Modelling and Analyzing Systems Biology Using Process Algebra

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MODÉLISATION ET ANALYSE DE PROCESSUS BIOLOGIQUES DANS DES ALGÈBRES DE PROCESSUS

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Abstract

The focus of this thesis is on modelling and analyzing systems biology using process algebra. We apply three process calculi into systems biology: the π-calculus and a variant, the $I\pi$-calculus; the κ-calculus and its finer-grained language, the $m\kappa$-calculus; and the *bigraphical reactive systems*.

There are three parts of my thesis. In Chapter 3 of the thesis we introduce the signal transduction with aberrance. A new extension of the π-calculus, the $I\pi$-calculus, is introduced to model signal transduction with aberrance. The calculus is obtained by adding two aberrant actions into the π-calculus. It is well-defined and biologically visible.

The $I\pi$-calculus shows its expressive capability. However, one may need to record more information about its terms in the process of simulation, especially in the simulation of aberrant biochemical processes. Therefore, two auxiliary systems, a tag system and a typing system, are introduced to help understanding the $I\pi$-calculus model. The tag system is more intuitive. But it may be redundant in the recordings of information of terms. The simple typing system, however, is enough to deal with it. We show that the tag system is equal to the typing system in terms of expressive power.

In Chapter 4 of the thesis we propose a rigorous account of self-assembly in the protein-protein language, κ-calculus introduced by Vincent Danos and Cosimo Laneve. We make use of reversible rules to embed the κ-calculus into a finer-grained language, the $m\kappa$-calculus. We prove that this simulation is correct mathematically.

In Chapter 5 of the thesis we use *bigraphs* to model and analyze systems biology. First we give an example to show how to model the biochemical processes using *Bigraphical reactive systems* (BRSs for short). We take the normal *ras* activation as our instance. Then the expressive power of the bigraphical models is discussed. We indicate how the κ-calculus, the protein-protein language, can be translated into BRSs by one example, which indicates that BRSs can be a suitable model in biological studying as well.

In summary, we give three process calculi to model systems biology. We extend the π-calculus to model the aberrant signal transduction; prove the correctness of self-assembly in κ-calculus; and make an attempt of modelling *ras* activation using BRSs. These results lay out some foundations for future interdisciplinary study of systems biology and process algebra. They also highlight the robustness of process algebra in modelling and analyzing systems biology.

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Main Notations

Below are the important notations used in this thesis, with the section number of their first appearance.

Metavariables		
$a, b, \cdots$	actions	2.1.1
$fn(\cdot)$	free names	2.2.1
Process constructions		
0	inaction	2.1.1
π	prefix	2.1.1
$P_1 + P_2$	nondeterministic choice	2.1.1
$P_1 \mid P_2$	parallel composition	2.1.1
$(\nu x)P$	restriction	2.1.1
$A(\rho)$	proteins	2.2.1
S, S'	solutions	2.2.1
$\mathfrak{S}$	reaction systems	2.2.1
$\Gamma, \Delta, \cdots$	environments	3.5.1
$\mathsf{I}, \mathsf{J}, \cdots$	interfaces	2.3.1
$\mathbf{K}$	constant	3.3.1
$K\lfloor \tilde{a} \rfloor$	constant application	3.3.1
$i_a, i_b, \cdots$	tags of actions	3.4.1
$I_P, I_Q, \cdots$	tags of processes	3.4.1
$M, N, \cdots$	terms	3.5.1
$[\![\mathsf{R}]\!]_g$	graphic-on-sites	4.3.1
$\mathsf{K}, \mathsf{L}, \cdots$	controls	5.1.1

Miscellaneous symbols

$\S$	sucide capability	3.3.1
$\sharp$	propagation capability	3.3.1
$\uplus$	disjoint union	3.4.1
$\mathfrak{r}$	κ-reactions	4.3.1
$[\![\cdot]\!]_m$	translation from κ to $m\kappa$	4.3.1
$\Box$	bigraphs	5.1.1

Relations

$\longrightarrow$	reduction rule	2.1.1
$\leq$	growth relation	2.2.1
$\longrightarrow_\kappa$	κ-transition	2.2.1
$\models$	matching	2.2.1
$\vdash$	type assertions	3.5.1
$\succ$	signal ordering	4.3.1
$\rightleftharpoons$	reversible translations	4.3.1
$\equiv$	structural congruence	2.1.1
$\longrightarrow_c$	translation relation	4.4
$\longrightarrow_{m\kappa}$	$m\kappa$-transition	4.4

Contents

Chapter 1

Introduction

Concurrency theory, as an area of research in computer science, emerged in the early seventies of the last century. It is concerned with the modelling and verification of concurrent systems which can be viewed as a collection of sequential processes, possibly running on different processors, that interact and exchange results with each other and with the external environment [RDN96]. *Process algebra* (or *process calculi*) is a subarea in concurrency theory. Origins are traced back to the early eighties of the twentieth century, and developments since that time are surveyed in [Bae04]. Process algebra is an algebraic approach to the study of concurrent processes. Its tools are algebraic languages for the specification of processes and the formulation of statements about them, together with calculi for the verification of these statements.

Systems biology [Kit01] is the study of an organism, viewed as an integrated and interacting network of genes, proteins and biochemical reactions which give rise to life. Instead of analyzing individual components or aspects of the organism, such as sugar metabolism or a cell nucleus, systems biologists focus on all the components and the interactions among them, all as part of one system [Wol]. These interactions are ultimately responsible for an organisms and functions. For example, the immune system is not the result of a single mechanism or gene. Rather the interactions of numerous genes, proteins, mechanisms and the organisms external environment, produce immune responses to fight infections and diseases.

Due to the nature of biological systems and concurrent systems, several authors have argued that process calculi could be the right abstraction to support *dynamic* bioinformatics and open new scenarios in the computer science and biology research [Car04e, Car05a, Car05b, DL04, RS, RS02].

1.1 Background

Systems biology has become more and more popular in the last few years. Systems biology is systems-level understanding of biological systems that takes into account complex interactions of gene, protein, and cell elements [BB01, MM87]. It aims

to integrate high-throughput biological studies to understand how biological systems function by studying the relationships and interactions between various parts of a biological system [LBZ+00a, LMF+00] (e.g. metabolic pathways, organelles, cells, physiological systems, organisms etc.)

In short, systems biology is defined as an approach to biology where organisms and biological processes should be analyzed and described in terms of their components and their interactions in a framework of mathematical models.

Systems biology begins with the insight that biological processes must be understood in terms of the components that participate in the processes, and that the complexity of biological systems makes it difficult to understand the workings of the system by simple qualitative arguments. Mathematically strict models must be formulated. This is required both in order to be able to capture the actual behavior of the system with acceptable precision, but also to be able to analyze the fundamental behavior of the system [Pri05].

There exist some models of the whole system so far. The models may be very simple (Boolean on/off) [RV90, PRS98], or very complex (including detailed descriptions of interactions at a molecular level) [Pau01, Pau02]. The important issue is that it should be possible to analyze the model, either by some mathematical approach, or to simulate it, in order to evaluate its correspondence with the observed facts.

As we mentioned, one important goal of systems biology is to understand life processes in sufficient detail to make predictions about their behavior. How to do it? A general biomolecular system is made up of bio-components which are taken as computational devices. The whole system achieves its function by the interactions among these components. All of these characteristics are analogous to the properties of process algebra.

The term *process algebra* was coined in 1984 by Bergstra and Klop [BK84]. A process algebra is a structure in the sense of universal algebra that satisfies a particular set of axioms. It offers description techniques to model a complex computing system, which is involving communicating and concurrently executing components. It mixes the areas of computer science and discrete maths, including system design notations, logic, concurrency theory, specification and verification, operational semantics, algorithms, complexity theory, and, of course, algebra. It develops a behaviorial theory of computing processes and allows for description and verification techniques in the same formal system.

CCS (Calculus of Communicating Systems) [Mil89], *CSP* (Communicating Sequential Processes) [Hoa85] and *ACP* (Algebra of Communicating Processes) [BK84, BK92], were proposed in the 1980's for describing and analyzing concurrent systems, and became the most successful process algebras. All of them were built around the central idea of interaction or communication between processes. In these formalisms, complex systems are built from simple subcomponents structurally, by a small set of primitive operators.

CCS [Mil80, Mil89] considers the problems caused by non-determinism. *CSP* [Hoa85] does away completely with global variables, and adopts the message passing paradigm of communication.

The limitation of these traditional process algebras is that they are not able to effectively specify mobile systems, i.e., systems with a dynamically changing configuration of communication links .

Milner, Parrow and Walker developed the π-calculus [Mil99, MPW92] on the basis of *CCS*, which achieves mobility by a powerful name-passing mechanism. The π-calculus [SW01] aims at the challenge of defining an underlying model, with a small number of basic concepts, in terms of which *interactional* behavior can be rigorously described.

In a word, the π-calculus includes the syntax which attempts to systemize descriptive grammar and the semantics which offer explanations of systematic relationship. The dynamic is introduced into the π-calculus by allowing dynamic creation of processes and for names to be passed among different processes, which is also one of the reasons why the π-calculus is a suitable model for systems biology.

A variant of the π-calculus, called the stochastic π-calculus [Pri95] is developed to be applied in the biological domain [Reg01, PC]. It essentially selects the enabled action to be performed according to the Gillespie algorithm [Gil76, Gil77] developed to simulate chemical reactions. Preliminary results in this field have been obtained in modelling a set of interesting biological systems and some analysis and simulation have been carried out [RSS00, RSS01, PRSS01, Reg01].

Applying existing calculi defined with computer systems to systems biology is a common strategy. However, some researchers have adopted the opposite strategy. They have defined calculi which come from biology so that they are better suited to modelling, analyzing and simulating living systems, for instance, see [Car04a, Car04b, Car04c, Car04d, DL04]. Then we can apply the new family of calculi to computer systems to see whether the bio-mimetic approach can further inspire and enhance our comprehension of how computer artificial systems can be modelled, designed and implemented.

The κ-calculus [DL04, DK03] is one kind of these process calculi. It aims at idealizing protein-protein interactions, essentially as a particular restricted kind of graph rewriting operating on graphs-on-sites. Biological reactions are modelled by two kinds of rewriting rules: one is monotonic and the other is antimonotonic. The former represents complexation, and the latter represents decomplexation.

Bigraphical reactive systems (BRSs for short) is a model for computer systems with mobile placing and linking [JM04, Mil01a, Mil01b, Mil05a, Mil05b, Mil]. It aims to unify calculi such as the π-calculus, *Petri nets* [Mil04] and so on. It models spatial activity as well.

BRSs are graphical models of computation which capture the properties of *locality* and *connectivity* [JM03, JM04]. They are reconfigurable as well since the nodes in graphs may represent a great variety of computational objects.

1.2 Objectives of Thesis

This thesis focuses on modelling and analyzing of systems biology. It is very important to make abstractions for modelling and analyzing the dynamic evolution of the systems in time and space. That is, we need a behavioral theory of biomolecular systems. As we have seen, computer and biomolecular systems have some resemblance. For example, they both start from a small set of elementary components. Computers are networked to perform larger and larger computations, and cells form multicelluar organisms. Therefore, we believe that we can find an appropriate computing model to deal with the biomolecular systems.

In research of systems biology, the basic studying approach is described by the following steps;

- to build a model of biological system;
- to perform experiments to test this model;
- to modify this model according to the results of experiments;
- to use this model.

Since concurrent processes are analogous to biological processes , it is worthy of making an attempt for modelling systems biology using processes calculi.

In this thesis, we apply three process calculi into systems biology.

(1) The π-calculus is suitable to model various molecular systems, including transcriptional circuits, signal transduction and metabolic pathways etc. [RS]. However, the modelling of cases with aberrance in molecular systems was not considered so far. We extend this calculus by adding two aberrant actions into the π-calculus. The new calculus, called the $I\pi$-calculus, is applied to model aberrant biochemical processes. Actually, to study the aberrant biochemical processes is important. For example, aberrant signal transduction is the cause of many diseases challenged by modern medicine, including cancers, inflammatory diseases, cardiovascular disease and neuropsychiatric disorders.

We use the following techniques for investigation in this thesis.

(a) To make an abstraction from the real biological system using our language. As the other processes calculi, our model should include a syntax, a semantics and satisfy some algebraic properties.

(b) To introduce auxiliary systems to help understand the model.
Besides making the model more clear, we hope that such auxiliary systems will be useful to prove much stronger properties in the future study, for example, the qualitative analysis, as well.

(c) Our model should be dynamic, i.e. it should be evolutive in view of deeper studying.

(2) The κ-calculus is a protein-protein language introduced by Vincent Danos [DK03]. We focus on its property of self-assembly. Self-Assembly is a very important property in biology. It is a method of integration in which the components spontaneously assemble, typically by bouncing around in a solution or gas phase until a stable structure of minimum energy is reached. The κ-calculus captures this property by means of a translation into a finer-grained language, the $m\kappa$-calculus [DL04].

In this thesis, we mainly study the correctness of self-assembly.

(a) The graphical explanation of the monotonic protocol of integration:
the graphical explanation makes it easier to understand how to divide one biochemical reaction into several basic reactions (or integrate one biochemical reaction from several basic reactions).

(2) The proof of correctness:
we have to prove that the translation process implements higher-level reactions correctly by means of the simple, local interactions of the $m\kappa$-calculus. To prove it, we need to know its mathematical structure and properties.

(3) *Bigraphical reactive systems* (BRSs for short) were first introduced by Robin Milner etc. [JM03, JM04, Mil, Mil05b, Mil05a, Mil04]. The bigraphical model aims at offering further generality both in the treatment of mobility and in behavioral theory. Therefore, BRSs can be applied into systems biology. As a general model, it not only has general properties of models, but also has its particular properties.

We give one biological example to show the expressive power of the bigraphical models in this thesis.

We argue that processes calculi can provide the much-needed abstraction for biomolecular systems. Based on the studying of these three process calculi in this thesis, we know:

(1) process calculi can be used to model biomolecular systems;

(2) process calculi can simulate the behavior of biomolecular systems;

(3) each process calculus can capture special properties of biomolecular systems;

(4) there exists one general model which includes the other models of the other process calculi;

(5) our models are configurable, extensible according to the different needs of biological studying.

1.3 Some Provisos

In this thesis, we make some tacit assumptions about our models. Actually the real biomolecular systems are very complicated. Our goal is to grasp some special properties of biomolecular systems. If the model is too complicated, we cannot get any good properties. So we need to simplify our theoretical models. The level of abstraction in the models are different. For example, the π-calculus is based on the level of functional domains of proteins, the κ-calculus id based on the level of proteins, and BRSs are based on different levels according to our assumptions.

In this thesis, we follow some principles in order to simplify models.

(1) Decision of the level of modelling: this is the first step of modelling.
For example, if we work at the level of the functional domains of proteins, our biological reactions are reactions of domains; if we work at the level of proteins, our biological reactions are those of proteins; if we work at the level of complexes, our biological reactions are those of complexes.

(2) Simplification of components: once we decide some kind of units as the level of modelling, we take them as primitive processes. We ignore or simplify the smaller units which are lower than this level. We also ignore the other parts in larger units except primitive processes.
For example, if proteins are taken as primitive processes, we won't consider the amino acids which constitute proteins; if the domains of proteins are taken as primitive processes, we regard a protein as a group of its domains. If we focus on complexes of proteins, we regard a complex as a group of proteins, regardless of the domains of proteins.

(3) Simplification of reactions: the reactions in biomolecular systems are taken as the binding (or interactions) of computing processes.
For example, in the κ-calculus, we take monotonic reactions as bindings of solutions; in the $I\pi$-calculus, we take reactions as interactions between processes; in BRSs, we take reactions as the change of bigraphs.

(4) We do not consider the factors of environments, including the temperature, consistency, time and so on. We just consider the possibility of reactions between two (or more) of entities.

1.4 Outline of the Thesis

The material presented in Chapter 2 is meant to prepare the technical development in the rest of the thesis. We introduce some basic notions about process calculi, with the π-calculus , the κ-calculus and *bigraphs* as our templates. We then focus on the biochemical process; we review signal transduction.

In Chapter 3, we introduce a calculus for formal molecular processes. We focus on the signal transduction with aberrance. The model for normal signal transduction is in [RSS00] [RSS01]. An extended calculus, $I\pi$-calculus, is given to model the signal transduction with aberrance. The calculus is obtained by adding two aberrant actions into the π-calculus. It is well-defined and biologically visible.

The $I\pi$-calculus shows its capability of description. However, one may need to record more information about its terms in the process of simulation, especially, in the simulation of aberrant biochemical processes. Therefore, two auxiliary systems, a tag system and a typing system are introduced to help understand the $I\pi$-calculus model. The tag system is easy to understand, more intuitive. But it would be redundant in the recordings of information of terms. The typing system, however, is simple enough to deal with it.

In the end of this chapter, we show that the tag system is equal to the typing system in terms of expressive power.

In Chapter 4, we introduce the language for formal proteins, κ-calculus. In the κ-calculus, reactions are modelled at the proteins level. The κ-model is well-defined and biological visible. A finer-grained language, $m\kappa$-calculus, is introduced as well. In the $m\kappa$-calculus, reactions are restricted to at most binary interactions.

Self-assembly is an important property in biology. In the κ-calculus, we propose propose a rigorous account of self-assembly. We construct one monotonic reversible protocol to embed the ka-calculus into a finer-grained language, the mka-calculus. Some mathematical properties are discussed. We prove that this simulation is correct mathematically.

In Chapter 5, we recall the basic informal notions of bigraphical reactive systems [JM04, JM03]. The example of *ras* activation is given, which shows that the description capability of bigraphical reactive systems is powerful.

We can use this general model it based on our practical needs. On the one hand, we can model different objects as bigraphs. For instance, we can take proteins as bigraphs if we need to study; we also can take the proteins as nodes if we need to know more about proteins. On the other hand, we can simplify our model. For instance, as one goal of experiments, we just want to know the connection among the proteins, we can only consider the link graph, while if we want to know the information of locality of reactants, we can consider the place graph.

As we have mentioned, bigraphical reactive systems are a general model. And some process calculi can be translated into it. In the end of this chapter, we illustrate through an example how the κ-calculus, the protein-protein language, can be translated into BRSs, which shows that the bigraphical reactive system is suitable model in biological studying as well.

In Chapter 6, we summarize the contributions of this thesis and discuss some directions for potential future work.

Provenance of the material This thesis is partially based on published material (mainly in Chapter 3). The presentation of the $I\pi$-calculus which is for describing

the aberrant signal transduction appeared in [ZLF04]; the simple typing system on this calculus with its properties appeared in [ZLF05]; and an analysis of a biological property for an aberrant signal (*the aberrant protein ras*) in this calculus appeared in [ZLF06].

Chapter 2

Preliminaries

This chapter introduces some basic notions about process calculi. Process calculi are our language tools to model systems biology. They are used in the following chapters. For more details, see [MPW92, Mil99, DL04, JM04]. Some knowledge about signal transduction in systems biology is introduced as well. Especially, we introduce the well-studied signal transduction, *RTK-MAPK*. Activation of the protein *ras* will be our main biological example in this thesis. For more details about signal transduction, see [LBZ+00b, Pta02, VV95, Wol, Kit01].

2.1 The π-calculus

The π-calculus is a mathematical model of processes whose interconnections change as they interact. We call these processes that change their interconnections structure when they execute *mobile processes*. A program in this calculus specifies a community of interacting processes.

Intuitively, each computational process is defined by its potential communication activities and may be composed in sequence or in parallel with other processes. Communication occurs via channels, denoted by their names, that represent atomic access capabilities. Computation is modelled as synchronous binary communication between processes over their channels. The only content of messages transmitted in communication is channel name or tuples of channel names, which may be used for further communication.

2.1.1 The π-calculus: Definitions

We presuppose that an infinite set N of *names* is given. Formally, the π-calculus consists of three components:

- A *syntax* for writing formal descriptions of a concurrent system;
- An *operational semantics* consisting of reduction rules, which describe the potential changes of the system induced by communication.

- A set of *congruence laws* that determine when two syntactic expressions are equivalent.

Processes evolve by performing actions. The capabilities for actions are expressed via *Prefixes*.

Definition 2.1 (Syntax) *Prefixes and processes of the π-calculus are given by*

$$\begin{array}{lcl} \pi & ::= & \overline{a}b \mid a(x) \mid \overline{a} \mid a \\ P & ::= & 0 \mid \pi.P \mid P_1 + P_2 \mid (P_1|P_2) \mid (\nu x)P \end{array}$$

In prefixes, $\overline{a}b$ expresses the action to send the name b via the name a; and $a(x)$ expresses the action to receive any name via a; a expresses the communication on channel name a; and $\overline{a}$ expresses the communication on co-name $\overline{a}$.

We give a brief interpretation of processes. 0 is inaction; it does nothing. The process $\pi.P$ has a single capability π, moreover, the process P cannot proceed until the capability π has been exercised. The capabilities of the sum $P_1 + P_2$ are those of P_1 together with those of P_2. When a sum exercised one of its capabilities, the others are rendered void. In the composition $(P_1 \mid P_2)$, the components P_1 and P_2 can proceed independently and can interact via shared names. In the restriction $(\nu x)P$, the scope of name x is restricted to P.

Definition 2.2 (Structural congruence) *Structural congruence, $\equiv$, is the smallest congruence on processes that satisfies axioms as follows;*

$$\begin{array}{rcll} P \mid Q & \equiv & Q \mid P & \\ (P \mid Q) \mid R & \equiv & P \mid (Q \mid R) & \\ P + Q & \equiv & Q + P & \\ (P + Q) + R & \equiv & P + (Q + R) & \\ (\nu a)0 & \equiv & 0 & \\ (\nu a)(\nu b)P & \equiv & (\nu b)(\nu a)P & \\ ((\nu a)P) \mid Q & \equiv & (\nu a)(P \mid Q) & \text{if } a \notin fn(Q) \end{array}$$

Definition 2.3 (Semantics) *Reduction rules, $\longrightarrow$, are defined by Table. 2.1.*

The rule *Com-N*(communication-names) deals with the interaction in which one sends a message with a channel while the other receives a message with the same channel so that they have an interaction.The rule *Com-SN*(communication-single name) deals with the interaction on the same channel so that they have an interaction though there is nothing to send. The reduction rules are closed under summation (the rule *Sum*), composition (the rule *Comp*), restriction (the rule *Res*) and structural congruence (the rule *Stc*).

$$\text{Com-N}\ \frac{}{(\overline{a}(b).Q + R_1) \mid (a(x).P + R_2) \xrightarrow{\tau} Q|P\{b/x\}}$$

$$\text{Com-SN}\ \frac{}{(\overline{a}.Q + R_1) \mid (a.P + R_2) \xrightarrow{\tau} Q \mid P}$$

$$\text{Sum}\ \frac{P \longrightarrow P'}{P + Q \longrightarrow P'} \qquad \text{Comp}\ \frac{P \longrightarrow P'}{P \mid Q \longrightarrow P' \mid Q}$$

$$\text{Res}\ \frac{P \longrightarrow P'}{(\nu a)P \longrightarrow (\nu a)P'} \qquad \text{Stc}\ \frac{Q \equiv P, P \longrightarrow P', P' \equiv Q'}{Q \longrightarrow Q'}$$

Table 2.1: Reduction rules.

2.1.2 A Simple Example

We give a simple example to illustrate reduction rules. Consider the following process Q ;

$$Q = (a(x).b(y).0 + \overline{d}v.0) \mid \overline{c}t.0 \mid \overline{a}w.c(z).\overline{b}u.0$$

So, we have $Q \xrightarrow{\tau} \xrightarrow{\tau} \xrightarrow{\tau} 0 \mid 0 \mid 0 \equiv 0$. See Table 2.2.

$$\text{Com}\ \frac{\text{Com-N}\ \dfrac{}{(a(x).b(y).0 + \overline{d}v.0) \mid \overline{a}w.c(z).\overline{b}u.0 \xrightarrow{\tau} b(y).0 \mid c(z).\overline{b}u.0}}{(a(x).b(y).0 + \overline{d}v.0) \mid \overline{c}t.0 \mid \overline{a}w.c(z).\overline{b}u.0 \xrightarrow{\tau} b(y).0 \mid \overline{c}t.0 \mid c(z).\overline{b}u.0}$$

$$\text{Com}\ \frac{\text{Com-N}\ \dfrac{}{\overline{c}t.0 \mid c(z).\overline{b}u.0 \xrightarrow{\tau} 0 \mid \overline{b}u.0}}{b(y).0 \mid \overline{c}t.0 \mid c(z).\overline{b}u.0 \xrightarrow{\tau} b(y).0 \mid 0 \mid \overline{b}u.0}$$

$$\text{Com}\ \frac{\text{Com-N}\ \dfrac{}{b(y).0 \mid \overline{b}u.0 \xrightarrow{\tau} 0 \mid 0}}{b(y).0 \mid 0 \mid \overline{b}u.0 \xrightarrow{\tau} 0 \mid 0 \mid 0}$$

Table 2.2: The reduction of Q.

2.2 The κ-calculus

The κ-calculus was introduced by Vincent Danos and Cosimo Laneve. [DL04] It is a language of formal proteins. Reactions are modelled at proteins level, bonds

are represented by means of shared names, and reactions are required to satisfy a requirement of *monotonicity* or *antimonotonicity*. In this section, we briefly introduce basic notions about the κ-calculus.

2.2.1 The κ-calculus: Definitions

We assume an infinite countable set $\mathcal{P}$ of protein names, an infinite countable set $\mathcal{E}$ of edge names. We take a *signature* map $\mathfrak{s}$ from $\mathcal{P}$ to natural numbers $\mathbb{N}$.

Let $A, B, \cdots$ range over protein names and $x, y, \cdots$ range over edge names. For each protein name A, $\mathfrak{s}(A)$ is the number of sites of A, and for any $1 \leqslant i \leqslant \mathfrak{s}(A)$, the pair (A, i) will accordingly be called a *site* of A.

An *interface* is a partial map from $\mathbb{N}$ to $\mathcal{E} + \{h, v\}$ usually ranged over by ρ, σ and similar symbols. The domain and range of an interface ρ will be respectively denoted by $\mathtt{dom}(\rho)$ and $\mathtt{ran}(\rho)$, and the set of names free in ρ, written $\mathrm{fn}(\rho)$, is obtained as $\mathtt{ran}(\rho) \cap \mathcal{E}$. We will only ever deal with interfaces with finite domain. The empty interface will be denoted $\varnothing$.

Definition 2.4 (Solutions) *Solutions of κ-calculus are defined as follows:*

$$S ::= 0 \mid A(\rho) \mid S, S \mid (\mathtt{new}\ x)(S)$$

Intuitively, the constructs of the κ-calculus solutions have the following meaning: 0 is the empty solution. The protein $A(\rho)$ with $A \in \mathcal{P}$ and ρ an interface with domain $s(A)$ is the primitive solution. A group of solutions S, S' is a complex of simple ones. In the restriction solution $(\mathtt{new}\ x)(S)$ with $x \in \mathcal{E}$, the new operator "$\mathtt{new}$" is a binder and S is the *scope* of the binder $(\mathtt{new}\ x)$.

The set $fn(S)$ of free names is defined inductively as follows;

$$\begin{array}{rcl} \mathrm{fn}(0) & \equiv & \varnothing \\ \mathrm{fn}(A(\rho)) & \equiv & \mathrm{fn}(\rho) \\ \mathrm{fn}(S, S') & \equiv & \mathrm{fn}(S) \cup \mathrm{fn}(S') \\ \mathrm{fn}((x)(S)) & \equiv & \mathrm{fn}(S) \backslash \{x\} \end{array}$$

Next we introduce an equivalence relation between solutions, called the *structural congruence*.

Definition 2.5 *Structural congruence, written $\equiv$, is the least equivalence closed under syntactic constructions, containing α-equivalence (injective renaming of bound variables), taking "," to be associative (as the choice of symbol suggests) and commutative, with 0 as neutral element, and satisfying the scope laws:*

$$\begin{array}{rcll} (x)(y)(\mathsf{S}) & \equiv & (y)(x)(\mathsf{S}), & \\ (x)(\mathsf{S}) & \equiv & \mathsf{S} & \textit{when } x \notin \mathrm{fn}(\mathsf{S}), \\ (x)(\mathsf{S}),\ \mathsf{S}' & \equiv & (x)(\mathsf{S},\ \mathsf{S}') & \textit{when } x \notin \mathrm{fn}(\mathsf{S}'). \end{array}$$

$$\text{create}\ \frac{x \in \tilde{x}}{\tilde{x} \vdash \imath \leq \imath^x}$$

$$\text{hv–switch}\ \frac{}{\tilde{x} \vdash \overline{\imath} \leq \imath} \qquad \frac{}{\tilde{x} \vdash \imath \leq \overline{\imath}}\ \text{vh–switch}$$

$$\text{reflex}\ \frac{\tilde{x} \cap \mathrm{fn}(\rho) = \varnothing}{\tilde{x} \vdash \rho \leq \rho} \qquad \frac{\tilde{x} \vdash \rho \leq \sigma \qquad \tilde{x} \vdash \rho' \leq \sigma'}{\tilde{x} \vdash \rho + \rho' \leq \sigma + \sigma'}\ \text{sum}$$

Table 2.3: The growth relation

We now construct the *growth relation* on partial interfaces. This relation is parameterized by a set of names, written $\tilde{x}$ below, which represent edges grown out by a reaction. It is written $\leq$ and is defined inductively by the clauses given in Table 2.3.

