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Final Report – Summary

Aiming at identifying patterns and general laws in data coming from biological systems, we focus on three different specific biological systems and their dynamics. We expect that common mechanisms and structures will be found at different levels (single cell, tissue, complex organisms) of biological systems and that is why we work at various scales. In order to keep the big picture in mind, I find it important to address a wide range of biological questions and also not to focus on a single model organism while working in Systems Biology. My specific projects, which share similar concepts and tools, are related to each other in the sense that they all concern biological networks of interactions and their dynamics. Moreover they also have more specific connections, the microbiome is affected by circadian rhythms and oscillations can be involved in the process of cell differentiation (e.g. in angiogenesis).

Keywords: Systems Biology, mathematical modeling, cell differentiation, circadian clock, microbiome.

——— Project # 1 : Neural induction in *Ciona Instestinalis* ———

Switch-like behavior are ubiquitous in biological networks of interactions. We focused on neural induction in *Ciona Intestinalis* embryos. At the stage of 32 cells, two pairs of cell respond to an extra-cellular signal called morphogen. Preliminary experimental data obtained by our collaborator Y. Hitoyoshi suggest that the cells respond in a switch like manner to this morphogen. We constructed a mathematical model of the signaling pathway consisting in the succession of a covalent modification cycle and a MAPK cascade. By performing a large random scanning over physiological values of parameters, we showed that the switch-like behavior is coming from the ultrasensitivity in the covalent modification cycle. These results need to be confirmed by performing other independent analysis.

——— Project # 2 : Cyanobacterial Circadian Clock ———

Almost all living organisms rely on an internal, or circadian, clock to orchestrated their genome activity. We study the simplest known circadian clock, the one of cyanobacteria. Based on previous works, we adapted our model of this clock to take into account the effect of light on the clock mechanism. We constructed phase dose response curves that predict the new phase of the clock after a dark pulse applied at different time and of different intensities.

——— Project # 2 : Microbiome ———

Can we predict how the microbiome behaves over time? How does the microbiome react to perturbations (for instance to antibiotics, or to the removal or addition of different species)? Karoline Faust (KUL) initiated a project about the microbiome predictability that I joined together with Didier Gonze (ULB), Franziska Bauchinger, Stefanie Widder (Vienna University) and Leo Lahti (Wageningen University). I am focussing on deterministic dynamics and comparing/improving existing techniques. We are interested in the network structure of microbial interactions and the biological functions of the sub-structures in the network. My collaborators will also study the stochastic and self-organized critical models. Comparison of the fits obtained for each type of model on synthetic data will determine if we can detect the underlying dynamics of the system via our techniques. If we can – as we expect – we will then analyze the real data mentioned above. A toy model that might help us understanding the microbiota is the study of microbial communities that live on cheese rinds since they are cultivable in the lab. This project is still on-going.

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Final Report

1 Objectives of the proposal

For a long time I have been fascinated by man's capacity for abstraction and deduction. Through reasoning, he succeeds in deciphering the world around him and understanding it beyond what he can comprehend through his five senses. This is what led me to study Physics. During my last post-doc, I became interested in questions about living systems and connecting my theoretical work directly to experiments. Today, Biology provides us with a huge number of quantitative experiments, which opens up the possibility of addressing fundamental questions about life quantitatively. Strong analytical backgrounds are needed for that and many theoretical physicists worldwide have turned to Systems Biology.

Aiming at identifying patterns and general laws in data coming from biological systems, we focus on three different specific biological systems and their dynamics. We expect that common mechanisms and structures will be found at different levels (single cell, tissue, complex organisms) of biological systems and that is why we work at various scales. In order to keep the big picture in mind, I find it important to address a wide range of biological questions and also not to focus on a single model organism while working in Systems Biology. My specific projects, which share similar concepts and tools, are related to each other in the sense that they all concern biological networks of interactions and their dynamics. Moreover they also have more specific connections, the microbiome is affected by circadian rhythms [1] and oscillations can be involved in the process of cell differentiation (e.g. in angiogenesis [2]).

