Spatiotemporal dynamics of signalling networks

Chloé Weckel

2022/2023
Master 2 Mathematics, Modelling and Learning
University of Paris Cité

Supervisor : Doctor Romain YVINEC
romain.yvinec@inrae.fr
Centre INRAE Val de Loire
Site de Tours
37380 Nouzilly

Academic supervisor : Doctor Marcela SZOPOS

01/02/2023-30/06/2023

Summary

In order to coordinate their actions and accomplish physiological functions, cells communicate to each others using extra-cellular signals, such as long-range hormonal signals. Ath the level of individual cells, these signals induce biological responses by triggering complex signaling cascades containing a series of molecules and biochemical reactions. One signaling cascade is typically activated by the interaction between an extracellular signal and its cognate receptor. The receptors of interest in this project are gonadotrophins receptors, that belong to the family of G-protein coupled receptors (GPCRs). Understanding cell signalling events and their spatiotemporal dynamics is a key step to develop pharmacological approaches. In fact, GPCRs are a wide range of targets for drugs because they control many biological systems as reproduction, embryonic development or neuronal system. For many hormones, including the reproductive hormones, GPCRs induce a physiological response at the plasma membrane but also within intracellular vesicles. The number of vesicles in the cell, their size but also their specific characteristics have dynamic evolution that shapes the signaling cascade. The spatiality and intensity of response have a physiological interest and suggest to take into account the entire intracellular response for pharmacological drug development. A key aspect of the spatial structure of the signaling cascade is the receptors trafficking, going back and forth from plasma membrane to dynamic vesicles. Modelling routing of these receptors could improve knowledge on this system and provide mechanistic interpretation to in-vitro biological experiences. Overall, the aim of my internship is to characterize the spatial and temporal dynamics of GPCR-induced signaling pathways.

Signaling cascades are translated in a mathematical model using the framework of chemical reaction networks. This formalism consists of species, reactions and kinetic rates to obtain an oriented graph on linear combination of species called complexes. The principle of this theory is based on this abstraction. Vesicles and plasma membrane can be considered as fixed compartments and then the traffic of receptors can be translated as reaction, understood as spatial transition from one compartment to another. Such approach, called compartmental or structured model, considers a globalization of quantities in the different types of compartments. This method permits to use the theory of chemical reaction networks to study the model behavior. During my internship, different models were written with two or three compartments including parameters of interest : recycling, internalization, dissociation and association rates. To obtain analytically models, various biologically motivated mathematical assumptions were used. Finally, thanks to interpretive expressions, the influence of parameters was studied and reveals that the signaling response dynamics might be influenced in distinct opposite ways by the receptor trafficking according to the ligand-receptor interaction parameters. Individual-based models have a greater modeling flexibility and allow to represent more complex phenomenon. The model I developed during my internship is hybrid : a mixture of stochasticity and determinism. Events of receptor trafficking and vesicles' size are random, but the biochemical reactions, including ligand-receptor interaction, are still assumed deterministic. In a certain way, these assumptions are closer to biological reality. This model leads to study Piecewise Deterministic Markov Processes and to write an algorithm in order to simulate the individual-based model. The biggest advantage of this approach is to be able to simulate the number of vesicles according to time.

The first perspective of my work is to calibrate models I developed in order to compare these models with biological data thanks to a statistical method of parameter estimation. The comparison of the deterministic and stochastic approach is not straightforward as several mechanisms can not be directly compared. Still, one possibility would be to use moment closure method in order to obtain a reduce set of ODEs, easier to analize and to compare to ODEs coming from reaction networks. Finally, many model extensions can be considered, including new molecular players that interact with the receptor trafficking machinery, or considering continuous space diffusion of effectors molecules that provide a coupling of signaling cascade at the scale of the whole cell.

Acknowledgements

My sincerest thanks go to Romain YVINEC for having accompanied me throughout this internship through numerous discussions, explanations, advice and proofreading, but above all for his constant benevolence.

I would also like to thank Marcela SZOPOS for sharing her love of research and for agreeing to supervise this internship from an academic point of view.

Within the mathematics team, I would like to particularly thank Léo DARRIGADE for his support throughout the internship, as well as for all the discussions on the world of research. I would also like to thank Misbah RAZZAQ and Nicolas AZZOPARDI for their advice during this period, and Frédérique CLEMENT for attending all my presentations and helping me to write this report.

Within the biology team, my thanks go to Frédéric JEAN-ALPHONSE for his availability, his sharing and all the biological discussions during which mathematics and biology meet and try to understand each other. I'm also grateful to Juliette GOURDON for taking the time to show me and explain the biological experiments, and to Camille GAUTHIER for her biological advice and proofreading.

Finally, I'd like to thank the whole BIOS team for their warm welcome, such a caring team in which everything is done to make you feel good and find your place. It is, I think, difficult to find such a close-knit interdisciplinary team that knows how to take an interest and find interest in the different fields. Thank you for all the moments of sharing within the laboratory, during walks or sports sessions, but also all the privileged moments outside INRAE.

In office 271, I'd like to thank Noa, Romane and Louis for making my discovery of Tours so incredible, and the days in the laboratory, but also the evenings so enjoyable.

Contents

Presentation of the laboratory

My intership took place at the Institut National de Recherche pour l'Agriculture, l'Alimentation et l'Environnement (INRAE) and more precisely in the Val de Loire Center, in Tours, in the team BIOlogy of GPCR Signaling Systems (BIOS) under the supervision of the Doctor Romain Yvinec. The team is part of a unit called Reproductive and Behavior unit. The academic supervisor is the Doctor Marcela Szopos.

BIOS is an interdisciplinary team composed of biologists, computer scientists and mathematicians managed by Romain Yvinec and Lucie Pellissier. The principle researches axes of the team revolve around the G protein coupled receptors (GPCRs), a family of receptors which generates many different cellular responses. The physiological response depends on the nature of the ligand. The team is focused on hormones involved in reproduction and social interactions. One part of the team study more preciously the biological responses induced by hormones which have a key role in social interactions. They try to identify social markers but also to improve sociability. Applications of this research range from the study of the autism spectrum disorder to animal welfare. The other part of the team works on reproduction and tries to develop non-hormonal control of reproduction (both for human and animals) but also to fight against infertility. The last part of the team uses multi-scale modelling of these different systems to predict biological responses which depend on space, time or intensity. Particularly, they develop mathematical models to understand the signaling response induced by GPCRs thanks deterministic, stochastic and hybrid approaches but also with artificial intelligence.

My internship concerned the project carried by the Doctor Frédéric Jean-Alphonse, on compartmentalized signalling. The principle question of this project is to determine how the routing of receptors and the signaling involved by the receptor from the intracellular compartments participate in the cellular and physiological response. This project is also in collaboration with the MUSCA team (MUltiSCAle population dynamics for physiological systems) based in INRIA Saclay (Institut National de Recherche en Informatique et Automatique). Biologists try to develop fluorescent sensors to follow in real time the signaling from the different compartments. In collaboration with biologists, some mathematical tools are developed (biochemical reaction networks, coagulation-fragmentation processes, individual-based model) to take into account compartmentalization.

I Biological introduction

I.1 Biological notions

G protein-coupled receptors (GPCRs) are a family of receptors implied in lots of biological mechanisms. These transmembrane proteins play a key role in the cell signaling in response of some ligands like hormones, ions or neurotransmitters. GPCRs are a wide range of targets for drugs because they control many biological systems as reproduction, embryonic development or neuronal system. Cells communicate to each others using extra-cellular signals, such as long-range hormonal signals. The ligand binding to its cognate receptor leads to a signaling cascade and a biological response. Many types of GPCRs exist which don't activate the same pathway. They are called GPCRs because after their activation, they recruit a heterotrimeric protein : the protein G composed of three parts α, β, γ. Then, after this interaction, this protein will be dissociated in two sub-units : the G_α and the combined $G_{\beta,\gamma}$ which will induce different signalling cascades.

Within the G protein-coupled receptors, one type of them specifically recognizes reproductive hormones called gonadotrophins hormones. The endocrine system composed of the hypothalamic-pituitary axis triggers especially the production of gonadotrophins hormones. Among them the luteinizing hormone (LH) and the follicle stimulating hormone (FSH) have a crucial role in the reproduction. These hormones are secreted in the pituitary gland and control the secretion of sexual steroid hormones (testosterone, estrogens...). For example, in males, FSH regulates the spermatogenesis in Sertoli cells, whereas LH takes action on Leydig cells to induce the synthesis of testosterone. For females, LH and FSH act in different phases of the ovulation. Understanding how work these hormones can permit to find some infertility treatments or non-hormonal contraception.

Gonadotrophin receptors are mostly combined to the $G_{\alpha s}$ sub-unit and will activate the adenylate cyclase (AC)/cyclic adenosine monophosphate (cAMP)/protein kinase A (PKA) signalling pathway. cAMP is produced by AC and activates PKA. Then, the production of cAMP is regulated by the phosphodiesterase (PDE). In fact, the key role of PDE is to degrade cAMP. During a long period, the biological community thought that the trigger of this signalling cascade only happened at the plasma membrane. Then, receptors were internalized and desensitized. Since few years ago, the vision of the GPCR signalling evolved. In fact, some biological studies showed that the cAMP production is also possible in organelles inside the cell [3] and more preciously in compartments called endosomes which have a key role in the intracellular transport of the molecules. Moreover, the reaction happens also in the Golgi apparatus or nuclear membrane. This property suggests an important spatial component of signalling and the production of cAMP in different intracellular membrane seems essential to ensure a correct physiological response [8].

For gonadotrophin receptors, it has been recently shown that there were rapidly internalized at first in little endosomes called Very Early Endosomes (VEE). They can also be internalized in Early Endosomes (EE) which are more used for transport to lysosome whereas VEE seems to play a direct role in the signalling inside the cell. Then, receptors can also be recycled back to the plasma membrane. However, to date, many uncertainties remain about the system of internalization or recycling of these receptors (Fig.1). Is there a recycling of EE and VEE? Is VEE can become an EE ? Moreover, VEE are distinct from EE by their size: VEE are smaller than EE [1]. The size characterization of the VEE and EE for these hormones leads to a mathematical model where compartments are represented by a volume and a certain quantity of molecules [2]. cAMP molecules might be produced in each compartment. Nevertheless, cAMP response seems to be separated in two time scales: a short acute response at the plasma membrane and a prolonged response which appeared after a few minutes of internalization in endosome [7]. Biologists can estimate the cAMP quantity thanks to BRET (Biology Resonance Emission Transfer) measurements. The ratio of BRET is proportional to the quantity of cAMP in the cell. This time dependence is typically summarized into dose response (using the area under the

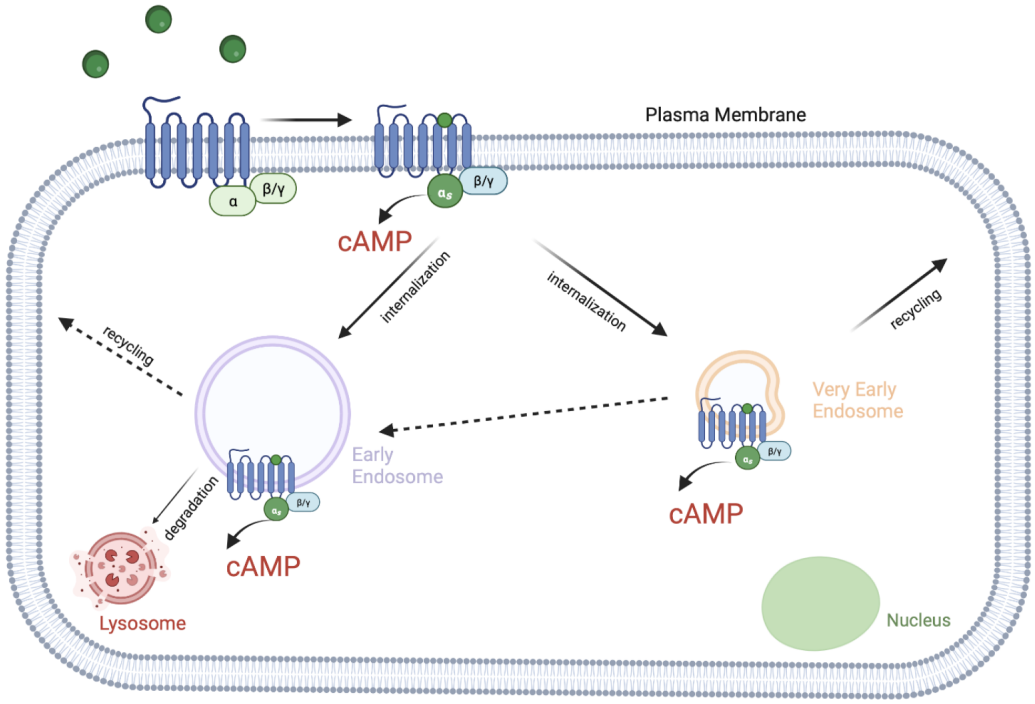

Figure 1: **Biological scheme about the routing of GPCRs.** This figure represents the mechanism of the internalization and recycling of the GPCRs activated by LH in the cell during a cellular response. The dotted lines represent the unknowns. The scheme was made thanks to Biorender.

curve, or response at a given final time)... The dose response represents the cAMP quantity in function of different doses of ligand in log scale (Appendix A-Fig.16). The biological response in function of time can have different profiles detailed in [5] and more precisely four different time course shapes are typically observed: the straight line is when signaling is unregulated; the association exponential curve is the most common observed when there are second messenger molecules; the rise-and-fall to baseline curve is when responses decline (desensitization) and the rise-and-fall to steady-state curve is observed when there are events of internalization and when the signaling is persistent inside the cell. The authors made parallel with biological conditions in function of different effectors molecules, if there is degradation or not but also if there is persistent signalling by internalized receptors. The notion of interaction between a ligand and its cognate receptor (Fig.3) was studied from a mathematical point of view at least since 1983 by Black and Leff [33]. Then, lots of mathematical models emerged to improve knowledge on this system [5], [12] and some models were developed in consideration with internalization of complex of ligand-receptors [5], [10], [25].

I.2 Modelling strategy

In view of its biological relevance, it seemed interesting to explore the traffic mechanism of these receptors and precisely to review the influence of routing parameters: internalization or recycling. Mathematical tools can be helpful to develop different models and then to propose alternative hypotheses to be confronted with biology. As a first approach, we consider the different types of endosomes and the plasma membrane as fixed compartments, among which molecules (*e.g.* receptors, ligand, complex ligand-receptors) can transition from one to the other: it is called routing. The model consider then that each compartment produces cAMP proportionally to the quantity of complex ligand-receptor that is present in it at a rate k^+ and degrades cAMP at a rate k^- (Fig.2). The initial quantities, the

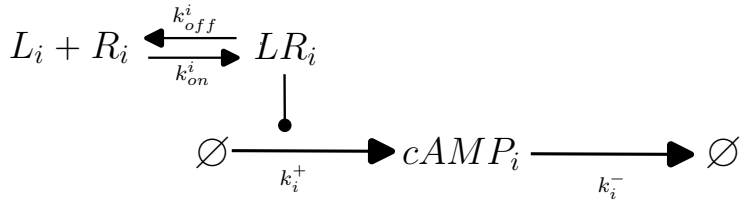

Figure 2: **cAMP modelling.** cAMP is produced in each compartment i at a rate k_i^+ and degraded at a rate k_i^-.

variables and the parameters of the model are introduced in the following table (Table.1).

Terms	***Unit***	***Description***
Initial quantities		
R_0	mol	The total initial quantity of receptors.
L_0	mol	The total initial quantity of ligand.
Variables		
R_i	mol	The amount of receptors in compartment i.
L_i	mol	The amount of ligand in compartment i.
LR_i	mol	The amount of complex ligand-receptors in compartment i.
$cAMP_i$	mol	The amount of cAMP in compartment i.
Parameters		
k^i_{off}	s^{-1}	The speed of dissociation of the complex ligand-receptors in compartment i.
k^i_{on}	$mol^{-1}.s^{-1}$	The speed of association of the complex ligand-receptors in compartment i.
$K^i_D = \frac{k^i_{off}}{k^i_{on}}$	mol	The constant of dissociation of the complex ligand-receptors in compartment i.
k_{xik}	s^{-1}	The rate of molecules x from compartment i to compartment k
k^i_{deg}	s^{-1}	The rate of the degradation of the ligand in compartment i.
k^+_i	s^{-1}	The rate of production of cAMP in compartment i.
k^-_i	s^{-1}	The rate of degradation of cAMP in compartment i.

Table 1: **Table of the initial quantities, the variables and the parameters.** x represents lr, r or l and $cAMP_i$ is proportional to the intensity of the biological response.