Similarly, we can extend the growth relation to groups of pre-proteins ($A(\rho)$ is a pre-protein if ρ is a partial interface of A, namely, $dom(\rho) \subseteq \mathfrak{s}(A)$.) as shown in Table 2.4.

$$\text{nil}\ \frac{}{\tilde{x} \vdash 0 \leq 0} \qquad \frac{\tilde{x} \vdash \mathsf{S} \leq \mathsf{T} \qquad \tilde{x} \vdash \rho \leq \sigma \qquad \mathrm{dom}(\sigma) \subseteq \mathfrak{s}(A)}{\tilde{x} \vdash \mathsf{S}, A(\rho) \leq \mathsf{T}, A(\sigma)}\ \text{group}$$

$$\frac{\tilde{x} \vdash \mathsf{S} \leq \mathsf{T} \qquad \mathrm{fn}(\sigma) \subseteq \tilde{x} \qquad \mathrm{dom}(\sigma) = \mathfrak{s}(A)}{\tilde{x} \vdash \mathsf{S} \leq \mathsf{T}, A(\sigma)}\ \text{synth}$$

Table 2.4: Extended growth relation

Definition 2.6 (graph-likeness) *A solution* S *is said to be graph-like if:*
— free names occur at most twice in S*;*
— binders in S *bind either zero or two occurrences.*

Definition 2.7 (Connectedness) *We define inductively when a term is* connected*:*
— $A(\rho)$ *is connected;*
— if S *is connected so is* $(x)(\mathsf{S})$*;*
— if S *and* S' *are connected and* $\mathrm{fn}(\mathsf{S}) \cap \mathrm{fn}(\mathsf{S}') \neq \varnothing$ *then* S, S' *is connected;*
— if S *is connected and* $\mathsf{S} \equiv \mathsf{T}$ *then* T *is connected.*

Definition 2.8 *Let* $[\![\cdot]\!]_g$ *be the following function from graph-like solutions to graphs with sites:*

1. $[\![A(\rho)]\!]_g$ *is the graph with a single node labeled* A*, sites in* $\{1, \ldots, \mathfrak{s}(A)\}$*, bound sites* k *being labeled by* $\rho(k)$*, and free sites being in the state prescribed by* ρ*;*

2. $[\![S, S']\!]_g$ *is the union graph of* $[\![S]\!]_g$ *and* $[\![S']\!]_g$*, with sites labeled with the same name being connected by an edge, and their common name erased;*

3. $[\![(x)(S)]\!]_g$ *is* $[\![S]\!]_g$*.*

Definition 2.9 (Reactions) *Let* L, R *be two pre-solutions,*

- $L \to (\tilde{x})R$ *is said to be a* monotonic reaction *if:*
  — $\tilde{x} \vdash L \leq R$,
  — *both* L *and* $(\tilde{x})R$ *are graph-like,*
  — *and* R *is connected.*

- $(\tilde{x})L \to R$ *is said to be an* anti-monotonic reaction *if:*
  — *its dual* $R \to (\tilde{x})L$ *is monotonic.*

A reaction which is either monotonic or antimonotonic is called a biological *reaction and* L *and* R *are referred to respectively as its* reactant *and* product*.*

Definition 2.10 (matching) *Given a monotonic reaction* $L \to (\tilde{x})R$*, with:*
— $L = A_1(\rho_1), \ldots, A_n(\rho_n)$
— *and* $R = A_1(\sigma_1), \ldots, A_m(\sigma_m)$*,*
one says that a pair of solutions S, T matches $L \to (\tilde{x})R$*, written* $S, T \models L \to (\tilde{x})R$*, if there exists a renaming* r *and partial interfaces* $\xi_1, \ldots, \xi_m$ *such that:*

1. *for all* i*,* $r(\tilde{x}) \cap \mathrm{fn}(\xi_i) = \varnothing$*,*

2. $S = A_1(r \circ \rho_1 + \xi_1), \ldots, A_n(r \circ \rho_n + \xi_n)$
   and $T = (r(\tilde{x}))(A_1(r \circ \sigma_1 + \xi_1), \ldots, A_m(r \circ \sigma_m + \xi_m))$*.*

Matching is defined by symmetry for anti-monotonic rules, that is $S, T \models (\tilde{x})L \to R$ *if and only if* $T, S \models R \to (\tilde{x})L$*.*

Definition 2.11 *Let* $\mathfrak{R}$ *be a set of biological reactions, the associated* $\mathfrak{R}$-system *is the pair* $(\mathfrak{S}, \to)$*, where* $\mathfrak{S}$ *is the set of solutions and* $\to_\kappa$*, called the* transition *relation, is the least binary relation over* $\mathfrak{S}$ *such that:*

$$\text{mon}\ \frac{S, T \models L \to_\kappa (\tilde{x})R \ \in \mathfrak{R}}{S \to_\kappa T} \qquad \frac{S, T \models (\tilde{x})L \to_\kappa R \ \in \mathfrak{R}}{S \to_\kappa T}\ \text{antimon}$$

$$\text{new}\ \frac{S \to_\kappa T}{(x)(S) \to_\kappa (x)(T)} \qquad \frac{S \to_\kappa T}{S, S' \to_\kappa T, S'}\ \text{group}$$

$$\text{struct}\ \frac{S \equiv S' \quad S' \to_\kappa T' \quad T' \equiv T}{S \to_\kappa T}$$

In the rest of the thesis, we call solutions in the κ-calculus *κ-solutions*, which are different from solutions in the $m\kappa$-calculus ($m\kappa$-solutions)(see Chapter 4.2)).

2.2.2 Simple Examples

We use ovals to represent proteins; the rings on the ovals to represent free sites; dots with links to represent bounded sites. The links with names x, y represent edges connecting two sites.

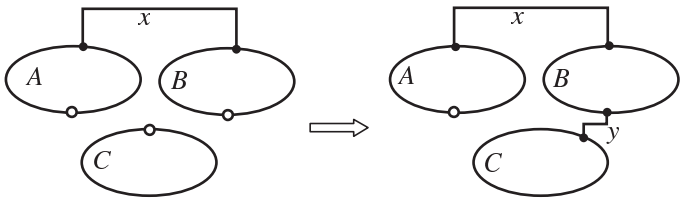


Figure 2.1: A monotonic κ-reaction.

Example 1 Fig. 2.1 shows a monotonic κ-reaction. The monotonic κ-reaction in Fig. 2.1 creates a new edge y. The formal expression can be written as follows:

$$A(1^x + 2), B(1^x + 2), C(1) \rightarrow (z)(A(1^x + 2), B(1^x + 2^z), C(1^z))$$

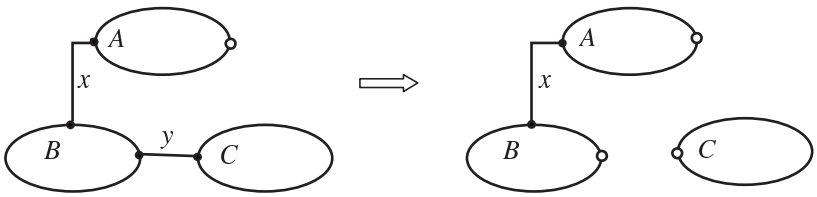


Figure 2.2: An antimonotonic κ-reaction.

Example 2 Fig. 2.2 shows an antimonotonic κ-reaction. The antimonotonic κ-reaction in Fig. 2.2 dismisses the edge y. The formal expression can be written as follows:

$$(xy)A(1^x + 2), B(1^x + 2^y), C(1^y) \rightarrow (x)(A(1^x + 2), B(1^x + 2), C(1))$$

Example 3 Fig. 2.3 shows a κ-reaction. The formal expression can be written as follows:

$$A(1^x + 2), B(1^x + 2), C(1) \rightarrow (y)(A(1 + 2), B(1 + 2^y), C(1^y))$$

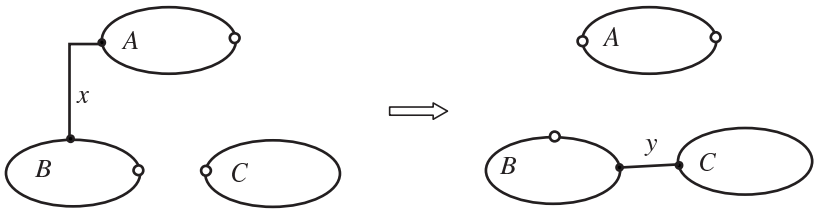


Figure 2.3: A κ-reaction.

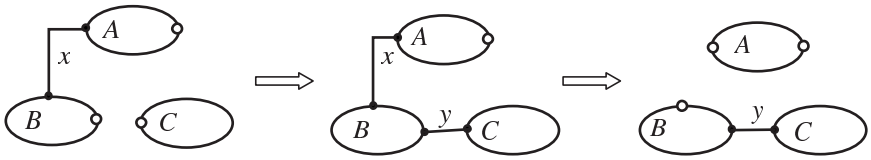


Figure 2.4: The decomposition of the κ-reaction in Fig. 2.3.

In fact, this κ-reaction can be decomposed as a monotonic κ-reaction followed by an antimonotonic κ-reaction 2.4.

$$A(1^x + 2), B(1^x + 2), C(1) \to (y)(A(1^x + 2), B(1^x + 2^y), C(1^y))$$

$$(x)A(1^x + 2^y), B(1^x + 2^y), C(1) \to (A(1 + 2), B(1 + 2^y), C(1^y))$$

Because the intermediate product which this decomposition is making explicit connected, it seems reasonable to consider the sequence as a synchronous composition.

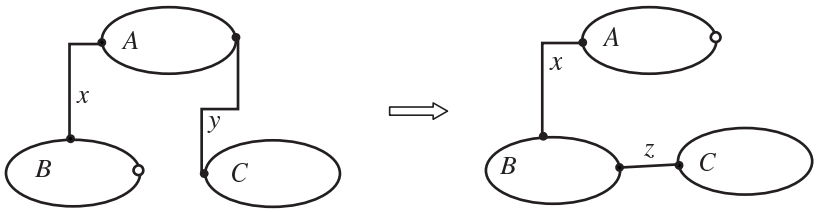


Figure 2.5: An edge-flipping κ-reaction.

Example 4 Fig. 2.5 shows an edge-flipping κ-reaction. The formal expression can be written as follows:

$$A(1^x + 2^y), B(1^x + 2), C(1^y) \to (z)(A(1^x + 2), B(1^x + 2^z), C(1^z))$$

It is different from Example 3. Since there is no free site for C to bind with B, this reaction only is decomposed as an antimonotonic κ-reaction and a monotonic one 2.6.

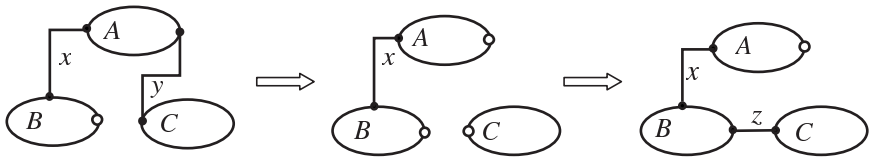


Figure 2.6: The decomposition of an edge-flipping κ-reaction in Fig. 2.5.

$$A(1^x + 2^y), B(1^x + 2), C(1^y) \rightarrow (A(1^x + 2), B(1^x + 2), C(1))$$

$$A(1^x + 2), B(1^x + 2), C(1) \rightarrow (z)(A(1^x + 2), B(1^x + 2^z), C(1^z))$$

We notice that there is no intermediate product which is connected.

2.3 Bigraphical Reactive Systems

Bigraphical reactive systems (BRSs) were introduced by Robin Milner and Ole Høgh Jensen [JM03, JM04]. A bigraphical reactive system involves *bigraphs*; it also allows bigraphs to configure themselves. *BRS*s are graphical models of computation in which both *locality* and *connectivity* are prominent. Actually, *BRS*s aim to provide a uniform way to model spatially distributed systems that both compute and communicate.

2.3.1 Bigraphs

A bigraph, just as its name implies, involves two graphs. One is the *place graph* in which the nesting of nodes represents locality. The other is the *link graph* in which the edges connect nodes.

Bigraphs have the following features . First, nodes may occur inside other nodes in bigraphs, so a bigraph has depth. Second, nodes have *ports* that may be connected by *links*, so a bigraph has connectivity. So far, we have two kinds of structure in bigraphs, *place graphs* and *link graphs*. A *Place graph* is the nesting structure of nodes. A *Link graph* is the linked structure of links which is independent of locality.

Bigraphs have another feature, that is, the notion of holes. The holes in bigraphs denote places at which other bigraphs can be inserted.

Bigraphs are taken as arrows of one kind of precategory. Actually, every bigraph is parametric in general. It has *inner* faces (written as I) with its parameter(s) and

outer faces (written as J) indicating kinds of hole(s) in which it, in turn, may be replaced.

Definition 2.12 (Interface) *Interfaces have the form* $< m, \vec{X}, X >$*, where* m *is the depth (the number of sites),* X *is the set of names, and* $\vec{X}= (X_0, X_1, ...X_{m-1})$ *is a vector of* m *disjoint subsets of* X *indicating the local names associated with each site. Names in* X *but not in* $\vec{X}$ *are global names.*

In Fig 2.7, I $=< 3, < \{u\}, \{v\} >, \{u, v\} >$. Since there are trees in which only three bigragph can be inserted, $m = 3$. Since there are two local names u and v on two holes respectively. There is no inner name any more, $\vec{X}=< \{u\}, \{v\} >$ and $X = \{u, v\}$. J $=< 1, < \varnothing >, \{x, y, z\} >$. Since it can be inserted in the bigger bigraph as a bigraph, $m = 1$. Since three names x,y and z are free names which can be used to link to another bigraph, $X = \{x, y, z\}$ and $\vec{X}=< \varnothing >$ because there is no local name.

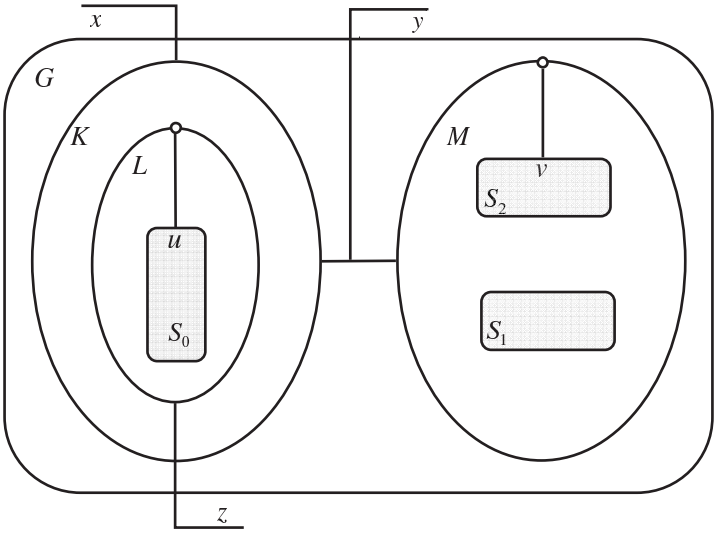


Figure 2.7: A simple bigraph.

Bigraph is a map from innerface I to outerface J. I and J are interfaces defined in Definition 2.12.

For instance, in Fig 2.7, this bigraph is represented formally as;

$$G :< 3, < \{u\}, \{v\} >, \{u, v\} > \longrightarrow < 1, < \varnothing >, \{x, y, z\} >$$

2.3.2 A Simple Example

Fig 2.7 shows a simple bigraph. Each node is assigned a *control*, such as K, L, G and M, which tell us what bigraphical reactive system kind of node it is. Each control has an *arity*, a finite ordinal. For instance, the control K has arity two. The

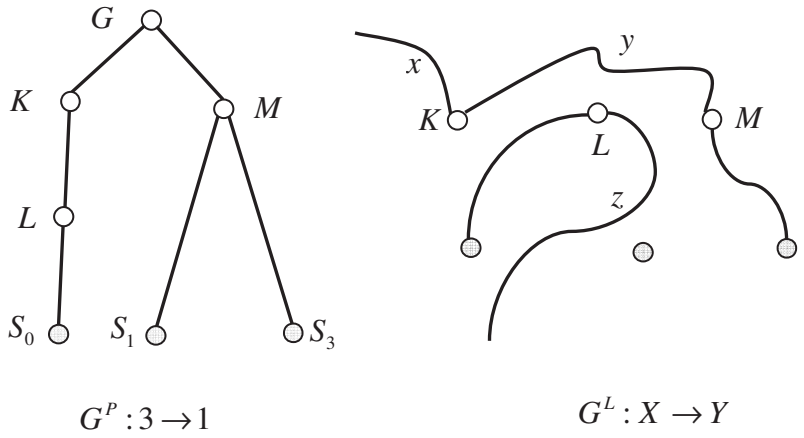


Figure 2.8: The *place graph* and the *link graph*.

names x and y denote links which allow a bigraph to be linked into larger bigraph. The grey box represents a hole where another bigraph may be inserted.

As we have mentioned, this bigraph can be divided into two graphs, the *place graph* and the *link graph*; see Fig. 2.8. The place graph and the link graph share a node set, but are otherwise independent structures.

2.4 Systems Biology

In this section, we give an informal introduction to *signal introduction* from the view of biology. Then the well-studied signal transduction, *RTK-MAPK*, in which the protein *ras* is activated, is introduced informally. The activation of the protein *ras* will be taken as our main example in the following chapters.

2.4.1 Signal Transduction

Signal transduction is a biological system at the cellular level. It refers to the movement of signals from outside the cell to inside. The movement of signals can be simple, like that associated with receptor molecules of the acetylcholine class: receptors that constitute channels which, upon ligand interaction, allow signals to be passed in the form of small ion movement, either into or out of the cell. These ion movements result in changes in the electrical potential of the cells that, in turn, propagate the signal along the cell. More complex signal transduction involves the coupling of ligand-receptor interactions to many intracellular events. These events include phosphorylations by tyrosine kinases and (or) serine (threonine) kinases. Protein phosphorylations change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cells.

Signal transducing receptors are of three general classes:

(1) Receptors that penetrate the membrane and have intrinsic enzymatic activity. Receptors that have intrinsic enzymatic activity include those that are tyrosine kinases, tyrosine phosphatases, guanylate cyclases and serine (threonine) kinases. Receptors with intrinsic tyrosine kinase activity are capable of autophosphorylation as well as phosphorylation of other substrates.
Additionally, several families of receptors lack intrinsic enzyme activity, yet are coupled to intracellular tyrosine kinases by direct protein-protein interactions .

(2) Receptors that are coupled, inside the cell, to *GTP*-binding and hydrolyzing proteins (termed *G*-proteins). Receptors of the class that interact with *G*-proteins all have a structure that is characterized by seven transmembrane spanning domains. These receptors are termed serpentine receptors.

(3) Receptors that are found intracellularly and upon ligand binding migrate to the nucleus where the ligand-receptor complex directly affects gene transcription.

In this section, we focus on a well-studied signal transduction, the *RTK-MAPK* pathway.

We denote *Receptor Tyrosine Kinases* as *RTK*s. Proteins encoding *RTK*s contain four major domains:

- an extracellular ligand binding domain.
- an intracellular tyrosine kinase domain.
- an intracellular regulatory domain.
- a transmembrane domain.

RTK proteins are classified into families based upon structural features in their extracellular portions (as well as the presence or absence of a kinase insert) which include the cysteine rich domains, immunoglobulin-like domains, leucine-rich domains, Kringle domains, cadherin domains, fibronectin type III repeats, discoidin I-like domains, acidic domains, and *EGF*-like domains. Based upon the presence of these various extracellular domains the *RTK*s have been sub-divided into at least 14 different families.

Many receptors that have intrinsic tyrosine kinase activity as well as the tyrosine kinases that are associated with cell surface receptors contain tyrosines residues, that upon phosphorylation, interact with other proteins of the signaling cascade. These other proteins contain a domain of amino acid sequences that are homologous to a domain first identified in the *c-Src* proto-oncogene. These domains are termed *SH2* domains. Another conserved protein-protein interaction domain identified in many

signal transduction proteins is related to a third domain in *c-Src* identified as the *SH3* domain.

The interactions of *SH2* domain containing proteins with *RTK*s or receptor associated tyrosine kinases lead to tyrosine phosphorylation of the *SH2* containing proteins. The result of the phosphorylation of *SH2* containing proteins that have enzymatic activity is an alteration (either positively or negatively) in that activity.

*MAPK*s were identified by virtue of their activation in response to growth factor stimulation of cells in culture, hence the name *mitogen activated protein kinases*. *MAPK*s are also called *ERK*s for extracellular-signal regulated kinases. Maximal *MAPK* activity requires that both tyrosine and threonine residues are phosphorylated. This indicates that MAP kinases act as switch kinases that transmit information of increased intracellular tyrosine phosphorylation to that of serine/threonine phosphorylation.

*MAPK*s are, however, not the direct substrates for *RTK*s nor receptor associated tyrosine kinases but are in fact activated by an additional class of kinases termed *MAP kinase* kinases (*MAPK* kinases) and *MAPK kinase* kinases (*MAPKK* kinases). One of the *MAPK* kinases has been identified as the proto-oncogenic serine/threonine kinase, *RAF*. Another *MAPK* kinases which are activated by *RAF* have been identified as *MEK1* and *ERK1*. Next this cascade culminates in activation of the threonine and tyrosine protein kinase,*ERK* (*MAPK*). Activated *ERK* translocates to the nucleus, where it phosphorylates and activates transcription factors, such as *AP-1*, leading to the novo gene expression.

2.4.2 The Graphical Expression of *RTK-MAPK*

In this section, we give a coarse description of signal transduction, *RTK-MAPK*. That is to say, it is described from protein level, not its domains (e.g. domain *SH2, SH3* etc.). Interactions are represented among proteins not domains of proteins.

In brief, the *RTK-MAPK* pathway is composed of 14 kinds of proteins. These bind and form complexes, modify certain residues on their counterparts (mostly by phosphorylation and dephosphorylation), change their confirmation and activity, and translocate between different cellular compartments (cytosol, nucleus and membrane). A change in gene expression patterns is the end result computed by this network of interactions. Fig. 2.9 [RSS00] is the graphical expression of signal transduction *RTK-MAPK*.

An informal description is as follows;
A protein ligand molecule (*GF*) with two identical domains is a protein (outside signal) which will be sent to signal transduction, *RTK-MAPK*. It binds two receptor tyrosine kinase (*RTK*) molecules on their extracellular part. The bound receptors form a dineric complex, and cross-phosphorylate and activate the protein tyrosine kinase in their intracellular part. The activated receptor can phosphorylate various targets, including its own tyrosine. The phosphorylated tyrosine is identified and bound by an adaptor molecule, *SHC*. A series of protein-protein binding events follows, lead-

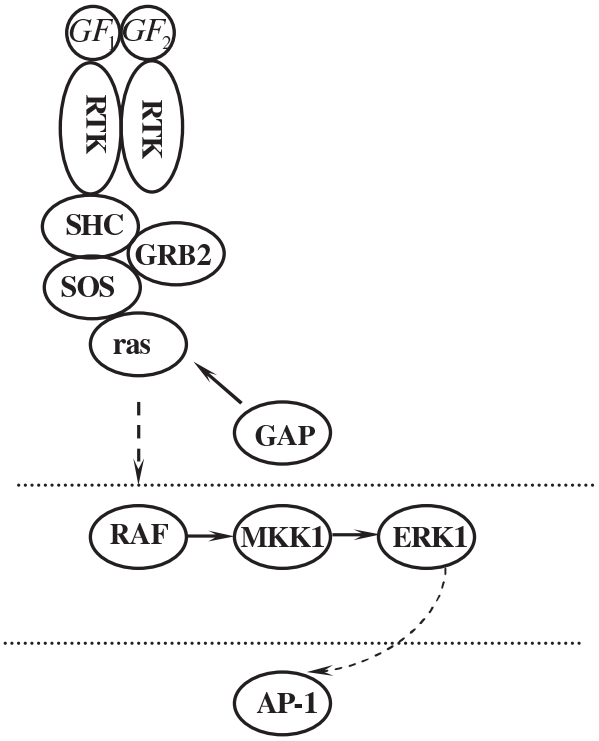


Figure 2.9: Signal Transduction *RTK-MAPK*.

ing to the formation of a protein complex *SHC-GRB2*, *SOS* and *ras* at the receptor intracellular side. Within this complex, the *SOS* protein activates the *ras* protein, which in turn recruits the serine threonine protein kinase, *RAF* to the membrane, where it is subsequently phosphorylated and activated. A cascade of phosphorylations or activations follows, from *RAF* to *MEK1* to *ERK1*. This cascade culminates in activation of the threonine and tyrosine protein kinase, *ERK*. Activated *ERK* translocates to the nucleus, where it phosphorylates and activates transcription factors, such as *AP-1*, leading to the novo gene expression.

Chapter 3

A Calculus For Formal Molecular Processes

In this chapter, we study how to model signal transduction by using process algebra. We consider mainly the case of signal transduction with aberrance. We provide a tag system and a simple typing system to mark aberrance. We prove that the tag system is equivalent to the simple typing system in the capability of labelling the existence of aberrance.

This chapter is organized as follows. First we recall some basic principles for modelling signal transduction using process algebra. In Section 3.2, we briefly recall the simple process of signal transduction with aberrance. In Section 3.3, we define the $I\pi$-calculus which is a variant of the π-calculus. The calculus is obtained by adding two aberrant actions into the π-calculus. In Section 3.4 and Section 3.5, we introduce a tag system and a simple typing system respectively based on the $I\pi$-calculus. Some properties of these two systems are discussed. In Section 3.6, we compare the tag system and the simple typing system and prove that they are equivalent in the capability of labelling existence of aberrance.

3.1 Basic Principles for the Model

Biomolecular processes are responsible for most of information processing in living cells. They are carried out by networks of interacting protein molecules. Systems biology aims to study systems consisting of these biomolecular processes. However the dynamic nature of these systems, their complexity, high connectivity and modularity further complicate this task. So we need novel approaches to study them. Formal approaches are thought to be feasible in studying of systems biology.

In the opinion of Aviv Regev etc. [RSS01], an appropriate formal approach for studying biomolecular processes should fulfill certain goals:

- It could provide a unifying view of dynamic behaviors it underlies. Preferably, this representation will be biologically visible, i.e. it should correspond well to

informal concepts and ideas of molecular biology.

- The formally represented data should be amenable to computer execution and analysis. Thus, the dynamic behavior of the system could be followed by simulation studies, including mutational analysis and simulated evolution. Alternatively, the system's behavior may be formally verified.
- The formal approach should facilitate comparative studies of a system's structure, dynamics and function within and between species.
- The formalism should be scalable and modularized to higher levels of organization.

Process algebras, which allow us to represent and analyze dynamic computational systems, seem appropriate formal approaches which satisfy the goals above.

Comparing to other methods, such as continuous mass-action differential equations, discrete Monte-Carlo simulations or Petri nets, the π-calculus, one of the process algebras, represents molecular systems as mobile communicating systems which are both highly detailed and biologically visible.

Aviv Regev etc. show that the π-calculus is suitable for modelling various molecular systems, including transcriptional circuits, metabolic pathways and signal transduction networks [RSS01]. For example, the π-calculus representations of signal transduction unify dynamic behavior and function of pathways with molecular details that underly their behavior. They provide a comprehensive model of signal transduction. The π-calculus programs are amenable to computer analysis and execution, analogous to mutational manipulation and experimentation, as well as to formal comparison and verification.

At the modelling level, within the particular framework of the π-calculus, we set five principles for this correspondence.

First, as our primitive *process*, we choose the functional signaling *domain*. We call the set of elements (amino acids for example) which have the similar function in a molecule a *functional domain*. A molecule can have several functional domains since it has different functions. To capture the functional and structural independence of domains in signaling molecules, a molecule is modelled as the composition of domains, a complex is modelled as the composition of molecules, and a more complicated complex is modelled as the composition of simple complexes. For example, in the well-studied signal transduction, *RTK-MAPK*, we view such signal transduction as a process.

$$RTK\_MAPK \quad ::= \quad Free\_ligand \mid \cdots \mid RTKs \mid ras \mid \cdots \tag{3.1}$$

where the molecule *Free_ligand*, the complex of some receptor tyrosine kinases (*RTK*s), the protein molecule *ras* etc. form the components of *RTK-MAPK*.

The complex is composed of several molecules, each of which is modelled as a process as well. For example,

$$RTKs \;::=\; RTK_1 \mid RTK_2 \mid \cdots \tag{3.2}$$

The complex of *RTKs* is composed of several receptors tyrosine kinases (denoted as RTK_1,RTK_2, $\cdots$).

A protein molecule is composed of several domains, each of which is modelled as a process as well.

$$Free\_ligand \;::=\; Free\_binding\_domain \mid Free\_extracell\_domain \tag{3.3}$$

Since *Free_ligand* has two domains which have different functions, we can think that the molecule *Free_ligand* is composed of these two domains.

Second, we model the *motifs* and *residues* of domains as communication *channels* that construct a process. Motifs and residues are interacting portions of a domain but are not independently functional, just as channel names are communication ports of the process, but are not processes in their own right. For example, we take the residue $\overline{ligand\_binding}$ of the domain $Free\_ligand\_domain$ of the protein $Free\_ligand$ as the channel name,

$$Free\_binding\_domain \;::=\; \overline{ligand\_binding}.\cdots \tag{3.4}$$

$$Free\_extracell\_domain \;::=\; ligand\_binding.\cdots \tag{3.5}$$

In biological reactions, two molecules (or two domains) interact with each other based on their structural and chemical complementarity. Two complementary motifs are denoted by a global name and co-name pair, for example, $\overline{ligand\_binding}$ and $ligand\_binding$ in (3.4),(3.5).

Third, molecular interaction and modification are modelled as communication and the subsequent change of channel names. Different types of molecular changes, such as chemical modification, conformation changes, or binding, affect further interaction in an analogous manner to the change in communication capabilities following channel names passing in mobile systems. These residues as channels and interaction as communication, yield a model of the molecular realm which is both highly visible and detailed. For example,

$$Free\_ligand\_domain \mid Free\_extracell\_domain \;\xrightarrow{\tau}\; \cdots \tag{3.6}$$

Two processes $Free\_ligand\_domain$ and $Free\_extracell\_domain$ make an interaction by the name $\overline{ligand\_binding}$ and co-name $ligand\_binding$ (3.6).

Fourth, mutually exclusive interactions are summed together, by $+$. Biochemical interaction events may occur in sequence, in parallel with other independent

occurrences, or in a mutually exclusive, competitive fashion. For instance,

$$\begin{aligned} Free\_extracell\_domain \quad ::= \quad & ligand\_binding.trk\_binding.\cdots \\ & + antagonist\_binding \end{aligned} \tag{3.7}$$

A sequence of interactions in which a molecule may participate is denoted by means of a prefix operator.

Finally, a pathway is not merely a bag of molecules and their domains. It is composed of defined *compartments*. First, parallel domains of a single molecule are linked together by a single *backbone*. Then, distinct multi-molecular complexes form. Finally, molecules are separated into higher-order cellular compartments. In all three cases molecules which share a common compartment may interact with each other, while molecules excluded from the compartment may not. We represent compartments by restricted communication scopes. For instance, a receptors tyrosine kinase (RTK) of the complex RTKs can be denoted as follows;

$$\begin{aligned} RTK \quad ::= \quad & \nu(backbone)(Extracell\_domain \mid \\ & Transmem\_domain \mid Intracell\_domain) \end{aligned} \tag{3.8}$$

In the models presented in this thesis, we will comply with these principles.

3.2 Signal Transduction with Aberrance

Signal transduction is the key to uncover the wild growth of cells. When the whole signal transduction works perfectly, decisions of growth and death of cells are also made by rule and line. When some signals mutate aberrantly, the growth of a cell is not controlled anymore by growth factors outside.

