Ovidiu Lipan
Center for Biotechnology
and Genomic Medicine
Medical College of Georgia
1120 15th St., CA-4139
Augusta, GA 30912
Office:(706) 721-7602
Email:olipan@mcg.edu

October 12, 2005

Dr. Yves Brun, Systems Biology or Microbiology Faculty Search
Department of Biology, Indiana University
Jordan Hall 142
1001 E 3rd St
Bloomington IN 47405-7005

Dear Dr. Brun:

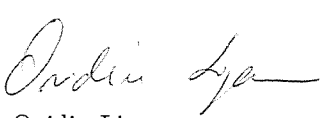
I am responding to your advertisement at http://www.bio.indiana.edu, regarding a tenure-track position at the Assistant Professor level computational Systems Biology. From my initial studies and work in mathematical physics, I moved to biological physics in 2000. I chose to start my faculty career in a Medical School to better learn and understand the experimental aspects of biology and their applications to medical problems. My reasons to depart from the Medical School are: (1) My research program is better fulfilled if I will work in collaboration with computational biologists from Biocomplexity Institute (2) I achieved the maturity and knowledge to teach biological physics. I am now looking for students interested in attending such lectures. Among my strengths are:

- I have 5 years experience in collaborating with biologists
- In my recent papers I solved a theoretical model for signal propagation in nonlinear genetic regulatory networks. At present, understanding the behavior of genetic network is fundamental for Systems and Synthetic Biology
- My teaching experience covers a large variety of undergraduate students, like engineers, physics majors, pre-meds, medical students and non-science majors
- Presently I advise a first year graduate student in genomic medicine with a background in chemistry. She came to my office when she was an undergraduate student. She is now improving her computational skills and is already actively involved in wet lab experiments.
- I am enthusiastic about participating in the development of a curriculum for biological physics

I have the maturity, skills, and abilities in developing collaborative research programs. I strongly believe that my research on signal propagation in genetic networks will attract Indiana University students. The subject is on the cutting edge of biological physics. Students can work from computer simulations and analytical computations through wet lab tests of their predictions.

Thank you for your consideration. I look forward to hearing from you soon.

Sincerely,



Ovidiu Lipan

Ovidiu Lipan, Statement of research interests

I. BACKGROUND

The central question for my research interests is this: how signal propagates in a nonlinear stochastic gene regulatory network?

The answer to this question is fundamental for Systems and Synthetic Biology. Genes interact to each other through a highly interconnected network. This network it is compared sometimes with a large scale electronic circuit. Receptors on the surface of the membrane of a cell behave like an electronic gate. However, contrary to electronic circuits which are deterministic in nature, the molecular interactions inside a genetic network are stochastic. When two proteins interact to form a complex, we speak about the probability for the formation of the complex. This probability is a nonlinear function of the number of molecules that interact.

The time evolution of the probability for the system to be in a specified state is given by a Master Equation, [1]. Although the Master Equation has been intensively studied for the past 70 years, it has only recently been implicated for gene regulatory networks at both the experimental and theoretical levels. The importance of stochastic fluctuations was emphasized by Arkin, Ross and McAdams in 1998 [2], by proving that phenotype variation can occur in a population of cells that have identical genetic background. Thus, pure statistical mechanisms can open different pathways within genetic networks. Thattai and van Oudenaarden, [3], studied in 2001 a stochastic gene regulatory network with transition probabilities being linear functions of the state q. They found a relation between the size of the fluctuation of the molecule numbers and the biochemical parameters. These theoretical results where then confirmed by experimental measurements.

From another perspective, highthroughput tools (microarray, proteomics) are available now to screen large sets of molecules simultaneously. Systems biology [4] is a newly created term to represent an analytical approach to describe the relationships among complex molecular machines. Hood and Perlmutter (2004) [5], argue that the inability from the pharmaceutical industry to visualize the complexity of biological systems has impeded the identification of novel therapies. Thus, various types of data must be integrated into a network model depicting how a particular biological system operates.

II. RESEARCH PLAN

In Lipan and Wong (2005), [6], we solved the problem of signal propagation through a linear stochastic gene regulatory network. Lipan and Achimescu, [7], also solved the signal propagation into a nonlinear network, presented recently as a poster at the 3rd International Conference on Pathways, Networks, and Systems: Theory and Experiments, October 2-7, Rhodes Greece 2005.

- The entire theory is presented in the attached manuscripts. However, to get an impression of the problem, we present a regulatory element important in cancer research.

