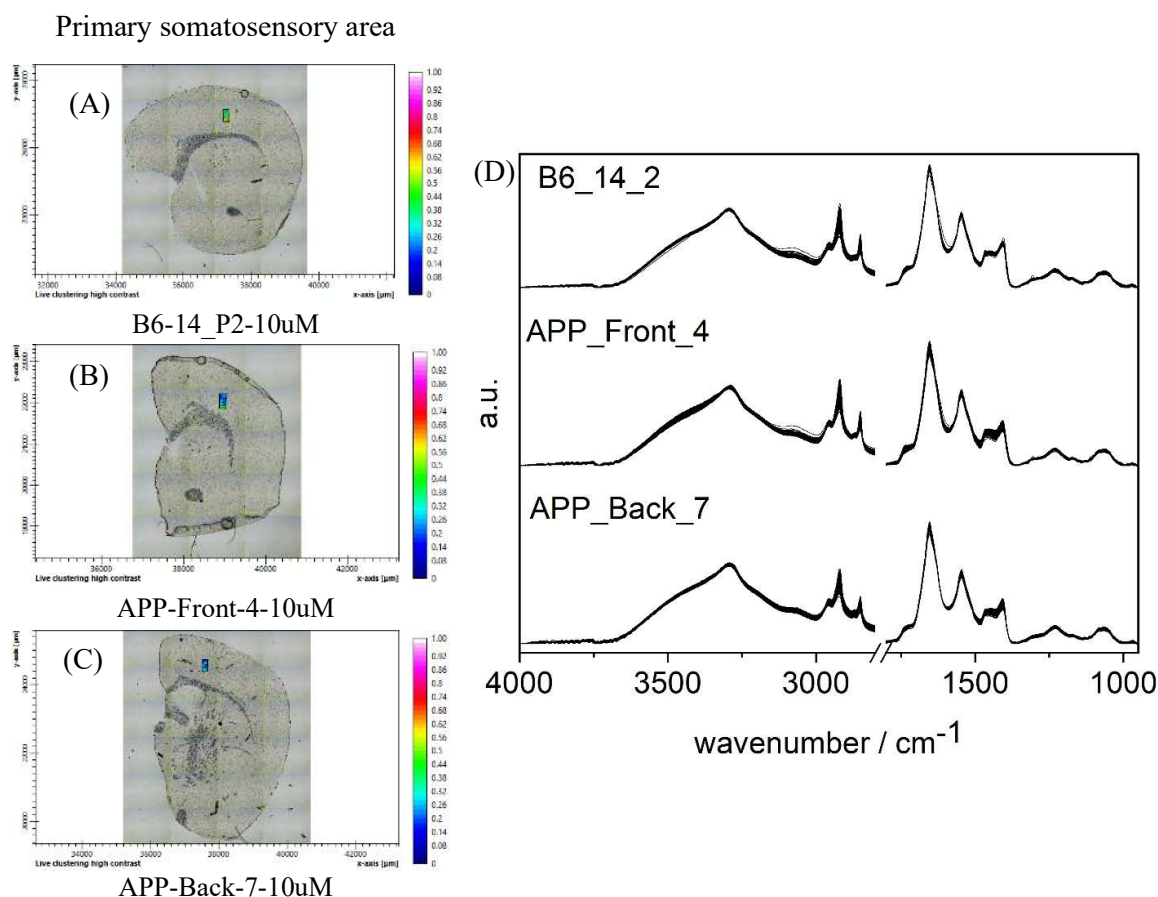


Figure 36. Optical images of the half-coronal brain sections, highlighting the analyzed areas in the primary somatosensory region, are shown for (A) B6-14-2, (B) APP-Front-4, and (C) APP-Back-7. (D) shows the corresponding absorption spectra obtained from FTIR mapping of B6-14-2, APP-Front-4, and APP-Back-7.

The analyzed FTIR mappings of the primary somatosensory region are shown in Figure 36: (A) B6-14-2, (B) APP-Front-4, and (C) APP-Back-7. The corresponding absorption spectra obtained from FTIR mapping of B6-14-2, APP-Front-4, and APP-Back-7 are presented in Figure 36(D). While the FTIR mappings of supplemental somatosensory region are shown in Figure 37:

(A) B6-14-3, (B) APP-Front-2, and (C) APP-Back-6 and (D) their corresponding absorption spectra.

Supplemental somatosensory area

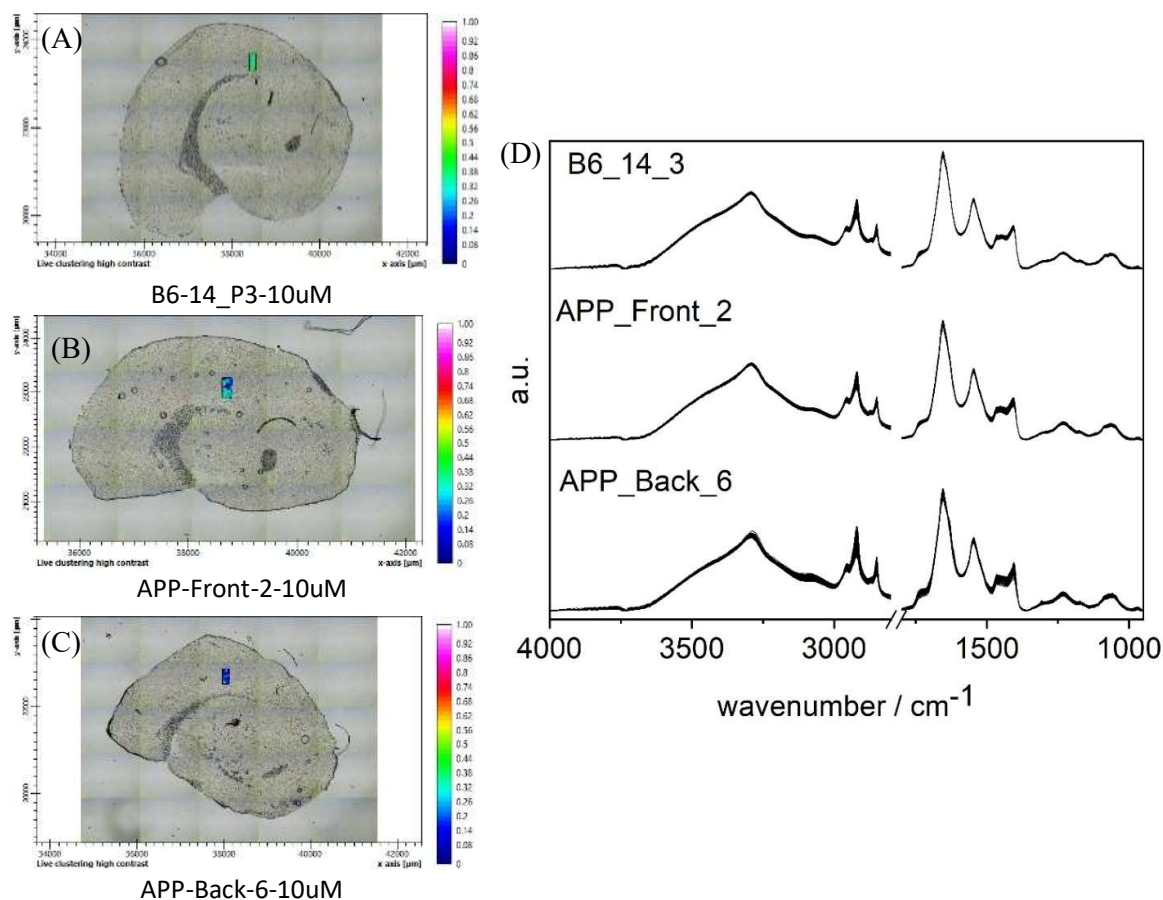


Figure 37. Optical images of the half-coronal brain sections, highlighting the analyzed areas in the supplemental somatosensory region, are shown for (A) B6-14-3, (B) APP-Front-2, and (C) APP-Back-6. (D) shows the corresponding absorption spectra obtained from FTIR mapping of B6-14-3, APP-Front-2, and APP-Back-6.

The characterization of non-AD and AD mouse brain tissues was analyzed using PCA. PCA scores are summarized in Supplementary Tables S16-S31. PCA captures the most significant variations. Scores present the relation to the PCs, helping to identify grouping, trends, or outliers. Loadings reveal which spectral regions contribute most to each PC. Positive and negative values in PC loadings arise because the data is mean-centered, allowing variation above and below the average. Positive loadings indicate variables that increase with a principal component, while negative loadings indicate those that decrease⁶⁸.

PC1 captured the highest variance, reflecting the greatest change in absorption intensity, as shown in Figure 38. Areas of highest variance were shaded in blue and red, indicating differences in chemical components. The PCA score plot uses shading to represent the distribution of principal component (PC) scores across the samples. These scores indicate how each sample projects onto the principal components, revealing similarities or differences between samples. The

score values for each PC were normalized to a range between -1 and 1 for (as shown in Figure 38B) visualization purposes⁸⁴. This ensures that both the positive and negative contributions of each loading are represented in the plot. The scale bar on the right side of the PCA score plot indicates the loading values using blue and red colors: blue represents positive values, while red indicates negative values.

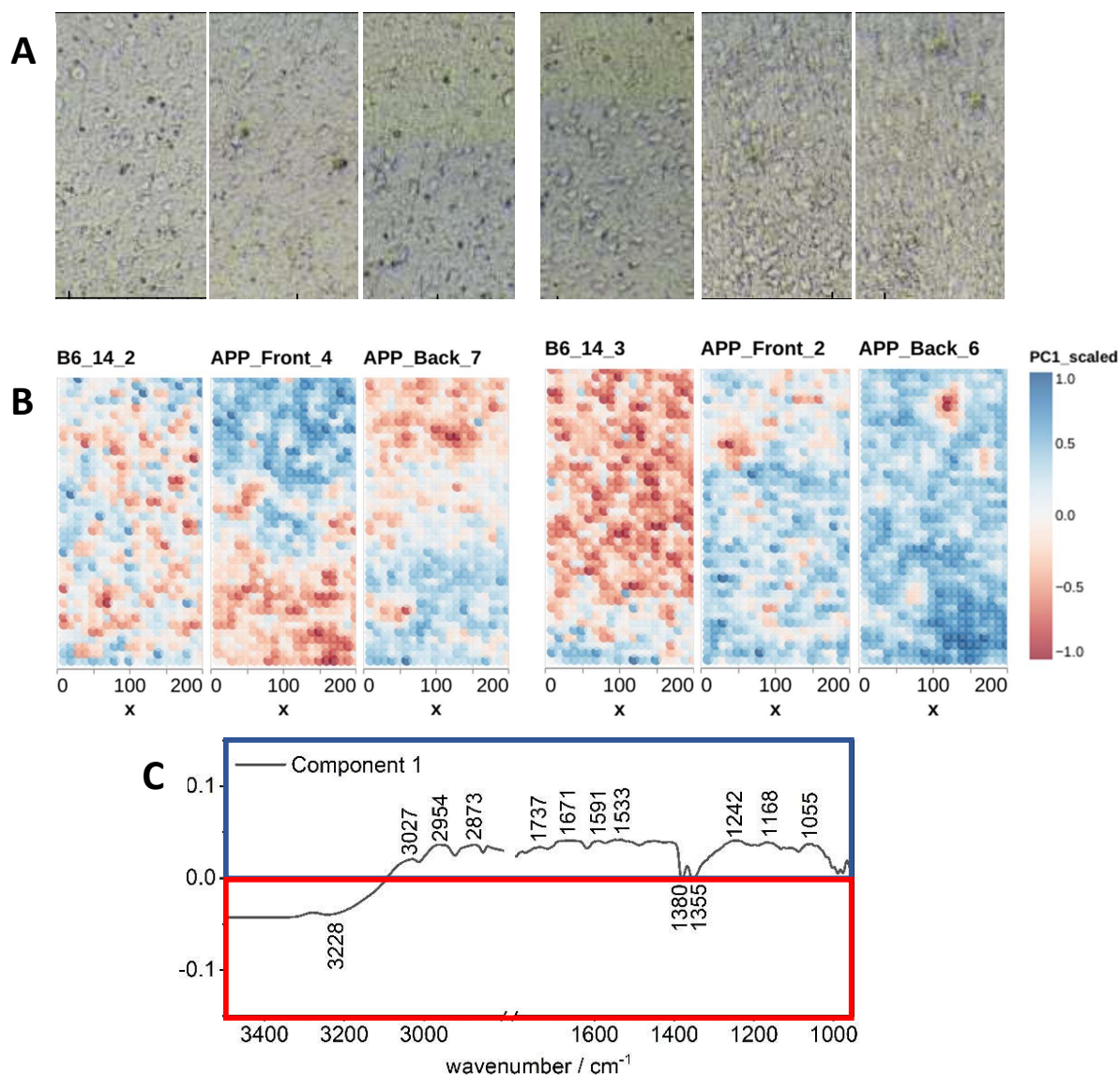


Figure 38. (A) Optical images of the analyzed areas in the primary somatosensory and supplemental somatosensory region (B) PC1 score calculated from absorption spectra of FTIR mapping observed on cerebral cortex. (C) PC1 loading, highlighting the variables that increase with positive values (blue shading in Figure 38B) and the variables that decrease with negative values (red shading in Figure 38B).

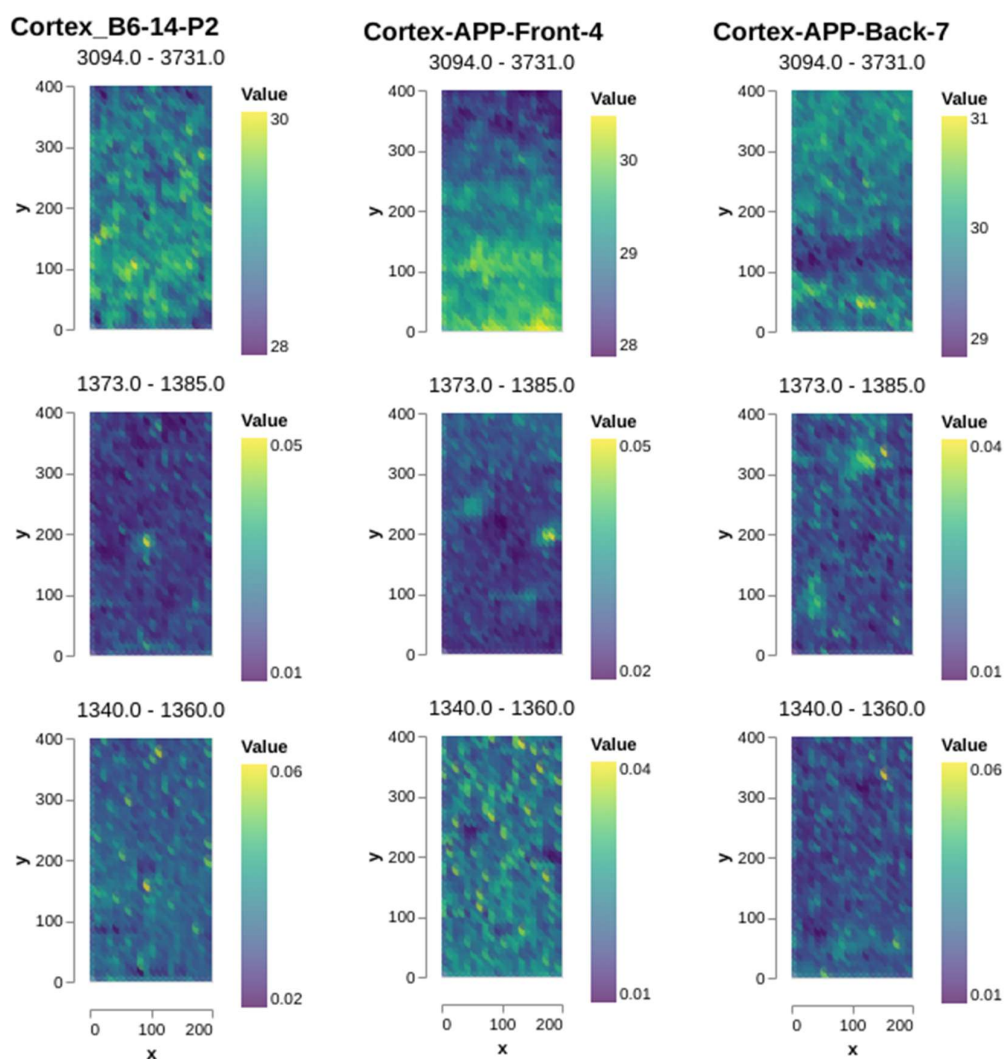


Figure 39. Chemical images analyzed on the cerebral cortex at primary somatosensory region using the wavenumber range corresponding to the negative values of the PC1 loading. Scale bar = 100 μm . Color bar: yellow indicates high intensity; purple indicates low intensity.

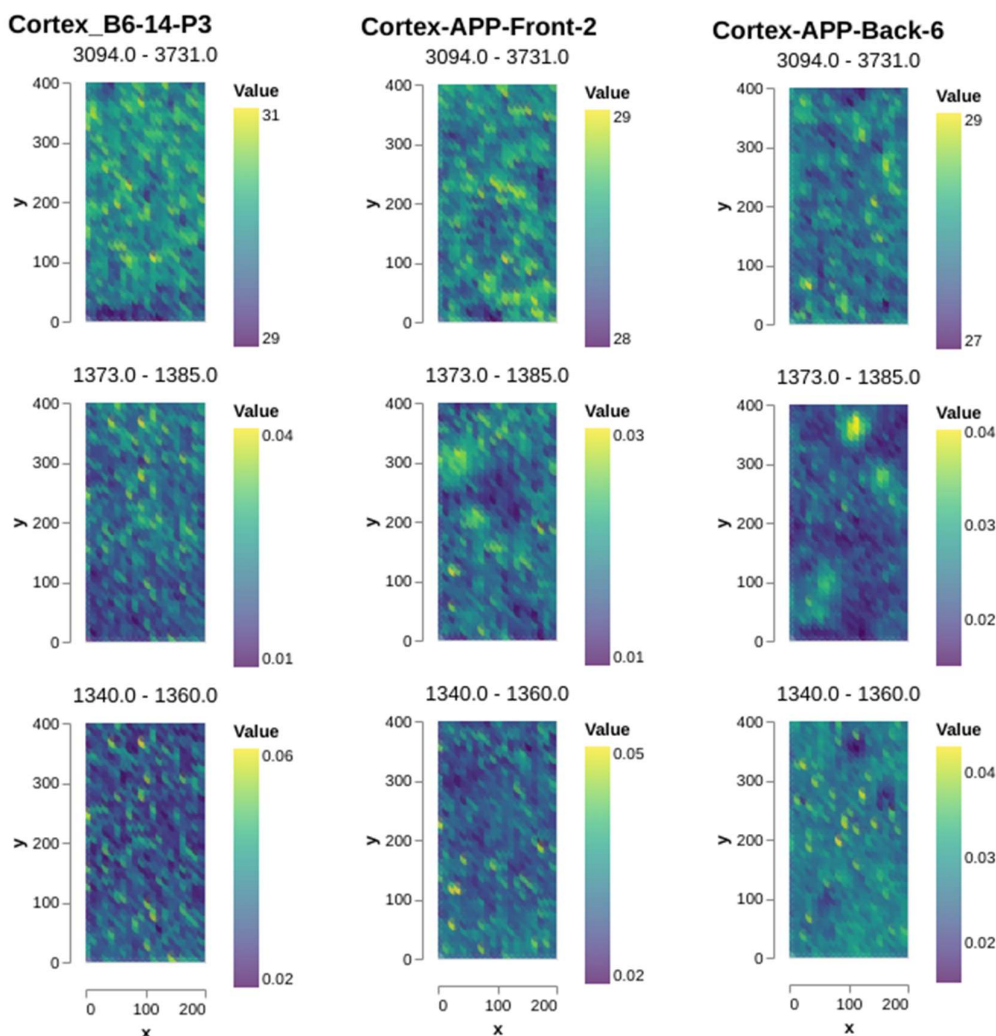


Figure 40. Chemical images analyzed on the cerebral cortex at supplemental somatosensory region using the wavenumber range corresponding to the negative values of the PC1 loading. Scale bar = 100 μm . Color bar: yellow indicates high intensity; purple indicates low intensity.

Areas of highest variance were shaded in blue and red as shown in Figure 38B, indicating differences in chemical components. Figure 39 and Figure 40 present the chemical composition changes identified using the wavenumber range corresponding to the negative values of PC1 (Figure 38C), which align with the red shading observed in Figure 38B. This red shading represents the largest absorption changes associated with O-H and N-H stretching ($3094\text{-}3731\text{ cm}^{-1}$), CH_3 symmetric bending ($1373\text{-}1385\text{ cm}^{-1}$)¹⁰⁰, and CH bending ($1340\text{-}1360\text{ cm}^{-1}$)¹⁰⁰ at plaque positions. The chemical images reveal the accumulation of signals at $1373\text{-}1385\text{ cm}^{-1}$ and a decrease in intensity at $1340\text{-}1360\text{ cm}^{-1}$ in the same areas, indicating both compositional gain and loss. This may correlate with the mechanism of lipid extraction from the bilayer lipid membrane, where the toxic peptide binds to lipids and subsequently extracts them from the membrane⁵⁶.