The main question of the project is to study the effect of the internalization and recycling on the production of cAMP in the cell from mathematical models. This work is applied on LH receptors but can be extended to other ligands and receptors. Compartmentalization could influence cellular signaling. Mathematics can be used to model transitions between each compartment and to understand the traffic influence. In [4], authors delineated how endocytocis regulates the signaling of the tyrosine kinase receptor (RTK). At first, a deterministic approach is developed by ordinary differential equations derived from a chemical reaction network, inspired by [10] or [25]. This formalism is said structured because the population of molecules is distributed into different structures (or compartments). These models allow to write a function of cAMP at steady state and to make an analogy with the dose response obtained in biology from approximately 30 minutes of reaction. This function depends on L_0, the initial quantity of ligand injected. It can predict the behaviour of the system in function of variations of the different parameters but also the maximal cAMP quantity obtained. A current limitation of the reaction network approach is that it doesn't take into account individual behaviours. A second approach, individual-based model, is presented. In fact, each compartment has its own life and so its own properties (cAMP production, lifetime, initial quantities,...). To take into account singularities of each compartment, a stochastic approach is proposed thanks to Piecewise Deterministic Markov

Processes and is consisted of two embedded models: deterministic and stochastic. The stochastic part models the compartment dynamics (creation of new compartments with given molecules quantities, recycling, etc.), while the deterministic models the reaction networks at play within each compartment.

In order to interpret the different models, some assumptions are advanced to simplify modelling. These hypotheses can be justified from a biological point of view.

1. **Time Scale Separation.** This hypothesis considers that reactions haven't the same speed. Some reactions are faster than others. In this problem, the association and dissociation of the LR complexes are faster than the internalization and recycling speed. The LR dissociation-association is thus taken at equilibrium.
2. **Ligand in excess at plasma membrane.** The quantity of ligand is considered in excess at plasma membrane compared to the quantity of receptors. This parameter is the only one that biologists can influence. They have no direct measurements of the quantity of receptors to the plasma membrane. To be sure to activate a reaction, they suppose that the administered doses of ligand are bigger that the quantity of receptors.
3. **Ligand in excess in all compartments.** The quantity of ligand is considered in excess in all compartments compared to the quantity of receptors. However, the quantity of ligand in the intracellular compartments is unknown and the hypothesis that it is in excess compared to receptors is to discuss. In fact, only the ligand-receptor complex is internalized, so the amount of ligand internalized may be limited by the amount of receptors.
4. **The initial quantity of receptors is very small.** This hypothesis considers that the initial quantity of receptors is very small and so tends to 0. It can be coherent if we considered that there are more ligands than receptors.
5. **The steps of recycling and dissociation of the complex LR are confused.** In fact, some biologists think that there is no dissociation of the complex but direct recycling of receptors to the plasma membrane and simultaneously degradation of the ligand.

Finally, this section introduced the biological problem and the strategy adopted. In section 2, we present mathematical preliminaries and more preciously two theories : the Chemical Reaction Network (CRN) and the Piecewise Deterministic Markov Processes (PDMP) and then in section 3, we exhibit the biological modelling by introducing different models.

II Mathematical preliminaries

II.1 Chemical Reaction Network theory (CRN)

This topic was first studied by Feinberg, Horn, and Jackson in the 1970s and is today a great advance to study directly biochemical networks. This theory is very used in applied mathematics and more especially for reaction's modelling like in Biology or Chemistry. This formalism uses a list of species (the biological entities), a list of reactions (that modifies the abundances of species) and a set of kinetic rates, deterministic or stochastic, that quantifies the speed at which each reaction proceeds. The set of reactions constitutes an oriented graph on linear combination of species (called complexes). This abstraction is the starting point of the chemical reaction network theory, which aims to characterize the dynamical behavior of each underlying dynamical systems, based on (mostly) the network topology. This theory gives some tools and theorems to ask directly about existence and uniqueness of steady-states or their stability without any calculation but just with the CRN's analysis. The presentation of this theory is inspired from the book of Martin Feinberg [13].

II.1.1 Chemical reaction network's introduction

Definition 1. *A chemical specie is a chemical substance that is involved in a reaction, and can be a product, a reactant or both.* $\mathcal{E} : \{E^1, ..., E^d\}$ *is the finite set of species with d, the total number of species.*

Definition 2. *Complexes are all vectors* $y^j \in \mathcal{C}$ *where* $C = \{y^1, ..., y^n\}$ *with n the total number of complexes. We identify* $y^j \in \mathcal{C}$ *as a vector in* $\mathbb{R}^d$*, with*

$$y^j = \begin{pmatrix} y_1^j \\ . \\ y_d^j \end{pmatrix} \text{ , } y_i^j = \textit{stoichiometry of } E^i \textit{ in } y^j$$

Often, we denote the source vector by y and the product vector by $y^{'}$ with y and $y^{'}$ belong to $\mathcal{C}$. We identify $\{e_{y^1}, ..., e_{y^n}\}$ as the canonical orthonormal basis of $\mathbb{R}^n$.

Definition 3. *A reaction* R_j *is given by* $R_j = y \to y^{'}$ *where* y *and* $y^{'}$ *are complexes in* $\mathcal{C}$ *defined as above. The set of all reactions are given by* $\mathcal{R} = \{y \to y^{'} : y, y^{'} \in \mathcal{C}\}$*, and* $|\mathcal{R}| = r$*.*

Definition 4. *The triple* $\{\mathcal{E}, \mathcal{C}, \mathcal{R}\}$ *is then called a chemical reaction network (CRN).*

We begin with an example that we will use on the next pages (Fig.3-A). In this example, there are

A

$$L + R \underset{k_{on}}{\overset{k_{off}}{\rightleftharpoons}} LR$$

B

$$R \underset{Lk_{on}}{\overset{k_{off}}{\rightleftharpoons}} LR$$

Figure 3: **Dissociation-association models.** Classic models about interaction between ligand and receptors when the ligand is considered as a constant (B) or not (A).

two reactions ($r = 2$), three species ($d = 3$) and two complexes ($n = 2$). We can write the reactants and products as column vectors, $y^1 = \begin{pmatrix} 1 \\ 1 \\ 0 \end{pmatrix}$ and $y^2 = \begin{pmatrix} 0 \\ 0 \\ 1 \end{pmatrix}$.

II.1.2 Deterministic mass-action

In a deterministic system, the concentration of each species is a function of time. We note $c_i(t)$ the concentration of species E^i per unit of volume. The concentration vector is finally $c = (c_1, ..., c_d)$. The mass action law states that the speed of the reaction is proportional to the product of the concentration of the different reactants.

Definition 5. *Kinetic rates are* $\kappa = \{\kappa_{y \to y'}, y \to y' \in \mathcal{R}\}$*.*

Definition 6. *The deterministic system associated to the CRN* $\{\mathcal{E}, \mathcal{C}, \mathcal{R}, \kappa\}$ *for the concentration of species c, is given by an initial condition* $x(0) = x_0 \in \mathbb{R}_+^d$ *and*

$$\frac{dc}{dt} = f(c(t)) = \sum_{y \to y' \in R} \kappa_{y \to y'} c^y (y' - y), \text{ with } c^y = \prod_{s \in \mathcal{E}} c_s^{y_s}. \tag{1}$$

In the previous example (Fig.3-A), the system obtained is :

$$\begin{cases} \frac{dL}{dt} = k_{off} LR(t) - k_{on} L(t) R(t), \\ \frac{dR}{dt} = k_{off} LR(t) - k_{on} L(t) R(t), \\ \frac{dLR}{dt} = k_{on} L(t) R(t) - k_{off} LR(t). \end{cases}$$

Remark 1. *The CRN's theory allows in some cases to analyze the long time behavior of an ODE regardless of its parameter values, and gives some nice properties to the model. In order to prove the existence and uniqueness of a solution, the deterministic theory can be used and in particular the Cauchy-Lipschitz theory. Chemical reaction networks contain polynomial functions which are locally Lipschitz. Then, thanks to Cauchy-Lispschitz, there exists a unique maximal solution. Global in time solution requires additionnal assumptions, to avoid degenerate cases like the CRN: $X + X \to 3X$.*

Definition 7. *(Complex composition matrix)*
$Y : \mathbb{R}^n \to \mathbb{R}^d$ is the linear application defined by $Y(e_y) = y$, $\forall y \in \mathcal{C}$. Y can be represented as a $\mathcal{M}_{d\times n}$ matrix, with:

$$Y_{ij} = y_i^j, \quad 1 \leqslant i \leqslant d, 1 \leqslant j \leqslant n. \tag{2}$$

Definition 8. *(Complex graph matrix)*
$A_\kappa : \mathbb{R}^n \to \mathbb{R}^n$ is defined by:

$$A_\kappa(c) = \sum_{y\to y' \in \mathcal{R}} \kappa_{y\to y'} c_y (e_{y'} - e_y). \tag{3}$$

A_κ can be represented as a $\mathcal{M}_{n\times n}$ matrix, with:

$$A_\kappa^{ij} = \begin{cases} \kappa_{j\to i} & \text{if } j \neq i, \\ -\sum_{l\neq j} \kappa_{j\to l} & \text{if } \quad j = i. \end{cases} \tag{4}$$

Definition 9. *(Propensity vector)*
The propensity vector $\Phi : \mathbb{R}^d \to \mathbb{R}^n$ is defined by $\Phi(c) = \sum_{y\in\mathcal{C}} c^y e_y$.
This vector is a vector of $\mathbb{R}^n$ with $\Phi(c) = \begin{pmatrix} c^{y^1} \\ . \\ c^{y^n} \end{pmatrix}$.

Finally, the system, $f(c) = \frac{dc}{dt}$ can be re-written as,

$$f(c) = Y \circ A_\kappa \circ \phi(c). \tag{5}$$

In particular, these definitions can be applied for the previous model (Fig.3-A) with, $Y = \begin{pmatrix} 1 & 0 \\ 1 & 0 \\ 0 & 1 \end{pmatrix}$, $A_\kappa = \begin{pmatrix} -k_{on} & k_{off} \\ k_{on} & -k_{off} \end{pmatrix}$, $\Phi(c) = \begin{pmatrix} L(t)R(t) \\ LR(t) \end{pmatrix}$, and finally thanks to (5),

$$Y \circ A_\kappa \circ \Phi(c) = \begin{cases} \frac{dL}{dt} = k_{off} LR(t) - k_{on} L(t)R(t), \\ \frac{dR}{dt} = k_{off} LR(t) - k_{on} L(t)R(t), \\ \frac{dLR}{dt} = k_{on} L(t)R(t) - k_{off} LR(t). \end{cases}$$

Definition 10. *(Reaction Stoichiometry matrix)*
The Reaction Stoichiometry matrix $\Gamma : \mathbb{R}^r \to \mathbb{R}^d$ is the linear application defined as a $\mathcal{M}_{d\times r}$ matrix, with:

$$\Gamma_{ij} = y_i' - y_i, \quad 1 \leqslant i \leqslant d, \quad 1 \leqslant j \leqslant r, \text{ where } y \to y' \in \mathcal{R} \text{ is the } j^{th} \text{ reaction.} \tag{6}$$

Definition 11. *(Complex Incidence matrix)*
$I : \mathbb{R}^r \to \mathbb{R}^n$ is the linear application defined as a $\mathcal{M}_{n\times r}$ matrix, where $I_j = e_k - e_i$, $1 \leqslant j \leqslant r$ and $y^i \to y^k \in \mathcal{R}$ is the j^{th} reaction.

Moreover, we define $k_j(c) = \kappa_j c^y$ where j is the number of the reaction.
With these definitions, we can verify that $\Gamma = YI$ and that the system, $f(c) = \frac{dc}{dt}$ can be re-written as,

$$f(c) = \Gamma \circ k(c(t)) = Y \circ I \circ k(c(t)). \tag{7}$$

For the example (Fig.3-A), these elements are : $\Gamma = \begin{pmatrix} -1 & 1 \\ -1 & 1 \\ 1 & -1 \end{pmatrix}$, $I = \begin{pmatrix} -1 & 1 \\ 1 & -1 \end{pmatrix}$,

$k(c(t)) = \begin{pmatrix} k_{on}L(t)R(t) \\ k_{off}LR(t) \end{pmatrix}$ and, $Y \circ I \circ k(c(t)) = \begin{cases} \frac{dL}{dt} & = k_{off}LR(t) - k_{on}L(t)R(t), \\ \frac{dR}{dt} & = k_{off}LR(t) - k_{on}L(t)R(t), \\ \frac{dLR}{dt} & = k_{on}L(t)R(t) - k_{off}LR(t). \end{cases}$

Remark 2. *If we have a graph with rates κ, there is an unique possible ODE system with respect this graph. However, if we have an ODE system, there are in general several graphs which are possible. There is no uniqueness of the graph.*

II.1.3 General properties of network

Definition 12. *A fixed point (resp. positive fixed point) is $c \in \mathbb{R}^d_{\geqslant 0}$ (resp. $c \in \mathbb{R}^d_{>0}$) such that $f(c) = 0$.*

Definition 13. *The stoichiometric subspace is $S = vect\{y' - y | y \to y' \in \mathcal{R}\}$ and $s = dim(S)$.*

Definition 14. *(Stoichiometric compatibility class) $\forall x \in \mathbb{R}^d$, $S_x = (x + S) \cap \mathbb{R}^d_+$.*

Remark 3. *A parallel could be made with the notion of compatibility class and the number of conservation laws. In all our models, the initial quantity of receptors is conserved. This means that, variables are bounded and the solution of the chemical reaction networks is global (Fig.3).*

Remark 4. *Moreover, conservation laws imply global solution. Finally, for all Cauchy Problem presented during the report with conservation laws, there exists a unique global solution.*

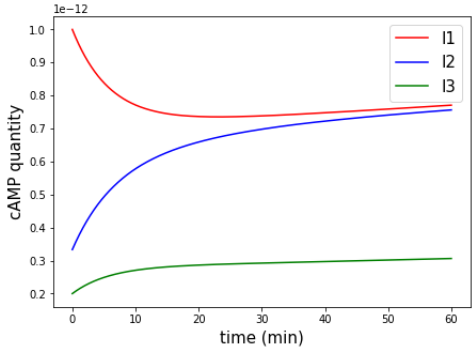

Figure 4: **Different stoichiometric compatibility classes.** The initial conditions 1 and 2 are in the same stoichiometric compatibility class whereas the initial condition 3 is in an other stoichiometric compatibility class.

Let us introduce some relations between complexes.
• We say y, y' are directly linked, denoted by $y \leftrightarrow y'$, if either $y \to y' \in \mathcal{R}$ or $y' \to y \in \mathcal{R}$.
• We say that y ultimately reacts to y' if either, $y = y'$ or if it exists $y^1, ...y^m \in \mathcal{C}$ such that $y = y^1 \to y^2 \to ... \to y^m = y'$. This is denoted by $y \Rightarrow y'$.

Definition 15. *(Linkage Class)*
The linkage relation is the equivalence relation on C, denoted by $y \sim y'$.
$y \sim y'$ if either $y = y'$ or if it exists $y^1, ..., y^m$, such that $y = y^1 \leftrightarrow y^2 \leftrightarrow ... \leftrightarrow y^m = y'$. The linkage classes are denoted by $L_1, ..., L_l$ and l is the number of linkage classes.

Definition 16. *(Strong Linkage Class)*
The strong linkage relation is the equivalence relation on C, denoted by $y \approx y'$.
$y \approx y'$ is if both $y \Rightarrow y'$ and $y' \Rightarrow y$. The strong linkage classes are denoted by $\bar{L_1}, ..., \bar{L_p}$ and p is the number of strong linkage classes.

Remark 5. *Every linkage class is an union of strong linkage class. Thus, $p \geqslant l$.*

Definition 17. *(Terminal Strong Linkage Class)*
The strong linkage class $\bar{L}$ is terminal if no complex in $\bar{L}$ reacts to a complex outside $\bar{L}$. The Terminal Strong Linkage classes are denoted by $T_1, ..., T_t$ and t is the number of Terminal Strong Linkage classes.

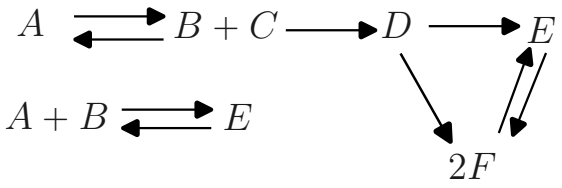

Figure 5: **CRN's example.**

In the previous example (Fig.5), there are two linkage classes ($l = 2$): $\{A, B + C, D, E, 2F\}$ and $\{A + B, E\}$, four strong linkage classes ($p = 4$) : $\{A, B + C\}, \{D\}, \{E, 2F\}$ and $\{A + B, E\}$, and two terminal linkage classes ($t = 2$) : $\{E, 2F\}$ and $\{A + B, E\}$.

Definition 18. *(Reversibility)*
$(\mathcal{E}, \mathcal{C}, \mathcal{R})$ is reversible if for any $y \to y' \in \mathcal{R}$, then $y \to y' \in \mathcal{R}$.
$(\mathcal{E}, \mathcal{C}, \mathcal{R})$ is weakly reversible if the strong linkage classes coincide with the linkage classes, or equivalently, if $y \Rightarrow y'$ then $y' \Rightarrow y$.

Definition 19. *(Complex balanced equilibrium and complex balanced)*
A complex balanced equilibrium is a concentration $c \in \mathbb{R}^d_{\geqslant 0}$ such that for all $z \in \mathcal{C}$,

$$\sum_{y : y \to z \in R} \kappa_{y \to z} c^y = \sum_{y' : z \to y' \in R} \kappa_{z \to y'} c^z.$$

Stated differently, this is when inflow rate is equal to the outflow rate for any complex z.
A chemical reaction network is said complex balanced if there exists a positive complex balanced equilibrium.

