Commentary

Borrowing to make ends meet

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"... the intimate contact between the molecules ... is possible only with similar geometrical configurations. To use a picture, I would say that the enzyme and the substrate must fit together like a lock and key."

Emil Fischer, 1895

No analogy has so profoundly influenced our thinking about the joining of biological molecules as Emil Fischer's lock and key. After a century, it still serves as an appropriate introduction to the exquisite fit that small molecule keys have for large molecule locks. Although the lock-and-key analogy is well known, the study from which it emerged is not. Fischer studied two different enzyme preparations, emulsin from yeast and maltase from bitter almonds, and examined their ability or lack of ability to hydrolyze synthetic glucose derivatives that had been prepared in his laboratory. When one derivative fit emulsin but not maltase and another fit maltase but not emulsin, the lock-and-key analogy was born. In addition to providing a useful analogy, Fischer's study demonstrated the ability of organic chemistry to produce small molecules that probed biological processes. Fischer's 1907 Faraday Lecture on "Synthetical Organic Chemistry in its Relation to Biology" can still be profitably read today.

The paper by Briesewitz *et al*. in this issue of the *Proceedings* (1) adds another interesting chapter to both Fischer's analogy and his organic chemistry-based approach. Ironically, their contribution involves the part of the small molecule that is not bound to the target protein—the molecular equivalent of the part of the key held in the hand. They use organic chemistry to modify this molecular feature so that it can bind another protein, borrow another protein in their words, to enhance or diminish the original binding. To abuse Fischer's analogy for the last time in this commentary, their approach reminds me of the key to my wife's car, which works when she has her hand on the protruding end but doesn't work when my hand is on it.

Briesewitz *et al*. need three components for their system—a target protein, a borrowed protein, and a small molecule that simultaneously binds both proteins. They made shrewd choices for each component. For the target they chose the SH2 domain, a small protein module widely distributed in key signaling pathways. For the borrowed protein, they used the human FK506-binding protein (FKBP) family of proteins that binds immunosuppressive drugs. Because small molecules that would bind tightly to both SH2 or FKBP had been made in many laboratories, molecular linkers with bipartite binding surfaces could be quickly synthesized from readily available pieces. Exploring the feasibility of such a system also requires the ability to vary components. The borrowed protein and the part of the small molecule that binds the borrowed protein were the variable components in their system.

SH2 domains are small protein modules with a wide distribution (2, 3). These domains bind peptide sequences containing a phosphotyrosine side chain, and high-resolution structures are available for a variety of SH2 domains with and without bound ligands. Most of the binding involves the phosphotyrosine itself, which is bound in a deep pocket lined with positively charged residues. The natural function of SH2 domains is to associate proteins into productive aggregates through the binding of a characteristic phosphotyrosine-containing sequence of one to the SH2 of another. SH2 domains are found in pathways involving phospholipid metabolism, protein phosphorylation and dephosphorylation, activation of small GTPases, gene expression, protein trafficking, and cytoskeletal architecture.

Because molecules with good affinity and high specificity for SH2 domains could be useful for diseases such as cancer, osteoporosis, inflammation, and immunosuppression, many laboratories have worked on developing them (4). In spite of this focused effort, there are no notable successes. Available molecules are plagued by low affinity or lack of specificity (and usually both). The authors used a phosphotyrosine-containing peptide that binds the Fyn SH2 domain used in their study.

The authors' approach to the variable part of the system was inspired by an unusual group of microbial natural products, the immunosuppressive agents FK506, rapamycin, and cyclosporin (5). These molecules, which were discovered through random screening efforts at three pharmaceutical companies, have given us the potent immunosuppressive drugs Sandimmune (cyclosporin A) and tacrolimus (FK506) used in organ transplant patients (5), as well as a promising approach to gene therapy based on rapamycin (6). Studies on the mechanisms of action of these molecules eventually led to the surprising conclusion that rather than binding one protein, they simultaneously bind two different proteins (6). A molecule uses one face to bind one protein, and this binary complex then binds another protein.

FK506 forms a tight binary complex with the small cytoplasmic FK506 binding protein FKBP12 (6). In forming a binary complex, FK506 uses roughly half of its atoms to bind in the deep binding pocket of FKBP12 (7). The high-resolution three-dimensional structure of the FKBP12–FK506 binary complex is known, and the structural basis for the binding affinity and specificity of this natural products is well understood. Of course one test of this understanding is the ability to prepare totally synthetic molecules that incorporate enough binding elements that they form binary complexes with FKBP12. Many academic and industrial laboratories took up the challenge of preparing such synthetic molecules. A notable success was achieved at the SmithKline Beecham laboratories, where relatively simple molecules with the ability to tightly bind FKBP12 were made (8). These simple synthetic tight binders were devoid of immunosuppressive activity because they lacked the molecular features to bind the additional protein needed, but they provided Briesewitz *et al*. with a small synthetic molecule to complement the natural product FK506.

FKBP12, which was discovered by using an affinity reagent based on FK506, turned out to be a member of a family of proteins that includes FKBP13, FKBP25, FKBP51, and FKBP52, among others. The structures and functions of these other FKBPs are not well known, but they all bind FK506 and

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FK506-type molecules. The binding pocket of the FKBPs is a strongly conserved group of aromatic side chains, and the binding pocket of FKBP52 is essentially identical to that of FKBP12 (9). The importance of this for the Briesewitz *et al.* study was that a small (FKBP12) and a large (FKBP52) borrowed protein with the same binding pocket were available.

In the end, Briesewitz *et al.* had one target (Fyn SH2), two borrowed proteins (FKBP12 and FKBP52), and two bipartite small molecules (Peptide–FK506 and Peptide–Synthetic), which gave rise to four possible target/bipartite small molecule/borrowed protein combinations. Of the four combinations, one had enhanced binding to SH2, one had decreased binding, and two had unchanged binding.

The most surprising result is enhanced binding. After all, as everyone who has tried to make a molecule that binds more tightly knows, there are lots of ways to make binding worse, few to make it better. The enhanced binding is most plausibly caused by favorable interactions between the target protein and borrowed protein, but this conclusion is in itself quite surprising. Protein–protein interactions typically involve the precise matching of large convoluted surface areas, and how could such an interaction arise between two proteins that have no measurable affinity for each other? The authors provisionally suggest—in the absence of structural data—that the plasticity of the protein surfaces allows them to find a mutually accommodating and energetically beneficial arrangement. Using a borrowed endogenous protein to enhance the affinity or selectivity of small molecules for SH2 domains is an exciting prospect.

Ultimately, the most important use of a borrowed endogenous protein surface may be for abolishing interactions. One can imagine schemes for deploying drugs against pathogens that utilizes a borrowed human protein to diminish the effects of toxic agents in human cells. For example, a highly toxic agent could be chemically fused to an FKBP12-binding molecule. Human cells, with their plentiful supply of FKBP12, could prevent the toxic agent from interacting with its target. The pathogen cells, lacking FKBP, would be exposed to the full force of the toxic agent.

Whatever the practical outcome of the Briesewitz *et al.* approach, the authors demonstrate still another way to use organic chemistry to influence biological processes.

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