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Letter of Recommendation for **Daniel B. Forger**:

I'm proud to say that Daniel Forger was my Ph.D. student. His thesis was on the mathematical modeling and computer simulation of circadian rhythms. Since receiving his Ph.D. in September, 2003, Forger has been working as a postdoc in the Laboratory of Justin Blau at the NYU Department of Biology, where he has been learning the experimental side of circadian rhythms research. Meanwhile, Forger and I continue to collaborate on extensions of the work begun in his thesis. I hope and expect that this will be a longterm collaboration.

Let me begin by noting that Forger has received two significant awards here at NYU. These are the Wilhelm Magnus Memorial Prize, which is awarded by the Courant Institute for a significant contribution to the mathematical sciences by a graduate student, and the Dean's Outstanding Dissertation Award, which is awarded by the Faculty of Arts and Science and therefore implies University-wide recognition of Forger's extraordinary Ph.D. thesis research.

Daniel Forger was a truly remarkable student. His most astonishing characteristic was the extent to which he already functioned as an independent scientist, not just in having an independent research program of his own design (although that is already remarkable), but also in terms of professional activities such as organizing sessions at meetings, not only writing papers but also contributing substantially to proposals and progress reports, representing a multi-institutional research group to a funding agency, coordinating research with other scientists around the world, etc.

For the last few years, Forger and I have been part of a DARPA-funded project on circadian rhythms. This project was centered at Harvard Medical School, and also involved an experimental group at the University of Massachusetts. The project had an international component through the participation of Albert Goldbetter and Jean-Christophe LeLoup, who are well known for their mathematical modeling in this area. Characteristically, they were recruited by Forger to join the project. Indeed, it seems to me that Forger has in many ways played the role that would be expected of the Principal Investigator of such a project. In particular, he created the mathematical model of the mammalian intracellular circadian clock that is the main goal of the project; on several occasions, he was the key speaker representing our group to DARPA; he was in constant touch with David Weaver of

the experimental group to coordinate the modeling and the experimental work; and he was very effective in communicating with other DARPA-funded groups such as John Tyson's, which are doing related work. My own involvement in this project (and indeed in this field of research) is entirely due to Forger's initiative, thus reversing the usual roles of advisor and student.

With regard to the research itself, Forger has a kind of genius for mathematical modeling. He is able to sift though large amounts of noisy and incomplete experimental data and to draw important conclusions from those data about the underlying mechanisms that must be at work. He refuses to accept easy ways to make a model produce a certain behavior if those ways are not supported by experiment. And he constantly questions whether conventional assumptions are really satisfied by the system under consideration, or whether that system might have special features that turn conventional wisdom on its head.

Let me now detail some of Forger's specific research accomplishments. In modeling circadian rhythms, there are two basic approaches. One is to treat the whole organism as a black box, and to introduce an abstract oscillator (typically, a two-variable van der Pol oscillator) to model the circadian clock. Despite its simplicity and lack of any explicit physiological basis, this approach is surprisingly effective in predicting the response of the organism to different temporal patterns of light exposure. The opposite approach is to make a detailed biochemical model (typically including gene regulation) of the intracellular circadian clock. It is noteworthy that Forger has made contributions within *both* of these modeling frameworks and moreover that he has shown how they are related. I doubt that there is anyone else in the circadian community about whom a similar claim could be made.

On the whole-organism level, Forger has made substantial contributions to the model of the circadian clock and its responses to light that has been put forth by the Division of Sleep Medicine, Harvard Medical School. This model is now embodied in software that can be used to predict the effects of various light-exposure schedules. Forger's particular contribution to this effort involved the way that light couples into the model, obviously a key component. He found a simpler coupling scheme than others that had previously been proposed, and showed that the more complicated schemes were actually inconsistent with the experimental data whereas the simpler scheme made accurate predictions. This is a key contribution to an important research project.