Definition 20. *(Deficiency)*
The deficiency of a network is defined as $\delta = n - l - s$ with n: the number of complexes, l: the number of linkage classes and s: the dimension of the stoichiometric subspace.

We can take the following CRN on example (Fig.6). This CRN is weakly-reversible. Moreover, there is just one linkage class ($l = 1$), the dimension of the stoichiometric subspace is $s = 2$, and there are three species ($n = 3$). Finally, the deficiency is $\delta = 0$. The deficiency of the CRN presented in (Fig.3) is 0 whereas the deficiency of the CRN (Fig.5) isn't 0.

II.1.4 Deficiency 0 theorem

In 1979, Feinberg proved the Deficiency Zero Theorem [20]. This theorem is used to directly prove from a Chemical Reaction Network that the mass actions systems have a unique stationary point within each positive stoichiometric compatibility class and this equilibrium is locally asymptotically stable. The unique equilibrium of deficiency 0 CRN has been proved globally stable in the case of a single linkage class [17]. Since then, many more generalized theorem have been made to expand the power of this theorem and to discover new properties on the CRN. However, many questions remain such that the existence of a global attractor for CRN with arbitrary number of linkage classes.

Theorem 1. *(Feinberg, 1979)*
Let $\{\mathcal{E}, \mathcal{C}, \mathcal{R}\}$ be a chemical reaction network with deterministic mass-action kinetics. Suppose it's a weakly reversible system which has zero deficiency, then within each positive stoichiometric compatibility class, there is precisely one equilibrium which is locally asymptotically stable.

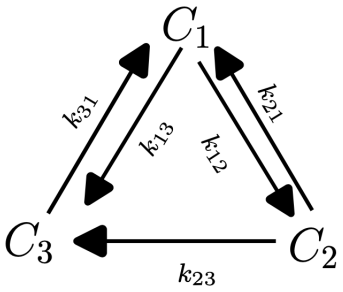

Figure 6: **Traffic model.** A simplify model which represents the traffic between three compartments.

Idea of the proof : Feinberg separated the proof in two part in five lectures. At first, he supposed that the differential equations for the mass action system admit a positive equilibrium (not necessarily one in each positive stoichiometric compatibility class) and proved the stability of the stationary point. In a second time, when the system is weakly reversible network of deficiency 0, he proved that the differential equations admit a positive equilibrium. All the demonstration is based on the kernel of the different matrix presented above and more preciously about the complex graph matrix, A_κ, in (3). One of the principle result used in the proof is the following corollary (Lecture 4).

Corollary 1. *Let $\{\mathcal{E}, \mathcal{C}, \mathcal{R}\}$ be a reaction network of deficiency zero, and let κ, the kinetic rates. If Y is the stoichiometric map for the network and A_κ is defined as in* (3)*,*

$$Ker(YA_\kappa) = Ker(A_\kappa). \tag{8}$$

Demonstration . • *If $x \in Ker(A_\kappa)$,*

$$\begin{aligned} x \in Ker(A_\kappa) &\Longleftrightarrow A_\kappa x = 0 \\ &\Longleftrightarrow YA_\kappa x = 0 \\ &\Longleftrightarrow x \in Ker(YA_\kappa). \end{aligned}$$

It's obviously that $Ker(A_\kappa)$ is contained in $Ker(YA_\kappa)$.
• *If $x \in Ker(YA_\kappa)$,*

$$\begin{aligned} x \in Ker(YA_\kappa) &\Longleftrightarrow YA_\kappa x = 0 \\ &\Longleftrightarrow A_\kappa x \in Ker(Y). \end{aligned}$$

Let $\Delta = \{e_{y'} - e_y \in \mathcal{R}^{\mathcal{C}} : y \sim y'\}$. Since A_κ takes values in $span(\Delta)$, we have the inclusion,

$$A_\kappa x \in Ker(Y) \cap span(\Delta). \tag{9}$$

Moreover, such $\delta = dim(Ker(Y) \cap span(\Delta))$, if the network has zero deficiency,

$$dim(Ker(Y) \cap span(\Delta)) = 0.$$

Finally, $A_\kappa x = 0$ and $x \in ker(A_\kappa)$.

Remark 6. *If a system admits one stationary point on a stoichiometric compatibility class, it converges for different initial conditions. However these initial conditions must be on the same stoichiometric compatibility class (Fig.4).*

From this first theorem, many people tried to modify and improve these results. I will just mention one of them which will be used for the analysis of the future models. Anderson in [17] proved that the stationary point is a global attractor when there is just one linkage class.

Theorem 2. *(Global Attractor [17])*
Let $\{\mathcal{E}, \mathcal{C}, \mathcal{R}, \kappa\}$ denote a complex-balanced system with one linkage class. Then, any complex-balanced equilibrium contained in the interior of a positive compatibility class is a global attractor of the interior of that positive class.

The proof of the global stability has not yet published in the general case even if the conjecture persists.

II.1.5 Time scale separation

In a system, all variables haven't the same speed. This theory allows to separate fast variables and slow variables [18],[19]. The system can be written in two ways :

$$\begin{cases} x' &= \frac{dx}{dt} = \epsilon f(x, y, \epsilon) \\ y' &= \frac{dy}{dt} = g(x, y, \epsilon) \end{cases} \text{ with } \epsilon \to 0$$

and with the following variables changes, $\tau = \dfrac{t}{\epsilon}$,

$$\begin{cases} x' &= \frac{dx}{d\tau} = f(x, y, \epsilon) \\ y' &= \frac{dy}{d\tau} = \frac{1}{\epsilon} g(x, y, \epsilon) \end{cases} \text{ with } \epsilon \to 0.$$

x are the slow variables and y are the fast variables. In fact, when $\epsilon \to 0$, $g(x, y, 0) = 0$. The fast variables reach very rapidly their stationary state. We have two cases if L is considered as a constant or not. We introduce some functions when the quantity of ligand is constant or not to take account of the time scale separation in the basic model (Fig.3).

• The quantity of ligand is not constant.
If we consider $k_{on}, k_{off} \sim \frac{1}{\epsilon}$ and let $\epsilon \to 0$, we obtain, at the limit that $\forall t \in \mathbb{R}_+$,

$$k_{on} L(t) R(t) = k_{off} LR(t), \tag{10}$$

$$\begin{cases} f(L_{tot}, R_{tot}, K_D) = LR(t) = \frac{1}{2}(L_{tot}(t) + R_{tot}(t) + K_D - \sqrt{(R_{tot}(t) - L_{tot}(t) + K_D)^2 + 4K_D L_{tot}(t)}), \\ g(L_{tot}, R_{tot}, K_D) = L(t) = \frac{1}{2}(L_{tot}(t) - R_{tot}(t) - K_D + \sqrt{(R_{tot}(t) - L_{tot}(t) + K_D)^2 + 4K_D L_{tot}(t)}), \\ h(L_{tot}, R_{tot}, K_D) = R(t) = \frac{1}{2}(R_{tot}(t) - L_{tot}(t) - K_D + \sqrt{(R_{tot}(t) - L_{tot}(t) + K_D)^2 + 4K_D L_{tot}(t)}). \end{cases} \tag{11}$$

• The quantity of ligand is constant.
By inspiring an article of Hoare [12], one can consider $L = L_0$, with $L_0 \in \mathbb{R}_+$ the initial quantity of ligand. If we consider $k_{on}, k_{off} \sim \frac{1}{\epsilon}$ and let $\epsilon \to 0$, we obtain, at the limit that $\forall t \in \mathbb{R}_+, L_0 \in \mathbb{R}_+$,

$$k_{on} L_0 R(t) = k_{off} LR(t), \tag{12}$$

$$\begin{cases} l(L_0, R_{tot}, K_D) = LR(t) = \frac{L_0}{K_D + L_0} R_{tot}(t), \\ p(L_0, R_{tot}, K_D) = R(t) = \frac{K_D}{K_D + L_0} R_{tot}(t). \end{cases} \tag{13}$$

II.1.6 General kinetics

Kinetics rates are not necessarily constant rates. They can have multiple forms, but they have to verify some properties [16], [21]. The first article, [16], defines different classes of kinetics : general kinetics, weak general kinetics and positive general kinetics. We will define more precisely what are general kinetics.

Definition 21. *Given a CRN, let $\mathcal{I}_{j,l}$ be the set of indices of species occuring on the left of reaction j and $\mathcal{I}_{j,r}$ be the set of indices of species occuring on the right of reaction j. f verifies the general kinetics if f is defined and C^1 on $\mathbb{R}^n_{\geqslant 0}$ for reaction j which is irreversible then:*

1. *$f_j \geqslant 0$ with $f_j = 0$ if and only if $x_i = 0$ for some $i \in \mathcal{I}_{j,l}$.*
2. *$\frac{df_j}{dx_i} \geqslant 0$ for each $i \in \mathcal{I}_{j,l}$. If $x_i > 0$ for all $i \in \mathcal{I}_{j,l}$, then $\frac{df_j}{dx_i} > 0$ for each $i \in \mathcal{I}_{j,l}$.*

It can be summarized very roughly as "reactions proceed if and only if all reactants are present, reaction rates are non decreasing with reaction concentration, and reaction rates increase strictly with reactant concentration if and only if all reactants are present." Banaji [21] provides general conditions under which, when kinetics are general kinetics, that the CRN admits an unique stationary point which is asymptotically stable on each stoichiometric compatibility class (theorem 2.1 in [21]). Moreover, f, g, h, l and p defined above in (11) and (13) can be used as general kinetics rates (see Appendix-B).

II.2 Piecewise Deterministic Markov Processes (PDMP)

II.2.1 Poisson process

Definition 22. *A point process on $\mathbb{R}_+$ is the data of a strictly increasing sequence of random variables $(T_n)_{n\geqslant 1}$ of positive reals and unbounded.*

Definition 23. *Let $(T_n)_{n\geqslant 1}$ be a point process, we call the counting function associated with $(T_n)_{n\geqslant 1}$, the function $t \in \mathbb{R}_+ \mapsto N_t$ defined by $N_t := sup\{n \geqslant 0, T_n \leqslant t\}$.*

Definition 24. *(Poisson process)*
We call Poisson process the given of a sequence of random variables $\omega \mapsto (T_n(\omega)_{n\geqslant 1})$ such that for any $\omega \in \Omega, (T_n(\omega)_{n\geqslant 1})$ is a point process for which the counting function $\omega \mapsto (N_t(\omega)_{t\geqslant 0})$ satisfies the two following conditions:

1. ***Hypothesis of independent increments**: the numbers of events that occur in disjoint time intervals are independent.*
   Let $0 < s < t$, $N_t - N_s$ is independent of F_s where $F_s = \sigma(N_r, 0 \leqslant r \leqslant s)$.
2. ***Stationarity hypothesis**: the distribution of the number of events occurring in a given time interval depends only on the length of the time interval.*
   If $s < t$, $N_t - N_s$ has the same law of N_{t-s}.

Proposition 1. *If (T_n) is a Poisson process, there is a constant $\lambda > 0$ such that for $s \geqslant 0$, N_s follows a Poisson distribution of parameter λs. λ is the process intensity and is the expected number of jumps per unit of time.*

II.2.2 Non-homogeneous Poisson process

Definition 25. *A non-homogeneous Poisson process $(N(t) : t \geqslant 0)$ is a Poisson process whose intensity is a function of time, $\lambda = \lambda(t)$ where $t \in \mathbb{R}_+$.*

Proposition 2. *The number of events N_t is then distributed according to a Poisson distribution of parameter $\tau(t) = \int_0^t \lambda(u)du$.*

Lewis in [22] proposed in 1979 a simple and relatively efficient method for simulating one-dimensional and two-dimensional non-homogeneous Poisson process. This method is based on the accept/reject principle. To simulate a non-homogeneous Poisson Process $(N(t) : t \geqslant 0)$ of intensity λ, he relied on the next theorem.

Theorem 3. *Consider a non-homogeneous Poisson process $(N^*(t) : t \geqslant 0)$ of intensity $\lambda^*(t)$. On a fixed interval $[0, t_0]$, the number of points, $N^*(t_0)$ has a Poisson distribution with parameter $\tau^*(t_0) - \tau^*(0)$. Let $X_1^*, .., X_{N^*(t_0)}^*$ be the points of the process. If for $t \in [0, t_0], \lambda(t) \leqslant \lambda^*(t)$. For $i = 1, ..., n$, delete the point X_i^* with probability $1 - \frac{\lambda(X_i^*)}{\lambda^*(X_i^*)}$; then the remaining points form a non-homogeneous Poisson Process $(N(t) : t \geqslant 0)$ with rate function $\lambda(t)$ on $[0, t_0]$.*

Algorithm 1 One-dimensional nonhomogeneous Poisson process.

1. Generate points in the nonhomogeneous Poisson process $(N^*(t) : t \geqslant 0)$. Let n^* be the number of points generated. If $n^* = 0$, exit because there are no points in the process $(N(t) : t \geqslant 0)$.
2. Denote the (ordered) points by $X_1^*, .., X_{n^*}^*$. Set $i = 1$ and $k = 0$.
3. Generate U_i, uniformly distributed between 0 and 1. If, $U_i \leqslant \frac{\lambda(X_i^*)}{\lambda^*(X_i^*)}$, $k \leftarrow k + 1$ and $X_k = X_i^*$.
4. Set $i \leftarrow i + 1$. If $i \leqslant n^*$, go to 3.
5. When $n = k$, return $X_1, ..., X_n$ and n.

II.2.3 Piecewise Deterministic Markov Processes

Piecewise Deterministic Markov Processes (PDMP) are used to simulate systems which include deterministic continuous dynamical systems perturbed by random discrete events in time. The perturbation can be a discontinuous jump or a change in the continuous motion. We can see this process like a stochastic hybrid model. It was literally introduced by Davis in 1984 [14]. Moreover in 1992, Lasota et al, [15], was one of the first to use this type of process to explore biological problems and more preciously the cell division. To introduce the piecewise deterministic Markov processes, one can rely on [28].
More preciously, let K be a countable set, $d : K \to \mathbb{N}$ and for each $k \in K$, P_k an open subset of $\mathbb{R}^{d(k)}$. The state-space E is:

$$E = \bigcup_{k \in K} P_k = \{z = (x, k); k \in K, x \in P_k\}.$$

We can also define $\mathcal{E}$ which denote the following class of measurables sets in E:

$$\mathcal{E} = \{\bigcup_{k \in K} A_k; A_k \in \mathcal{P}_k\}, \text{ where } \mathcal{P}_k \text{ denotes the Borel set of } P_k.$$

The PDMP is determined by the following objects:

- Vector fields $(H_k, k \in K)$ such that for all $k \in K$, all $x_0 P_k$, there is a unique global solution in P_k of
$$\begin{cases} \frac{dX_t}{dt} = H_k(X_t), \\ X_0 = x_0. \end{cases} \tag{14}$$
- A measurable function $\lambda : E \to \mathbb{R}_+$, such that for all $z = (k, x_0) \in E$, the function $t \mapsto \lambda(X_t)$ is locally integrable along the solution of equation (14).
- A transition measure $Q : \mathcal{E} \times E \to [0, 1]$, such that $Q(A; z)$ is a measurable function of $z \in E$ for each fixed $A \in \mathcal{E}$, and is a probability measure on $(E, \mathcal{E})$ for each fixed $z \in E$.

More preciously, the system (H_k, λ, Q) is a piecewise deterministic process which depends on k. At the jump time, the next value of the ODE is given by the transitional measure Q defined above.

