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Dear Colleagues,

I am happy to offer a letter of recommendation for Dr. Ovidiu Lipan who is applying for a faculty position in your department.

Ovidiu received strong graduate and postdoctoral training in mathematical physics at the University of Chicago and California Institute of Technology. From September 2000 to August 2003, he conducted research in computational biology in my laboratory. During that period, he completed two important pieces of works.

In the first work, he collaborated with Charles Weitz's group in the Neurobiology Department at the Harvard Medical School on the molecular mechanism of circadian rhythm in mouse, both in the suprachiasmatic center (SCN) in the brain, and in peripheral organs such as the eye, the heart and the liver. The basic circadian clock, involving two feedback loops and several key proteins, has been worked out during the past decade by Weitz, Kay, Takahashi and others. The goal of our project was to understand how this clock can exert such dramatic effects on the body, affecting everything from appetite to sleep pattern. In order to identify the downstream effectors of the circadian clock, the Weitz lab generated global gene expression profiles of cells from liver and heart. Ovidiu developed a very elegant scheme, based on the use of highly calibrated sensors for periodicity that was constructed by the use of a set of gold-standard circadian genes. This analysis provided convincing evidence supporting the conclusion that very large numbers of genes (more 8-10%) are regulated in the circadian cycle in both cell types but that the common circadian genes in these two tissues are much smaller in number and they tend to be in the core clock components. On the other hand, the distributions of the circadian regulated genes across functional categories are remarkably consistent between the two tissues. The important implication of this analysis is thus that although the same genes may be used to transduce and the circadian signal, they may control a large number of cellular processes and the control may be mediated by different genes in different tissues. This important finding was published in Nature and Ovidiu's contribution was invaluable. This work demonstrated Ovidiu's ability to work with bench biologists on the analysis of large scale functional genomics data to obtain significant scientific findings.

Ovidiu's main research interest, however, is in systems biology. His postdoctoral research addressed the question of whether the use of oscillatory perturbation may yield better experimental data for the study of a cellular system. To answer this question, he had to build up a substantial theoretical infrastructure. He represents the state of a cell by a vector containing the counts for various species of molecule—mRNA for gene 1, protein for gene1, mRNA for gene 2, protein for gene 2, etc. This state vector is time dependent and it evolves in a stochastic manner according to a continuous time Markov process where at any instant, the infinitesimal probability of increase (or decrease) of a particular count is dependent on the current counts in a linear manner. The coefficients of this linear dependency reflect how the levels of different mRNAs and proteins affect each other's production and degradation rates. Conceptually, these coefficients specify the structure of the "network" of genes and proteins that work together as a system. Although such a stochastic process has recently been used for modeling a cellular system by van Oudenaarden's group at MIT, the mathematical properties of this process have not been studied.

To study such a system experimentally, one can first perturb it in a certain way, say by expressing a particular gene at a high level (setting its mRNA at a high value from a certain time onward), or by inhibiting or degrading a particular protein from a certain time onward, or as in Ovidiu's approach, by varying the level of a mRNA or protein in a periodic manner. One then measures the state vector of the system as a function of time after the start of the perturbation. The specific perturbation is encoded as an "input" function (also called a signal generator) that specifies the values of the variables chosen to be perturbed, and the subsequent measurement of the other counts gives the "output" function. By choosing various input functions one hoped to estimate the network parameters from the output measurements through computational analyses. The computational reconstruction of the network depends on knowledge of the "transfer function' of the system that maps a given input function to the output. Since the output is a stochastic process this situation is much more difficult than the standard setting in systems theory. Ovidiu's first contribution was the derivation of a close-form solution of the Laplace transform of the first two moments of the process, given the Laplace transform of the input function. The result for the mean function was already derived by Thattai and Oudenaarden in 2001, but the result for the variances and covariances is new and much more complex, and it immediately suggests some very interesting properties of the network. For example, Ovidiu discovered that the system can exhibit a phenomenon that he called "pure fluctuation resonance": at a certain specific frequency (computable from the network parameters when the input is periodic), the covariance matrix may be at resonance with the input while the mean is not. When driven at such a frequency, the state vector will repeatedly exhibit large variation at regular time intervals, even though the mean level may show little changes over time.

More importantly, based on this mathematical framework, Ovidiu was able to answer his original question of whether periodic input may lead to more informative experiments than step or impulse inputs. Using the close-form transfer functions for the moments, he showed that when the input is of a step or impulse form, the estimation of the system parameters, given the measurements of the output moments, is extremely sensitive to measurement errors of the output moments. This sensitivity is eliminated by the use of periodic input. The importance of this result cannot be overstated. Almost all input perturbations currently in use in systems biology studies are of the step or impulse form. Ovidiu's analysis had shown that there is basically no hope of estimating the system accurately using this type of input. He offers an alternative way to move forward by proposing the use of periodic input. These results have recently been published in the Proceedings of the National Academy of Sciences. I expect they will stimulate experimental research on the implementation of periodic input perturbations.

One loose-end in Ovidiu's postdoc work was the assumption that the state vector affects the infinitesimal transition probabilities in a linear manner. As such, Ovidiu's original results cannot cover many nonlinear cellular systems. I am thus excited to learn that, after 18 months of intense effort, he is now able to handle dependency of the rational form. The rational form is of course much more general than the linear form, and will allow Ovidiu's theory to cover many important nonlinear systems including those with Hill-equation feedback and those with higher order reaction kinetics such as Michaelis-Menten type catalytic enzymatic processes. Thus this is a very significant advance that will enable the use of this approach in real applications. After studying his preprint, I am extremely impressed by the originality and depth in this new analysis, as well as by the scope of Ovidiu's vision. It will take another three pages to summarize the many important new findings in this paper. Instead, I would just say that it is the most profound work in the mathematical and theoretical analysis of biological systems that I have read in the past 10 years.

Ovidiu is currently exploring various experimental strategies to implement periodic perturbations. He made a construct with a heat shock promoter driving the expression of a transgene and then successfully tested the use of temperature to control the mRNA levels of the transgene in a periodic manner. He is doing this directly on mammalian cell lines and the preliminary results are very promising. Thus he has demonstrated the experimental feasibility of a periodic signal generator in mammalian cell culture. To measure output at the signal cell level, which is the situation where his theory is the most relevant, Ovidiu is using flow cytometry to measure events, such as transgene activity, within single cells. Although not trained as an experimental biologist, Ovidiu is familiar with experimental design and modern instrumentation because of his background in physics (he was a TA in the experimental physics course by James Cronin at the University of Chicago). In the past few years he had also gained considerable knowledge and proficiency in the molecular biology techniques needed for implementing his approach. This combination of very high level theoretical and mathematical prowess, and the willingness and ability to pursue experimental implementation of the theoretical ideas, is almost unique among his cohort. I can think of no one with a higher promise to become a leader in the new discipline of systems biology. It gives me great pleasure to recommend Ovidiu for a faculty position in your institution in the most enthusiastic term.

Sincerely,

Wing Hung Wong
Professor of Statistics and of Health Research and Policy and
by courtesy, of Biological Sciences