In terms of the relationship between the two types of modeling, Forger has shown, using the Goldbetter model as an example, that detailed biochemical models of the circadian clock can be reduced (to a good approximation) to two-variable models of the type that are typically used in whole-organism modeling. This is because the multivariable biochemical system spends most of its time (as Forger has shown) near a two-dimensional manifold within its multidimensional phase space. This remarkable connection has profound implications for a field that would otherwise be split between two irreconcilable approaches.

Construction of a detailed biochemical model of the mammalian intracellular circadian clock was the core of Forger's Ph.D. thesis. Some distinctive features of the model that he created are as follows: First, the model is constructed with careful attention to experimental data

and in consultation with experimentalists, whose ongoing investigations lead to ongoing refinement of the model. Second, the model is based on individual biochemical reactions rather than higher-level reactions that combine several individual reaction steps. Thus, for example, Michaelis- Menton kinetics, and Hill coefficients of traditional enzyme kinetics are *not* used; instead the individual reactions underlying such kinetics are considered explicitly in Forger's model. There are two reasons why this is important. One is that the assumptions that are traditionally used to justify the higher-level description may not be valid in the case of the reactions that comprise the biochemical circadian clock. Indeed Forger has shown simply by counting the numbers of molecules involved that these assumptions are highly suspect. Another benefit of considering the individual biochemical steps is that then there is a clear unambiguous path that connects a deterministic model of the system (described as a system of ordinary differential equations for the *concentrations* of the individual molecular species) and a stochastic model of the system (that tracks the *integer* numbers of molecules of each molecular species that may be present). With Michaelis-Menton kinetics, or other more complicated kinetics of that general type, there is an ambiguity in going from the deterministic to the stochastic description of the system. With Forger's approach, there is an unambiguous recipe that defines both the deterministic equations and the corresponding stochastic model once all of the individual reactions have been stated.

This brings us to the third main feature of Forger's thesis research, which is that his model comes in two versions: deterministic and stochastic. These two versions involve precisely the same reactions and parameters, but the stochastic version takes into account the effects of molecular noise, which the deterministic version neglects. Since the stochastic model is inherently more realistic, one might ask why use a deterministic model at all? One reason is that the deterministic model is cheaper to simulate and easier to analyze. Such simulation and analysis can be used to explore parameter space more thoroughly than would be possible with the stochastic model alone. Indeed, Forger has devised a simple but effective direct-search method for determining automatically the parameter values of the deterministic model that provide a best fit to the experimental data. A striking feature of this procedure is the way that Forger avoids the phase ambiguity that is inherent in fitting a model to *periodic* data. He overcomes this difficulty by considering transient experiments in which an animal that has been entrained by normal day/night light variation is suddenly put in constant-light conditions. Of course, any conclusions reached with the deterministic model must be regarded as preliminary, and have to be checked by running the (more expensive) stochastic model. But the deterministic model can be used to delineate those regions of parameter space where it is (most likely) worthwhile to run a stochastic simulation. Another reason why it is useful to have both versions is that the comparison between them elucidates precisely the effects of molecular noise.

Besides constructing a detailed biochemical model of the mammalian intracellular circadian clock, Forger has also worked out the "design principles" that underlie evolution's selection of the particular biochemical clock networks that we see today. One important design principle involves insensitivity to molecular noise, and Forger has proposed several mechanisms for this and demonstrated their effectiveness by computer simulation. An important example is the principle, discovered by Forger, that gene regulation can be made insensitive to molecular noise by increasing the binding and unbinding rates of protein-DNA interac-

tions. Since a given gene is invariably present in a *small* number of copies per cell, gene regulation is likely to be the greatest source of molecular noise in a biochemical network. Typically, molecular noise is overcome by the law of large numbers, when large numbers of molecules of each relevant type are involved, but this mechanism is unavailable in the case of genes. Remarkably, though, Forger has shown that large numbers of *biochemical events* can play the same role. Thus, rapid binding and unbinding of regulatory proteins reduces the molecular noise of gene regulation. This observation of Forger's explains why fast reactions on the time scale of minutes or even seconds might be useful in regulating a process, the circadian rhythm, that runs on a time scale of 24 hours.