III Biological modelling

To characterize the spatial and temporal dynamics of GPCR-induced signaling pathways, we model routing of these receptors to improve knowledge on this system and provide mechanistic interpretation to in-vitro biological experiences. To model the effect of internalization and recycling on the production of $cAMP$ (Fig.1), a first part with only two types of compartments is advanced in section 3.1. The type "endosomes" can correspond to a global term that correspond to both EE end VEE. However, to separate EE and VEE, some three compartment models are proposed in section 3.2. For the two compartment model, a deterministic approach and stochastic approach are developed whereas for the three compartment models, we only focus on the deterministic approach. To study the influence of parameters, the goal for all models is to write $cAMP$ in function of the initial quantity of ligand. This parameter is the only one for which biologists have access. An analogy is possible between the equilibrium obtained inside biological experience after 30 minutes and the steady states obtained by a mathematical model. On future models for two or three compartments, the $cAMP$ function is always increasing function of L_0. In the different models that we developed, we omitted the variable of $cAMP$. In fact, $cAMP$ is proportional to the quantity of ligand-receptor complexes LR and we can focus on the quantity of L, R and LR to determinate independently the amount of $cAMP$. Moreover, the $cAMP$ amount is convergent in the model describing the production and degradation of $cAMP$ function of LR (Fig.2) (Proposition.3). The exact solution is:

$$cAMP(t) = k^+ \int_0^t LR(s) e^{-k^-(t-s)} ds.$$

Proposition 3. *In the model describing the production and degradation of cAMP function of LR (Fig.2), the cAMP amount is convergent:*

$$\lim_{t \to +\infty} cAMP(t) = cAMP^*, \text{ with } cAMP^* \in \mathbb{R}_+.$$

Demonstration . *Thanks to mass conservation,* $R_0 = LR(t) + R(t)$ *and* $L_0 = LR(t) + L(t)$.

$$\begin{aligned}
\dot{LR} &= -k_{off} LR(t) + k_{on} L(t) R(t) \\
&= -k_{off} LR(t) + k_{on}(L_0 - LR(t))(R_0 - LR(t)) \\
&= k_{on} L_0 R_0 - LR(t)(k_{off} + k_{on}(L_0 + R_0)) + k_{on} LR^2(t) \\
&:= \alpha - \beta LR + \gamma LR^2 \\
&:= F(LR).
\end{aligned}$$

Such LR, *the amount of complex of ligand-receptors and* $LR^* \in \mathbb{R}_+$, *a constant.*

$$\begin{aligned}
(LR - LR^*)(F(LR) - F(LR^*)) &= (LR - LR^*)(-\beta(LR - LR^*) + \gamma(LR^2 - (LR^*)^2)) \\
&= -\beta(LR - LR^*)^2 + \gamma(LR^2 - (LR^*)^2)(LR - LR^*) \\
&= -\beta(LR - LR^*)^2 + \gamma(LR - LR^*)^2(LR + LR^*) \\
&= (LR - LR^*)^2(-\beta + \gamma(LR + LR^*)) \\
&\leqslant (LR - LR^*)^2(-\beta + 2\gamma R_0 \wedge L_0) \\
&\leqslant (-k_{off} - k_{on}|R_0 - L_0|)(LR - LR^*)^2.
\end{aligned}$$

Finally,

$$\begin{aligned}
&|F(LR) - F(LR^*)| \leqslant (-k_{off} - k_{on}|R_0 - L_0|)|LR - LR^*| \\
\Longleftrightarrow &|\dot{LR} - \dot{LR^*}| \leqslant (-k_{off} - k_{on}|R_0 - L_0|)|LR - LR^*|.
\end{aligned}$$

Thanks to the Grönwall lemma, $\forall t \in \mathbb{R}_+$,

$$|LR(t) - LR^*| \leqslant |LR(0) - LR^*|e^{-Kt}, \text{ with } K = k_{off} + k_{on}|R_0 - L_0| > 0$$

and $\lim_{t\to+\infty} LR(t) = LR^*$. *Finally, cAMP admits a finite limit:*

$$\begin{aligned}
cAMP(t) &= k^+ \int_0^t LR(s)e^{-k^-(t-s)}ds \\
&= k^+ \int_0^t (LR(s) - LR^* + LR^*)e^{-k^-(t-s)}ds \\
&= k^+ \int_0^t (LR(s) - LR^*)e^{-k^-(t-s)}ds + k^+LR^* \int_0^t e^{-k^-(t-s)}ds \\
&\leqslant k^+ \int_0^t |LR(0) - LR^*|e^{-Ks-k^-(t-s)}ds + k^+LR^* \int_0^t e^{-k^-(t-s)}ds \\
&\leqslant k^+|LR(0) - LR^*|e^{-k^-t} \int_0^t e^{-(K-k^-)s}ds + k^+LR^* \int_0^t e^{-k^-(t-s)}ds \\
&\leqslant k^+|LR(0) - LR^*|e^{-k^-t}\frac{1}{K-k^-}\left(1 - e^{-(K-k^-)t}\right) + \frac{k^+}{k^-}LR^*\left(1 - e^{-k^-t}\right) \\
&\leqslant k^+|LR(0) - LR^*|\frac{1}{K-k^-}\left(e^{-k^-t} - e^{-Kt}\right) + \frac{k^+}{k^-}LR^*\left(1 - e^{-k^-t}\right).
\end{aligned}$$

To conclude,

$$\lim_{t\to+\infty} cAMP(t) = \frac{k^+LR^*}{k^-} = cAMP^*.$$

The $cAMP$ convergence isn't explicit when networks are more complicated and take account into internalization or recycling of quantities.

III.1 Two compartment models

In this part, we will consider a simplified mathematical model of two types of compartments where 1 corresponds to plasma membrane and 2 is for endosomes (EE and VEE) in the hope to understand the role of the traffic in the cell signaling. The complex LR is internalized and then there is an event of dissociation/association of the complex. Only receptors are recycled and ligands in endosomes are degraded. It will be possible to interpret the function of cAMP dose response with various parameters and to find an optimum in the different cases.

III.1.1 Deterministic approach

This section will focus on the two compartment model and will study how and why going from model A to E (Fig.7) but also the results for each sub-model.
The first model (Fig.7-A) isn't weakly-reversible. The CRN's theory cannot help, but this network is also written by:

$$\begin{cases}
\frac{dL_1}{dt} = -k^1_{on}L_1R_1 + k^1_{off}LR_1, \\
\frac{dR_1}{dt} = -k^1_{on}L_1R_1 + k^1_{off}LR_1 + k_{r21}R_2, \\
\frac{dLR_1}{dt} = k^1_{on}L_1R_1 - k^1_{off}LR_1 - k_{lr12}LR_1, \\
\frac{dL_2}{dt} = -k^2_{on}L_2R_2 + k^2_{off}LR_2 - k^2_{deg}L_2, \\
\frac{dR_2}{dt} = -k^2_{on}L_2R_2 + k^2_{off}LR_2 - k_{r21}R_2, \\
\frac{dLR_2}{dt} = k^2_{on}L_2R_2 - k^2_{off}LR_2 + k_{lr12}LR_1, \\
x(0) = (L_0, R_0, 0, 0, 0, 0).
\end{cases}$$

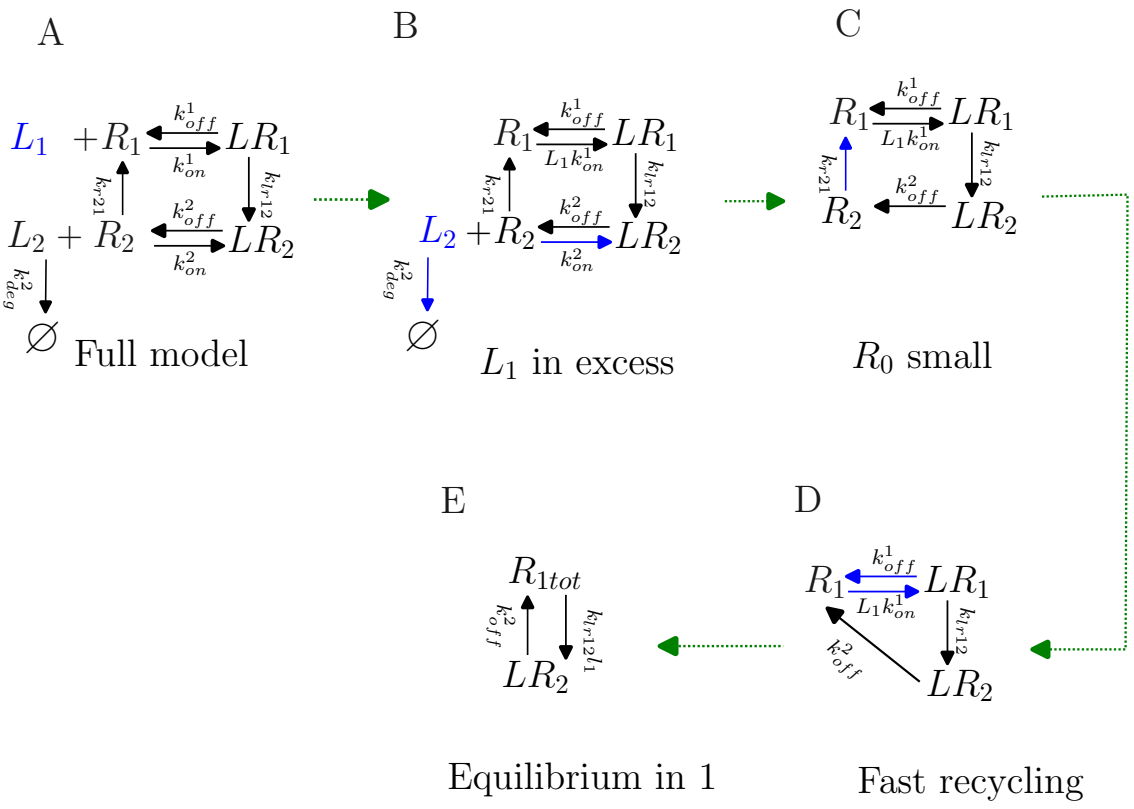

Figure 7: **Two compartment models.** 1 is for plasma membrane and 2, for endosome. (A) is the full model, in (B) the ligand is constant, in (C) the initial quantity of receptors is very small, in (D) the steps of recycling and dissociation of the complex LR are confused and finally in (E), the reactions of dissociation/association at the plasma membrane are very fast : it's the hypothesis of time scale separation.

We can show here by direct calculus that the unique stationary point is here degenerated because $L_1 = LR_1 = LR_2 = R_2 = 0$, $R_1 = R_0$. Because of this result, we made the first hypothesis that the ligand is considered in excess compared to the quantity of receptors. It allows to consider the ligand as a constant and to obtain the second model (Fig.7-B). As we can show by algebraic manipulations, a unique non-degenerate steady state exists and can be calculated explicitly:

$$\begin{cases} R_2 = \frac{k^2_{deg}K^2_D}{2}(-C + \sqrt{C^2 + 4R_0 k_{r21} k^2_{deg} K^2_D}), \\ LR_1 = \frac{k_{r21}}{k_{lr12}} R_2, \\ LR_2 = \frac{k_{r21}}{k^2_{off}} R_2 + \frac{k_{r21}}{k^2_{deg}K^2_D} R_2^2, \end{cases} \tag{15}$$

with $C = \frac{K^1_D}{k^1_{off}L_0}(1 + \frac{k^1_{off}}{k_{lr12}}) + \frac{1}{k_{lr12}} + \frac{1}{k_{r21}} + \frac{1}{k^2_{off}}$. Finally, like cAMP is produced in each compartment (Fig.2), one obtains:

$$cAMP(L_0, R_0) = \frac{k^+_1}{k^-_1}\frac{k_{r21}}{k_{lr12}} R_2 + \frac{k^+_2}{k^-_2}(\frac{k_{r21}}{k^2_{off}} R_2 + \frac{k_{r21}}{k^2_{deg}K^2_D} R_2^2),$$

and we show that this function is strictly increasing function of L_0. Unfortunately, it seems complicated to analyze the relations between species in this steady point.

If we consider that R_0 is very small, after a Taylor series approximation at order two, we can obtain different relations between species:

$$LR_1 = \frac{k_{off}^2}{k_{lr12}} LR_2,$$
$$LR_2 = \frac{k_{r21}}{k_{off}^2} R_2,$$
$$LR_1 = \frac{L_0}{K_D^1} R_1.$$

but above all,

$$LR_1 = \frac{R_0 L_0}{L_0\big[1 + k_{lr12}(\frac{1}{k_{r21}} + \frac{1}{k_{off}^2})\big] + K_D^1(\frac{k_{lr12}}{k_{off}^1} + 1)}. \tag{16}$$

This model corresponds to the steady state of the model (Fig.7-C). An analogy is possible with the result found in [10]. This last network has some others properties because it's a weakly reversible network and the deficiency is 0 ($n = 4$, $s = 3$ and $l = 1$). In fact, thanks to deficiency 0 theorem, there is a unique stationary point in each compatibility class and this stationary point is asymptotically stable (Theorem.1). In addition, this stationary point is globally stable (Theorem.2). However, it could be possible to study the matrix like this model is linear. Nevertheless, to obtain the global convergence with the study of the matrix, we must calculate the eigenvalues which might be complicated. Then, one can obtain an expressive formula to cAMP dose response which is strictly increasing in function of L_0:

$$cAMP(L_0, R_0) = (\frac{k_1^+}{k_1^-} + \frac{k_2^+}{k_2^-}\frac{k_{lr12}}{k_{off}^2})\frac{L_0 R_0}{L_0\big[1 + k_{lr12}(\frac{1}{k_{r21}} + \frac{1}{k_{off}^2})\big] + K_D^1(1 + \frac{k_{lr12}}{k_{off}^1})}. \tag{17}$$

Remark 7. *Equations* (16) *and* (17) *are interpretive expressions which have to be compared with:*

$$LR_1 = \frac{L_0 R_0}{L_0 + K_D^1} \text{ and, } cAMP = \frac{k_1^+}{k_1^-}\frac{L_0 R_0}{L_0 + K_D^1}. \tag{18}$$

These expressions are obtained when there is just one compartment and more preciously just reactions defined in the model dissociation/association (Fig.3).

The understanding of the influence of various parameters like internalization, recycling or the dissociation of the complex in the endosome (respectively k_{lr12}, k_{r21} and k_{off}^2) is possible with (17). The existence of optimum values is demonstrated to obtain the maximum quantity of cAMP and noted $cAMP^*$. We will note $f = cAMP(L_0, R_0)$. Moreover, if the ratio of the production and degradation are equal ($\frac{k_2^+}{k_2^-} = \frac{k_1^+}{k_1^-}$) at endosome and at the plasma membrane, the rates will be noted $\frac{k^+}{k^-}$ and conditions are a bit simplified.

A) Internalization

- If, $\frac{k_2^+}{k_2^-}\frac{L_0+K_D^1}{k_{off}^2} > \frac{k_1^+}{k_1^-}(\frac{L_0}{k_{off}^2} + \frac{K_D^1}{k_{off}^1})$ and if, $k_{r21} > \frac{k_1^+}{k_1^-}\frac{L_0}{\frac{k_2^+}{k_2^-}\frac{L_0+K_D^1}{k_{off}^2} - \frac{k_1^+}{k_1^-}(\frac{L_0}{k_{off}^2} + \frac{K_D^1}{k_{off}^1})} = S_{rec}$, f is strictly increasing function of k_{lr21} and the maximum is when $k_{lr12} \longrightarrow +\infty$,

$$cAMP^* = \frac{k_2^+}{k_2^-}\frac{L_0 R_0}{k_{off}^2}\frac{1}{L_0(\frac{1}{k_{r21}} + \frac{1}{k_{off}^2}) + \frac{K_D^1}{k_{off}^1}},$$

- else, f is strictly decreasing function of k_{lr21} and the maximum is when $k_{lr12} \longrightarrow 0$,

$$cAMP^* = \frac{k_1^+}{k_1^-}\frac{L_0 R_0}{K_D^1 + L_0}.$$

- If rates of cAMP are equal, if $k^2_{off} < k^1_{off}$, and $k_{r21} > \frac{L_0}{K^1_D(\frac{1}{k^2_{off}} - \frac{1}{k^1_{off}})} = S_{rec}$, f is strictly increasing function of k_{lr21} and the maximum value is when $k_{lr12} \longrightarrow +\infty$,

$$cAMP^* = \frac{k^+}{k^-}\frac{L_0R_0}{k^2_{off}}\frac{1}{L_0(\frac{1}{k_{r21}} + \frac{1}{k^2_{off}}) + \frac{K^1_D}{k^1_{off}}}, \text{ and if not,}$$

f is strictly decreasing function of k_{lr21} and the maximum value is when $k_{lr12} \longrightarrow 0$,

$$cAMP^* = \frac{k^+}{k^-}\frac{L_0R_0}{K^1_D + L_0}.$$

B) Recycling

The function is strictly increasing function of k_{r21} and the maximum is when $k_{r21} \longrightarrow +\infty$,

$$cAMP^* = (\frac{k^+_1}{k^-_1} + \frac{k^+_2}{k^-_2}\frac{k_{lr12}}{k^2_{off}})\frac{L_0R_0}{L_0\big[1 + k_{lr12}(\frac{1}{k^2_{off}})\big] + K^1_D(1 + \frac{k_{lr12}}{k^1_{off}})}.$$

C) Dissociation of complex LR in the endosome

- If $\frac{k^+_1}{k^-_1} > \frac{k^+_2}{k^-_2}\frac{L_0 + K^1_D(\frac{k_{lr12}}{k^1_{off}} + 1)}{L_0}$, and if $k_{r21} > \frac{k^+_2}{k^-_2}\frac{L_0k_{lr12}}{\frac{k^+_1}{k^-_1}L_0 - \frac{k^+_2}{k^-_2}(L_0 + K^1_D(\frac{k_{lr12}}{k^1_{off}} + 1))} = S_{rec}$, f is strictly increasing function of k^2_{off} and the maximum value is when $k^2_{off} \longrightarrow +\infty$,

$$cAMP^* = \frac{k^+_1}{k^-_1}\frac{L_0R_0}{L_0(1 + \frac{k_{lr12}}{k_{r21}}) + K^1_D(\frac{k_{lr12}}{k^1_{off}} + 1)},$$

- else, f is strictly decreasing function of k^2_{off} and the maximum value is when $k^2_{off} \longrightarrow 0$,

$$cAMP^* = \frac{k^+_2}{k^-_2}R_0.$$

- In addition, if rates of cAMP are equal, f is strictly decreasing function of k^2_{off} and the maximum value is $k^2_{off} \longrightarrow 0$,

$$cAMP^* = \frac{k^+}{k^-}R_0.$$

D) A fix ratio between recycling and internalization rates ($k_{r21} = Kk_{lr12}$, with $K \in \mathbb{R}_+$ and we note, $x := k_{lr12} = \frac{k_{r21}}{K}$)

