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## Systems Engineering to Systems Biology

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## NEWS AND VIEWS

# Systems engineering to systems biology

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The 'bottom-up' approach to systems biology entails quantitatively studying complex biological processes by analyzing their molecular components. A converse system biology approach is to infer properties of biological systems in a 'top-down' fashion, using a variety of network reverse engineering methods, data-driven modeling and data integration strategies. Application of a top-down approach to the quantitative biology of a small size system is however less common. In a recent publication, Mettetal *et al* (2008) have insightfully applied such a strategy to successfully decode critical properties of osmo-adaptation in the yeast *Saccharomyces cerevisiae*.

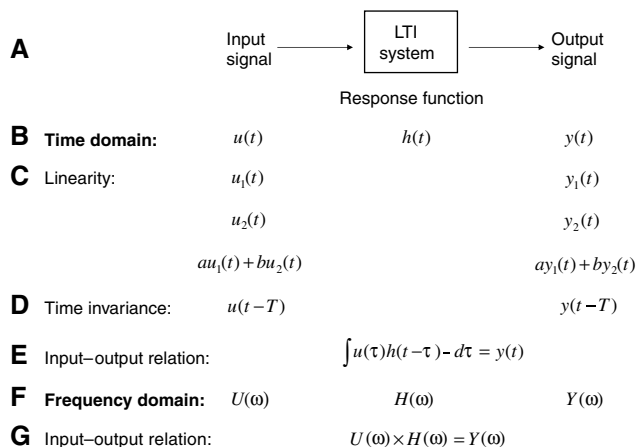
A biological system can, in principle, be dissected by successively inactivating each component individually and measuring how the overall input-output characteristics of the system change. For cellular systems, such dissection involves gene knockouts and/or knockdowns. However, given the complexity of living cells, it is difficult to link the function of single components (e.g. a protein) to observed outputs. Moreover, invasive knockout/knockdown often leads to undesired complications (e.g. lethality).

Mettetal *et al* followed a different systems reverse-engineering approach by which they considered the system first as a 'black box' and assumed it to be equivalent to a linear time-invariant (LTI) system (Oppenheim *et al*, 1997) (Figure 1A and B). An LTI system has two defining properties: first, the output from a set of inputs represents the linear sum of the outputs from each individual input (Figure 1C). Second, the generated output is independent of the time point at which the causal input was applied (Figure 1D). An LTI system is characterized by a single 'response function.' Once the response function is known, the output for any arbitrary input can be deterministically calculated. If the response function is unknown, which is generally the case, then one can methodically apply different inputs and observe changes in output to attempt to decode the response function (Figure 1E). For example, when a sinusoidal periodic input with a certain frequency is applied to an LTI system, the output will have the same frequency. Jean Baptiste Joseph Fourier (1768–1830)

was the first to suggest that almost any physical input function can be uniquely written as a linear combination of sinusoidal functions, the famous 'Fourier transform'. A Fourier transform describes the original function in the frequency domain instead of time domain, where the frequencies come from the sinusoids (Figure 1F). As an input to an LTI system can be expressed as a linear combination of sinusoids, the output can also be expressed with the same sinusoidal functions (with a possible time shift), whose coefficients are related in a precisely computable manner to the coefficients of input signal. In the frequency domain, the input-output relation becomes a straightforward multiplication rule, which makes it easier to determine the response function (Figure 1G). If a series of input signals each having a different frequency is applied, then in theory the response function can be fully described.

Input-output relationships can be defined and experimentally measured for a variety of biological systems and may thus be used to uncover hidden biological properties. Mettetal *et al* considered yeast as a 'black box' and chose the well-characterized yeast osmo-adaptation system, even though this regulatory network has great complexity with more than 50 known interactions among tens of proteins and metabolites (Klipp *et al*, 2005). Quantitative modeling of the osmo-adaptation pathway would require a huge amount of experimental data as well as intense computer simulations; yet measurement of many of the experimental parameters is problematic, making reliable predictions uncertain. Measuring input-output characteristics and applying LTI-based analysis reduces the underlying complex map to its simplest form, identifying key chemical reactions that dominate the response of the yeast cell to osmotic shock. The resulting reduced set of reactions permits accurate and experimentally verifiable predictions.