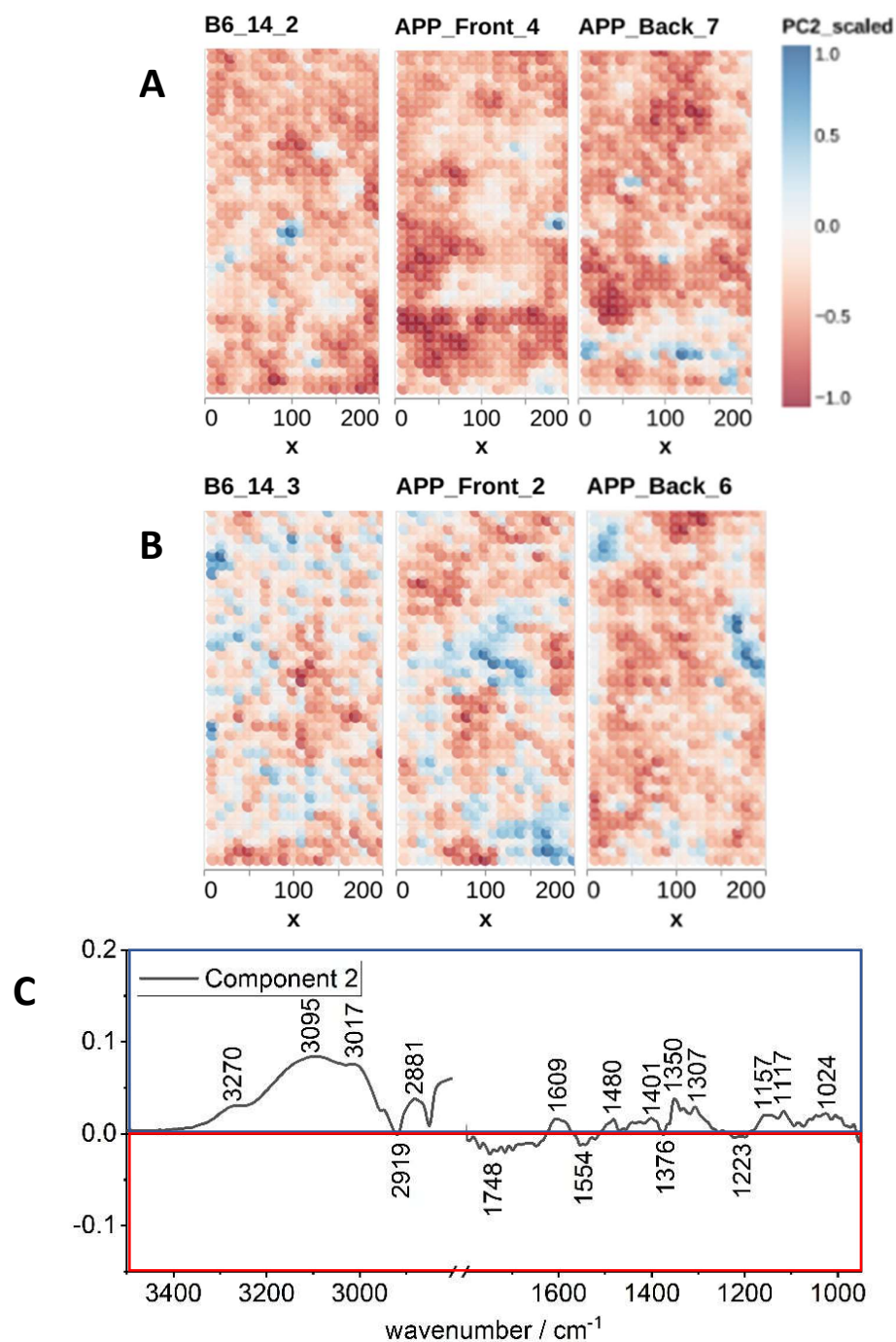


Figure 41. (A) PC2 mapping of cerebral cortex was shaded by PC2 score, this score was scaled from -1 (red shading) to +1 (blue shading). (B) PC2 loading obtained from PCA analysis, distinguishing the highest variance into positive value (blue shading, Figure41A and 41B) and negative value (red shading, Figure41A and 41B).

Figure 41A and 41B illustrate the PC2 scores of the cerebral cortex, with blue and red shading. The blue shading represents the distribution of positive values, while the red shading corresponds to the negative vector of the PC2 loading. The chemical image (Figure 42 and Figure 43) was plotted using wavenumbers corresponding to the positive vector of the PC2 loading (Figure 41C).

The chemical changes were analyzed specifically in the regions corresponding to the blue shading in Figure 41A and 41B. PC2 captured less variance compared to PC1; however, its highest contribution was observed in the 2923-3444 cm^{-1} range. This region is associated with asymmetric C-H₂ stretching centered at 2923 cm^{-1} (mainly lipids) and N-H stretching from amides and O-H stretching from water content and hydroxyl groups around 3300-3444 cm^{-1} . The chemical image shows an increased intensity in this region. Similarly, an increase was observed in the 2915-2800 cm^{-1} range, which corresponds to the asymmetric stretching of -CH₂ groups centered at 2915 cm^{-1} , symmetric stretching of -CH₂ groups centered at 2850 cm^{-1} (both primarily from lipids), and -CH₃ asymmetric stretching from methyl groups. This range is typically assigned to lipids and fatty acid chains, reflecting the hydrocarbon backbone of lipid structures.

Additionally, an increase in intensity was detected in the 1571-1624 cm^{-1} range, which is mainly associated with protein structures. This range includes part of the amide I band (primarily C=O stretching of peptide bonds), where 1620-1640 cm^{-1} corresponds to β -sheet structures, and 1571-1600 cm^{-1} is amide II band reflecting the formation of parallel β -sheets in the transition from A β oligomers to fibrils¹⁰¹⁻¹⁰².

In contrast, decreases in intensity were observed in the 1380-1510 cm^{-1} , 1232-1371 cm^{-1} , and 959-1193 cm^{-1} regions. The 1380-1510 cm^{-1} range includes the amide II band (associated with N-H bending and C-N stretching of proteins) and CH₂ bending vibrations of lipids and fatty acids, as well as symmetric -CH₃ bending from lipid and protein side chains, indicating lipid degradation and amino acids side chain environmental changes. The 1232-1371 cm^{-1} range corresponds to the amide III band (1232-1300 cm^{-1} , related to protein secondary structure changes) and lipid-associated CH₂ vibrations (1300-1371 cm^{-1} , reflecting membrane lipids). The 959-1193 cm^{-1} region is mainly assigned to C-O stretching vibrations (indicative of carbohydrates and glycoproteins), P=O stretching (associated with phospholipids and nucleic acids), and potential nucleic acid (DNA/RNA) alterations, reflecting membrane degradation and changes in glycoprotein and carbohydrate content.

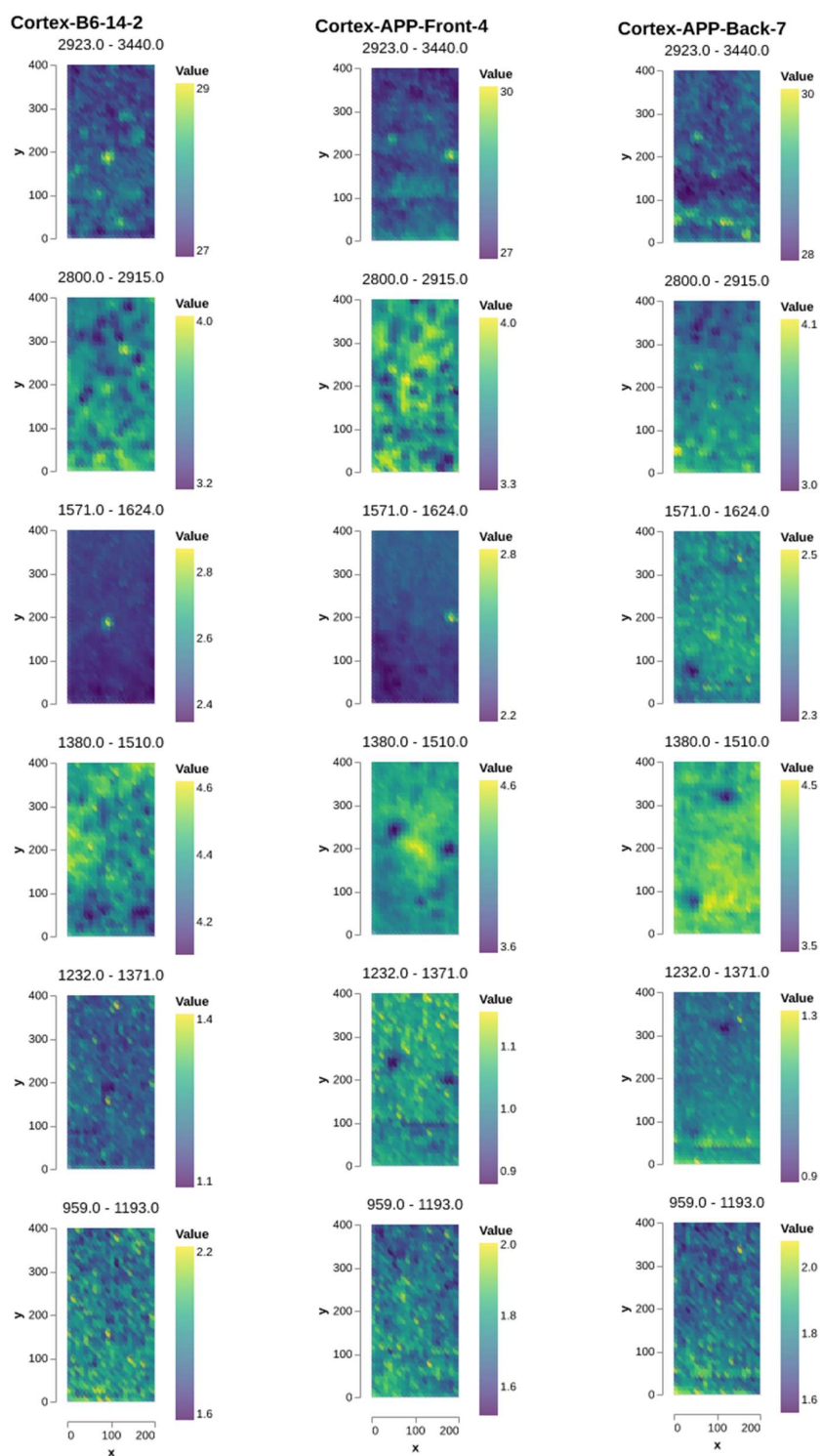


Figure 42. Chemical images analyzed on the cerebral cortex at primary somatosensory region using the wavenumber range corresponding to the positive values of the PC2 loading. Scale bar = 100 μ m. Color bar: yellow indicates high intensity; purple indicates low intensity.

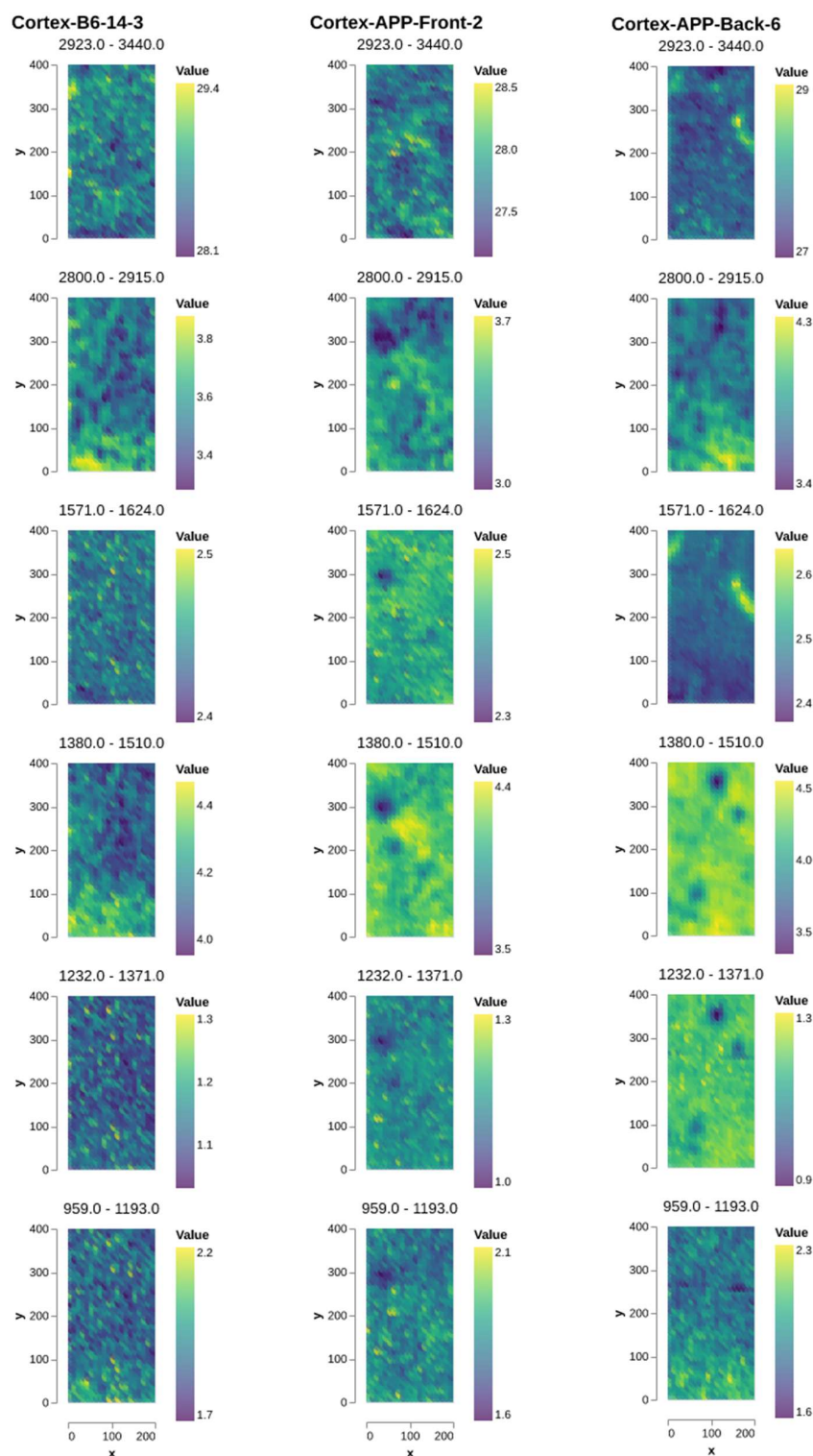


Figure 43. Chemical images analyzed on the cerebral cortex at supplemental somatosensory region using the wavenumber range corresponding to the positive values of the PC2 loading. Scale bar = 100 μ m. Color bar: yellow indicates high intensity; purple indicates low intensity.

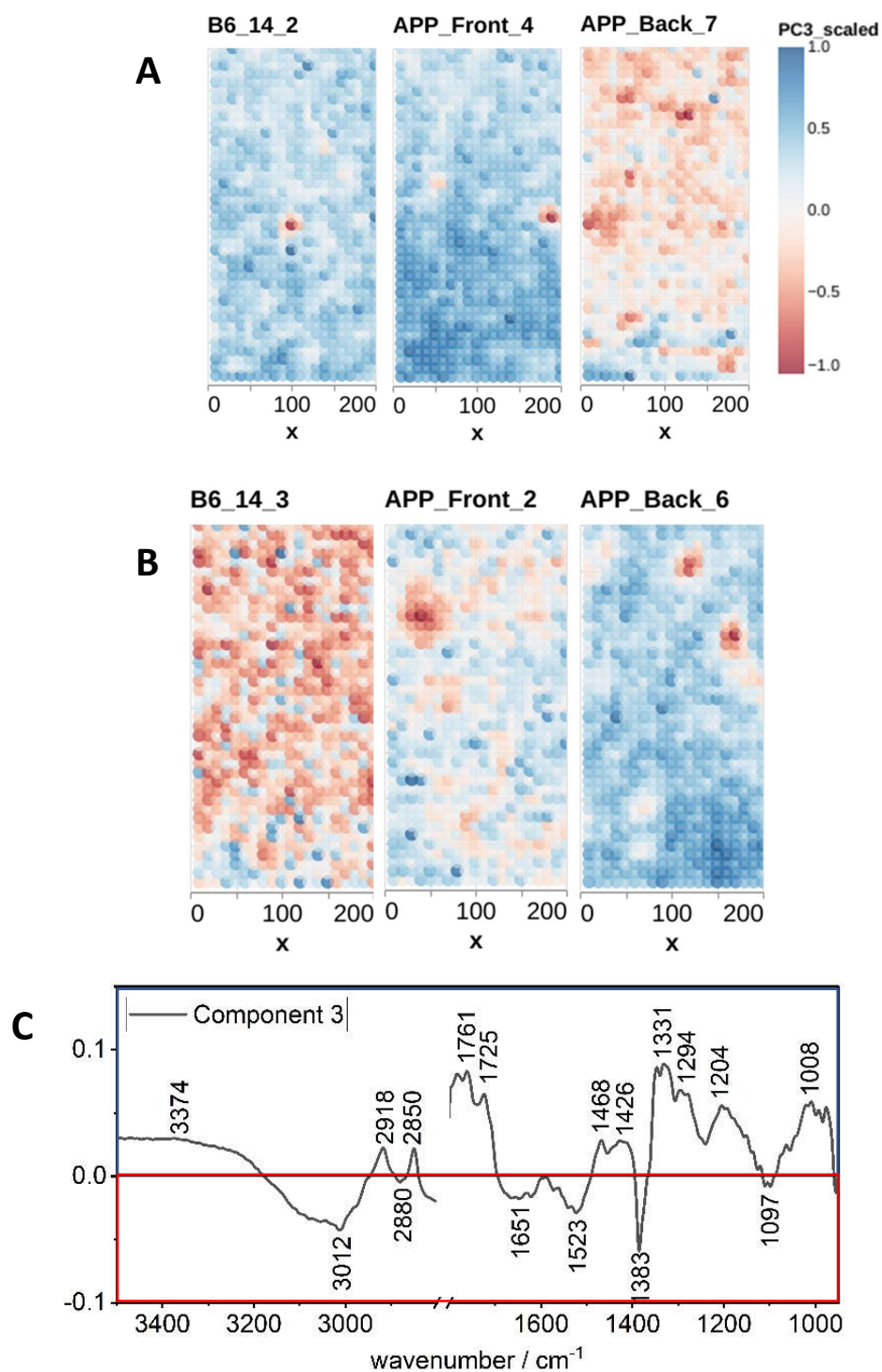


Figure 44. (A) PC3 mapping of the cerebral cortex, where the PC3 scores are shaded from -1 (red) to $+1$ (blue). (B) PC3 loading obtained from PCA, distinguishing the highest variance into positive values (corresponding to the blue shading in Figure 44A and 44B) and negative values (corresponding to the red shading in Figure 44A and 44B).

PC3 mapping and loading, shown in Figure 44, exhibit less variance compared to PC2. The chemical image was plotted using the negative vector of the PC3 loading (Figure 44C), representing the composition corresponding to the red-shaded areas in Figure 44A and 44B. This loading shows a strong distribution in the 1366-1395 cm^{-1} range. Additionally, a decrease in intensity was observed in the 2946–3178 cm^{-1} region (Figure 45 and Figure 46). A clear decrease in intensity was found in the 1490-1695 cm^{-1} region, where the areas of decreased intensity were of similar size across AD samples. However, no low-intensity regions were observed in the non-AD samples. This range corresponds protein structures, particularly the amide II and amide I bands, indicating changes in protein secondary structures. The increase in the 1087–1113 cm^{-1} region is attributed to PO_3^- symmetric stretching, indicating increased phosphorylation of proteins.

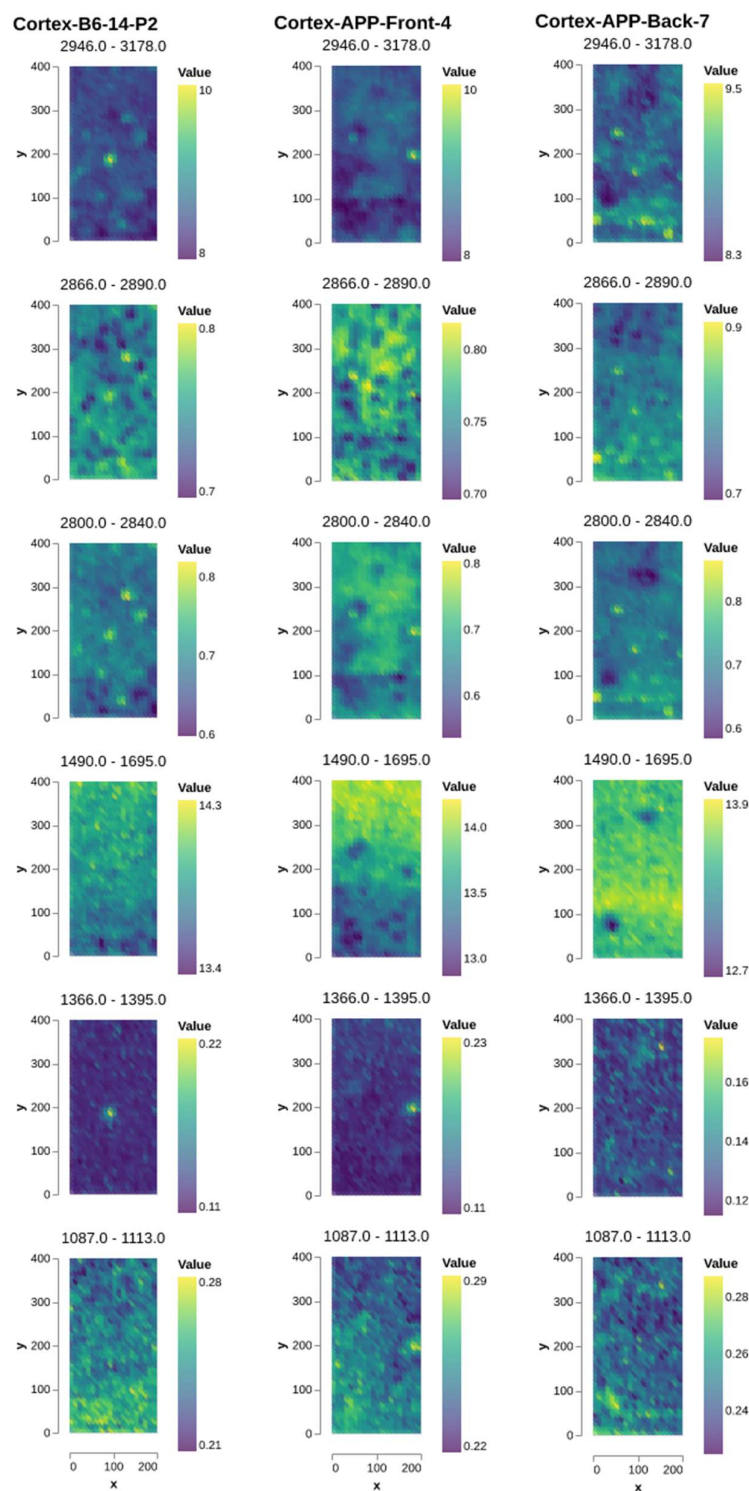


Figure 45. Chemical images analyzed on the cerebral cortex at primary somatosensory region using the wavenumber range corresponding to the negative values of the PC3 loading. Scale bar = 100 μm . Color bar: yellow indicates high intensity; purple indicates low intensity.

