Multiscale Modeling of Circadian Rhythms

The goal of this project is to use a novel method, coupled reaction theory, to bridge data-poor (parameters for mass action dynamics in metabolism) and data-rich scales (chemical potentials of metabolites, and metabolite, protein & transcript data) to enable predictive modeling across scales from enzymatic reactions (10^{-3} to 10^{0} s^{-1}) to gene and protein regulation (~20 minutes) to circadian rhythms (24 hours).

Model Credibility Plan

Our model credibility plan is focused on uncertainty quantification and sensitivity analysis. The largest uncertainties are due to the simulation parameters, which in this case are the chemical potentials of the metabolites. Chemical potentials are calculated from standard free energies of formation in solution using either component contribution methods or electronic structure calculations. Experimentally determined parameters are available for reactions of central metabolism, but these are *in vitro* assays, and *in vitro* conditions likely do not match *in vivo* conditions.

List of Planned Actions

Year 3 Actions:

- Evaluate the sensitive of metabolite level predictions to variances within the 95% confidence interval of the parameter space for standard free energies of reaction. Standard free energies of reaction are the main parameters used in these new models. NIST has compiled an accurate database of standard free energies of reaction for reactions of central metabolism. However, for other reactions, these parameters need to be estimated computationally using quantum chemistry or group contribution methods. We will evaluate the variability of the predictions from the simulation model due to variances in the standard free energies of reaction. The variance model to be used is the 95% confidence interval.
    - *Information obtained*: Dependence of predicted metabolite concentrations on uncertainties in the parameters.
    - *How this leads to a credible model*: Low variability in the predictions based on the uncertainties in the parameters provides credibility to the model outcomes.
- Evaluate the sensitivity of metabolite level predictions to uncertainties in the cells ionic strength. The parameters used in the simulations depend on the ionic strength of the surrounding environment. Cells can have varying ionic strengths depending on the solute and the size of the compartment in which they are found. This sensitivity analysis will establish the range of variability in the predictions due to uncertainties in the cell environment.
    - *Information obtained*: Dependence of parameters, predicted metabolite concentrations and reaction fluxes on variability of the ionic strength of the cell interior and its compartments.
    - *How this leads to a credible model*: Low variability in the predictions based on the uncertainties in the cell environment provides credibility to the model outcomes and phenotypes.

- Evaluate uncertainties in the metabolite levels, reaction fluxes and entropy production rates due to changes in reaction rate constants. Developing mass action ODE's for metabolic reactions has been hampered by the lack of available rate parameters. The method used in these models allows one to infer rate constants based on biological and physical principles. The idea is that the fitness landscape on which organisms compete and are selected constrains rate parameters to a narrow window which is bounded by biological and physical constraints. This evaluation will test that hypothesis.
    - *Information obtained*: Dependence of predicted metabolite concentrations, reaction rates, entropy production and total free energy over a wide range of rate constants.
    - *How this leads to a credible model*: That natural selection is based on maximizing entropy production rates has been proposed and discussed for nearly 100 years. However, the concept is notoriously difficult to test, in part because of its abstract nature. We will test these principles while recognizing that there are critical biological and physical constraints that need to be accounted for. We will provide tangible details of what it means to maximizing entropy production rates, and the consequences, favorable or unfavorable, for biological systems. These tests should help define the limits and assumptions of this principle.

Progress to-date: In September 2018, we successfully implemented and tested our first prototype code for carrying out tests of parameter variability (standard free energies of solvation) and variability in the cell environment.

CPMS Ten Simple Rules Table.

Number	Rules	Actions/Activities
1	Define context clearly	The context of the multiscale model has been defined to be that for simulation of coupled metabolic reactions. This is stated in publications and in the code repository.
2	Use appropriate data	The minimal data needed for the model can be generated in the code or acquired from third party tools (eQuilibrator or COBRA Toolbox). Additional data sources are being developed.
3	Evaluate within context	The model is being evaluated for its ability to produce reasonable reaction rates and metabolite concentrations for reactions of metabolism. At this point, the model has not been extended or evaluated beyond metabolism. It is not an appropriate modeling method for abiotic processes.
4	List limitations explicitly	We specifically list assumptions of the model in our manuscripts and README file in the code distribution.
5	User version control	We use Github for version control. Project code is available at https://github.com/PNNL-CompBio/Boltzmann
6	Document adequately	Documentation is on-going and when provided for MSM evaluation, the model simulation codes will be sufficiently documented.
7	Disseminate broadly	We have provided our model as (1) in an open access publication (2) containing computational notebooks (Jupyter) that describe data analysis and modeling concepts and (3) provide links to all the data and code on Github. We will follow the same process when we publish our credibility study.
8	Get independent reviews	Our model simulation and optimization codes are being evaluator by a third-party evaluator with expertise in mathematical biology.
9	Test competing implementations	We hope to develop and evaluate C and a version of in either MATLAB or Python to test consistency across different implementation.
10	Conform to standards	We will conform to SBML standards for models and IUPAC standards for thermodynamic parameters.