- If $\frac{k^+_1}{k^-_1} > \frac{k^+_2}{k^-_2}\frac{L_0 + K^1_D}{L_0 + \frac{K^1_D k^2_{off}}{k^1_{off}}}$, and if $K > \frac{L_0}{k^2_{off}}\frac{k^+_2}{k^-_2}\frac{1}{\frac{k^+_1}{k^-_1}(\frac{L_0}{k^2_{off}} + \frac{K^1_D}{k^1_{off}}) - \frac{k^+_2}{k^-_2}\frac{L_0 + K^1_D}{k^2_{off}}} = S_K$, f is strictly decreasing function of x and the maximum value is when $x \to 0$,

$$cAMP^* = \frac{k^+_1}{k^-_1}\frac{L_0R_0}{L_0(1 + \frac{1}{K}) + K^1_D}.$$

- else, f is strictly increasing function of x and the maximum value is when $x \to +\infty$,

$$cAMP^* = \frac{k^+_2}{k^-_2}\frac{L_0R_0}{L_0 + \frac{K^1_D k^2_{off}}{k^1_{off}}}.$$

- If the rates of cAMP are equal, if $k^1_{off} < k^2_{off}$, and if $K > \frac{L_0}{\frac{K^1_D}{k^1_{off}} - \frac{K^1_D}{k^2_{off}}} = S_K$, f is strictly decreasing in function of x and the maximum value is $x \longrightarrow 0$,

$$cAMP^* = \frac{k^+}{k^-} \frac{L_0 R_0}{L_0(1 + \frac{1}{K}) + K^1_D},$$

and if not, f is strictly increasing function of x the maximum value is $x \longrightarrow +\infty$,

$$cAMP^* = \frac{k^+}{k^-} \frac{L_0 R_0}{L_0 + \frac{K^1_D k^2_{off}}{k^1_{off}}}.$$

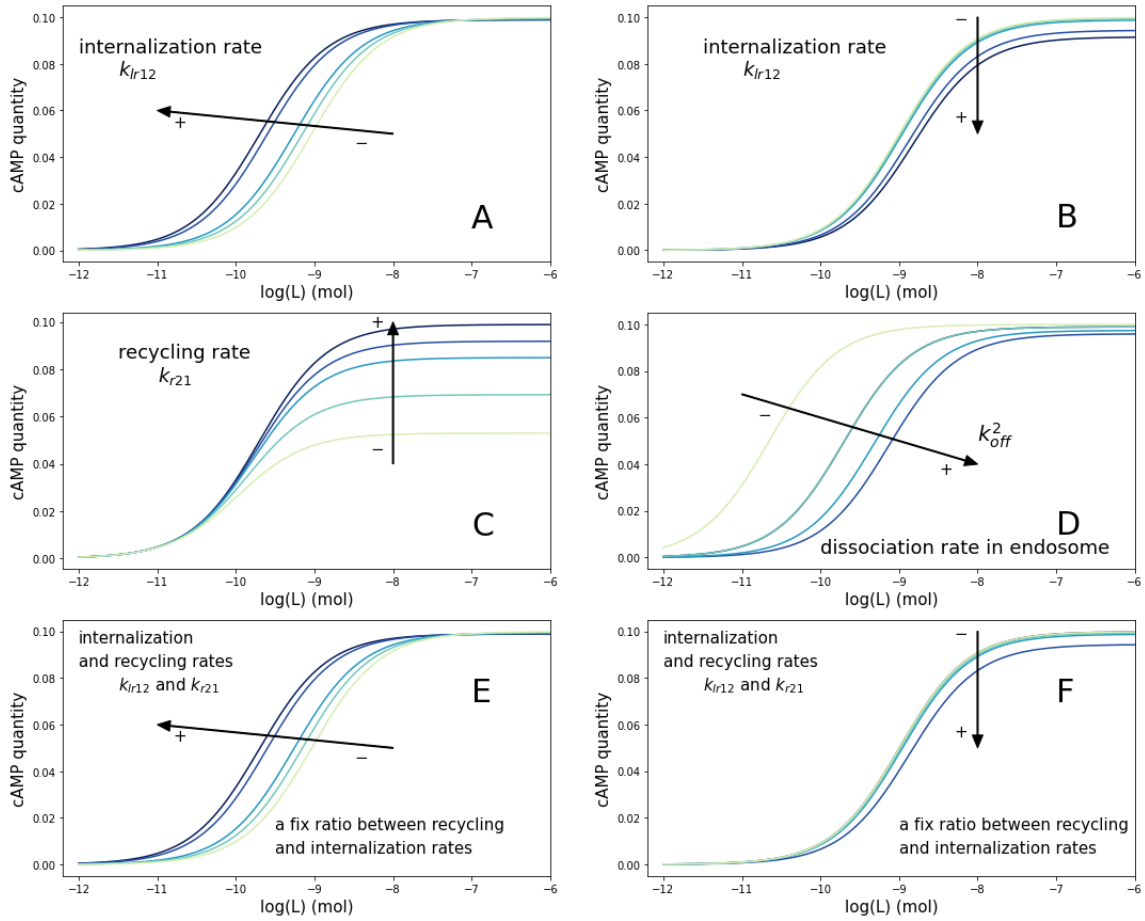

Figure 8: **Influence of parameters when production and degradation rates are equal in both compartments to focus on the effect of the others parameters.** (A): $k^2_{off} < k^1_{off}$ and $k_{r21} > S_{rec}$, f is strictly increasing function k_{lr12}; (B): $k^2_{off} > k^1_{off}$ and f is strictly decreasing function k_{lr12}; (C): f is strictly increasing function k_{r21}; (D): f is strictly increasing function k^2_{off}; (E): $k^1_{off} > k^2_{off}$, f is strictly increasing function k_{lr12} with $k_{r21} = K k_{lr12}$; (F): $k^1_{off} < k^2_{off}$ and $K > S_K$, f is strictly decreasing function x with $x = k_{lr12} = \frac{k_{r21}}{K}$.

Remark 8. *These optimums could permit to show that according to the different parameters the extreme value of parameters isn't the same to obtain the maximal cAMP quantity. For example, in*

the first case (A) when rates of cAMP are equal, if $k^2_{off} < k^1_{off}$, in function of the value of recycling, the maximum cAMP obtained is different and not for the same extreme value of internalization. In one case, the maximum is obtained when internalization rate is going to 0 whereas in the second case, the maximum is reached when internalization rate is increasing. If the complex LR_1 is more stable than LR_2 ($k^1_{off} < k^2_{off}$), the maximum of cAMP is reached with no internalization (and LR_2 is zero and LR_1 maximized). However, if LR_2 is more stable, and if recycling is fast enough, the maximum of cAMP is reached with infinite rate of internalization (and LR_1 is zero and LR_2 maximized). It is interesting to note the asymmetry of the conditions that come from the asymmetry of the reaction network at plasma membrane and at the endosome. Interestingly, the cAMP is always higher with higher recycling rate, as this tends to minimize the non cAMP-productive state R_2.

Biologists think that the dissociation in the endosomes (k^2_{off}) is slower than the dissociation in the plasma membrane (k^1_{off}). If biologists might be able to have some idea of the value of recycling parameters, this result could be used to determine if it's better to increase internalization or decrease internalization to achieve the maximal quantity of cAMP. In addition, available biological experiences (Appendix-A.Fig.16) seems to be in favor of behavior (A).

Moreover, when we consider that the dissociation of the complex and recycling are confused, one can obtain the model (Fig.7-D). In this model, the parameter k^2_{off} represents both recycling and dissociation. The system can be written as:

$$\begin{pmatrix} \dot{LR_1} \\ \dot{LR_2} \end{pmatrix} = \begin{pmatrix} -(k^1_{off} + k_{lr12} + k^1_{on}L_0) & -k^1_{on}L_0 \\ k_{lr12} & -k^2_{off} \end{pmatrix} \begin{pmatrix} LR_1 \\ LR_2 \end{pmatrix} + \begin{pmatrix} k^1_{on}L_0R_0 \\ 0 \end{pmatrix},$$

with $A = \begin{pmatrix} -(k^1_{off} + k_{lr12} + k^1_{on}L_0) & -k^1_{on}L_0 \\ k_{lr12} & -k^2_{off} \end{pmatrix}$. Finally, $det(A) > 0$ and $tr(A) < 0$, the real part of both eigenvalues are negatives. In addition, eigenvalues aren't real only if:

$$k^2_{off} = k^1_{off} + k_{lr12} + k^1_{on}L_0. \tag{19}$$

However, it seems to be a degenerative point because it's the only values of parameters for which the eigenvalues are complex. It appears when incoming flows are equal to outgoing flows.
The stationary point is:

$$\begin{cases} LR_1 = \frac{R_0L_0}{L_0(1+\frac{k_{lr12}}{k^2_{off}})+K^1_D(\frac{k_{lr12}}{k^1_{off}}+1)}, \\ LR_2 = \frac{k_{lr12}}{k^2_{off}}LR_1, \end{cases}$$

and the cAMP function is:

$$cAMP(L_0, R_0) = (\frac{k^+_1}{k^-_1} + \frac{k^+_2}{k^-_2}\frac{k_{lr12}}{k^2_{off}})\frac{R_0L_0}{L_0(1+\frac{k_{lr12}}{k^2_{off}}) + K^1_D(\frac{k_{lr12}}{k^1_{off}}+1)}. \tag{20}$$

The last model (Fig.7-E) is obtained thanks to the hypothesis of time scale separation. Like there is mass conservation, the explicit solution for LR_2 is:

$$LR_2(t) = \frac{k_{lr12}}{k^2_{off}}\frac{L_0R_0}{L_0(\frac{k_{lr12}}{k^2_{off}}+1) + K^1_D}(1 - e^{-t(k_{lr12}\frac{L_0R_0}{K_D{}^{1+L_0}} + k^2_{off})}).$$

As $LR_1 = \frac{k^2_{off}}{k_{lr12}}LR_2$, the explicit solution for cAMP is:

$$cAMP(L_0, R_0, t) = (\frac{k^+_1}{k^-_1} + \frac{k^+_2}{k^-_2}\frac{k_{lr12}}{k^2_{off}})\frac{L_0R_0}{K^1_D + L_0(1+\frac{k_{lr12}}{k^2_{off}})}(1 - e^{-t(k_{lr12}\frac{L_0R_0}{K_D{}^{1+L_0}} + k^2_{off})}). \tag{21}$$

From different hypotheses, the initial model was simplified to permit its analysis. All assumptions start from a biological idea and like models were not fitted with biological data, it's impossible to say

if a model is better than another. In fact, from a biological point of view, it's not necessarily the full model (Fig.7-A) which has the best interest. One interest of the reduction of the models is to decrease the number of parameters of the system.

III.1.2 Stochastic approach

A current limitation of the reaction network approach is that it doesn't take into account individual behaviours of intracellular vesicles, in particular their dynamic and intermittent behaviour, nor the fact that each individual vesicle induce a compartmentalized pool of molecules, separate from the bulk. In fact, individual-based models have a greater modeling flexibility and allow to represent more complex phenomenon, but the price to pay is the lack of tools to study analytically their behavior. Only a few models were interested in the dynamic of endosomes with a individual based model. Foret et al [30] looked about this evolution using a PDE based approach: they consider that endosomes have their own characteristics in time, size or chemical properties with a partial differential equations approach. Endosomes might undergo different events such creation, degradation, coagulation and fragmentation. However, this method is expensive to simulate when there are lots of structured variables (e.g. different molecules species or chemical properties). However, if there are lots of individuals which belong to the same type, a stochastic formalism is not the most appropriate. We expect numerical simulation to be faster than PDEs approach, especially when the number of endosomes (individuals) stay moderate. There's a compromise to find between the number of individuals and the number of the types of the species. In order to take into account the dynamic of each compartment, a stochastic approach has been considered thanks to a piecewise deterministic approach [31], [34] because the number of vesicles is not too large. The estimation of the cAMP production in each vesicle is possible (Fig.9) but also the number of endosomes at each time. The PDMP model (Appendix C-Fig.17) for two types of compartments is considered in which different events are possible : a creation of a new endosome, with the internalization of a certain quantity of LR from plasma membrane or the recycling of an existing endosome to the plasma membrane. To keep the mass conservation, the quantity of receptors and complex is recycled to the plasma membrane. To sum up, there are two states possible with different chemical reactions possible: the plasma membrane and the endosomes. However, there is a certain number of endosome which evolved in function on time and this evolution depends on the events number of recycling or internalization. In fact, when there is an internalization's event the number of endosomes increases whereas if an endosome is recycled, it decreases.

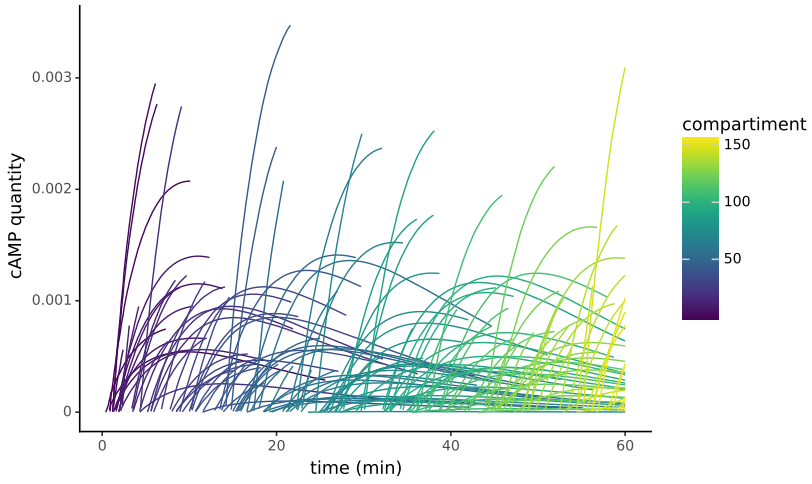

Figure 9: **cAMP quantity for each endosome.** One advantage of the stochastic model is to follow the individual dynamic of compartments. Each endosome produces its own cAMP quantity.

In order to simulate this model, an algorithm based on the next reaction method is used. In this method, each event has its own clock for its next occurence. For example, each endosome has its own time of recycling.The algorithm moves forward in time up to the smallest time of potential event. Iteratively, the algorithm tests whether or not the smallest time actually corresponds to an event, using the reject sampling that we detailed in the following algorithm (Algorithm.2). In function of this test, the status changes and a new potential occurence time is assigned for this event. The occurence time follows an exponential random law whose parameter is given by the upper bound of the rate function (Algorithm.2). This algorithm permits to determine the number of endosomes in function of time but also the cAMP total quantity (Fig.10). The creation of a new compartment depends of the quantity of ligand-receptor complex at the plasma membrane whereas the recycling of an endosome depends on the quantity of free receptors in the endosome. To boost the creation of a new compartment, one possibility is to raise the association rate. The quantity of complex will increase, and therefore, so will the number of internalization. To boost the recycling of an endosome, one possibility is to raise the dissociation rate and to decrease the association rate. In fact, in this case, the quantity of receptors is increasing and more endosomes are recycled. Moreover, the rates of internalization and recycling can also play a role: they influence at the next time of jump of each event. If they increase, the interval between two times of jumps is smaller. For example, if the recycling rate is decreasing, there are no more endosomes which are recycled.

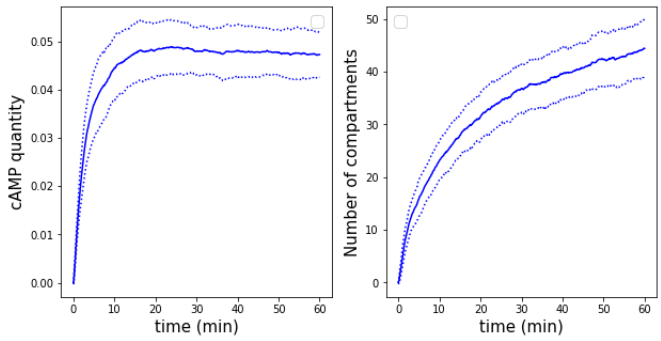

Figure 10: **PDMP simulations.**This figure is an average of 100 realisations of the algorithm. The dotted lines represent the standard deviation. The parameters of production and degradation of cAMP are equal at the plasma membrane and in endosomes. (A) represents the cAMP quantity total according to time and (B) represents the number of endosomes.

Remark 9. *From the modeling point of view, the choice of internalization and recycling functions is a work in progress. As a first choice, functions are in this case linear according to the quantity of complex LR_1 and an internalization rate for the internalization event and according to the quantity of receptors in endosome R_2 and a recycling rate for the recycling event. However, others functions could be chosen as saturation functions, functions which take into account more molecules,... Moreover, this system considers there is a law of conservation for the total number of receptors and that when there is recycling, the time of events depends only on receptors but receptors and complex are recycled to the plasma membrane. The modelling of this system has multiple possibilities.*

III.2 Three compartment models

To model more precisely, the biological system of the internalization and recycling of receptors (Fig.1), taking into account three types of compartments is necessary. In the future models, the plasma membrane is noted 1, Very Early Endosome is 2 and Early Endosome is 3. As the exact process of the mechanism of recycling or internalization of receptors is subject to many unknown, different models

Algorithm 2 Next reaction Method for the stochastic model with two types of compartments (Appendix-C.Fig.17).

