SYNTHETIC BIOLOGY

Bringing neural networks to life

A synthetic protein-based winner-take-all neural network controls cell fate decisions

By **Katie Galloway** *and* **Christopher Johnstone**

Native gene circuits that drive differentiation and maintain cell fate are constructed from layers of multi-input programs that compute cell responses to diverse stimuli. Although multilayer synthetic circuits have been constructed with DNA oligonucleotides in vitro (*1*), in bacteria (*2*), and in mammalian cells (*3*), adaptation of these methods to new tasks often requires reselection and reoptimization of circuit components. On page 1243 of this issue, Chen *et al.* (*4*) describe a protein-level synthetic circuit framework that implements a "winner-take-all" neural network capable of classifying the relative abundance of multiple inputs to modify the circuit output. This network replicates various classification circuits by adjusting the relative concentrations of a few components. By connecting this circuit to molecular pathways that regulate apoptosis, the authors demonstrate the promise of this approach for programming cell fate outcomes. This could be extended to design complex neural circuits that augment the computational capacity of cells.

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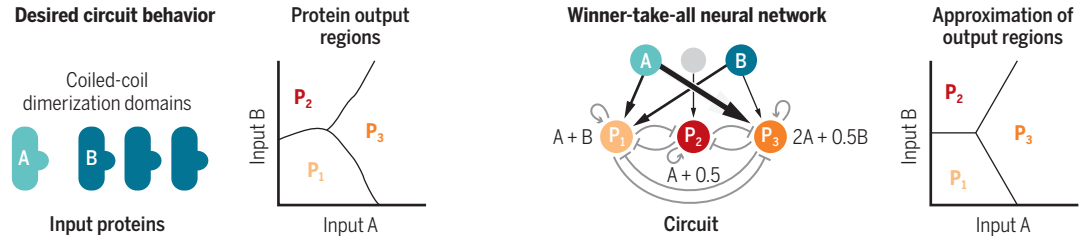
Developing logic circuits to sense, process, and integrate signals serves as a hallmark of synthetic biology. Primarily, logic has been implemented through engineering promoter systems for transcriptional control. However, transcription-based sequential logic-gate circuits often suffer from high background noise due to leakage (i.e., activity in the absence of input). In theory, adopting a neural network design could help to avoid the confounding effect of this basal gene expression. Specifically, winner-take-all neural networks can approximate any continuous classification function (*5*). For example, a DNA oligonucleotide winner-take-all network (*6*) was capable of classifying complex patterns into discrete categories, such as recognizing handwritten digits from 1 to 9, a task that would be difficult to implement with discrete biological logic gates. In addition, winner-take-all networks stabilize the circuit output using both positive and negative feedback, making the network robust to noise.

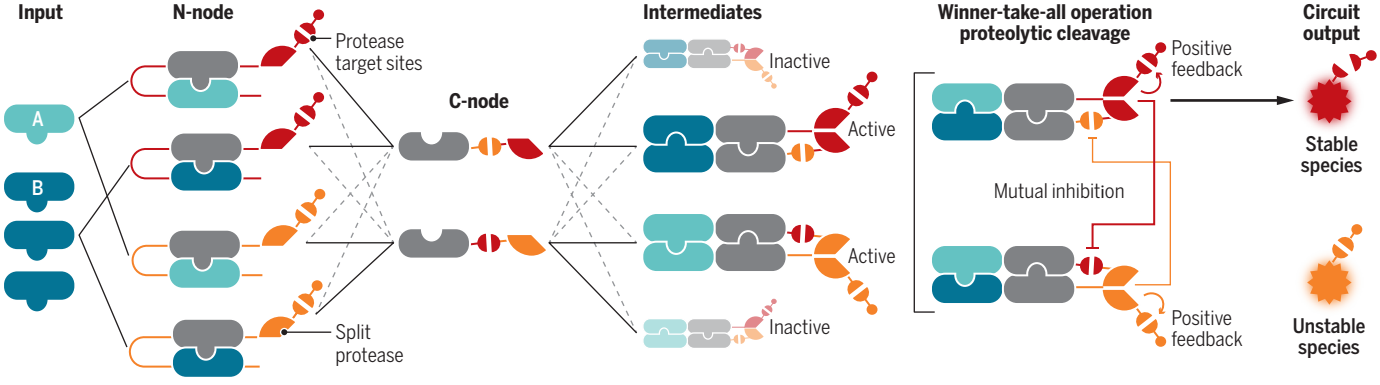
To implement a winner-take-all network in mammalian cells, Chen *et al.* integrated two modular protein systems: de novo–designed protein pairs (coiled-coil heterodimers) to encode specific orthogonal dimerization reactions (*7*), and split viral proteases to implement feedback (*8*). The resulting split proteases can only cleave their targets when partnered with their cognate pair through the heterodimers. At the output layer, split proteases target themselves and other proteases, economically providing both the positive and negative feedback necessary for winner-take-all behavior. By cleaving its own destabilizing degron (a motif that targets the protein for degradation), the protease stabilizes its species, while destabilizing other proteases and nonfunctional protease hybrids, resulting in mutual inhibition. When suf-

Molecular implementation of a protein-based winner-take-all neural network

Desired circuit behavior may depend on a complicated function of two or more inputs. After defining desired output regions that depend on the input concentrations, a winner-take-all neural network that approximates the desired output regions can be calculated.



