How Will Kinetics and Thermodynamics Inform Our Future Efforts to Understand and Build Biological Systems?

Nucleases: Up, with a Twist

Jennifer Doudna
University of California, Berkeley

When I started graduate school, we were taught that every protein has a single unique three-dimensional structure that defines its function. Thirty years later, it is now clear that many proteins include domains that assume different structures in response to binding partners or small molecules. Likewise, RNA molecules can form kinetically trapped intermediates that slowly refold into their functional forms. Only by discovering such kinetic and thermodynamic properties of molecules can we hope to understand molecular structure at a level necessary to engineer it. And we don't truly understand molecular behavior until we can engineer it.

A great recent example of this in my own lab is the finding that the CRISPR-Cas9 enzyme, an RNA-guided protein that operates as part of a bacterial adaptive immune system, cleaves DNA by using a mechanism of coordinated conformational changes. Cas9's recognition of a short DNA sequence (the "PAM") triggers local DNA unwinding and concomitant guide RNA-DNA hybridization. In turn, cognate base pairing along the length of the 20 base-pair RNA-DNA hybrid favors a conformational change in which the enzyme active sites are positioned for double-stranded DNA cutting. Understanding the kinetics of these protein structural changes, as well as the thermodynamics of RNA-DNA helix formation, are key both to understanding this amazing molecular machine and engineering it for precision genome editing.

Biology Built Outside the Cell

Roy Bar-Ziv
Weizmann Institute of Science

Feynman's famous quote "What I cannot create, I do not understand" can be regarded as an oxymoron when it comes to "building" a biological system. How can we possibly assemble a cell de novo, as opposed to manipulating a living cell? Estimates have put forth that a few hundred essential genes encapsulated inside a membrane vesicle, along with a minimal set of purified enzymes and metabolites, may suffice to boot up a cell. Arguably, however, there is more to building a cell than knowing the genes. We need to understand how the chemical and spatial organization of macromolecules in the cell optimizes the conditions for basic functions, such as machine assembly and self-replication.

Cells evolved to operate hundreds of parallel reactions in crowded compartments, with energy consumed to maintain order against entropy. One fundamental question and a challenge for the building of biological systems is just how a new ribosome is made from an old one. Reconstituting such a complex pathway outside a living cell calls for re-creating cellular conditions, for example, coupling the synthesis and assembly of ribosomal components in both time and space to maximize precision and yield. Recent work on the assembly of dense two-dimensional DNA compartments enables us to study spatiotemporal dynamics and collective behavior of gene expression, toward self-assembly in synthetic biological systems.

To Model What Is Measured

Johan Elf
Uppsala University

Kinetics and thermodynamics define the fundamental constraints that limit biological modeling to the physically possible. Such bounds are critical because questions regarding biological systems are often mercilessly open-ended, and the prospects of answering them are often hampered by the lack of sufficiently accurate measurements. Fortunately, more often than not, even simple kinetic, stochastic, and spatial considerations make it possible to discard seemingly sound cartoon models in favor of more probable alternatives.

In addition to the obvious aspect of adding a solid framework for rational reasoning about system models, kinetics and thermodynamics are key components in making quantitative predictions of what should be expected from experiments if a model is true, and more importantly, how accurate the measurements need to be to know whether the model is wrong. Prediction of specific experimental results must, however, go further than modeling the expected behavior of the biochemical processes, to include modeling the experimental system (e.g., the dynamics of any reporter molecules, the noise in the detection system, and the averaging over cells or molecules in different states).

As we model complex biological phenomena with increasing detail, the quantitative predictions of expected experimental results have to be sharpened alongside the measurement accuracy itself. In this sense we have a lot to learn from particle- or astrophysics, in which striking quantitative predictions have pushed the development of the measurement technology for a long time.

CellPress

Cell-Free Molecular Dissection

Vincent Noireaux
University of Minnesota

The precise characterization of complex dynamical interactions in biological networks holds great promise for both understanding the fundamentals of living cells and engineering biological systems in a controlled manner. Some central principles have found consensus, such as robustness through feedback and redundancy, but our ability to direct living systems by integrating molecular components into predictable and robust synthetic biological networks is still limited. One of the bottlenecks to this goal is our current approach to modeling such systems.

The theoretical analysis of networks in vivo often relies on rough estimates of many parameters or specific assumptions about molecular mechanisms. It is difficult to determine accurate kinetic and thermodynamic values describing the entangled nonlinear biological processes in a cell. While powerful predictive thermodynamics models have been proposed, novel experimental approaches are needed to get realistic estimates of kinetic parameters and develop authentic knowledge of biological networks dynamics.

Cell-free systems have become reliable platforms for tackling such quantitative descriptions. In vitro construction of biomolecular networks with known components has proven useful for capturing reaction mechanisms governing system behaviors. Together with computational modeling, cell-free systems demonstrate strong potential for systematically and accurately describing elementary biological networks and for unraveling hidden mechanisms that are hard to gauge in vivo. The new generation of cell-free platforms being developed can have a major contribution to engineering predictable biological systems.

Model Twice, Experiment Once

Julien Berro
Yale University

New hypotheses in biology are traditionally discussed over hand-drawn cartoons. As telling as a cartoon can be, it is not uncommon for the depicted mechanisms to violate the realities of stoichiometry, number of molecules, energetics, time dependence, or even geometry and dimensions. Theoretical and computational models taking into account kinetics and thermodynamics extend cartoon models and are informative at all stages of the research process.