2 Results and Methodology

——— Project # 1 : Neural induction in *Ciona Instestinalis* ———

A key question in Biology is how multicellular organisms develop. In particular, during embryonic development, how cell diversity and patterns are generated is not well understood. The idea is that graded signals, called morphogens, provide the information that regulates gene expression and cell types in embryos. The extracellular concentration gradient of morphogens is supposed to establish distinct levels of signaling within cells and thereby regulates target genes in a concentration-dependent manner [36]. This model implies that cells can detect distinct thresholds in the graded field of the incoming signals, and make decision in consequence. Very little is known about how cells exhibit these threshold responses. Three mechanisms generating a switch-like response in embryonic develpment proposed in the literature have been confirmed experimentally. The first one is a switch-like response to progesterone to resume meiosis I in the *Xenopus* oocyte. It is based on a positive feedback loop in the Mos-MEK-ERK2 cascade [42]. The second mechanism is zero-order ultrasensitivity in response to EGF-ERK signals in *Drosophila* that creates sharp boundaries between Yan-positive and Yan-negative domains, which determine pattering of the embryonic ventral ectoderm [43]. The last one specifies left and right axis in vertebrate embryos via the ubiquitous BMP/Smad1 signalling pathway [44].

We focus on neural induction in ascidian embryos, which provide a good system for this type of analysis as they develop with an invariant cell lineage, much like the *C. elegans*. One can trace the evolution of each cell individually. At the 32-cell stage, ascidian neural induction begins and is initiated by FGF9/16/20, which is expressed broadly in the vegetal hemisphere [45]. At this stage, the animal hemisphere consists of 16 ectoderm cells that are all competent to respond to neural inducers [46]. However, only two pairs of cells respond to this signal and exhibit ERK1/2 activation and otx gene expression, so they are specified as neural precursors. Importantly, other ectoderm cells do not show any ERK1/2 activation or otx expression. This indicates that ascidian neural induction operates in all-or-nothing manner. Why do only two pairs of cells respond to FGF signals even though all ectoderm cells are neural-competent? A partial answer comes from the fact that among the ectoderm cells, the neural precursors have the largest area of cell contact with the FGF-expressing vegetal cells [47].

Moreover, it has been shown experimentally that an antagonistic regulation by two extracellular signals, of a protein named Ras can activate when Ras binds to guanosine triphosphate (GTP), the Raf-Mek-Erk enzymatic cascade when Ras is in its active form, i.e. when it binds to GTP. The cascade in turns acts on the expression of a neuron marker, the Otx gene. The signaling pathway is therefore build up with two possible switches: the covalent modification cycle of Ras and the downstream phosphorylation cascade of Raf-MEK-ERK, see Figure 1. Understanding where the switch-like behavior is coming from in this pathway will be addressed via experiments and a mathematical model.

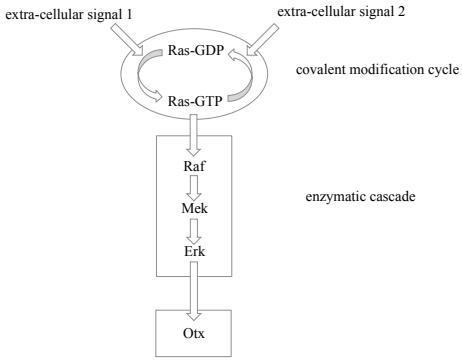

Figure 1: Schematic representation of the pathway leading to neural induction.

One cannot access directly all the protein concentrations experimentally and therefore the model will be crucial to answer that question. A simple mathematical model of this pathway can be obtained by putting together the covalent modification cycle equations (see equations [3-6] Goldbeter and Koshland, PNAS 1981) and the MapK cascade equations (see equations [1-35] Huang and Ferrell, PNAS 1996).

Experimentally, our collaborator can control the FGF signaling. We can therefore obtain a curve of ERK (or Otx) as a fonction of FGF and estimate the steepness of this curve. Preliminary experimental results show that ectopic activations of Otx are present when the ephrine signal is blocked. The level of these activations is not as high as when the ephrine is expressed, suggesting that the ERK/FGF curve is less steep in that case and that the system does not behave in a switch-like manner. We wonder if that information can help determining were in the pathway the switch-like behavior is coming from.

