October 15, 2005

Yves Brun,
Systems Biology/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 the advertisement for the tenure track faculty position as listed September 2nd issue of the science magazine. My significant interdisciplinary research experience combined with strong theoretical background would make me an ideal candidate for this position.

My research has centered around developing quantitative mathematical models of biological systems with a focus on regulatory and metabolic networks that are directly comparable with experimental data. I also have an interest in the development of effective symbolic and numerical computation methods to analyze these models.

My recent work is on a mathematical model of the pheromone signaling pathway of yeast *S. cerevisiae*. My goal in this project is to develop a computational model of this pathway to interpret time-dependent data for protein activity and investigate effects of noise due to a low copy number of molecular species in the pathway. I have been collaborating with Profs. Timothy Elston from UNC Pharmacology and Henrik Dohlman from UNC Biochemistry departments on this project. I will apply Monte Carlo methods to address how well the model parameters are constrained by the experimental data and what further experiments have to be done in order to extract as much useful predictive information as possible from the model.

My teaching responsibilities have covered a range of students and topics from rudimentary mathematics to intermediate undergraduate courses for majors.

More details of my research, teaching and education can be found in the accompanying materials. I look forward to an opportunity to visit your department and further discuss the position. Thank you for your consideration.

Sincerely,

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My research interests are mainly focused on mathematical modeling of biological/chemical systems with a focus on regulatory biochemical networks. High-throughput data acquisition technologies in molecular biology are able to provide tremendous amounts of data about individual components of the incredibly complex molecular cell machine. Although many intracellular regulatory networks have been extensively studied using advanced experimental techniques, it has turned out to be quite difficult to make predictions in terms of regulation of these networks. How individual components of this machine are assembled and dynamically regulated and how their behavior as a whole is related with the properties of the individual parts are still not completely understood. Mathematical modeling and computer simulations are promising tools to investigate and address such questions.

I am interested in developing quantitative mathematical models of biological systems that are directly comparable with experimental data. I also have an interest in the development of effective symbolic and numerical computation methods to analyze these models. In the following sections, I will briefly summarize each of my interests in detail then conclude with an outline of my future research plan.

Investigation of regulatory control mechanisms in the pheromone response pathway of yeast S. cerevisiae

My current postdoctoral position involves a group project on investigating cellular signal transduction systems. I have been collaborating with Profs. Timothy Elston and Henrik Dohlman on this project. Signal transduction pathways enable cells to receive, process and respond to biochemical stimuli. These pathways are generally highly nonlinear and often contain multiple feedback and feedforward loops and share common functional components. The broad questions I seek to address with mathematical models and computer simulations are: What biological functions do feedback and feedforward loops provide? How is pathway specificity achieved and how is signal intensity controlled?

To address these questions, we have chosen to study the mating response pathway of yeast *S.cerevisiae*. This system is one of the best characterized signaling pathways of any eukaryote and has strong structural and functional similarity to mammalian signaling pathways. Furthermore, the genetic perturbations of yeast cells are easy, and knock-out mutants and overexpressions are available for each component of this pathway.

The first part of this project concerns modeling of receptor and G protein activation in the pathway. We constructed a model based on ordinary differential equations to model the negative feedback loop in which increased synthesis of the RGS protein leads to signal inactivation. However, an inconsistency with the computational and experimental data suggested also the existence of a previously-unsuspected positive feedback loop. In testing the revised model we discovered that the RGS protein undergoes stimulus-dependent ubiquitination and degradation. This work was profiled in a news article published in Science STKE. This paper provides a clear example of the power of combining experimental and computational analysis to study G protein signaling.

Although distinct cellular signaling pathways often use common signaling proteins, how cell maintains signal specificity is still not completely understood. The mating and invasive signaling pathways of yeast are typical examples. Activation of these two pathways requires a distinct receptor and activates two distinct MAP kinases; however, both pathways use a common set of intermediary kinases. The second part of this project is to explore how signal specificity is maintained in the mating signaling pathways in yeast.

To investigate signal specificity mechanisms in the mating pathway, we have devised five different mathematical models, each corresponds to different biological mechanisms and then estimated the parameters involved in these models with *in vivo* experimental data for activities and abundances of some protein. We have seen that one of the models captures experimental data much better compared to other four models that suggests that activation of a MAP kinase phosphatase by the mating-specific MAP kinase (Fus3) leads to selective inhibition of the second MAP kinase (Kss1) preventing activation of invasive signaling pathways. This prediction has been tested experimentally and phosphorylation of the phosphatase by Fus3 has been demonstrated. The experiment to test how this phosphorylation event affects activity of the phosphatase is underway.

I am presently working to construct and test models of the entire pathway module by module and the ultimate goal of this project is to model the whole pathway and determine pathway components that are most important in terms of regulation of the signaling.

