Research Statement
Daniel Forger

My research is devoted to understanding biological clocks. I use techniques from many fields, including computer simulation, detailed mathematical modeling and mathematical analysis, to understand biological timekeeping. My research aims to generate predictions that can be experimentally verified.

Design Principles of Genetic Clocks

In 1960, Goodwin proposed the first mathematical model of a cellular clock. In this model, mRNA (X) codes for a protein (Y) which when activated (Z), can inhibit the production of the mRNA X. Equations for the rate of change of the concentration of X, Y and Z are given below:

\[
\begin{align*}
\frac{dX}{dt} &= f(Z) - \nu X, \\
\frac{dY}{dt} &= \alpha X - \beta Y, \\
\frac{dZ}{dt} &= \rho Y - \gamma Z
\end{align*}
\]  

(1)

where \(\nu, \beta\) and \(\gamma\) are the rates of clearance of the mRNA (X), inactive protein (Y), and active protein (Z). The production rates of the inactive and active protein are given by \(\alpha\) and \(\rho\). The nonlinearity \(f(Z)\) describes how the activated protein Z regulates the production of mRNA X.

Feedbacks networks similar to, but often more complex than, the Goodwin oscillator regulate most genes. Understanding which biochemical mechanisms cause oscillations in these networks is fundamental to understanding the basis of cellular timekeeping. It is one of the most studied problems in mathematical biology, and mainly studied with linear theory. I have recently proved several results about this system, and generalizations of it.

One such result is that if system (1) oscillates, the period of this oscillation is:

\[
\tau = \frac{2\pi}{\sqrt{\gamma \beta + \beta \nu + \nu \gamma}} \sqrt{\frac{\sum |a_j|^2 j^4}{\sum |a_j|^2 j^2}}
\]

(2)

where \(|a_j|\) is the magnitude of the jth Fourier coefficient of \(Z(t)\). Also:

\[
\left\langle \frac{df}{dZ} \right\rangle \left\langle \frac{Z}{f(Z)} \right\rangle = 1 - \left( \nu + \beta + \gamma \right) \left( \frac{1}{\nu} + \frac{1}{\beta} + \frac{1}{\gamma} \right)
\]

(3)
where $<>$ is the mean and $\left<\frac{df}{dZ}\right>_d$ is the weighted average derivative $rac{\int_a^b \frac{df}{dZ}\left(\frac{dZ}{dt}\right)^2 dt'}{\int_a^b \left(\frac{dZ}{dt}\right)^2 dt'}$.

These mathematical results have the following strong biological implications. (2) implies that the shortest possible period which can be observed in the Goodwin system is:

$$\frac{2\pi}{\sqrt{\gamma \beta + \beta \nu + \nu \gamma}}.$$  Important biological factors, such as transcription or translation rates, do not affect this minimum. As oscillating solutions of $Z(t)$ become less sinusoidal (i.e. contain higher harmonics), the period lengthens. The right hand side of (3) is $\approx -8$. The left hand side can be thought of as a measure of the sensitivity of $f$ to $Z$. Few known biochemical mechanisms are known to give such large magnitude sensitivity. This may explain why few genetic feedback networks show oscillations. Special biochemical mechanisms, with high sensitivity, are needed to achieve this sensitivity in order for genetic clocks to oscillate.

By studying the biochemistry of transcription regulation in circadian clocks, I have discovered a design principle that may provide the sensitivity required for oscillation. In these clocks, the repressors bind tightly to activators to stop transcription. Relatively simple mathematical analysis of this mechanisms shows that high sensitivity can be seen when the repressor and activator concentrations are similar. The report of this work is currently under revision at PNAS.

I have also found counterexamples to several supposed design principles of genetic clocks. A recent paper claimed that certain designs of genetic clocks acted as resonators (by being close to a Hopf bifurcation) and others acted as integrators (by being close to a SNIC bifurcation). With my post-doc Emery Conrad, we showed that these designs, as well as many others, could exhibit either behavior or bifurcation. The Journal of the Royal Society Interface recently published this work.

With a NIH R01 grant (scored in the top 1% of grants) Alex Ninfa and I are testing these design principles in living cells. Members of his lab are testing designs of several genetic networks, based on the design principles described above. In particular, we are building a genetic feedback loop with the promoter saturation to test whether it can promote oscillations. We have also tested which biochemical parameters determine the period of a previously built synthetic clock. We have also designed and built a developmental switch, based on two interlocked positive feedback loops. This switch is remarkably robust and operates at a wide range of parameter values. We plant to submit this work for publication soon.

Mathematical Modeling of the Mammalian Circadian Clock

Circadian (~24-hour) clocks control the timing of many processes within our body including sleep, hormone release and metabolism. Understanding the behavior of this clock has practical applications in the treatment of several key diseases including:
Alzheimer, Diabetes, Sleep Disorders and Cancer. The work above described research on design principles; here quantitative predictions are especially when designing treatments for disease or schedules for shift work. To make quantitative predictions, I was recently funded as an Air Force Young Investigator to develop biologically accurate models of circadian clocks.

My main goal in this research is to model the network of ~20,000 neurons in the suprachiasmatic nucleus (SCN) of the brain, which acts as a central pacemaker and directs timekeeping throughout the body. The basic units of this model are mathematical descriptions of 1) the biochemistry of circadian timekeeping within individual SCN neurons and 2) the electrical signals, which SCN neurons use to synchronize with each other. With efficient numerical methods, these two basic units can be combined to simulate the collective behavior of the whole SCN.

