Signaling Tug-of-War Delivers the Whole Message

Andrea Y. Weiße,[1] Ahmad A. Mannan,[2] and Diego A. Oyarzún[2,*]
[1]SynthSys - Centre for Synthetic and Systems Biology, University of Edinburgh, Edinburgh, EH9 3BF, UK
[2]Department of Mathematics, Imperial College London, London, SW7 2AZ, UK
*Correspondence: d.oyarzun@imperial.ac.uk
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How do cells transmit biochemical signals accurately? It turns out, pushing and pulling can go a long way.

Cells trigger many of their functions in response to environmental signals. In this issue of *Cell Systems*, Andrews et al. (2016) study how signaling reactions should be coupled to one another to accurately transmit signals from outside to inside the cell. They propose that a simple push-pull mechanism is sufficient for cells to produce precise readouts of external signals. In this mechanism, the active form of a signaling molecule "pushes" up the concentration of a molecule downstream, while the inactive form "pulls" it back. These opposing effects align the activity levels of signaling molecules along a pathway and result in a proportional relation between receptor activation and downstream signal strength. The study casts new light on design principles of cellular signaling, and opens up plenty of questions for further research.

Cells have membrane receptors that relay external signals into the intracellular space. External ligands bind to these receptors and trigger signaling reactions such as mitogen-activates protein kinase (MAPK) cascades (Figure 1A). Andrews et al. (2016) note an intriguing observation in the pheromone response pathway in yeast. This system initiates signaling upon binding of a pheromone to the G-protein-coupled receptor Ste2, which triggers a MAPK cascade on the scaffold protein Ste5 by recruiting it to the membrane. The last component of the cascade is Fus3, which carries the signal into the nucleus. Experimental evidence shows that Ste2 and Fus3 respond similarly to the pheromone, so that their individual response curves—active-Ste2 and active-Fus3 as functions of pheromone—closely resemble each other, a phenomenon termed "dose-response alignment."

Dose-response alignment avoids distortion of the transmitted signal, producing a linear relationship between receptor occupancy and signal strength at the end of the cascade. This strategy allows cells to transmit a broad range of sensed signals and enables finer control of cell function. Misalignment of the dose-response curves distorts the input signal and causes the cascade to act as a nonlinear amplifier of its input (Figure 1B). For example, if receptor occupancy is graded and the cascade output is more switch-like, the cascade acts as an ultrasensitive amplifier; conversely, if receptor occupancy is more switch-like than the cascade output, the signaling cascade will be largely insensitive and act as a saturator of its input.

Dose-response alignment has been observed across other signaling pathways such as insulin, acetylcholine, and angiotensin II systems (Yu et al., 2008), suggesting that it affects many cellular functions. But it is unclear how cells implement such precise alignment and, moreover, how the precision is conserved through the multiple signaling steps, each with different kinetics and protein abundances. Further, given the diversity of signaling systems, with specific components arranged in different architectures, it is challenging to pinpoint the general principles that guarantee response alignment.

Andrews et al. (2016) use computational optimization to find architectures that can produce dose-response alignment. The authors searched for optimal model parameters that minimize an objective function representing the mismatch between dose-response curves for various architectures. The advantage of optimization is that, instead of studying a signaling system with given kinetics, it allows sweeping over the whole space of kinetic parameters. Their procedure revealed two architectures that produce perfect dose-response

alignment: a push-pull system and a negative feedback from a saturated downstream enzyme. The push-pull strategy is commonly found in bacterial two-component regulatory systems, such as the EnvZ-OmpR system for osmoregulation in *E. coli*, while negative feedback has been reported in the yeast pheromone pathway itself, where Fus3 inhibits the recruitment of the scaffold Ste5 to the membrane (Yu et al., 2008). The negative feedback also bears similarities with regulation of the MAPK-ERK signaling pathway (Sturm et al., 2010), where ERK represses that activation of upstream signaling and linearizes the overall system response. The approach by Andrews et al. (2016) was able to identify architectures that are present in other systems found in nature, thus suggesting that optimization can effectively identify design principles that apply to a broad class of signaling pathways.

Beyond the study of natural systems, optimization is becoming increasingly relevant for the design of molecular circuits in synthetic biology. The field is moving from small-scale gene circuits to more complex systems that interface across layers of the cellular machinery (Oyarzún and Stan 2013). As the repertoire of biological parts grows, so does the number of ways in which they can be assembled, as well as the number of circuit architectures that produce the same function. Automated design techniques are proving powerful strategies for biological circuit design (Nielsen et al., 2016); these use optimization algorithms to navigate the design space and single out the best circuit blueprint for a desired function and implementation constraints.