Another important design principle is temperature compensation: the mammalian intracellular circadian clock runs at the same rate in hibernating mammals whose body temperatures are considerably lowered. This is remarkable when one considers the extreme sensitivity of typical biochemical reactions to temperature. Of course, temperature compensation of the circadian clock is even more important in species that do not regulate their body temperatures. Forger has figured out how temperature compensation might work, and has begun testing his hypothesis on drosophila in the laboratory of Justin Blau here at NYU.

Indeed, it is in the Blau laboratory that Forger is now a postdoctoral fellow. In this setting, he is learning laboratory skills and techniques that would have been inaccessible to him if he had confined his career to the mathematical side of mathematical biology.

Forger's research has not been limited to circadian clocks: He has also done a very nice research project on bistable neurons stimulated by noise. Bistable neurons have two dynamical states, quiescence and repetitive firing. Brief stimuli, such as post-synaptic potentials, can switch the system from one state to the other. Forger has studied the situation in which the stimuli are random, and has used techniques such as spike-triggered averaging to identify the maximally effective switching signal, which has quite different characteristics for the ON transition from those of the OFF transition. Notably, this study was conducted not only on several different mathematical models of bistable neurons, but also on actual squid giant axons that were stimulated in the same manner as the models. What emerges from the study is a general model of switching in bistable neurons, a substantial achievement. Forger's experience in computational and also in laboratory neural science is particularly relevant to the part of his future research plan concerning the link between biochemical circadian rhythms and the electrophysiology of such rhythms within the suprachiasmatic nucleus (SCN).

Forger also found the time to be an exceptionally good citizen while he was a student here at the Courant Institute. For example, he was one of the organizers of a seminar in which faculty are invited to speak to graduate students about their research. This was organized in such a way that groups of faculty with related interests speak on a given day, each professor giving a short talk that naturally relates to those of the others. This has been very successful. Forger also gave substantial amounts of time to advising Stuyvesant High School students who were working on the Intel (formerly Westinghouse) science competition. In one case, Forger and I co-advised such a student, Albert Leung, who became a finalist with a project on circadian rhythms. In another case, that of Varun Narendra, Forger did essentially all of

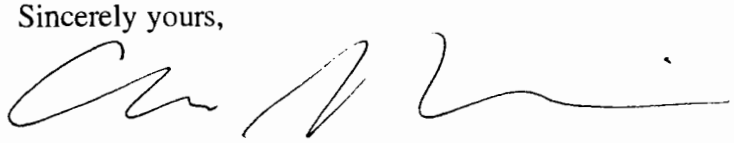
the advising by himself, and the student did a great project on mathematical modeling of Gaucher's disease. That student, too, became an Intel finalist!

Forger himself is a friendly energetic person who has tremendous drive and enthusiasm for scientific research. He is open and generous with his time and ideas. Forger is also a great speaker, whose talks truly engage the audience. I have heard him speak quite a few times, to several different kinds of audiences, and am impressed by his ability to communicate not only the substance but also the excitement of what he is doing. His ability to communicate across disciplinary boundaries is perhaps the best I have ever seen, and it is important to note that this refers to communication in both directions: Forger's ability to listen across disciplinary boundaries is as exceptional as his ability to talk across them.

Considering both his wonderful ability in giving technical talks to diverse audiences, and also his success in working one-on-one with students as in the case of the Intel competition advisees, is seems safe to predict that Forger will be a superb teacher. His lively manner and profound interdisciplinary insight will make him an inspiration to his students.

By creating the most detailed and realistic model yet of the mammalian intracellular circadian clock, Daniel Forger has made a major scientific contribution in his Ph.D. thesis. When this work is put in the context of his other research and professional activities, detailed above, it is clear that Forger is already a world leader of the circadian rhythms community. This field is an important one for practical reasons (jet lag, human performance in relation to sleep, ...) as well as scientific ones (gene regulation, interaction of biochemical and neuronal networks, coordination of large numbers of disparate nonlinear oscillators, ...) It is therefore a pleasure to recommend Daniel Forger for a faculty position in the strongest possible terms.

Sincerely yours,



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