The genetic network is inspired by the E2F1, a member of the E2F family of transcription factors [8]. In addition to its established proliferative effect, E2F1 has also been implicated in the induction of apoptosis through p53-dependent and p53-independent pathways [9]. The mRNA level, Fig.1, is regulated by 3 molecules: E2F1, pRb and DP1. The number of mRNA produced depends on the input signals $G_1(t), G_2(t)$ and $G_3(t)$. The dimers a and b are intermediates between the input signals and the output mRNA. The molecules c and d represent the the DNA binding of a and b respectively. In the manuscript, precise rules are presented for drawing diagrams like in Fig.1. There is a one-to-one correspondence between a diagram and the mathematical model that captures the biological information. Such a correspondence is lacking in the present day description of genetic pathway by biologists.

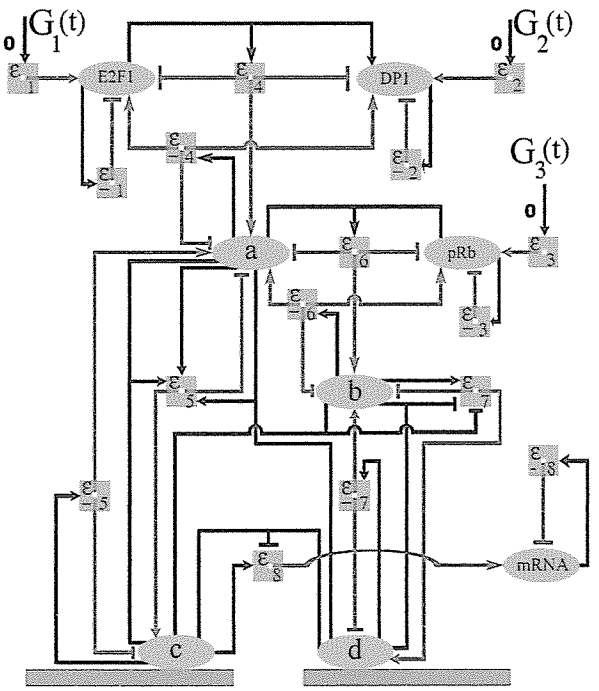


FIG. 1: Molecular diagram for E2F1 regulatory element

- The diagram represents a stochastic process, and thus goes beyond the classical picture of an electronic circuit. Solving the equations for the molecular diagram in Fig.1, we obtain the expression of the mean values and standard deviation of the mRNA.

Without going into any detailed description of the notations, the reader can understand the result by looking at the following formulas, II.1, and reading them from the right to the left. The variables $g_1(s), g_2(s)$ and $g_3(s)$ come from the Laplace transform of the input signals $G_1(t), G_2(t)$ and $G_3(t)$. The precise form of these signals is determined by either the experimentalist or by the exterior networks that act upon the regulatory element under study. In other words, $g_1(s), g_2(s)$ and $g_3(s)$ are independent of the structure presented in Fig.1. Contrary, the transfer functions for the mean values $H_r^1(s)$,$H_r^2(s)$,$H_r^2(s)$ and $H_{rr}^1(s)$,$H_{rr}^2(s)$,$H_{rr}^2(s)$ for the standard deviation depend only on the structure of the regulatory element. These transfer functions once computed describe the system for any kind of input signals.

- To make an analogy, the transfer functions are like the value R of a resistor and the input signals like the current intensity I. The output voltage V is given by $V = RI$. The analog of the voltage are the quantities $X_{r,1}$ and $X_{rr,1}$ from which the mean value and standard deviation for the number of mRNA molecules can be computed.

$$\begin{aligned} X_{r,1}(s) &= H_r^1(s)g_1(s) + H_r^2(s)g_2(s) + H_r^3(s)g_3(s)\,, \\ X_{rr,1}(s) &= H_{rr}^1(s)g_1(s) + H_{rr}^2(s)g_2(s) + H_{rr}^3(s)g_3(s)\,, \end{aligned} \tag{II.1}$$

$$H_r^1(s) = \frac{0.06\,s^2 + 0.65\,s + 0.87}{(s+1.57)\,(s+1.48)\,(s+1.38)}\,, \qquad H_{rr}^1(s) = \frac{0.0002\,s^2 + 0.0228\,s + 0.0340}{(s+80.9)\,(s+1.57)\,(s+1.48)}\,,$$