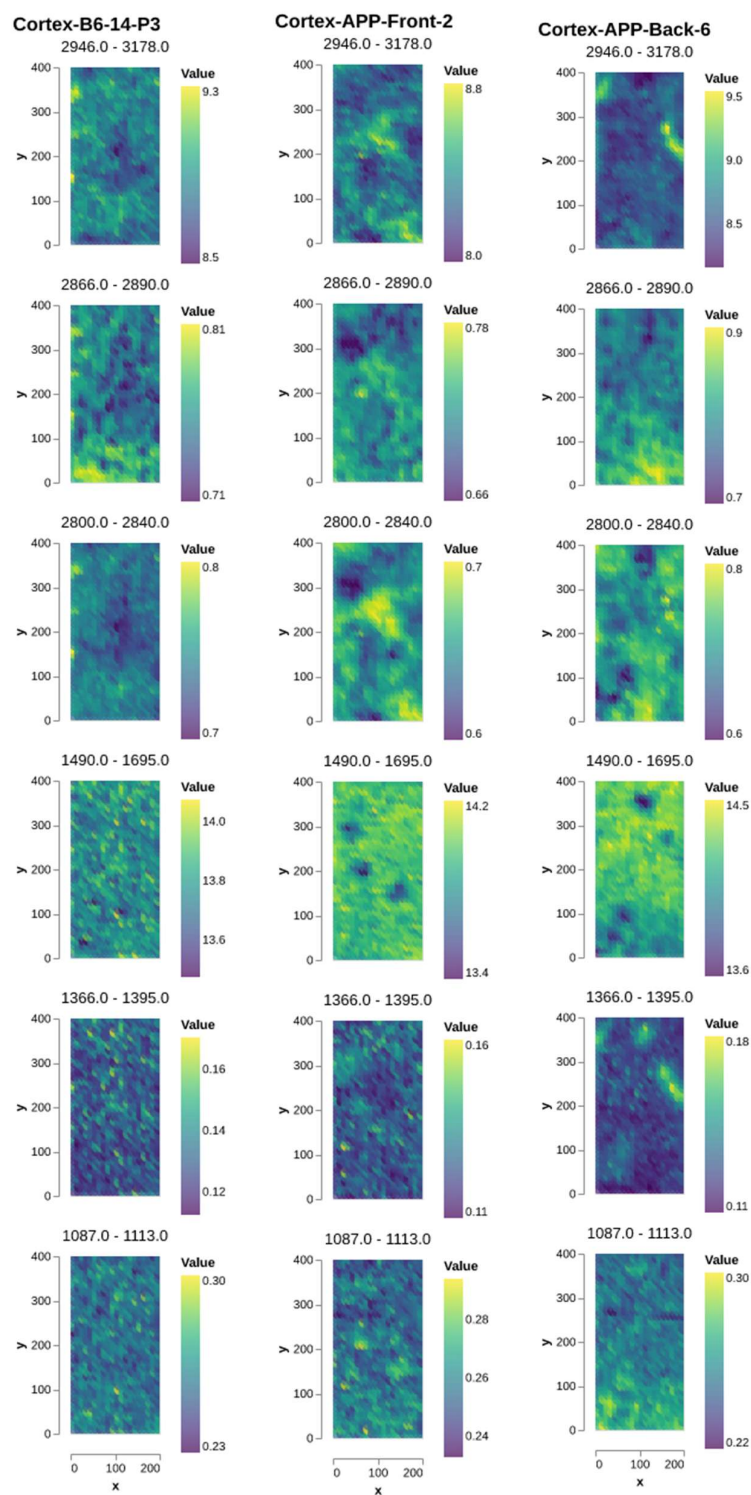


Figure 46. Chemical images analyzed on the cerebral cortex at supplemental somatosensory region using the wavenumber range corresponding to the negative values of the PC3 loading. Scale bar = 100 μ m. Color bar: yellow indicates high intensity; purple indicates low intensity.

2.2 Aggregation of A β ₄₀ fibrils

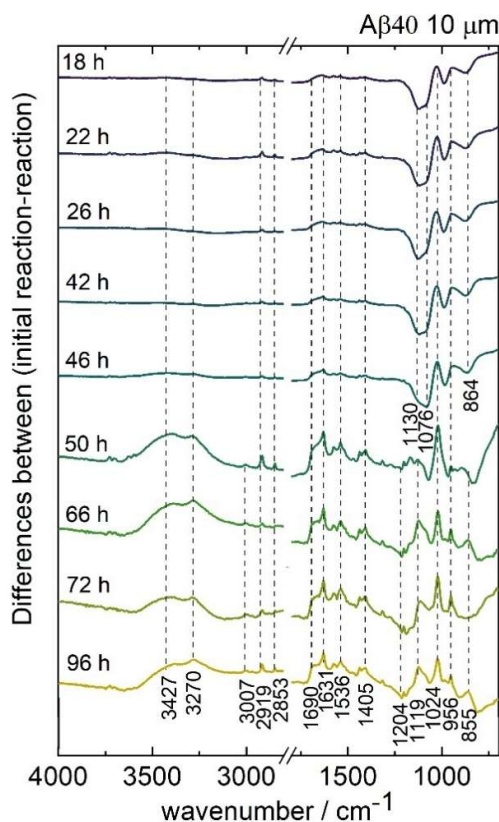


Figure 47. Difference FTIR spectra between the initial state and the reaction (reaction at 1 h minus x h), recorded from dried films during the aggregation of monomeric A β ₄₀ at a concentration of 10 μ M.

The transition from monomeric A β ₄₀ to fibrillar A β ₄₀ at a concentration of 10 μ M was studied using FTIR spectroscopy. Measurements were performed at different time points: 1 h, 18 h, 22 h, 26 h, 42 h, 46 h, 50 h, 66 h, 72 h, and 96 h, in order to investigate time-dependent conformational changes. The FTIR spectra obtained from this reaction are shown in Figure 34s. To analyze the kinetics of the aggregation process, difference spectroscopy was applied by subtracting the FTIR spectrum at 1 h (initial state) from the spectra recorded at each subsequent time point (reaction time point - reaction at 1 h). The interpretation of the difference spectra is as follows: A positive value indicates that the absorption intensity of the 1 h reaction is higher than that of the reaction at the given time point. Conversely, a negative value indicates that the absorption intensity of the reaction at that time point is higher than that of the initial 1 h reaction.

The difference spectroscopy shown in Figure 47 reveals the highest changes in the regions of 1180-1060 cm^{-1} , 1041-1014 cm^{-1} , 956-900 cm^{-1} , and 856-698 cm^{-1} , respectively. These regions correspond to C-O stretching vibrations, C-O-C side chain linkages, backbone C-C and C-O vibrations, and aromatic ring out-of-plane bending, respectively. The most pronounced changes were observed at the time points of 18 h, 22 h, 26 h, 42 h, and 46 h.

A decrease was observed in 3646-3146 cm^{-1} , 3007 cm^{-1} , 2919 cm^{-1} , 2853 cm^{-1} as well as 1702-1371 cm^{-1} regions starting from the 18-hour time point. This observed decreases in the N-H, and C-H stretching regions, as well as in the amide I (C=O stretch) and II bands (N-H bend and C-N stretch). As the reaction time progressed beyond 50 hours, the increase in the 1364-1143 cm^{-1} corresponding to C-N stretching and C-H bending vibrations. Thus, this 1364-1143 cm^{-1} region supports the side chain reorganization during fibril A β_{40} aggregation. The decrease in intensity in the 1180-1060 cm^{-1} region during the aggregation of monomeric A β_{40} into fibrils is attributed to the reduced mobility and exposure of C-O functional groups as β -sheet-rich fibril structures form, leading to decreased vibrational activity detectable by FTIR ¹⁰³.

When comparing the molecular components of plaques in AD cortical tissue (Figure 45 and 46) with fibrillar A β_{40} at a concentration of 10 μM (Figure 47), a notable similarity is observed in their spectral features. Both show a loss of intensity in the O-H, N-H, and C-H stretching regions, as well as in the amide I and amide II bands, reflecting a reduction in hydration and changes in protein secondary structure. In contrast, both systems exhibit a gain in the C-N stretching and C-H bending regions: specifically, 1364-1143 cm^{-1} in fibrillar A β_{40} and 1366-1395 cm^{-1} and 1087-1113 cm^{-1} in AD brain tissue.

2.3 Disaggregation of A β_{40} fibrils in presence of GNP-functionalized with CP-2

Figure 48 presents the differences FTIR spectra between initial reaction and reaction measured in dried film of disaggregation of A β_{40} fibrils in presence of GNP-functionalized with CP-2 at 10 μM . A decrease in intensity in the 1180-1060 cm^{-1} region was observed at every time point during the 96-hour reaction period. This observation is consistent with the previous study of the transition from monomeric A β_{40} to fibrillar A β_{40} , where a similar decrease in this region was attributed to the reduced mobility and exposure of C-O groups upon fibril formation. Therefore, the persistent decrease in intensity from the earliest stages of the reaction between A β_{40} fibrils and GNP-functionalized with CP-2 suggests that GNP-functionalized with CP-2 does not revert fibrillar A β_{40} back to the initial monomeric form during the reaction.

At 42 hours of reaction, a decrease in intensity was observed in the regions of 3400-2800 cm^{-1} , 1700-1441 cm^{-1} , and 1135-1050 cm^{-1} , indicating a loss of functional group vibrations. This loss is characteristic of the monomeric A β_{40} -to-fibrillar A β_{40} transition, similar to the pattern observed at 46 hours (as shown in Figure 47). However, after 46 hours, an increase in the regions of 3660-2800 cm^{-1} and 1679-1589 cm^{-1} was detected which GNP-functionalized with CP-2 was able to partially restore the signals associated with O-H, N-H, and C-H stretching vibrations as well as the amide I and amide II bands. Furthermore, the intensities in these regions continued to increase with longer reaction times, indicating progressive recovery of these functional groups during the disaggregation process.

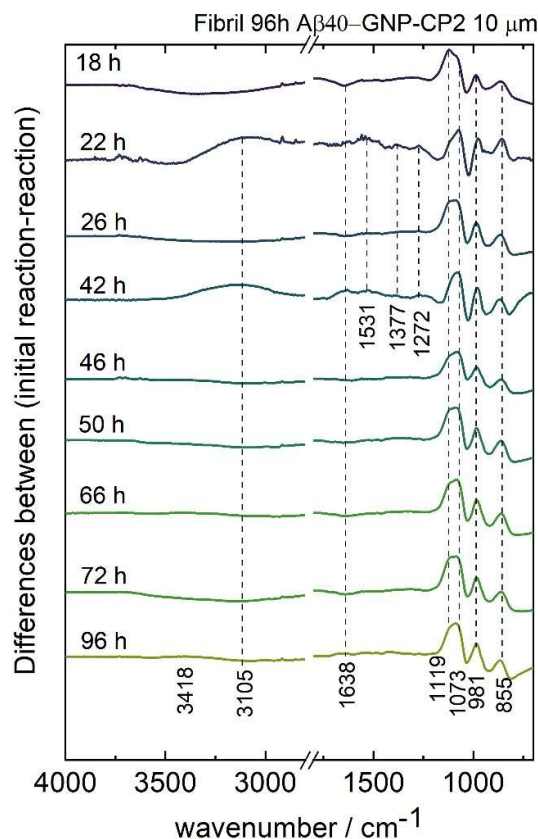


Figure 48. Difference FTIR spectra between the initial state and the reaction, recorded from dried films during the disaggregation of A β_{40} fibrils in presence of GNP-functionalized with CP-2 at 10 μ M.

2.4 Inhibition of A β_{40} monomeric in presence of GNP-functionalized with CP-2

Figure 49 presents the difference FTIR spectra between the initial reaction and subsequent time points, measured from dried films of A β_{40} monomer inhibition in the presence of CP-2-functionalized CP-2 at a concentration of 10 μ M. At 17 hours of reaction, an increase in difference intensity was observed in the regions of 3640–2800 cm^{-1} , 1636–1623 cm^{-1} and 1156–1050 cm^{-1} . Between 22 and 42 hours, a decrease within these regions was detected. This three times point shows the similar pattern observed at 42 hours of disaggregation of A β_{40} fibrils in presence of GNP-functionalized with CP-2 (as shown in Figure 48).

The increase in C–O vibrations within the 1165–1050 cm^{-1} region was observed starting at 46 hours of reaction, which may reflect signals originating from monomeric A β_{40} species. Furthermore, from 66 hours onward, spectral features in the 1492–935 cm^{-1} region became clearly evident. This region is associated with the fibrillar conformation of A β_{40} , as identified in previous studies on the monomeric A β_{40} -to-fibrillar A β_{40} transition (Figure 47). These findings therefore suggest that GNP-functionalized with CP-2 effectively delays the formation of fibrillar A β_{40} structures up to 50 hours.

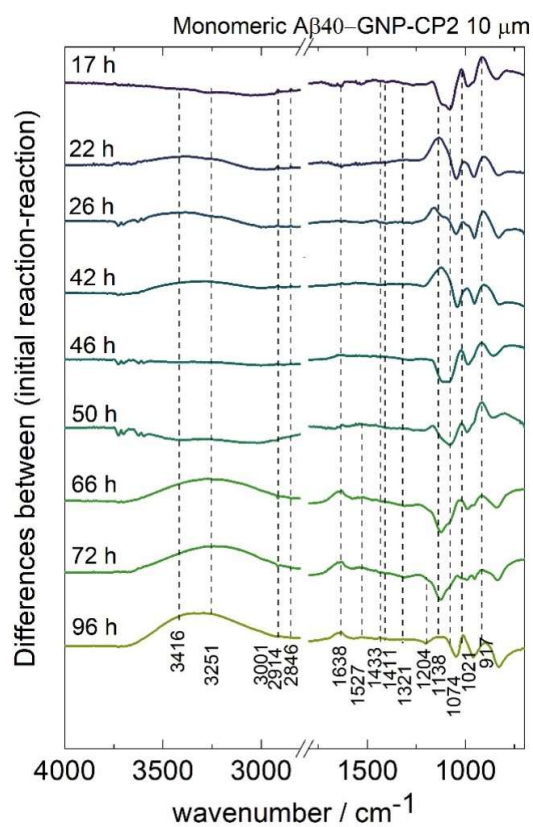


Figure 49. Difference FTIR spectra between the initial state and the reaction, recorded from dried films during the inhibition of A β ₄₀ monomeric in presence of GNP-functionalized with CP-2 at 10 μ M.

Chapter IV

Conclusions and Perspectives

FTIR spectroscopy is able to identify biochemical changes of lipids, proteins, phosphate, and carbohydrates in blood, MS brain tissue as well as AD brain tissue. We further establish the use of random forest algorithm to discriminate the signature of mice sera spectra between healthy, MS and treatment. The multivariate analysis has been applied for the brain tissue especially in AD.

1. MS of mice brain tissue and sera

The RRMS mouse model exhibits substantial alterations in the infrared spectrum of serum. Notably, a significantly higher contribution of β -sheets is observed in RRMS mice compared to naïve controls. Similarly, a marked decrease in lipid-associated absorption is detected in RRMS mice. Although various treatments reduce the clinical scores in RRMS mice, only the 20 kIU dose of IFN β significantly improves the β -sheet/ α -helix ratio within the amide I band.

In contrast, while XBD173 alone leads to lower clinical scores, it does not appear to significantly affect the serum spectra. However, the combination of a lower dose of IFN β (10 kIU) with XBD173 (10 mg/kg) produces an effect on the lipid signatures in the serum that is comparable to the 20 kIU IFN β treatment.

The significant increase in β -sheet protein content in the serum does not correlate directly with the improvement in clinical scores, as clinical benefits are observed with both treatments, whereas only IFN β reduces the β -sheet contribution. On the other hand, the alteration in serum lipid composition aligns more closely with the evolution of clinical scores.

Importantly, combining 10 mg/kg XBD173 with a lower dose of IFN β exerts a synergistic effect, resulting in lower clinical scores in RRMS mice. XBD173 therapies demonstrate superior effects, particularly in the carbohydrate and RNA regions, whereas IFN β therapies exhibit less pronounced effects in these domains. These findings support the potential of XBD173 as a promising candidate for combination therapy with existing disease-modifying treatments in RRMS.

The effects of the various treatments—vehicle, IFN β , XBD173 (10 mg/kg), and the combination of XBD173 with IFN β —were also assessed via a specific spectral ratio: the total area of phosphate, carbohydrate, and nucleic acid regions relative to the CH-vibration lipid region.

This ratio, measured in both serum and cerebellum tissue, may serve as a potential biomarker linking systemic serum changes to cerebellar tissue alterations following treatment.

To determine demyelinating activity from a molecular perspective, FTIR spectroscopy will first be performed on brain tissue to assess the overall biochemical composition in the corpus callosum, cerebellum, and cortex. Consecutive tissue sections will then be used for immunohistochemical staining. Immunohistochemistry and pathological classification will reveal the location of lesions—including active white matter lesions, inactive white matter, and remyelinated plaques—providing insight into the progression of multiple sclerosis (MS). From a systemic perspective, absorbance spectra will be used to characterize whole brain tissue in comparison to blood signatures. This integrated approach will help classify the stage of MS and identify the optimal therapeutic dose for each individual.

2. AD mice brain tissue and Cyclic D,L- α -Peptide

PCA analysis was applied in order to capture the highest variance. The chemical images reveal the accumulation of signals at 1373-1385 cm^{-1} and a decrease in intensity at 1340-1360 cm^{-1} in the same areas, indicating both compositional gain and loss. At AD plaque position shows a strong distribution in the 1366-1395 cm^{-1} , a decrease in intensity was observed in the 2946–3178 cm^{-1} region, protein structures, particularly the changes in protein secondary structures of amide II and amide I bands. Additionally, the increase in the 1087–1113 cm^{-1} region is attributed to PO_3^- symmetric stretching.

The molecular components of fibrillar $\text{A}\beta_{40}$ at a concentration of 10 μM , a loss of intensity in the O-H, N-H, and C-H stretching regions, as well as in the amide I and amide II bands, reflecting a reduction in hydration and changes in protein secondary structure. In contrast, both systems exhibit a gain in the C-N stretching and C-H bending regions: specifically, 1364–1143 cm^{-1} in fibrillar $\text{A}\beta_{40}$. Thus, a notable similarity between plaques in AD cortical tissue and fibrillar $\text{A}\beta_{40}$ at a concentration of 10 μM is observed in their spectral features.

GNP-functionalized with CP-2 was able to disaggregate the fibrillar $\text{A}\beta_{40}$ and partially restore the signals associated with O-H, N-H, and C-H stretching vibrations as well as the amide I and amide II bands. Furthermore, GNP-functionalized with CP-2 does not revert fibrillar $\text{A}\beta_{40}$ back to the initial monomeric form during the reaction during the disaggregation process. For the inhibition of $\text{A}\beta_{40}$ monomeric, in the presence of GNP-functionalized with CP-2 effectively inhibits the formation of fibrillar $\text{A}\beta_{40}$ structures up to 50 hours.

From an analytical perspective, studying the effectiveness of the treatment should take into account concentrations of GNPs functionalized with CP-2 as low as the nanomolar range. These measurements should be conducted in solution using infrared analogs of surface-enhanced Raman spectroscopy (SERS), allowing for sensitive detection and evaluation of molecular interactions.

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Appendix A

Spectroscopic studies of multiple sclerosis of mice sera

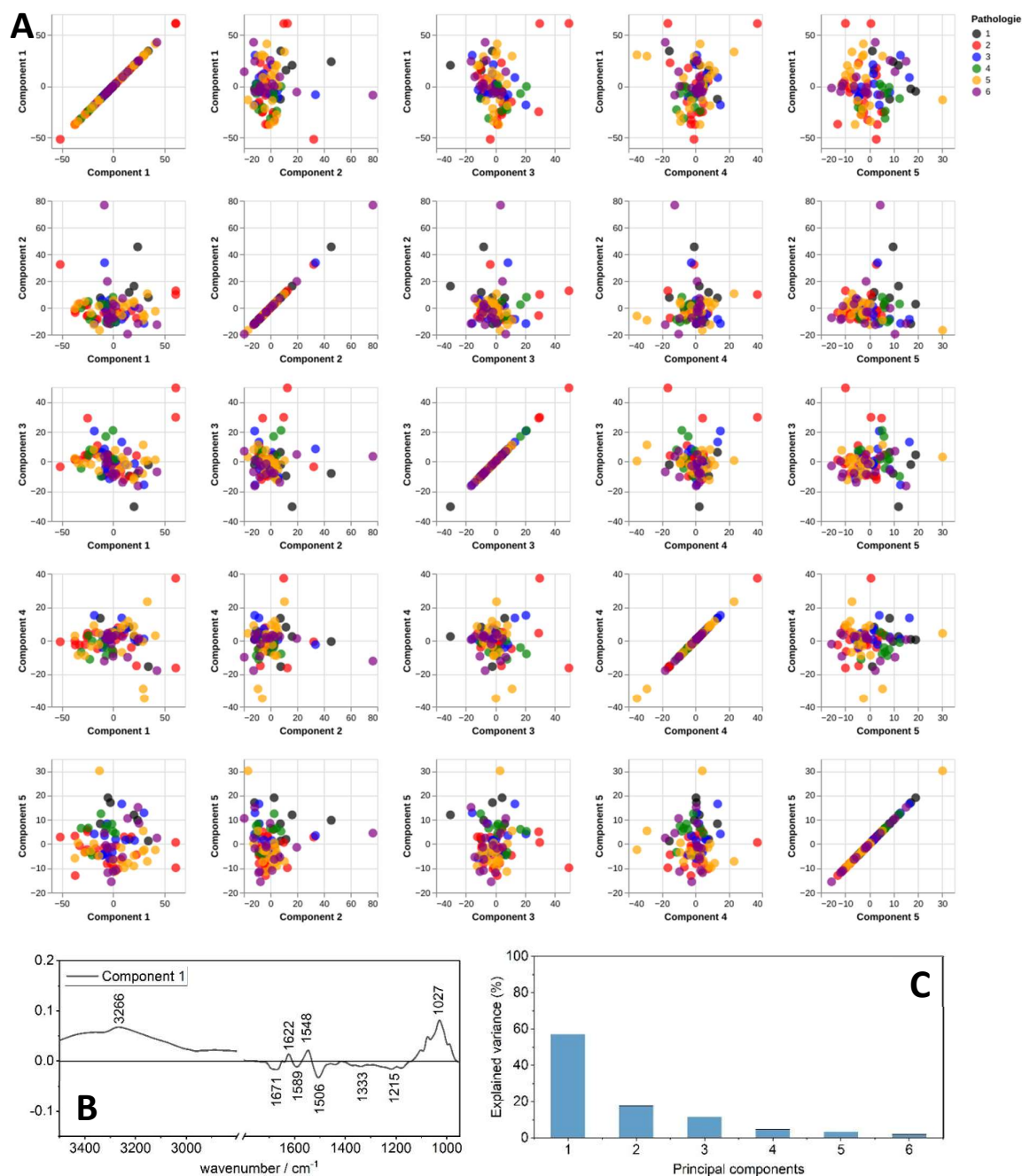


Figure S1. PCA of mice sera analyzed in $3750\text{--}2800\text{ cm}^{-1}$ and $1800\text{--}700\text{ cm}^{-1}$. The colors symbolize pathologies of Naïve in black, Vehicle in red, IFN β 10kIU in blue, IFN β 20kIU in green, 10mg/kg XBD173 in orange and the mixture of 10 mg/kg XBD173 + IFN β 10kIU in purple.

