SYSTEMS BIOLOGY

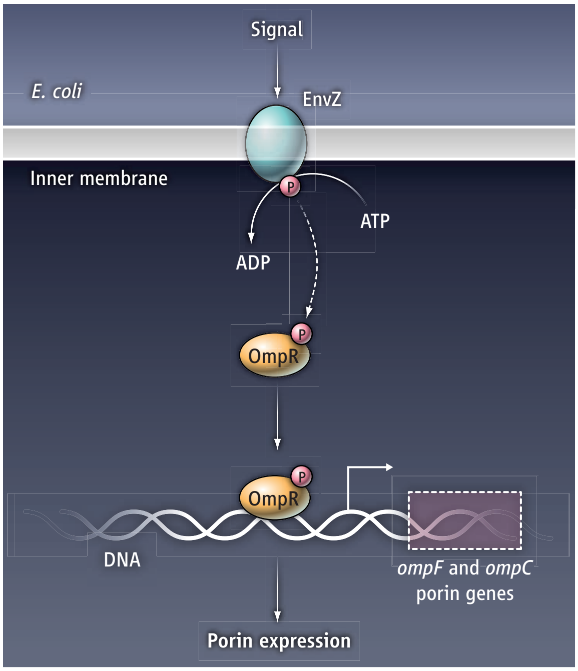
Biological Systems Theory

The robustness of a biochemical network can be inferred mathematically from its architecture.

Jeremy Gunawardena

Mathematical models are fashionable in systems biology, but there is a world of difference between a model and a theorem. When researchers build models, they make assumptions about a specific experimental setting and have to choose values for rate constants and other parameters. A theorem, by contrast, can apply to a setting of arbitrary molecular complexity, such as a biochemical network with many components. In a recent study, Shinar and Feinberg (*1*) formulate a theorem that shows when such biochemical networks exhibit "absolute concentration robustness."

Robustness is a widely used concept in biology but notorious for being poorly defined. In the Shinar and Feinberg theorem, absolute concentration robustness means that the concentration of one of the network components remains exactly the same in any positive steady state of the network. This is true no matter how much of each of the other components is present. The robust concentration value depends only on the parameters of the network, not on amounts. Remarkably, Shinar and Feinberg discovered a condition that depends only on the structure of the biochemical reaction network, and not on parameter values, under which absolute concentration robustness occurs.



Biochemical network dynamics. A two-component regulatory system allows *E. coli* to respond to changes in environmental osmolarity. The histidine kinase EnvZ responds to a stimulus by transferring phosphate from ATP to a histidine residue. Phosphorylated EnvZ then transfers its phosphate to an aspartic acid on the response regulator, OmpR. The regulator then affects gene expression. Shinar and Feinberg's theorem can be applied to show that the concentration of phosphorylated OmpR is the same in any positive steady state (absolute concentration robustness), irrespective of the amounts of network components present.

What makes this theorem of interest not just to mathematicians but also to biologists is that it distills and clarifies previous studies of bifunctional enzymes in the model bacterium *Escherichia coli* (*2*–*4*). The EnvZ-OmpR two-component osmoregulator (see the figure) and the glyoxylate bypass regulator (which allows two-carbon sugars like acetate to be used for biosynthetic purposes) both use bifunctional enzymes. In each case, the concentration of one of the key components in the biochemical network has been shown experimentally to have a high degree of robustness to changes in the amounts of the network components (*2*, *3*). With some plausible assumptions about the biochemistry, the theorem of Shinar and Feinberg explains why. Coincidentally, shortly after Shinar and Feinberg proposed their theorem, a new bifunctional metabolic enzyme (fructose 1,6-bisphosphate aldolase/phosphatase) was discovered among the archaea (*5*). Such enzymes occur in all walks of life. Thus, the theorem provides a starting point for understanding the advantages that bifunctionality might bring.

Early embryologists were astounded by the robustness of development. Conrad Waddington conceived of the developmental process as a ball rolling down a landscape of branching valleys, with distinct differentiated states at the ends of valleys (*6*). This "epigenetic landscape" arises from the underlying network of gene and protein interactions, and its ridges and contours suggested how development could be robust to perturbations that pushed the ball off course without taking it across the ridges ("canalization"). This is a form of dynamical robustness; it is very far from a steady state.

Remarkably, Waddington's intuitive conception was formalized by the French mathematician René Thom, in what became known as "catastrophe theory" (*7*). This interdisciplinary marriage was one of the highlights of the 1960s explosion of interest in theoretical biology (*8*). Waddington is now an icon of evolutionary developmental biology, and the concepts he introduced are central to the field (*9*). As for catastrophe theory, it has had its fans as well as its detractors, but its biological roots are rarely remembered. Waddington and Thom did not know the molecular details, and their mathematics could not be guided by experiments and data. That is what distinguishes the systems biology of Shinar and Feinberg from the theoretical biology of the 1960s. Now that many of the molecular components are known, we can start to explain how robustness emerges from molecular mechanisms (*1*, *2*, *4*, *5*).