The biology community is already getting accustomed to developing quantitative models after performing an experiment, in an effort to fit and interpret experimental data or evaluate their compatibility with existing hypotheses. Developing such models before performing experiments is even more powerful. Pre-testing hypotheses in silico avoids wasting precious reagents and time at the bench, for example, by rejecting hypotheses that violate elementary principles of thermodynamics or by helping researchers design experiments and identify conditions that will produce the most informative data able to discriminate between alternative molecular mechanisms.

Mechanical engineers routinely use computer simulations to design and test prototypes before building cars and planes. Best practice in carpentry is to measure twice and cut once. With growing amounts of kinetics and thermodynamics information, biologists will model twice and experiment once—and this ratio is probably off by at least an order of magnitude.

Design Principles at Work

Leonor Saiz
University of California, Davis

The quest for understanding and building biological systems often seeks to uncover the key design principles at work. Why are systems "designed" the way they are? Answers to this question often provide avenues to imitate nature in developing new systems as well as fundamental knowledge for altering existing ones. At the center of most answers, there is evolution. But evolution has to play within the thermodynamic constraints. These constraints are responsible for phenomena as widespread as non-specific interactions, noise in transcription regulation, and errors in DNA replication.

Evolution has devised complex mechanisms to efficiently function within the thermodynamics confines. DNA looping, for instance, effectively increases the strength of the specific binding of transcription factors without the non-specific side effects and without using energy currencies like ATP. These constraints can also simplify the description of many systems, as illustrated by recent modular approaches that faithfully characterize complex gene regulation systems with just a few parameters. Kinetic mechanisms can be stacked on top of thermodynamic processes to increase efficiency, such as the reduction of thermodynamic errors in kinetic proofreading, or to obtain more complex types of behavior, such as oscillations and adaptation in signal transduction.

The key for the success of our future efforts is the multi-scale integration of these principles in combined experimental-computational approaches across levels of biological organization.

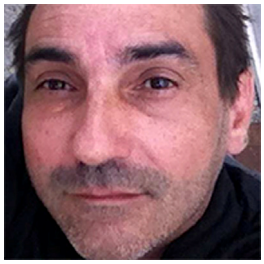
Cell Life: Flow of Energy

Dimitrios Vavylonis
Lehigh University

Thermodynamics, kinetics, and mechanics govern the statistical behavior of biomolecules and are central to understanding how cells work. Cellular functions rely on specificity of molecular interactions, forces, self-organization, and feedback control mechanisms. Constant flow of energy and cooperative interactions among biomolecules are essential elements for sensing and response, adaptability, and structure formation at the micron scale. This is the case in subcellular systems such as membrane trafficking, cytoskeletal networks, Rho GTPase signaling, chromosomal organization, and intracellular phase separation. An ongoing challenge in developing a deeper understanding of these phenomena is quantifying the key mechanistic interactions at both biochemical and collective levels. A large community of researchers, to which I belong, use the feedback among quantitative cell experiments, modeling, and in vitro analysis and reconstitution to make progress on this front.

What about molecular-level physics and chemistry in larger systems: multicellular, communities, the brain, etc.? They should be very relevant there too: some aspects of these systems are optimized around a biochemical or physical limit (such as efficiency of oxygen delivery) while others are specifically robust or flexible with respect to molecular composition and interactions. Following evolution's mechanisms, synthetic biological systems could be constructed to exhibit similar properties when built layer upon layer starting from kinetics and thermodynamics.

Machine Learning Kinetics



Jean-Loup Faulon
INRA, Jouy-en-Josas; University of Manchester

The principles of engineering are now an integral part of biological sciences. For instance, we have engineering recipes available for the biosynthesis of hundreds of molecules. Yet, the bio-production process is costly; few products have reached the market and much effort is still dedicated toward increasing bio-production yield. That endeavor requires precise handling of thermodynamics and enzyme kinetics.

Biochemical engineers wish to identify thermodynamically feasible pathways and efficient enzymes catalyzing them. That information is sometimes difficult to retrieve from the existing heterogeneous databases. This is a job that, in turn, machine learning handles well. There are plenty of examples in which machine learning has been successful with biological data. Some of these studies were focused on searching for enzyme sequences catalyzing desired reactions and inferring enzyme kinetics parameters and reaction thermodynamics feasibility.

Beyond these initial attempts, machine learning could also be used to exploit both the successful and the not so successful data that biochemical engineers are acquiring on a daily basis.

Ultimately, one could foresee the building of biology systems being driven by an active machine learning process that would learn from data acquired in a first round of experiments, including failure data, and determine the key components (e.g., specific reactions and enzymes yielding increased performances) to be built and tested in a second spin of an iterative cycle between data acquisition and learning.

Strong and Weak Interactions

Polly Fordyce
Stanford University

Biological systems make extensive use of weak and transient interactions to dynamically regulate and modulate cellular behaviors. In our efforts to understand and build biological systems, we often focus on only the strongest and most long-lived molecular interactions. This focus stems, at least in part, from limitations in current measurement technologies. Incorporating redundant weak interactions into designed systems would likely enhance both robustness and evolvability by mimicking natural systems more closely but requires technological advances to illuminate the weak interactions that have traditionally been the most elusive.

Powerful screening platforms have revealed the vast array of molecular parts in the cell and begun to map the strongest interactions between them. We must now develop technologies that move beyond mapping the cast of characters and toward quantifying their interaction strengths and time-scales with a wide dynamic range. This is particularly true when considering reaction kinetics. Although cells are inherently non-equilibrium systems, nearly all high-throughput biophysical technologies are restricted to measuring interactions at steady state. With this critical thermodynamic and kinetic information in hand, we can then develop quantitative, predictive models of how interactions drive behavior. By including the full spectrum of interactions—both strong and weak—we will enhance our ability to both explain phenomena we observe and design phenomena we desire.