Table S1. PCA and loading plot of cerebellum mice brain tissue. The scale values for each component were scaled to between -1 to 1 for the visualization purpose of PCA. This ensures that both the positive and negative components of each loading are included in the visualization. The scale bar on the right side of the PCA plot indicates the value of the PCA using blue and red color. The blue color represents the positive values of loading and the negative value presented by red. The loading provides the component related to FTIR wavenumber which reveals the biochemistry of the sample.

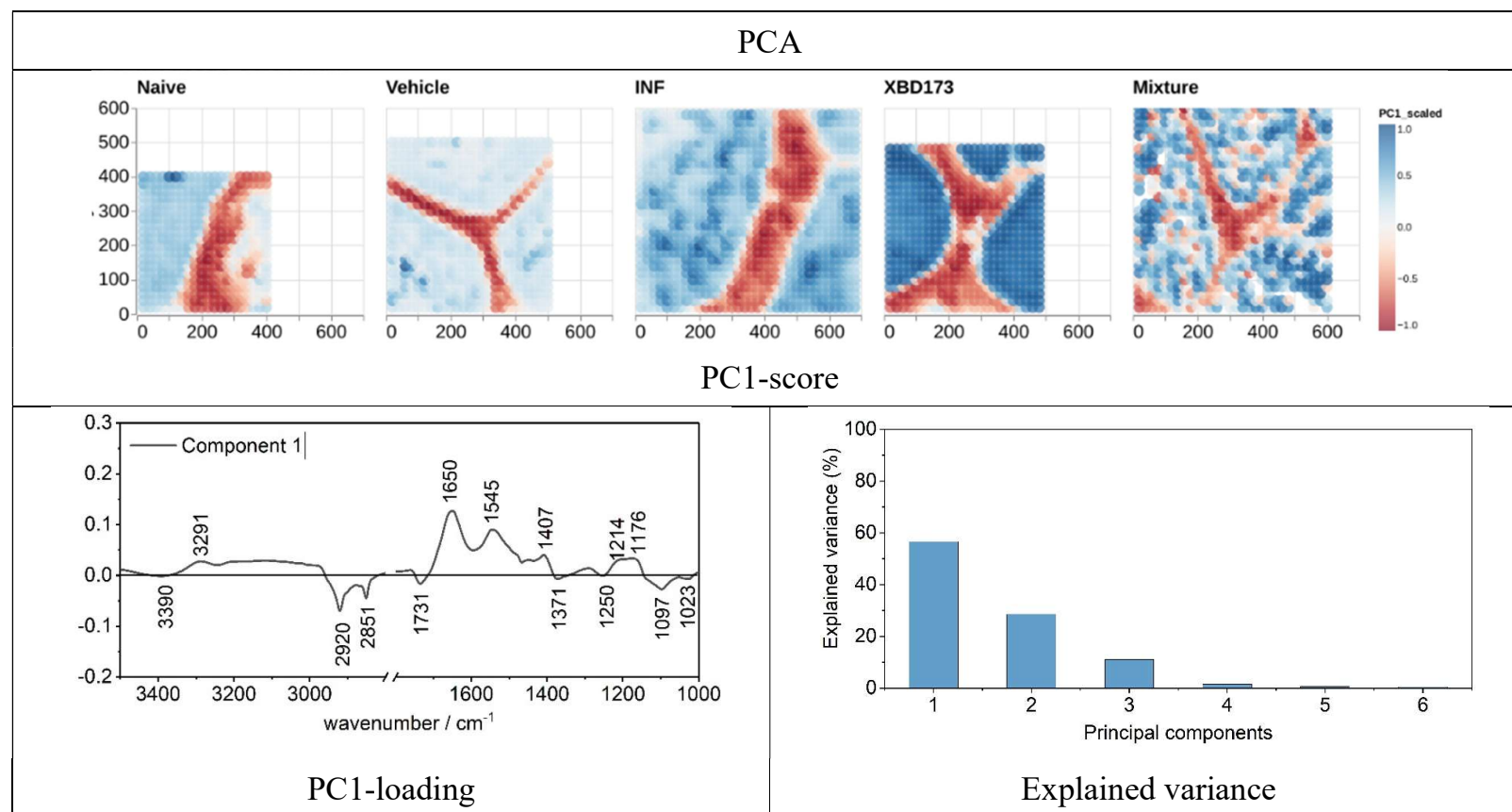


Table S2. Chemical image of cerebellum mice brain. The scale bar on the right side of the chemical image plot indicates the value of the integrated wavelength ratio using brown and light brown color. The lighter color represents the lower values of integrated ratio.

Integrated wavelength (cm^{-1})	Chemical image
$\text{Olefinic ratio} = \frac{\text{=CH stretching/CH-vibration lipid region} = \frac{[2996 - 3025]}{[2800 - 2996]}}$	
$\text{Lipids length} = \frac{\text{total CH}_2/\text{CH-vibration lipid region} = \frac{[2910 - 2944] + [2860 - 2842]}{[2800 - 2996]}}$	
$\text{Methylated content} = \frac{\text{total CH}_3/\text{CH-vibration lipid region} = \frac{[2944 - 2970] + [2880 - 2860]}{[2800 - 2996]}}$	
$\text{Carbonyl ratio} = \frac{\text{C=O stretching/CH-vibration lipid region} = \frac{[1715 - 1755]}{[2800 - 2996]}}$	

Integrated wavelength (cm ⁻¹)	Chemical image
$\beta\text{-sheet}/\alpha\text{-helix ratio} = \frac{[1622 - 1642]}{[1643 - 1674]}$	
$\text{Phosphate ratio} = \frac{\text{PO}_2^- / \text{CH-vibration lipid region} = [1203 - 1280]}{[2800 - 2996]}$	
$\text{Carbohydrate ratio} = \frac{(\text{glycogen, collagen, amino acids, ribose}) / \text{CH-vibration lipid region} = [1140 - 1183]}{[2800 - 2996]}$	
$\text{Nucleic acid ratio} = \frac{(\text{Nucleic acids, polysaccharide, carbohydrate}) / \text{CH-vibration lipid region} = [994 - 1140]}{[2800 - 2996]}$	

Table S3. The area ratio of olefinic ratio, lipids length, methylated content and carbonyl ratio of cerebellum white matter (up) and granular and molecular layer (down) was calculated by integrated wavelength from Table 3S. The significance was analyzed at $p < 0.05$ using the Kruskal-Wallis H test, followed by post hoc analysis using Dunn's test with p -values adjusted by the Holm method⁸⁰.

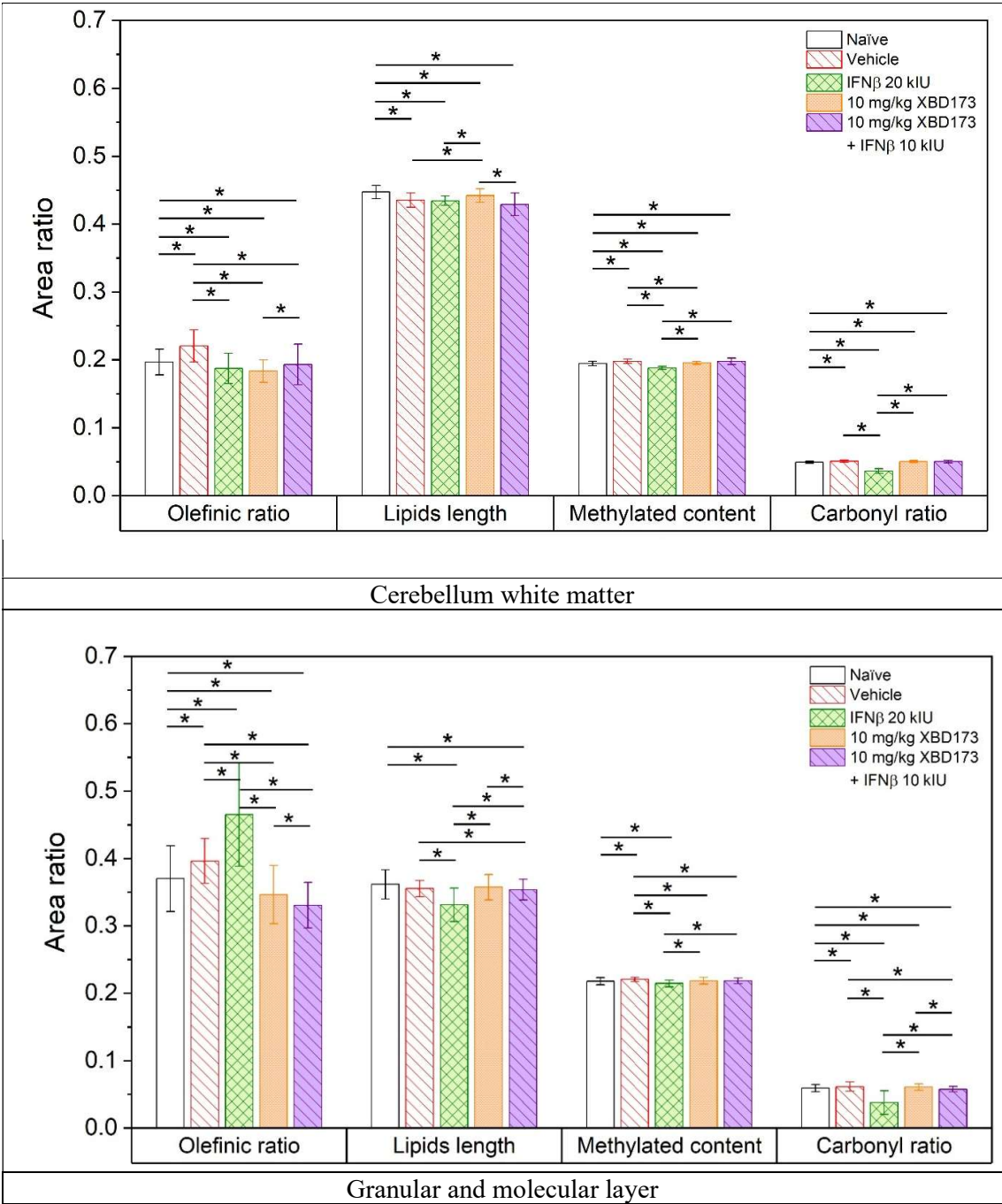


Table S4. The area ratio of beta sheet/alpha helix, phosphate ratio, carbohydrate ratio and nucleic acids ratio of cerebellum white matter (up) and granular and molecular layer (down) was calculated by integrated wavelength from Table 3S. The significance was analyzed at $p < 0.05$ using the Kruskal-Wallis H test, followed by post hoc analysis using Dunn's test with p -values adjusted by the Holm method⁸⁰.

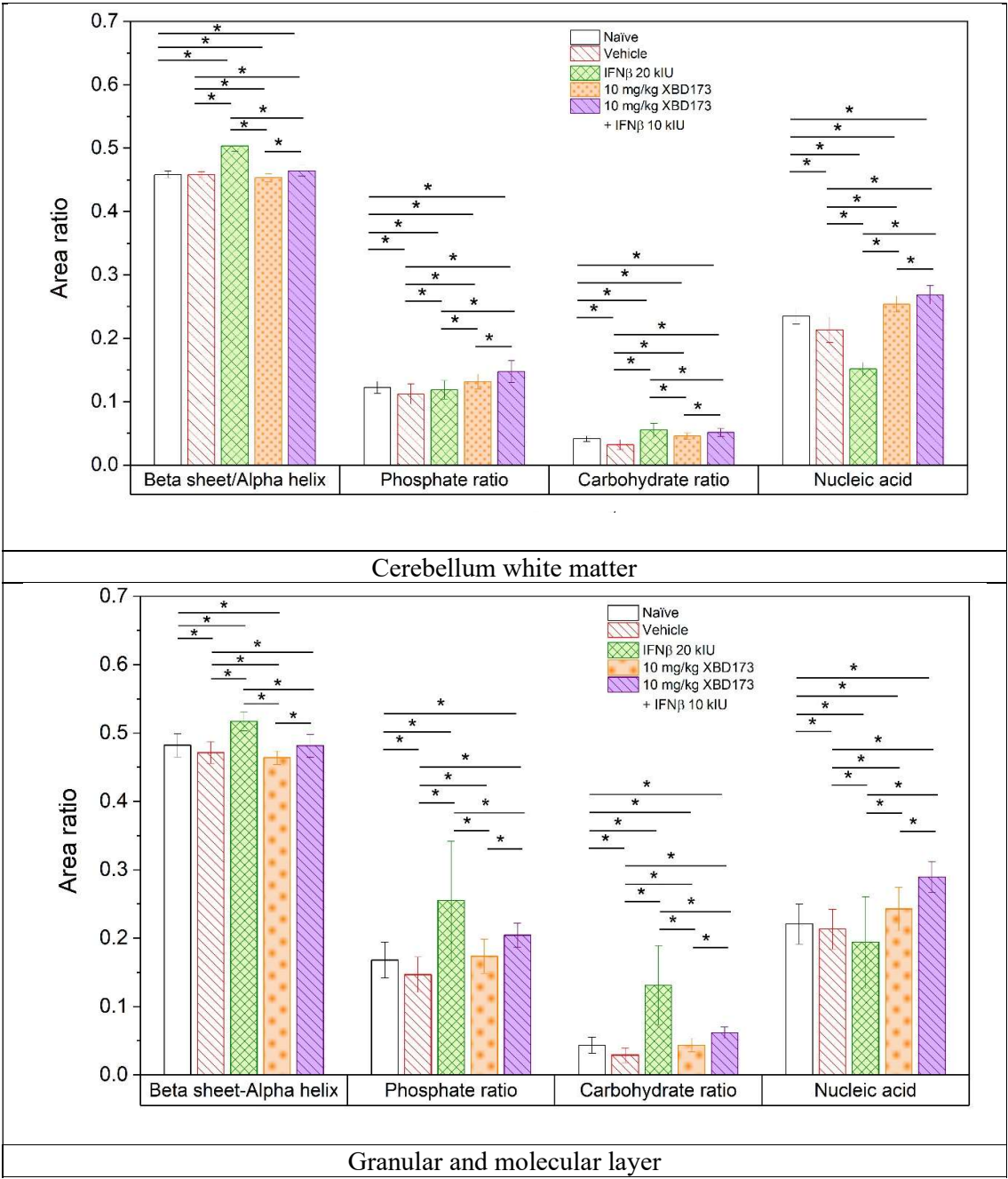
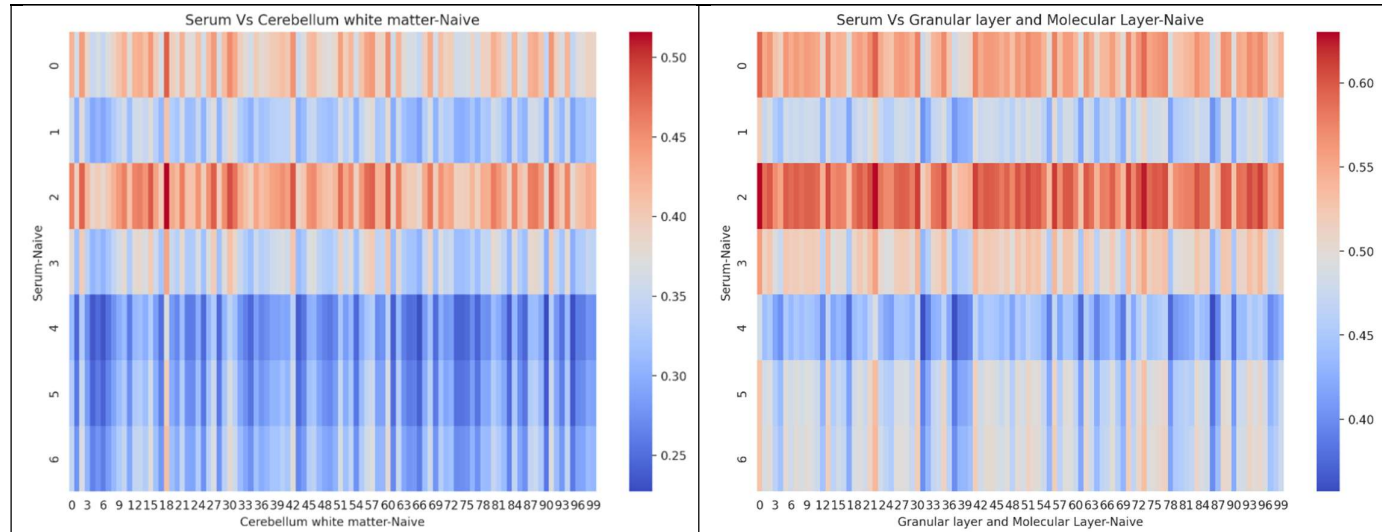
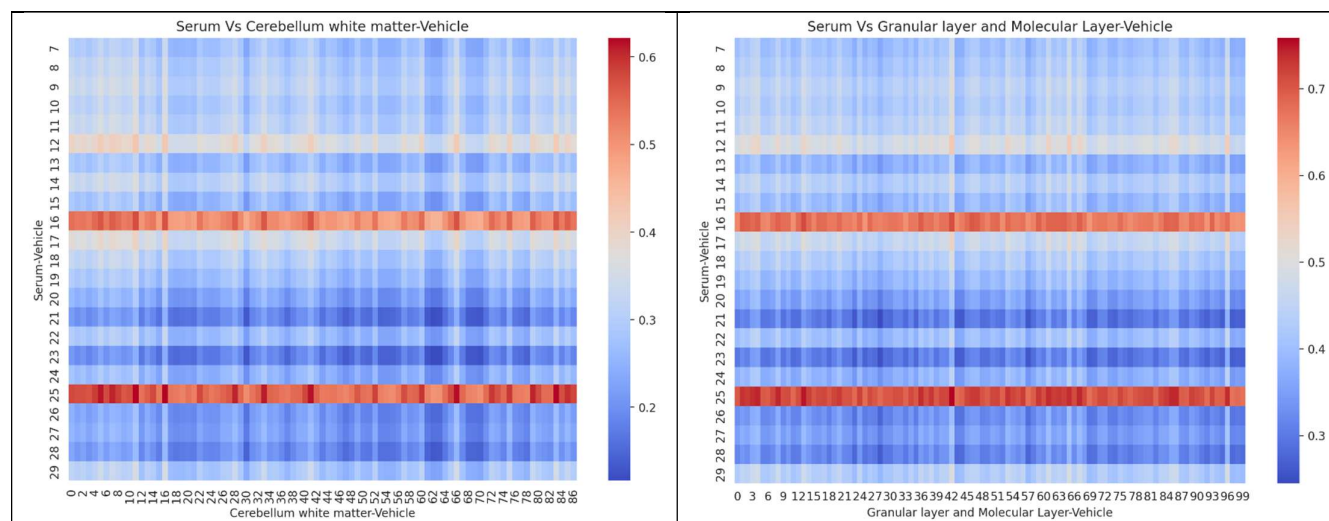


Table S5. The average Spearman correlation of pair test between each serum against 100 spectra obtained from cerebellum white matter of Naïve (left) and against 100 spectra obtained from granular layer and molecular layer of Naïve (right)



Index	Samples	Cerebellum white matter of Naïve			Granular and molecular layer of Naïve		
		Average Correlation	Lower 95% CI	Upper 95% CI	Average Correlation	Lower 95% CI	Upper 95% CI
0	NA_1_5N1S7	0.395533	0.348039	0.450483	0.544063	0.484102	0.584674
1	NA_2_5B1S7	0.332423	0.289953	0.381915	0.467252	0.410659	0.506774
2	NA_3_5V1S7	0.430543	0.381782	0.486152	0.581348	0.520732	0.619342
3	NA_4_5R1S7	0.350128	0.301947	0.406391	0.504822	0.439533	0.546462
4	NA_5_1S21	0.280697	0.231706	0.338217	0.431842	0.366381	0.476893
5	NA_6_1S22	0.295895	0.241395	0.361556	0.473124	0.397695	0.522146
6	NA_7_1S23	0.313805	0.261175	0.376597	0.478554	0.409364	0.523753

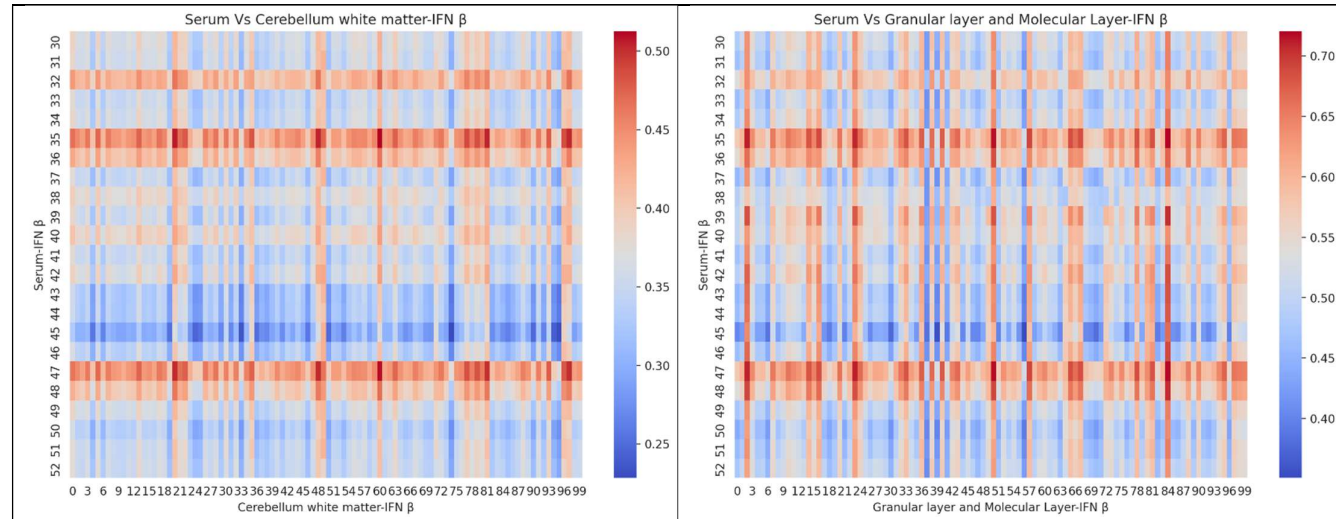
Table S6. The average Spearman correlation of pair test between each serum against 100 spectra obtained from cerebellum white matter of Vehicle (left) and against 100 spectra obtained from granular layer and molecular layer of Vehicle (right)



