Multi-scale Modeling of Circadian Rhythms: From Metabolism to Regulation and Back

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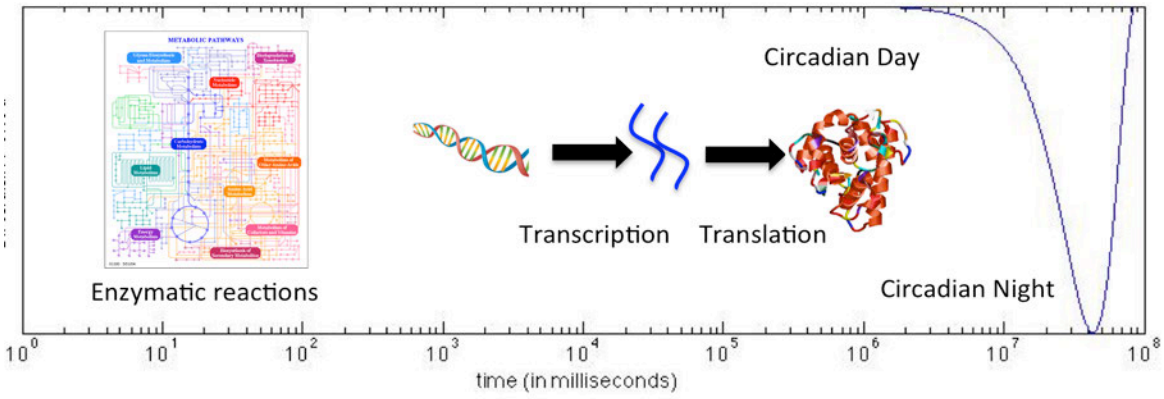
Goals: The goal of this research is to develop and implement a new computational and theoretical method for modeling biological systems that fills a gap in modeling mass action dynamics. Based on statistical thermodynamics, the method bridges data-poor scales (parameters for mass action kinetics) and data-rich scales (chemical potentials of metabolites, and metabolite, protein & transcript data) to enable predictive modeling from enzymatic reactions (10^{-3} to 10^{0} s^{-1}) to gene and protein regulation (~20 minutes) to circadian rhythms (24 hours). We are:

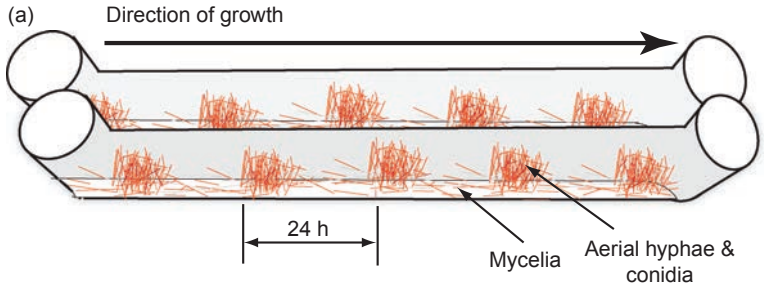
- Implementing an approach to the law of mass action that uses chemical potentials rather than rate constants. This approach involves a rescaling of the fast degrees of freedom, resulting in a compression of the time-dependence to fewer relative scales. Steady state processes can be 'telescopically' modeled to address the scale of interest while collapsing faster scales.
- Using the new method to understand the relationship between central metabolism and circadian rhythms in *Neurospora crassa* by using a multi-scale model of metabolism that will include regulation of the circadian clock.

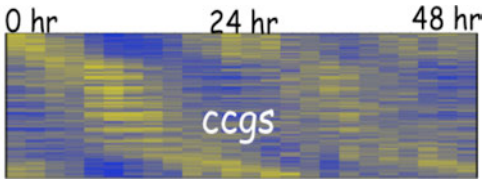
Abstract. Predictive modeling relies on solving equations in which the necessary equation parameters are either based on first principles, such as Hamiltonian systems, or on empirical data such diffusion constants or rate parameters. This requirement has hampered the predictive modeling of biological systems in that the relevant scales (e.g. those in metabolism) are too large and complex to be modeled by first principles, and the necessary rate constants are not generally available. We will address this challenge by implementing a new approach to the law of mass action that does not require rate parameters but instead uses chemical potentials (1). This new approach is possible because of advances in statistical thermodynamic methods in the last 20 years. Due to the statistical formulation of the theory, the tools are capable of direct integration of metabolomics and proteomics data. We will use these tools to fundamentally understand the relationship between metabolism (2) and molecular circadian clocks with regard to the role of the circadian clock in increasing the metabolic efficiency of the cell (3).



Timescales that the simulations using statistical thermodynamics will cover. Enzymatic reactions occur on the millisecond to second timescale while gene and protein expression occur on the minute to ~30 minute scale and the circadian rhythm occurs over a period of 24 hours.

Circadian clocks lie at the epicenter of cellular physiology for both fungal and mammalian cells, both of which share clocks with equivalent regulatory architecture (4). At the core of these clocks, a heterodimeric transcription factor (TF) drives expression of genes whose protein products feed back, physically interact with, and depress the activity of their heterodimeric activator. This negative feedback loop, yielding oscillatory TF activity, is the basis of fungal and animal circadian rhythms. Output from the clock occurs when these TFs regulate genes whose products do not impact the core feedback loop. Cellular clocks in mammalian cells regulate ~15% of genes. In the aggregate nearly all human genes are clock-regulated in some cell type, yielding the profoundly rhythmic metabolism that is characteristic of humans and that has a major impact on both normal and disease physiology including sleep/wake cycles. *Neurospora crassa* is the best studied cellular circadian system and is a well-established model for eukaryotic including mammalian clocks. In addition, *Neurospora* is engineered to overproduce cellulases, essential components in biomass deconstruction for biofuel precursors. *Neurospora* provides an extremely tractable system in which to pioneer modeling of these cellular clocks and their influence on metabolism. As shown below, stages in the circadian cycle are well marked by visual observation and transcriptomics. All *Neurospora* genes encoding enzymes have been mapped to their corresponding steps in metabolism. In a data set well beyond anything available in any other circadian system, the entire assemblage of clock-controlled genes has been described, and data are in hand to delineate all clock-controlled proteins, including enzymes, and the clock-controlled metabolites to which they give rise (5). Each step from gene to protein to metabolite is regulated and the entire assemblage can be modeled using this unparalleled data set.





Heat map showing rhythmic expression of the *Neurospora* genome over 48 hrs in constant darkness.

References

1. Cannon WR & Baker SE (2016) Non-Steady State Mass Action Dynamics Without Rate Constants: Dynamics of Coupled Reactions using Chemical Potentials. *submitted*.
2. Dreyfuss JM, *et al.* (2013) Reconstruction and validation of a genome-scale metabolic model for the filamentous fungus Neurospora crassa using FARM. *PLoS Comput Biol* 9(7):e1003126.
3. Hurley JM, Loros JJ, & Dunlap JC (2016) The circadian system as an organizer of metabolism. *Fungal Genet Biol* 90:39-43.
4. Baker CL, Loros JJ, & Dunlap JC (2012) The circadian clock of Neurospora crassa. *FEMS Microbiol Rev* 36(1):95-110.
5. Hurley JM, *et al.* (2014) Analysis of clock-regulated genes in Neurospora reveals widespread posttranscriptional control of metabolic potential. *Proc Natl Acad Sci U S A* 111(48):16995-17002.

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