Determining the Internal Allosteric Architecture of DHFR With Saturation Mutagenesis

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Allosteric regulation involves the transfer of information between distant regions of a protein. However, the thermodynamic coupling pattern between amino acids that enables this transmission remains unclear. Statistical analyses of amino acid co-evolution suggest one hypothesis: that allostery is mediated by sparse, cooperative networks of amino acids embedded in the protein structure. To test this idea, I am using deep mutational scanning to comprehensively map the positions contributing to allostery in a synthetic allosteric switch: the metabolic enzyme Dihydrofolate Reductase (DHFR), which is regulated by the light-sensing domain LOV2.

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LONG range interactions between amino acids are important for allosteric regulation, information transmission, and catalysis. One approach for inferring the underlying pattern of interactions between amino acids is Statistical Coupling Analysis (SCA). The premise behind SCA is that functional interactions between amino acids link the evolution of those residues, and this co-evolution can be detected in multiple sequence alignments that are representative of a protein family's long-term evolutionary record. In prior research, SCA was used to identify a co-evolving network of residues inside DHFR (the sector), and it was shown that sector-connected suface sites are hotspots for introducing allosteric regulation [1]. However, further work is needed to test if sector positions do indeed form the structural basis for allosteric information transfer within a protein.

One method for testing these proposed allosteric pathways is through double-mutant cycles. In this experiment, a wild-type protein, two single mutants, and a protein containing both mutants are compared. If the change in free energy associated with the double mutant protein differs from the sum of changes in the single mutants, then the two mutations are thermodynamically coupled [2]. However, the difficulty of examining all residue combinations in a protein increases exponentially with the number of residues. One study on a small, 9-residue section of a protein involved 65,840 double-mutant cycles [3]. To overcome this combinatorial complexity, we have designed a new strategy to measure the effect of all single mutations in DHFR within the context of a light-induced allosteric perturbation.

Specifically, we generated saturation mutagenesis libraries for the DHFR enzyme in the context of two DHFR/LOV2 fusions: one in which LOV2 is connected at a sector position and provides ~2-fold regulation of DHFR activity by light, and one in which LOV2 is not sector-connected and does not regulate DHFR activity. Library competition in light/dark environments allows the detection of mutant fitness (and contribution to allostery) through the application of next-generation sequencing, where the relative frequency of each mutation is coupled to its fitness.

The results of these experiments in progress will provide a complete map of which positions inside DHFR contribute to allostery in the DHFR/LOV2 switch. Furthermore, it will provide insight into how allostery evolves: is there a path of single mutant variation that can lead to optimization of regulation in the sector-connected and/or non-sector-connected fusions? Finally, these data will provide a rich test set for evaluating computational methods to model allosteric pathways inside the protein.

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