Two kinds of mechanisms are changed in signal transduction with aberrance. One is that some aberrant proteins make the cell release growth factors into the environment. These factors can stimulate the cell which sets them free, and make it grow. In fact, one normal protein makes the release growth factors into the environment. These factors will stimulate some other protein, and make it grow. In this way, the growth of one protein depends on growth factors from other proteins, not growth factors from itself.

The other mechanism is aberrance of the *ras* protein. The normal *ras* protein in the inactive state is waiting for the signal. It is activated when it receives the signal, and then sends the signal to the other proteins. After that, it could be inactivated to return the initial state. This kind of inactivity ensures that the cell just can send finitely many signals at a time.

An aberrant *ras* protein has some difference with a normal *ras* protein. The aberrant *ras* protein can be activated and send a signal to the others, just as a normal *ras* protein. The aberrant *ras* protein however cannot be inactivated any more. That

means, it will be always in the active state and always send the signal to the others, even when there is no real signal coming.

A biochemical process is like a team which works well. They trust each other. A protein will make the decision to send signals if and only if it receives necessary signals. It just checks signals it receives but doesn't care signals his father receives.

For instance, in Fig. 3.1, proteins A_1, A_2, B_1, B_2, C are in normal state. When the whole process proceeds normally, proteins B_1 and B_2 receive signals (S_{A_1} and S_{A_2}) from proteins A_1 and A_2 respectively and send signals (S_{B_1} and S_{B_2}) to the protein C. When C receives all the signals (S_{B_1} and S_{B_2}), it sends the signal S_C to the new one. When the protein B_2 is in the aberrant state (we denote the aberrant B_2 as B_2'), it can also send the signal S_{B_2} to C though there is no signal S_{A_2} (in Fig. 3.1, the dashed arrow from A_2 to B_2' represents no signal from A_2 to B_2'). The protein C makes the decision to continue just by checking signals S_{B_1} and S_{B_2}. It doesn't care whether the signal S_{A_2} actually occurred.

Formally, we take the whole biochemical process as a graph (e.g. Fig. 3.1). Suppose that S and P are boolean functions from proteins. $S(C) = T$ means the protein C sends all the signals to the next ones successfully, and $S(C) = F$ means that some signals fail to be sent. $P(C) = T$ means that the protein C receives all the signals from its predecessors successfully, and $P(C) = F$ means that some signals fail to be received.

Proposition 3.1 *If a protein C is normal, then*

$$P(C) = T \Leftrightarrow S(C) = T$$

PROOF: Assume that $P(C) = T$, that is to say, the protein C receives all necessary signals form his predecessors, then C could send signals to the next ones since C is in the normal state. On the other hand, if $P(C) = F$, because C is in the normal state, it cannot send signals to the next ones, then $S(C) = F$.

For a protein C, if it satisfies $P(C) = F$ but $S(C) = T$, then C is in the aberrant state. The corollary is from proposition 3.4.

3.3 The *Interference* π-calculus

The π-calculus has been applied to model biochemical networks. In these applications the modelling is done without considerations to exceptions. In order to describe more complex biochemical systems, the $I\pi$-calculus, the *Interference pi calculus*, is introduced for description of signal transduction with aberrance. The calculus is obtained by adding aberrant actions into the π-calculus

3.3.1 The Interference π-calculus: Definition

In process algebra, processes evolve by performing actions. Actions capabilities are introduced by prefix capabilities. In the $I\pi$-calculus, we introduce two capabilities in addition to prefixes defined by the π-calculus.

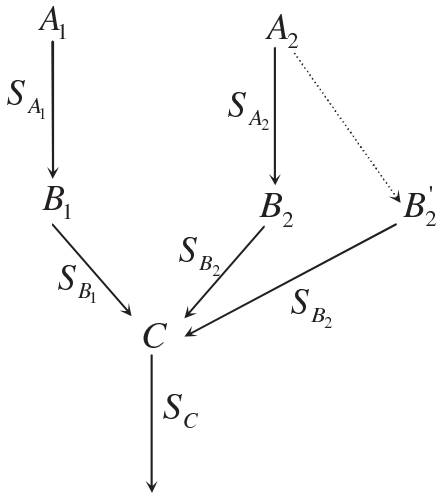


Figure 3.1: Normal States and Aberrant States.

Let $a, b, \cdots$ range over names. We also define two symbols § and ♯ to represent the aberrance capability. Here § represents the suicide capability and ♯ the propagation capability. When a process has the suicide capability, it terminates its action immediately. And when a process has the propagation capability, it will duplicate its action infinitely.

Definition 3.1 (Prefix) *The prefixes of $I\pi$-calculus are defined as follows:*

$$\pi_0 ::= \overline{a}(b) \mid a(x) \mid \overline{a} \mid a \qquad \pi_i ::= \pi_0 \mid \S(\pi) \mid \sharp(\pi)$$

where $\pi ::= \pi_0 \mid \pi_0.\pi$.

The syntactic category π_0 is like in the π-calculus [SW01]. The category π stands for sequences of π-calculus capabilities. Finally the category π_i allows for aberrant capabilities in addition to the normal ones. The two aberrance are relative to sequences of normal capabilities.

Definition 3.2 (Process) *The $I\pi$-calculus processes are defined as follows:*

$$P ::= 0 \mid \pi_i.P \mid \pi_i.P + \pi_i'.P' \mid P|P' \mid (\nu a)P$$

Hence the syntax of $I\pi$-calculus is the same as that of π-calculus except for its richer set of capabilities.

The standard π-calculus [Mil89] defines replication. In [SW01], it is shown that any process involving recursive definitions is representable using replication, and, conversely, that replication is redundant in the presence of recursion. In our language, to model the biological processes more naturally, we choose recursion instead of replication.

To give recursive definitions of processes, we introduce process *constants*, ranged by K, and add two new forms. First, we have recursive definitions of the form

$$K \triangleq (\tilde{x}).P$$

where $fn(P) \subseteq \tilde{x}$. Secondly, we have a new form of process, the *constant application*,

$$K\lfloor \tilde{a} \rfloor$$

We refer to $K\lfloor \tilde{a} \rfloor$ as an instance of the process constant K.

The structural congruence $\equiv$ is the least equivalent relation on closed processes that satisfies the following equalities:

$$\begin{array}{rcll} P \mid Q & \equiv & Q \mid P & \\ (P \mid Q) \mid R & \equiv & P \mid (Q \mid R) & \\ P + Q & \equiv & Q + P & \\ (P + Q) + R & \equiv & P + (Q + R) & \\ (\nu a)0 & \equiv & 0 & \\ (\nu a)(\nu b)P & \equiv & (\nu b)(\nu a)P & \\ ((\nu a)P) \mid Q & \equiv & (\nu a)(P \mid Q) & \text{if } a \notin \text{fn}(()Q) \end{array}$$

The reaction relation, introduced initially by Robin Milner [Mil89], is a concise account of computation in the π-calculus. In addition to the well-known interaction rule (*Com-N*), our reaction relation also includes two new rules about reactions with aberrance (Pre-§ and Pre-♯).

τ is used to represent the *silent action*. α stands for actions, τ, ♯, and §.

$$\frac{}{\S(\pi).P \xrightarrow{\S} 0}\ \text{Pre-§ ;} \qquad \frac{}{\sharp(\pi).P \xrightarrow{\sharp} \pi.\sharp(\pi).P}\ \text{Pre-♯ ;}$$

$$\frac{}{(\overline{a}(b).Q + R_1) \mid (a(x).P + R_2) \xrightarrow{\tau} Q|P\{b/x\}}\ \text{Com-N;}$$

$$\frac{}{(\overline{a}.Q + R_1) \mid (a.P + R_2) \xrightarrow{\tau} Q \mid P}\ \text{Com-SN}$$

$$\frac{P \xrightarrow{\alpha} P'}{P + Q \xrightarrow{\alpha} P'}\ \text{Sum;} \qquad \frac{P \xrightarrow{\alpha} P'}{P \mid Q \xrightarrow{\alpha} P' \mid Q}\ \text{Comp;}$$

$$\frac{P \xrightarrow{\alpha} P'}{(\nu a)P \xrightarrow{\alpha} (\nu a)P'}\ (\alpha \neq a)\quad \text{Res;} \qquad \frac{Q \equiv P \qquad P \xrightarrow{\alpha} P' \qquad P' \equiv Q'}{Q \xrightarrow{\alpha} Q'}\ \text{Stc.}$$

$$\frac{}{K\lfloor \tilde{a} \rfloor \longrightarrow P\{\tilde{a}/\tilde{x}\}}\ (K \triangleq (\tilde{x}).P)\ \text{R-Const}$$

Table 3.1: Reaction rules of $I\pi$-calculus.

The first two rules deal with reactions with aberrance: the former says that the resulting process is terminated; the latter declares that the resulting process may duplicate its action infinitely. The following rules are like in the π-calculus. The last rule is for constant applications. For example, assume that the constant A_0 is defined

as follows,

$$A_0 \triangleq (x, y, z).(x(u).A_1\lfloor x, u\rfloor + y(w).A_0\lfloor x, y, w\rfloor)$$

The instance $A_0\lfloor a, b, c\rfloor$ can be applied the rule *R-Const*.

$$A_0\lfloor a, b, c\rfloor \longrightarrow (a(u).A_1\lfloor a, u\rfloor + b(w).A_0\lfloor a, b, w\rfloor)$$

3.3.2 A Model about *ras* Activation

In order to illustrate the use of our calculus, we consider an example in signal transduction pathway with aberrance. We focus our attention on the well-studied *RTK-MAPK* pathway. In biology, pathways of molecule interactions provide communication between the cell membrane and intracellular endpoints, leading to some change in the cell. Here we choose a small yet important part, *ras* activation, for explanation.

Fig. 3.2 gives an example of *ras* activation of the signal transduction pathway, *RTK-MAPK* (in Fig. 3.2, the protein *ras* is denoted as *RAS*). At the normal state, the protein-to-protein interactions bring the protein *SOS* close to the membrane, where the protein *ras* can be activated. The protein *SOS* activates the protein *ras* by exchanging *ras*'s *GDP* with *GTP*. More precisely, growth factor and cytokine activation of many tyrosine kinase and kinase-linked receptors recruits many proteins to the plasma membrane including *ras*-specific guanine nucleotide releasing proteins *GNRP*. Under the influence of a *GNRP*, *ras* proteins bind *GTP*, resulting in activation of the *ras* signal. Active *ras* interacts the next protein *RAF* in this signal transduction. After that, the protein *GAP* inactivates the active protein *ras* and makes it in the initial state, the inactive state.

Within the framework of the $I\pi$-calculus, we comply with the principles we mentioned in Section 3.1. Aviv Regev etc. have given the representation of normal *RTK-MAPK* using the π-calculus [RSS00, RSS01].

The interpretation of *ras* in the $I\pi$-calculus can be done in the following manner: The system defined in (3.9) is a collection of concurrently operating molecules, seen

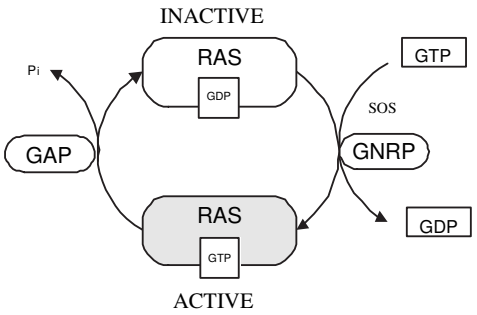


Figure 3.2: *ras* Activation

as processes with potential behavior.

$$Sys \;::=\; ras \mid SOS \mid GAP \mid RAF \tag{3.9}$$

where *Sys* is the abbreviation of our system. It includes four main proteins: *ras, SOS, GAP, RAF*. This system, in fact, is the subsystem of (3.2).

A protein molecule is composed of several domains, each of which is modelled as a process as well. In (3.10) through (3.13) the detailed $I\pi$-calculus programs for proteins *ras*, SOS, RAF and GAP are given:

$$\begin{aligned} ras &::= INASWI\_I \mid INASWI\_II && (3.10)\\ SOS &::= S\_SH3\_BS \mid S\_GNEF && (3.11)\\ RAF &::= R\_Nt \mid R\_ACT\_BS \mid R\_M\_BS \\ &\quad \mid INA\_R\_Ct \mid R\_ATP\_BS && (3.12)\\ GAP &::= sg(c\_ras).\overline{c\_ras}(gdp).GAP && (3.13) \end{aligned}$$

(3.10) says that *ras* is composed of two domains *INASWI_I* and *INASWI_II*. We use *"IN"* to record the fact that two domains are in the inactive state. The protein *SOS* has two domains *S_SH3* and *S_GNEF* where the domain *S_GNEF* participates in interactions of our system *Sys*, and the other domain *S_SH3* does not. Similarly, only the domain *R_Nt* of the protein *RAF* participates in interactions of our system *Sys*. The function of the protein *GAP* is to sends the *residue GDP* to the protein *ras*. So we can take it as one domain. (3.13) says chemical components *sg, c_ras, gdp* compose of the protein *GAP* where *gdp* (i.e *GDP* in Fig. 3.2) is the chemical component which makes the protein *ras* stay in the inactive state.

The molecules (or domains) interact with each other based on their structural and chemical complementarity. Interaction is accomplished by motifs and residues that constitute a domain. These are viewed as channels or communication ports of the molecule:

$$\begin{aligned} INASWI\_I &::= \overline{bbone}.ACTSWI\_I && (3.14)\\ INASWI\_II &::= \overline{sg}(rs\_1).rs\_1(x).ACTSWI\_II && (3.15)\\ S\_GNEF &::= bbone.S\_GNEF \mid sg(c\_ras).\overline{c\_ras}(gtp).S\_GNEF && (3.16) \end{aligned}$$

In (3.16), the motif *bbone* is the abbreviation of *backbone* which is the interacting portion of the domain *S_GNEF*. The *residue gtp* (i.e *GTP* in Fig. 3.2) is the chemical component which makes the protein *ras* stay in the active state. In (3.15), the motif $\overline{sg}$ of the domain *INASWI_II* is the complementary of the motif *sg* of the protein *GAP*. In (3.14), the motif $\overline{bbone}$ is the complementary of the motif *bbone* of the domain *S_GNEF*.

Hence, the following interactions are possible:

$$INASWI\_I \mid S\_GNEF \xrightarrow{\tau} ACTSWI\_I \mid S\_GNEF \mid \ldots \tag{3.17}$$

$$INASWI\_II \mid S\_GNEF \xrightarrow{\tau}{}^{*} ACTSWI\_II[gtp/x] \mid S\_GNEF \mid \ldots \tag{3.18}$$

The interaction (3.17) shows that the domain $INASWI\_I$ of *ras* is activated by the domain of S_GNEF of SOS. The interaction (3.18) shows that the domain $INASWI\_II$ of *ras* is activated by the domain S_GNEF of SOS by passing the residue *gtp*. Hence the protein *ras* is activated.

The detailed $I\pi$ programs for the domains, $ACTSWI\_I$, $ACTSWI\_II$ of the protein *ras* and the domain R_Nt of RAF are defined in (3.19) through (3.21):

$$ACTSWI\_I ::= \overline{s}(rs\_2).\overline{rs\_2}.bbone.INASWI\_I \quad (3.19)$$

$$ACTSWI\_II ::= \overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{bbone}.INASWI\_II \quad (3.20)$$

$$R\_Nt ::= s(c\_ras).c\_ras.ACTR\_Nt \quad (3.21)$$

(3.19) says that the domain $ACTSWI\_I$ can interact with another domain which has the complementary motif s.

The processes so defined have the following interactions:

$$ACTSWI\_I \mid R\_Nt \longrightarrow^* bbone.INASWI\_I \mid ACTR\_Nt \quad (3.22)$$

$$ACTSWI\_II \mid GAP \longrightarrow^* \overline{bbone}.INASWI\_II[gtp/x] \mid GAP \quad (3.23)$$

$$bbone.INASWI\_I \mid \overline{bbone}.INASWI\_II \longrightarrow INASWI\_I \mid INASWI\_II \quad (3.24)$$

During the process of *ras* activation, the interaction (3.22) occurs firstly, then interactions (3.23) and (3.24) occur. (3.22) says that the domain $ACTSWI\_I$ of *ras* interacts with the domain R_Nt of RAF, that is to say, the protein *ras* sends some signal to the protein *RAF*. (3.23) says that GAP inactivates the domain $ACTSWI\_II$ of *ras* by passing the *residue GDP*. (3.24) says that the domains of *ras* interact with each other and that *ras* rollbacks to the initial inactivated state. One process of *ras* activation is over. Then the protein *ras* will wait another signal from the protein SOS, and begin the next activation.

When *ras* mutates aberrantly, it does not have any effect on the *ras*'s binding with *GTP* but will reduce the activity of the *GTP* hydrolase of *ras* and lower its hydrolysis of *GTP* greatly; in the meantime *ras* will be kept in an active state, that is, the protein *ras* keeps activating the molecule, inducing the continual effect of signal transduction, which results in cell proliferation and tumor malignancy.

During the process with aberrance, the protein *GAP* looses its capability of sending the *residue GDP* to the protein *ras*. The structure and chemistry of the motif *sg* of *GAP* are changed, and it cannot be complementary to the motif $\overline{sg}$ of the domain $ACTSWI\_II$ of the protein *ras*. (3.25) defines the $I\pi$ representation of *GAP* in the aberrant state.

$$GAP ::= \S(sg(c\_ras)).\overline{c\_ras}(gdp).GAP \quad (3.25)$$

$$GAP \longrightarrow 0 \quad (3.26)$$

$$ACTSWI\_II \mid GAP = ACTSWI\_II \mid 0 \quad (3.27)$$

(3.26) shows that *GAP* loses its function and does nothing, meaning that it cannot inactivate the domain $ACTSWI\_II$ of *ras*. Then the interaction (3.24) will not occur, that is to say, the protein *ras* will always stay in active state.

During the process with aberrance, the domain $ACTSWI\_I$ of the protein *ras* duplicates its capability of sending signals to the domain R_Nt of the protein *RAF* by interactions. Now $ACTSWI\_I$ can be written as follows;

$$ACTSWI\_I ::= \sharp(\overline{s}(rs\_2).\overline{rs\_2}).bbone.INASWI\_I$$

Hence, the following interactions are possible:

$$\begin{aligned} ACTSWI\_I \mid R\_Nt \quad \xrightarrow{\tau}{}^{*} \quad & \sharp(\overline{s}(rs\_2).\overline{rs\_2}).bbone.INASWI\_I \\ & \mid ACTR\_Nt \end{aligned} \tag{3.28}$$

The interaction (3.28) says that the domain $\sharp(\overline{s}(rs\_2).\overline{rs\_2}).bbone.INASWI\_I$ always has the capability of sending signals to the domain R_Nt which has the complemental motif *s*. The interaction (3.24) never occurs.

In the π-calculus, it is a little difficult to represent the lost of capability and the propagation of some capabilities. Hence, the π-model could not easily describe this aberrant case. $I\pi$-calculus, on the contrary, can describe it quite precisely but simply.

3.4 The $I\pi$-calculus with a Tag System

Even in one aberrant biomolecular process, there are some proteins are aberrant while the others are normal. If all the proteins have some additional information about their states (normal or aberrant) in a formal model, the whole model will be clearer. Therefore, the idea of giving a tag to each protein for informing its state (normal or aberrant) is spontaneous.

In this section, we introduce such a tag system to make the model of the $I\pi$-calculus clearer.

3.4.1 A Tag System

We assume a set $\mathbb{R}^+ = \{i : i \geq 0\}$ for tags. Let $i_a, i_b, \cdots$ range over tags.

The syntax of the $I\pi$-calculus is modified as follows: we write a pair $\langle i_{\pi_i}, \pi_i \rangle$ instead of the prefix π_i, where $i_{\pi_i} \in \mathbb{R}^+$ is the tag of π_i. When $\pi_i = \pi_0$, $i_{\pi_i} > 0$ is the tag of π_0; when $\pi_i = \S(\pi)$ or $\pi_i = \sharp(\pi)$, $i_{\pi_i} = 0$. As we have mentioned, π is the collection of some π_0s. For example, $\pi = \pi_0^1.\pi_0^2$, we define the tag of π as a set of tags of π_0^1 and π_0^2, that is, $I_\pi = \{i_{\pi_0^1}, i_{\pi_0^2}\}$.

The expression of a process is also a pair $\langle I_P, P \rangle$ where I_P is the tag of the process P. The tag of one process is defined inductively by the following rules(Table 3.2).

We use the symbol $\uplus$ to denote multiset union. For example, let $A = \{a, b, c, d\}$ and $B = \{b, c, e, f\}$, then $A \uplus B = \{a, b, c, d, b, c, e, f\}$.

We write $\biguplus_{n=1}^{\infty} I_P \triangleq I_P \uplus I_P \uplus \cdots$.

Let $K_i = \tilde{x_i}.P_i$ be constants, where $i = 1, 2, \ldots, n$. We use $\widetilde{K}$ to represent $(K_1, K_2, \ldots, K_n)$, and $\widetilde{P}$ to represent $(P_1, P_2, \ldots, P_n)$. The expression "$Let\ \widetilde{K} = \widetilde{P}\ in\ K_i$" means $K_i = \tilde{x_i}.P_i$. We denote this expression as let.

For each recursive process P, we define an environment ρ mapping the constants that appear free in P to sets of tags. Let $\{K^n\}$ be a set if free constants in P. Define the tag of the process P:

$$I_{P,\rho} = \rho(\{K^n\}) \uplus I_{P \setminus \{K^n\}}$$

Next we compute the tag of a recursive process, that is, the tag of the expression *let*. We define a function F on sets of tags.

$$F(J_1, J_2, \ldots J_n) = I_{\widetilde{P},\rho'}$$

where ρ' extends ρ with $\rho'(K_1) = J_1, \rho'(K_2) = J_2, \ldots, \rho'(K_n) = J_n$.

Proposition 3.2 (The Least Fixed Point) *The function F defined above has the least fixed point.*

PROOF: According to the least fixed point theorem, we just need to prove F is continuous, that is, F reverses increasing chains. Suppose that $\tilde{J} = (J_1, J_2, \ldots, J_n)$, $\widetilde{J'} = (J'_1, J'_2, \ldots, J'_n)$. $\tilde{J} \subseteq \widetilde{J'}$ if and only if $J_i \subseteq J'_i$, where $i = 1, 2, \ldots, n$. We have $F(\tilde{J}) = I_{\widetilde{P},\rho'} = (\rho'(K_1) \uplus I_{P_1 \setminus \{K_1\}}, \rho'(K_2) \uplus I_{P_2 \setminus \{K_2\}}, \ldots, \rho'(K_n) \uplus I_{P_n \setminus \{K_n\}}) = (J_1 \uplus I_{P_1 \setminus \{K_1\}}, J_2 \uplus I_{P_2 \setminus \{K_2\}}, \ldots, J_n \uplus I_{P_n \setminus \{K_n\}}) \subseteq (J'_1 \uplus I_{P_1 \setminus \{K_1\}}, J'_2 \uplus I_{P_2 \setminus \{K_2\}}, \ldots, J'_n \uplus I_{P_n \setminus \{K_n\}}) = F(\widetilde{J'})$.

So the least fixed point of the function F is the sup of $\{\varnothing, F(\varnothing), F^2(\varnothing), \ldots, F^n(\varnothing), \ldots\}$. We compute it as follows:

$F(\varnothing) = I_{\widetilde{P},\rho'} = (\uplus I_{P_1 \setminus \{K_1\}}, \ldots, \uplus I_{P_n \setminus \{K_n\}})$

$F^2(\varnothing) = I_{\widetilde{P},\rho''} = (\overset{2}{\underset{m=1}{\uplus}} I_{P_1 \setminus \{K_1\}}, \ldots, \overset{2}{\underset{m=1}{\uplus}} I_{P_n \setminus \{K_n\}})$

$\ldots$

$F^n(\varnothing) = I_{\widetilde{P},\rho^n} = (\overset{n}{\underset{m=1}{\uplus}} I_{P_1 \setminus \{K_1\}}, \ldots, \overset{n}{\underset{m=1}{\uplus}} I_{P_n \setminus \{K_n\}})$

$\ldots$

Therefore the least fixed point of F is

$$lfp(F) = (\overset{\infty}{\underset{m=1}{\uplus}} I_{P_1 \setminus \{K_1\}}, \ldots, \overset{\infty}{\underset{m=1}{\uplus}} I_{P_n \setminus \{K_n\}}) \triangleq (I_1, I_2, \ldots, I_n)$$

According to Proposition 3.2, for the expression *let*, $I_{let,\rho} = I_i = \overset{\infty}{\underset{m=1}{\uplus}} I_{P_i \setminus \{K_i\}}$. Especially, when a process P has no free constants K, $I_P = I_{P,\varnothing}$, where $\varnothing$ is an empty environment.

Example 1 Let $K_0 = (x, y).(x(y).K_0\lfloor x, y \rfloor)$. The process P is a constant application, that is, $P = K_0\lfloor a, b \rfloor$. According to the rule *Const-t*, the tag of the process P is:

$$I_P = \overset{\infty}{\underset{n=1}{\uplus}} I_{P \setminus \{K_0\},\rho} = \overset{\infty}{\underset{n=1}{\uplus}} I_{(a(b).0)} = \overset{\infty}{\underset{n=1}{\uplus}} \{i_a\}$$

The last part of this derivation can be justified according to rules *0-t, N-t*.

Example 2 Let $K_0 = (x, y).(x(y).K_0\lfloor x, y \rfloor + K_1\lfloor x, u, v \rfloor)$. The process P is a constant application, that is, $P = K_0\lfloor a, b \rfloor$. According to the rule *Const-t*, the tag of the process P is:

$$I_P = \overset{\infty}{\underset{n=1}{\uplus}} I_{P \setminus \{K_0\},\rho} = \overset{\infty}{\underset{n=1}{\uplus}} I_{(a(b).0 + K_1\lfloor a,u,v \rfloor)} = \overset{\infty}{\underset{n=1}{\uplus}} (\{i_a\} \uplus I_{K_1\lfloor a,u,v \rfloor})$$

The last part of this derivation can be justified according to rule *sum-t*.

$$\frac{P = 0}{I_P = \varnothing}\ \text{0-t} \qquad \frac{P = \pi_0.Q}{I_P = \{i_{\pi_0}\} \uplus I_Q}\ \text{N-t}$$

$$\frac{P = \S(\pi).Q}{I_P = \{0\}}\ \S\text{-t} \qquad \frac{P = \sharp(\pi).Q}{I_P = \biguplus_{n=1}^{\infty}(\{0\} \uplus I_\pi) \uplus I_Q}\ \sharp\text{-t}$$

$$\frac{P = Q + R}{I_P = I_Q \uplus I_R}\ \text{Sum-t} \qquad \frac{P = Q|R}{I_P = I_Q \uplus I_R}\ \text{Com-t}$$

$$\frac{P = (\nu x)Q}{I_P = I_Q}\ \text{Res-t} \qquad \frac{P = K\lfloor \tilde{a} \rfloor}{I_P = I_{P,\rho}}\ (K \triangleq \tilde{x}.P)\ \text{Cons-t}$$

Table 3.2: Tags of processes.

Let I_P, I_Q be the tags of the processes P and Q. We define

$$\langle I_P, P\rangle \equiv \langle I_Q, Q\rangle \Leftrightarrow P \equiv Q \ \&\ I_P = I_Q$$

Since we have reaction rules of $I\pi$-calculus (see Table 3.1), we can assign tags to a reaction rule $P \longrightarrow Q$. These "dynamic tags" can be computed by Table 3.3.

We write $P \longrightarrow_I Q$ if $P \longrightarrow Q$ and I is the tag of this derivation according to Table 3.3.

$$\frac{}{\{0\} \setminus \{0\} = \varnothing}\ \text{Pre-§;}$$

$$\frac{}{\biguplus_{n=1}^{\infty}(\{0\} \uplus \{i_{\pi_i}\}) \uplus I_P \setminus \{0\} = \{i_{\pi_i}\} \uplus I_P \uplus \biguplus_{n=1}^{\infty}(\{0\} \uplus \{i_{\pi_i}\}) \uplus I_P}\ \text{Pre-♯;}$$

$$\frac{}{(\{i_x\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_x\} \uplus I_P \uplus I_{R_2}) \setminus (\{i_x\} \uplus I_{R_1} \uplus I_{R_2}) = I_Q \uplus I_P}\ \text{Com-N;}$$

$$\frac{}{(\{i_x\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_x\} \uplus I_P \uplus I_{R_2}) \setminus (\{i_x\} \uplus I_{R_1} \uplus I_{R_2}) = I_Q \uplus I_P}\ \text{Com-SN;}$$

$$\frac{I_P \setminus \{i_y\} = I_{P'}}{I_P \uplus I_Q) \setminus \{i_y\} \uplus I_Q = I_{P'}}\ \text{Sum;} \qquad \frac{I_P \setminus \{i_y\} = I_{P'}}{I_P \uplus I_Q \setminus \{i_y\} = I_{P'} \uplus I_Q}\ \text{Com;}$$

$$\frac{I_Q = I_P \qquad I_P \setminus \{i_x\} = I_{P'} \qquad I_{P'} = I_{Q'}}{I_Q \setminus \{i_x\} = I_{Q'}}\ \text{Str;}$$

$$\frac{}{I_{P[\tilde{a}/\tilde{x}]} = I_{P,\rho}}\ K \triangleq \tilde{x}.P\ \ \text{R-const.}$$

Table 3.3: Tags for reduction rules.

Proposition 3.3 *If* $P \longrightarrow_I Q$, *then* $I_P \setminus I = I_Q$.

PROOF: According to Table 3.3, we check tags of processes as follows:

(1) Assume that $\S(\pi).P \xrightarrow{\S} 0$ by the rule *Pre*-§, where $I = \{0\}$. The tag of the process $\S(\pi).P$ is $\{0\}$ by rule §-*t* in Table 3.2. Then we have $\{0\} \setminus \{0\} = \varnothing$, where $\varnothing$ is the tag of the process 0.

(2) Assume that $\sharp(\pi).P \xrightarrow{\sharp} \pi.\sharp(\pi).P$ by the rule *Pre*-♯, where $I = \{0\}$. The tag of the process $\sharp(\pi).P$ is $\overset{\infty}{\underset{n=1}{\uplus}}(\{0\} \uplus I_\pi) \uplus I_P$. Then we have $\overset{\infty}{\underset{n=1}{\uplus}}(\{0\} \uplus I_\pi) \uplus I_P \setminus \{0\} = I_\pi \uplus \overset{\infty}{\underset{n=2}{\uplus}}(\{0\} \uplus I_\pi) \uplus I_P$, where $I_\pi \uplus \overset{\infty}{\underset{n=2}{\uplus}}(\{0\} \uplus \{I_\pi\}) \uplus I_P$ is the tag of the process $\pi.\sharp(\pi).P$.