$$H_r^2(s) = \frac{0.01\,s^2 + 0.18\,s + 0.26}{(s+1.57)\,(s+1.48)\,(s+3.79)}\,, \qquad H_{rr}^2(s) = \frac{0.01\,s^2 + 0.14\,s + 0.29}{(s+1.57)\,(s+11.30)\,(s+3.01)}\,,$$

$$H_r^3(s) = \frac{-0.16\,s^2 - 2.16\,s - 0.52}{(s+0.043)\,(s+1.48)\,(s+22.8)}\,, \qquad H_{rr}^2(s) = \frac{0.006\,s^2 - 0.066\,s - 0.023}{(s+1.57)\,(s+13.08)\,(s+0.043)}\,.$$

We proved by this example that is possible to combine nonlinearity and stochasticity and study the mRNA control from the point of view of input-output relations.

III. BROADER IMPACT

The results of this research are highly significant to biology, applied mathematics, statistics, physics, engineering and physical chemistry. Our theory can be applied to any stochastic system with a discrete state space that is described by a Master Equation. The Master Equation for discrete states plays a central role in diverse areas such as statistical physics, chemical reactions

and Markov Chain Monte Carlo. Nonequilibrium statistical physics of discrete systems driven by external signals have not been fully explored, especially for systems with many degrees of freedom. Biomedical engineering is entering a new phase. At present, engineered genetic circuits are capable of performing switching functions or follow a predictable dynamical behavior. In the near future, simple, but useful molecular amplifiers, transducers and switches will be built out of standard components. The engineering challenge is to design reliable biomolecular devices that function over a wide range of time-scales and under a variety of stimuli. The theory we propose offers a promising way for creating algorithms for genetic circuitry design. This project will have a high impact on education and training. Students working on this project will be able to participate in multidisciplinary research which is an essential aspect of training the next generation of scientists. The field of gene regulatory networks is just beginning.

---

[1] van Kampen NG (1992) Stochastic Processes in Physics and Chemistry. NorthHolland, Amsterdam. 465 p.

[2] Arkin A, Ross J, McAdams HH (1998) Stochastic kinetic analysis of developmental pathway bifurcation in phage l-infected Escherichia coli cells. Genetics 149: 16331648.

[3] Thattai M, van Oudenaarden A, (2001) Intrinsic noise in gene regulatory networks. Proc. Natl. Acad. Sci. USA 98: 8614 – 8619.

[4] Westerhoff HV, Palsson BO (2004) The evolution of molecular biology into systems biology. Nature Biotechnology V. 22 N.10: 1249 – 1252.

[5] Hood L, Perlmutter PM (2004) The impact of systems approaches on biological problems in drug discovery. Nature Biotechnology V. 22 N.10: 1215 – 1217.

[6] Lipan O, Wong WH, The use of oscillatory signals in the study of genetic networks. Proc. Natl. Acad. Sci. USA. 102: 7063 – 7068.

[7] Lipan O.,Achimescu S., Modulation of nonlinear stochastic gene regulatory network by input signals. Poster at the 3rd International Conference on Pathways, Networks, and Systems: Theory and Experiments, October 2-7, Rhodes Greece 2005.

[8] Muller H, Helin K. (2000) The E2F transcription factors: key regulators of cell proliferation. Biochim. Biophys. Acta 1470: M1-M12.

[9] Phillips AC, Vousden KH (2001) E2F-1 induced apoptosis. Apoptosis 6: 173 – 182.

Statement of teaching philosophy

Ovidiu Lipan

Socrates said that the idea should be born in the student's mind and the teacher should act as a midwife. The student thus must be creatively implicated in her own training. A teacher should guide the student to discover her inner potentials that will later make the student a happy person in a job that fits her. In my personal experience in teaching I followed this principle, adapting it to different situations.

In the last 5 years I learned from biologists, taught biologists and worked on research projects with biologists. Thus, I became convinced that new programs that involve courses from biology, physics and mathematics must be offered to a new generation of students. The computational knowledge of the present-day biologist was acquired through lectures in biostatistics. Although statistics plays and will play an important role in experimental data analysis, ideas from physics and mathematics will be necessary to lay down the principles of the newly born Systems Biology and Synthetic Biology. Today, a typical experimental design in biology is to compare treated with non-treated cells. The new generation of students should learn from physics many other experimental design procedures. They should also learn that when planning an experiment it is more useful to have a mathematical model for the biological system, and not only a model for the experimental errors.