To untangle the complexity of cellular systems, it is useful to find suitable descriptions that encompass the most fundamental aspects of their architecture but avoid reliance on exhaustive

mechanistic details of all biochemical interactions. At the core of Andrews et al. (2016) is the idea that signaling pathways can be seen as input-output systems. These descriptions are popular in many engineering disciplines, and there is a reason why engineers love them. Input-output models are high-level descriptions that highlight the dependencies among system components rather than their individual details. When studying biochemical networks that are interwoven with feedback and feedforward interactions, input-output thinking allows us to zoom out and lump several processes into a "black box" (Del Vecchio et al., 2016). This is particularly useful for revealing system-level principles when the biochemical details are difficult to measure, unknown, or not relevant to the phenomenon of interest.

Signaling pathways are particularly amenable to input-output descriptions, as they can be thought of as separate modules: a sensing module representing receptor-ligand binding, a signal transmission module composed of signaling cascades, and a nuclear internalization module (Figure 1B). A word of caution, however: "inputs" and "outputs" are just abstractions, and thus, they are only meaningful in the context of the particular scientific question at hand. This is a subtle but important distinction, especially when comparing design principles across different pathways or organisms. For example, there are other instances of linear input-output responses in the EGF and Epo systems (Sturm et al., 2010; Oyarzún et al., 2014; Becker et al., 2010) that are conceptually similar to the results by Andrews et al. (2016) but slightly different when the input-output definitions are taken into account. While Andrews et al. (2016) describe a linear relationship between receptor occupancy and downstream phosphorylation, Sturm et al. (2010) reported linearity between ligand dose and phosphorylation of downstream

Figure 1. Input-Output View of Signaling Pathways
(A) Typical signaling system composed of a membrane-bound receptor and an intracellular signaling cascade. Signaling systems have different dose-response curves from ligand concentration to occupied receptors and downstream signaling.
(B) Block diagram of a signaling system. When the dose-response curves are perfectly aligned, the signaling cascade maps receptor occupancy into downstream activation in a linear fashion. Misalignment of the dose-response curves in (A) causes the cascade to behave as a nonlinear amplifier with varied input-output responses.

signaling molecules, whereas Oyarzún et al. (2014) and Becker et al. (2010) showed a linear response between ligand dose and the time-integrated phosphorylation of membrane receptors. Although all these results may be equally described as "linear input-output behavior," this can be misleading unless we specify how inputs and outputs were defined. Therefore, to ascertain the generality of design principles in different networks, care must be taken to use comparable input-output definitions across all systems.

The work by Andrews et al. (2016) brings to the surface several questions for future investigations. It remains to be determined if the push-pull and feedback topologies are robust to parameter variability or whether other signaling architectures can implement a more robust (but imperfect) alignment. This could be studied with Pareto optimality, which allows finding optimal trade-offs between mutually conflicting objectives. The analysis could also be extended to a larger family of signaling
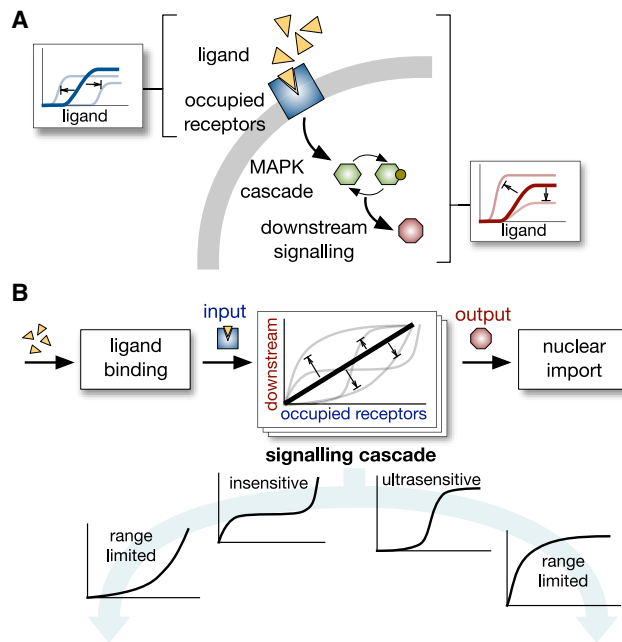
architectures by including both parameters and architectures in the optimization search. Such problems can be addressed with mixed-integer optimization algorithms, which can account for the network topology itself as an optimization variable. It is further unclear what types of cell responses benefit from dose-response alignment as opposed to, for example, signal amplification or saturation. Some strategies may outperform others depending on environmental conditions and intracellular trade-offs that limit cellular physiology (Weiße et al., 2015). Information theory can also provide a general toolkit to study signal transmission and has opened new avenues to understand cellular pathways (Cheong et al., 2011). Optimization and information theory are just some of the disciplines that can help uncover the complexities of cellular signaling. Novel approaches are much needed if we wish to truly understand signaling in the context of larger systems, such as microbial communities, developmental pathways, and disease-relevant networks, all of which are at the core of future progress in therapeutics and biotechnology.