A winner-take-all network is implemented through coiled-coil input proteins that interact with N-nodes, allowing specific interactions between N-nodes and C-nodes to form intermediates. Through protease-driven mutual inhibition and stabilizing positive feedback, one type of protease (red) dominates the winner-take-all network and activates the stabilized circuit output (red).



GRAPHIC: K. HOLOSKI/*SCIENCE*

ficient stabilized proteases accumulate, the circuit settles into a steady state that is detected through the degradation of genetically encoded fluorescent reporters by the accumulated proteases. Taken together, these components theoretically provide all necessary dynamics for a winner-take-all circuit, dubbed a "perceptein" network (see the figure). After validating that individual dimerization, degradation, and proteolytic cleavage components perform as expected, Chen *et al*. implemented a two-input classifier. The authors measured classifier performance with their integrated reporter. Then, to show biological control, the authors demonstrated state-specific regulation of apoptosis by introducing a caged caspase-3 that gets activated upon cleavage by the output of the network.

The use of designed coiled-coil heterodimers to modulate how split proteases pair could allow this neural network architecture to be adapted to a larger number and range of other inputs. For example, beyond simple two- or three-input classifier circuits, protease-based winner-take-all networks have the potential to scale with the number of orthogonal proteases that are integrated into the circuit. For adoption into cell and gene therapies that require low immunogenicity, viral proteases may be replaced by recently engineered human proteases (*9*). Furthermore, by demonstrating control of apoptosis, Chen *et al*. provide a kill switch for the rational control of cell therapies, which may improve the safety and performance of these therapies.

To achieve different classification tasks, the relative strength of interactions between components in a neural network must be tunable. Accordingly, Chen *et al*. designed the network such that the relative molar fractions of circuit components are modified simply by changing the relative amounts of the delivered components. Although these delivery ratios (e.g., transfection ratios) are rationally selected in this study, screening of transfection ratios by poly-transfection could accelerate the exploration of circuit function. Poly-transfection (*10*) supports optimization of multicomponent circuits by sorting for a desired phenotype from a large library of transfection ratios. As both the number of simulation parameters needed and cell type– and cell state–specific effects grows with the size of a network, using poly-transfection as a "training" method to identify optimal neural network parameters may be beneficial. In addition, scaling winner-take-all neural networks to larger classifiers and generalizing across different cell types may require new, expanded data-driven models. For example, data-driven machine learning approaches have recently been proposed that replace the traditional design-build-test-learn cycle of circuit design with high-throughput library screening (*11*, *12*). A unified design methodology built around winner-take-all neural networks could account for both designed and cell limitation–derived interactions, improving forward design and performance.

Notably, competition for cellular resources can function as an unintended winner-take-all coupling. The number of RNA polymerases, ribosomes, degradation machinery, and other cofactors required for a circuit of interest is limited per cell, so competition for resources leads to mutual inhibition between highly expressed circuit components. The effects of resource competition can be especially pronounced in transfection systems, where plasmid copy number can exceed a hundred copies. Although resource competition can impede performance, winner-take-all coupling has been harnessed to enhance circuit behavior (*13*). By localizing computation to the protein level, protein-based circuits mitigate the effects of direct competition for transcriptional resources, which may confound designs. However, as these networks are integrated into stable cell lines, the effects of hidden transcriptional coupling may emerge and provide opportunities to rebalance weights within the network to ensure robust winner-take-all computation (*14*, *15*).

Augmenting the computational abilities of cells with engineered circuits will advance sophisticated cellular systems for sensing, recording, and responding to diverse extracellular and intracellular cues. Harnessing synthetic computation systems into therapeutically relevant cell types presents a new challenge for engineering the next generation of "smart" cell-based therapies. ■

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PHOTONICS

Optical devices as thin as atoms

Controlling exciton resonances in two-dimensional materials can create dynamic flat optics

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The functional properties of traditional optical structures such as lenses are linked to their inherent three-dimensional (3D) shape, which is often fixed upon fabrication. Their 3D nature also prevents integration of these optical elements into tightly packed systems. Metasurfaces—2D metamaterials that can control the propagation and scattering of light—are promising for compact, flat optics. These materials leverage strong interactions of light with metal and semiconductor nanostructures to control the flow of light with geometric features. However, dynamically tuning the optical properties of metasurfaces remains difficult because they tend to be only weakly dependent on external stimuli such as an electrical field. Reducing the dimension of a metasurface to the atomic scale could enable dynamic tuning and strong light-matter interaction through the quantum size effect, which allows materials to more effectively absorb and emit light at a specific wavelength.

2D van der Waals (vdW) materials—stacked atomically thin sheets of semimetals, semiconductors, or insulators—host a variety of intriguing phenomena that arise from the extreme localization of charge carriers (electrons or holes) by quantum confinement. These include quantum many-body effects (complex behaviors that arise from interacting quantum particles) and exotic optical properties that are not found in bulk materials. The ultrathin layered materials can strongly interact with light and "resonate"; this resonance is an

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