My objective as a teacher of computational Systems Biology at Indiana University is to actively participate in shaping this new generation of students. I envision biology and physics fusing in the minds of these students, who will then be able to employ established laws of natural phenomena to decipher biological connections. This is not an idealistic dream, as I presently advise a first year graduate student in genomic medicine with a background in chemistry. She came to my office when she was an undergraduate student. She is now improving her computational skills and is already actively involved in wet lab experiments.

The majority of the student body learns what we test for. To achieve the objective of fusing biology and physics, my tests for biological physics will focus on a variety of experimental designs taken from Physics, Systems Biology, and Synthetic Biology. The tests will emphasize dynamics of gene expression and their products, the mathematical model for understanding the dynamics, experimental evidence for model validation.

Besides having its scientific, cultural and social values, teaching is a great source of enjoyment in my life. I present below my teaching methods, as I used them in class.

My teaching experience started with teaching analytical mechanics to first year mechanical engineering students. The subject was not on their focus agenda; therefore I chose to shape my presentation in algorithmic form, so they could apply it step by step. Then, by many examples taken from mechanics I explained the power of the method. At the middle of the semester the students realized that they absorbed a subject that initially looked impenetrable; they were happy about their success, as if they had climbed a difficult mountain. Thus, I realized two key aspects: one was the advantage of taking small steps utilizing familiar examples; the second was to capture students' attention by anchoring the lecture in present times, improvising and using examples from what is happening in the class, or about what you see through the window at that moment, and relaxing the audience with anecdotes. In this way the lecture is fresh and alive.

For five years I was teaching assistant at University of Chicago and I deeply enjoyed it. To know my students better and understand each individual background, I asked to be assigned to teach

laboratory physics. In a lab the interaction between a teacher and a student is more direct than in a seminar lecture. A teacher can observe and talk with each student individually. At the end of first quarter I knew my students very well. I learned that a teacher should spend time with her students and that the student must sense that the teacher cares for her and is interested in her scientific development. I saw what a significant impact 30 seconds of my time had on a student. There are two conditions here though: in those 30 seconds the teacher should act like she has infinite time for the student and the teacher's mind should be completely focused on the student, not anticipating the next task instead.

At Chicago, I also taught "physics for poets". I did not realize at the beginning what was the meaning of "poets"; in essence, they were students that disliked physics. The first class was an attack on the system that forced them to take a science class. Of course, the rhetoric was not phrased plainly like "I do not like this system", but asked questions like:" Why do we need logarithms for?" This question was in fact very good, but the students were not prepared to listen to my answer. Thus, I started instead to discuss their hatred of science. My goal was to separate science from negative emotions. By the middle of the quarter, the class was working well.

While teaching at Caltech, the challenge was to teach a subject the students already knew, without making them feel bored. I chose to use their knowledge as examples to introduce advanced concepts. For example, students were impressed by the visual power of the phase-plane description of a free falling particle. Teaching them, I didn't expose the entire secret from the beginning. I let them guess the result and then use it in many other circumstances, so that they will learn the way to generalize from simple examples.

With my move into computational biology, from Caltech to Harvard, I plunged into a biological environment where mathematical physics was a strange newcomer. However, the new biotechnology equipments had made algorithms and mathematical theories to become as needed as a bottle of reagent. I had to explain to biologists, for example, how Fourier decomposition works, without formulas; only by graphs, pictures and real data measured in lab. This first encounter with the biological way of thinking helped me to be able to teach formal lectures to first year graduate medical students at the Medical College of Georgia. Here the goal was to be very clear, in the form of an input-output presentation. The algorithms were never discussed in details; only what is the input data and how to interpret the output.

Being an assistant professor in a Medical School, I had the opportunity to teach one lecture in Histology. I structured the information to fit an analytical way of thinking establishing classification parameters and organizing tree-rooted graphs. Computer visuals and interactive tools helped me enhance the clarity of the presentation. The clear and organized lecture pleased the students and the faculty responsible for the entire course.

While the enthusiasm I gathered from understanding the freedom of mathematical creation and the beauty of discovery is as indispensable to good teaching as it is contagious, I learned that great teaching is also made with well-rounded knowledge of the subject, including its historical development. The teacher can thus present each theorem or method as a natural consequence of a need that appeared at some point in the history of science.

Biological Physics is a perfect field to teach a science in the making, as its new developments have appeared as a need. The above-referenced characteristics constitute, I believe, my strengths as a teacher.