1) The basic building block of this model is a mathematical description of the timing system within a cell. Originally developed with Charles Peskin, recent versions of this model contain more accurate descriptions of the detailed biochemistry (particularly transcription regulation and post-translations modifications), and have been fit to experimental data using improved search (Nealder-Mead) algorithms. Deterministic (ODE) and stochastic (Kinetic Monte Carlo) formulations of the model have been developed. With hundreds of chemical species to track, the model is one of the most biologically detailed mathematical descriptions of any cellular system.

Several collaborations with experimentalists have tested model predictions. One particularly important model prediction concerned Familial Advanced Sleep Phase Syndrome (FASPS), a genetic disorder that causes patients to wake up very early. The model predicted that, contrary to a widely held view, FASPS was caused by increased (rather than decreased) kinase activity. Data from the Virshup lab confirmed this prediction. These results, published in PNAS, were featured in many newspaper articles and even a television news story. The Faculty of 1000 selected it as a “Must Read.” Future work is now being funded by a collaborative NIH R01 grant (scored within the top 3% of grants).

2) With Michigan undergraduate Choon Kiat Sim, I developed the first mathematical model of the electrical activity of SCN neurons. The model contains a similar structure to the Hodgkin-Huxley model, but with an additional calcium current. By carefully reading over 100 experimental papers, we were able to obtain precise measurements for many of the model’s rate constants. The rest were fit in a similar way to the Nobel Prize winning method of Hodgkin and Huxley. Of particular interest is the mathematical structure of the model, which contains a stable steady state and a stable limit cycle in close proximity. We showed that this can allow signals to start or stop the firing of SCN neurons. To date, five experimental groups have offered collaborations to test model predictions. When the Journal of Biological Rhythms published this paper, it was the most read original research article in the journal.
My current research seeks to join our biochemical model of the SCN and the
electrophysiological model of SCN neurons to develop a biologically accurate multi-scale
model of the SCN. This new model tracks the behavior of every molecule within the
biochemical clock, as well as every electrical signal (action potential) of every SCN
neuron. Bioinformatics graduate student Casey Diekman and I have developed a model
of this network of ~20,000 neurons. Two behaviors typically emerge in these network
simulations. If the intracellular circadian clocks of SCN neurons indicate approximately
the same time of day throughout the network, the neurons quickly join one of a small
number of subpopulations that send off electrical signals synchronously. If, however, the
intracellular circadian clocks disagree, firing throughout the network appears random.
We are currently exploring the causes of this behavior and its biological implications.

On a 24-hour time scale, post-doc Richard Yamada and I have developed approximations
of the average electrical behavior of SCN neurons with the intent to couple the
intracellular circadian clocks within the ~20,000 neurons of the SCN. The stochasticity
of intracellular reactions is key to reproducing the behavior of the overall network. In our
research thus far, we have found that some coupling mechanisms can exploit this
stochasticity to allow for noise-induced oscillations. This may explain why circadian
clocks oscillate in the presence of genetic mutations, even when our deterministic models
predict that oscillations should cease. The Takahashi lab has tested several of these
predictions. We plan to submit a high impact paper soon.

Computational Neuroscience

Over 50 years ago, Hodgkin and Huxley published a model of the electrical behavior of
the squid giant axon. This Nobel Prize winning model has become the cornerstone of
computational neuroscience and is the subject of thousands of studies. When I was a
graduate student, I had an opportunity to test the model’s most basic prediction: As the
applied current to the axon increases, the system goes through a subcritical Hopf
bifurcation, which leads to oscillations. Amazingly, the actual squid giant axon did not
display this behavior and never exhibited oscillations.

In a recent collaboration with electrophysiology John Clay (NIH) and neurologist
David Paydarfar (U. Mass), we remeasured and refit the ionic currents in the axon. We
discovered that the linear approximation used to fit the potassium current was not
accurate. Using a more accurate relationship (the Goldman-Hodgkin-Katz equation), we
were able to refit the model and reproduce the actual behavior seen in the squid giant
axon. The Journal of the Royal Society Interface recently published our findings.

Depending on initial conditions, many neurons can exhibit either 1) repetitive firing of
action potentials or 2) quiescence. In the presence of noise, these neurons can display a
pattern of “Random Bursting” where the neurons alternate between these states.
Paydarfar, Clay and I studied this behavior in both squid giant axons and several
mathematical models. In our research, we determined what properties of the input signal
to a squid giant axon cause transitions from repetitive firing to quiescence and what
properties of the input signal cause transitions from quiescence to repetitive firing.
Moreover, we also found several interesting properties about the number of action potentials that are fired in each burst and what these bursts encode. The Journal of Neurophysiology published this work.

We next turned to a general problem of neuronal computation: “How does the shape of an input stimulus affect the firing of a neuron?” We first demonstrate that how a neuron can be surprisingly precise in distinguishing between stimulus shapes. We find that the optimal stimulus shapes to excite a neuron are complex and depend on network properties. Different optimal shapes are found when a neuron is within an excitatory network or in an inhibitory network. The time scale of post-synaptic potentials is also a key factor in determining the optimal signal shape. We validate our experimental results using the calculus of variations on the Hodgkin-Huxley equations: we were the first to calculate optimal stimulus on these stiff and complex equations. These results have broad implications, and high impact, particularly for researchers studying neuronal computation and the design of medical devices used in Deep Brain Stimulation to treat Parkinson Disease. This research has recently been submitted.