Index	Samples	Cerebellum white matter of Vehicle			Granular and molecular layer of Vehicle		
		Average Correlation	Lower 95% CI	Upper 95% CI	Average Correlation	Lower 95% CI	Upper 95% CI
7	VE_1_1B1S8	0.277525	0.226522	0.331628	0.407326	0.370819	0.458662
8	VE_2_1R1S8	0.300367	0.251238	0.351098	0.423453	0.388746	0.474639
9	VE_3_1V1S8	0.315260	0.265874	0.366896	0.443205	0.408600	0.490389
10	VE_4_1B3S7	0.294437	0.242537	0.350512	0.430194	0.393460	0.478494
11	VE_5_1R3S7	0.313680	0.262702	0.367948	0.448536	0.413007	0.493815
12	VE_6_1V3S7	0.369782	0.320813	0.420996	0.491375	0.456395	0.540708
13	VE_7_1N3S7	0.264063	0.213400	0.315879	0.384861	0.349240	0.438473
14	VE_8_1RT3S7	0.312628	0.264116	0.361845	0.436018	0.402376	0.483129
15	VE_9_1B2S7	0.259939	0.206617	0.317930	0.396376	0.358100	0.449093
16	VE_10_1V2S7	0.514879	0.467674	0.569984	0.669780	0.636061	0.698355
17	VE_11_1RT2S7	0.347430	0.300202	0.395093	0.465105	0.432300	0.514521
18	VE_12_1R2S7	0.301439	0.250367	0.355333	0.427715	0.391775	0.478624
19	VE_13_1N2S7	0.270692	0.220153	0.323219	0.395374	0.359882	0.446945
20	VE_14_2S2B7	0.228966	0.175371	0.286138	0.356151	0.316066	0.414091

21	VE 15 2S2N7	0.183742	0.130288	0.240080	0.303296	0.261514	0.364357
22	VE 16 2S3RT4	0.268431	0.214279	0.327730	0.402872	0.363400	0.457183
23	VE 17 2S3B4	0.178560	0.124882	0.235236	0.303789	0.264709	0.361638
24	VE 18 2S3NT3	0.248825	0.195313	0.306512	0.381076	0.341878	0.436441
25	VE 19 2S3VT3	0.556971	0.509003	0.619531	0.711374	0.677997	0.742577
26	VE 20 2S4RT3	0.211933	0.158647	0.267111	0.330601	0.291350	0.389996
27	VE 21 2S4VT3	0.222434	0.169464	0.279002	0.353264	0.315198	0.407793
28	VE 22 2S2NT8	0.196115	0.142621	0.252770	0.320494	0.279911	0.380479
29	VE 23 2S4N4	0.289458	0.234138	0.351677	0.434677	0.396189	0.486069

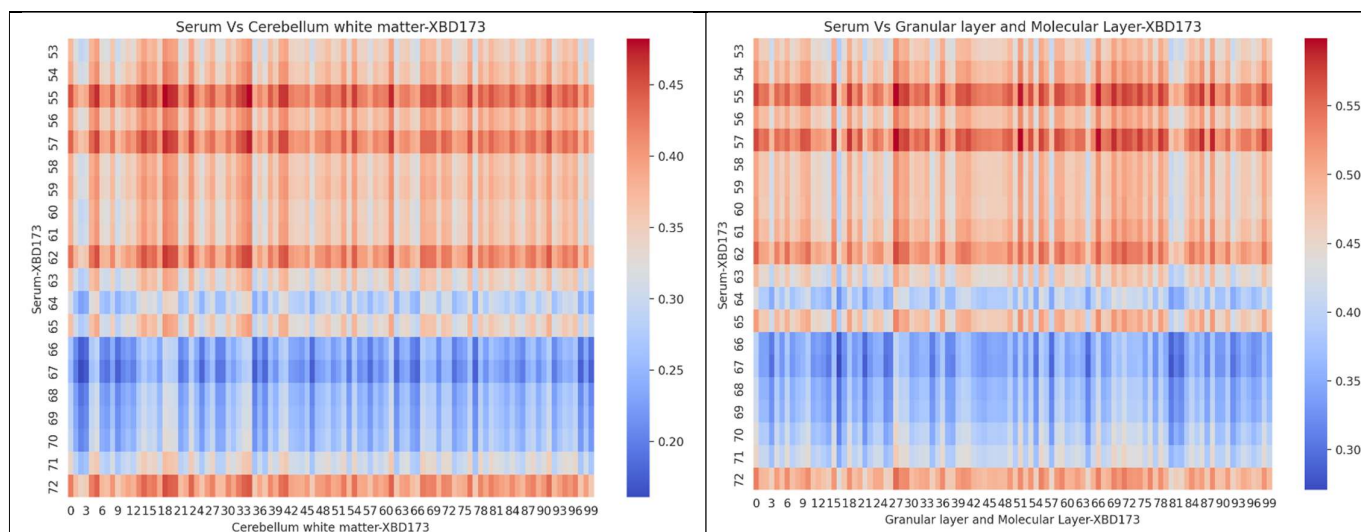
Table S7. The average Spearman correlation of pair test between each serum against 100 spectra obtained from cerebellum white matter of IFN β (left) and against 100 spectra obtained from granular layer and molecular layer of IFN β (right)



Index	Samples	Cerebellum white matter of IFN β			Granular and molecular layer of IFN β		
		Average Correlation	Lower 95% CI	Upper 95% CI	Average Correlation	Lower 95% CI	Upper 95% CI
30	IF_1_3RT3S8	0.364823	0.319323	0.415756	0.534001	0.451645	0.634327
31	IF_3_3VT3S8	0.360072	0.311048	0.415350	0.520052	0.435139	0.625662
32	IF_4_3N3S8	0.417848	0.378358	0.464586	0.572609	0.505054	0.639067
33	IF_5_3V3S8	0.352700	0.305951	0.405366	0.512183	0.429982	0.613719
34	IF_6_3R3S8	0.359478	0.312205	0.412349	0.533338	0.447893	0.639119
35	IF_7_3B3S8	0.443907	0.391076	0.504425	0.617845	0.537198	0.709767
36	IF_8_3R7S7	0.406630	0.364387	0.457922	0.586033	0.508548	0.667586
37	IF_9_3V7S7	0.350402	0.299742	0.407626	0.511674	0.423399	0.621920
38	IF_10_3B7S7	0.375466	0.341055	0.418674	0.524326	0.460343	0.585224
39	IF_11_3R6S7	0.357112	0.303088	0.418294	0.556715	0.458156	0.681005
40	IF_12_3N6S7	0.382739	0.341603	0.431392	0.546902	0.471334	0.630774
41	IF_13_3B6S7	0.352049	0.302194	0.409187	0.521598	0.433398	0.631092
42	IF_14_3V6S7	0.366950	0.311669	0.430225	0.546871	0.454157	0.657526
43	IF_15_4S3B1	0.329435	0.269404	0.401887	0.514472	0.415705	0.636742

44	IF 16 4S3V1	0.329445	0.272907	0.394300	0.518085	0.419212	0.640487
45	IF 17 4S3BT1	0.301082	0.238107	0.379009	0.472041	0.378085	0.607776
46	IF 18 4S3VT2	0.339951	0.282296	0.408411	0.521711	0.422614	0.645950
47	IF 19 4S3RT1	0.441609	0.389965	0.501187	0.619098	0.538944	0.709843
48	IF 20 4S3NT2	0.400111	0.346962	0.461297	0.583319	0.496103	0.688242
49	IF 21 4S3B2	0.361993	0.312917	0.416891	0.524107	0.441000	0.626626
50	IF 22 4S3R1	0.337026	0.285525	0.397168	0.496657	0.409858	0.605009
51	IF 23 4S3BT2	0.351228	0.299111	0.409404	0.513348	0.425934	0.619661
52	IF 24 4S3R2	0.352300	0.307569	0.402993	0.521281	0.440494	0.619153

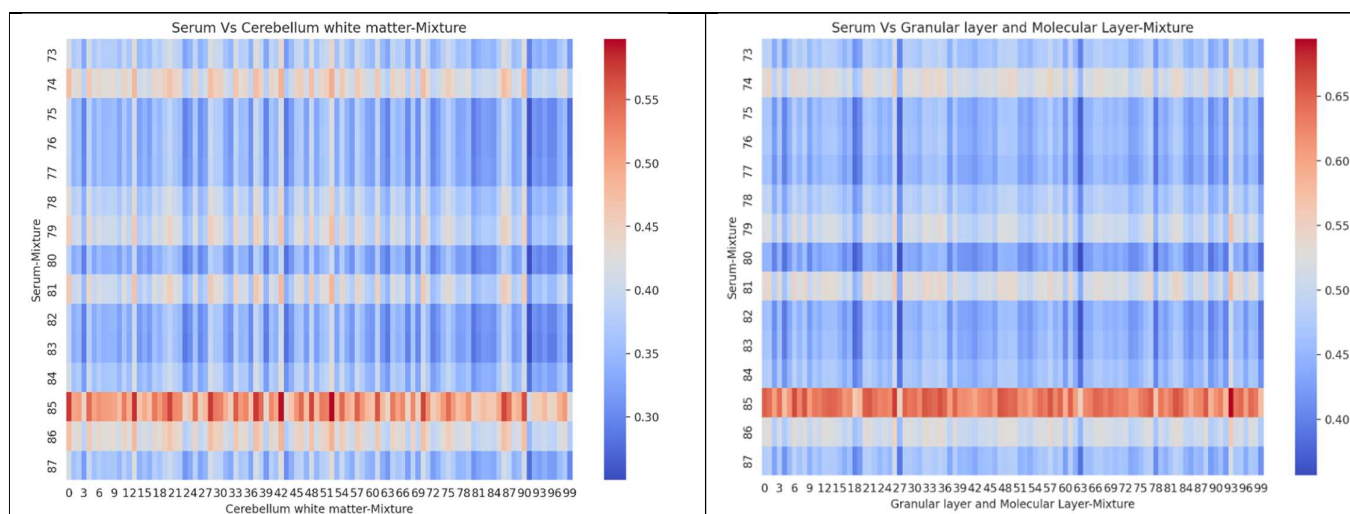
Table S8. The average Spearman correlation of pair test between each serum against 100 spectra obtained from cerebellum white matter of XBD173 (left) and against 100 spectra obtained from granular layer and molecular layer of XBD173 (right)



Index	Samples	Cerebellum white matter of 10mg/kg XBD173			Granular and molecular layer of 10mg/kg XBD173		
		Average Correlation	Lower 95% CI	Upper 95% CI	Average Correlation	Lower 95% CI	Upper 95% CI
53	XB 1 2VT2S8	0.349748	0.304234	0.396038	0.463571	0.406742	0.509065
54	XB 2 2BT2S8	0.364601	0.320197	0.411068	0.484622	0.424288	0.533748
55	XB 3 2RT2S8	0.427411	0.387405	0.470364	0.541283	0.485047	0.589457
56	XB 4 2R2S8	0.374955	0.333330	0.417241	0.481100	0.427809	0.526200
57	XB 5 2V2S8	0.415574	0.374803	0.460360	0.539629	0.477089	0.592827
58	XB 6 2N2S8	0.359769	0.317601	0.402864	0.469543	0.413925	0.516436
59	XB 7 2B2S8	0.365547	0.323176	0.408196	0.470899	0.417699	0.515689
60	XB 8 2R5S7	0.353129	0.306064	0.401304	0.468823	0.412592	0.513758
61	XB 9 2V5S7	0.356377	0.310448	0.404878	0.481770	0.419201	0.531893
62	XB 10 2B5S7	0.406005	0.360627	0.453144	0.515156	0.463925	0.557776
63	XB 11 2R4S7	0.336790	0.290726	0.383426	0.448401	0.392511	0.493210
64	XB 12 2B4S7	0.288825	0.240699	0.336719	0.393032	0.341424	0.438501
65	XB 13 2V4S7	0.337362	0.288237	0.389387	0.470777	0.405338	0.521276
66	XB 14 3S3N5	0.239093	0.187622	0.290683	0.349149	0.295734	0.397652

67	XB 15 3S3VT5	0.226771	0.174317	0.279769	0.346300	0.286922	0.397235
68	XB 16 3S3RT5	0.255838	0.204815	0.307345	0.366923	0.313203	0.415470
69	XB 17 3S3BT5	0.260979	0.211293	0.311068	0.371425	0.317173	0.419526
70	XB 18 3S4BT5	0.268712	0.218140	0.320562	0.392833	0.331019	0.441065
71	XB 19 3S4VT5	0.312586	0.265022	0.359631	0.413634	0.363294	0.456017
72	XB 20 3S4V5	0.397760	0.354456	0.441367	0.496062	0.450181	0.536926

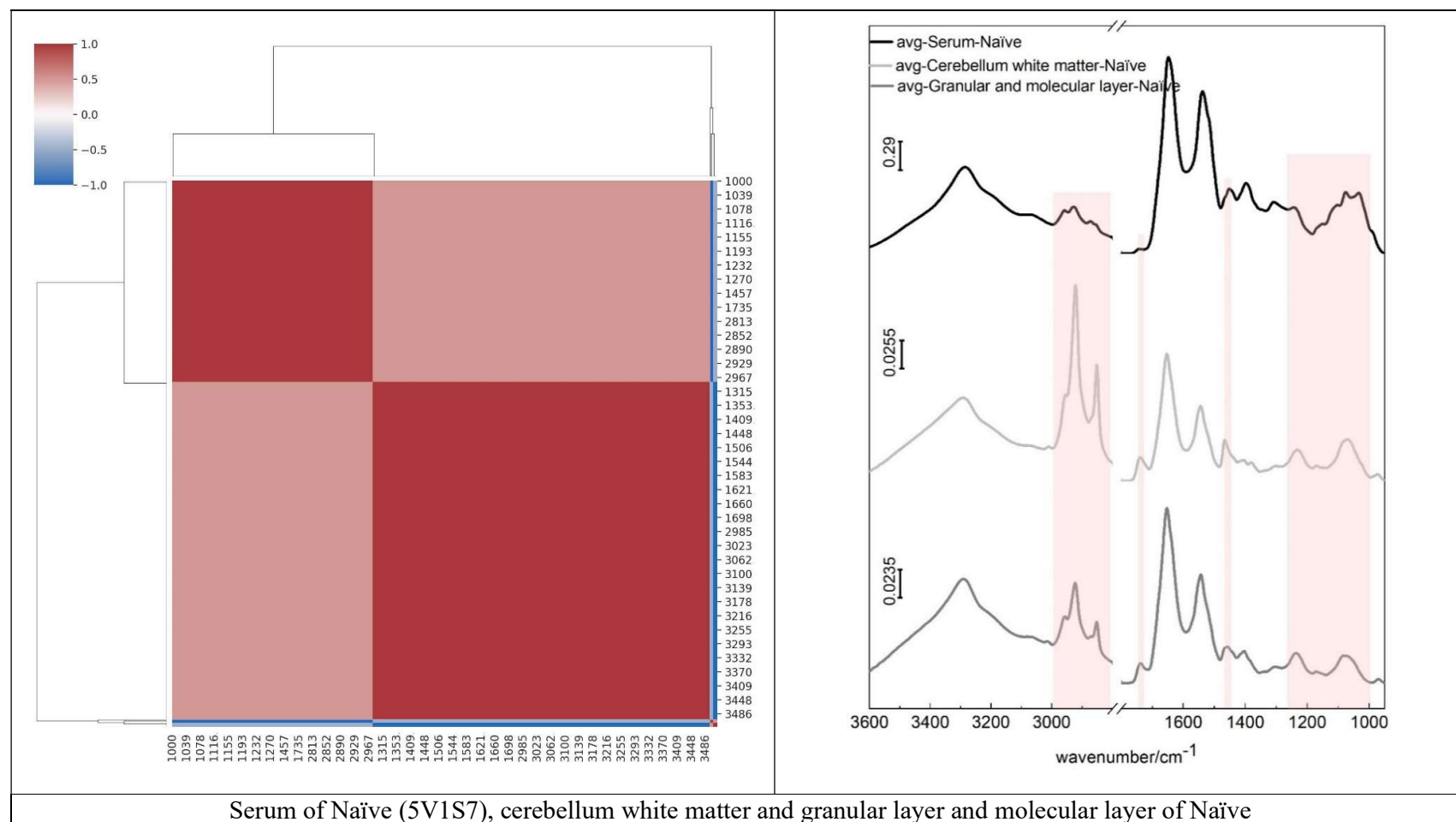
Table S9. The average Spearman correlation of pair test between each serum against 100 spectra obtained from cerebellum white matter of the mixture of 10 mg/kg XBD173 + IFN β 10kIU (left) and against 100 spectra obtained from granular layer and molecular layer of the mixture of 10 mg/kg XBD173 + IFN β 10kIU (right)



Index	Samples	Cerebellum white matter of the mixture of 10 mg/kg XBD173 + IFN β 10kIU			Granular and molecular layer of the mixture of 10 mg/kg XBD173 + IFN β 10kIU		
		Average Correlation	Lower 95% CI	Upper 95% CI	Average Correlation	Lower 95% CI	Upper 95% CI
73	IX 1 4RT4S7	0.373419	0.321221	0.429009	0.468496	0.415198	0.497797
74	IX 2 4NT4S8	0.426451	0.374473	0.480614	0.517755	0.468078	0.545813
75	IX 3 4BT4S8	0.344624	0.288778	0.404787	0.449022	0.389332	0.480377
76	IX 4 4V4S8	0.345542	0.288809	0.407384	0.452187	0.391544	0.484704
77	IX 5 4R4S8	0.345718	0.292608	0.402135	0.443341	0.386581	0.473948
78	IX 6 4VT4S8	0.380539	0.329454	0.435035	0.473446	0.422820	0.503080
79	IX 7 4N4S8	0.400192	0.345041	0.459953	0.501382	0.443925	0.533529
80	IX 8 4B4S8	0.335762	0.283804	0.390339	0.428002	0.372983	0.460840
81	IX 10 4B9S7	0.400549	0.341499	0.466966	0.514759	0.452036	0.547096
82	IX 11 4R9S7	0.339243	0.284843	0.397352	0.439663	0.381914	0.470318
83	IX 12 4N9S7	0.337236	0.280553	0.399390	0.445013	0.384325	0.477252
84	IX 13 4N8S7	0.363094	0.309821	0.419393	0.459250	0.403742	0.488589
85	IX 14 4V8S7	0.502967	0.439257	0.578961	0.628035	0.563077	0.664078

86	IX_15_4R8S7	0.425564	0.376143	0.477692	0.509885	0.463763	0.539858
87	IX_16_4B8S7	0.361565	0.308047	0.418493	0.459649	0.404001	0.489274

Table S10. Heatmap plot of the correlation between serum, average spectrum of cerebellum white matter and granular and molecular layer of Naïve (5V1S7) analyzed by Spearman's rank correlation coefficient.



Remark : The scale bar on the left side of the heatmap indicates the Spearman correlation coefficients. The correlation coefficient closer to +1 indicates the higher association (red). On the other hand, if close to -1, the association is inverse (blue).

Table S11. Heatmap plot of the correlation between serum, average spectrum of cerebellum white matter and granular and molecular layer of Vehicle (2S3VT3) analyzed by Spearman's rank correlation coefficient.

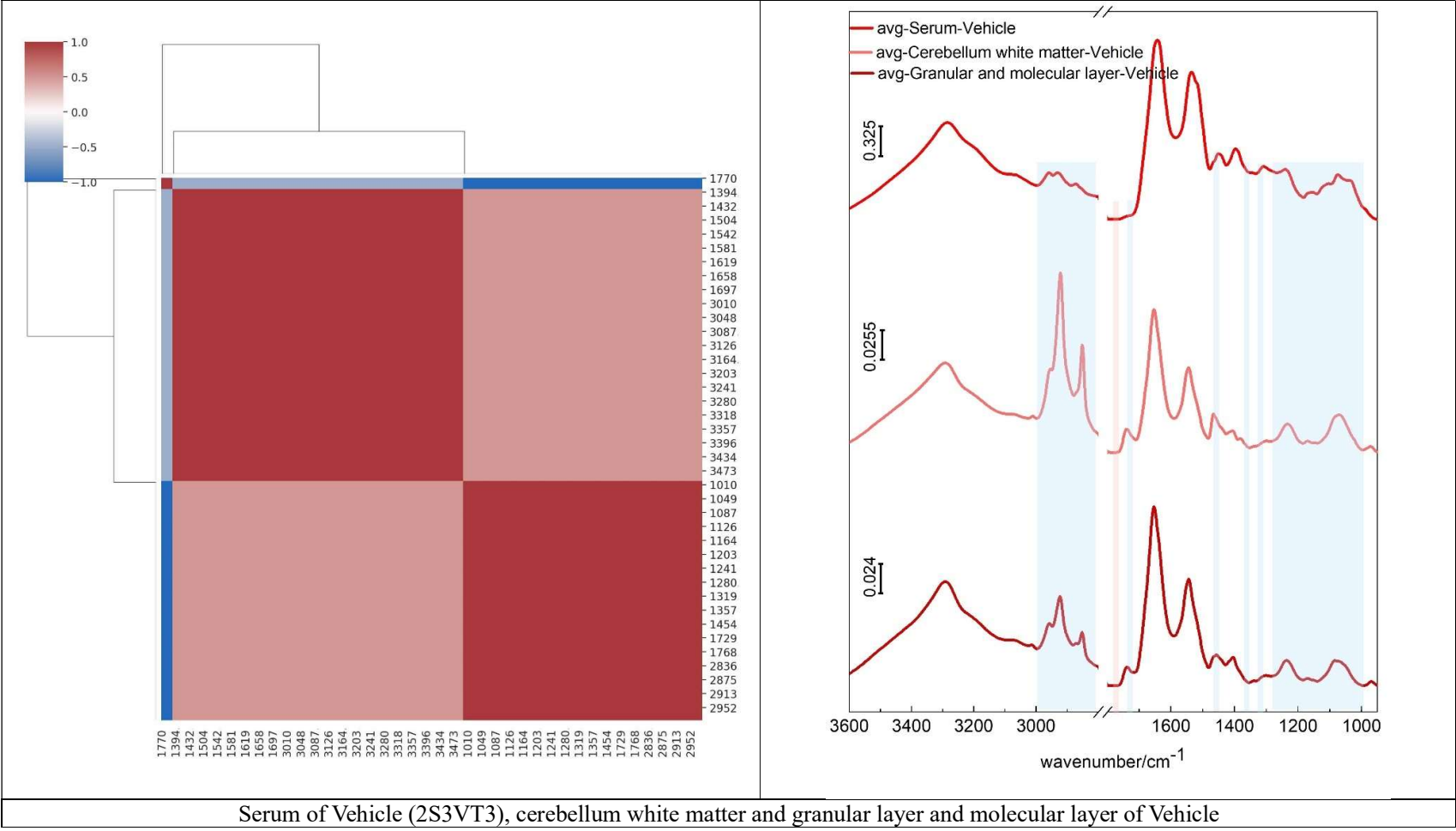


Table S12. Heatmap plot of the correlation between serum, average spectrum of cerebellum white matter and granular and molecular of IFN β 20kIU (4S3RT1) analyzed by Spearman's rank correlation coefficient.

