Recruitment of proteins to modulate protein–protein interactions

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The use of a 'borrowed' protein surface to either enhance or inhibit the interaction of a small ligand with its protein target has been reported recently. This approach represents a general method for modulating protein–protein interactions and may find many applications in both biology and medicine.

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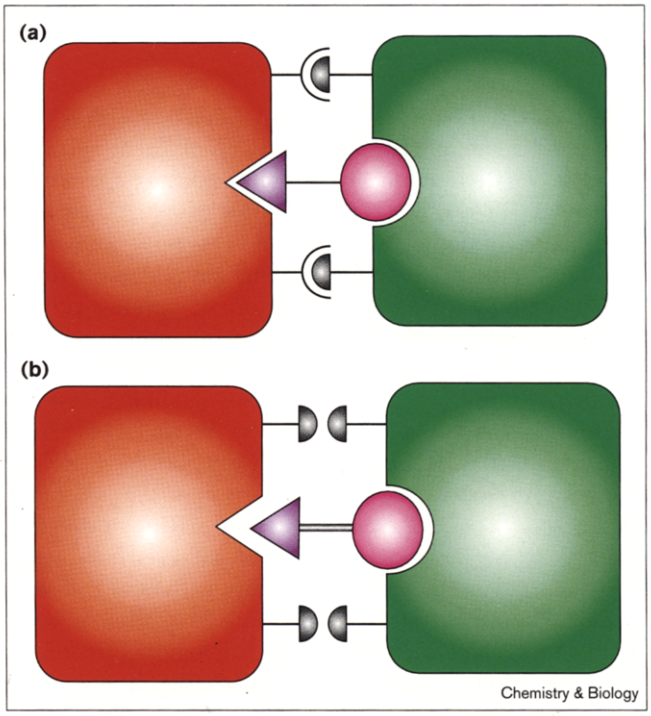
Protein–protein interactions are involved in the regulation of almost all physiological processes, including signal transduction and transcription. Biologists and chemists have long been interested in finding general methods to disrupt or enhance interactions between proteins to modulate cellular processes and to discover new drug candidates. Significant difficulties have been encountered, however, for reasons that in retrospect seem obvious. Protein–protein interactions rarely rely upon individual amino acid residues. Instead, they are often mediated by many amino acid residues spread around a large surface, which complicates the use of small molecules to regulate such interactions. The interaction of SH2 domains with their phosphotyrosine-containing ligands involves only 4–5 amino acids [1], but, even when so few amino acids are involved, finding small molecular antagonists with high affinity and specificity is not a straightforward process.

Nature has encountered and found a way to overcome this problem. The immune system recognizes antigens through the binding of peptides derived from the invading viruses or bacteria to the major histocompatibility complex (MHC) protein. The peptide–MHC complex is then recognized by the T-cell antigen receptor (TCR), triggering T-cell activation. Although the peptide antigen cannot bind to the TCR with appreciable affinity, the peptide–MHC complex is capable of high-affinity binding to the TCR [2]. An even more striking example is provided by the unique mode of action of a family of immunosuppressive natural products including cyclosporin A (CsA), FK506 and rapamycin. These natural products cannot bind to their respective targets alone. Once inside the cell, these compounds bind to abundant intracellular immunophilin receptors — cyclophilin (in the case of CsA) or FKBP (for FK506 and rapamycin). The cyclophilin–CsA and FKBP–FK506 complexes bind and inhibit the phosphatase activity of calcineurin [3] and the FKBP–rapamycin complex inhibits the kinase known as FRAP/RAFT/RAPT/TOR [4–7]. A family of man-made dimeric immunophilin ligands, known as chemical inducers of dimerization, have already found widespread use in the modulation of various cellular processes and have great potential in gene therapy [8–9]. More recently, it was reported that another natural product, brefeldin A, may work in a similar fashion. Brefeldin A inhibits the activity of the GTP/GDP exchange factor for the ADP-ribosylation factor (ARF) by trapping the complex formed between the exchange factor and the GDP-bound form of ARF, although it remains to be determined whether the relatively small brefeldin A simultaneously interacts with both proteins [10,11]. These examples suggest that small molecules need not act alone to exert their biological effects but can recruit other binding proteins for help to achieve high-affinity and high-specificity interactions with a given protein target. It is likely that there are other ligands yet to be identified that work in a similar fashion.