- L_0 and R_0: initial quantities of ligand and receptors;
- $x_1^0 = (L_0, R_0, 0, 0)$;
- f_{inter}: the function associated to internalization with $f_{inter} = k_{inter} L R_1$;
- H_{inter} with $\forall x \in (\mathbb{R}_+)^4, f_{inter}(x) \leqslant H_{inter}$;
- f_{rec}: the function associated to recycling with $f_{rec} = k_{rec} R_2$;
- H_{rec} with $\forall x \in (\mathbb{R}_+)^4, f_{rec}(x) \leqslant H_{rec}$;
- ODE_i: the ODE's associated to the plasma membrane ($i = 1$) and the endosome ($i = 2$);
- S_1: the time of the internalization and S_2^l: the time of the recycling of the endosomes l;
- t_{max}: the final time of the reaction;
- t_1: the initial time of the reaction;
- k: the number of compartments at each time;
- j: the label attributed to each compartment ($j = 0$ is for plasma membrane).

$k = 0$;
$t_2 = 0$;
$S_1 \sim \mathcal{E}(H_{inter})$ and $S_2^l \sim \mathcal{E}(H_{rec})$ for all endosomes present at initial time.

while $t_2 < t_{max}$ **do**:

- $Z \leftarrow t_1 + vect(S_1, S_2), \quad t_2 \leftarrow min(Z), \quad i \leftarrow index[min(Z)]$;
- $x_1(t) = ODE_1(x_1^0, t_1, t_2)$ and $x_2^l(t) = ODE_2(x_2^{0,l}, t_1, t_2)$ with l all endosomes present between $[t_1, t_2]$;
- $x_1^0 \leftarrow x_1(t_2)$ and $x_2^{0,l} \leftarrow x_2^l(t_2)$ with l all endosomes present between $[t_1, t_2]$;
- Generate U, uniformly distributed between 0 and 1;
- $t_1 \leftarrow t_2$

if $i = 0$ **then**:
if $U \leqslant \frac{f_{inter}(x_1^0)}{H_{inter}}$ **then**:

- $k \leftarrow k + 1, \quad j \leftarrow j + 1$;
- $\alpha \sim \mathcal{N}(0, 0.1)$ and $\alpha \in [0, 1]$;
- Creation of a new endosome j of initial conditions : $x_2^{0,j} = (0, 0, \alpha L R_1, 0)$ and $x_1^0 = x_1^0 - x_2^{0,j}$;
- $S_1 \sim \mathcal{E}(H_{inter})$ and $S_2^j \sim \mathcal{E}(H_{rec})$;

else $S_1 \sim \mathcal{E}(H_{inter})$;
end if
else $i = j$ with j the label of the endosome which can be recycled;
if $U \leqslant \frac{f_{rec}(x_2^{0,j})}{H_{rec}}$ **then**:

- $k \leftarrow k - 1$;
- $x_1^0[R] \leftarrow x_1^0[R] + x_2^{0,j}[R] + x_2^{0,j}[LR]$;
- Delete $x_2^{0,j}$ and t_2;

else $S_2^j \sim \mathcal{E}(H_{rec})$
end if
end if
end while.

with three types of compartments will be presented. The models differ especially in function of which quantities are recycled. In fact, it is above all this property for which biologists are unsure. We develop five types of models. Moreover, for each model, we consider two cases: when the ligand concentration varies according to time (Case 1 : Fig.3-A) and when the ligand is considered in excess and is approximated by a constant in each compartment (Case 2: Fig.3-B) or not. We finally consider, for certain models, a further reduction when we separate the faster (ligand-receptor association-dissociation) and slower (traffic) reactions to obtain an interpretive CRN. We will call the reduced model: the quasi-steady state model, which is common terminology in the reaction network field. Let us introduce the

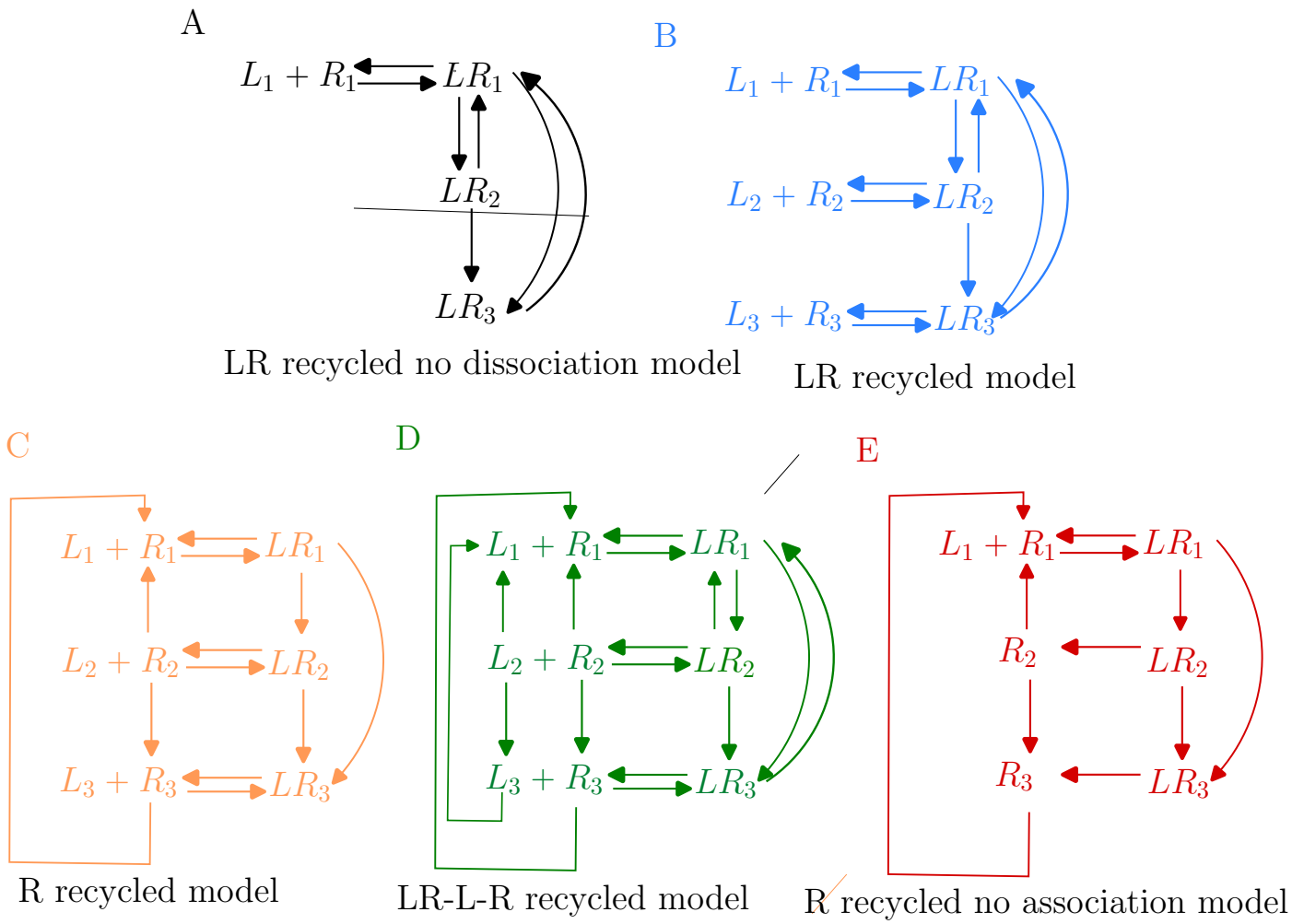

Figure 11: **Three compartment models.** LR recycled no dissociation model (A), LR recycled model (B), R recycled model (C), LR-L-R recycled model (D) and R recycled no association model (E).

different models (Fig.11) which consider all that $cAMP$ is produced and degraded as presented above (Fig.2).

- **LR recycled no dissociation model (A)**: There is no dissociation of the LR complexes in intracellular compartments. The quantity of ligands and receptors in these compartments are insignificant compared to the quantity at the membrane and just LR complexes are recycled.
- **LR recycled model (B)**: There is dissociation of the LR complexes in all compartments and the LR complexes are recycled.

- **R recycled model (C)**: This model is maybe the most realistic from a biological perspective. The LR complexes are internalized, dissociated in ligand and receptors and only receptors are recycled. The reaction of association is possible.
- **LR-L-R recycled model (D)**: All species are recycled and can go from a compartment to another, except the ligand and receptors from plasma membrane to intracellular compartments.
- **R recycled no association model (E)**: This model doesn't consider about the ligand in the compartments as suppose some biologists. The LR complexes are internalized, dissociated and these receptors are recycled.

III.2.1 Traffic model

A simplified model (Fig.6) with three compartments C_1, C_2 and C_3 is looking to illustrate the routing of compartments. This network can be written as:

$$\begin{cases} \frac{dC_1}{dt} = -(k_{12}+k_{13})C_1 + k_{21}C_2 + k_{31}C_3, \\ \frac{dC_2}{dt} = k_{12}C_1 - (k_{21}+k_{23})C_2, \\ \frac{dC_3}{dt} = k_{13}C_1 + k_{23}C_2 - k_{31}C_3. \end{cases}$$

The stationary point and cAMP function are:

$$\begin{cases} C_1 = \frac{C_0}{1+a+b}, \\ C_2 = \frac{aC_0}{1+a+b}, \\ C_3 = \frac{bC_0}{1+a+b}, \end{cases} \quad \text{and, } cAMP(C_0) = (\frac{k_1^+}{k_1^-} + a\frac{k_2^+}{k_2^-} + b\frac{k_3^+}{k_3^-})\frac{C_0}{1+a+b}, \tag{22}$$

with C_0 the initial condition ($C_0 = C_1(0)+C_2(0)+C_3(0)$), $a = \frac{k_{12}}{k_{21}+k_{23}}$ and $b = \frac{1}{k_{31}}(\frac{k_{23}k_{12}}{k_{21}+k_{23}}+k_{13})$. This function is strictly increasing function of C_0. The key parameters a and b can informally understood as "$a = \frac{\text{input in 2}}{\text{output of 2}}$" and "$b = \frac{\text{input in 3 by 2 + input in 3 by 1}}{\text{output of 3}}$", which sum up the traffic of compartments. We will find these two parameters in many following models at the equilibrium.

III.2.2 LR recycled no dissociation model

This section is in reference to (Fig.11-A). There is no dissociation of the LR complexes in intracellular compartments and LR complexes are recycled.

• **Case 1:** At first, one obtains different properties.

-*CRN study:* Thanks to the deficiency 0 theorem [20], each compatibility class admits one asymptotically locally stable. Moreover, the stationary point is globally stable because there is just one linkage class [17].

-*Stationary point and cAMP function:*

$$\begin{cases} LR_1 = (\frac{(L_0-R_0)(1+a+b)-K_D+\sqrt{\Delta}}{(1+a+b)})(\frac{R_0}{(L_0-R_0)(1+a+b)+K_D+\sqrt{\Delta}}), \\ LR_2 = aLR_1, \\ LR_3 = bLR_1, \end{cases}$$

$$\text{and, } cAMP(L_0,R_0) = (\frac{k_1^+}{k_1^-} + a\frac{k_2^+}{k_2^-} + b\frac{k_3^+}{k_3^-})\frac{R_0}{(1+a+b)}\frac{(L_0-R_0)(1+a+b)-K_D+\sqrt{\Delta}}{(L_0-R_0)(1+a+b)+K_D+\sqrt{\Delta}}, \tag{23}$$

with $a = \frac{k_{lr12}}{(k_{lr21}+k_{lr23})}$, $b = \frac{1}{k_{lr31}}(k_{lr13} + \frac{k_{lr23}k_{lr12}}{k_{lr21}+k_{lr23}})$ and,
$\Delta = ((R_0-L_0)(1+a+b)+K_D)^2 + 4(1+a+b)L_0K_D$. In addition, this function is strictly increasing function of L_0.

• **Case 2:** Moreover, if the ligand is considered in excess at the plasma membrane, the cAMP function has a simplified expression.

-*CRN study:* Thanks to the deficiency 0 theorem [20], each compatibility class admits one asymptotically locally stable stationary point. Moreover, the stationary point is globally stable because there is just one linkage class [17].
-*Stationary point and cAMP function:*

$$\begin{cases} LR_1 = \frac{R_0 L_0}{K_D 1 + L_0(1+a+b)}, \\ LR_2 = \frac{a R_0 L_0}{K_D 1 + L_0(1+a+b)}, \\ LR_3 = \frac{b R_0 L_0}{K_D 1 + L_0(1+a+b)}, \end{cases} \quad cAMP(L_0, R_0) = (\frac{k_1^+}{k_1^-} + a\frac{k_2^+}{k_2^-} + b\frac{k_3^+}{k_3^-})\frac{R_0 L_0}{K_D + L_0(1+a+b)}, \tag{24}$$

with $a = \frac{k_{lr12}}{(k_{lr21}+k_{lr23})}$ and $b = \frac{1}{k_{lr31}}(k_{lr13} + \frac{k_{lr23}k_{lr12}}{k_{lr21}+k_{lr23}})$. In addition, the function is strictly increasing function of L_0.

III.2.3 LR recycled model

The LR recycled model (Fig.11-B) considers the dissociation of the LR complexes in all compartments and the recycling of LR complexes. Thanks to the deficiency 0 theorem, each compatibility class admits one asymptotically locally stable stationary point. Moreover, the stationary point is globally stable because there is just one linkage class [17].

III.2.4 R recycled model

In the R recycled model, (Fig.11-C), just receptors are recycled to the plasma membrane.
• **Case 1:**
-*CRN study:* In this model, the deficiency 0 theorem isn't applicable because the systems aren't weakly-reversible.
-*Stationary point :* $LR_1 = LR_2 = LR_3 = R_2 = R_3 = 0$ and $R_1 = R_0$ The ODE system is degenerated and admits an equilibrium point which is located on the edges of the system. This result is obtained because the ligand at the plasma membrane isn't recycled and isn't degraded. In order to study the cAMP dose response at the steady state, this model isn't applicable in our case.
• **Case 2:** If the ligand is considered in excess in all compartments, the model has some nice properties. In this case, it seems interesting to apply the time scale hypothesis and to look a model with association-dissociation reactions (Fig.12-A) and a quasi steady-state model (Fig.12-B) because they haven't exactly the same steady state. It permits to compare the cAMP dose response with the time scale hypothesis. The function l_i and p_i are defined in (13) and can be used as general kinetics.
-*CRN study:* In the model with association-dissociation reactions, the stationary point is globally stable because there is just one linkage class and kinetics are law mass action and in the quasi steady-state model, the stationary point is asymptotically locally stable thanks to the deficiency 0 theorem.
-*Stationary point and cAMP function for the quasi steady-state model:*

$$\begin{cases} LR_1 = \frac{R_0 L_0}{K_D^1 + L_0(1+a+b)}, \\ LR_2 = \frac{L_2}{K_D^2 + L_2} a \frac{R_0 L_0}{K_D^1 + L_0(1+a+b)}, \\ LR_3 = \frac{L_3}{K_D^3 + L_3} b \frac{R_0 L_0}{K_D^1 + L_0(1+a+b)}, \end{cases}$$

$$cAMP(L_0, R_0) = (\frac{k_1^+}{k_1^-} + a\frac{k_2^+}{k_2^-}\frac{L_2}{K_D^2 + L_2} + b\frac{k_3^+}{k_3^-}\frac{L_3}{K_D^3 + L_3})\frac{R_0 L_0}{K_D^1 + L_0(1+a+b)}, \tag{25}$$

with, $a = \frac{k_{lr12}}{k_{lr23}\frac{L_2}{K_D^2+L_2} + (k_{r21}+k_{r23})\frac{K_D^2}{K_D^2+L_2}}$ and $b = \frac{K_D^3+L_3}{k_{r31}K_D^3}\big[k_{lr13} + a(\frac{L_2}{K_D^2+L_2}k_{lr23} + k_{r23}\frac{K_D^2}{K_D^2+L_2})\big]$. This function is a strictly increasing function of L_0.
-*Stationary point and cAMP function for the model with association-dissociation reactions*:

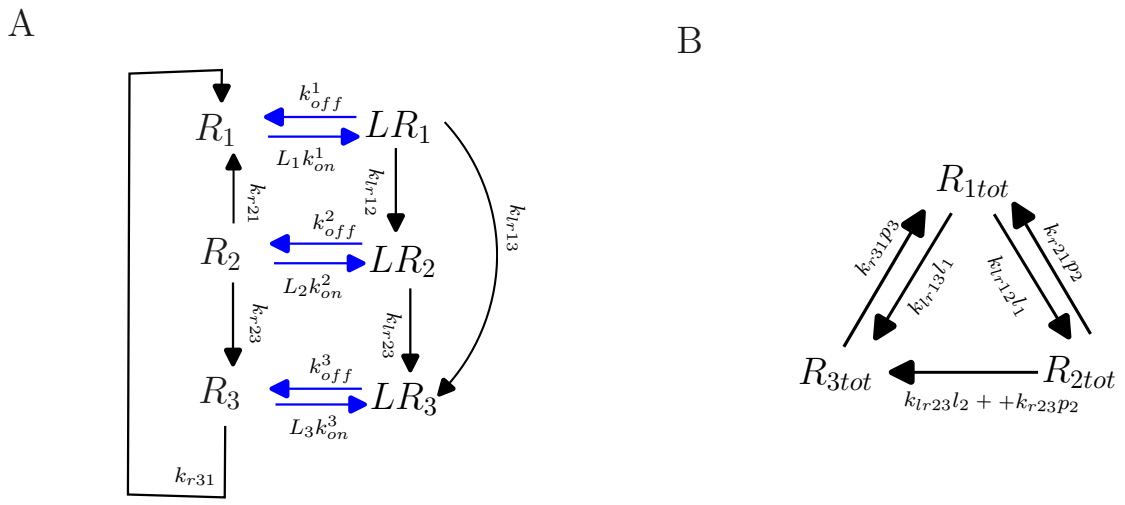

Figure 12: **R recycled model with ligand in excess in all compartments (Case 2).** The model with association-dissociation reactions (A) and the quasi steady-state model (B).

$$\begin{cases} LR_1 = a\frac{R_0L_0}{K_D^1+L_0k}, \\ LR_2 = bc_1\frac{R_0L_0}{K_D^1+L_0k}, \\ LR_3 = c\frac{R_0L_0}{K_D^1+L_0k}, \end{cases}$$

$$cAMP(L_0,R_0) = (\frac{k_1^+}{k_1^-}a + \frac{k_2^+}{k_2^-}cc_1 + \frac{k_3^+}{k_3^-}c)\frac{R_0L_0}{K_D1+L_0k},$$

with $k = a + c_1(1+b) + c_2 + c$, $a = \frac{k^1_{off}}{k_{lr12}+k_{lr13}+k^1_{off}}$, $b = \frac{L_2}{K_D^2} + \frac{k_{r21}+k_{r23}}{k_{off^2}}$,
$c_1 = \frac{k_{lr12}a}{b(k^2_{off}+k_lr23)-k^2_{on}L_2}$, $c_2 = k^1_{off}(1-a-\frac{k_r21}{k^1_{off}}c_1)$ and $c = c_2(\frac{L_3}{K_D^3} + \frac{k_{r31}}{k^3_{off}}) - k_{r23}c_1$. In addition, this function is a strictly increasing function of L_0.