(3) Assume that $(\overline{a}(b).Q + R_1) \mid (a(x).P + R_2) \xrightarrow{\tau} Q|P\{b/x\}$ by rule *Com-N*, where $I = \{i_a\} \uplus \{i_a\} \uplus I_{R_1} \uplus I_{R_2}$. The tag of the process $(\overline{a}(b).Q + R_1) \mid (a(x).P + R_2)$ is $(\{i_a\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_a\} \uplus I_P \uplus I_{R_2})$. We have $(\{i_a\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_a\} \uplus I_P \uplus I_{R_2}) \setminus (\{i_a\} \uplus \{i_a\} \uplus I_{R_1} \uplus I_{R_2}) = I_Q \uplus I_P$, where $I_Q \uplus I_P$ is the tag of the process $Q|P\{b/x\}$.

(4) Assume that $(\overline{a}.Q + R_1) \mid (a.P + R_2) \xrightarrow{\tau} Q|P$ by rule *Con-SN*, where $I = \{i_a\} \uplus \{i_a\} \uplus I_{R_1} \uplus I_{R_2}$. The tag of the process $(\overline{a}.Q + R_1) \mid (a.P + R_2)$ is $(\{i_a\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_a\} \uplus I_P \uplus I_{R_2})$. We have $(\{i_a\} \uplus I_Q \uplus I_{R_1}) \uplus (\{i_a\} \uplus I_P \uplus I_{R_2}) \setminus (\{i_a\} \uplus \{i_a\} \uplus I_{R_1} \uplus I_{R_2}) = I_Q \uplus I_P$, where $I_Q \uplus I_P$ is the tag of the process $Q|P$.

(5) In the rule *Sum*, suppose that for the reaction $P \longrightarrow_I P'$, $I_P \setminus I = I_{P'}$, then for the reaction $P + Q \longrightarrow_{I'} P'$, where $I' = I \uplus I_Q$, we have $(I_P \uplus I_Q) \setminus (I \uplus I_Q) = I_{P'}$.

(6) In the rule *Com*, suppose that for the reaction $P \longrightarrow_I P'$, $I_P \setminus I = I_{P'}$, then for the reaction $P|Q \longrightarrow_{I'} P'$, where $I' = I$, we have $(I_P \uplus I_Q) \setminus I = I_{P'} \uplus I_Q$.

(7) In the rule *Res*, suppose that for the reaction $P \longrightarrow_I P'$, $I_P \setminus I = I_{P'}$, then for the reaction $(\nu a)P \longrightarrow_{I'} (\nu a)P'$, where $I' = I$, we have $I_P \setminus I' = I_{P'}$.

(8) In the rule *Comp*, suppose that $I_Q = I_P$, $I_{Q'} = I_{P'}$ and for the reaction $P \longrightarrow_I P'$, $I_P \setminus I = I_{P'}$, then for the reaction $Q \longrightarrow_{I'} Q'$, where $I' = I$, we have $I_Q \setminus I' = I_{Q'}$.

(9) Assume that for the recursive process $K \triangleq \tilde{x}.P$, $K\lfloor\tilde{a}\rfloor \longrightarrow P\{\tilde{a}/\tilde{x}\}$ by the rule *R-Const*. It is trivial to prove.

In Section 3.3.2, we have given an example to show how to model an aberrant signal transduction using the $I\pi$-calculus. We can annotate this example using the $I\pi$-calculus with the tag system. In the new model, we give a tag for each *motif* and *residue*, then according to the rules in Table 3.2, we give the tag of each *domain*

and *process*, which makes the model clearer since we have the state of each action and each process. If a prefix has tag 0, then it is in aberrant state; if 0 belongs to the tag of one process, then this process has aberrant actions. For any biomolecular interaction, the change of tags of processes (reactant and products) follows the rules of Table 3.3. Hence, we can check the existing aberrance, using the tag system of the $I\pi$-calculus. See Appendix A.

3.4.2 Tag Systems for Quantitative Analysis?

In Section 3.4.1, tags are just numbers which have not any meaning. We distinguish the aberrant processes and normal ones by checking the number 0 is in tags of processes or not.

In fact, the occurrence of aberrance is affected by temperature, environment, and concentration, etc. Naturally, if our tags come from biological results, that is, tags are gotten by some biological rules, then we want the tags to reflect this information. For example, suppose that the protein A is in the normal state when the temperature is in some interval $[n, m]$ in the environment. When the real environmental temperature is far away from this interval, the protein A will be in aberrant state. Based on this case, we can express it as tags formally:

For each motif or residue a, the tag i_a of a can be defined as a function from temperatures to $\mathbb{R}^+ = \{i : i \geq 0\}$:

$$i_a(l) = \begin{cases} 0 & n \leq l \leq m \\ n - l & l \leq n \\ l - m & m \leq l \end{cases}$$

For a slightly more complicated example, we may want to account for the fact that the temperature interval $[n, m]$ at which the protein A is in the normal state can change according to time, that is to say, n, m, are functions of time instead of constants. At time t_0, when the temperature is in the interval $[n(t_0), m(t_0)]$ in the environment, the protein A is in the normal state. Hence, the tag i_a of a can be modified as follows:

$$i_a(l, t_0) = \begin{cases} 0 & n(t_0) \leq l \leq m(t_0) \\ n(t_0) - l & l \leq n(t_0) \\ l - m(t_0) & m(t_0) \leq l \end{cases}$$

In this thesis, we just gave the simple modification of tag systems. We believe that richer tags systems will allow to record more information on the reactants of the biological processes, and hence will allow to account for quantitative aspects of systems biology in the framework of process calculi.

3.5 The $I\pi$-calculus with a Typing System

In this section, we introduce a simple typing system for the $I\pi$-calculus, with the similar aim to make the model of $I\pi$-calculus easier to understand.

3.5.1 A Typing System

As we have mentioned, for a biochemical network with aberrance, we hope to know what is brought on by aberrance. So in the $I\pi$-calculus, we need to control the information flow when modelling an aberrant biochemical network. This section describes rules for controlling information flow in the $I\pi$-calculus. There are several ways of formalizing those ideas, like the tag system introduced in Section 3.4. Here we embody them in a typing system for the $I\pi$-calculus.

In order to represent the aberrance of signal transduction we classify signals into three classes:

- A *Normal* signal is one that takes part in the normal processes.
- An *Aberrant* signal is one that takes part in the aberrant processes.
- An *Unknown* signal could be any signal.

To simplify we define a reflexive order relation $<:$ among these three classes:

Normal $<:$ *Unknown*;
Aberrant $<:$ *Unknown*.

A name Γ is denoted as *environment*, and P as *processes*. The typed system has three kinds of assertions:

- "$\vdash \Gamma$ well formed" means that the environment Γ is well-formed.
- "$\Gamma \vdash a : T$" means that the name a is of the class T in Γ.
- "$\Gamma \vdash P : Ok$" means that the process P typechecks in the environment Γ.

Typing rules are given under an environment Γ. An environment is a list of distinct names with associated classifications.

Definition 3.3 (Typed Environment) *Typed environments are given by the following rules:*

$$\frac{}{\vdash \varnothing \ well\ formed} \ \textit{Environment Empty}$$

$$\frac{\vdash \Gamma \ well\ formed, M \notin \Gamma}{\vdash \Gamma, M : T \ well\ formed} \ \textit{Environment Name}$$

Having defined the environments, one can define rules for terms and processes.

Definition 3.4 (Terms) *The rules for terms of typing system are as follows:*

$$\frac{\Gamma \vdash M : T \qquad T <: R}{\Gamma \vdash M : R}\ \textit{Level Subsumption}$$

$$\frac{\vdash \Gamma\ well\ formed \qquad M : T\ in\ \Gamma}{\Gamma \vdash a : T}\ \textit{Level Name}$$

The rule Level Subsumption says that a term of level *Normal* or *Aberrant* has level *Unknown* as well.

Definition 3.5 (Processes) *The rules for typing processes are as follows:*

$$\frac{\Gamma \vdash a : Normal \qquad \Gamma \vdash b : Normal \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \overline{a}(b).P : Ok}\textit{T-out}$$

$$\frac{\Gamma \vdash a : Normal \qquad \Gamma \vdash x : Unknown \qquad \Gamma \vdash P : Ok}{\Gamma \vdash a(x).P : Ok}\textit{T-in}$$

$$\frac{\Gamma \vdash a : Normal \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \overline{a}.P : Ok}\textit{T-sout} \qquad \frac{\Gamma \vdash a : Normal \qquad \Gamma \vdash P : Ok}{\Gamma \vdash a.P : Ok}\textit{T-sin}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash b : Normal \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \S(\overline{a}(b).\pi).P : Ok}\textit{T-kout}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash x : Unknown \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \S(a(x).\pi).P : Ok}\textit{T-kin}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \S(\overline{a}.\pi).P : Ok}\textit{T-ksout}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \S(a.\pi).P : Ok}\textit{T-ksin} \qquad \frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \sharp(a.\pi).P : Ok}\textit{T-psin}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash b : Normal \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \sharp(\overline{a}(b).\pi).P : Ok}\textit{T-pout}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash x : Unknown \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \sharp(a(x).\pi).P : Ok}\textit{T-pin}$$

$$\frac{\Gamma \vdash a : Aberrant \qquad \Gamma \vdash P : Ok}{\Gamma \vdash \sharp(\overline{a}.\pi).P : Ok}\textit{T-psout} \qquad \frac{\Gamma \vdash P : Ok \quad Q \equiv P}{\Gamma \vdash Q : Ok}\textit{T-stc}$$

$$\frac{\vdash \Gamma\ well\ formed}{\Gamma \vdash 0 : Ok}\textit{T-nil} \qquad \frac{\Gamma, a : Normal \vdash P : Ok, \quad a \notin dom(\Gamma)}{\Gamma \vdash (\nu a)P : Ok}\textit{T-res}$$

$$\frac{\Gamma, a : Aberrant \vdash P : Ok, \quad a \notin dom(\Gamma)}{\Gamma \vdash (\nu a)P : Ok}\ \textit{T-ares}$$

$$\frac{\Gamma \vdash P[\tilde{a}/\tilde{x}, 0/K] : Ok}{\Gamma \vdash K\lfloor \tilde{a} \rfloor : Ok}\ K \triangleq \tilde{x}.P \quad \textit{T-const}$$

$$\frac{\Gamma \vdash P : Ok \quad \Gamma \vdash Q : Ok}{\Gamma \vdash P \mid Q : Ok}\textit{T-com} \qquad \frac{\Gamma \vdash P : Ok \quad \Gamma \vdash Q : Ok}{\Gamma \vdash P + Q : Ok}\textit{T-sum}$$

$$\frac{\Gamma \vdash x : Unknown \quad \Gamma \vdash b : Normal \quad \Gamma \vdash P : Ok}{\Gamma \vdash P\{b/x\} : Ok}\textit{T-Sub}$$

The original idea of this simple typing system is from [Aba99].

We give some properties about this simple typing system. The details of proofs are given in Appendix B.

Proposition 3.4 (Strengthening) *Assume that the name m is not free in the process P and that $n \neq m$. The following properties hold:*

- *If $\Gamma, m : T \vdash N : S$, then also $\Gamma \vdash n : S$.*
- *If $\Gamma, m : T \vdash P : Ok$, then also $\Gamma \vdash P : Ok$.*

Proposition 3.4 enables us to condense an environment, moving out the declaration of a name that is not used.

Proposition 3.5 (Weakening) *Assume that m is not defined in the environment Γ. The following properties hold:*

- *If $\Gamma \vdash n : S$, then $\Gamma, m : T \vdash n : S$.*
- *If $\Gamma \vdash P : Ok$, then $\Gamma, m : T \vdash P : Ok$.*

Proposition 3.5 declares that anything that can be proved in a given environment can also be proved with more assumptions.

Proposition 3.6 *Assume that $\vdash \Gamma$ well formed and that names in $dom(\Gamma)$ are all $normal$. Then the following properties hold:*

- *If m is a name and $m \in dom(\Gamma)$, then $\Gamma \vdash m : Normal$.*
- *if P is a process with $f_n(P) \cup f_v(P) \subseteq dom(\Gamma)$, then $\Gamma \vdash P : ok$.*

Proposition 3.6 says that a name is defined in an environment, then the name is of the class T in the environment; if names of one process are defined in an environment, then the process P typechecks in the environment.

3.5.2 A Simple Example

As we know, the typing system extracts information that is useful for reasoning about the behavior of programs. In this section, we still take *ras* activation (Section 3.3.2) as our basic example. We add a type for each motif and residue. Our example is type checked according to the typing rules.

Let Γ be an environment, where some names are $Unknown$ levels:

$$x_{INASWI\_II} : Unknown, \quad x_{ACTSWI\_II} : Unknown, \quad .$$

Bound names with their $Normal$ levels:

$$\begin{array}{rll} c\_ras_{GAP} : Normal, & c\_ras_{S\_GNEF} : Normal, & c\_ras_{R\_Nt} : Normal \\ gdp_{GAP} : Normal, & bbone_{INASWI\_I} : Normal, & sg_{INASWI\_II} : Normal, \\ rs\_1_{INASWI\_II} : Normal, & bbone_{S\_GNEF} : Normal, & sg_{S\_GNEF} : Normal, \\ gtp_{S\_GNEF} : Normal & rs\_2_{ACTSWI\_I} : Normal, & bbone_{ACTSWI\_I} : Normal, \\ sg_{ACTSWI\_II} : Normal, & bbone_{ASCTWI\_II} : Normal, & r\_swi\_1_{ACTSWI\_II} : Normal, \\ s_{R\_Nt} : Normal. & & \end{array}$$

Bound names with there $Aberrant$ levels:

$$s_{ACTSWI\_I} : Aberrant, \quad sg_{GAP} : Aberrant.$$

The subscript of a name represents the domain to which the name belongs.

According to Section 3.3.2, domains *INSWI_I* and *INASWI_II* of the protein *ras* are actually recursive processes:

$$INASWI\_I \quad ::= \quad bbone.\sharp(\overline{s}(rs\_2).\overline{rs\_2}).bbone.INASWI\_I \tag{3.29}$$

$$\begin{array}{rcll} INASWI\_II & ::= & \overline{sg}(rs\_1).rs\_1(x).\overline{sg}(r\_swi\_1). & \\ & & r\_swi\_1(x).\overline{bbone}.INASWI\_II & (3.30) \end{array}$$

Since

$$\begin{array}{rl} \Gamma \ \vdash & bbone_{INASWI\_I} : Normal, s_{ACTSWI\_I} : Aberrant, \\ & rs\_2_{ACTSWI\_I} : Normal, bbone_{ACTSWI\_I} : Normal \end{array}$$

for the domain *INASWI_I* (3.29), applying rules *T-nil, T-sin, T-pout, T-sout,* and *T-const* in turn, we prove it as follows:

$$\frac{\vdash \Gamma \ well\ formed}{\Gamma \vdash 0 : Ok}\text{T-nil}$$

$$\frac{\Gamma \vdash bbone_{ACTSWI\_I} : Normal \qquad \Gamma \vdash 0 : Ok}{\Gamma \vdash bbone.0 : Ok}\text{T-sin}$$

$$\frac{\Gamma \vdash s_{ACTSWI\_I} : Aberrant \quad \Gamma \vdash rs\_2_{ACTSWI\_I} : Normal \quad \Gamma \vdash bbone.0 : Ok}{\Gamma \vdash \sharp(s(rs\_2).\overline{rs\_2}).bbone.0 : Ok}\text{T-pin}$$

$$\frac{\Gamma \vdash bbone_{INASWI\_I} : Normal \quad \Gamma \vdash \sharp(s(rs\_2).\overline{rs\_2}).bbone.0 : Ok}{\Gamma \vdash bbone.\sharp(s(rs\_2).\overline{rs\_2}).bbone.0 : Ok}\text{T-sin}$$

$$\frac{\Gamma \vdash bbone.\sharp(s(rs\_2).\overline{rs\_2}).bbone.0 : Ok}{\Gamma \vdash INASWI\_I : Ok}\text{ T-const}$$

Since

$$\begin{aligned}\Gamma \quad \vdash \quad & sg_{INASWI\_II} : Normal, rs\_1_{INASWI\_II} : Normal,\\ & x_{INASWI\_itII} : Unknown, sg_{ACTSWI\_II} : Normal,\\ & r\_swi\_1_{ACTSWI\_II} : Normal, x_{ACTSWI\_II} : Normal\\ & bbone_{ACTSWI\_II} : Normal\end{aligned}$$

for the domain *INASWI_II* (3.30), applying rules *T-nil, T-sout, T-in, T-out, T-in, T-out* and *T-const* in turn, we prove it as follows:

$$\frac{\vdash \Gamma \ well\ formed}{\Gamma \vdash 0 : Ok}\text{T-nil}$$

$$\frac{\Gamma \vdash bbone_{ACTSWI\_II} : Normal \quad \Gamma \vdash 0 : Ok}{\Gamma \vdash \overline{bbone}.0 : Ok}\text{T-sout}$$

$$\frac{\begin{array}{c}\Gamma \vdash r\_swi\_1_{ACTSWI\_II} : Normal\\ \Gamma \vdash x_{ACTSWI\_II} : Unknown\\ \Gamma \vdash \overline{bbone}.0 : Ok\end{array}}{\Gamma \vdash r\_swi\_1(x).\overline{bbone}.0 : Ok}\text{T-in}$$

$$\frac{\begin{array}{c}\Gamma \vdash sg_{ACTSWI\_II} : Normal\\ \Gamma \vdash r\_swi\_1_{ACTSWI\_II} : Normal\\ \Gamma \vdash r\_swi\_1(x).\overline{bbone}.0 : Ok\end{array}}{\Gamma \vdash \overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{bbone}.0 : Ok}\text{T-out}$$

$$\frac{\begin{array}{c}\Gamma \vdash rs\_1_{INASWI\_II} : Normal \\ \Gamma \vdash x_{INASWI\_II} : Unknown \\ \Gamma \vdash \overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{bbone}.0 : Ok\end{array}}{\Gamma \vdash rs\_1(x).\overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{bbone}.0 : Ok}\text{T-in}$$

$$\frac{\begin{array}{c}\Gamma \vdash sg_{INASWI\_II} : Normal, \\ \Gamma \vdash rs\_1_{INASWI\_II} : Normal, \\ \Gamma \vdash rs\_1(x).\overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{bbone}.0 : Ok\end{array}}{\Gamma \vdash \overline{sg}(rs\_1).rs\_1(x).\overline{sg}(r\_swi\_1).r\_swi\_1(x).bbone.0 : Ok}\text{T-out}$$

$$\frac{\Gamma \vdash \overline{sg}(rs\_1).rs\_1(x).\overline{sg}(r\_swi\_1).r\_swi\_1(x).\overline{\overline{bbone}}.0 : Ok}{E \vdash INASWI\_II : Ok}\text{ T-const}$$

Hence, $\Gamma \vdash ras = INASWI\_I \mid INASWI\_II : Ok$.

Only the functional domain *S_GNEF* of the protein *SOS* takes part in our system. It is represented as a recursive process in Section 3.3.2.

$$S\_GNEF = bbone.S\_GNEF \mid sg(c\_ras).\overline{c\_ras}(gtp).S\_GNEF$$

Since

$$\begin{array}{rl}\Gamma & \vdash \quad bbone_{S\_GNEF} : Normal, sg_{S\_GNEF} : Normal, \\ & \quad c\_ras_{S\_GNEF} : Normal, gtp_{S\_GNEF} : Normal\end{array}$$

applying rules *T-nil, T-sin, T-out T-in, T-com* and *T-const*, we prove it as follows:

$$\frac{\vdash \Gamma \text{ well formed}}{\Gamma \vdash 0 : Ok}\text{T-nil}$$

$$\frac{\Gamma \vdash bbone_{S\_GNEF} : Normal \qquad \Gamma \vdash 0 : Ok}{\Gamma \vdash bbone.0 : Ok}\text{T-sin}$$

$$\frac{\Gamma \vdash c\_ras_{S\_GNEF} : Normal \qquad \Gamma \vdash gtp_{S\_GNEF} : Normal \qquad \Gamma \vdash 0 : Ok}{\Gamma \vdash (\overline{c_{ras}}(gtp).0 : Ok}\text{T-out}$$

$$\frac{\Gamma \vdash sg_{S\_GNEF} : Normal \quad \Gamma \vdash c\_ras_{S\_GNEF} : Normal \quad \Gamma \vdash (\overline{c_{ras}}(gtp).0 : Ok}{\Gamma \vdash sg(c\_ras).(\overline{c_{ras}}(gtp).0 : Ok} \text{T-in}$$

$$\frac{\Gamma \vdash bbone.0 : Ok \quad \Gamma \vdash sg(c\_ras).(\Gamma \vdash \overline{c_{ras}}(gtp).0 : Ok}{\Gamma \vdash bbone.0 \mid sg(c\_ras).(\overline{c_{ras}}(gtp).0 : Ok} \text{ T-com}$$

$$\frac{\Gamma \vdash bbone.0 \mid sg(c\_ras).(\overline{c_{ras}}(gtp).0 : Ok}{\Gamma \vdash S\_GNEF : Ok} \text{ T-const}$$

Only the functional domain *R_Nt* of the protein *RAF* takes part in our system. It is represented in Section 3.3.2 as follows:

$$R\_Nt = s(c\_ras).c\_ras.ACTR\_Nt$$

Since

$$E \vdash s_{R\_Nt} : Normal, c\_ras_{R\_Nt} : Normal$$

where the domain $ACTR\_Nt$ does not take part in our system, and we assume that the environment Γ has $ACTR\_Nt : Ok$.

applying rules *T-sin, T-in*, we prove it as follows:

$$\frac{c\_ras_{R\_Nt} : itNormal \quad \Gamma \vdash ACTR\_Nt : Ok}{\Gamma \vdash c\_ras.ACTR\_Nt : Ok} \text{ T-sin}$$

$$\frac{s_{R\_Nt} : Normal \quad c\_ras_{R\_Nt} : Normal \quad \Gamma \vdash ACTR\_Nt : Ok}{\Gamma \vdash s(c\_ras).c\_ras.ACTR\_Nt : Ok} \text{ T-in}$$

Hence, $\Gamma \vdash R\_Nt : Ok$

The protein *GAP* is represented as a recursive process in Section 3.3.2.

$$GAP = \S(sg(c\_ras).\overline{c\_ras}(gdp)).GAP$$

Since

$$\Gamma \vdash sg_{GAP} : Aberrant, c\_ras_{GAP} : Normal, gdp_{GAP} : Normal$$

applying rules *T-nil, T-kin* and *T-const*, we prove it as follows:

$$\frac{\vdash \Gamma \ well \ \ formed}{\Gamma \vdash 0 : Ok} \text{ T-nil}$$

$$\frac{\Gamma \vdash sg_{GAP} : Aberrant \quad \Gamma \vdash c\_ras_{GAP} : Normal \quad \Gamma \vdash 0 : Ok}{\Gamma \vdash \S(sg(c\_itras).\overline{c\_ras}(gdp).0 : Ok} \text{ T-kin}$$

$$\frac{\Gamma \vdash \S(sg(c\_itras).\overline{c\_ras}(gdp).0 : Ok}{\Gamma \vdash GAP : Ok} \text{ T-const}$$

In our system

$$Sys = ras \mid SOS \mid RAF \mid GAP$$

the domain S_SH3_BS of the protein SOS, domains R_ACT_BS, R_M_BS, INA_R_Ct, and R_ATP_BS of the protein RAF do not take part in our system. We assume that the environment Γ has $S\_SH3\_BS : Ok$, $R\_ACT\_BS : Ok$, $R\_M\_BS : Ok$, $INA\_R\_Ct : Ok$, and $R\_ATP\_BS : Ok$.

Therefore, it is easy to find that $E \vdash Sys : Ok$ by rule *T-Com*.

3.6 Comparing Tags and Types

In Section 3.4 and Section 3.5, we have given a tag system and a typing system, which help us to understand the $I\pi$-calculus. In this section, we compare these two systems and show that the tag system is equal to the typing system in terms of expressive power.

Every term has a tag in the tag system. A tag for a term and a set of tags for a process allow us record states of the whole biochemical process. We can use the language of set theory to record the process. It is natural and simple to understand. For instance,

$$A ::= a.b.0$$

a has the tag i_a, b has the tag i_b, 0 has the tag $\varnothing$. Then A has the tag $\{i_a, i_b\}$.

We find, however, when a biochemical process is very complex, we have to take a large amount of space to record its tag. The tag of the process could be a large set containing all the tags of all the terms. Actually, in the qualitative analysis of biochemical processes, we don't care what is the tag. We just want to know what happens with the aberrant ones, therefore, in the tag of the process. Of course, we believe that tags are important in the quantitative analysis.

The strong points of the typing system are obvious. As the other type systems, this typing system for $I\pi$-calculus is useful for several reasons:

(1) to detect programming errors statically;
For example, the following errors in the process P can be found by our simple typing system:

(a) When $\Gamma \vdash a : Normal$, P has the form of $\S(\pi).Q$ or $\sharp(\pi).Q$.

(b) When $\Gamma \vdash a : Aberrant$, P has the form of $\pi_0.Q$.

where $\pi_0 = \overline{a}(b) \mid a(x) \mid a \mid \overline{a}$, and $\pi = \pi_0 \mid \pi$.

(2) to extract information that is useful for reasoning about the behavior of programs;

(3) to improve the efficiency of code generated by a compiler;

(4) to make programs easier to understand.

Specially, comparing to the tag system, this typing system is much simpler. For instance, taking

$$A ::= a.b.0$$

again, we have that $a : normal, b : normal, 0 : ok \vdash A : ok$. The process A is checked simply which it is enough to help our simulation. Of course, if we want to analyze the biochemical process quantitatively, this simple typing system is not enough.

Although these two systems have their shortcomings and strong points respectively, they are equal in terms of expressive power.

Proposition 3.7 (Signal checking) *Let i_a be the tag of the name a. Then*

- $i_a = 0$ *if and only if* $a : Aberrant$;
- $i_a > 0$ *if and only if* $a : Normal$.

The proof of this proposition is trivial by the definition of tags.

Now, we bring out the key theorem of this chapter, presented as follows. We relate the tag system and the typing system by proving that they both capture the presence of an aberrant component in the system.

Theorem 3.1 (Correspondence) *Let I_P be the tag of P. If $\Gamma \vdash P : Ok$, the following statements are equivalent:*
(a) $0 \in I_P$
(b) P *has a subprocess of the form* $\S(\pi).Q$ *or* $\sharp(\pi).Q$
(c) There is a typing judgement $\Gamma \vdash P : Ok$ *such that in the proof of this typing judgement there is a proof of a judgement of the form* $\Gamma' \vdash m : Aberrant$

Proof: It is easy to prove that if P has a subprocess of the form $\S(\pi).Q$ or $\sharp(\pi).Q$, then $0 \in I_P$.

Suppose that $0 \in I_P$. According to Table 3.2, (b) is proved.

Next we prove that (b) is equal to (c). Suppose that P has a subprocess of the form $\S(\pi).Q$ or $\sharp(\pi).Q$. It suffices to consider the following cases:

(1) Case $\S(\pi).Q$. Since $\Gamma \vdash \S(\pi).Q : Ok$, this assertion must be established through rules *T-kout*, *T-kin*, *T-ksout*, and *T-ksin*. Then there is a name m in π such that $\Gamma \vdash m : Aberrant$.

(2) Case $\sharp(\pi).Q$. Since $\Gamma \vdash \sharp(\pi).Q : Ok$, this assertion must be established through rules *T-pout*, *T-pin*, *T-psout*, or *T-psin*. Then there is a name m in π such that $\Gamma \vdash m : Aberrant$.

(3) Case $R' + R$. Since $\Gamma \vdash R' + R : Ok$, this assertion must be established through the rule *T-Sum*. Then we have $\Gamma \vdash R' : Ok$ and $\Gamma \vdash R : Ok$. Because $R' + R$ has a subprocess of the form $\S(\pi).Q$ or $\sharp(\pi).Q$, we have R' or R has a subprocess of the form $\S(\pi).Q$ or $\sharp(\pi).Q$. Without loss of generality, we consider R' has a subprocess of the form $\S(\pi).Q$. Then there is a name m in R' such that $\Gamma' \vdash m : Aberrant$ by assumption, of course, $m \in R' + R$ and $\Gamma' \vdash m : Aberrant$. Case $R' \mid R$ is similar to prove.

(4) Case a constant application ($P = K\lfloor \tilde{a} \rfloor$) for a recursive definition ($K \triangleq \tilde{x}.P'$). Since $\Gamma \vdash P : Ok$, this assertion must be established through the rule *T-Const*. Then we have $\Gamma \vdash P'[\tilde{a}/\tilde{x}, 0/K] : Ok$. Because P has the form of $\S(\pi).Q$ or $\sharp(\pi).Q$, that is, $P'[\tilde{a}/\tilde{x}, 0/K]$ has the form of $\S(\pi).Q$ or $\sharp(\pi).Q$. By assumption, there is a name m in $P'[\tilde{a}/\tilde{x}, 0/K]$, of course in P, such that $\Gamma' \vdash m : Aberrant$.

Next we prove the converse. It also suffices to consider the following cases:

(1) The base case is that P is the form of $\S(\pi).Q$ or $\sharp(\pi).Q$ by the rules *T-kout*, *T-kin*, *T-ksout*, *T-ksin*, *T-pout*, *T-pin*, *T-psout*, and *T-psin*. Then (b) is proved.

(2) Assume that P is the form of $R' + R$ and processes R' and R satisfy the property (c). Since $\Gamma \vdash P : Ok$, that is $\Gamma \vdash R' + R : Ok$, we have $\Gamma \vdash R' : Ok$, and $\Gamma \vdash R : Ok$. Because there is a term $m \in P$ such that $\Gamma' \vdash m : Aberrant$, we can know that $m \in R'$ or $m \in R$ such that $\Gamma \vdash m : Aberrant$. Without loss of generality, we consider $m \in R'$. From the assumption, we know R' has the subprocess of the form of $\S(\pi).Q$ or $\sharp(\pi).Q$, then of course, P has the subprocess of the form of $\S(\pi).Q$ or $\sharp(\pi).Q$. The case that P is the form of $Q \mid R$ is similar to discuss.

