Protein Design

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Allosteric Switches: Remote Controls for Proteins**

*Thorsten Berg**

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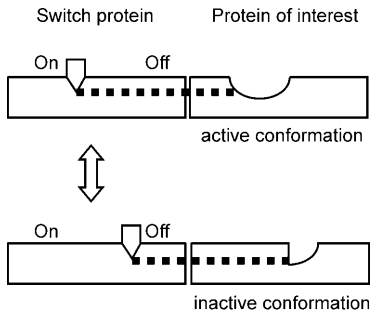
Chemical biology has multiple aims,[1] one of which is the identification of small-molecule modulators for individual functions of as many human proteins as possible. Various approaches towards this goal have been developed, which converge to create a toolbox of small molecules for chemical biologists.[2–4] This endeavor constitutes a long and winding road, however, on which shortcuts are welcome. One intriguing shortcut would be to control the activity of any protein of interest by fusing it to a universally applicable "switch" protein, for which an experimentally accessible control mechanism exists (Scheme 1). Control of the protein of interest by means of the switch protein would be exerted allosterically. Allostery can be described as the phenomenon by which a change in protein structure at one site leads to a change in protein structure, and thereby function, at a distant site.[5] Important functional domains of a protein, such as the catalytic center of an enzyme, can thus be linked with remote sites at the protein surface. In the article highlighted here, Ranganathan et al. hypothesize that when two proteins are fused at such remote surface sites, the activities of one protein might control the activity of the other.[6]



Scheme 1. Design principle of fusion proteins in which the activity of a protein of interest is controlled allosterically through a switch protein. The path of allostery is represented by the dotted lines.

To demonstrate the feasibility of their idea, Ranganathan et al. created fusion proteins in which the LOV2 domain from plant phototropin was employed as a "switch" protein to regulate the enzymatic activity of dihydrofolate reductase (DHFR).[6] LOV (light, oxygen, and voltage) domains are photosensors that bind flavin chromophores. Detailed experimental evidence indicates that light absorption of a flavin mononucleotide bound to the phototropin LOV2 domain triggers a conformational change of the helical extensions at both the N terminus and the C terminus (the "Jα helix") of the LOV2 domain, leading to release of the Jα helix from the LOV2 domain (Scheme 2).[7–9] The βF-βG loop of DHFR is remote from the active site, but its dynamics control enzymatic activity.[10] Hence, both proteins have known surface-exposed sites spatially removed from, but functionally linked to, the ligand binding site of the LOV2 domain or the enzymatic center of DHFR, and these remote sites were chosen as connecting points in the fusion protein. The LOV2 domain and its helical extensions were inserted into the βF-βG loop (Scheme 2).

If the authors' hypothesis is correct, the light-induced conformational change occurring adjacent to the LOV2 domain should alter the enzymatic DHFR activity of the flavin mononucleotide bound fusion molecule in some way. In fact, the authors observed a twofold increase in the enzymatic activity of one of the fusion molecules upon exposure to light as compared to in the dark at 17 °C, and a 1.6-fold increase at 25 °C. Consistent with these data, the rate-limiting release of the enzymatic product tetrahydrofolate was increased by a factor of approximately 1.3 when exposed to light. Even though the effects are relatively small, studies with mutant fusion proteins showed that they were indeed dependent on known signaling mechanisms in the two proteins, thus supporting the notion of an allosteric switch in the fusion molecule.

The major hurdle for attempts to design artificial allosteric switches seems to be the identification of appropriate sites for fusion of the two protein domains. They should be removed from the "center of action" of a protein, that is, an enzyme's active site or the ligand-binding domain of a non-enzymatic protein, but they must still be physically linked to it through interacting amino acids which provide a path of allostery through the protein. Although Ranganathan et al. chose two protein domains that had been intensively experimentally characterized with respect to their allosteric proper-

[*] Priv.-Doz. Dr. T. Berg
Department of Molecular Biology
Max Planck Institute of Biochemistry
and Cluster for Integrated Protein Science Munich (CIPSM)
Am Klopferspitz 18, 82152 Martinsried (Germany)
Fax: (+49) 89-8578-2454
E-mail: berg@biochem.mpg.de
Homepage: http://www.biochem.mpg.de/ullrich/junior_groups/thorsten_berg/index.html