III.2.5 LR-L-R recycled model

The LR-L-R recycled model considers that all quantities are recycled to the plasma membrane (Fig.11-D). The complete model doesn't say much because it isn't weakly reversible and calculus is complicated. However, if the flows are equals : $k_{ik} = k_{lrik} = k_{lik} = k_{rik}$, this model presented in (Fig.13) is obtained and it's possible to calculate the steady state.

• **Case 1:**

-*CRN study:* There are no direct theorems linked with the CRN theory to prove the existence and uniqueness of one stationary point in the initial and quasi steady-state model. However, by the Jacobian Matrix, the stationary point is asymptotically locally stable for the quasi steady-state model. The function f_i, g_i and h_i are defined in (11) and can be used as general kinetics (proof in Appendix B).

-Stationary point and cAMP function for the quasi steady-state model:

$$\begin{cases} LR_1 = (\frac{(L_0-R_0)(1+a+b)-K_D+\sqrt{\Delta}}{(1+a+b)})(\frac{R_0}{(L_0-R_0)(1+a+b)+K_D+\sqrt{\Delta}}), \\ LR_2 = \frac{1}{4K_D^2}(-1+\sqrt{1+\frac{4aLR_1}{K_D^2}})^2, \\ LR_3 = \frac{1}{4K_D^3}(-1+\sqrt{1+\frac{4bLR_1}{K_D^3}})^2, \end{cases}$$

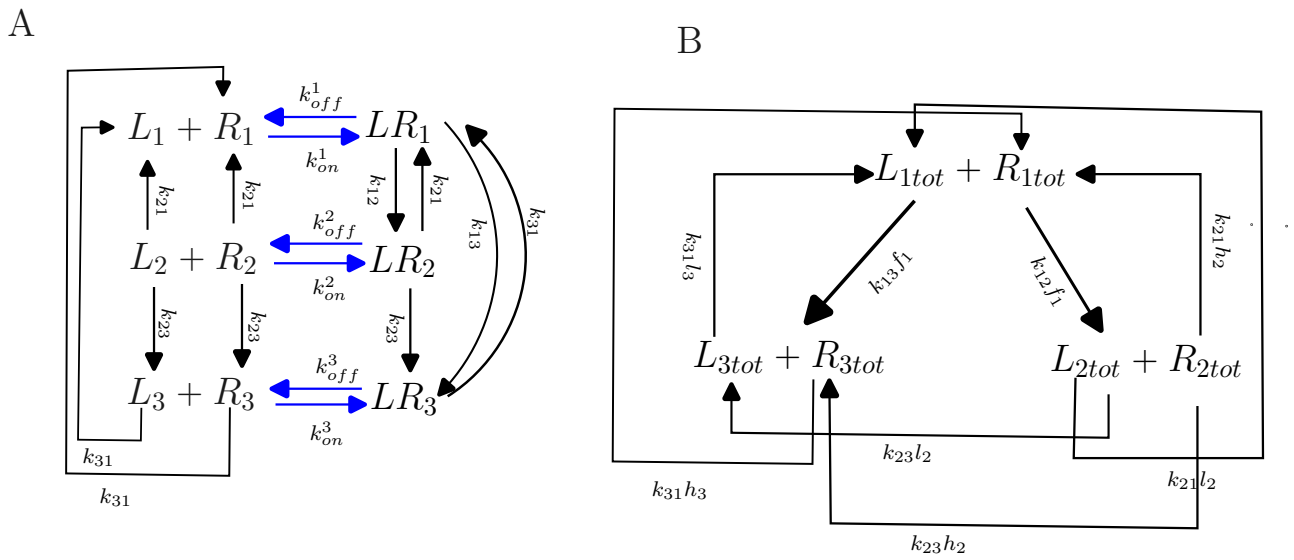

Figure 13: **LR-L-R recycled model with time-dependent ligand concentration (Case 1).** The model with association-dissociation reactions (A) and the quasi steady-state model (B) with equal flows.

$$cAMP(L_0, R_0) = LR_1\frac{k_1^+}{k_1^-} + \frac{k_2^+}{k_2^-}LR_2 + \frac{k_3^+}{k_3^-}LR_3, \tag{26}$$

with $a = \frac{k_{12}}{(k_{21}+k_{23})}$, $b = \frac{1}{k_{31}}(k_{13} + \frac{k_{23}k_{12}}{k_{21}+k_{23}})$ and $\Delta = ((R_0 - L_0)(1 + a + b) + K_D)^2 + 4(1 + a + b)L_0K_D$. In addition, the function is strictly increasing function of L_0.

Remark 10. *We found the same stationary point for LR_1 that the LR recycled no dissociation model (Fig.11-A).*

• **Case 2:** the ligand is considered in excess and two models are studied: the model with association-dissociation reactions (Fig.14-A) and the quasi steady-state model (Fig.14-B).
-*CRN study:* In the model with association-dissociation reactions, the stationary point is globally stable because there is just one linkage class and kinetics are law mass action and in the quasi steady-state model, the stationary point is asymptotically locally stable thanks to the deficiency 0 theorem.
-*Stationary point and cAMP function for quasi steady-state model:*

$$\begin{cases} LR_1 = \frac{R_0L_0}{K_D^1 + L_0(1+a+b)}, \\ LR_2 = \frac{L_2}{K_D^2+L_2}\frac{aR_0L_0}{K_D^1+L_0(1+a+b)}, \\ LR_3 = \frac{L_3}{K_D^3+L_3}\frac{bR_0L_0}{K_D^1+L_0(1+a+b)}, \end{cases}$$

$$cAMP(L_0, R_0) = \frac{R_0L_0}{K_D^1 + L_0(1 + a + b)}(\frac{k_1^+}{k_1^-} + a\frac{k_2^+}{k_2^-}\frac{L_2}{K_D^2 + L_2} + b\frac{k_3^+}{k_3^-}\frac{L_3}{K_D^3 + L_3}), \tag{27}$$

with $a = \frac{k_{lr12}}{(k_{lr23}+k_{lr21})\frac{L_2}{K_D^2+L_2} + (k_{r23}+k_{r21})\frac{K_D^2}{K_D^2+L_2}}$ and,
$b = (\frac{1}{k_{lr31}\frac{L_3}{K_D^3+L_3} + k_{r31}\frac{K_D^3}{K_D^3+L_3}})(k_{lr13} + \frac{k_{lr12}}{(k_{lr23}+k_{lr21})\frac{L_2}{K_D^2+L_2} + (k_{r23}+k_{r21})\frac{K_D^2}{K_D^2+L_2}})$. This function is strictly increasing function of L_0.

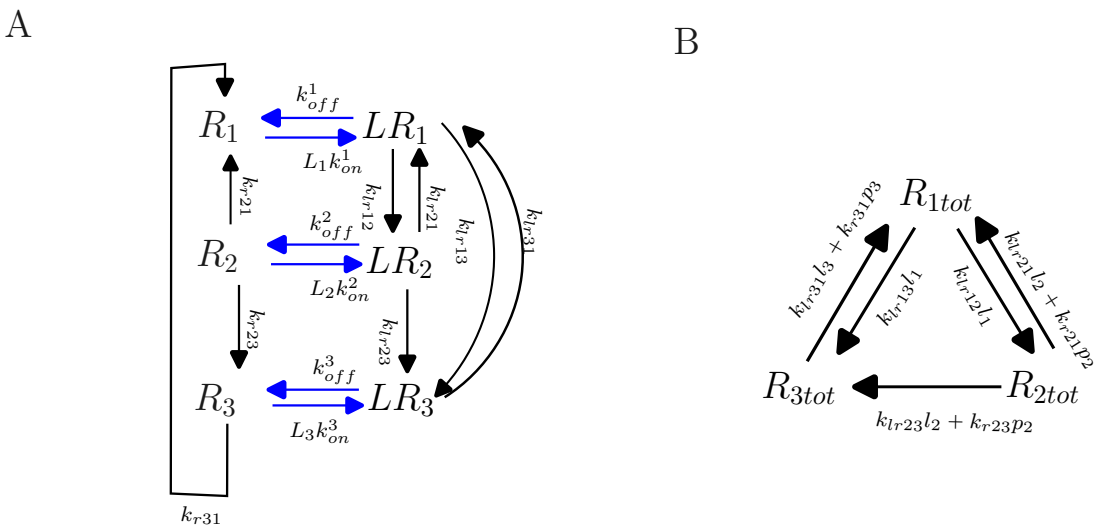

Figure 14: **LR-L-R recycled with ligand in excess in all compartments (Case 2)**. The model with association-dissociation reactions (A) and the quasi steady-state model (B).

III.2.6 R recycled no association model

The R recycled no association model (Fig.11-E) is equivalent to the model with two compartments (Fig.7-C). If the ligand isn't a constant, the stationary point is degenerated, but if we consider ligand in excess, the model has nice properties (Case 2).
-*CRN study:* Thanks to the deficiency 0 theorem [20], each compatibility class admits one asymptotically locally stable. Moreover, the stationary point is globally stable because there is just one linkage class [17].
-*Stationary point and cAMP function:*

$$\begin{cases} LR_1 = \frac{R_0L_0}{K^1_D(1+\frac{k_{lr12}}{k^1_{off}})+L_0\Big[1+\frac{k_{lr12}}{k^2_{off}+k_{lr23}}(1+\frac{k^2_{off}}{k_{r21}+k_{r23}}+\frac{k_{lr23}}{k^3_{off}}+\frac{1}{k_{r31}}(k_{lr23}+\frac{k_{r23}k^2_{off}}{k_{r21}+k_{r31}}))\Big]}, \\ LR_2 = \frac{k_{lr12}}{k^2_{off}+k_{lr23}}LR_1, \\ LR_3 = \frac{k_{lr23}k_{lr12}}{k^3_{off}(k^2_{off}+k_{lr23})}LR_1, \end{cases}$$

$$cAMP(L_0,R_0) = \frac{R_0L_0(\frac{k^+_1}{k^-_1}+\frac{k_{lr12}}{k^2_{off}+k_{lr23}}\frac{k^+_2}{k^-_2}+\frac{k_{lr23}k_{lr12}}{k^3_{off}(k^2_{off}+k_{lr23})}\frac{k^+_3}{k^-_3})}{K^1_D(1+\frac{k_{lr12}}{k^1_{off}})+L_0\Big[1+\frac{k_{lr12}}{k^2_{off}+k_{lr23}}(1+\frac{k^2_{off}}{k_{r21}+k_{r23}}+\frac{k_{lr23}}{k^3_{off}}+\frac{1}{k_{r31}}(k_{lr23}+\frac{k_{r23}k^2_{off}}{k_{r21}+k_{r31}}))\Big]}. \quad (28)$$

In addition, the function is strictly increasing depends on L_0.

Thanks to these various models, one obtains some interpretive formulas to compare with the expression when there is just one compartment (18). The higher differences between models are what quantities are recycled: receptors, complex, all quantities and the association/dissociation of complexes. However, the best model from a biological point of view seems to be the last one (Fig.11-E: R Recycled no association model), when only receptors are recycled and when there is no more association in endosomes. In this case, the interpretive formula looks like (16). Nevertheless, analytic expressions are complicated to interpret as there are several parameters. The cAMP functions are all

strictly increasing function of L_0 but not necessarily of the others parameters. Models could be fitting with biological data even if the dose response will not allow us to discriminate the different models. The study of the dynamic properties might be interesting to select models.

IV Conclusion

The routing of endosome seems to influence intracellular response by the production of cAMP. The two different dynamic approaches, deterministic or with a random formalism permitted the study of this problem in two ways. Each approach has its own limits and advantages. The first approach is called structured or compartmental because it considers a globalization of quantities in the different types of compartments. This method follows the general behaviour of a dynamic population along the time through chemical reaction systems. Moreover, in this case, the analysis of the system is possible thanks Chemical Reaction Network theory. This approach permitted the development of many networks about the routing of endosomes for gonadotrophin receptors which lead to the study of their long time behavior, their steady states and their stability. Some analytical expressions are deduced from this analysis and can be compared to biological problems. In fact, the effect of parameters was studied and reveals that the signalling response dynamics might be influenced in distinct opposite ways by the receptor trafficking according to the ligand-receptor interaction parameters. However, to develop these models different assumptions were advanced which could be discussed. In fact, considering that the ligand is in excess in all types of compartments doesn't seem realistic and is complicated to verify by biological experiences. The different networks aren't comparable because they don't look exactly the same quantities and the same transitions between compartments. The current limitation of this formalism is that it doesn't consider the specific behaviour of variables. The individual-based model with a random formalism takes into account the evolution of each individual. This approach is maybe closer of the biological problem because this method considers that the size of a new endosome but also the events of creation or degradation of these vesicles are random. Finally, the piecewise deterministic Markov process gives an idea of the number of compartments in function of time whereas this number isn't accessible in the first approach.

The first limit of this work is that models weren't calibrated with biological data. In fact, like biologists are developing intracellular sensors, they are not yet capable to address these sensors to VEE and EE and to obtain precisely cAMP quantities produced in each compartment. The perspective of this work is finally to calibrate mathematical models with biological data with a statistical approach of parameter estimation. Such calibration may help to rule out or to favour some of the models we developed, and would help gaining biological knowledge on the exact molecular trafficking involved in signalling cascade. All models consider the mass conservation for receptors to obtain analyzable networks and interpretive expressions. However, from a biological point of view, a quantity of receptors is degraded. An improved model with an additional compartment (lysosome) may be developed. In addition, to get an individual-based model with three compartments, an algorithm in this case could be written. Studying in a long time, stochastic processes is also conceivable. The comparison of the ODE and PDMP models (Fig.15) may be not relevant. To compare them, an intermediate step should be considered: a moment closure step. This theory permits thanks to moment of order one (mean) or order two (variance) to obtain a reduce set of ODEs, easier to analyze and to compare to ODEs coming from reaction networks. It makes the average of the stochastic approach and allows the comparison between equations from moment closure and from deterministic models. Then, the analysis of the moment closure equations could be done. In addition, the interpretation of the parameters in the three compartment models might be possible but maybe with long and complex calculations.

Moreover, this mathematical model is simplified for signalling cascade. To be more precise, it could be interesting to take a deeper look after the mechanisms of activation of the G-protein [23], the β-arrestin role [24] but also to take into account other molecules of the cascade (ATP, AC, PDE,...). The

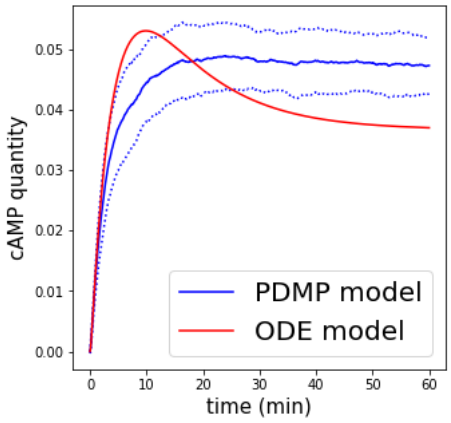

Figure 15: **Comparison of the cAMP quantity total for both approaches.** In blue, the PDMP model and in red, the ODE model.

conformation of receptors and properties of ligands can also be a way of study: active or non-actives receptors [25], dimmers receptors [6], promiscuous between proteins [32], inverse agonisms [26] and biaised-agonisms [27]. Moreover, some receptors can have a constitute activity and the synthesis of new receptors is also possible. Endosomes have also properties like the pH in function of their maturation. It could be a parameter to consider to discriminate compartments [29]. Endosomes can also product reactions together (fusion, fission, degradation,..) and by considering it, a model to follow the number of endosomes which contained a number of molecules [30] was created. Another interesting molecule is APPL1 because biologists showed that this molecule is necessary for VEE recycling [11]. Other mathematical modelling could be envisaged by considering more compartments: lysosomes, nucleus, Golgi apparatus [3] or the spatial dynamic of cAMP. In fact, cAMP can move inside the cell.

