PROTEIN BIOCHEMISTRY

Hotwiring protein regulation

An algorithm for identifying allosteric mechanisms allows researchers to assemble a functional multidomain protein and may offer new evolutionary insights.

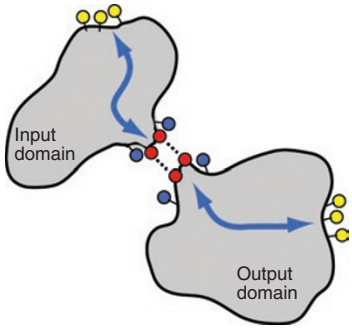
The principle of allosteric regulation, in which ligand-binding at one protein site triggers functional changes at another remotely positioned site, is familiar even to beginning students of biology. However, the underlying physical mechanisms of allosteric regulation remain largely mysterious, and much of what is known is a result of the painstaking analysis of individual proteins.

This frustrated Rama Ranganathan of the University of Texas Southwest Medical Center, who sought a more generic strategy to characterize such mechanisms. Ranganathan also thought that existing methods tended to approach proteins as purpose-built machines rather than points along an evolutionary continuum, shaped by forces of natural selection. "There was a sense that the evolutionary design of proteins might be rather different than what we might imagine by thinking about the protein like it was some kind of engineered object," he says.

To address these considerations, his group had previously developed a statistical coupling analysis (SCA)-based computational approach for identifying regulatory networks of amino acids based on evolutionary conservation across families of proteins. SCA proved to be a potent analytical tool, and the team found that as long as their starting dataset of proteins was sufficiently large, they could use SCA to accurately identify discrete, co-evolved circuits of residues that form the mechanisms of allosteric regulation in a variety of protein domains.

Ranganathan's group now provide a striking new demonstration of the effectiveness of SCA, using data from their method to assemble an operational synthetic 'circuit' from two functionally distinct protein domains. The result was a chimeric protein in which activation of the first, 'input' domain is transmitted to the second, 'output' domain via direct physical linkage of the two domains' allosteric pathways.

Their chimera incorporated a light-activated domain (LOV2) from a plant protein, phototropin, as its input, which they then linked to the bacterial metabolic enzyme dihydrofolate reductase (DHFR). In one construct, DHFR and LOV2 were connected at a regulatory site identified via SCA (site A); in another, LOV2 was attached to an alternate site on DHFR considered irrelevant to regulation (site B). Light-stimulated enzymatic activation took place in the properly connected site-A chimera but not the site-B chimera, providing support for the SCA approach and the concept of surface 'hotspots' for allosteric control.



In a synthetic protein circuit, a signal received at active site residues (yellow) of an 'input' domain triggers allosteric mechanisms (blue arrow) that generate a response at a remote site on the domain (red); this response is directly transmitted to a complementary site on the 'output' domain, initiating a secondary allosteric pathway that induces activation of the output domain. Reprinted with permission from AAAS.

Ranganathan calls his article an "idea paper" that defines principles he intends to explore more deeply in future work: "What if we took membrane receptors and control elements, and really asked, 'Can I engineer regulatory control into proteins such as kinases or ion channels through knowledge of their basic evolutionary design?'"

But right now, Ranganathan is most excited by the possibility that this work may offer insights into how complex proteins emerged in nature—through stepwise linkage and optimization of connections between conserved allosteric circuitry contained within individual protein domains. "If this has some impact on our thinking about the way evolution works," he says, "that would be in my view the most far-reaching and important aspect of the work."

Michael Eisenstein

RESEARCH PAPERS
Lee, J. *et al.* Surface sites for engineering allosteric control in proteins. *Science* **322**, 438–442 (2008).