(3) Assume that P is a constant application ($P = K\lfloor \tilde{a} \rfloor$) for a recursive definition ($K \triangleq \tilde{x}.P'$) and $P'[\tilde{a}/\tilde{x}, 0/K]$ satisfies the property (c). Since $\Gamma \vdash P : Ok$, it must be that $\Gamma \vdash P'[\tilde{a}/\tilde{x}, 0/K] : Ok$ by the rule *T-Const*. If there is a name $m \in P$ such that $\Gamma' \vdash m : Aberrant$, we can know that $m \in P'[\tilde{a}/\tilde{x}, 0/K]$. Then $P'[\tilde{a}/\tilde{x}, 0/K]$ has the subprocess of the form of $\S(\pi).Q$ or $\sharp(\pi).Q$ by assumption. That is P has the subprocess of the form of $\S(\pi).Q$ or $\sharp(\pi).Q$.

With our brief typing system, we can verify the aberrant ST pathways without complex tags.

Chapter 4

A Language for Formal Proteins

In this chapter, we study a language for formal proteins, the κ-calculus. We consider the problem of *self-assembly* which we formalize in terms of a translation from the κ-calculus (playing the role of a coarse-grained language) to a finer-grained (sub)language, the $m\kappa$-calculus. The mathematical properties of self-assembly are discussed. The correctness of this translation is proved using these properties.

The contents of this chapter are organized as follows. In Section 4.1 we give a κ-model of *ras* activation. In Section 4.2, we recall some basic notions about the $m\kappa$-calculus. In Section 4.3 we introduce the property self-assembly. The graphic explanation of the reversible and non reversible rules of translation from the κ-calculus to the $m\kappa$-calculus is given. In Section 4.4 we show some mathematical properties of self-assembly, including *confluence*, *strong normalization*. The correctness of this translation is proved using these mathematical properties.

4.1 The κ-model of *ras* Activation

In Section 2.2.1, we have recalled some basic notions of the κ-calculus. In this section, we give an example to show how to model the process of *ras* activation using the κ-calculus.

The process of *ras* activation was introduced in Section 3.3.2. Here we denote proteins as primitive proteins; complexes of proteins as solutions; and residues of functional domains as sites of proteins. Reactions for activation are denoted by monotonic reactions, and reactions for inactivation are denoted by antimonotonic reactions.

First, the protein *ras* is activated by the protein *SOS* (reaction (4.1)).

$$\begin{aligned} ras(bbone + sg + rs\_1 + \overline{bbone2}), SOS(bbone + sg + c\_ras) \longrightarrow \\ (xy_1y_2)(ras(bbone^x + sg^{y_1} + rs\_1^{y_2} + bbone2), \\ SOS(bbone^x + sg^{y_1} + c\_ras^{y_2})) \end{aligned} \tag{4.1}$$

Different from the example in Section 3.3.2, we do not denote functional domains in proteins. Actually, in reaction (4.1), *bbone* is the residue of one functional domain ($INASWI\_I$) and *sg, rs_1, bbone2* belong to the other domain ($INASWI\_II$) in the protein *ras*. Reaction (4.1) says that the domain $INASWI\_I$ of the protein *ras* is activated by the binding of *bbone*, and the other domain $INASWI\_II$ is activated by bindings of *sg, rs_1*. Then the protein *ras* is activated.

Reaction (4.2) shows how the activated protein *ras* activates the next protein *RAF*. The protein *RAF* is activated by binding sites *s, rs_2* of the protein *ras* with sites *s, c_ras* of the protein *RAF*.

$$\begin{aligned} ras(bbone^{x} + sg^{y_1} + rs\_1^{y_2} + s + rs\_2), RAF(s + c\_ras) \longrightarrow \\ (z_1 z_2)(ras(bbone^{x} + sg^{y_1} + rs\_1^{y_2} + s^{z_1} + rs\_2^{z_2}), RAF(s^{z_1} + c\_ras^{z_2})) \end{aligned} \tag{4.2}$$

Reaction (4.3) says that when the protein *GAP* takes part in the reactions, the activated protein *ras* begins to loose its activity. This reaction is taken by unbinding sites *sg, rs_1* of the protein *ras* from sites of *sg, c_ras* of the protein *SOS*.

$$\begin{aligned} (y_1 y_2)(ras(bbone^{x} + sg^{y_1} + rs\_1^{y_2} + bbone2), \\ SOS(bbone^{x} + sg^{y_1} + c\_ras^{y_2}), GAP(sg + c\_ras) \longrightarrow \\ (ras(bbone^{x} + sg + rs\_1 + \overline{bbone2}), \\ SOS(bbone^{x} + sg + c\_ras), GAP(\overline{sg} + \overline{c\_ras})) \end{aligned} \tag{4.3}$$

Reaction (4.4) says that the protein *ras* goes back to the initial state, that is, the inactive state. The process of *ras* activation is over.

$$\begin{aligned} (x)(ras(bbone^{x} + sg + rs\_1 + \overline{bbone2}), SOS(bbone^{x})) \longrightarrow \\ ras(bbone + sg + rs\_1 + \overline{bbone2}), SOS(bbone) \end{aligned} \tag{4.4}$$

This example is simple yet biologically visible. We simplify complicated biological reactions and only use the binding or unbinding to represent reactions, which capture the information about which proteins take part in the reactions though we omit details of reactions.

4.2 The $m\kappa$-calculus

The $m\kappa$-calculus is a finer-gained language which describes a less idealized formal biology. The syntax of the $m\kappa$-calculus is the same as the κ-calculus discussed

in Section 2.2.1 except for *group names*, and the semantics of the reaction rules in the $m\kappa$-calculus are more restricted.

We call basic components in the $m\kappa$-calculus *agents*. We assume an infinite countable set $\mathcal{P}$ of agents names, an infinite countable set $\mathcal{E}$ of edge names and an infinite countable set $\mathcal{G}$ of group names. Edge and group names are supposed to be disjoint. We take the same *signature* map $\mathfrak{s}$ from $\mathcal{P}$ to natural numbers $\mathbb{N}$ as in κ-calculus.

Let $A, B, \cdots$ range over protein names, $x, y, \cdots$ range over edge names, and r, $r', \cdots$ range over group names. For each agent name A, $\mathfrak{s}(A)$ is the number of sites of A, and for any $1 \leqslant i \leqslant \mathfrak{s}(A)$, each pair (A, i) will accordingly be called a *site* of A.

An *extended interface*, is a finite map from $\mathbb{N}$ to $(\mathcal{E} + \mathcal{G} + \{h, v\}) \times \mathbb{N}$, ranged over by θ and similar symbols. The integer part of $\theta(i)$ is referred to as a *log*.

An *agent* is a pair written $A(\theta)$ with $A \in \mathcal{P}$ and θ an extended interface defined on $\mathfrak{s}(A)$. Solutions are built as in Section 2.2. All concepts about the edges is extended to the edges and group names. For instance, interval extrusion $\mathsf{S}, (x)(\mathsf{S}') \equiv (x)(\mathsf{S}, \mathsf{S}')$ applies both for x in $\mathcal{E}$ and $\mathcal{G}$, with the usual side-condition that $x \notin \mathrm{fn}(\mathsf{S})$.

For example, for an agent A, if $\mathfrak{s}(A) = 3$, and $\theta(1) = x, 1$, $\theta(2) = r, 2$ and $\theta(3) = h, 0$, then one may simply write $A(1^{x,1} + 2^{r,2} + \bar{3}^{0})$. We will also indulge sometimes in not writing a log when it is 0, so that for instance $C(1^{x,4} + 2^{r} + 3)$ will stand for $C(1^{x,4} + 2^{r,0} + 3^{0})$. A convenient consequence of this notational abuse is that κ-proteins become a particular case of $m\kappa$-agents.

In the $m\kappa$-calculus, group names are used as the means to built transient cooperative structures in our low-level systems. Logs are the additional information on sites. They are used to effect that the agent can log what's up on this connection. The logs can be forgotten by means of the following *projection* map:

$$(\imath^{x,n})^{-} = \imath^{x} \qquad (\imath^{r,n})^{-} = (\imath^{v,n})^{-} = \imath^{v} \qquad (\imath^{h,n})^{-} = \imath^{h}$$

This projection extends in the obvious way to interfaces, agents and solutions.

Definition 4.1 (Interaction) *Let L,R, be two $m\kappa$-solutions, $L \longrightarrow R$ is said to be an interaction if:*
— both L and R consist of at most two agents
—$fn(L) \supseteq fn(R)$
— L does not contain any "ν" on group names

Definition 4.2 (Monotonic Interaction) *An $m\kappa$-interaction $L \longrightarrow R$ is said to be monotonic (resp. anti-monotonic) if its projection $(L)^{-} \longrightarrow (R)^{-}$ is a monotonic (resp. anti-monotonic) κ-reaction.*

Thus, in the $m\kappa$-calculus we consider only interactions which are simple and close to biological interactions. These interactions are taken as atomic events. And they can encode a given higher level reaction.

4.3 The Self-assembly Protocol for the κ-calculus

Self-assembly is a very important property in biology. It is a method of integration in which components spontaneously assemble, typically by bouncing around in a solution or gas phase until a stable structure of minimum energy is reached. Self-assembly is crucial to the assembly of bio-molecular nano-technologies, and is thus a promising method for assembling atomically precise devices. Components in self-assembled structures find their appropriate location based solely on their structural properties (or chemical properties in the case of atomic or molecular self-assembly). Self-assembly is by no means limited to molecules or the nano-scale and can be carried out on just about any scale, making it a powerful bottom-up method.

In our specific case, we can synthesize processes given a higher level description in the κ-calculus. For each of interacting proteins, in a purely decentralized way and with binary synchronization as the only means of communication, proteins are going to behave according to the original higher level description.

4.3.1 The Monotonic Protocol

The purpose of this subsection is to decompose a κ-reaction in the $m\kappa$-calculus: given a κ-reaction $\mathfrak{r}$, one wants to define an associated family, $[\![\mathfrak{r}]\!]_m$, of $m\kappa$-interactions capable of simulating $\mathfrak{r}$ in a sense that will be made precise below.

We follow a protocol that gradually recruits reactants and constructs the products by the only means of binary and unary interactions. Hence, we need some statically predetermined structure in which we can know which agents may or may not be managed in one step in the protocol.

Definition 1 (Micro-scenario) *Let* $\mathfrak{r} = \mathsf{L} \to (\tilde{x})\mathsf{R}$ *be a monotonic* κ*-reaction, a* micro-scenario *for* $\mathfrak{r}$ *is a triple* $(\mathcal{F}_\mathfrak{r}, \mathcal{T}_\mathfrak{r}, \mathsf{init})$ *such that:*
— $\mathcal{F}_\mathfrak{r}$ *is (isomorphic to) an acyclic orientation of* $[\![\mathsf{R}]\!]_g$*, called a flow graph;*
— $\mathcal{T}_\mathfrak{r}$ *is a tree spanning* $\mathcal{F}_\mathfrak{r}$*;*
— init*, also written* $\mathsf{init}(\mathcal{F}_\mathfrak{r})$*, is the common root of* $\mathcal{F}_\mathfrak{r}$ *and* $\mathcal{T}_\mathfrak{r}$*;*
— *and* init *belongs to* $[\![\mathsf{L}]\!]_g$ *(up to the isomorphism above).*

We have some remarks on *micro-scenario*.

(1) We assume that $\mathcal{F}_\mathfrak{r}$ is an oriented graph-with-sites with integers as nodes. For different κ-reactions, their micro-scenarios are chosen to use disjoint set of nodes. In this way, when agents are recruited and handed a node of $\mathcal{F}_\mathfrak{r}$, they can identify which global κ-reaction they take part in, and what role they have in it.

(2) Such micro-scenarios always exist [DL04]. There always is a micro-scenario for any given monotonic κ-reaction: since $[\![\mathsf{R}]\!]_g$ is connected by monotonicity, any node of $[\![\mathsf{L}]\!]_g$ can be chosen as the root and one could even assume $\mathcal{T}_\mathfrak{r}$ to be

depth-first. Because any connected graph admits an acyclic orientation which can be obtained, for instance, by choosing an arbitrary root, constructing a depth-first tree spanning the graph, and directing all remaining edges according to the tree ordering.

(3) We suppose that $[\![\mathsf{R}]\!]_g$ doesnt have loops. In fact, there could be loops in $[\![\mathsf{R}]\!]_g$, that is edges from a node to itself. The techniques described here adapt very easily to this case, since loops are purely local to a node.

(4) The *micro-scenario* is not unique. We could do with more than one initiator, without a spanning tree, etc [**?**]. Even in the restricted kind of micro-scenarios that we are considering, there is room for different choices.

(5) The spanning tree $\mathcal{T}_\mathfrak{r}$ serves as a way of imposing a "parental priority" between the contacts in our protocol. We will give explanation later.

The graph $\mathcal{F}_\mathfrak{r}$ can be decided as a map over sites, defined as follows:

$$\begin{array}{ll} \mathcal{F}_\mathfrak{r}(a,i) = (b,j) & \textit{if } (a,i),(b,j) \textit{ are connected in } \mathcal{F}_\mathfrak{r} \\ \mathcal{F}_\mathfrak{r}(a,i) = \bot & \textit{if } (a,i) \textit{ is free in } \mathcal{F}_\mathfrak{r} \end{array}$$

We write $\mathcal{F}_\mathfrak{r}^\star$ for the inverse of $\mathcal{F}_\mathfrak{r}$.

Intuitively, a site is an output if it belongs to the domain of $\mathcal{F}_\mathfrak{r}$, and an input if it belongs to the range of $\mathcal{F}_\mathfrak{r}$. In other words, a site (a,i) is called an output if $\mathcal{F}_\mathfrak{r}(a,i) \neq \bot$ and an input if $\mathcal{F}_\mathfrak{r}^\star(a,i) \neq \bot$.

Definition 2 (input/output interfaces) *For $n \in \mathbb{N}$, $a \in \mathcal{F}_\mathfrak{r}$ and $\widetilde{x}$ a tuple indexed over the set of inputs (resp. outputs) of a and with values in $\mathcal{E}$, we define the* input *(resp.* output*) interfaces:*

$$\begin{array}{rcl} \wedge_a^{\widetilde{x},n} & := & \displaystyle\sum_{\{i \,|\, \mathcal{F}_\mathfrak{r}^\star(a,i) \neq \bot\}} i^{x_i,n} \\ \vee_a^{\widetilde{x},n} & := & \displaystyle\sum_{\{i \,|\, \mathcal{F}_\mathfrak{r}(a,i) \neq \bot\}} i^{x_i,n} \end{array}$$

These interfaces will serve us to denote the fact that all input (or output) parts bear a certain log n.

A protein with name A is translated as an agent of the same name but with one more *auxiliary* site, written $*$:

$$[\![A(\rho)]\!]_m = A(* + \rho).$$

This translation extends to κ-solutions and likewise, if S is a κ-solution, we write $[\![\mathsf{S}]\!]_m$ to denote its translation. That special site $*$ is used to log what little additional information one needs, that is the role of A in a given reaction (i.e. to which node it corresponds in $\mathcal{F}_\mathfrak{r}$) and a group name identifying uniquely the current attempted

high-level reaction. To ease reading, we have systematically abbreviated $A(*^{r,a} + \theta)$ as $A^{r,a}(\theta)$ and also made use of the notation for input and output interfaces introduced above.

Thereafter and for the rest of the paper, we suppose that it is given a monotonic κ-reaction $L \longrightarrow_\kappa (\upsilon x)R$, a choice of *micro-scenarios* has been made.

The monotonic protocol consists in a first phase of *recruitment* and a subsequent phase of *completion*. Recruitment begins with a signal sent by a specific agent called the *initiator*. Then one sends and propagates two kinds of signals: *forward* signals to recruit the necessary reactants, and *backward* signals to report success back to the initiator. At the end of this first phase, the initiator knows that the global κ-reaction can be completed, and in the completion phase, this information is propagated to the other reactants.

$$\frac{\mathcal{F}^\star_{\mathfrak{r}}(a, \_) = \bot}{A(\triangle^{v} + \vee^{\widetilde{x}}) \rightleftharpoons (r)(A^{r,a}(\triangle^{v,1} + \vee_a^{\widetilde{x},0}))}\ \text{init}$$

$$\frac{\mathcal{T}_{\mathfrak{r}}(a,i) = (b,j),\ y \in \mathrm{fn}(\mathfrak{r})}{A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{y,0}), B(j^{y} + \vee^{\widetilde{z}}) \rightleftharpoons A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{y,1}), B^{r,b}(j^{y,1} + \vee_b^{\widetilde{z},0})}\ \text{FC}_1$$

$$\frac{\mathcal{T}_{\mathfrak{r}}(a,i) = (b,j),\ y \notin \mathrm{fn}(\mathfrak{r}),\ b \in \mathsf{L}}{A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{v,0}), B(j^{v} + \vee^{\widetilde{z}}) \rightleftharpoons (y)(A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{y,1}), B^{r,b}(j^{y,1} + \vee_b^{\widetilde{z},0}))}\ \text{FC}_2$$

$$\frac{\mathcal{T}_{\mathfrak{r}}(a,i) = (b,j),\ y \notin \mathrm{fn}(\mathfrak{r}),\ b \in \mathsf{R} \setminus \mathsf{L}}{A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{v,0}) \rightleftharpoons (y)(A^{r,a}(\wedge_a^{\widetilde{x},1} + i^{y,1}), B^{r,b}(\wedge_b^{\widetilde{x}\setminus y,0} + j^{y,1} + \vee_b^{\widetilde{0},z}))}\ \text{FC}_3$$

$$\frac{\mathcal{T}^c_{\mathfrak{r}}(a,i) = (b,j),\ x \in \mathrm{fn}(\mathfrak{r})}{A^{r,a}(\wedge_a^{\widetilde{y},1} + i^{x,0}), B^{r,b}(j^{x,0} + \vee_b^{\widetilde{z},0}) \rightleftharpoons A^{r,a}(\wedge_a^{\widetilde{y},1} + i^{x,1}), B^{r,b}(j^{x,1} + \vee_b^{\widetilde{z},0})}\ \text{LC}_1$$

$$\frac{\mathcal{T}^c_{\mathfrak{r}}(a,i) = (b,j),\ x \notin \mathrm{fn}(\mathfrak{r})}{A^{r,a}(\wedge_a^{\widetilde{y},1} + i^{v,0}), B^{r,b}(j^{v,0} + \vee_b^{\widetilde{z},0}) \rightleftharpoons (x)(A^{r,a}(\wedge_a^{\widetilde{y},1} + i^{x,1}), B^{r,b}(j^{x,1} + \vee_b^{\widetilde{z},0}))}\ \text{LC}_2$$

Table 4.1: $\downarrow^0_1$

$$\frac{\mathcal{F}_{\mathfrak{r}}(a,i)=(b,j)}{A^{r,a}(\wedge_a^{\widetilde{x},1}+i^{y,1}),B^{r,b}(j^{y,1}+\vee_b^{\widetilde{z},2})\rightleftharpoons A^{r,a}(\wedge_a^{\widetilde{x},1}+i^{y,2}),B^{r,b}(j^{y,2}+\vee_b^{\widetilde{z},2})}\ \mathsf{R}$$

Table 4.2: $\downarrow\!\!\uparrow_2^1$

$$\uparrow_{12};\downarrow_{20}\frac{\mathcal{F}^{\star}_{\mathfrak{r}}(a,\_)=\bot}{A^{r,a}(\triangle^{v,1}+\vee_a^{\widetilde{x},2})\to A^{r,a}(\triangle^{v,3}+\vee_a^{\widetilde{x},2})}\ \text{phase-shift}$$

$$\frac{\mathcal{F}_{\mathfrak{r}}(a,i)=(b,j)}{A^{r,a}(\wedge_a^{\widetilde{x},3}+i^{y,2}),B^{r,b}(j^{y,2}+\vee_b^{\widetilde{z},2})\to A^{r,a}(\wedge_a^{\widetilde{x},3}+i^{y,3}),B^{r,b}(j^{y,3}+\vee_b^{\widetilde{z},2})}\ \text{propagation}$$

Table 4.3: $\downarrow_3^2$

$$\frac{}{A^{r,a}(\wedge_a^{\widetilde{x},3}+\vee_a^{\widetilde{y},3})\to A(\wedge^{\widetilde{x}}+\vee^{\widetilde{y}})}\ \text{exit}$$

Table 4.4: Exit

We give some explanation for the protocol as follows:

(1) Table 4.1 includes the rule *initiation* (*init* for short), *first contacts* (FC_1,FC_2, FC_3), and *late contacts* (LC_1, LC_2). Table 4.2 includes the rule *response* (R for short). Table 4.3 includes the rule *phase-shift* (*ps* for short) and the rule *propagation*. Table 4.4 includes the rule *exit*.

(2) The graphical explanation of the protocol will be given in the next section.

(3) The spanning tree $\mathcal{T}_{\mathfrak{r}}$ assures that only the parent according to $\mathcal{T}_{\mathfrak{r}}$ is allowed to wake a child and recruit it, while all the other reactants have to use further interactions. Some self-deadlocks may happen if we do not do this. For instance, given a monotonic κ-reaction and a solution as follows:

$$\begin{array}{rcl}\mathsf{t} & = & A(1^x+2),B(1^x+2),C(1+2)\to\\ & & (yz)(A(1^x+2^y),B(1^x+2^z),C(1^y+2^z))\\ \mathsf{T} & = & (xyz)(A(1^x+2),B(1^x+2),C(1+2),C(1+2))\end{array}$$

if there is no priority between contacts, then A and B might recruit distinct Cs in T, and so, in some sense, the recruitment defeats itself all alone.

Hence, if we have a tree to sort out who is doing the first contact, and who is not, then the problem is solved.

(4) Reversible rules before the rule **phase-shift** give the group the ability to escape deadlocks. Agents can test whether they can take a step backward without inconsistencies.

4.3.2 A Graphical Explanation of the Protocol

In this section, we give a graphical description of this protocol, which we hope make it easier to understand.

We use ovals to represent proteins; arrows $\downarrow$ with names to represent the binding from one output of one protein to one input of another protein; the box to represent the group r. When the arrow $\downarrow$ is included into the box completely, it says the log of this site is 1, otherwise, the log is 0. The symbol $\uparrow$ represents the feedback respectively, that is , the logs of the correlative input and output are 2. Similarly, the log on site is 3 when there are three arrows $\downarrow\uparrow\downarrow$.

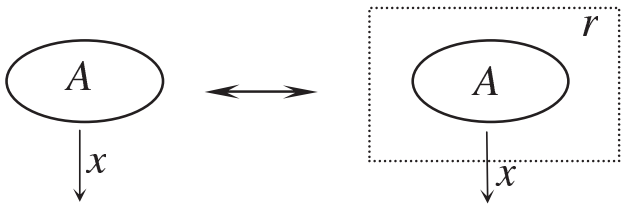


Figure 4.1: The Rule **init**.

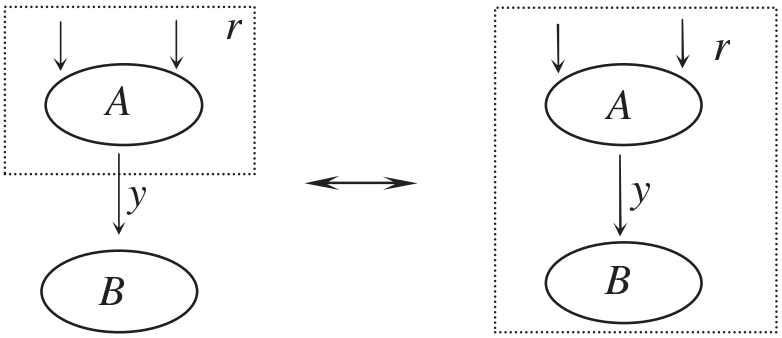


Figure 4.2: The Rule $\mathbf{FC}_1$.

A is the initial protein, so it has not inputs. The rule **init** (Fig. 4.1) says, as the initial protein, A makes a group (r) to begin to recruit the next proteins.

The rule $\mathbf{FC}_1$ (Fig. 4.2) says that when all inputs of the protein A are recruited into the group r, A can recruit a new protein B through one of its outputs.

The difference between the rule $\mathbf{FC}_2$ (Fig. 4.3) and the rule *FC1*, is that the protein A and B have not connection on sites before the recruitment in the rule $\mathbf{FC}_2$.

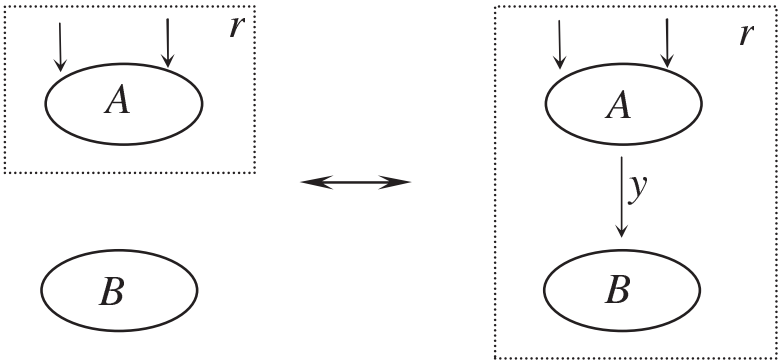


Figure 4.3: The Rule $\mathbf{FC}_2$.

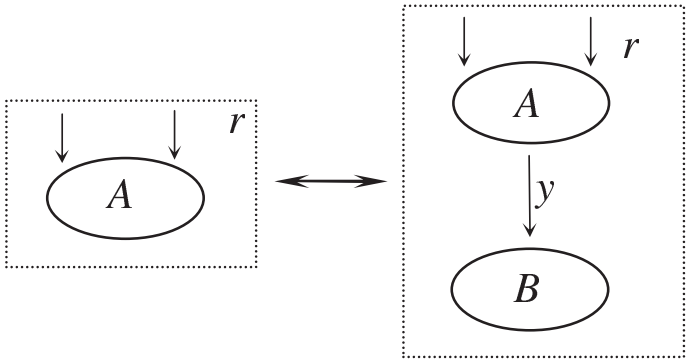


Figure 4.4: The Rule $\mathbf{FC}_3$..

The difference between the rule $\mathbf{FC}_3$ (Fig. 4.4) and the rules $\mathbf{FC}_1$ and $\mathbf{FC}_2$, is that the recruited protein B is new in the rule $\mathbf{FC}_3$.

The difference between the rules $\mathbf{LC}_1$, $\mathbf{LC}_2$ (Fig. 4.5 and Fig. 4.6) and the rules $\mathbf{FC}_1$,$\mathbf{FC}_2$ is the protein B has already been recruited through one of its other input ports in the rules $\mathbf{LC}_1$, $\mathbf{LC}_2$.

The rule **R** (Fig. 4.7) says that, when all outputs of the protein B have received the feedback, the B can propagate it to its input sites.

The rule **phase-shift** (Fig. 4.8) says that, when the initial protein A receives all the feedback from his outputs, it can initiate the succeeding phase of sending a successful message to all the proteins which have been recruited.

The rule **propagation** (Fig. 4.9) says that, when the protein B receives the successful message from inputs, he will send it to the next proteins from his outputs.

The rule **exit** (Fig. 4.10) says that, the proteins which take part in the reaction exit when the recruitment is over, which is witnessed by the fact that all input and output sites have the new log 3 (new $\downarrow\updownarrow\downarrow$ in the figure).

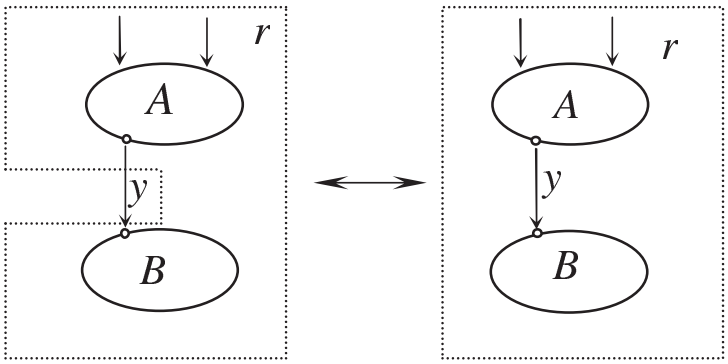


Figure 4.5: The Rule $\mathbf{LC}_1$..

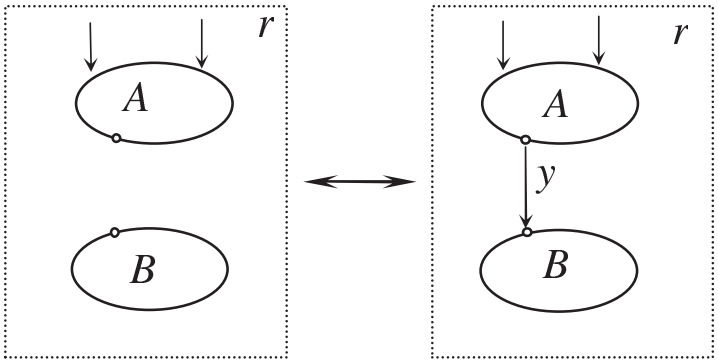


Figure 4.6: The Rule $\mathbf{LC}_2$..

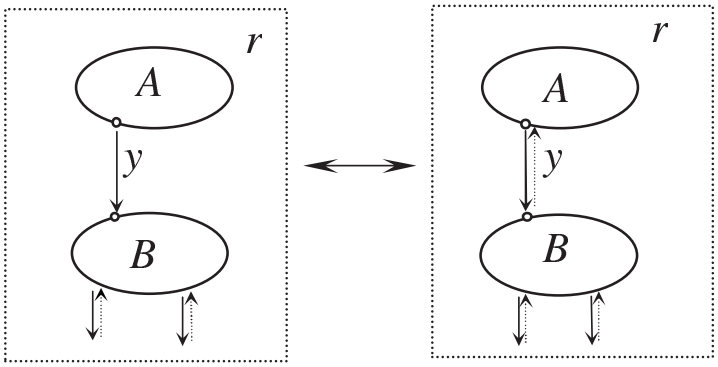


Figure 4.7: The Rule **R**.

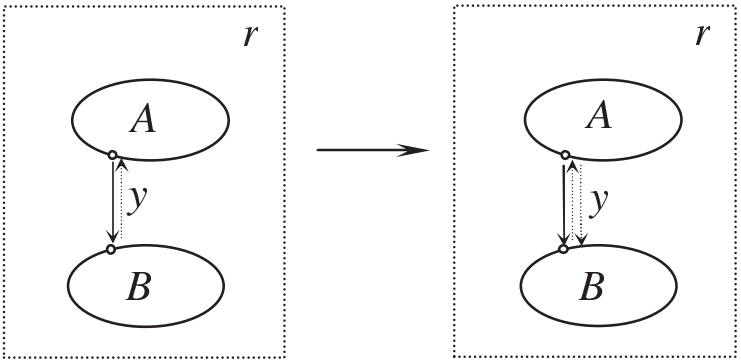


Figure 4.8: The Rule **phase-shift**.