This internship brought me a lot of skills in mathematics because I learnt new mathematical theories (CRN, PDMP, moment closure) and I have deepened my knowledge of modelling. Many discussions with biologists gave me an opening on this domain and the desire to continue in biological application. Like biologists were in the same team, it was really interesting to discuss with them to consider real biological problems and then to think about modelling. I learnt how to translate biological problem in mathematics. The research world is fascinating and possibilities are plentiful. It's difficult to stop because we could always go further. Particularly, I learnt to structure my research. Moreover, during my internship, I assisted to several presentations in mathematics but also in biology and I had the opportunity to present my work in front of the BIOS team but also in front of the MUSCA team at INRIA Saclay. I improved my English through presentations and discussions.

Bibliography

[1] F. Jean-Alphonse et al. (2014). Spatially Restricted G Protein-coupled Receptor Activity via Divergent Endocytic Compartments.*Journal of Biological Chemistry*, 289 (7): 3960-3977.

[2] C. Alamichel et al. (2023). Modeling compartmentalization within intracellular signaling pathway. hal-04098543

[3] S. E. Crilly et al (2021). Compartmentalized GPCR Signaling from Intracellular Membranes. *The Journal of Membrane Biology*, 254 (3): 259-271.

[4] J.C. Weddell and P.I. Imoukhuede. (2017). Integrative meta-modeling identifies endocytic vesicles, late endosome and the nucleus as the cellular compartments primarily directing RTK signaling. *Integrative Biology*, 9(5): 464-484.

[5] S.R.J. Hoare et al. (2020). Analyzing kinetic signaling data for G-protein-coupled receptors. *Scientific Reports*, 10(1): 12263.

[6] C. White and L.J. Bridge. (2017). Ligand Binding Dynamics for Pre-dimerised G Protein-Coupled Receptor Homodimers: Linear Models and Analytical Solutions. *Bulletin of Mathematical Biology*, 81(9): 3542-3574.

[7] J.P. Vilardaga et al. (2014). Endosomal generation of cAMP in GPCR signaling. *Nature Chemical Biology*, 10(9): 700–706.

[8] A.D. White et al. (2021). Spatial bias in cAMP generation determines biological responses to PTH type 1 receptor activation. *Science Signaling*, 14(703): eabc5944.

[9] J. Leelawattanachai et al. (2009). Modeling and genetic algorithm optimization of early events in signal transduction via dynamics of G-protein-coupled receptors: Internalization consideration. *Applied Mathematics and Computation*, 207(2): 528-544.

[10] M. R. Birtwistle and B. N. Kholodenko. (2009). Endocytosis and signalling: A meeting with mathematics. *Molecular Oncology*, 3(4): 308-320.

[11] S. Sposini et al. (2017). Integration of GPCR Signaling and Sorting from Very Early Endosomes via Opposing APPL1 Mechanisms. *Cell Reports*, 21(10): 2855-2867.

[12] S. R.J. Hoare et al. (2018). Kinetic operational models of agonism for G-protein-coupled receptors. *Journal of Theoretical Biology*, 446: 168-204.

[13] M. Feinberg. Foundations of Chemical Reaction Network Theory. (2019) *Applied Mathematical Sciences*. 202.

[14] M.H.A. Davis. (1984). Piecewise-deterministic markov processes: A general class of non-diffusion stochastic models. *Journal of the Royal Statistical Society, Series B (Methodological)*, 46(3): 353–388.

[15] A. Lasota et al. (1992). The statistical dynamics of recurrent biological events. *Journal of Mathematical Biology*, 30(8): 775–800.

[16] M. Banaji and C. Pantea. (2016). Some results on injectivity and multistationarity in chemical reaction networks. *SIAM Journal on Applied Dynamical Systems*, 15(2): 807-869.

[17] D.F. Anderson. (2011). A Proof of the Global Attractor Conjecture in the Single Linkage Class Case. *SIAM Journal on Applied Mathematics*, 71 (4): 1487-1508.

[18] E. Benoit. (2008). Notes sur les techniques d'étude des champs lents-rapides.

[19] H.G. Othmer and C.H. Lee. (2008). A multi-time-scale analysis of chemical reaction networks: I. Deterministic systems. *Journal of Mathematical Biology*, 60 (3): 387-450.

[20] M.Feinberg. (1979). Lectures on Chemical Reaction Networks.

[21] P. Donnell and M. Banaji. (2013). Local and Global Stability of Equilibria for a Class of Chemical Reaction Networks. *SIAM Journal on Applied Dynamical Systems*, 12 (2): 899-920.

[22] P.A.W Lewis and G.S Shedler. (1979). Simulation of non-homogeneous Poisson processes by thinning. *Naval Research Logistics Quarterly*, 26 (3): 403-413.

[23] C.Y. Chen. (2003). Modelling of signalling via G-protein coupled receptors: pathway-dependent agonist potency and efficacy. *Bulletin of Mathematical Biology*, 65(5): 933-958.

[24] K. Eichel and M. von Zastrow. (2018). Subcellular Organization of GPCR Signaling. *Trends in Pharmacological Sciences*, 39 (2): 200-208.

[25] J. Leelawattanachai et al. (2009). Modeling and genetic algorithm optimization of early events in signal transduction via dynamics of G-protein-coupled receptors: Internalization consideration. *Applied Mathematics and Computation*, 207(2): 528-544.
[26] L.J.Bridge. (2010). Modeling and Simulation of Inverse Agonism Dynamics. *Methods in Enzymology*, Volume 485.
[27] L.J. Bridge et al. (2018). Modelling and simulation of biased agonism dynamics at a G protein-coupled receptor. *Journal of Theoretical Biology*, 442: 44-65.
[28] R. Yvinec. Piecewise deterministic Markov processes, applications in biology. 3rd cycle. Minicours chercheur (Laboratoire Jacques Louis-Lions, Université Paris VI), 2015, 24 p.hal-02795547.
[29] M. Castro et al. (2021). Fusion and fission events regulate endosome maturation and viral escape. *Scientific Reports*, 11(1): 7845.
[30] L. Foret et al. (2014). Theory of cargo and membrane trafficking.
[31] M. Voliotis et al.(2016). Stochastic Simulation of Biomolecular Networks in Dynamic Environments. *PLOS Computational Biology*, 12(6): e1004923.
[32] H.E. Klumpe et al. (2023). The computational capabilities of many-to-many protein interaction networks. *Cell Systems*, 14(6): 430-446.
[33] J.W. Black and P.Leff. (1983). Operational models of pharmacological agonism. *Proceedings of the Royal Society of London*, B(220): 141-162.
[34] L. Duso and C. Zechner. (2020). Stochastic reaction networks in dynamic compartment populations. *Proceedings of the National Academy of Sciences*, 117 (37): 22674-22683.

A Biological figures

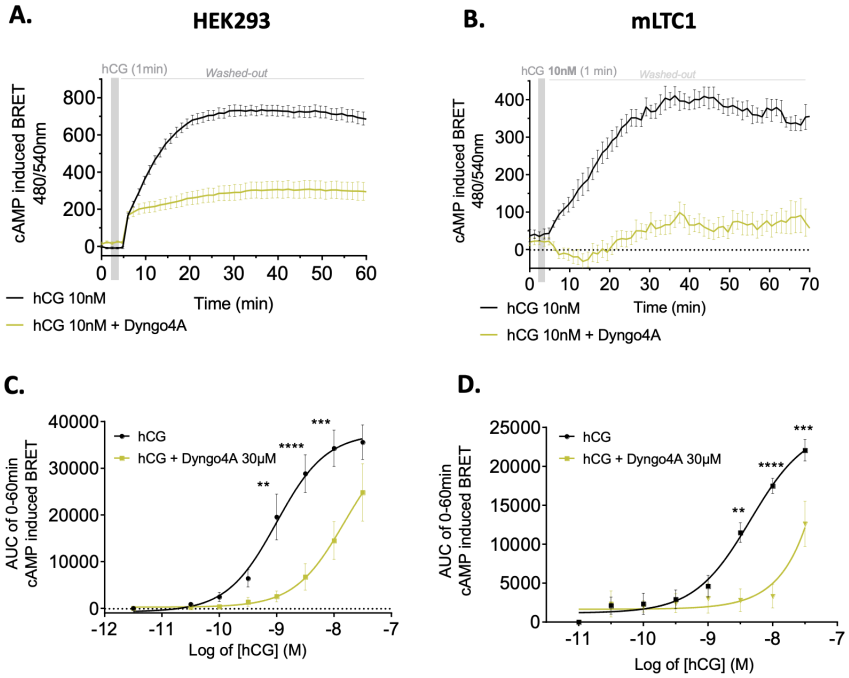

Figure 16: **[Confidential] The intracellular production of cAMP represents a major part of the production of cAMP total.** These figures were realised by Juliette Gourdon, in the PhD in BIOS team under the supervision of Frédéric Jean-Alphonse. The aim of these figures is to show the influence of internalization and to compare cAMP production with internalization or not. Dyngo4A inhibits the internalization. (A) and (C) represent responses in HEK293 cells whereas (B) et (D) represent biological responses in mLTC1 cells. (A) and (B) represent the production and degradation of cAMP according to time with Dyngo4A (in yellow) or not (in dark). (C) and (D) represent the dose response of cAMP obtained with the area under the curve with Dyngo4A (in yellow) or not (in dark).

B General kinetics

This section will prove that the functions f, g, h, l and p can be used for general kinetics. However, we detail only the proof for f because it's the same principle for the others.

Demonstration . $\forall t \in \mathbb{R}_+$,

1. *f is defined and C^1 on $\mathbb{R}_+$.*
2. *$f = 0 \iff R_{tot} = 0$ or $L_{tot} = 0$.*

$$R_{tot} = 0 \rightarrow f = L_{tot} + K_D - \sqrt{(-L_{tot} + K_D)^2 + 4K_D L_{tot}} = 0,$$
$$L_{tot} = 0 \rightarrow f = R_{tot}(t) + K_D - \sqrt{(R_{tot}(t) + K_D)^2} = 0.$$

3. f is strictly positive if $L_{tot} \neq 0$ and $R_{tot} \neq 0$.

$$
\begin{aligned}
f > 0 \Longleftrightarrow & \frac{1}{2}(L_{tot} + R_{tot} + K_D - \sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}) > 0 \\
\Longleftrightarrow & L_{tot} + R_{tot} + K_D > \sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}} \\
\Longleftrightarrow & (L_{tot} + R_{tot} + K_D) > \sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}. \\
& \textit{If, } L_{tot} + R_{tot} + K_D > 0 \textit{ , we obtain :} \\
\Longleftrightarrow & (L_{tot} + R_{tot} + K_D)^2 > (R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot} \\
\Longleftrightarrow & (2L_{tot}(2R_{tot} + 2K_D) > 4K_D L_{tot} \\
\Longleftrightarrow & 4L_{tot} R_{tot} > 0 \textit{ that is always true if } L_{tot} \neq 0 \textit{ and } R_{tot} \neq 0.
\end{aligned}
$$

4. $\frac{\partial f}{\partial L_{tot}} \geqslant 0$ and $\frac{\partial f}{\partial R_{tot}} \geqslant 0$.

- $\frac{\partial f}{\partial L_{tot}} = \frac{1}{2}(1 - \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot})}} \geqslant 0$ *?*
  - ***Case 1:*** $L_{tot} - R_{tot} + K_D \geqslant 0$,

$$
\begin{aligned}
& \frac{\partial f}{\partial L_{tot}} \geqslant 0 \\
\Longleftrightarrow & 1 - \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot})}} \geqslant 0 \\
\Longleftrightarrow & \sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}} \geqslant L_{tot} - R_{tot} + K_D \\
\Longleftrightarrow & (R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot} \geqslant (L_{tot} - R_{tot} + K_D)^2 \\
\Longleftrightarrow & (2R_{tot} - 2L_{tot})(2K_D) + 4K_D L_{tot} \geqslant 0 \\
\Longleftrightarrow & 4K_D R_{tot} \geqslant 0 \textit{ that is always true because } R_{tot} \geqslant 0.
\end{aligned}
$$

  - ***Case 2:*** $L_{tot} - R_{tot} + K_D \leqslant 0$,

$$
\begin{aligned}
& L_{tot} - R_{tot} + K_D \leqslant 0 \\
\Longleftrightarrow & \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}} \leqslant 0 \\
\Longleftrightarrow & 1 - \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}} \geqslant 1 \\
\Longleftrightarrow & \frac{1}{2}(1 - \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot})}}) \geqslant \frac{1}{2} > 0.
\end{aligned}
$$

- $\frac{\partial f}{\partial L_{tot}} = \frac{1}{2}(1 - \frac{L_{tot} - R_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot})}} \geqslant 0$ *?*
  - ***Case 1:*** $R_{tot} - L_{tot} + K_D \geqslant 0$,

$$
\begin{aligned}
& \frac{1}{2}(1 - \frac{R_{tot} - L_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}} \geqslant 0 \\
\Longleftrightarrow & 1 \geqslant \frac{R_{tot} - L_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}} \\
\Longleftrightarrow & \sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}} \geqslant R_{tot} - L_{tot} + K_D \\
\Longleftrightarrow & (R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot} \geqslant (R_{tot} - L_{tot} + K_D)^2 \\
\Longleftrightarrow & 4K_D L_{tot} \geqslant 0 \textit{that is always true.}
\end{aligned}
$$

– ***Case 2:*** $R_{tot} - L_{tot} + K_D \leqslant 0$,

$$\begin{aligned} & R_{tot} - L_{tot} + K_D \leqslant 0 \\ \iff & \frac{R_{tot} - L_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}} \leqslant 0 \\ \iff & \frac{1}{2}(1 - \frac{R_{tot} - L_{tot} + K_D}{\sqrt{(R_{tot} - L_{tot} + K_D)^2 + 4K_D L_{tot}}}) \geqslant \frac{1}{2} > 0. \end{aligned}$$

C Piecewise Deterministic Markov Process model

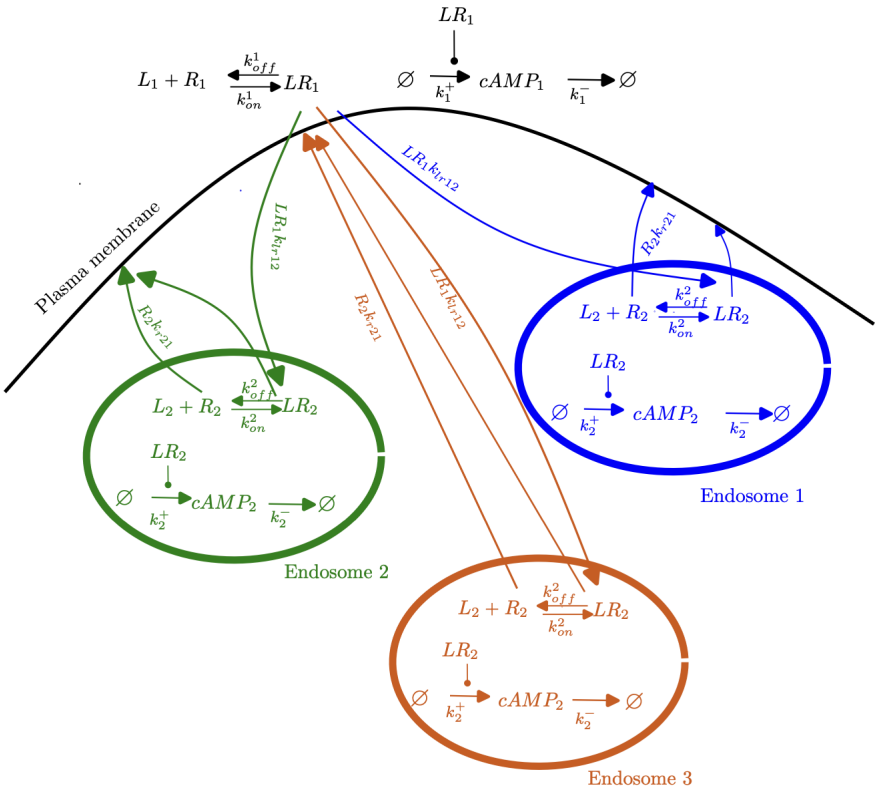

Figure 17: **PDMP model for two types of compartments.** This figure represents a schematic point of view the piecewise deterministic Markov process for this model. Recycling and internalization events have a random time which depends respectively on the quantity of receptors in each endosome and the quantity of complex at the plasma membrane. Each compartment posses its own ordinary differential equations system.