We randomly scanned the parameter space over physiological values, see Table 1. We kept the sets of parameters that fall into the two following categories:

- case 1: covalent modification cycle is ultrasensitive (criteria: $0.001 < \frac{k+d}{aW_{tot}} < .1$) and the mapk cascade just transmit the signal (criteria: Hill coefficient of the mapK cascade is between 0 and 2). [1]
- case 2: covalent modification cycle is not ultrasensitive and the switch-like behaviour comes from the mapk cascade.

Over 1000 sets of parameters, we got 53 sets that fit in case 1 and 29 in case 2. Then we varied the ephrine concentration for all the sets of parameters in case 1 and case 2, and plotted the Hill coefficient of the input/output curve as a fonction of the ephrine concentration, see Figure 2. We conclude that it is more likely that case 1 is the correct one, since the Hill coefficient decreases when the ephrine level decreases. We plotted also all ERK/FGF curves for case 1 and case 2 in Figure 3.

Another experimental observation is that the intensity of the ERK signaling increases when the ephrine signaling is suppressed. Could we explain that phenomena too? We plotted the maximal value of ERK reached divided by the ERK total concentration as a function of the sos level. In both case 1 and case 2, we see that the level of ERK reaches ERK total when one decreases the ephrine level.

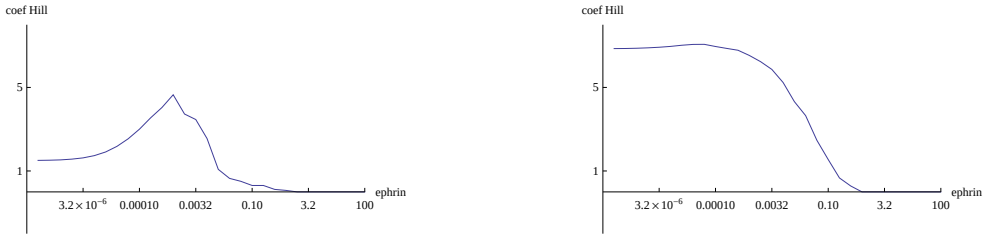

Figure 2: Hill coefficient as a function of ephrin signal. The Hill coefficient characterizes the stimulus/response curves of the ERK response as a function of the FGF stimulus (extra-cellular signal 1). Coefficients have been computed for various *fixed* values of ephrin (extra-cellular signal 2). On the left panel, the curve represents the mean obtained by averaging over parameter sets corresponding to a steep stimulus/response curve in the covalent modification cycle and mild response of the MAPK cascade, the right panel corresponds to the opposite case. Experimentally, reducing the ephrin level leads to ectopic activation of ERK response and a loss of steepness in the ERK response. We therefore expect that the switch like behavior finds its origin in the covariant modification cycle.

[1] The Hill coefficient n_H is defined by the following equation,

$$S_{0.9}/S_{0.1} = 81^{1/n_H}$$

where $S_{0.9}$ represents the input signal needed to obtain 90% of the maximal output while $S_{0.1}$ the one to get 10 %.

Parameter	Minimum value	Maximum value
WT	0,00023 μM	0,023 μM
a_{11}, a_{12}	1000 $\mu M^{-1} min^{-1}$	100000 $\mu M^{-1} min^{-1}$
$d_{11} = d_{12}$	1000 min^{-1}	100000 min^{-1}
$k_{11} = k_{12}$	0,01 min^{-1}	1 min^{-1}
mos_{tot}	0,0003 μM	0,03 μM
$e2_{tot}$	0,00003 μM	0,003 μM
mek_{tot}	0,12 μM	12 μM
$mekpase_{tot}$	0,00003 μM	0,003 μM
$mapk_{tot}$	0,12 μM	12 μM
$mapkpase_{tot}$	0,012 μM	1,2 μM
a_i $(i = 1, .., 10)$	100 $\mu M^{-1} min^{-1}$	10000 $\mu M^{-1} min^{-1}$
d_i $(i = 1, .., 10)$	15 min^{-1}	1500 min^{-1}
k_i $(i = 1, .., 10)$	15 min^{-1}	1500 min^{-1}

