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What can biochemical networks compute?

Limits on the computational expressivity of non-equilibrium biophysical processes
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Recommended with a Commentary by Krishna Shrinivas, Northwestern University

Inspiration

Living cells must constantly make choices and decisions. These range from an *E. coli* choosing which way to swim to increase odds of finding food (glucose) to human immune cells that must discriminate between foe (pathogen) and friend (self). Much of this information processing is driven by interactions and reactions between biomolecules in the crowded cellular environment. A natural question follows: what strategies enable, and how do physical processes constrain, this form of biological computation?

This has led to inquiries that have provided important insights over the years, with far-from-equilibrium operation of biochemical cascades (from ATP hydrolysis for example) playing a central role. For example, Ninio and Hopfield (*1*, *2*) proposed that kinetic proofreading pathways could use energy consumption to introduce delays in reactions to improve discrimination amongst competing binding targets. In his original paper, Hopfield notes: *"In each case, known reactions which otherwise appear to be useless or deleterious complications are seen to be essential to the proofreading function."* Over the years, this framework has been employed to understand how our DNA is replicated with minimal errors, how proteins are made correctly, and in fact, how a human immune T cell tells apart a friend from foe (*3*). Similarly, Goldbeter and Koshland (*4*) showed that the balance between protein states – for example the addition and removal of phosphorylation – can be driven out of equilibrium to transform graded input signals into sharp changes in the state of the protein. Here, sensitivity to input signal can arise from dynamic, and indeed futile, cycles of reversible reactions rather than evolved cooperativity or allostery from a molecule's stable structure. These frameworks thus help expand our understanding of *where* and *how* information processing occurs in living cells.

Comment

In this comment, I recommend the paper by Floyd and coauthors that follows this tradition of inquiry to pose the following question: if a biochemical cascade must use information (from input stimuli) to make decisions (run output programs), what are the capabilities and limits to this *biochemical classification*? To explore this, they introduce a generic description of reaction cascades as Markov jump processes. Briefly, the model is characterized by multiple states (protein conformations or phospho-states) and reactions between states, explicitly including irreversible energy-consuming ones. Starting off at a particular state, the Markov process stochastically jumps between connected states, with the likelihood of the jump related to reaction rate. Over time, the system settles into a steady-state distribution that describes the relative probability of being in each state. In their framework, input signals bias transitions between a pair of states – for example, recognition of extracellular glucose could tilt the balance between phosphorylated versus unphosphorylated protein states, and different inputs can bias different reactions. High occupancy of an output state models the subsequent decision, perhaps through the establishment of downstream gene regulatory programs. To achieve a desired classification task, the authors use a learning algorithm inspired by contrastive learning techniques (*5*) to train their model. How good of a classifier, or expressive, can such a biochemical cascade be?

At steady state, the occupancy of all states of *any* Markov model can be described using the Matrix Tree theorem. At its essence, this theorem calculates the probability of being in a particular state by carefully counting how many ways there are for reaction fluxes to flow into that state relative to all other states. As an analogy, if the model describes a group of cities (states) connected by highways (transitions or reactions), the theorem helps estimate the fraction of time a wandering traveler spends in each city by accounting for global connectivity and the varying rates of traffic along every highway. Using this theorem, the authors show that when any input signal biases a reaction, the occupancy of *any* state can only change in one direction, i.e. changing the rate of flow of traffic along a particular highway can only change the fraction of time spent at any city up or down but not both. This limits any output–input response function to be purely *monotonic.* Hence, no band-pass filters or other non-convex decision boundaries are possible. This result holds irrespective of the size or complexity of the reaction network—in other words, it represents a hard limit.

Fortunately, not all is lost! The above result arises from a constraint on how an output state varies with input signal i.e. a derivative, when all other inputs are held constant. The authors propose to relax this latter assumption by invoking input *multiplicity* i.e. an input stimulus can *simultaneously* bias multiple reactions. For example, an input signal, say glucose concentration, can simultaneously transduce multi-site phosphorylation. In the traffic analogy, changing an input signal changes rates of traffic along multiple different highways simultaneously. With this feature, they show that non-convex classification tasks are within reach through numerical experiments. Intriguingly, the number of turns such an input-output response function can

potentially take - one way to estimate how complex a decision boundary can be modeled, grows linearly with multiplicity. Crucially, the authors find that enhanced flexibility still fundamentally relies on non-equilibrium driving forces and equilibrium parameters alone are insufficient.

Beyond this flexibility, the authors share a vignette on how biochemical cascades provide *sensitivity* through assaying sharpness in response. Through a scenario modeled on the Goldbeter-Koshland cascade, they show that network topology (the possible sets of reaction transitions) can dramatically alter the potential steepness in response. Densely connected networks where states can transition to each other are less favorable to ones where the set of transitions are much sparser. Intuitively, this arises from the ability to find direct strong routes from inputs to outputs – more likely in a sparsely connected map of highways than a densely interconnected traffic pattern – which can be exploited to amplify or sharpen responses.

Perspective

Overall, this paper adds to a growing body of work that clarifies capability and constraint in biophysical computation. Recent studies highlight that reversible binding can possess remarkable versatility and flexibility in input-output classification (*6)*. Away from thermodynamic bulk limits, Poole and colleagues outline how stochastic noise from molecular fluctuations can be harnessed for computation in particular types of reaction networks (*7*). Beyond reactions in well-mixed systems, phase transitions and self-assembly that arise from multicomponent molecular interactions can drive classification through spatial patterning of macrostates (*8,9*).

Finally, in this paper, the authors highlight an interesting analogy between Markovian cascades and machine learning models, specifically the attention-mechanism in neural networks called Transformers (these are the architectures that help power ChatGPT and AlphaFold). In both cases, comparison (or "attention") occurs between competing alternatives: input tokens in a neural network or spanning trees corresponding to distinct reaction pathways in a Markov process. In the biochemical setting, effective feature representations are learned as non-linear products of reaction fluxes along these pathways. However, unlike transformers, in Markov models, the space of learnable encodings is constrained by network topology, kinetics, and steady-state thermodynamic constraints. In parallel, emerging work on non-linear electrical resistor networks (*10*) shows that these physical, analog systems are capable, in principle, of approximating any continuous function, much like certain types of neural networks.

There remains more to be learned about where and how computation can occur in physical and living systems.

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