So far, the steady-state theorem of Shinar and Feinberg has been applied only to bifunctional enzymes. A robust variable is an example of an "invariant": a polynomial equation in the component concentrations that holds in any steady state (*10*). It is a particularly simple polynomial because it is linear and has only one variable. However, networks can have more complex invariants, as in the case of multisite protein phosphorylation (*10*). In this case, the invariant characterizes the network structure. If it is not satisfied at steady state, then additional reactions are present and the way in which the invariant breaks down can help to identify the new reactions. What is common to both absolute concentration robustness and invariants is that mass-action kinetics, which is the most systematic way to work out the rates of production and consumption of network components, always gives rise to polynomial rate equations from which invariants can be calculated. It becomes increasingly difficult to manipulate polynomials when there are several variables and higher degrees, as happens with mass-action biochemistry, but a branch of mathematics called computational algebraic geometry provides the tools for doing exactly that (*10*).

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Absolute concentration robustness and invariants are systems properties that arise from the coordinated behavior of all the components in the network. Such integrative analysis is still in its infancy. Does some evolutionary advantage accrue to the organism from absolute concentration robustness? This property can be verified experimentally for certain networks and we can speculate about why it is needed, yet the answer lies not in the network but in the environments in which the organism exists. *E. coli* is a gut microbe. Did absolute concentration robustness evolve in osmoregulation and in utilization of two-carbon sugars because of the intestinal physiology and ecology of the gut microbiota, or is it merely a spandrel in some molecular architecture that we cannot yet perceive (*11*)?

"Systems" thinking has always been present in biology, even if its importance has waxed and waned with changes in experimental capabilities. The disciplinary histories of embryology, ecology, genetics, physiology, immunology, and neuroscience, among others, suggest that mathematical tools become important when attention shifts from identifying the components to understanding their collective function. What is different today is that the molecular details are at the bottom of the biological hierarchy. Molecular biology was reductionism's finest hour. Now, there is nowhere left to go but up.

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APPLIED PHYSICS

The Road Ahead for Metamaterials

Metamaterials enable us to design our own "atoms" and thus create materials with new properties and functions.

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Metamaterials are artificial media structured on a size scale smaller than the wavelength of external stimuli. Whereas conventional materials derive their electromagnetic characteristics from the properties of atoms and molecules, metamaterials enable us to design our own "atoms" and thus access new functionalities, such as invisibility and imaging, with unlimited resolution.

The next stage of this technological revolution will be the development of active, controllable, and nonlinear metamaterials surpassing natural media as platforms for optical data processing and quantum information applications (*1*). Metamaterials are expected to have an impact across the entire range of technologies where electromagnetic radiation is used, and will provide a flexible platform for modeling and mimicking fundamental physical effects as diverse as superconductivity and cosmology and for templating electromagnetic landscapes to facilitate observations of phenomena that would otherwise be difficult to detect.

In terms of a Tree of Knowledge with roots embedded deep in the soil of microwave technology (see the figure), metamaterials became a big issue when the tree brought forth the "forbidden fruit" of negative-index media. Agressively contested when it frst appeared, the concept of negative index is now widely accepted and the focus of research has moved toward application. Other developments have been metamaterials with strong magnetic response and "magnetic mirror" functionality at optical frequencies, the discovery of structured surfaces exhibiting directional asymmetry in transmission, and metal structures invisible to electromagnetic radiation. Substantial effort has gone into the development of chiral "stereo" metamaterials for controlling the polarization state of light and achieving negative refraction. Metamaterials exhibiting properties suitable for use in delay lines and sensors have also been demonstrated across the entire spectral range, from microwaves to optics. Another active area of research has been in waveguide applications. The fascinating "transformation optics" idea of controlling the fabric of "electromagnetic space" (and thus light propagation) by filling it with metamaterial is being developed, requiring media with coordinate-dependent parameters, and offers cloaking and light-channeling solutions such as sophisticated lenses and "mirage" devices.

In developing active gain-assisted metamaterials, the main goal is the compensation of losses that dampen the coupled oscillations of electrons and light (known as plasmons) in the nanostructures. These losses render photonic negative-index media useless. One solution is to combine metamaterials with electrically and optically pumped gain media such as semiconductor quantum dots (*2*), semiconductor quantum wells, and organic dyes (*3*) embedded into the metal nanostructures. Electrically and optically pumped semiconductor gain media and emerging technology of carbon monolayers (graphene) could be expected to provide loss compensation from optical to terahertz spectral ranges. Alloys and band-structure engineering of metals also promise better plasmonic media.

Another grand goal is to develop a gain-assisted plasmon laser, or "lasing spaser" device (*4*): a flat laser with its emission fueled by plasmonic excitations in an array of coherently emitting metamolecules. In contrast to conventional lasers operating at wavelengths of suitable natural molecular transitions, the lasing spaser does not require an external resonator and its emission wavelength can be controlled by metamolecule design. Finally, the use of nanostructured high-permittivity materials offers the possibility of tailoring the electric and magnetic response in metamaterials consisting only of dielectric, thus removing the issue of losses at the source (*5*).

The development of nanoscale all-optical data processing circuits depends on the availability of fast and highly responsive nonlinear media that react to light by changing their refractive index and absorption. In all media where functionality depends on electronic or molecular anharmonicity, stronger responses come at the expense of longer reaction times, a constraint that is practically impossible to break. The plasmonic nonlinearity of metals constituting metamaterial nanostructures is extremely fast and could provide terahertz modulation, but requires high intensities to

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