Cells need accurate mechanisms to control how and when to initiate responses to external stimuli. The work by Andrews et al. (2016) provides new insights into how signaling pathways can transduce external cues. Their approach harnesses the power of optimization and input-output thinking to uncover principles of biological organization, a strategy that can greatly benefit other areas of basic biological research, as well as the design of biomolecular networks in synthetic biology.

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Personalized Disease Models on a Chip

Nalin Tejavibulya[1] and Samuel K. Sia[1,*]
[1]Department of Biomedical Engineering, Columbia University, 351 Engineering Terrace, 1210 Amsterdam Avenue, New York, NY 10027, USA
*Correspondence: ss2735@columbia.edu
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Organs-on-chips are beginning to serve as a useful platform for individualized disease models in a way that minimizes patient-to-patient variability.

Organs-on-chips offer the potential to recapitulate human physiology by culturing human cells in precise three-dimensional architecture and compartments, fed with native-like chemical and mechanical cues. In this issue of *Cell Systems*, Benam et al. (2016) take a step toward demonstrating one such organ-on-chip system as an effective disease model. They engineered an apparatus to "breathe" cigarette-smoke-filled air over a human lung-on-a-chip, and measured systems-level responses from human small airway epithelial cells. Comparison of responses with or without stimulus revealed nuanced differences and potentially novel biomarkers.

Early work on organs-on-chips leveraged advances in tissue engineering and microfabrication to form miniaturized compartments containing multiple human cell types (Sin et al., 2004). Such systems offer precise spatial architecture and dynamic physiochemical environments compared to homogeneous 2D or 3D cell cultures and systematic perturbations on human cells compared to live animal models (Bhatia and Ingber, 2014). The breathing human lung-on-chip system, which was first introduced by the same research group (Huh et al., 2010), recapitulated the epithelium between the air sac and the bloodstream and took advantage of a mechanically flexible

material in the microfluidic chip to incorporate a breathing motion. Subsequently, organs-on-chips have been employed in the study of kidney (Jang et al., 2013), gut (Kim and Ingber, 2013), liver (Bhise et al., 2016; Ebrahimkhani et al., 2014), heart (Grosberg et al., 2011), and skin (Abaci et al., 2016). These studies have ranged from subjecting cells to cycles of mechanical stretch and release (to mimic the forces of peristalsis in a gut) to examining cellular injury from chemotherapy.

In this study, Benam et al. (2016) incorporated a microrespirator (which mimics the inward and outward air movement by the rib cage and diaphragm) and airway (lined by human bronchiolar epithelium cells from healthy subjects or patients with chronic obstructive pulmonary disease). They also added a smoke machine, which exposed the cells to whole cigarette smoke, and applied horizontal shear forces across cell surfaces. The researchers found that this method produced more precise results than Transwell methods, which require that the epithelium be submerged in a cell culture medium in order to be exposed to cigarette smoke extract in solution.

Compared to studies with live animal models or human subjects, an important advantage of this organ-on-chip system is patient-normalized comparison of bio-

logical responses. Here, cells lining the microfluidic channels are sourced from the same individual and cultured in the presence or absence of an environmental perturbation (smoke exposure). Such patient-matched comparisons have the potential to uncover phenotypic differences masked in clinical studies, which insufficiently account for inter-individual variability among multiple study groups and different subjects.

Key results of the study include the identification and validation of genes upregulated upon smoke exposure, including a confirmation of the upregulation of Cytochrome P450 Family 1 Subfamily B. In addition, the researchers performed time-lapse imaging and applied spectral analysis to determine the beating frequency of cilia on epithelial cells. The study showed that smoking produced a heterogeneous effect on ciliary beating across the epithelium, with some areas beating normally and other areas beating at reduced rates. The researchers also identified about 10 genes that could distinguish responses to smoke exposure in diseased, compared to healthy, lung epithelium. More work will have to be done to validate these biomarkers and determine whether the patient-matched model leads to new biological insights, but in this manner, the researchers augmented the likelihood of identifying