Table 1: Random scan of parameters between min and max values : we choose the same values as in the Ferrell paper for the mapk cascade. We relabelled the parameters a_1, d_1, k_1, a_2, d_2, k_2 of Goldbeter's paper by a_{11}, d_{11}, k_{11}, a_{12}, d_{12}, k_{12}. We also set $k_{11} = k_{12} = d_{11} = d_{12}$ in the covalent modification cycle.

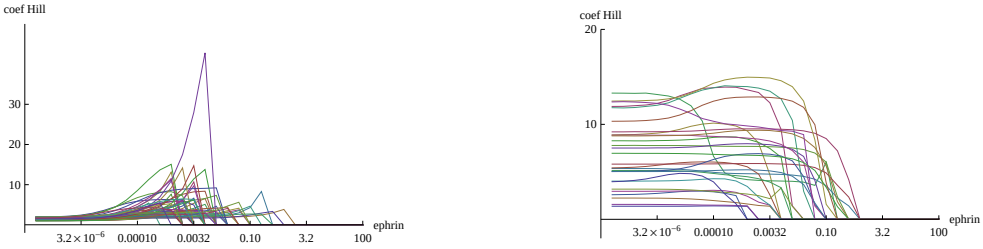

Figure 3: Hill coefficient as a function of ephrin signal, without averaging.

——— Project # 2 : Cyanobacterial Circadian Clock ———

Circadian clocks [3, 4] are endogenous oscillators that keep track of time in living organisms and allow them to synchronize their physiological activities with the time of the day. They play a role in most living organisms from bacteria to humans. They regulate temporal activities such as sleeping, feeding, regulating body temperature, producing hormones, but also cell division and other biological activities. The alternation of day and night is one of the most predictable environmental change; anticipation of these changes permit to living systems to best adapt to their environment. In humans, disruption in circadian rhythm homeostasis can lead to various health problems such as seasonal affective disorder and delayed sleep phase syndrome and is possibly directly connected to cancer. The **simplest circadian clock** is the one of **cyanobacteria** also known as blue-green algae.

A circadian clock possesses three defining properties:

* As mentioned above, they are **endogenous**. This means that circadian rhythms persist in the absence of external cues (for instance under constant light exposition).
* They are **temperature compensated**, *i.e.* they keep their circadian periodicity over a large range of temperature. This phenomenon is unexpected since the velocity of almost all chemical reactions increase with the temperature as dictated by Arrhénius law.
* They can be **entrained** by external signals, *i.e.* the rhythm of the clock can be reset by exposure to external stimuli such as light and heat.

The cyanobacteria clock consists of a core oscillator made of proteins whose concentrations oscillate with a period of about 24 hours. The clock is characterized by the amplitude, period and phase of the oscillations of the proteins. The phase is the relative positioning of the curve in reference to a specific time-point, *e.g.* the time placed under constant light. The basic mechanism behind these oscillations is a negative feedback on the production of proteins by the proteins themselves. This mechanism relies on the rhythmic activity of the genes and is called **Transcription Translation Regulation** (TTR). Cyanobacteria provide us with the simplest experimental model to study these clocks. Interestingly, for these unicellular organisms, the clock can be reproduced *in vitro* by mixing three proteins named Kai-A, Kai-B, Kai-C and Adenosine triphosphate (ATP) [5]. This oscillator is called **Post-Translational Regulation** (PTR) because it does not involve the replication machinery of DNA and RNA but biochemical interactions between proteins. For the cyanobacteria, as well as for other organisms, we have therefore two mechanisms to generate oscillations: the TTR system and the PTR system.