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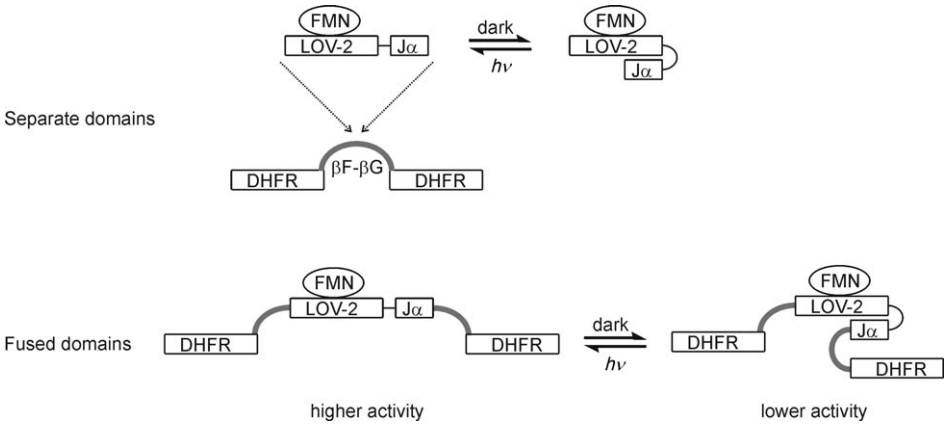

Scheme 2. Design of the DHFR–LOV2-Jα fusion protein. The plant phototropin LOV2 domain and its C-terminal helical extension, the Jα helix, undergo an allosteric conformational change when a bound flavin mononucleotide (FMN) absorbs light. The βF-βG loop of dihydrofolate reductase (DHFR) is removed from the active center but functionally linked to it by allosterism. In the fusion protein, the phototropin LOV2 domain and its helical extensions at the N terminus and the C terminus are inserted into the βF-βG loop of DHFR. Exposure to light increases the enzymatic activity of the fusion protein.

ties, the same sites had been identified in the highlighted publication as removed from, but functionally connected to, the ligand binding pocket of phototropin or the enzymatic center of DHFR by a computational approach also developed by Ranganathan et al., dubbed statistical coupling analysis (SCA).[11] This method analyzes the occurrence of covariations of amino acids in evolutionary conserved protein families and assigns them functional significance for allostery. The approach is based on two assumptions: firstly, evolution is regarded as the result of a large-scale mutagenesis experiment with protein functionality as the selection constraint. Amino acids conserved throughout a protein family are therefore likely to be of functional significance. Secondly, if two amino acids are energetically coupled, then a deviation of the amino acid distribution at one position found in a subgroup of all analyzed protein family members is expected to correlate with an altered amino acid distribution at the second position. Statistical analysis of all pairs of energetically coupled amino acids throughout an entire protein family can be exploited to delineate a path through the protein which links obviously relevant sites such as the catalytic center of an enzyme to a distant surface-exposed site. The ability of SCA to predict physically connected networks of amino acids that link distant functional sites had previously been demonstrated for numerous protein families;[12–15] its application to Per/Arnt/Sim (PAS) domains (which comprise the phototropin LOV2 domain), and the family of dihydrofolate reductases correctly identified the allosteric sites known from biochemical experiments.[6] This suggests that the approach should be applicable to protein domains for which no prior experimental data with respect to allosteric sites are available. Methods other than SCA to identify allosteric intramolecular signaling pathways include molecular dynamics simulations of protein dynamics and allostery,[16] and anisotropic thermal diffusion.[17] Analysis of protein dynamics of a PDZ domain by NMR spectroscopy revealed ligand-dependent side-chain dynamics, and thus intramolecular signaling.[18] In principal, these methods should also be suitable for identifying connecting points for allosteric fusion proteins.

Even though the allosteric regulation of a protein domain by a fused "switch" protein as highlighted here is an artificial system, this principle is also found in nature. As an example, the enzymatic activity of the catalytic domains of protein kinases can be enhanced allosterically by the binding of a ligand to the adjacent SH2 domain, thereby coupling enzymatic activation to substrate recognition.[19,20] Moreover, nature has extended the principle of allosteric regulation even across protein–protein interfaces: the affinity of the PDZ domain of the cell polarity protein Par6 for its ligands is regulated by the binding of Par6 to the guanine nucleotide-binding protein Cdc42.[21,22] Statistical coupling analysis presented in the highlighted paper was able to delineate a continuous network of amino acids connecting the nucleotide binding pocket of Cdc42 and the Par6 PDZ domain across the protein–protein interface.[6] This points out the possibility of designing artificial systems based on the transfer of allosteric effects across protein–protein interfaces, and maybe even across interfaces between different sorts of biopolymers?

Future studies will need to explore which protein domains are amenable to fusion through allosteric surface sites, and whether bidirectional systems can be designed in which each domain can be used to regulate the activity of the other domain. It would furthermore be interesting to explore whether larger allosteric effects are obtainable, for example, by optimizing the position and length of the fusion partners. Allosteric networks with increased complexity could be generated by linear or circular coupling of three or more allosteric protein domains. Substantial progress will be required in this field in order to devise useful artificial allosteric systems, but an important step has been taken.

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