Inspired by the unique mode of action of CsA, FK506 and rapamycin, Crabtree, Wandless and colleagues [12] came upon the idea of using a bifunctional ligand dimer to 'borrow' the surface of immunophilins to alter the affinity of the interaction between a given ligand and its target. The protein target they chose was the SH2 domain from the protein tyrosine kinase Fyn. SH2 domains, one of the most widely studied protein modules, are involved in the recognition of phosphotyrosine-containing sequences present in many signaling proteins [13]. These domains are responsible for mediating protein–protein interactions in response to the activation of protein tyrosine kinases. As a result, SH2 domains from various signaling proteins have been popular targets for drug discovery. The SH2 domain of Fyn binds to phosphotyrosine-containing peptides such as pYEEI [1]. Crabtree, Wandless and colleagues [12] therefore attached pYEEI to either FK506 (FK–pYEEI) or a simpler synthetic FKBP ligand, known as SLF (SLF–pYEEI) [14]. The linker used was designed on the basis of the crystal structures of both the Fyn SH2 domain and FKBP12 to optimize surface contact between the Fyn SH2 domain and FKBP. When the binding affinities between the two pYEEI-containing conjugates were measured in the absence or the presence of FKBP12 and FKBP52, some surprising observations were made. The presence of FKBP52 enhanced the binding of FK–pYEEI to the Fyn SH2 domain by about threefold, whereas the presence of FKBP12 made little difference. In contrast,

Figure 1



Two opposite effects of a borrowed protein surface on small ligand–receptor interactions. **(a)** An enhanced interaction. **(b)** A weakened interaction. The ligand of interest is represented by a purple triangle and the borrowed ligand is represented by a pink circle.

the addition of FKBP12 decreased the affinity of SLF–pYEEI by almost sixfold, whereas addition of FKBP52 had little effect. Although the changes in affinity seem somewhat small, these are the results from the very first set of conjugates tested, without further optimization. The results therefore signal a new direction for the modification of small ligands that may eventually be capable of efficiently modulating protein–protein interactions.

Among the most important questions raised from this study are why and how the FK–pYEEI–FKBP52 complex had increased affinity for the Fyn SH2 domain, whereas the other complexes had either a decrease or no change in affinity. These differences are likely to be attributable to the protein–protein contacts between FKBP52 and the Fyn SH2 domain, as the authors suggested [12]. A comparison of the structure of FK–pYEEI with that of SLF–pYEEI suggests another possibility; the portion of FK506 that is missing in SLF in the calcineurin effector domain may also make fruitful contacts with residues in the SH2 domain. A cocrystal structure of the trimeric complex consisting of the Fyn SH2 domain, FK–pYEEI and FKBP52 will be quite informative, revealing the relative contributions of various components of this complex to the enhanced affinity. The structure of the complex containing the Fyn SH2 domain, SLF–pYEEI and FKBP12 could be equally revealing as to why this complex had little change in affinity. As for the decrease in affinity observed for the SLF–pYEEI–FKBP12 complex, it may be rationalized in large part by the difference in distance between the tetrapeptide and α-dicarbonylamide in FK506 or SLF. To a first approximation, the linker is about four atoms closer to the dicarbonyl pipcolinic ester in SLF–pYEEI than in FK–pYEEI, thus bringing the Fyn SH2 domain closer to FKBP12, leading to steric clashes between the Fyn SH2 domain and FKBP12, and a decrease in affinity between Fyn SH2 and SLF–pYEEI.

By converting a single ligand into a bifunctional dimer and using the attached ligand to recruit a presenting protein, the authors effectively transformed a simple, straightforward small ligand–protein interaction into a more sophisticated trimolecular complex involving both ligand–protein and protein–protein interactions. Once the ligands are decided upon, the nature and length of the linker connecting the two small ligands will determine the distance and orientation of the target protein with respect to the presenting protein. Crabtree, Wandless and colleagues [12] took advantage of the availability of the crystal structures of the FKBP–FK506 complex and the Fyn SH2 domain to design a linker that allowed a favorable interaction between FKBP52 and the Fyn SH2 domain. For protein targets for which no crystal structural data is available, one will have to take a trial-and-error approach by testing a number of possible candidates.

In addition to FKBPs, quite a few other cellular proteins can also be borrowed to present dimeric ligands. For this approach to work *in vivo*, however, the binding of the bifunctional ligand to the presenting protein *per se* should not interfere with other cellular processes. The heat-shock proteins, an abundant family of proteins, fit this criterion. The interactions of heat-shock proteins with small ligands do not usually have significant cellular consequences [15]. Although plasticity on protein surfaces allows presenting proteins and target proteins to find favorable interactions with one another, to what extent such interactions can occur between any two given proteins remain to be seen. One general approach to discovering suitable presenting proteins could involve using a library of ligands conjugated to pYEEI in place of FK506 or SLF and screening in a cellular context for those that have higher affinity without even predetermining which proteins are recruited. Once such a ligand is discovered, the corresponding presenting protein can then be identified and characterized.

In essence, the surface-borrowing approach is to create more than one point of contact to a given protein target, thereby enhancing or weakening the interaction between a ligand and its target protein (Figure 1). In principle, it is related to the use of secondary binding sites on proteins to produce new multimeric ligands that have higher affinity

for the target protein [16,17]. What is unique about the protein-leasing approach is that the presenting protein associated with the bifunctional ligand provides a much larger surface area than the surface area that can be presented by individual small ligands alone. The approach extends the power of small ligands in biology and medicine [18]. The search for new synthetic dimeric ligands for controlling protein–protein interactions will provide new challenges, as well as opportunities, for chemists and biologists for some time to come.

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