This leads to the natural question: "Why do we need the TTR mechanism in cyanobacteria circadian clock given the existence of the PTR oscillator?". We answered this question by showing that the TTR oscillator is necessary to stabilize the phase of the oscillators but not to maintain the oscillations [6]. More precisely, we constructed a model for the wild strain based on the *in vitro* model of [7] with the addition of degradation and production processes and a simple TTR term. We also constructed two models for the genetically modified cyanobacteria that have either the TTR or PTR circuit abrogated. Next, we compared the robustness of the different models with respect to changes in parameter space and their robustness against molecular noise [8]. The cyanobacteria clocks with

the TTR circuit abrogated can still produce sustained oscillations – but they get desynchronized faster that the oscillations of the wild strain – while the ones without PTR circuit cannot. The TTR circuit is able to buffer the circadian oscillator against stochastic fluctuations in clock components, and also against sustained changes in parameters such as the cellular growth rate and protein translation and degradation rates. We therefore concluded that the TTR oscillator is necessary to stabilize the phase of the oscillators but not to maintain the oscillations [6]. This architecture may represent a general solution used in other circadian circuits.

The clock regulatory network is summarized in schema 4.

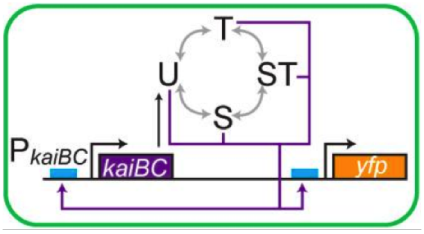

Figure 4: Schema of the cyanobacterial circadian clock model. The core protein of the clock is termed KaiC and has four phosphoforms: unphosphorylated KaiC (U), KaiC phosphorylated only at Serine residu (S) or Threonine residu (T) and finally doubly phosphorylated KaiC (ST or D). The *in vitro* mechanism is represented by grey arrows and the negative feedback regulation of KaiC on its own production in represented in purple. Figure from [6]. Light enters the clock by altering the phosphorylation rates of KaiC.

We have incorporated the effect of light and studied the effect of dark pulses applied at various times and of different intensities. We wrote a Matlab code to compute systematically the phase shift of the clock after dark pulses applied at different time and of various intensities. An example of phase dose response plot is presented in Figure 5. Next step will be to model adaptation to jet lag by computing the phase shift after change to various time zones. In particular, we will study the transient period and determine how to recover as fast as possible. Adaptation to seasons will also be investigated. We will be particularly interested in the relation between energy consumption and entrainment properties.

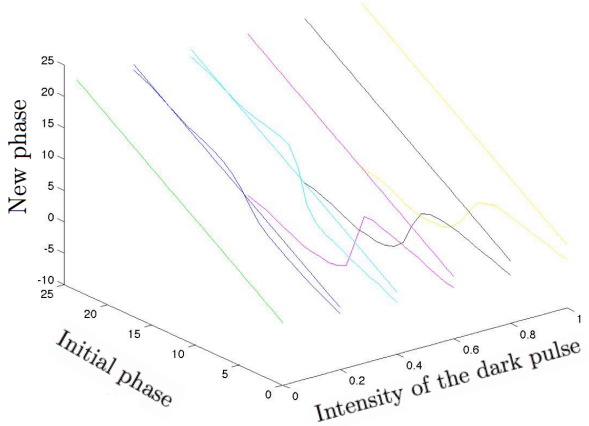

Figure 5: New phase of the cyanobacterial circadian clock after a 4 hour dark pulse applied with various intensities (reflected in the ratio of ADP over ATP), as a function of the initial phase.