Yeast cells primarily respond to osmotic pressure by changing the concentration of osmolyte glycerol. The high-osmolarity glycerol (HOG) pathway controls the glycerol level, maintaining osmotic balance by tuning the export rate of glycerol through the cell membrane. In Mettetal *et al* (2008),



**Figure 1** (A) A linear time-invariant (LTI) system takes an input signal and converts it to an output signal. The system is characterized by a response function. (B) Input signal, output signal and response function can be written as a function of time. (C) An LTI system is linear, that is, an output to a set of inputs can be formulated as the sum of outputs to each input. (D) An LTI system is time-invariant, that is, the output that an input generates is independent of the time that the input is applied. (E) Input–output relation is mediated through the response function. However, it is harder to decode response function from this relation. (F) All the functions can be transformed to the frequency domain using Fourier transforms. (G) In the frequency domain, input–output relation becomes a multiplication rule, which makes it easier to decode the response function.

extracellular osmolyte concentration inside a flow chamber was modulated using a computer-controlled valve creating square-wave osmolar shocks with variable frequencies. The output signal measured was the activity of mitogen-activated protein kinase Hog1. Upon osmotic shock, cytoplasmic Hog1 becomes activated and migrates to the nucleus. The ratio of nuclear versus cytoplasmic Hog1 levels constituted the measured output. The HOG pathway is favorable for input–output analysis, as both input and output are easily manipulated and measured, most molecular components are known and the system relies on multiple negative feedback circuits with unclear properties.

Mettetal *et al* (2008) measured input–output signals with square-wave stimuli with frequencies ranging from 2 to 128 min, transformed the data into frequency domain and calculated the response function to osmolar shocks. This decoded response function could subsequently predict the output to any input signal, for instance a step function like osmolar shock. Moreover, they corrected the response function with nonlinearities to avoid non-biological signal output predictions.

What does the inferred response function tell us about what is inside the ‘black box’ and the underlying biology? The available knowledge on the overall wiring of the HOG pathway allowed interpreting the response function as being the result of a combination of two dominant-negative feedback mechanisms. These feedback loops control the levels of phosphorylated Hog1 and of intracellular osmolyte concentration. The

authors predicted that one feedback loop is Hog1-dependent, whereas the second one is Hog1-independent, although both affect the function of membrane protein Fps1. To test this prediction, they repeated the input–output analysis with a mutant strain unable to induce high Hog1 activity, finding that mainly the Hog1-dependent feedback loop is critical for rapid regulation of osmotic pressure upon osmotic shock. Apart from the modeled Hog1-dependent and Hog1-independent feedback loops, there is another slower feedback loop based on gene expression. However, as yeast cells can adapt to osmotic shock within 15 min, much shorter than the time required for induction of gene expression, the authors hypothesize that changes in gene expression provide a longer timescale feedback response to osmolar shock. They confirm this hypothesis by inhibiting new protein production.

Mettetal *et al* (2008) successfully apply engineering principles that have seldom been used to understand biological systems. Importantly, they demonstrate that significant insight can be derived about the dynamical properties of a system without the need for an extensive quantitative characterization of all individual parameters and molecular interactions of the system. Limitations of LTI-based methodology might keep it from being applied to every cellular system. First, the linearity property has to be checked, as most biological systems would violate it. However, many systems have a linear regime and many experiments could be performed within this regime to avoid nonlinear effects; nonlinear correction factors could be added as was done for osmo-regulation. Second, the time-invariance property should be established before using LTI system analysis. Moreover, for all this to succeed, one needs to be able to translate the characteristics of LTI system into biology using knowledge about underlying components and pathways.

Systems engineering has been long studied, so perhaps now more techniques from this mature systems field can be adapted to biology, permitting precise control over biological input and output signals to reveal structures hidden under complicated network maps.

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