Investigation of effects of inherent time delays and bistability in genetic networks: Lac operon in *E.coli*

Prior to my current position, I worked with Prof Michael Mackey at McGill University on a project about investigation of effects of inherent time delays and bistability in lac opereon, a genetic circuit in *E.coli.*

The lac operon is a classic example of an inducible genetic network in *E.coli.* It consists of a small promoter–operator region and three larger structural genes. Preceding the lac operon is a regulatory operon lacI that is responsible for producing a repressor protein. This control system functions in the following manner: In the presence of glucose, LacI represses the lac operon. However, in the absence of glucose, but in the presence of external lactose, lactose is transported into the cell by a permease. Intracellular lactose is then broken down into glucose, galactose, and allolactose by the enzyme β-galactosidase. The allolactose feeds back to bind with the lactose repressor and enables the transcription process to proceed.

It is known that the lac operon regulatory pathway is capable of showing bistable behavior. I worked on how bistability arises in this system and how time delays due to the transcriptional and translation process, two relatively slow processes in this circuit, affect the dynamics of this system.

To investigate the role of time delays which are often ignored in previous models on the dynamics of the system we developed a model of ordinary differential equations with discrete time delay. Our model is five dimensional and has three delay terms. We numerically investigated the model using published parameter values and demonstrated that this system is indeed capable of bistability under physiological conditions.

To illuminate the origin of bistability further, we assumed a constant amount of lactose inside the cell. This assumption reduced the five dimensional model to three dimensions by eliminating the equations for the permease and external lactose concentrations. By numerically solving the time-delay differential equations of the reduced model and by performing a local stability analysis we showed that it behaved similarly to the complete model and displayed bistability. This last result supports the conclusion that the β-galactosidase regulatory pathway is the most essential of the regulatory mechanisms in the lac operon. We showed that time delays do not affect the stability of the system in the sense that there is no evidence for a Hopf bifurcation. Although one was reported in a model developed for the regulation of lac operon by Mahaffy and Simeonov(1999). The analysis of our reduced model gives no grounds to expect a Hopf bifurcation or oscillatory behaviour. On the other hand, we have also showed the time delays could kick the system to the upper or the lower stable steady states when external lactose concentration in the bistable region.

We have published our analysis in two papers (Yildirim and Mackey, 2003 and Yildirim et al., 2004). Soon after our second paper, Ozbudak et al. (2004) published a paper in Nature that quantifies dynamics of lac systems in single cell experiments. Our estimate for the external lactose concentration for full induction of the system matches very well with the experimental results in this paper. They nicely showed that lac system can show bistability in a physiological meaningful parameter regime and showed that 30 μM is enough to fully induce this system which is in our estimate range of 20-60 μM depending upon how fast bacteria grows.

Computer algebra approach to *in vitro* **multi enzyme systems**

Throughout my graduate study at Ataturk University in Turkey I have worked on *in vitro* biochemical reaction networks catalyzed by multi enzymes with aid of mixed symbolic and numerical computation techniques. Symbolic computation techniques performed by computer algebra systems have found broad applications in many areas of science. It has led to new approaches for problem-solving and provides tools that enable an automatic and computerized solution of problems in ways that are not possible with conventional computing systems.

To this extent, I am interested in automatic derivation of rate equations for various enzyme binding mechanisms. This is a tedious job especially for the reactions involving more than one substrate and/or product due to the nonlinearities involved and a number of unknown parameters. Several methods have been developed to address this problem, and the most accepted one is the graphical method developed by

King and Altman(1956). Although this method is quite general it is unpractical for predicting the general behavior associated with broad classes of binding mechanisms. I have developed an algorithm that uses symbolic computation tools that derive rate equations and applicable to all reactions mechanism and multi enzyme systems under quasi steady state assumption of intermediate metabolites. This method uses Groebner bases algorithms(Burchberger, 1985) that transforms a set of multivariate polynomial F into another set G of polynomials with certain nice algebraic and geometric properties, called a Groebner basis, such that F and G are equivalent (i.e. generate the same ideal).

My dissertation is a nice application of symbolic and numeric computation techniques to analyze kinetics and control of multi enzyme systems. I particularly worked on the three step conversion of glucose and creatine into NADPH using *in vitro* measurements of NADPH over time. In this system, each of three enzymes creatine kinase(CK), hexokinase(HK) and glucose 6 phophate dehydrogenase(G6PDH) has two substrates and two products. After deriving rate equations symbolically with regard to the binding mechanisms I have successfully estimated the kinetic parameters from *in vitro* experimental data and calculated metabolic control coefficients that determine how flux through the pathway is controlled by these three enzymes and how individual metabolites concentration depend on the system parameters. As opposed to widespread belief of the existence of a unique rate-limiting step in the metabolic pathways, we have seen that overall flux is distributed among three enzymes. The enzyme of the first reaction CK mostly controls the overall flux (76.2%). The second enzyme HK that catalyses the middle reaction controls only 6.1% of the flux while the third enzyme G6PDH 17.6% of the flux.

Future Projects

The experience of working closely with biochemist during my graduate and post graduate study provided me a great experience. Over the past two years I have been working on the pheromone signaling pathway of yeast and I plan to continue working on this project. The ultimate goal of this project is the development of a realistic mathematical model to simulate the whole pathway with experimental data for protein levels obtained from either population or single cell measurements.