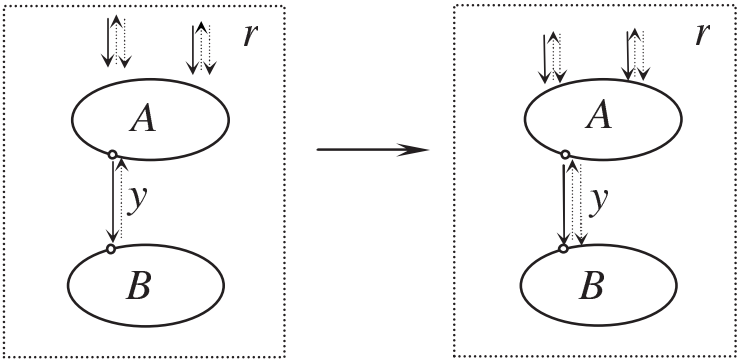


Figure 4.9: The Rule **propagation**.

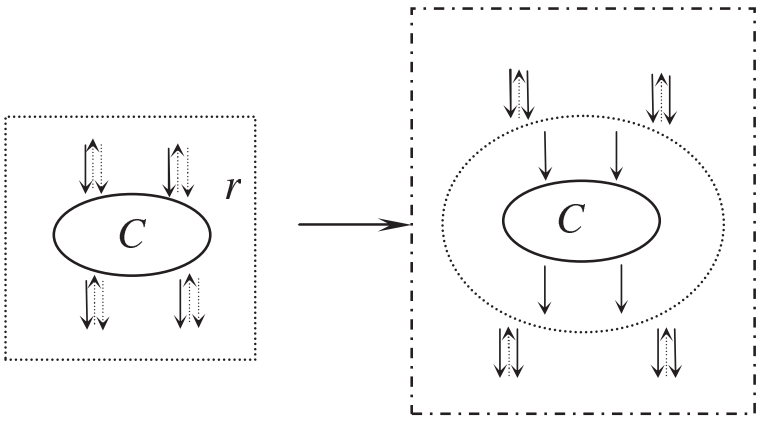


Figure 4.10: The Rule **Exists**.

4.4 Mathematical Properties of Self-assembly

In this section, we discuss mathematical properties of self-assembly, which culminate in the proof of the correctness of self-assembly.

In the monotonic protocol, we denote the set of forward rules before the rule **phase-shift** (**ps** for short) as *pre-ps* (i.e. all forward rules in Table 4.1 and Table 4.2) and the set of backward rules as *pre-ps*$^{-1}$ (i.e. all backward rules in Table 4.1 and Table 4.2); denote the set of rules after the rule **ps** as *post-ps* (i.e. all rules in Table 4.3 and Table 4.4 except for the rule **ps**). The binary relation $\longrightarrow_c$ on $m\kappa$-solutions is the union of *pre-ps*$^{-1}$ and *post-ps*.

Definition 4.3 *In the $m\kappa$-calculus, let $\mathfrak{R}$ be a set of biological reactions, the associated* $\mathfrak{R}$-system *is the pair $(\mathfrak{S}, \rightarrow_{m\kappa})$, where $\mathfrak{S}$ is the set of $m\kappa$-solutions and $\rightarrow_{m\kappa}$, called the* transition *relation, is the least binary relation over $\mathfrak{S}$ such that:*

$$\text{mon}\ \frac{S, T \models L \rightarrow_{m\kappa} (\tilde{x})R \ \in \mathfrak{R}}{S \rightarrow_{m\kappa} T} \qquad \frac{S, T \models (\tilde{x})L \rightarrow_{m\kappa} R \ \in \mathfrak{R}}{S \rightarrow_{m\kappa} T}\ \text{antimon}$$

$$\text{new}\ \frac{S \rightarrow_{m\kappa} T}{(x)(S) \rightarrow_{m\kappa} (x)(T)} \qquad \frac{S \rightarrow_{m\kappa} T}{S, S' \rightarrow_{m\kappa} T, S'}\ \text{group}$$

$$\text{struct}\ \frac{S \equiv S' \quad S' \rightarrow_{m\kappa} T' \quad T' \equiv T}{S \rightarrow_{m\kappa} T}$$

We shall use the following notation: If T is a solution, then T^r denotes the subsolution consisting of all the proteins of T of group r.

Definition 4.4 *Consider the following properties of a solution T:*

INV_p *Logs 1 and 2 come in pairs, i.e. if one input site i named x has log n, then the corresponding output site j named x has log n.*

INV_0 *If log 0 occurs on some protein of T of group r, then at least one log 1 appears on a protein of that group.*

INV_1 *If log 1 occurs on some output of a protein of T, then all inputs of that protein have log 1.*

INV_2
1. *If log 2 appears on an output of a protein of T , then no input of that protein has log 0*
2. *if log 2 appears on an input of of a protein of T, then all outputs of that protein have log 2.*

INV_3 *If log 3 occurs on some protein of T of group r, then no log 0 nor 1 appear on any protein of that group.*

We write $T \models INV$ if T satisfies all these conditions.

Lemma 4.1 *Let S be κ-solution. $[\![S]\!]_m \models INV$.*

PROOF: This property holds vacuouly since $[\![S]\!]_m \models INV$ has no logs.

Lemma 4.2 *For any κ-solution S and $m\kappa$-solution T, we have:*
$$((\![S]\!]_m, T) \models \text{INV}) \Longrightarrow (T \models \text{INV})$$

PROOF: The statement follows obviously from the observation that the invariants concern only the proteins engaged in a group.

Lemma 4.3 *1. If $T_1 \longrightarrow_{m\kappa} T$, and $T_1 \models INV$, then $T \models INV$;*

2. $T_1 \longrightarrow_c T$, and $T_1 \models INV$, then $T \models INV$

PROOF: The statement follows easily from the following observations (we consider each invariant in turn):

INV_p All the rules (in either direction for the reversible ones) but $init$, $phase - shift$ and $exit$ are about replacing simulatneously the (identical) logs at the two ends of an edge by a nw log. So the invariant is maintained. The three remaining rules do not make any changes to logs 2 or 1 of edges.

INV_0 All the redexes have at least one log 1,2, or 3.

INV_2 One checks easily that all the proteins which are appear in the rules and belong to a group either have no output 2 or have no output 0, except possibly protein B in rule R (in either sense) or *propagation*, but no 0 is introduced on the other side, so the invariant is preserved.

INV_3 The set of proteins of group r on the left or on the right side of each rule of groups $\downarrow\uparrow_1^0$ and $\downarrow\uparrow_2^1$ has at least one log 1 or 0, hence INV_3 says that log 3 does not appear on those proteins (and does not appear either on the left hand site of $init$). Since no 3 is introduced by these rules (in either direction), the invariant is maintained vacuously. The remaining (irreversible) rules do not introduce any logs 0 or 1, hence they preserve the invariant.

INV_1 One checks easily that all the proteins which are appear in the rules and belong to a group either have all their input logs at 1 or have not output log at 1 (for rule *propagation*, we use the fact that INV_3 is satisfied (this is the reason why we treat this case last).

Corollary 4.1 *If $[\![S]\!]_m \longrightarrow^*_{m\kappa} T$, then $T \models INV$.*

PROOF: By lemmas 4.1 and 4.3.

Lemma 4.4 *If $T \models INV$, and T is a* normal form *(*NF *for short) with respect to $\longrightarrow_c$, then T is the form of $[\![S]\!]_m$, where S is a κ-solution.*

PROOF:

If T is not the form of $[\![S]\!]_m$, then it has at least one log 0, 1, 2, or 3. We show that T contains a redex.

(1) Suppose T has one log 3 in some group r. Suppose first that there is no log 2 in T^r. Then the protein where this log occurs cannot have logs 1,0, nor 2: hence all its logs are 3 and this protein can exit. If some log 2 appears, take a minimal one, which by INV_p is on an output of some protein A. By INV_3 and minimality, all the inputs of A must be 3. Hence we can apply the propagation rule.

(2) Suppose that T has no log 3 and at least one log 2. Take a minimal such 2, which by INV_p is on an output of some protein A while the other end of the edge has also log 2 and is on an input of some protein B, Then INV_2 guarantees that all input sites of A have log 1 (no 0 nor 3, nor 2 by minimality), and that all outputs of B have log 2: hence there is a R redex.

(1) Suppose that T has no log 3 nor 2, but at least one log 1. Take a maximal such 1. There are two cases.

- This 1 is on the input of the root. Then the root is an $init^{-1}$ redex, since all its output logs are 0 by maximality and assumption.
- Otherwise, by INV_p this log 1 is on an intput of some protein B while the other end of the edge is on on output site of some protein A. Then INV_1 guarantees that all input sites of A have log 1. On the ther hand all output sites of B have log 0 (by maximality and assumption). Hence A, B form a redex for one of the FC^{-1} or LC^{-1} rules.

(0) Hence we know that all logs, if any, are 0. But this contradicts INV_0.

Lemma 4.5 (Strong Normalization) *The reduction system $\longrightarrow_c$ is strongly normalizing.*

PROOF: Let T be a solution, with n_i occurrences of log i ($i = 0, 1, 2, 3$). We set

$$\rho(T) = p_0 n_0 + p_1 n_1 + p_2 n_2 + p_3 n_3$$

for some natural numbers p_0, p_1, p_2, p_3 such that $0 < p_0 < p_1 < p_2 > p_3 > 0$ It is easily checket that if $T \longrightarrow_c T'$ then $\rho(T') < \rho(T)$, and strong normailization follows.

Lemma 4.6 (Local Confluence) *The reduction system $\longrightarrow_c$ is locally confluent.*

PROOF:

Let $T \longrightarrow_c T'_1$ and $T \longrightarrow_c T'_2$, where the respective reductions involve subsolutions T_1 and T_2, respectively. The local confluence property is obvious if T_1 and T_2 are disjoint subsolutions. Hence we concentrate our intention on the possible overlappings. Then all the proteins of T_1 and T_2 bear the same group name: this

follows immediately from the fact that this property holds in T_1 and T_2 separately, and from the overlapping. Hence we may restrict our attention to the case when the two reductions take place in the simulation of the same macro-reduction step.

Corollary 4.2 (Confluence) *The reduction system $\longrightarrow_c$ is confluent.*

PROOF: This corollary is from lemma 4.5 and lemma 4.6.

Lemma 4.7 *Let S be a κ-solution and T be a $m\kappa$-solution. If $[\![S]\!]_m \longrightarrow_c{}^* T$, then $[\![S]\!]_m = T$.*

PROOF: Because all logs of $[\![S]\!]_m$ are 0, we cannot fire any reduction rule. From $[\![S]\!]_m \longrightarrow_c{}^* T$, we have $[\![S]\!]_m = T$.

Corollary 4.3 *Let S and S' be κ-solutions, if $[\![S]\!]_m \longrightarrow_c{}^* [\![S']\!]_m$, then $S = S'$.*

PROOF: According to lemma 4.7. we have $[\![S]\!]_m = [\![S']\!]_m$. Then $S = S'$.

By the corollary 4.1, lemma 4.4 and lemma 4.6,

Lemma 4.8 *For any $m\kappa$-solution T which satisfies $[\![S]\!]_m \longrightarrow^*_{m\kappa} T$, there exists an unique κ-solution which is denoted as T^c and called the* clean-up *of T such that $T \longrightarrow^*_c [\![T^c]\!]_m$.*

PROOF: Assume that there exist two κ-solutions S, S' such that $T \longrightarrow^*_c [\![S]\!]_m$ and $T \longrightarrow^*_c [\![S']\!]_m$. By corollary 6, there is a $m\kappa$ solution T' such that $[\![S]\!]_m \longrightarrow^*_c T'$ and $[\![S']\!]_m \longrightarrow^*_c T'$. We have $[\![S]\!]_m = T' = [\![S']\!]_m$ by lemma 4.7. And $S = S'$ by corollary 4.3.

Lemma 4.9 *If $T_1 \models INV$ and $T_1 \longrightarrow_{ps} T$, then $T_1^c \longrightarrow_\kappa T^c$.*

PROOF: The rule of *ps* is applied on its root protein A in the κ-reaction $L \longrightarrow_\kappa (\upsilon x)R$ with the group name r and the alias a. Since the invariants, we take T_1 contains the proteins of L with the group name r such that their logs are 2. Suppose that ${T_1}^1$ is the form of these proteins. We write $T_1 = {T_1}^1, T'$. We can apply the rules of *pre-ps*$^{-1}$, ${T_1}^1 \longrightarrow^*_c [\![L]\!]_m$. Similarly, we write $T = T^1, T'$ and ${T_1}^1 \longrightarrow_{ps} T^1$. We have $T^1 \longrightarrow^*_c [\![(\upsilon x)R]\!]_m$ by applying the rules of *post-ps*.

Finally, we have ;

$$T_1 = {T_1}^1, T' \longrightarrow^*_c [\![L]\!]_m, T' \longrightarrow^*_c [\![L]\!]_m, [\![T'^c]\!]$$

hence ${T_1}^c = L, T'^c$.

$$T = T^1, T' \longrightarrow^*_c [\![(\upsilon x)R]\!]_m, T' \longrightarrow^*_c [\![(\upsilon x)R]\!]_m, [\![T'^c]\!]$$

hence ${T_1}^c = (\upsilon x)R, T'^c$. According to corollary 4.2, $T' \models INV$. Then the last part of these two derivations can be justified. So we have ${T_1}^c \longrightarrow_\kappa T^c$.

About the notion *clear-up*, we have the following proposition;

Lemma 4.10 *If $[\![S]\!]_m \longrightarrow^*_{m\kappa} T$, then $S \longrightarrow^*_\kappa T^c$.*

PROOF: To proof this proposition, we reduce on the depth of $[\![S]\!]_m \longrightarrow^*_{m\kappa} T$.

If $T = [\![S]\!]_m$, it it trivial since $[\![S]\!]^c_m = S$. If $[\![S]\!]_m \longrightarrow^*_{m\kappa} T_1 \longrightarrow_{m\kappa} T$, and suppose that $S \longrightarrow^*_\kappa T_1^c$, it is sufficient to consider the following two cases;
(1) $T_1 \longrightarrow_{m\kappa} T$ by the rule except the rule *ps*. In this case, we have $T_1^c = T^c$ since $\longrightarrow_c$ is *confluent* and *normal form*.
(2) $T_1 \longrightarrow_{ps} T$. Then by the lemma 4.9, ${T_1}^c \longrightarrow_\kappa T^c$ according to the supposition, we have $S \longrightarrow^*_\kappa T_1^c \longrightarrow_\kappa T^c$.

Theorem 4.1 (Correctness) *Suppose that T is the $m\kappa$-solution and S is the κ solution. If $[\![S]\!]_m \longrightarrow^*_{m\kappa} T$, then there exists a κ solution S' such that $S \longrightarrow^*_\kappa S'$ and $T \longrightarrow^*_c [\![S']\!]_m$.*

PROOF:

Let S' be T^c. By lemma 4.10, we prove our theorem. We prove it by induction for the steps of $m\kappa$-reactions.

The base case is that $[\![S]\!]_m = T$. Let $S' = S$. It is trivial to prove.

The second case is that $[\![S]\!] \longrightarrow_{m\kappa} T$. Let $S' = S$. Because all the logs of $[\![S]\!]$ are 0, we get T only by firing one $m\kappa$-reaction rule before *phase shift*.Then we can fire the reversible rule from T to $[\![S]\!]$, that is , $T \longrightarrow_c [\![S]\!]$.

If $T_1 \longrightarrow_{m\kappa} T$, and T_1 satisfies the correctness(that is, assume that if $[\![S]\!] \longrightarrow_{m\kappa}{}^* T_1$, then there exists a κ solution S_1' such that $S \longrightarrow_\kappa{}^* S'$ and $T \longrightarrow_c{}^* [\![S_1']\!]$.), by induction, we need consider the two cases as follows;
(1) If T_1 fires one of rules before *phase shift*, then T can fires the reversible rule before *phase shift* to T_1 respectively, that is, $T \longrightarrow_c T_1 \longrightarrow_c{}^* S_1'$.
(2) If T_1 fires one of rules after *phase shift*, that is $T_1 \longrightarrow_c T$, and by induction, $T_1 \longrightarrow_c{}^* [\![s_1']\!]$, then according to corollary 4, there exists a $m\kappa$-solution T' such that $T \longrightarrow_c{}^* T'$ and $[\![S_1']\!] \longrightarrow_c{}^* T'$. According to lemma 5, $T' = [\![S_1']\!]$. So $T \longrightarrow_c{}^* [\![S_1']\!]$. Therefore T satisfies the correctness.

Corollary 4.4 *Let S and S' be κ-solutions, if $[\![S]\!]_m \longrightarrow_{m\kappa}{}^* [\![S']\!]_m$, then $S \longrightarrow_\kappa{}^* S'$.*

PROOF: By theorem 4.1, there exists a κ- solution S'' such that $S \longrightarrow_\kappa{}^* S''$ and $[\![S']\!]_m \longrightarrow_c{}^* [\![S'']\!]_m$. By corollary , $S' = S''$.

Chapter 5

A More General System

In this chapter, we study a more general system, bigraphical reactive systems (*BRS*s). We choose one biological example as our example to show the expressive power of the *BRS*s. Translating κ-reactions to bigraphical reactions, we show informally that the *BRS*s are more general systems.

The contents of this chapter are organized as follows. In Section 5.1, we discuss the dynamics of *BRS*s. In Section 5.2, we choose the protein *ras* activation as an example to show the expressive power of the *BRS*s. In Section 5.3, we translate κ-reactions to bigraphical reactions to show informally that the *BRS*s are more general systems.

5.1 The Dynamics in Bigraphical Reactive Systems

In Section 2.3, we have recalled basic notions of bigraphical reactive systems informally. In this section, we continue discussing the dynamics of *BRS*s.

5.1.1 The Dynamics of Bigraphs

The *dynamics* of bigraphs is dedicated to reconfigurations of bigraphs [JM04]. They depend upon both structural components; and there are one or more *reaction rules* to support them. Each such rule has a *redex* and *reactum*. The redex is a precondition for a reaction, represented by a pattern of nesting and linkage; the reactum is a postcondition indicating how the reaction will change that pattern. Places at which reactions may occur are determined by controls. A control K has three states as follows;
—A control K may be *atomic*, meaning that nothing may be nested within a K-node;
—A control K may be *active*, meaning that reactions may occur within a K-node;
—A control K may be *passive*, meaning that a control K must be destroyed before its inhabitant nodes can react.

A control K is called non-atomic if it is active or passive.

A reaction is modelled as communication and the subsequent change of channel names and nodes.

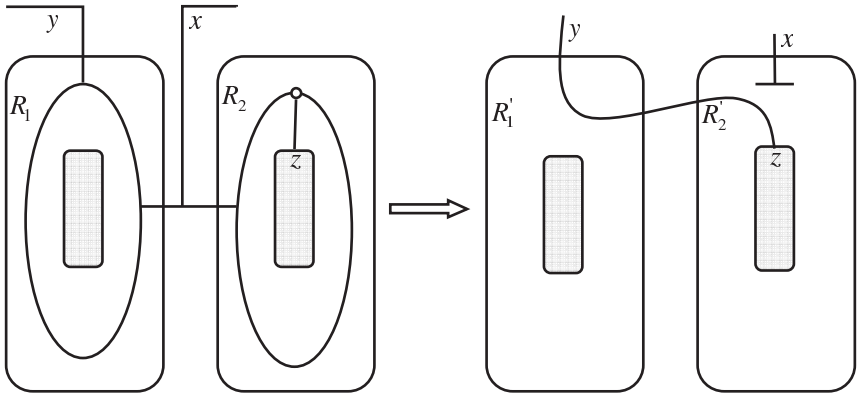


Figure 5.1: The communication rule.

Fig. 5.1 shows a typical communication rule of the π-calculus;

$$\overline{x}y.P \mid x(z).Q \longrightarrow P \mid Q\{y/z\}$$

The rule above says that the process $\overline{x}y.P$ can send name y via the name x while the other process $x(z).Q$ receives this name y via the same name x.

In bigraphical expressions, we take boxes as bigraphs, grey boxes as holes where other bigraphs can be inserted, ovals denoted by controls as nodes, thin lines denoted by names as links, rings as binding ports.

In formal graphical expressions of bigraphs, we have some notions for expression.

- We use N_{xy} to represent the node with the control N has two ports linked by x and y.
- We use the symbol $\|$ to separate the bigraphs in the redex or the reactum, for example, $R_1 \| R_2$ represents the redex in Fig. 5.1.
- We use the complex of nodes and holes with their ports to represent bigraphs, for example, we use $Send_{xy}\Box$ to represent the bigraph R_1.
- We use $|$ to represent the separation of nodes or links in one bigraph. For example the expression $N_x|M_y$ says that in one bigraph, there exist two nodes N and M which have ports linked by x and y respectively.

According our notions, the formal graphical expression of Fig. 5.1 is as follows;

$$Send_{xy}\Box \parallel Get_{x(z)}\Box \longrightarrow \Box \parallel x|\{y/z\}\Box \tag{5.1}$$

In Fig. 5.1, R_1 and R_2 are the redex before communication, R_1' and R_2' are reactum after communication. We write the redex of communication as a pair $R_1 \parallel R_2$ the reactum as a pair $R_1' \parallel R_2'$. The node (the oval in R_1), with the control $Send$, has two ports which are linked by x and y respectively. The node (the oval in R_2), with the control Get, has two ports as well; one is linked by x, and the other is the *binding* port which is linked with other nodes or holes. (In Fig. 5.1, we use a ring to indicate a binding port.) After the reaction, since nodes with controls $Send$ and Get are destroyed, the common name x is unattached in the reactum. The expression $\{y/z\}$ is represented by a curving link.

Hence, the process $\overline{x}(y).P$ is represented as R_1 in Fig. 5.1, and as $Send_{xy}\Box$ in the formal graphical expression (5.1). The process $x(y).Q$ is represented as R_2 in Fig. 5.1, and as $Get_{x(z)}\Box$ in the formal graphical expression (5.1). After communication, the process P is represented as a bigraph R_1' in Fig. 5.1, and as $\Box$ in the formal graphical expression (5.1). The process $Q[y/z]$ is represented as a bigraph R_2' in Fig. 5.1, and as $x \mid \{y/z\}\Box$ in the formal graphical expression (5.1).

As Robin Milner etc. mentioned [JM04], rules in the π-calculus, the *ambient* calculus etc., for instance, a reaction rule of the asynchronous π-calculus, a reaction rule for replication and a reaction rule for summation in the π-calculus, some reaction rules in the *ambient* calculus, can be translated into *BRS*s.

5.1.2 The Dynamics of Place Graphs and Link Graphs

Since a bigraph is represented as a combination of two independent mathematical structures: a place graph and a link graph, we can discuss the dynamics of place graphs and link graphs respectively. That is to say, a reaction rule of bigraphs is represented as a combination of a reaction rule of link graphs and a reaction rule of place graphs. This kind of separability of bigraphs can simplify our models. According to our practical goals, we can choose place-graphical models or link-graphical models.

We use $\triangle$ to represent the bigraphs (we take bigraphs as roots), ∇ to represent the hole (e.g. grey boxes in bigraphs), $\circ$ to represent nodes, and edges represent the inclusion relation of bigraphs, nodes and holes.

Fig. 5.2 shows the reaction rule of place graphs taking place in Fig 5.1.

In Fig. 5.2, "$R_1 \triangle$" represents the bigraph R_1 in Fig. 5.1. "$R_2 \triangle$" represents the bigraph R_2 in Fig. 5.1. "$\circ$" on the left represents the node with the control *Send* of R_1 in Fig. 5.1. "$\circ$" on the right represents the node with the control *Get* of R_2 in Fig. 5.1. The "∇" linked with the left "$\circ$" (*Send*) is the hole (the grey box) of the oval in bigraph R_1 in Fig. 5.1. The "∇" linked with the right "$\circ$" (*Get*) is the hole (the grey box) of the oval in bigraph R_2 in Fig. 5.1. The edge from "$R_1 \triangle$" to the "$\circ$" on the left says that the node (*Send*) $\circ$ belongs to the bigraph R_1 in Fig. 5.1. Fig. 5.2 declares the inclusion relation of bigraphs, nodes and holes in Fig 5.1: the bigraph R_1 has a node with control *send*, this node has a hole; the bigraph R_2 has a node with control *Get*, this node has a hole. After communication, the bigraph R_1' hasn't nodes any more

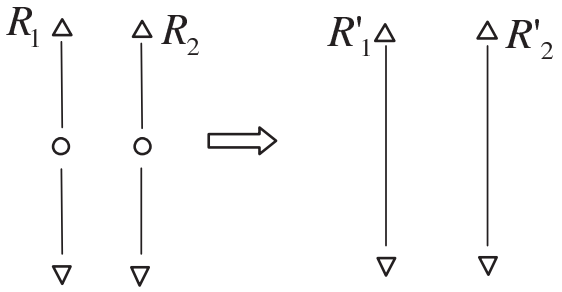


Figure 5.2: The dynamics of place graphs..

and has a hole. the bigraph R'_1 also has one hole.

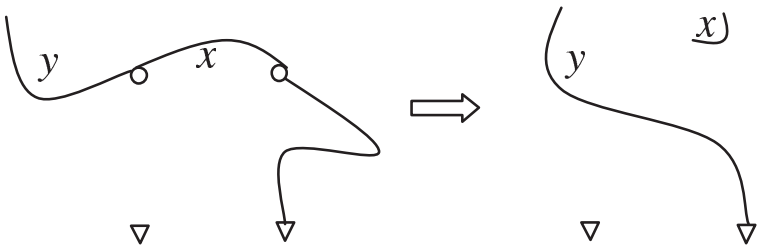


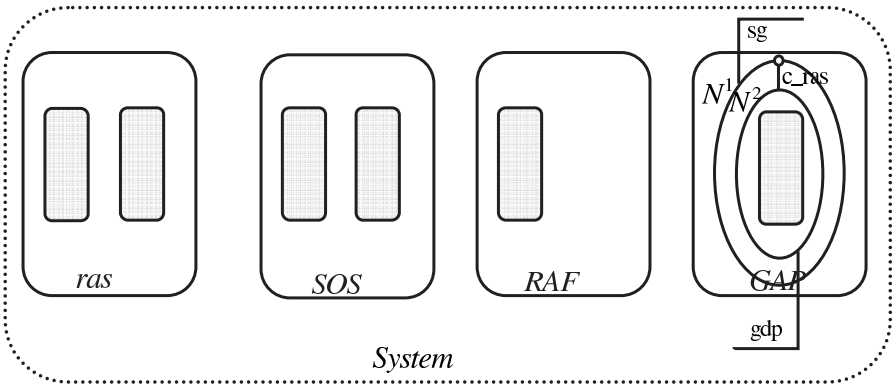
Figure 5.3: The dynamics of link graphs..

Fig. 5.3 shows the reaction rule of link graphs. It records the track of links in the reaction of Fig. 5.1. The link graph shares the same set of nodes and holes with the place graph. In fig. 5.3, the " ∘ " on the left still represents the node with the control *Send* in the bigraph R_1 in Fig. 5.1. It has two links x and y, and x is also connected to the right node (the node with the control *Get* in the bigraph R_2 in Fig. 5.1). The ∇ on the left is the hole of the node with control *Send* in the bigraph R_1 in Fig. 5.1. Since there is no link between the node with the control *Send* and its hole in the bigraph R_1 in Fig. 5.1, there is no link between the left ∇ and the left "∘" in Fig. 5.3. Comparing to the left ∇, the right ∇ is linked with the right "∘" since there is one link between the node with the control *Get* and its hole in the bigraph R_2 in Fig. 5.1. After communication, the link y is connected to the hole in the bigraph R_2 in Fig. 5.1. The link x is independent.

5.2 A Bigraphical Model of *ras* Activation

In this section, we still choose *ras* activation as our example (see 3.2) to show the expressive power of the *BRS*s.

In this model, we give some explanations as follows: first, in formal bigraphical expressions, we use the notation introduced in Section 5.1.1.



$$Sys := RAS \mid\mid SOS \mid\mid RAF \mid\mid GAP$$

Figure 5.4: The bigraphical representation of the system.

Second, in bigraphical expressions, we use the notation introduced in Section 5.1.1.

Third, from the angle of modelling, we take proteins and their functional domains as bigraphs, components *residues* of domains as links. Nodes with controls are suppositional, that is, there is no direct respondence in biology, they just make it easier to understand the biochemical process.

Finally, molecular interactions and modification are modelled as communication and the subsequent change of names and nodes.

As mentioned in Section 3.3.2, the whole system consists of four proteins (*ras, SOS, RAF* and *GAP*).

Fig. 5.4 shows the system as a bigraph in which there are four subbigraphs representing four kinds of proteins. The grey boxes represent their domains (subsubbigraphs) respectively. (Here we only give domains which take part in this system.)

Next we use reaction rules in *BRS*s to model biological reactions.

Fig. 5.5 and Fig. 5.6 show how the protein *SOS* activates the protein *ras*. It is implemented in two steps. One is the reaction between one domain (S_GNEF) of *SOS* and one domain ($INASWI\_I$) of *ras*. The other is the reaction between the domain (S_GNEF) of *SOS* and the other domain ($INASWI\_II$) of *ras*. N^i denotes nodes in formal graphical expressions.

In Fig. 5.5, the node N^1 in the bigraph $INASWI\_I$ has the common link $bbone$ with the node N^2 in the bigraph S_GNEF. After the reaction, nodes N^1 and N^2 are destroyed and the link $bbone$ is unattached.

In Fig. 5.6, the node N^1 in the bigraph $INASWI\_II$ has the common link sg with the node N^3 in the bigraph S_GNEF. After one reaction, nodes N^1 and N^2 are destroyed and the link sg are unattached. The link rs_1 is sent to the node N^4 in the bigraph S_GNEF. Since there is the common link rs_1 between nodes N^2 and N^4, there is one reaction which sends the link gdp from the node N^4 in the bigraph S_GNEF to the node N^2 in the bigraph $ACTSWI\_II$.