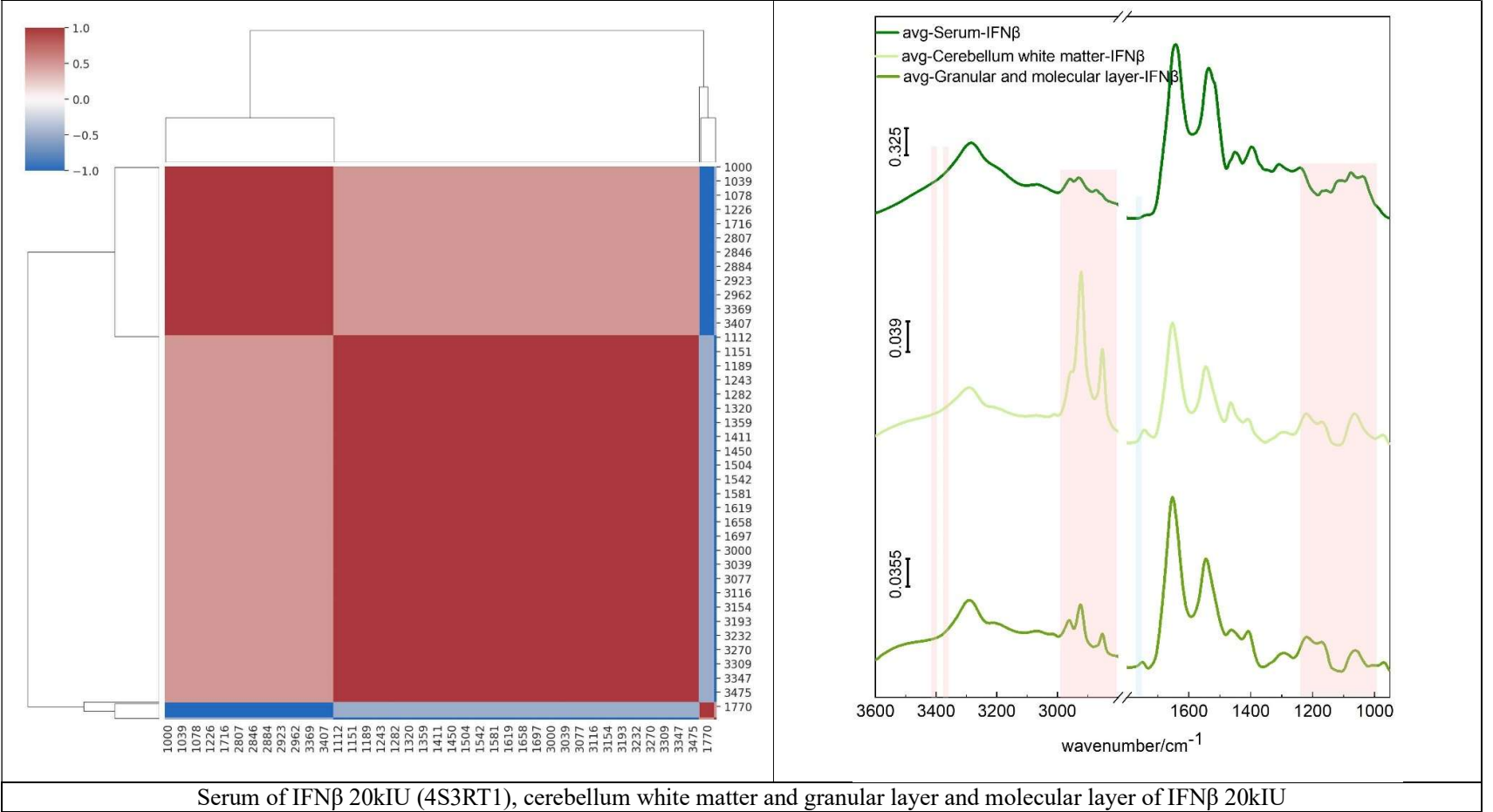


Table S13. Heatmap plot of the correlation between serum, average spectrum of cerebellum white matter and granular and molecular of 10 mg/kg XBD173 (2RT2S8) analyzed by Spearman's rank correlation coefficient.

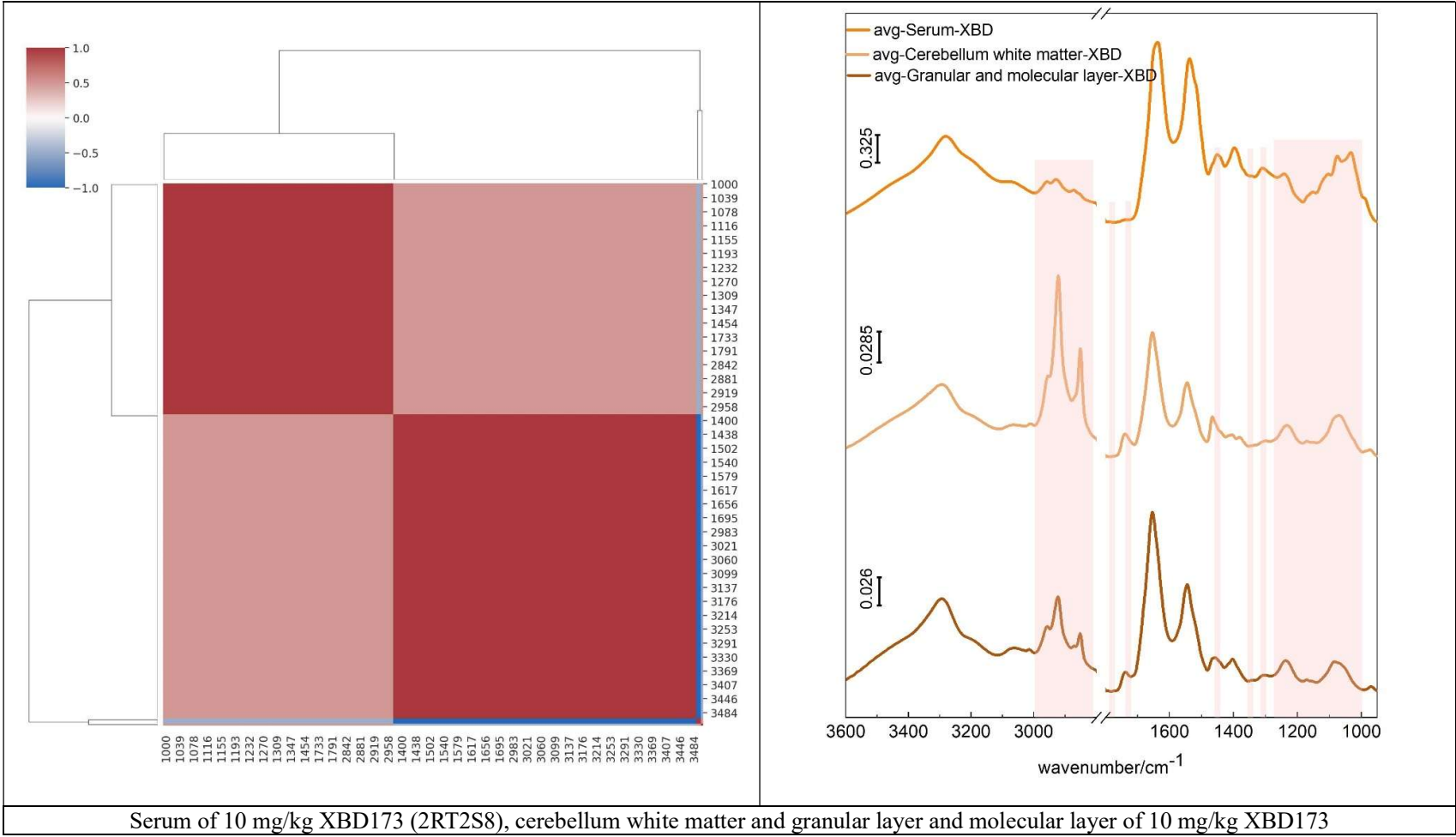
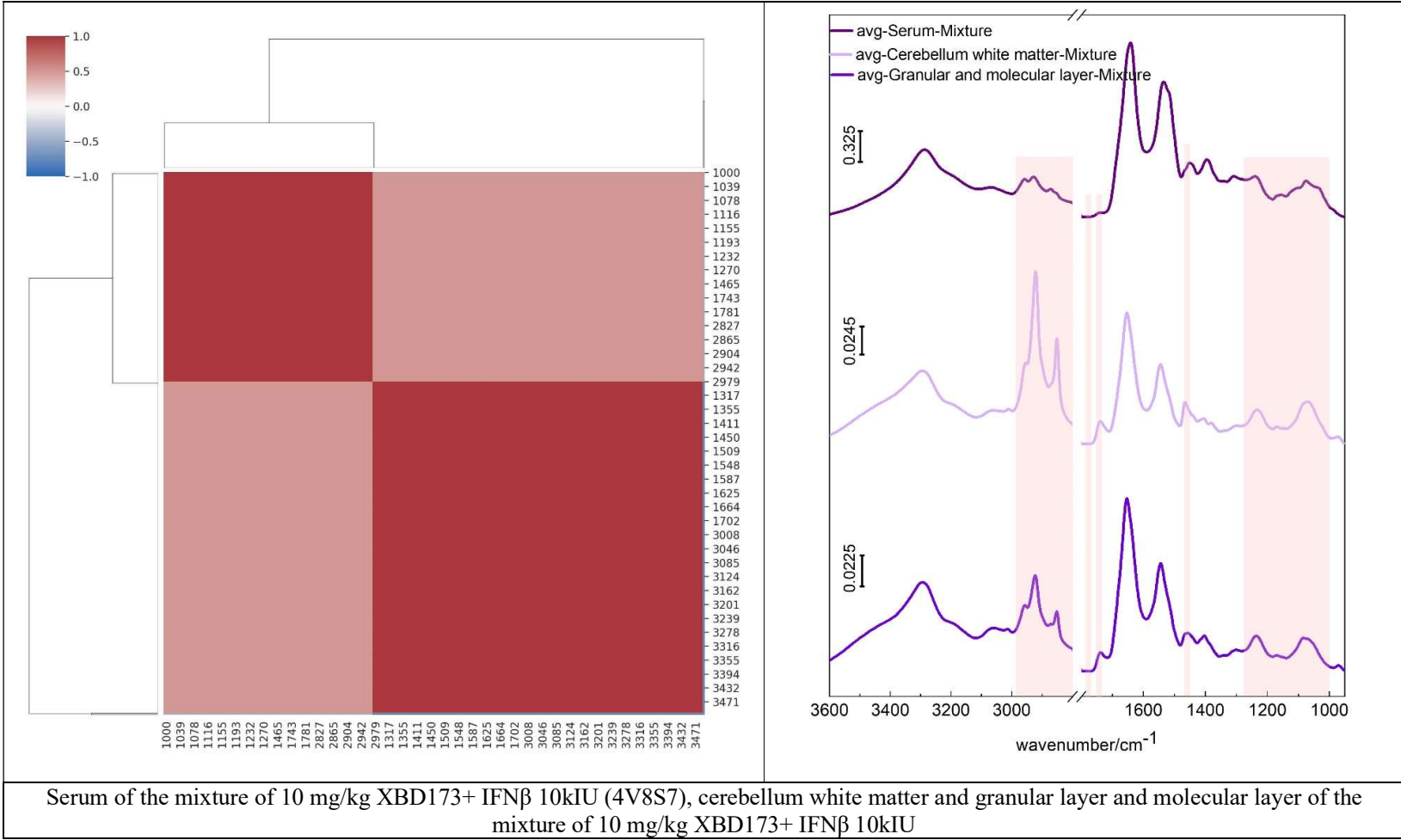


Table S14. Heatmap plot of the correlation between serum, average spectrum of cerebellum white matter and granular and molecular of the mixture of 10 mg/kg XBD173+ IFN β 10kIU (4V8S7) analyzed by Spearman's rank correlation coefficient.



Appendix B

Spectroscopic studies of Cyclic D,L- α -Peptide and AD mice brain tissue

Table S15. Analyzed images of B6 and APP in cortical layers II–III.

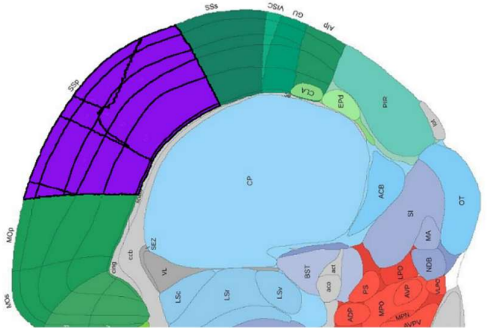
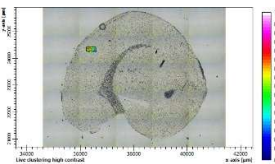
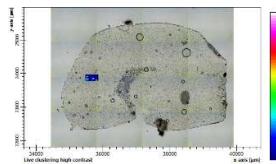
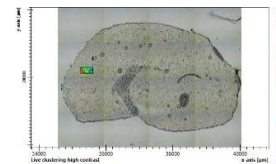
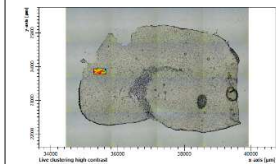
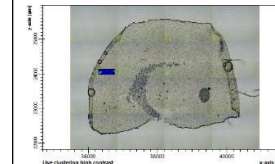
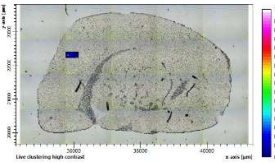
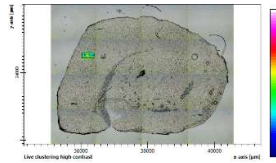
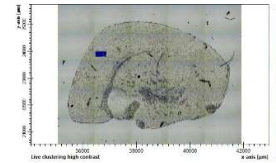
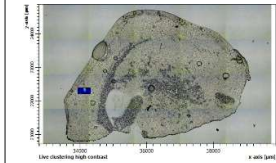
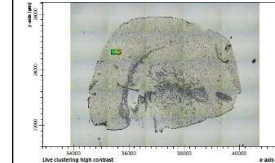
 Source : https://atlas.brain-map.org/atlas?atlas=602630314#atlas=602630314&plate=576987833&structure=369&x=5275.628255208333&y=3743.999883863661&zoom=-5&resolution=35.59&z=3		The cerebral cortex is divided into four lobes: the frontal, temporal, parietal, and occipital lobes, each responsible for a multitude of functions. It contains sensory, motor, and important association areas. The thalamus receives somatosensory information and relays it to the primary somatosensory cortex, located in the postcentral gyrus of the parietal lobe. Other important primary cortical sensory areas include the auditory cortex in the temporal lobe and the visual cortex in the occipital lobe¹⁰⁴.		
				
B6-14-10uM	APP-Front-1-10uM	APP-Front-2-10uM	APP-Front-3-10uM	APP-Front-4-10uM
				
APP-Back-5-10uM	APP-Back-6-10uM	APP-Back-7-10uM	APP-Back-8-10uM	APP-Back-9-10uM

Table S16. PC-1 mapping was generated by filling areas based on PC-1 scores, with blue representing the positive vector of the PC-1 loading and red representing the negative vector. Chemical images of B6 and APP-Front were analyzed in cortical layers II–III using the wavenumber range corresponding to the negative vector of the PC-1 loading.

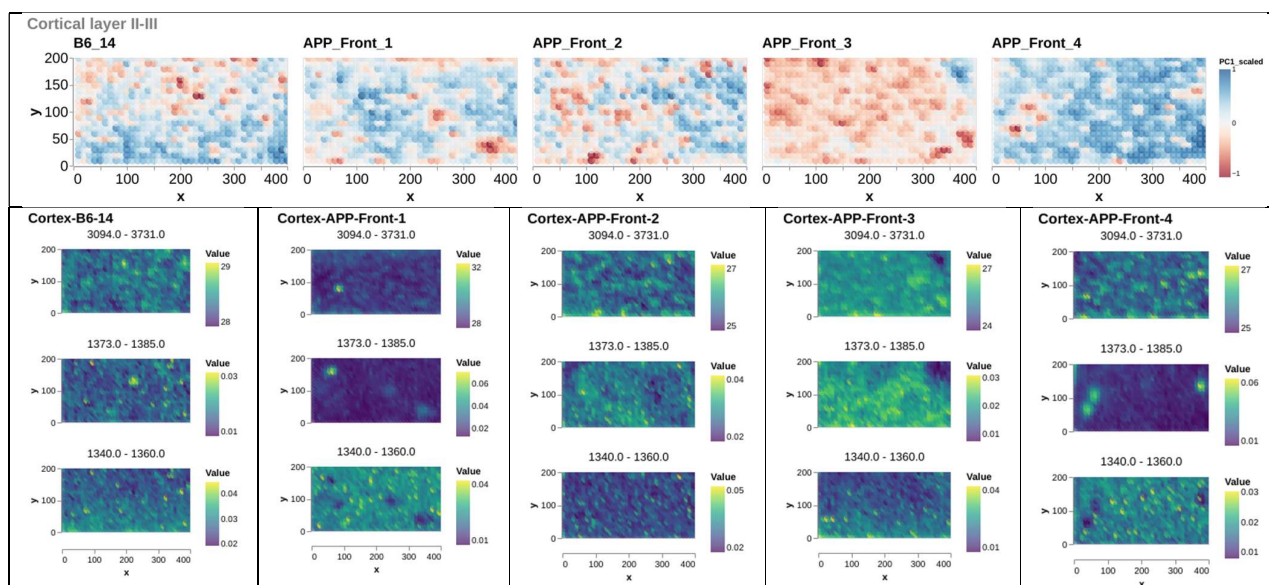


Table S17. PC-2 mapping was generated by filling areas based on PC-2 scores, with blue representing the positive vector of the PC-2 loading and red representing the negative vector. Chemical images of B6 and APP-Front were analyzed in cortical layers II–III using the wavenumber range corresponding to the positive vector of the PC-2 loading.

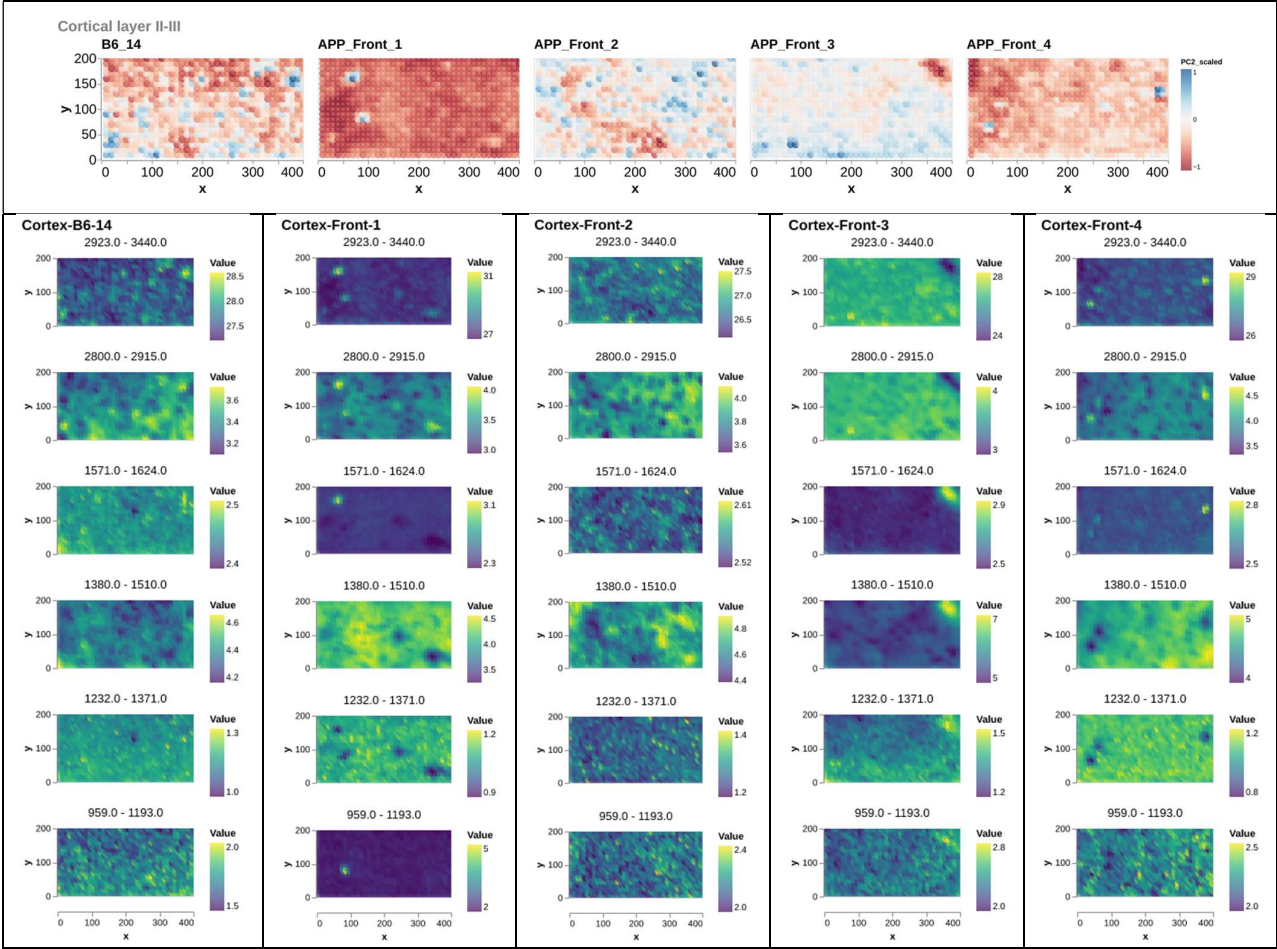


Table S19. PC-1 mapping was generated by filling areas based on PC-1 scores, with blue representing the positive vector of the PC-1 loading and red representing the negative vector. Chemical images of B6 and APP-Back were analyzed in cortical layers II–III using the wavenumber range corresponding to the negative vector of the PC-1 loading.