I am also interested in stochastic modeling of biochemical networks, and the natural and artificial regulation of such networks. Deterministic models overlook the random nature of biochemical reactions. However, stochasticity is a significant effect in genetic regulatory networks because of the low copy numbers of molecular species such as DNA and mRNA. To consider the effects of fluctuations requires the use of stochastic models. Stochastic models can capture important behavior that is not seen in deterministic models (Kepler and Elston, 2001). I would like to use stochastic models to elucidate effects of the fluctuations on the dynamics of such systems.

Estimation of parameter values in models of biochemical networks is often difficult because the data are noisy and our knowledge of the network topology and participating molecular species is incomplete. Additionally, determining how well the model parameters are constrained by the experimental data is as crucial as finding optimal fits to the data. Both of these issues can be addressed using Monte Carlo methods. Because these methods determine which parameters are least constrained by the data, that often suggest new avenues for experimental analysis to measure unknown parameter values. I plan to use Monte Carlo methods for model driven experimental design and robustness analysis of these systems.

I also would like to continue my investigation of multiple steady states in biological/chemical systems. Although the capacity to achieve more than one internal steady state for a set of parameters is common in biological/chemical systems, experimentalist often believes the systems under investigation always have single stable steady state. Among the various patterns of behaviour emerging from regulation associated with nonlinear kinetics, bistability is extremely interesting. It allows a true discontinuous switching (with hysteresis) between alternate steady states that can convert graded inputs into switch-like responses. This permits a discontinuous evolution of the system along different possible pathways, which can be either reversible or irreversible, and may provide the system with a memory. Bistability is often produced by positive feedback or mutual inhibition but it has recently been shown that direct feedback is not necessary to have multiple steady states in a MAPK model when dual phophylation of the MAPK is taken into account (Markevich et al, 2004). Equations that describe steady states of these systems can often be written as a system of nonlinear multivariate polynomial equations systems. Computer algebra systems that perform symbolic computations provide powerful tools particularly when working with polynomial systems. I plan to explore necessary and sufficient conditions for existence of multiple steady states in chemical/biological systems with symbolic computation tools. Questions to be addressed are whether a system cable of showing bistability. If so, what parameters or parameter combinations provide multiple

steady states. Numerical methods generally based on some continuation methods do not answer these questions.

In summary, there is a wealth of new experimental techniques in molecular and cell biology, such as biosensors and microarrays that have created an incredible amount of novel data. I plan to continue applying mathematical, statistical and computational methods and tools to interpret and analyze these new data.

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TEACHING STATEMENT

NECMETTIN YILDIRIM

Teaching is an important part of being a professor at every academic institution. Teaching mathematics several years at various levels provided me with many great experiences in terms of teaching methods, use of teaching tools in the classroom, assessment of student's success and student–professor interactions.

My teaching method depends on the course I teach but effective motivation, which involves good interaction, awakening and nurturing interest and using technology effectively in the class, constitutes an integral part of my teaching philosophy. It is widely believed mathematics is inapproachable to all but a few. One of the most challenging things is to overcome the lack of confidence some students have in their math abilities and show them how math is useful and how it makes life easier for us. There are many methods I use to engage the students' interest in the subject of math. The most straightforward way to do this is to show them how math actually permeates with our everyday life. I find that it increases students' understanding when I show them how the topics of interest have applications in other sciences. I want to convince them that math is a language and that uses numbers, theorems and functions instead of letters.

There are many reasons why I enjoy teaching. First of all, I am not only teaching it to others, I am exploring the math myself. After years in undergraduate and graduate training, I still think that there is much to explore. Second, I believe mathematics is one of the most enjoyable things to teach, since a math instructor not only carries mathematical knowledge, but is given an opportunity to stimulate logical thinking and reasoning among the students. One can forget all equations and formulae once the class is over, but, students who have learned "how to think like a mathematician" will have a precious skill for the rest of their life.

Teaching is a dynamic profession. In teaching a science that is at a crossroad, like math, one should know how to deliver the material to students. Students and instructor both have the responsibility to adapt to change and new materials in a class. As an instructor, it is my job to provide well-prepared and up to date material, encourage students to learn, and do the necessary work. Careful preparation of class material is the first step in being able to explain complicated topics clearly.

Since my research interest is in applied math, I often incorporate applications and ideas from other sciences. I believe that applications of formulae and complicated theorems to another science and seeing concrete results of their effort will show them that math is more than just two plus two. The teaching methods I use change course to course. In advance classes, students are prone to learn more effectively with take-home examinations or projects. I always encourage students to complete projects that involve real life problems. Wh en students realize that they can use simple math to understand and solve problems relevant to their lives, they will discover that math is stimulating. I also try to give plenty of time to students for discussion inside or outside the class.

My principal philosophy is to understand the interest, strengths and weaknesses of each individual in my class because I believe teaching works best when adjusted to each student's needs. My enthusiasm for math and teaching are my strengths. My major goal as an instructor and research advisor is to transfer to students understanding and joy of applied math.

Thank you for your consideration,

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