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Bridging the Synthetic and Biopolymer Worlds with Peptide-Drug Conjugates

Roberts and Li break the standard library mold by generating hybrid libraries of small molecules tethered to peptides. Hybrid libraries harness larger chemical and structural diversities and thus represent a new frontier in lead drug discovery.

At first glance, the report by Li and Roberts published in this issue of *Chemistry & Biology* would appear to be a move in the wrong direction [1]. Who would want to mess up a perfectly good small molecule by tethering it to a peptide with the expected effective loss in pharmacokinetics? The answer is affinity. The two researchers have developed a clever way to boost drug affinities by tapping into the massive combinatorial potential of peptide libraries. The technique holds promise for rapidly optimizing the binding affinities of lead molecules without being confronted with the sometimes excruciating task of preparing large libraries of small molecule analogs.

To accomplish their goal, Li and Roberts literally had to bridge two important but distinct areas of drug lead discovery: biopolymer libraries and small molecules. Biopolymer libraries, which can offer dazzling molecular diversities approaching a trillion or more different compounds, are central to this new technology. Libraries of biopolymers, such as peptides, RNA, or DNA, are readily accessible to researchers and are a standard tool used to generate ligands for a particular target or to screen for a lead compound or polypeptide drug with improved affinity. The tremendous diversity of such libraries can often provide a starting point for such screens with relatively little synthetic effort [2]. On the other hand, combinatorial libraries of small molecules are much more laborious to prepare and rarely approach the levels of diversity possible with biopolymers. However, one crucial advantage of using small molecules is the ability to explore the physical and chemical parameters of complementary molecular diversity well beyond the 20 naturally occurring amino acids or 5 nucleotides. Until now, combinatorial small molecule and biopolymer libraries have been considered separate approaches to generate molecular diversity, but this perception will change as a consequence of this innovative research producing libraries comprised of small molecule-peptide hybrids.

Though many methods for constructing peptide libraries are available, the best physically connect each peptide with its encoding DNA. Phage display is one example of this methodology. Here, for each individual constituent of the library, the peptide is attached to the surface of a bacteriophage, a virus that infects bacteria, and the DNA encoding the displayed peptide is encapsulated by the phage particle (a convenient vehicle for site-directed mutagenesis and sequencing to determine the identity of the displayed peptide). Library diversity is also vital for its value as a research tool. An underappreciated source for generating diversity, whether DNA, RNA, or peptide, is chemically synthesized degenerate oligonucleotides, made possible by phenomenally efficient phosphoramidite coupling reactions. However, when library diversities grow larger than about one million different peptides, assaying or screening each peptide individually becomes impractical, and at this stage strategies are required to select peptides with desirable properties (e.g., binding to a particular receptor) before amplifying the selectants. Another advantage of phage display is that peptides selected for their desired properties can be readily amplified in an *E. coli* host. By repeating the process of peptide selection and amplification multiple times, the staggering varieties of peptides found in the original library can be narrowed down to a few peptides with sought-after characteristics.

Li and Roberts use an alternative to phage display in this study. They utilized the mRNA display format that traces its roots to studies of "polysomes" (multiple ribosomes translating an mRNA transcript) carried out in the 1970s. This technology was improved in a number of ways in the 1990s, including the optimization of mRNA 5′ and 3′ end sequences and the addition of chaperones to the in vitro translation mixture, to facilitate the synthesis of peptide libraries with diversities greater than 10^{12} different peptides [3, 4]. Perhaps the most significant advance in the context of library synthesis was made by Roberts and Szostak, who developed a technique to covalently connect an mRNA to its peptide translation product [5]. To this end, the antibiotic puromycin and a short DNA linker are first appended to the 3′ end of synthetic mRNAs. When the ribosome translates this modified mRNA, it stalls upon reaching the DNA linker, and the puromycin at the tail of the mRNA/DNA hybrid

enters the A site of the ribosome and forms an amide bond with the translated peptide. The result is a stable mRNA/DNA/peptide hybrid in which the translated peptide is physically attached to the RNA from which it was translated and can, in turn, be amplified by PCR between rounds of peptide selection.

Peptide-small molecule hybrid libraries could be conceived in two ways. One possibility is that the hybrid library centers on a small lead molecule which is combined with a very large number of peptide appendages. The alternative is that a large peptide library is produced containing combinations of the normal complement of 20 proteinogenic amino acids plus a noncoded residue/small molecule. Roberts previously reported the preparation of peptide libraries containing noncoded amino acids (the equivalent of the small molecule in the present report) that were prepared by suppression mutagenesis [6]. This technique requires a substantial synthetic effort in the preparation of the requisite suppressor tRNA, and furthermore, it is perhaps not generally recognized that the efficiency of the suppression itself, i.e., introduction of the noncoded amino acid via "reading" of a stop codon signal by the synthetic acylated tRNA, is highly variable. This element of capriciousness makes this route inefficient; thus, resorting to a more traditional method of chemical posttranslational modification of natural or introduced cysteine residues within a peptide as a means of attaching the small molecule in the hybrid library is likely to be easier and more reliable than suppression mutagenesis.

The molecular bridge connecting the small molecule and the biopolymer favored by Li and Roberts features well-established and robust crosslinking chemistry: a highly electrophilic bromoacetamide bearing the β-lactam core of penicillin is attacked by the nucleophilic free thiol of a cysteine in the peptide. The chemoselectivity of cysteine is unrivaled among the 20 naturally occurring amino acids and has been the basis for numerous schemes for covalent modification of proteins, from native chemical ligation to proximity probes. In their report, an elegant series of controls demonstrate the specificity of this chemistry for the targeted cysteine only. Using this strategy, a molecule with 100 times greater affinity than penicillin for the *Staphlococcus aureus* penicillin binding protein 2a (PBP2a) was obtained from a hybrid library of approximately a trillion different peptide-drug conjugates. In addition to providing significant technological advances over the phage display and standard mRNA display library protocols described, the hybrid library has generated inhibitors to PBP2a that could be useful for overcoming β-lactam resistance in methicillin-resistant *S. aureus*.

The results presented by Li and Roberts may be most memorable for their promise of harnessing the vast diversities of peptide/small molecules in a library format rather than for the specific achievement of tethering penicillin to a peptide library. By appending the peptide to a site on penicillin known to tolerate (and benefit from) additional functionality, the results of the experiment are perhaps not too surprising. The two researchers argue convincingly that the modest affinities achieved in their experiment are not indicative of the actual improvement gained from the tethered peptide, and that it is the 100-fold increase in affinity that demonstrates the value of their technique. Regardless, this experiment builds a bridge to a new frontier of peptide-small molecule hybrid libraries that holds far-reaching and exciting possibilities for rapidly optimizing small molecule binding affinities without the need to synthesize large libraries of small molecules.

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Selecting Selective Suppressors of Selective Uptake

Scavenger receptor BI (SR-BI) is a high-density lipoprotein (HDL) receptor that mediates the selective uptake of HDL cholesteryl ester (CE) and the bidirectional flux of free cholesterol (FC). The identification of selective uptake inhibitors holds promise for mechanistic studies of SR-BI and for discovery of pharmaceuticals useful in therapy of atherosclerosis.

The selective uptake of HDL CE is a major pathway by which plasma HDL cholesterol is delivered to the liver and steroidogenic cells [1–3]. In contrast to the LDL receptor pathway in which LDL particles are endocytosed and degraded in cells to release cholesterol [4],