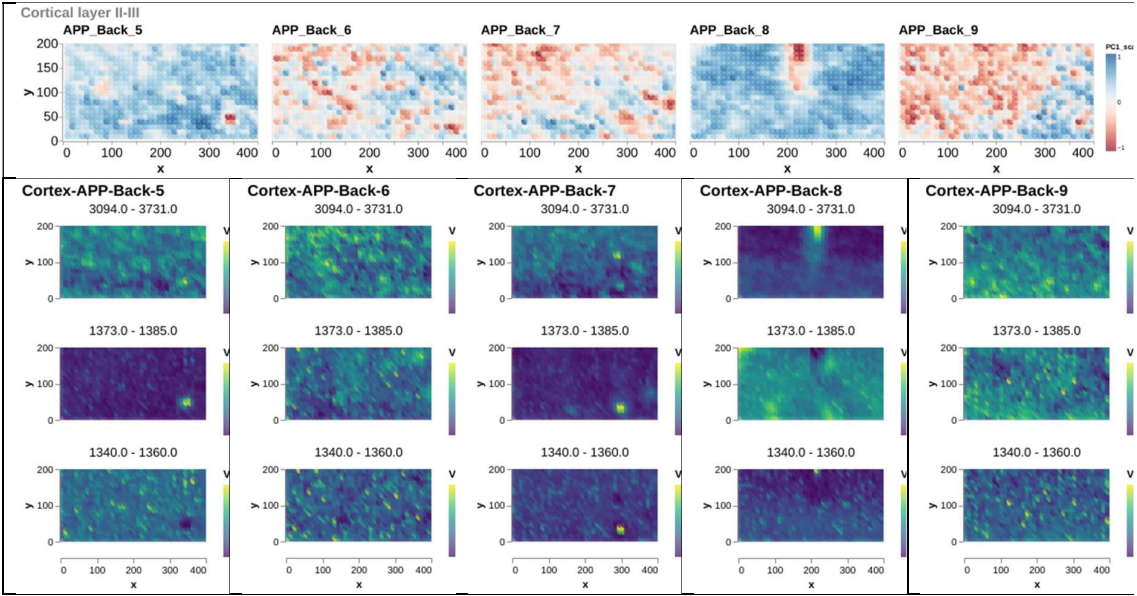


Table S22. Analyzed images of B6 and APP in cortical layers IV–V.

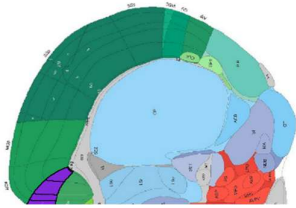
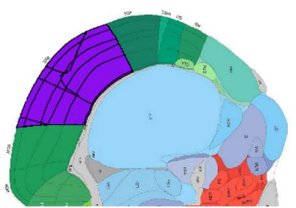
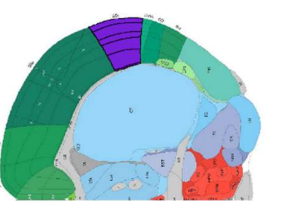
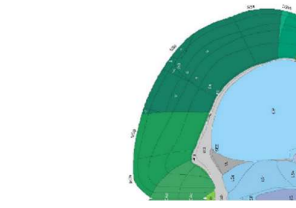
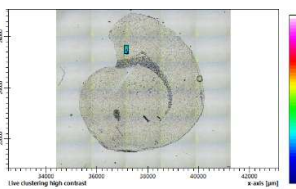
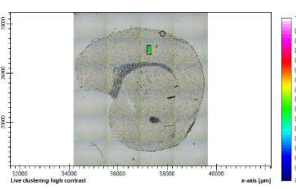
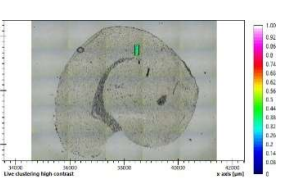
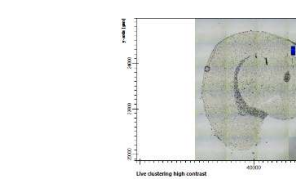
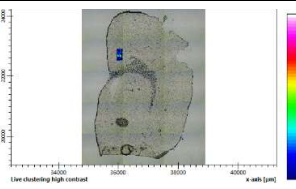
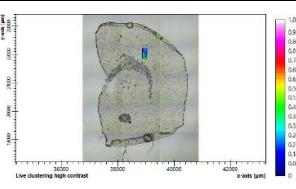
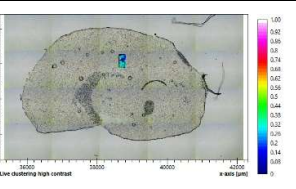
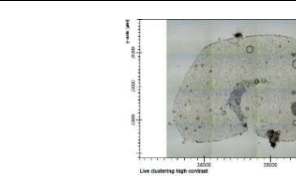
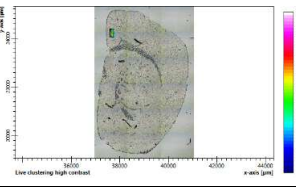
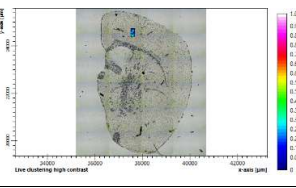
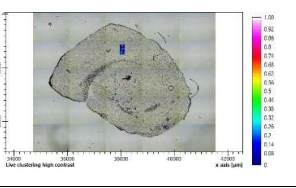
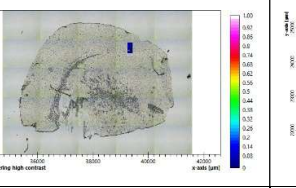
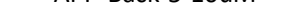
Anterior cingulate area	Primary somatosensory area	Supplemental somatosensory area	Piriform area	
				
				
B6-14_P1-10uM	B6-14_P2-10uM	B6-14_P3-10uM	B6-14-10uM	
				
APP-Front-3-10uM	APP-Front-4-10uM	APP-Front-2-10uM	APP-Front-1-10uM	
				
APP-Back-5-10uM	APP-Back-7-10uM	APP-Back-6-10uM	APP-Back-9-10uM	APP-Back-8-10uM
				

Table S23. PC-1 mapping was generated by filling areas based on PC-1 scores, with blue representing the positive vector of the PC-1 loading and red representing the negative vector. Chemical images of B6 were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-1 loading.

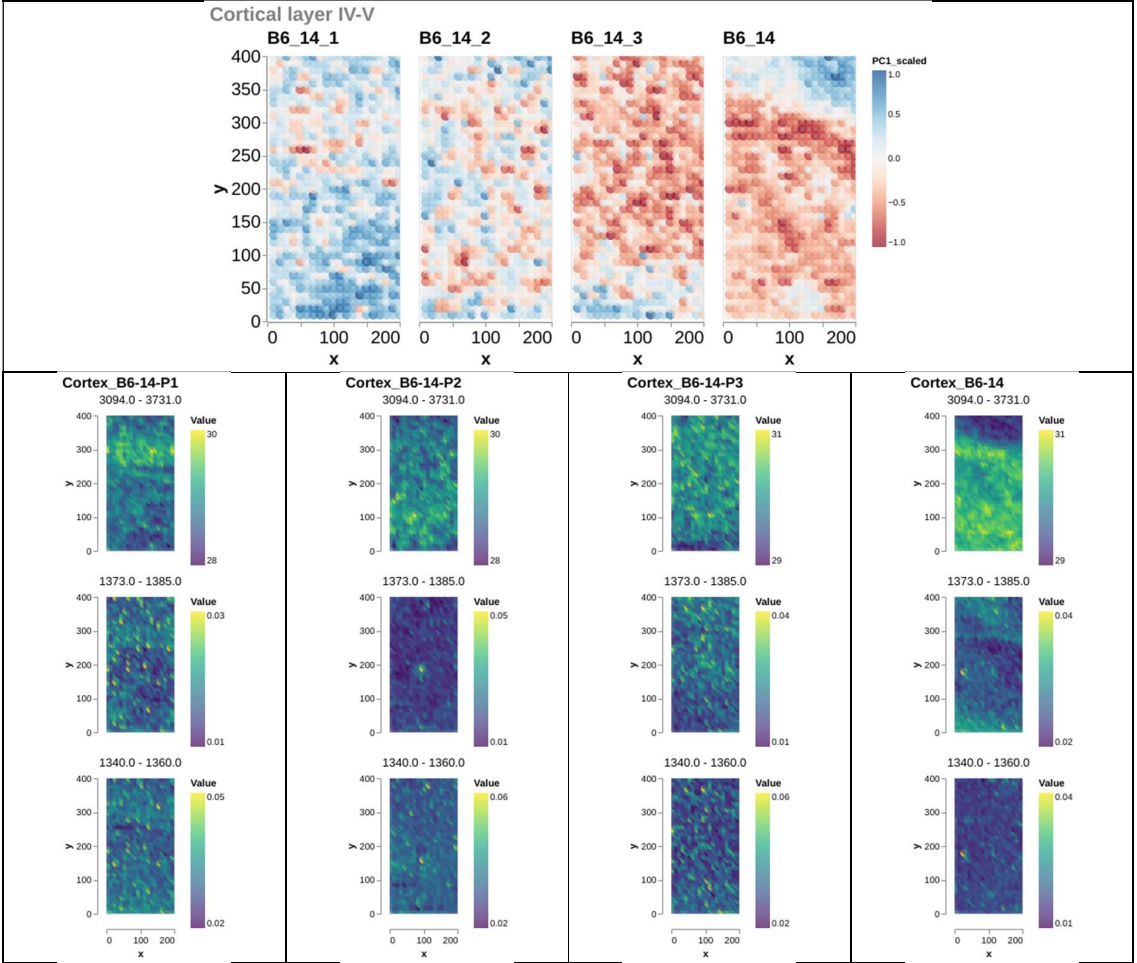


Table S25. PC-3 mapping and chemical images of B6 were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-3 loading.

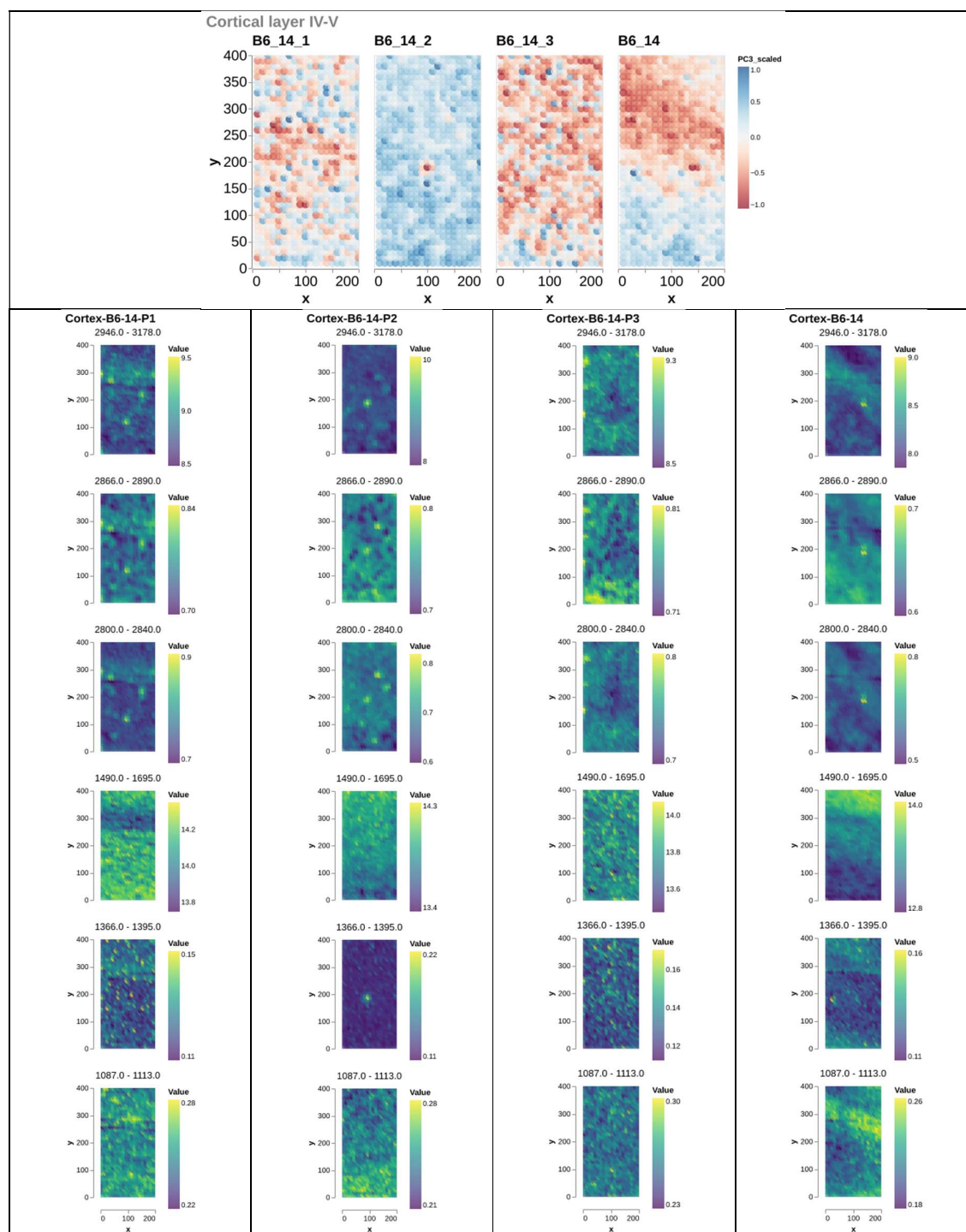


Table S26. PC-1 mapping and chemical images of APP-Front were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-1 loading.

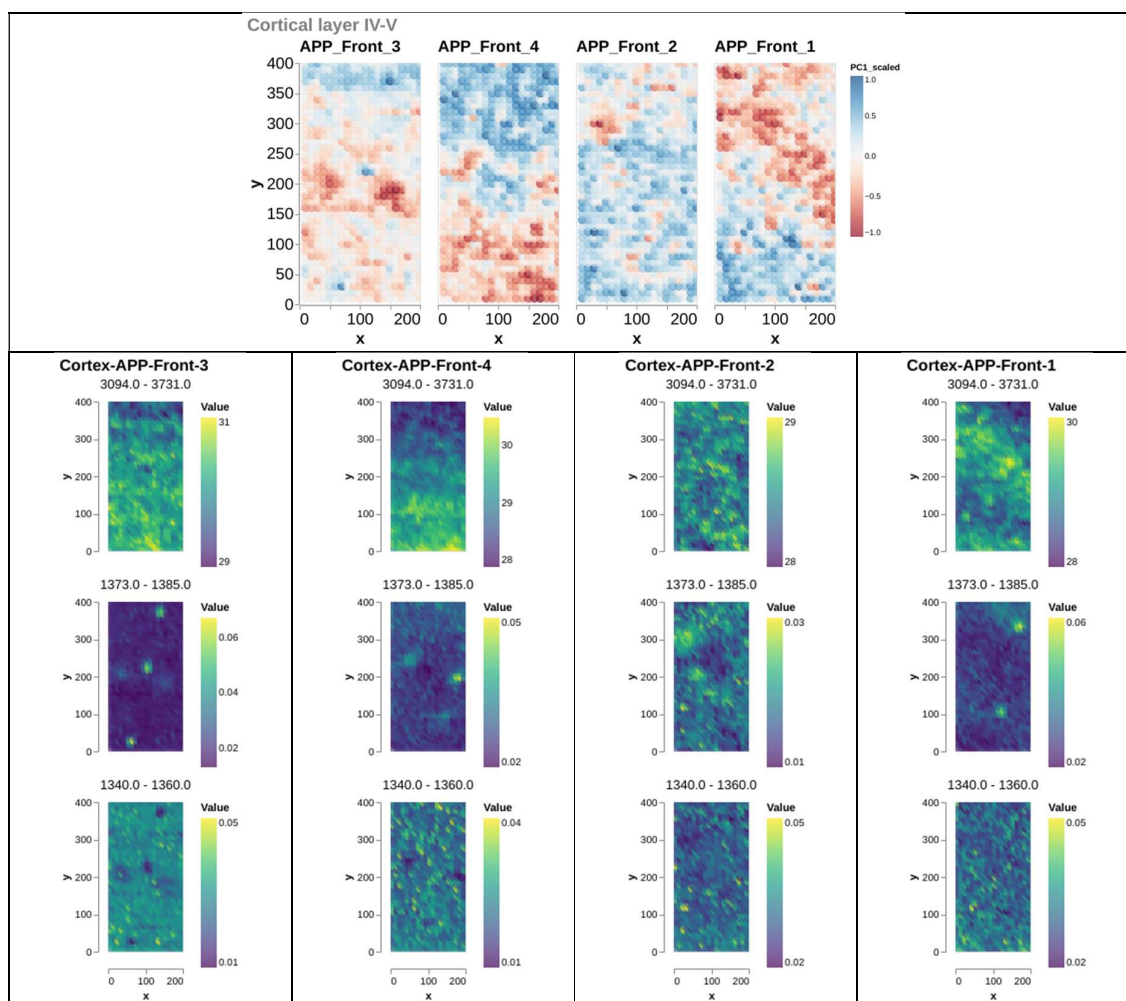


Table S27. PC-1 mapping and chemical images of APP-Front were analyzed in cortical layers IV–V using the wavenumber range corresponding to the positive vector of the PC-2 loading.

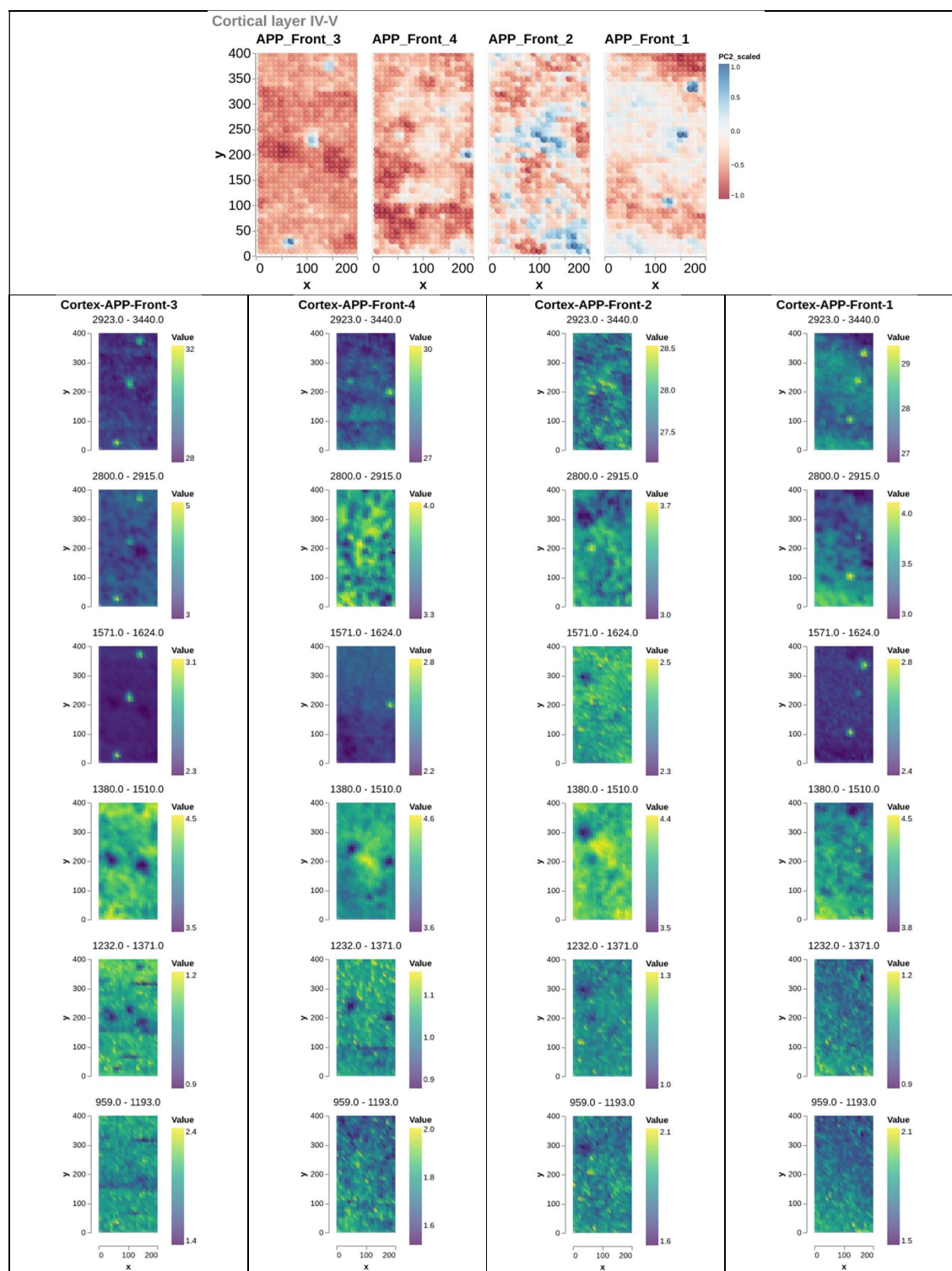


Table S28. PC-3 mapping and chemical images of APP-Front were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-3 loading.

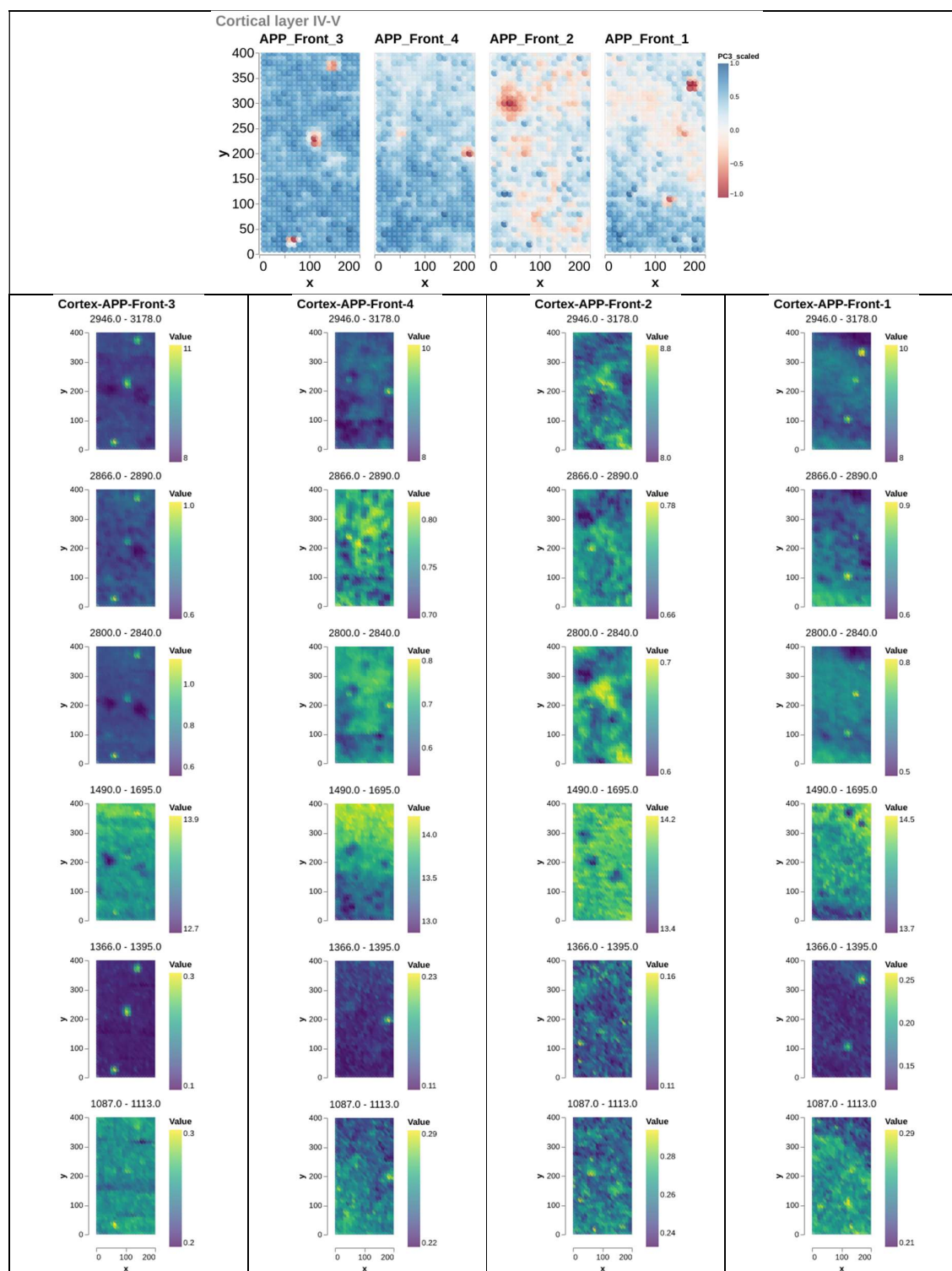


Table S29. PC-1 mapping and chemical images of APP-Back were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-1 loading.