Fig. 5.7 shows the reaction between the domain $ACTSWI\_I$ of *ras* and the do-

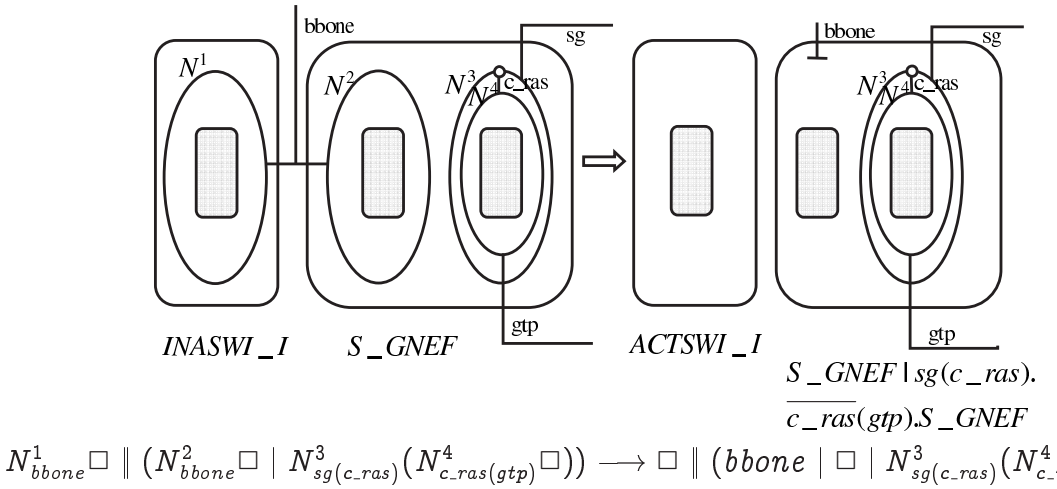


Figure 5.5: *Ras* Activation-1.

main R_Nt of *RAF*. It shows how the protein *ras* activates the next protein *RAF* in the signal transduction *RTK-MAPK*.

In fig. 5.7, the node N^1 in the bigraph $ACTSWI\_I$ has the common link s with the node N^4 in the bigraph R_Nt. After one reaction, nodes N^1 and N^4 are destroyed and the link rs_2 is sent to the node N^5. Since nodes N^2 and N^5 have common link rs_2, there is another reaction in which nodes N^2 and N^5 become destroyed and the link rs_2 is unattached.

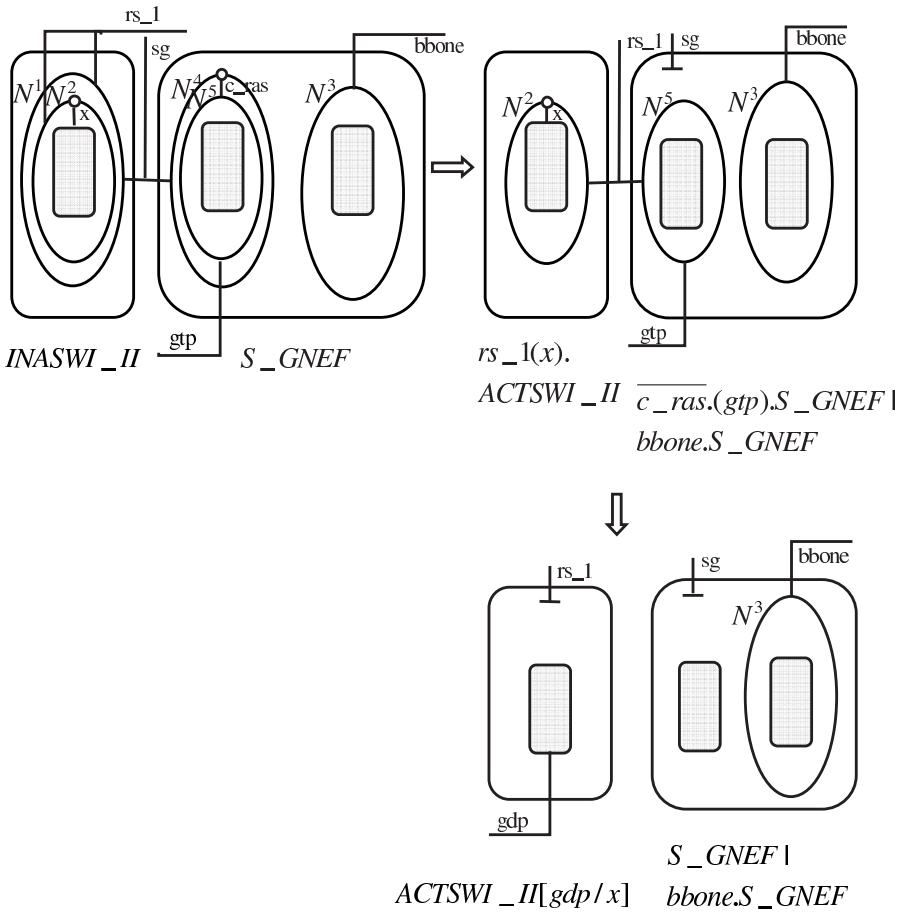
Fig. 5.8 and Fig. 5.9 show the inactivation of the active protein *ras*. After the reaction showed in Fig. 5.7, the protein *ras* is inactivated by the protein *GAP*. Fig. 5.8 shows the reaction between the domain ($ACTSWII\_II$) of *ras* and *GAP*.

In Fig. 5.8, the node N^1 in the bigraph $ACTSWI\_II$ has the common link sg with the node N^4 in the bigraph GAP, so there is a reaction. After reaction, nodes N^1 and N^4 are destroyed and the link r_swi_1 is sent to the node N^5. Since nodes N^2 and N^5 have the common link r_swi_1, a reaction is possible in which nodes N^2 and N^5 are destroyed and the link gdp is sent to the node N^3 in the bigraph $bbone.INASWI\_II$.

Fig. 5.9 shows the reaction between the domains ($bbone.INASWI\_I$ and $\overline{bbone}.INASWI\_II$) of *ras*. It shows that the protein *ras* returns to the initial inactive state.

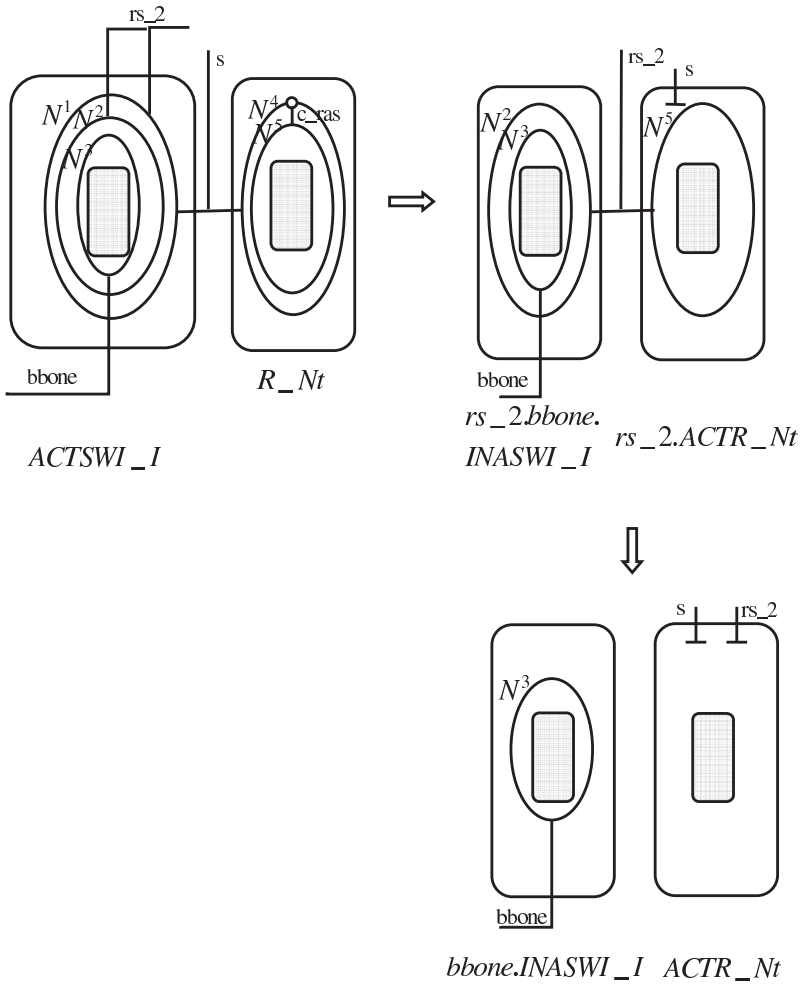
In our model, we separate the whole proceeding of *ras* activation into several reaction rules (from Fig. 5.5 to Fig. 5.9). We represent each protein or domain as a bigraph. Actually, according to our requirement, we can choose different biological objects as bigraphs. For example, we can take a complex as a bigraph, which makes the whole model simpler.

On the other hand, we also can separate each reaction rule in *BRS*s into two reaction rules in place graphs and link graphs respectively. The place-graphical model and the link-graphical model of *ras* activation are shown in Appendix A.2.



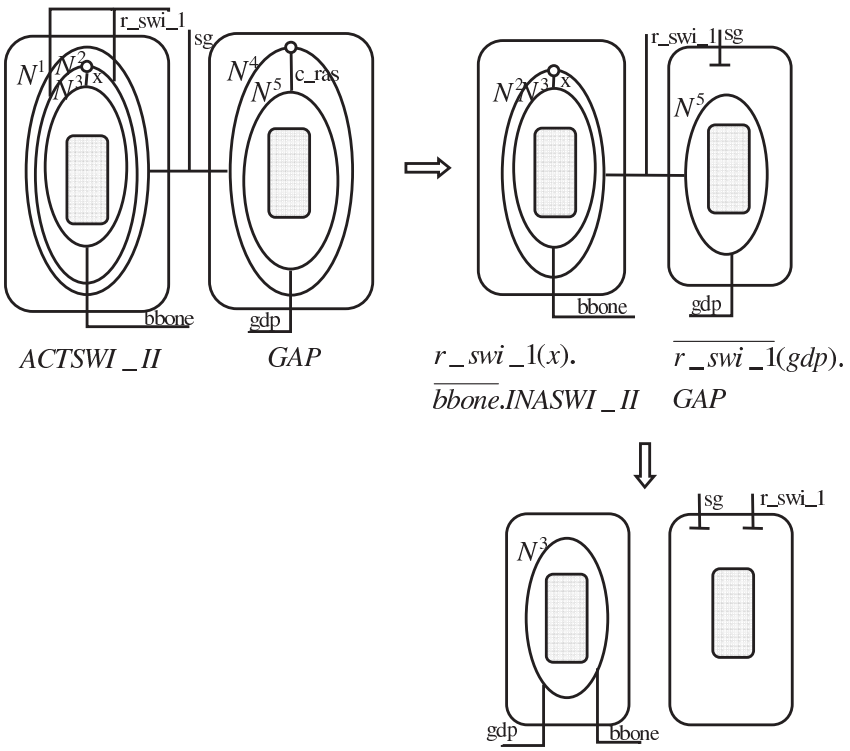
$$N^1_{sg(rs\_1)}(N^2_{rs\_1(x)}\Box) \parallel (N^4_{sg}(c\_ras)(N^5_{c\_ras(gtp)}\Box) \mid N^3_{bbone}\Box) \longrightarrow$$
$$N^2_{rs\_1(x)}\Box \parallel sg \mid N^5_{[rs\_1/c\_ras](gtp)}\Box \mid N^3_{bbone}\Box \longrightarrow$$
$$rs\_1 \mid [gtp/x]\Box \parallel (sg \mid \Box \mid N^3_{bbone}\Box)$$

Figure 5.6: *Ras* Activation-2.



$$(N^1_{s(rs\_2)}(N^2_{rs\_2}(N^3_{bbone}\Box)) \parallel N^4_{s(c\_ras)}(N^5_{c\_ras}\Box) \longrightarrow N^2_{rs\_2}(N^3_{bbone}\Box) \parallel (s \mid N^5_{[rs\_2/c\_itras]}\Box)$$
$$\longrightarrow N^3_{bbone}\Box \parallel (rs\_2 \mid s \mid \Box)$$

Figure 5.7: Signal Transfer.



$$N^1_{sg(r\_swi\_1)}(N^2_{r\_swi\_1(x)}(N^3_{bbone}\Box)) \parallel N^4_{sg(c\_ras)}(N^5_{c\_ras(gdp)}\Box) \longrightarrow$$
$$N^2_{r\_swi\_1(x)}(N^3_{bbone}\Box) \parallel (sg \mid N^5_{[r\_swi\_1/c\_ras](gdp)}\Box) \longrightarrow$$
$$N^3_{[gdp/t]bbone}\Box \parallel (r\_swi\_1 \mid sg \mid \Box)$$

Figure 5.8: *ras* inactivation-1.

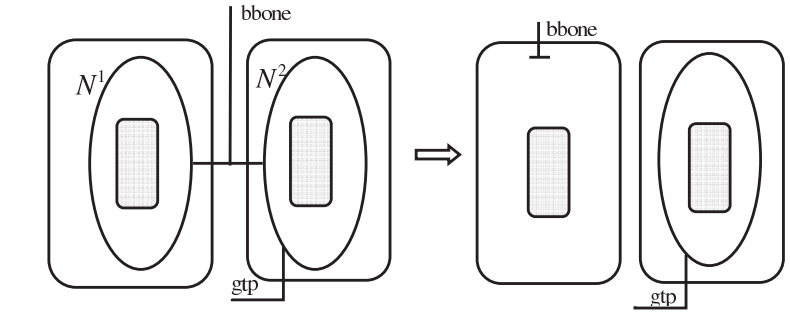


Figure 5.9: *ras* inactivation-2.

5.3 An Example

Bigraphs are graphical structures. The κ-calculus introduced in Chapter 4 idealizes protein-protein reactions. Strictly speaking, it is some kind of graphical rewriting operating on graphs-on-site. It has a graphical structure as well.

It is easy to translate κ-reactions to bigraphical reactions. We take solutions as bigraphs, proteins as nodes, sites of proteins as ports, and bindings as links. For instance, we consider a monotonic κ-reaction as follows;

$$A(1^x + 2), B(1^x + 2), C(1) \to (y)(A(1^x + 2), B(1^x + 2^y), C(1^y)) \tag{5.2}$$

This monotonic κ-reaction (5.2) says that, when the site 1 of the protein A binds to the site 1 of the protein B via the name x, the site 2 of the protein B will bind to the site 1 of the protein C via the name y.

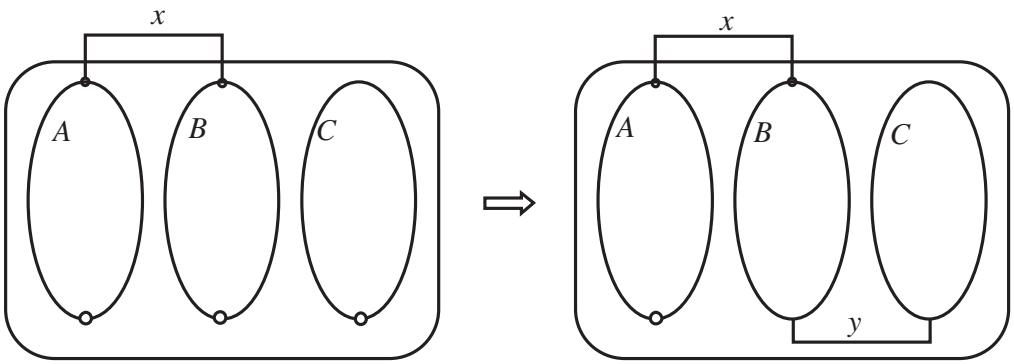


Figure 5.10: A κ-reaction.

Fig. 5.10 shows how to represent such a reaction in *BRS*s. We take proteins A, B, and C as nodes in a bigraph, and sites of proteins as ports of nodes in the bigraph. The edge x connecting sites of proteins is represented as a link in the bigraph. The monotonic κ-reaction is actually the generation of a new link y in bigraphical reaction. The formal bigraphical expression of Fig. 5.10 is:

$$A_x \mid B_x \mid C \longrightarrow A_x \mid B_{xy} \mid C_y$$

This example gives us an evidence that the κ-calculus can be translated into the bigraphical reactive system.

Remarkably, this example is very simple. It gives us an evidence that κ-calculus can be translated in this way into the bigraphical reactive system.

Chapter 6

Conclusions and Future Work

In this thesis, we have studied three process calculi to model systems biology. We extended the π-calculus to model the aberrant signal transduction; proved the correctness of self-assembly in κ-calculus; and made an attempt of modelling *ras* activation using *BRS*s. These results lay out some foundations for future studies of systems biology and process algebra. They also highlight the robustness of process algebra in modelling and analyzing systems biology.

In the rest of this chapter we discuss possible future work, including several problems that have been left open.

Generalization of Results In Chapter 3, the $I\pi$-calculus was obtained by adding two aberrant actions (the suicide action $\S$ and the propagation action $\sharp$) to the π-calculus. Does there only exist these two kinds of aberrance in biology? Or could other aberrance be represented by these two actions? We don't know yet. Our motivation started from one detailed case, the aberrant protein *ras* activation in signal transduction. Compared with computer systems, biological systems are so complicated that our model cannot verify all of them.

In Chapter 4, biological reactions are represented by way of binding or unbinding in κ-calculus. We focused on the monotonic or antimonotonic reactions, which have been proved to have good mathematical properties. We did not find a good way to deal with other more complicated reactions so far.

In a word, we have the following challenges:

- A list for general features of systems biology? The summary of features of systems biology is necessary because our models stem from these properties.
- A general model for biological processes with aberrance? The $I\pi$-calculus is the first step. On the one hand, since systems biology has various parts, for instance, metabolic pathways, organelles, cells, physiological systems, organisms and so on, we should find appropriate models for these parts respectively. Then we shall try to unify them. On the other hand, maybe the $I\pi$ calculus is the one that can unify the others. It needs to be proved.

- A model for general biological systems? Since *BRS*s aim to unify the existing process algebras, it may be that model. But this needs future study.

In future work, we would like to go beyond case studies, and we are looking for generalization of results.

Models for quantitative analysis All models in this thesis are for qualitative analysis. They capture the relations among components in biological system. Quantitative analysis is another very important direction in systems biology. We can know more accurately relation among the components and the relation with the environment outside by quantitative analysis. Aviv Regev etc. have taken the *Stochasitic* π-calculus as a quantitatively analytic model to model signal transduction *RTK-MAPK* and have got some useful results.

The models we designed lack of quantitative analysis. In Chapter 3, we introduced a tag system as an auxiliary system to the $I\pi$-calculus. The tag system is based on set theory. And tags can represent quantitative information. So we believe that the tag system will be useful in the quantitative analysis.

As one direction in future work, we need to get hold of some points as follows:

- Objects. We cannot analyze quantitatively all the elements in one model. But we can focus on one or some of them. For instance, Aviv Regev etc. focus on effect of concentration of proteins in the model of *stochastic* π-calculus. Temperature, the number of molecules , intensity of pressure, etc. should be considered if necessary.

- Method. We believe that there exist many other models to make quantitative analysis, other than the tag system. Simplicity and feasibility will be an important criterion.

- Results. Results of models should be consistent with results of biology. This is also the criterion to evaluate our models.

The theoretical development of models Deeper studying of models is necessary. The algebraic properties of models enrich the theory of models themselves. However, when focusing on models for systems biology, those properties which can verify properties of systems biology are more important. The use of formal and algorithmic approaches has greatly accelerated progress in the sequence and structure branches of biology. Adopting a common representation language for biochemical processes may similarly accelerate progress in understanding their function and evolution.

So far, we just considered the expressive power of models. We ensured our model can describe the biological process. For models themselves, we know that the π-calculus, the κ-calculus and *BRS*s have rich good algebraic properties. How

to transfer this in the systems biology? That is to say, how do we ensure some biological properties using these algebraic properties?

Biological properties in systems biology are different from our computing models; for example, (1) complicated concurrence;(2) enormous entities;(3) the indetermination of factors. All of these are our obstacles in future work.

Implementation of Models The implementation of models is another future research direction. Aviv Regev etc. have developed a computer application, called *PiFCP*. *PiFCP* is based on the *Logix* system, which implements *Flat Concurrent Prolog*. Our research opens up new possibilities in the study of biochemical systems . However, there are no related automatic tools yet. Designing and implementing such a tool will be our next work.

Appendix A

Some Additional Examples

A.1 The $I\pi$-model with the Tag System about the Protein *ras*

In Section 3.3.2, we have used the $I\pi$-calculus to model the protein *ras* activation. Here we revisit this example with the tag system. Tags of motifs and residues show that motifs and residues are normal or aberrant, and tags of proteins help to justify whether there exit aberrant components in proteins. By the respective change of tags in reduction rules, it is clearer to know the movement of aberrant components during the process of *ras* activation.

A.1.1 The Normal State of *ras*

First, the system is defined in A.1

$$\begin{aligned} < I_{Sys}, Sys > \quad ::= \quad & < I_{ras}, ras > | < I_{SOS}, SOS > | < I_{GAP}, GAP > \\ & | < I_{RAF}, RAF > \end{aligned} \tag{A.1}$$

where the tag of the system is:

$$I_{Sys} \quad ::= \quad I_{ras} \uplus I_{SOS} \uplus I_{GAP} \uplus I_{RAF} \tag{A.2}$$

The four proteins, *ras*, *SOS*, *GAP*, *RAF*, in this system are given through (A.3)

to (A.6)

$$
\begin{aligned}
< I_{ras}, ras > \ ::= \ & < I_{INASWI\_I}, INASWI\_I > \\
& |< I_{INASWI\_II}, INASWI\_II > \qquad \text{(A.3)} \\
< I_{SOS}, SOS > \ ::= \ & < I_{S\_SH3\_BS}, S\_SH3\_BS > |< I_{S\_GNEF}, S\_GNEF > \qquad \text{(A.4)} \\
< I_{RAF}, RAF > \ ::= \ & < I_{R\_Nt}, R\_Nt > |< I_{R\_ACT\_BS}, R\_ACT\_BS > \\
& |< I_{R\_M\_BS}, R\_M\_BS > |< I_{INA\_R\_Ct}, INA\_R\_Ct > \\
& |< I_{R\_ATP\_BS}, R\_ATP\_BS > \qquad \text{(A.5)} \\
< I_{GAP}, GAP > \ ::= \ & < i_{sg}, sg(c\_ras) > . < i_{c\_ras}, \overline{c\_ras}(gdp) > . \\
& < I_{GAP}, GAP > \qquad \text{(A.6)}
\end{aligned}
$$

Respectively, the tags are given as follows:

$$
\begin{aligned}
I_{ras} \ ::= \ & I_{INASWI\_I} \uplus I_{INASWI\_II} \qquad \text{(A.7)} \\
I_{SOS} \ ::= \ & I_{S\_SH3\_BS} \uplus I_{S\_GNEF} \qquad \text{(A.8)} \\
I_{RAF} \ ::= \ & I_{R\_Nt} \uplus I_{R\_ACT\_BS} \uplus I_{R\_M\_BS} \uplus I_{INA\_R\_Ct} \uplus I_{R\_ATP\_BS} \qquad \text{(A.9)} \\
I_{GAP} \ ::= \ & \biguplus_{n=1}^{\infty} (\{i_{sg}, i_{c\_ras}\}) \qquad \text{(A.10)}
\end{aligned}
$$

Since *GAP* is represented by a recursive process in our model, by the rule *t-const* in Table 3.2, the tag of *GAP* is (A.10).

(A.3) shows that the protein *ras* has two domains which are represented as follows:

$$
\begin{aligned}
< I_{INASWI\_I}, INASWI\_I > \ ::= \ & < i_{bbone}, \overline{bbone} > . < i_s, \overline{s}(rs\_2) > . \\
& < i_{rs\_2}, \overline{rs\_2} > . < i_{bbone}, bbone > . \\
& < I_{INASWI\_I}, INASWI\_I > \qquad \text{(A.11)} \\
< I_{INASWI\_II}, INASWI\_II > \ ::= \ & < i_{sg}, \overline{sg}(rs\_1) > . < i_{rs\_1}, rs\_1(x) > . \\
& < i_{sg}, \overline{sg}(r\_swi\_1) > . < i_{r\_swi\_1}, \\
& r\_swi\_1(x) > . < i_{bbone}, \overline{bbone} > . \\
& < I_{INACTSWI\_II}, INACTSWI\_II > \qquad \text{(A.12)}
\end{aligned}
$$

where domains *INASWI_I* and *INASWI_II* are recursive processes. Then the tags are given in (A.13) and (A.14).

$$
\begin{aligned}
I_{INASWI\_I} \ ::= \ & \biguplus_{n=1}^{\infty} \{i_{bbone}, i_s, i_{rs\_2}, i_{bbone}\} \qquad \text{(A.13)} \\
I_{INASWI\_II} \ ::= \ & \biguplus_{n=1}^{\infty} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} \qquad \text{(A.14)}
\end{aligned}
$$

The domain *S_GNEF* of the protein *SOS* is represented in (A.15):

$$
\begin{aligned}
< I_{S\_GNEF}, S\_GNEF > \ ::= \ & < i_{bbone}, bbone > . < I_{S\_GNEF}, S\_GNEF > | \\
& < i_{sg}, sg(c\_ras) > . < i_{c\_ras}, \overline{c\_ras}(gtp) > . \\
& < I_{S\_GNEF}, S\_GNEF > \qquad \text{(A.15)}
\end{aligned}
$$

where the respective tag is in A.16.

$$\begin{aligned} I_{S\_GNEF} &::= \overset{\infty}{\underset{n=1}{\uplus}}(\{i_b\} \uplus (\{i_{sg},,i_{c\_ras},\}) \\ &= \overset{\infty}{\underset{n=1}{\uplus}}\{i_b, i_{sg},,i_{c\_ras},\} \end{aligned} \tag{A.16}$$

The domain *R_Nt* of *RAF* is defined in (A.17):

$$\begin{aligned} < I_{R\_Nt}, R\_Nt > ::= & < i_s, s(c\_ras) > . < i_c, c\_ras > . \\ & < I_{ACTR\_Nt}, ACTR\_Nt > \end{aligned} \tag{A.17}$$

The respective tag is in (A.18).

$$I_{R\_Nt} = \{i_s, i_{c\_ras}\} \uplus I_{ACTR\_Nt} \tag{A.18}$$

The protein *SOS* activates the protein *ras*. There are two steps: one is that the domain $bbone.S\_GNEF$ of the protein *SOS* interacts with the domain $INASWI\_I$ of the protein *ras,* the other is that the domain $sg(c\_ras).\overline{c\_ras}(gtp).S\_GENF$ of the protein *SOS* interacts with the domain $INASWI\_II$ of the protein *ras*. Then the following interactions are possible:

$$\begin{aligned} & < I_{INASWI\_I}, INASWI\_I > | < I_{S\_GNEF}, S\_GNEF > \xrightarrow{\tau_{I_1}} \\ & < i_s, \overline{s}(rs\_2). < i_{rs\_2}, \overline{rs\_2} > . < i_{bbone}, bbone > . \\ & < I_{INASWI\_I}, INASWI\_I > | < I_{S\_GNEF}, S\_GNEF > | \ldots \end{aligned} \tag{A.19}$$

$$\begin{aligned} & < I_{INASWI\_II}, INASWI\_II > | < I_{S\_DNEF}, S\_GNEF > \xrightarrow{\tau_{I_2}}\xrightarrow{\tau_{I_3}} \\ & < i_{sg}, \overline{sg}(r\_swi\_1). < i_{r\_swi\_1}, r\_swi\_1(x) > . < i_{bbone}, bbone > . \\ & < I_{INASWI\_II}, INASWI\_II > | < I_{S\_GNEF}, S\_GNEF > | \ldots \end{aligned} \tag{A.20}$$

Where

$$I_1 = \{i_{bbone}, i_{bbone}\} \tag{A.21}$$

$$I_2 = \{i_{sg}, i_{sg}\} \tag{A.22}$$

$$I_3 = \{i_{r\_swi\_1}, i_{c\_ras}\} \tag{A.23}$$

According to Table 3.3, the change of tags in interactions follows some rules.

$$\begin{aligned}
\biguplus_{n=1}^{\infty}\{i_{bbone}, i_s, i_{rs_2}, i_{bbone}\} &\uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_{sg}, , i_{c_ras}, \} \setminus \{i_{bbone}, i_{bbone}\} \\
&= \\
\{i_s, i_{rs_2}, i_{bbone}, i_{sg}, , i_{c_ras}, \} &\uplus \biguplus_{n=2}^{\infty}\{i_{bbone}, i_s, i_{rs_2}, i_{bbone}\} \uplus \biguplus_{n=2}^{\infty}\{i_{bbone}, i_{sg}, , i_{c_ras}, \} \\
&= \\
\biguplus_{n=1}^{\infty}\{i_{bbone}, i_s, i_{rs_2}, i_{bbone}\} &\uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_{sg}, , i_{c_ras}, \} \qquad \text{(A.24)}
\end{aligned}$$

$$\begin{aligned}
\biguplus_{n=1}^{\infty}\{i_{sg}, i_{rs_1}, i_{sg}, i_{r_swi_1}, i_{bbone}\} &\uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_{sg}, i_{c_ras}, \} \setminus \{i_{sg}, i_{sg}, i_{r_swi_1}, i_{c_ras}\} \\
&= \\
\{i_{sg}, i_{rs_1}, i_{bbone}, i_{bbone}\} &\uplus \biguplus_{n=2}^{\infty}\{i_{sg}, i_{rs_1}, i_{sg}, i_{r_swi_1}, i_{bbone}\} \uplus \biguplus_{n=2}^{\infty}\{i_{bbone}, i_{sg}, i_{c_ras}, \} \\
&= \\
\biguplus_{n=1}^{\infty}\{i_{sg}, i_{rs_1}, i_{sg}, i_{r_swi_1}, i_{bbone}\} &\uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_{sg}, i_{c_ras}, \} \qquad \text{(A.25)}
\end{aligned}$$

After interactions (A.19) and (A.20), the protein *ras* is activated and interact with the new protein *RAF*. In fact, it is the interaction between the domain $\overline{s}(rs\_2).\overline{rs\_2}.bbone.INASWI\_I$ of the protein *ras* and the domain R_Nt of the protein *RAF*.

$$\begin{aligned}
&< i_s, \overline{s}(rs_2). < i_{rs_2}, \overline{rs_2} > . < i_{bbone}, bbone > . \\
&\quad < I_{INASWI_I}, INASWI_I > | < I_{R_Nt}, R_Nt > \xrightarrow{\tau}{}^{*} \\
&\quad < i_{bbone}, bbone > . \\
&< I_{INASWI_I}, INASWI_I > | < I_{ACTR_Nt}, ACTR_Nt > \qquad \text{(A.26)}
\end{aligned}$$

Respectively, the change of tags in the interaction(A.26) is as follows:

$$\begin{aligned}
\{i_s, i_{rs_2}, i_{bbone}\} \uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_s, i_{rs_2}, i_{bbone}\} &\uplus \{i_s, i_{c_ras}\} \uplus I_{ACTR_Nt} \\
\setminus \{i_s, i_{rs_2}, i_s, i_{c_ras}\} &= \\
\{i_{bbone}\} \uplus \biguplus_{n=1}^{\infty}\{i_{bbone}, i_s, i_{rs_2}, i_{bbone}\} &\uplus I_{ACTR_Nt} \qquad \text{(A.27)}
\end{aligned}$$

Next, the protein *GAP* inactivates the protein *ras*, then the protein *ras* comes back to the initial state, that is, the inactive state. The detailed $I\pi$-programme of this inactivation is as follows:

$$\begin{aligned}
&< i_{sg}, \overline{sg}(r_swi_1). < i_{r_swi_1}, r_swi_1(x) > . < i_{bbone}, bbone > . \\
&\quad < I_{INASWI_II}, INASWI_II > | < I_{GAP}, GAP > \xrightarrow{\tau}{}^{*} \\
&< i_{bbone}, bbone > . < I_{INASWI_II}, INASWI_II > | < I_{GAP}, GAP > \qquad \text{(A.28)}
\end{aligned}$$

The protein *GAP* actually interacts with one domain $\overline{sg}(r\_swi\_1).r\_swi\_1(x).bbone.INASWI\_II$ of the active protein *ras* (A.28). Then the domain $bbone.INASWI\_II$

interacts with another domain $\overline{bbone}.INASWI\_I$ of the protein *ras* (A.29), which the protein *ras* would be in inactive state.

$$\begin{aligned} &< i_{bbone}, \overline{bbone} > . < I_{INASWI\_I}, INASWI\_I > | < i_{bbone}, bbone > . \\ &\quad < I_{INASWI\_II}, INASWI\_II > \xrightarrow{\tau} < I_{INASWI\_I}, INASWI\_I > \\ &\quad\quad | < I_{INASWI\_II}, INASWI\_II > \end{aligned} \tag{A.29}$$

(A.30) and (A.31) show the relations of tags with respect to reactions (A.28) and (A.29) respectively.

$$\begin{aligned} \overset{\infty}{\underset{n=1}{\uplus}} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} \uplus \overset{\infty}{\underset{n=1}{\uplus}} \{i_{sg}, i_{c\_ras}\} \setminus \{i_{sg}, i_{r\_swi\_1}, i_{sg}, i_{c\_ras}\} &= \\ \{i_{sg}, i_{rs\_1}, i_{bbone}\} \uplus \overset{\infty}{\underset{n=2}{\uplus}} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} \uplus \overset{\infty}{\underset{n=2}{\uplus}} \{i_{sg}, i_{c\_ras}\} &= \\ \overset{\infty}{\underset{n=2}{\uplus}} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} \uplus \overset{\infty}{\underset{n=2}{\uplus}} \{i_{sg}, i_{c\_ras}\} & \quad (A.30) \\ \{i_{bbone}\} \uplus \overset{\infty}{\underset{n=1}{\uplus}} \{i_{bbone}, i_s, i_{rs\_2}, i_{bbone}\} \uplus & \\ \{i_{bbone}\} \uplus \overset{\infty}{\underset{n=1}{\uplus}} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} \setminus \{i_{bbone}, i_{bbone}\} &= \\ \overset{\infty}{\underset{n=1}{\uplus}} \{i_{bbone}, i_s, i_{rs\_2}, i_{bbone}\} \uplus \overset{\infty}{\underset{n=1}{\uplus}} \{i_{sg}, i_{rs\_1}, i_{sg}, i_{r\_swi\_1}, i_{bbone}\} & \quad (A.31) \end{aligned}$$

From (A.24), (A.25), (A.27) , (A.30), (A.31), we observe that, during the process of activation of the protein *ras*, 0 never occurs in the tag of the system (*Sys*).