——— Project # 3 : Microbiome ———

Did you know that nine cells out of ten in our bodies are not human? These cells are microbes and they work with our organism to help digestion and the immune system. They are also related to obesity [9], chronic inflammatory diseases [10], and some cancers [11]. Bacteria are the most abundant organisms on Earth, but only one percent of bacterial species can be cultured, and even less has been sequenced. Technological advances enable us today to sequence bacteria without having to culture them. The sequencing of bacteria present in our bodies opens up the possibility to understand and eventually control our microbiome (the ensemble of genes carried by the microbiota) [12].. Can we predict how the microbiome behaves over time? How does the microbiome react to perturbations (for instance to antibiotics, or to the removal or addition of different species)? What is a healthy microbiome? Karoline Faust (KUL) initiated a project about the microbiome predictability that I joined together with Didier Gonze (ULB), Franziska Bauchinger, Stefanie Widder (Vienna University) and Leo Lahti (Wageningen University). The key challenge we face is to infer the dynamics of the microbiota from its time series. Among the longest public metagenomic time series available, we have at our disposal 185 daily data points for 3 body sites of 2 persons [13], and gut samples taken from persons daily for one year with one perturbation [14]. We started investigating whether these time series fit better predictions forecasted by a determinist system (i.e., generalized Lotka-Volterra equations), stochastic system (i.e., the Hubbell neutral model [15]) or self-organized critical model [16], see Figure 6. We have to overcome difficulties coming from the fact that the time series we have at hand are short and are not always equally spaced in time. We are developing methods based on approaches used in ecology, bioreactors and models of bacterial interactions. I focus on deterministic dynamics and compare/improve techniques introduced by Fisher [17], Stein [18], Marino [19] and Sughiara [20]. We are interested in the network structure of microbial interactions and the biological functions of the sub-structures in the network. My collaborators will also study the stochastic and self-organized critical models. Comparison of the fits obtained for each type of model on synthetic data will determine if we can detect the underlying dynamics of the system via our techniques. If we can – as we expect – we will then analyze the real data mentioned above. A toy model that might help us understanding the microbiota is the study of microbial communities that live on cheese rinds since they are cultivable in the lab [21]. The long-term goal of this project is to design probiotic and prebiotic therapies. In the future, we hope to understand and be able to act on disturbed microbiota related to some cancers, auto-immune disease (multiple sclerosis, lupus, rheumatoid arthritis), antibiotic associated diarrhea, diabetes, eczema, allergies, gastric ulcers, hardening of the arteries, dental cavities, acne, obesity and even autism and depression [9, 10, 11].

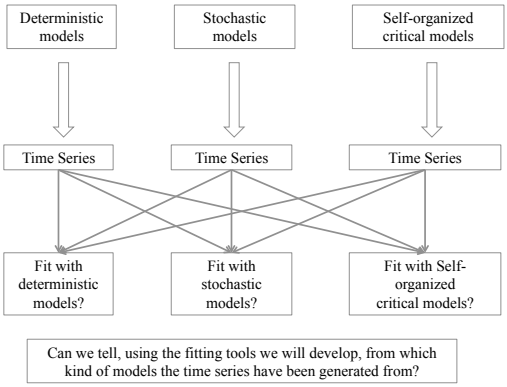

Figure 6: : First steps in the microbiome projet, (1) generate synthetic data (time series) with the different models (2) develop fitting tools to (3) apply these fitting tools to the synthetic data (4) can we tell from which kind of model they come from? If yes, we will turn to real data.

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3 Valorisation/Diffusion (including Publications, Conferences, Seminars, Missions abroad...)

Conferences and Seminars:

- Robust circadian oscillations in growing cyanobacteria require transcriptional feedback, 9th European Conference on Mathematical and Theoretical Biology, June 2014.
- Nonlinear analysis techniques for time series data, VUB, April 22, 2014.
- The Cyanobacterial Circadian Clocks, Leloir Institute (Argentina), December 17, 2014.
- Clocks, Switches and Ecosystems in Biology, KUL, April 23, 2015.

Missions abroad: I visited the Leloir Institute of Buenos Aires of Prof. M. Yanovsky. He is conducting research on circadian rhythms in plants and the effect of the seasons, and we could potentially collaborate. I also participated to the 9th European Conference on Mathematical and Theoretical Biology in June 2014, http://ecmtb2014.org. I gave a seminar at a mini-symposium and also at Chalmers University during that stay.

4 Future prospects for a permanent position in Belgium

I obtained an Assistant Professor position at the Vrije Universiteit van Brussel. I applied to the Chercheur Qualifié at the Fonds National Recherche Scientifique and obtained an A+ mark. Also, I declined a postdoctoral position at the Vlaamse Institut for Biologie at the Katholieke Universiteit van Leuven.