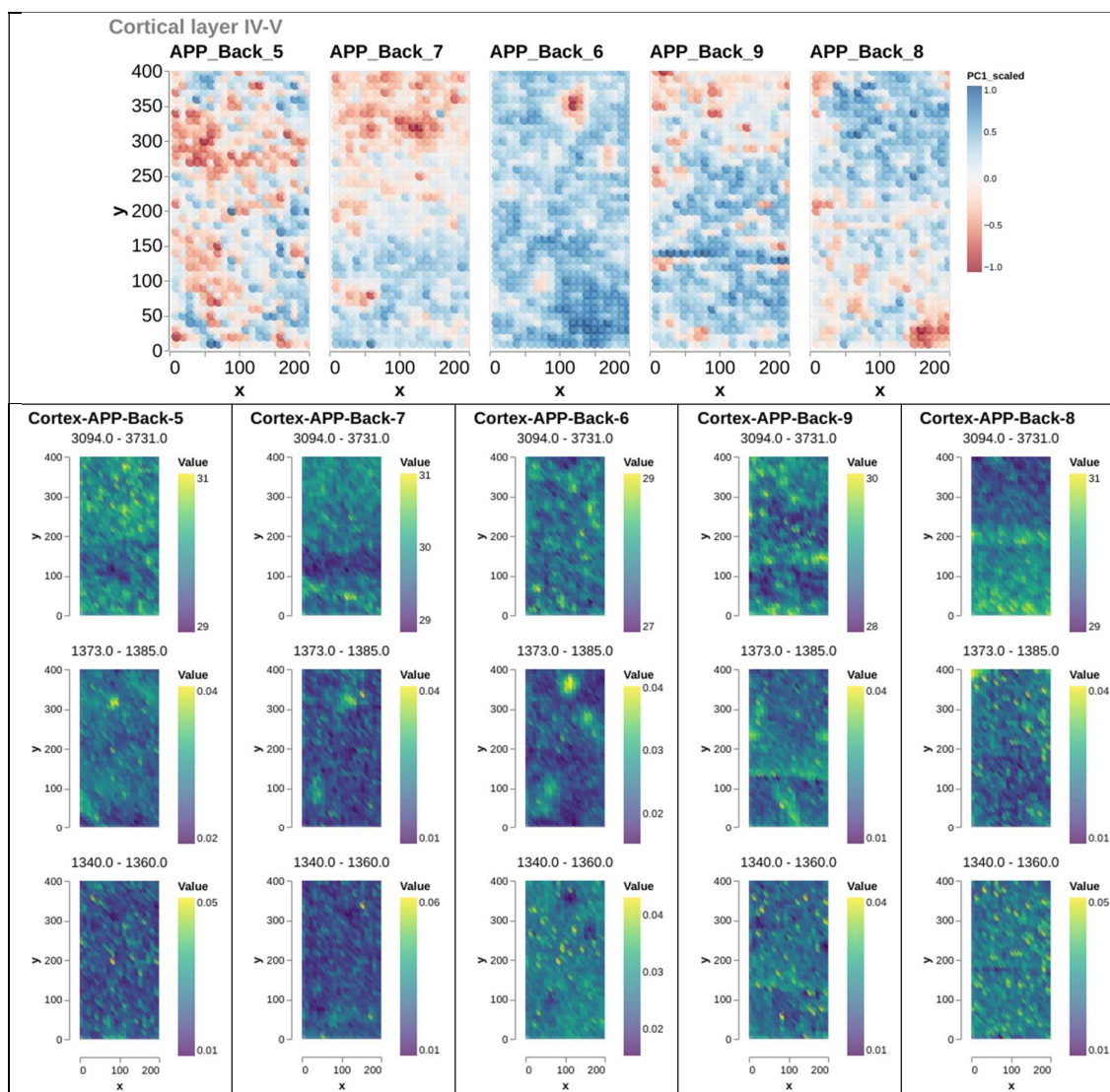


Table S30. PC-2 mapping and chemical images of APP-Back were analyzed in cortical layers IV–V using the wavenumber range corresponding to the positive vector of the PC-2 loading.

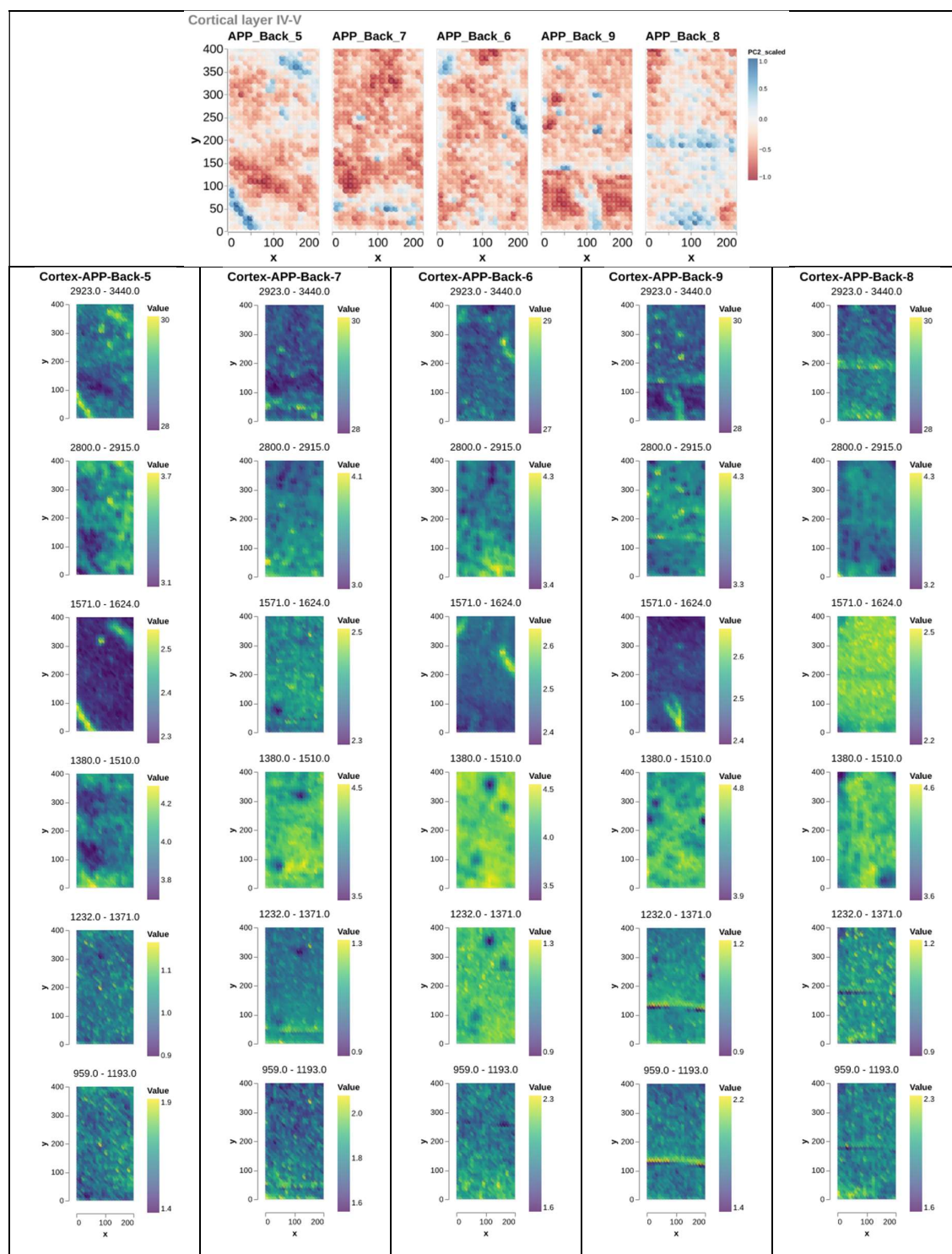


Table S31. PC-3 mapping and chemical images of APP-Back were analyzed in cortical layers IV–V using the wavenumber range corresponding to the negative vector of the PC-3 loading.

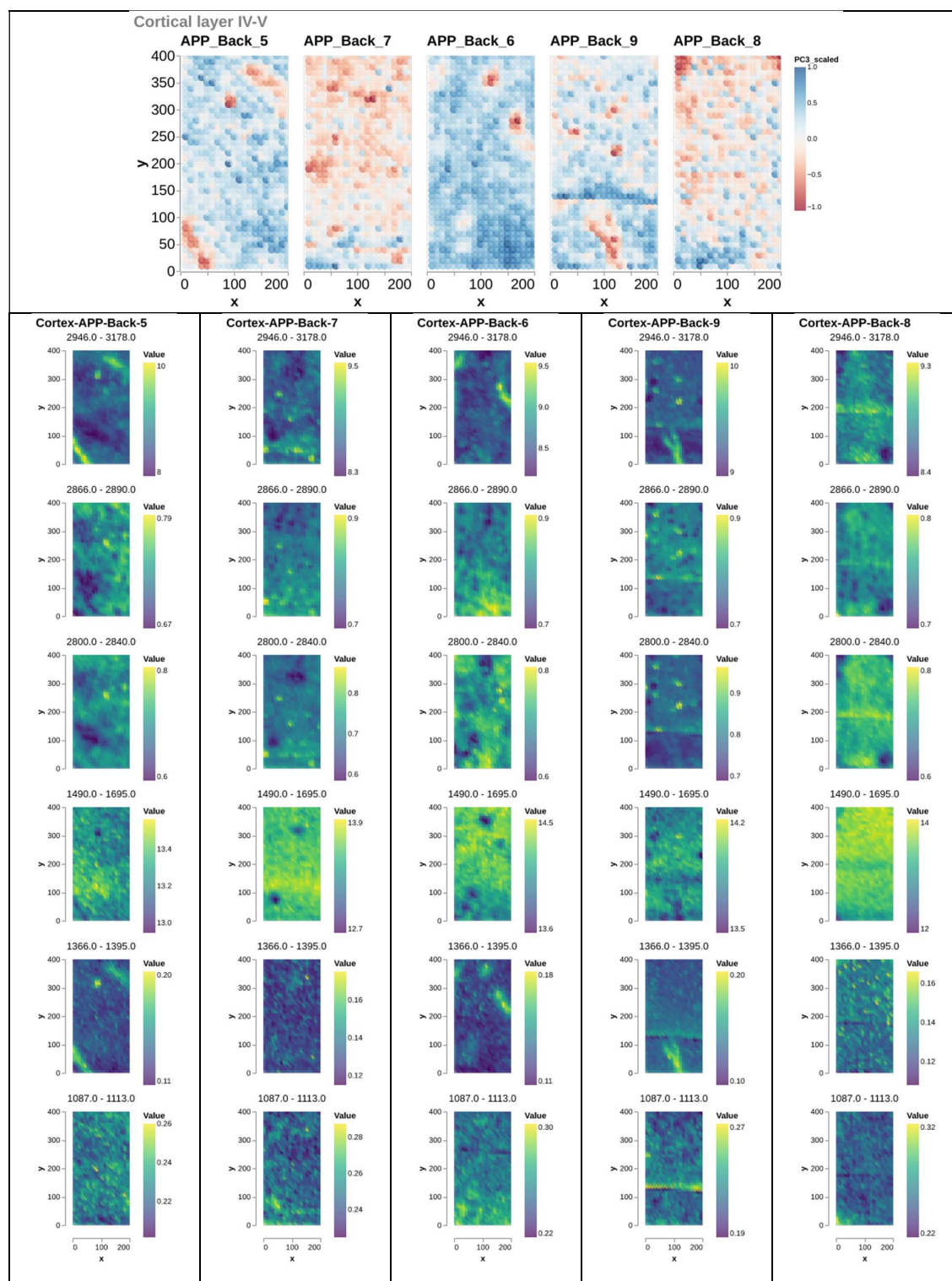


Table S32. Particle size and zeta potential of GNPs functionalized with CP-2 were obtained from Prof. Shai Rahimipou.

GNPs Mercaptodecanoic acid linker

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 42.54	Peak 1: 32.56	68.1	17.82
Pdl: 0.448	Peak 2: 209.6	22.3	93.17
Intercept: 0.872	Peak 3: 4626	9.6	824.8

Result quality : Refer to quality report

Size Distribution by Intensity

Record 343: GNPs mercaptoundecanoic ligands 1
Record 344: GNPs mercaptoundecanoic ligands 2
Record 345: GNPs mercaptoundecanoic ligands 3

Average particle size 42.54 nm

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): -16.3	Peak 1: -4.55	52.5	5.76
Zeta Deviation (mV): 14.5	Peak 2: -29.1	47.5	8.58
Conductivity (mS/cm): 0.185	Peak 3: 0.00	0.0	0.00

Result quality : See result quality report

Zeta Potential Distribution

Record 340: GNPs mercaptoundecanoic ligands 1
Record 341: GNPs mercaptoundecanoic ligands 2
Record 342: GNPs mercaptoundecanoic ligands 3

Average zeta potential -16.3 mV

GNPs Mercaptodecanoic acid linker + CP2

Results

	Size (d.nm):	% Intensity:	St Dev (d.nm):
Z-Average (d.nm): 103.0	Peak 1: 138.4	96.9	81.27
Pdl: 0.282	Peak 2: 4222	3.1	997.7
Intercept: 0.901	Peak 3: 0.000	0.0	0.000

Result quality : Good

Size Distribution by Intensity

Record 376: GNPs Mercaptoundecanoic with CP-2 1
Record 377: GNPs Mercaptoundecanoic with CP-2 2
Record 378: GNPs Mercaptoundecanoic with CP-2 3

Average particle size 103 nm

Results

	Mean (mV)	Area (%)	St Dev (mV)
Zeta Potential (mV): 5.15	Peak 1: -0.795	74.2	7.80
Zeta Deviation (mV): 13.0	Peak 2: 23.7	25.8	5.09
Conductivity (mS/cm): 0.247	Peak 3: 0.00	0.0	0.00

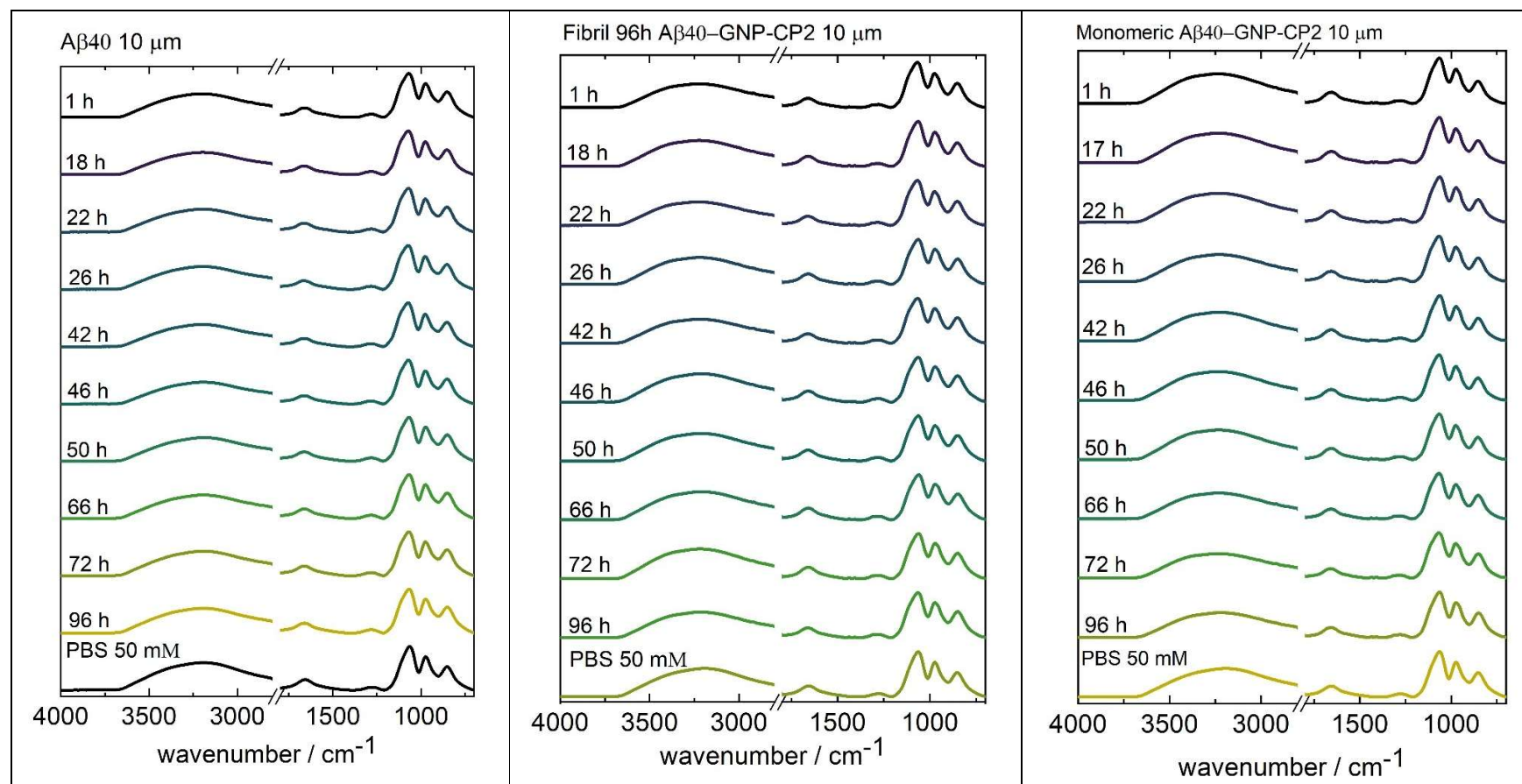
Result quality : See result quality report

Zeta Potential Distribution

Record 379: GNPs Mercaptoundecanoic with CP-2 1
Record 380: GNPs Mercaptoundecanoic with CP-2 2
Record 381: GNPs Mercaptoundecanoic with CP-2 3

Average zeta potential 5.15 mV

Table S33 FTIR spectra were measured from dried films of: (i) aggregated monomeric A β ₄₀, (ii) disaggregation of A β ₄₀ fibrils in the presence of GNPs functionalized with CP-2, and (iii) inhibition of A β ₄₀ monomers in the presence of GNPs functionalized with CP-2 at 10 μ M.



Résumé

Cette thèse se concentre principalement sur deux maladies neurologiques : la sclérose en plaques et la maladie d'Alzheimer. La première partie de la thèse aborde la sclérose en plaques récurrente-rémittente (SEP-RR), une maladie auto-immune inflammatoire chronique du système nerveux central (SNC) caractérisée par la démyélinisation et l'inflammation. Deux molécules, l'interféron- β (IFN- β) et le XBD173, ont été utilisées pour traiter l'encéphalomyélite auto-immune expérimentale (EAE), un modèle murin de la SEP-RR. L'association de 10 mg/kg de XBD173 avec une dose plus faible d'IFN β exerce un effet synergique, entraînant une diminution des scores cliniques chez les souris atteintes de SEP récurrente-rémittente (RR).

La deuxième partie de la thèse porte sur la maladie d'Alzheimer (MA), un trouble neurodégénératif progressif. Au niveau des plaques amyloïdes d'AD, une forte distribution est observée dans la région 1366–1395 cm^{-1} , tandis que l'augmentation observée dans la région 1087–1113 cm^{-1} est attribuée à l'élongation symétrique du groupement PO_3^- . Les nanoparticules d'or (GNP), en combinaison avec le CP, ont permis de désagréger les fibrilles d'A β 40. En outre, les GNP fonctionnalisées avec le CP-2 ne ramènent pas l'A β 40 fibrillaire à sa forme monomérique initiale au cours du processus de désagrégation.

Mots clés : Sclérose en plaques (SEP), Interféron- β (IFN- β), XBD173, Maladie d'Alzheimer (MA), Peptides cycliques (PC), Amyloïde bêta (A β)

Résumé en anglais

This thesis focuses primarily on two neurological disorders: Multiple Sclerosis and Alzheimer's disease. The first part of the thesis addresses relapsing-remitting multiple sclerosis (RRMS), a chronic autoimmune disease of the central nervous system (CNS) characterized by demyelination and inflammation. Two molecules, interferon- β (IFN- β) and XBD173, have been used to treat experimental autoimmune encephalomyelitis (EAE), a mouse model of RRMS. A combining 10 mg/kg XBD173 with a lower dose of IFN β exerts a synergistic effect, resulting in lower clinical scores in RRMS mice.

The second part of the thesis focuses on Alzheimer's disease (AD), a progressive neurodegenerative disorder. At the AD plaque position, a strong distribution is observed in the 1366–1395 cm^{-1} region, and the increase in the 1087–1113 cm^{-1} region is attributed to PO_3^- symmetric stretching. Gold nanoparticles (GNP) in combination with the CP was able to disaggregate the fibrillar A β 40. Furthermore, GNP-functionalized with CP-2 does not revert fibrillar A β 40 back to the initial monomeric form during the reaction during the disaggregation process.

Keywords : Multiple sclerosis (MS), Interferon- β (IFN- β), XBD173, Alzheimer's disease (AD), Cyclic peptides (CP), Amyloid-beta (A β)