A.1.2 The Aberrant State of *ras*

When *ras* mutates aberrantly, (A.32) defines the $I\pi$ representation of *GAP* in the aberrant state. (A.36) shows that *GAP* loses its function and does nothing, meaning that it cannot inactivate the domain $\overline{sg}(r\_swi\_1).r\_swi\_1(x).bbone.INASWI\_II$ of *ras*.

$$\begin{aligned} < I'_{GAP}, GAP > &::= < 0, \S(sg(c\_ras)) > . < i_{c\_ras}, \overline{c\_ras}(gdp) > . \\ &\quad < I'_{GAP}, GAP > & (A.32) \\ I'_{GAP} &::= \{0\} & (A.33) \end{aligned}$$

$$\begin{aligned} < I'_{GAP}, GAP > &\longrightarrow < \varnothing, 0 > & (A.34) \\ I'_{GAP} \setminus \{0\} &= \varnothing & (A.35) \end{aligned}$$

The aberrant protein *ras* has the aberrant domain $INASWI\_I$:

$$\begin{aligned} < I'_{INASWI\_I}, INASWI\_I > &::= < i_{bbone}, \overline{bbone} > . < 0, \sharp(\overline{s}(rs\_2).\overline{rs\_2}) > . < i_{bbone}, \\ &\quad bbone > . < I'_{INASWI\_I}, INASWI\_I > & (A.36) \\ I'_{INASWI\_I} &::= \{i_{bbone}\} \uplus \overset{\infty}{\underset{n=1}{\uplus}} \{0, i_s, i_{rs\_2}\} & (A.37) \end{aligned}$$

Hence, the interactions (A.28)and (A.29)cannot happen. While the interaction (A.26) is changed as follows:

$$\begin{aligned} &< 0, \sharp(\overline{s}(rs\_2). < i_{rs\_2}, \overline{rs\_2}) > . < i_{bbone}, bbone > . \\ &\quad < I'_{INASWI\_I}, INASWI\_I > | < I_{R\_Nt}, R\_Nt > \xrightarrow{\tau}{}^{*} \\ &< 0, \sharp(\overline{s}(rs\_2). < i_{rs\_2}, \overline{rs\_2}) > . < i_{bbone}, bbone > . \\ &\quad < I'_{INASWI\_I}, INASWI\_I > | < I_{ACTR\_Nt}, ACTR\_Nt > \qquad \text{(A.38)} \\ \overset{\infty}{\underset{n=1}{\uplus}} \{0, i_s, i_{rs\_2}\} \uplus (\{i_s, i_{c\_ras}\} \uplus I_{ACTR\_Nt}) \setminus \{0, i_s, i_{rs\_2}, i_s, i_{c\_ras}\} \quad &= \\ \overset{\infty}{\underset{n=1}{\uplus}} \{0, i_s, i_{rs\_2}\} \uplus I_{ACTR\_Nt} \qquad \text{(A.39)} \end{aligned}$$

We can find, on one hand, 0 occurs in the tag of the system which says that aberrance exits in the system. On the other hand, only one 0 is in the tag of *GAP* which says that *GAP* has the suicide capability, and unlimited 0s are in the tag of $INASWI\_I$ which says that $INASWI\_I$ has the propagation capability. We cannot check whether $INASWI\_I$ has the suicide capability or not only by the tag system.

A.2 The Link and Place Graphical Model of *ras* Activation

In this section, we give the link graphical and place graphical model of *ras* activation, respectively. The reaction rules of *BRS*s in Section 5.2 are combinations of reaction rules of link graphs and reaction rules of place graphs.

According to Fig. 5.4, the place graph of the system is shown in Fig. A.1.

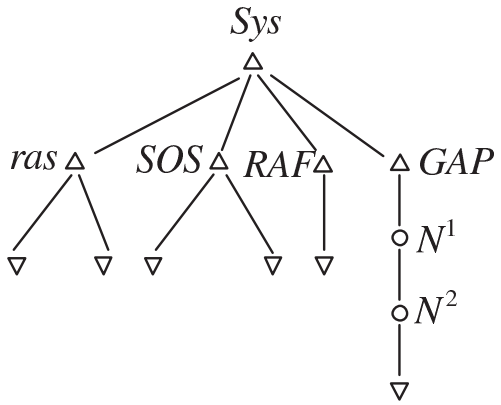


Figure A.1: The place graph of the system.

The system in Fig. A.1 includes four bigraphs (proteins).

Fig. A.2 and Fig. A.3 are the place graphs and the link graphs when the protein *SOS* activates the protein *ras*.

In Fig. A.2, the place graph says that the domain $INASWI\_I$ has one subdomain ("∇") which is controlled by one node N^1 ("$\circ$"), and the domain S_GNEF has two subdomains in which one is controlled by nodes N^2 and the other is controlled by nodes N^3 and N^4. After the reaction, nodes N^1 and N^2 are destroyed.

The link graph says that, before the reaction, nodes N^1 and N^2 are bounded by $bbone$. After the reaction, since nodes N^1 and N^2 are destroyed, the link $bbone$ becomes independent.

In Fig. A.3, the place graph says that the domain $INASWI\_II$ has one subdomain ("∇") which is controlled by nodes N^1 and N^2, and the domain S_GNEF has two subdomains in which one is controlled by node N^3 and the other is controlled by nodes N^4 and N^5. After two reactions, nodes N^1, N^2, N^4 and N^5 are destroyed.

The link graph says that, before the reaction, nodes N^1 and N^4 are bounded by sg. After one reaction, since nodes N^1 and N^2 are destroyed, the link sg is independent and the link rs_1 is sent to the node N^5 by the link c_ras. Then nodes N^2 and N^5 have the common link c_ras. After the other reaction, the link gtp is sent to the subdomain (the left "∇") by the link x. Nodes N^2 and N^5 are destroyed and the link rs_1 becomes independent.

In the bigraphical example of *ras* activation, reaction rules of place graphs show the occurrences of biological reactions, and link graphs show the direction of biological reactions. In other words, reaction rules of place graphs ensure the occurrence of biological reactions, while reaction rules of link graphs describe how biological reactions occur.

Fig. A.4 are reactions that the activated *ras* sends the signal to the next protein *RAF* in place graphs and in the link graphs respectively.

In Fig. A.4, the place graph says that the domain $ACTSWI\_I$ has one subdomain controlled by three nodes N^1, N^2 and N^3, and the domain R_Rt has one subdomain controlled by two nodes N^4 and N^5. After two reactions, nodes N^1, N^2, N^4 and N^5 are destroyed.

The link graph says that nodes N^1 and N^4 have the common link s and there is one reaction such that N^1 and N^4 are destroyed, the link s is independent, and the link rs_2 is sent to the node N^5 by the link c_ras. So there is another reaction such since nodes N^2 and N^5 have the common link rs_2. After the second reaction, nodes N^2 and N^5 are destroyed and the link rs_2 becomes independent.

Fig. A.5 and Fig. A.6 are the reactions showing how the activated *ras* returns back to the initial inactive state in place graphs and in link graphs respectively.

In Fig. A.5, the place graph says that, the domain $ACTSWI\_II$ has one subdomain controlled by nodes N^1, N^2 and N^3, and the domain GAP has one subdomain controlled by nodes N^4 and N^5. After two reactions, nodes N^1, N^2, N^4 and N^5 are destroyed.

The link graph says that, nodes N^1 and N^4 have the common link sg and there is a reaction such that the link r_swi_1 is sent to the node N^5 by the link c_ras. Then

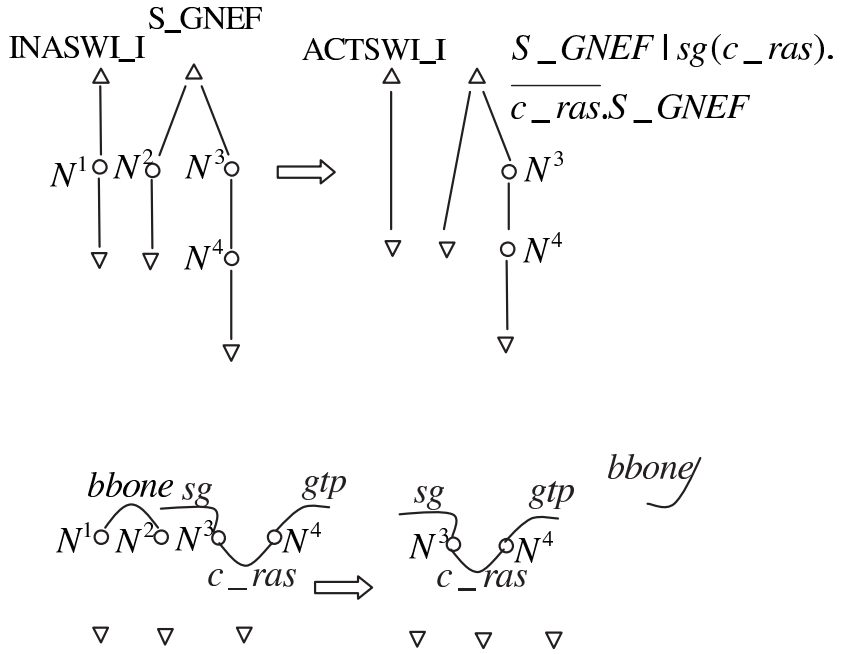


Figure A.2: The place graph and the link graph of *ras* activation-1.

since nodes N^2 and N^5 have the common link r_swi_1, there is another reaction such that the link gdp is sent to the node N^3 by the link x. During the two reactions, nodes N^1, N^4, N^2 and N^5 are destroyed and links sg and r_swi_1 are independent.

In Fig. A.6, the place graph says that, the domain $bbone.INASWI\_I$ has one subdomain controlled by one node N^1, and the domain $\overline{bbone}.INASWI\_II[gtp/x]$ has one subdomain controlled by the node N^2. After reaction, the node N^1 is destroyed while the node N^2 becomes active. The link graph says that, one reaction occurs since nodes N^1 and N^2 have the common link $bbone$. After the reaction, the node N^1 is destroyed and the link $bbone$ becomes independent.

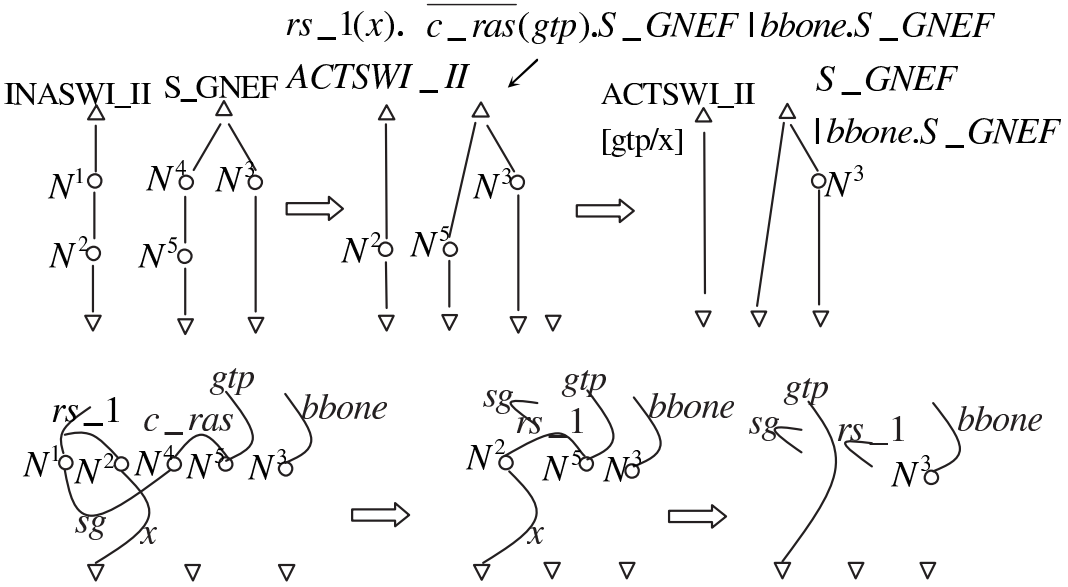


Figure A.3: The place graph and the link graph of *ras* activation-2.

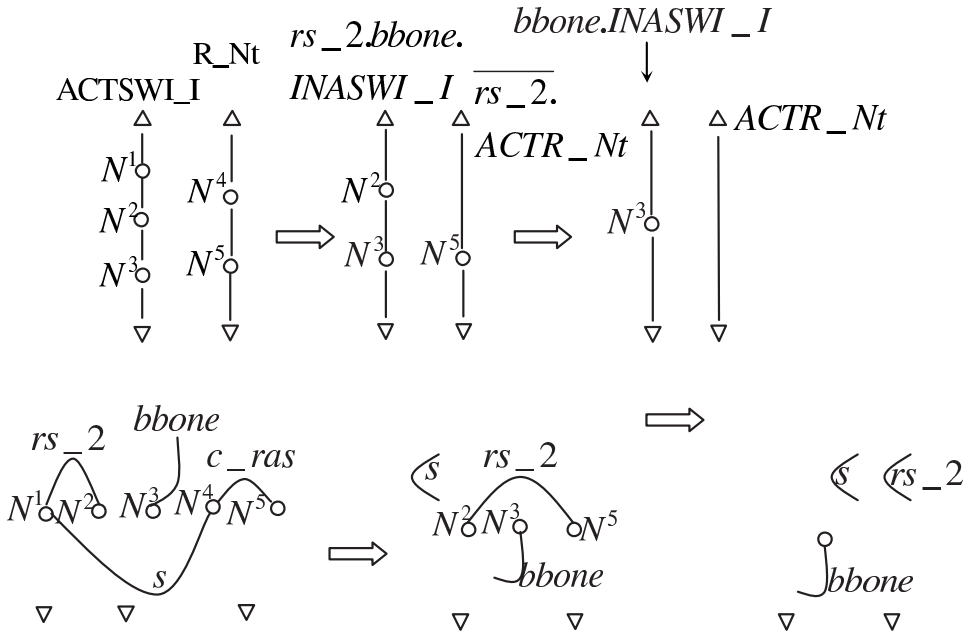


Figure A.4: The place graph and the link graph of signal transfer.

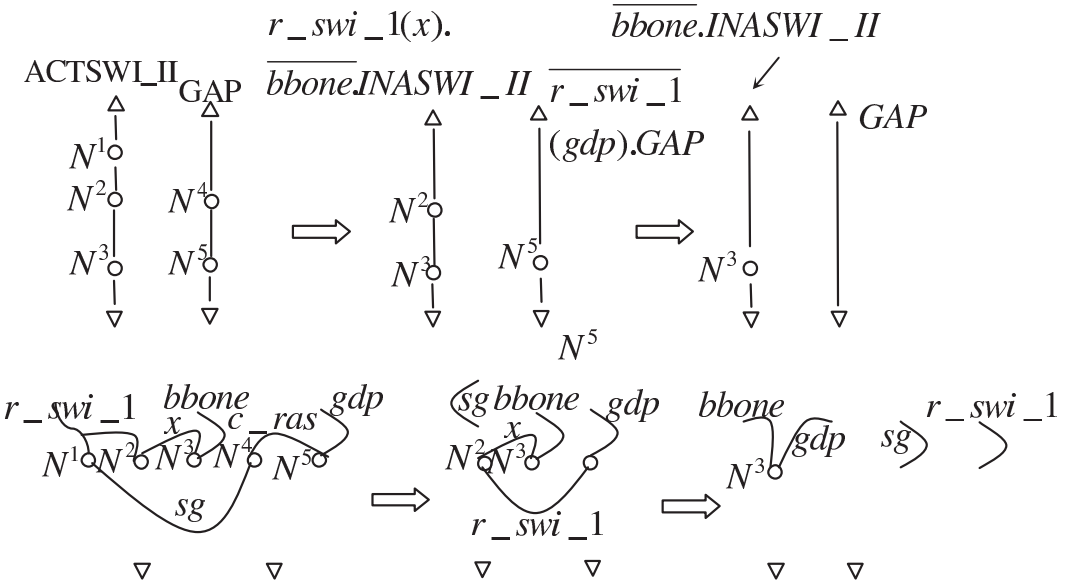


Figure A.5: The place graph and the link graph of *ras* inactivation-1.

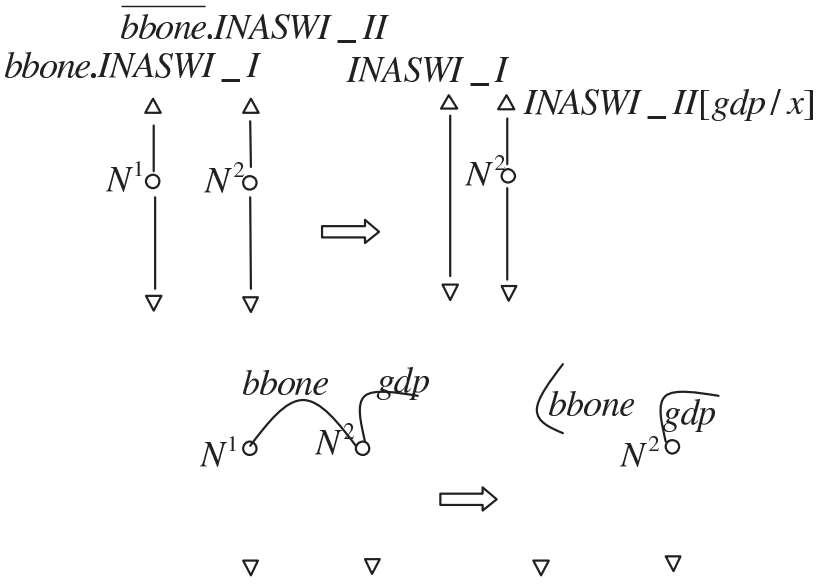


Figure A.6: The place graph and the link graph of *ras* inactivation-2.

Appendix B

Some Proofs in the Thesis

B.1 The Proof of Proposition 3.4

Proposition 3.3 [Strengthening] Assume that the term M is not free in the process P and that $N \neq M$. The following properties hold:

(1) If $\Gamma, M : T \vdash N : S$, then also $\Gamma \vdash N : S$.

(2) If $\Gamma, M : T \vdash P : Ok$, then also $\Gamma \vdash P : Ok$.

Proof: (1) The judgement $\Gamma, M : T \vdash N : S$ must be established through the rule *Level Term* with $\vdash \Gamma, M : T$ *well formed*, and $N : S$ in $\Gamma, M : T$. Then the judgment $\vdash \Gamma, M : T$ *well formed* must be established through the rule *Environment Term* with $\vdash \Gamma$ *well formed*. Because $N \neq M$, we have the fact that $N : S$ in Γ. Hence we have $\Gamma \vdash N : S$ using the rule *Level Term*.

(2) The second proposition is obtained by induction over the structure of P.

(a) Case 0. The judgement $\Gamma, M : T \vdash 0 : Ok$ must be established through the rule T-*nil* with $\vdash \Gamma, M : T$ *well formed*. The judgment $\vdash \Gamma, M : T$ *well formed* must be established through the rule *Environment Term* with $\vdash \Gamma$ *well formed*. So $\Gamma \vdash 0 : Ok$ by the rule T-*nil* again.

(b) Case $\pi_i.P$. The judgement $\Gamma, M : T \vdash \pi_i.P : Ok$, must be established through one of the rules (*T-out, T-in, T-sout, T-sin, T-kout, T-kin, T-ksout, T-ksin, T-pout, T-pin,T-psout* and *T-psin*) with $\Gamma, M : T \vdash a : R$, ($\Gamma, M : T \vdash b :$ *Normal* or $\Gamma, M : T \vdash x :$ *Unknown*), and $\Gamma, M : T \vdash P : Ok$, where $R \in \{Normal, Aberrant\}$. By induction hypothesis we have $\Gamma \vdash P : Ok$. Hence we have $\Gamma \vdash \pi_i.P : Ok$ also by one of the rules above.

(c) Case $P \mid Q$. The judgement $\Gamma, M : T \vdash P \mid Q : Ok$ must be established through the rule *T-com* with $\Gamma, M : T \vdash P : Ok$ and $\Gamma, M : T \vdash Q : Ok$. By induction hypothesis we have $\Gamma \vdash P : Ok$, and $\Gamma \vdash Q : Ok$, we have $\Gamma \vdash P \mid Q : Ok$ also

by the rule $T\text{-}com$. Similarly, we can get the same results for $P + Q$ using the rule, $T\text{-}sum$.

(d) Case $(\nu a)P$. The judgement $\Gamma, M : T \vdash (\nu a)P : Ok$ must be established through one of rules $T\text{-}res$ and $T\text{-}ares$ with $\Gamma, M : T, a : R \vdash P : Ok$, where $R \in \{Normal, Aberrant\}$ By induction hypothesis we have $\Gamma, a : R \vdash P : Ok$, we have $\Gamma \vdash (\nu a)P : Ok$ using the rule $T\text{-}res$ or $T\text{-}ares$ again.

B.2 The Proof of Proposition 3.5

Proposition 3.4 [Weakening] Assume that M is not defined in the environment Γ,

(1) If $\Gamma \vdash N : S$, then $\Gamma, M : T \vdash N : S$.

(2) If $\Gamma \vdash P : Ok$, then $\Gamma, M : T \vdash P : Ok$.

Proof: (1) The judgement $\Gamma \vdash N : S$ must be established through the rule $Level\ Terms$ with $\vdash \Gamma\ well\ formed$, and $N : S$ is in Γ. Because M is not defined in the environment Γ, we have $\vdash \Gamma, M : T\ well\ formed$ by the rule $Environment\ Term$. Since $N : S$ is in Γ, of course, $N : S$ is in $\Gamma, M : T$, hence we have $\Gamma, M : T \vdash N : S$ using the rule $Level\ Term$.

(2) It is obtained by induction over the structure of P.

(a) Case 0. The judgement $\Gamma \vdash 0 : Ok$ must be established through the rule T-nil with $\vdash \Gamma\ well\ formed$. For M is not defined in the environment Γ, then we have $\vdash \Gamma, M : T\ well\ formed$ by the rule $Environment\ Term$. So $\Gamma, M : T \vdash 0 : Ok$ by the rule T-nil again.

(b) Case $\pi_i.P$. The judgement $\Gamma \vdash \pi_i.P : Ok$ must be established through one of the rules ($T\text{-}out$, $T\text{-}in$, $T\text{-}sout$, $T\text{-}sin$, $T\text{-}kout$, $T\text{-}kin$, $T\text{-}ksout$, $T\text{-}ksin$, $T\text{-}pout$, $T\text{-}pin$, $T\text{-}psout$ and $T\text{-}psin$) with $\Gamma \vdash a : R$, ($\Gamma \vdash b : Normal$ or $\Gamma \vdash x : Unknown$) and $\Gamma \vdash P : Ok$ where $R \in \{Normal, Aberrant\}$. By induction hypothesis we have $\Gamma, M : T \vdash P : Ok$. Hence we have $\Gamma, M : T \vdash \pi_i.P : Ok$ also by the rules above.

(c) Case $P \mid Q$. The judgement $\Gamma \vdash P \mid Q : Ok$ must be established through the rule $T\text{-}com$ with $\Gamma \vdash P : Ok$ and $\Gamma \vdash Q : Ok$. By induction hypothesis we have $\Gamma, M : T \vdash P : Ok$, and $\Gamma, M : T \vdash Q : Ok$, then we have $\Gamma, M : T \vdash P \mid Q : Ok$ also by the rule $T\text{-}com$. Similarly, we can get the same results for $P + Q$ using the rule $T\text{-}sum$.

(d) Case $(\nu a)P$. The judgement $\Gamma \vdash (\nu a)P : Ok$ must be established through one of rules $T\text{-}res$ and $T\text{-}ares$ with $\Gamma, a : R \vdash P : Ok$, where $R \in \{Normal, Aberrant\}$. By induction hypothesis we have $\Gamma, a : Normal, M : T \vdash P : Ok$, we have $\Gamma, M : T \vdash (\nu a)P : Ok$ also by the rule $T\text{-}res$ or $T\text{-}ares$.

B.3 The Proof of Proposition 3.6

Proposition 3.5 Assume that $\vdash \Gamma$ well formed and that terms in $dom(\Gamma)$ are all $normal$. Then the following properties hold:

(1) If M is a term and $M \in dom(\Gamma)$, then $\Gamma \vdash M : Normal$.

(2) if P is a process with $f_n(P) \cup f_v(P) \subseteq dom(\Gamma)$, then $\Gamma \vdash P : ok$.

Proof: (1) The former proposition is obtained trivially by the rule $Level\ Term$.

(2) The latter proposition is obtained by induction over the structure of P.

(a) The base case is that $\Gamma \vdash 0 : Ok$ by the rule T-nil.

(b) Case $\pi_i.Q$ where $f_n(\pi_i.Q) \cup f_v(\pi_i.Q) \subseteq dom(\Gamma)$. Then $f_n(Q) \cup f_v(Q) \subseteq dom(\Gamma)$. By induction hypothesis we have $\Gamma \vdash Q : Ok$, hence we have $\Gamma \vdash \pi_i.Q : Ok$ using one of the rules $Level\ Subsumption$, T-out, T-in, T-$sout$, T-sin, T-$kout$, T-kin, T-$ksout$, T-$ksin$, T-$pout$, T-pin, T-$psout$, and T-$psin$.

(c) Case $R \mid Q$ where $f_n(R \mid Q) \cup f_v(R \mid Q) \subseteq dom(\Gamma)$. Then $f_n(R) \cup f_v(R) \subseteq dom(\Gamma)$ and $f_n(Q) \cup f_v(Q) \subseteq dom(\Gamma)$. By induction hypothesis we have $\Gamma \vdash R : Ok$, and $\Gamma \vdash Q : Ok$, and hence we have $\Gamma \vdash R \mid Q : Ok$ using the rule T-com. Similarly, we can get the same result for $P + Q$ using the rule T-sum.

(d) Case $(\nu a)Q$ where $fn((\nu a)Q) \cup fv((\nu a)Q) \subseteq dom(\Gamma)$. If $a \in dom(\Gamma)$, then $fn(Q) \cup fv(Q) \subseteq dom(\Gamma)$, where Γ can be written as $\Gamma', a : Normal$ and $a \notin dom(\Gamma')$. By induction hypothesis we have $\Gamma \vdash P : Ok$, that is, $\Gamma', a : Normal \vdash Q : Ok$. We have $\Gamma' \vdash (\nu a)Q : Ok$ using the rule T-Res. By Proposition 3.5, we have $\Gamma \vdash (\nu a)Q : Ok$. If $a \notin dom(\Gamma)$, then $fn(Q) \cup fv(Q) \subseteq dom(\Gamma) \cup \{a\}$. From $\vdash \Gamma\ well\ formed$ and $a \notin dom(\Gamma)$, we get $\vdash \Gamma, a : Normal\ well\ formed$. By induction hypothesis we have $\Gamma, a : Normal \vdash Q : Ok$, hence we have $\Gamma \vdash (\nu a)Q : Ok$ using the rule T-Res.

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