Innovations

Phage on display
Dyax Corp.

Big numbers are now almost routine in the pharmaceutical industry, as the screening of huge libraries of chemicals becomes a common event. Phage display, the technology that is at the core of Dyax Corp. (Cambridge, Massachusetts), is perhaps the most extreme example of the numbers game.

The numbers in phage display come from the millions of different sequences that can be inserted in the gene for a coat protein of a phage (a virus that infects bacteria). The phage then obligingly make and display the hybrid proteins, and researchers can select phage with proteins that bind the specified target, with no need for microtiter plates, and the identity of the binding agent encoded in the viral DNA. The procedure sounds simple, and it is. "Phage display is so important in biological research because it's very cheap," says George Smith of the University of Missouri at Columbia.

The genesis of an idea

Smith was the first to use phage as a carrier of foreign sequences. Inspired by work showing that gene *III* of the filamentous phage fd was modular, he inserted foreign DNA into gene *III*. The hybrid phage were still capable of infecting bacteria, and the foreign sequences could be recognized by antibodies.

"In 1984, I had tried to patent the idea of phage display without much of an idea of what we would use it for," says Smith. Although his 1985 *Science* paper suggested cloning genes using antibody-based selection of hybrid phage, by this time he had failed to follow through with the patent process. A 1988 paper in *Gene* outlined a practical approach to the cloning idea and suggested a new application. After a lunchtime discussion of Mario Geysen's construction of libraries of synthetic peptides, Smith proposed inserting thousands or millions of different sequences into phage to make libraries that could be searched for novel binding activities. This is what we now call phage display, and Robert Ladner, now the Chief Scientific Officer at Dyax, had been having similar thoughts. "Ladner had the idea of phage display independently," says Smith, "and he filed a patent a few weeks before the 1988 paper." At around 350 pages, Ladner's patent was no bedtime read. "We went through all of the steps in what some people thought of as nauseating detail," he says. "But I don't consider prolixity in this case to be a pejorative."

Certainly there is no abuse coming from Smith's corner. "Ladner is a really inventive guy," he says. "He's had all these great ideas and is not well recognized for them."

Peptide therapeutics

Since the main phage display patents were issued, Dyax has been busy licensing the technology, with over 25 companies licensed to date.

The licenses are non-exclusive, so Dyax continues to develop therapeutics. Ladner has found several picomolar inhibitors of kallikrein and plasmin (proteases implicated in surgical bleeding) and human neutrophil elastase (hNE), a culprit in diseases including cystic fibrosis (CF), bronchitis, and emphysema. In CF, for example, the underlying defect in chloride channel functioning may cause increased recruitment of neutrophils to the lungs, and so excessive hNE production. The debris from the resultant proteolysis attracts more neutrophils and continues the cycle of inflammation and mucus production. "If you can break that cycle you wouldn't have to dissolve the mucus," says Ladner, "because you would prevent the production of the mucus in the first place."

Ladner uses small, well-structured proteins as scaffolds for the variant sequences, then adds the whole unit to gene *III*. Structural biologists have found that only a few critical amino acids specify the way that many proteins fold. This frustrates many a structure prediction, but allows Ladner to change many residues with few global consequences on the structure. "Because you can change the side-groups," he says, "you can get binding to pretty much anything you want."

The scaffold for the hNE inhibitors is a Kunitz domain, the type of domain found in proteins such as bovine pancreatic trypsin inhibitor (BPTI). Other libraries have been based on cyclic peptides constrained by a single disulfide, and a trypsin inhibitor from the pumpkin *Cucurbita maxima*.

The constrained sequences are preferable to unstructured peptides for a number of reasons: entropic change upon target binding is minimized, hydrophobic residues can be presented, binding characteristics are retained whether or not the sequence is embedded in the phage coat, the structure is easier to determine, and the same peptide sequences with different templates give libraries with different properties.

But who wants peptides?

The pharmaceutical industry, an enterprise based primarily on small, cell-permeant chemicals, was skeptical of the initial versions of combinatorial chemistry. That method has only become widespread with the generation of chemical rather than peptide libraries, but phage display is stuck with proteins and peptides. "Phage

Innovations is a monthly column that surveys the science done at biotechnology companies.

display, where the structures are peptides, is not very well suited to drug development," says Smith. "It's pretty hard to turn a peptide into a drug."

But Ladner believes a change in attitude is in order. "Most US pharmaceutical companies are still run by chemists," he says. "Even Amgen [a company that produces two protein pharmaceuticals with sales of ~$2 billion per year] would rather have a pill than an injectable protein. We think they are excessively cautious. People don't appreciate how effective proteins could be. There are some nightmare stories with the production of large, glycosylated proteins, but we stick with small proteins."

"If you're not willing to use a protein [as a therapeutic]," he continues, "you don't have to throw away phage display. This is by far the cheapest way to generate structure–activity information." By looking at the shape of the best ten or one hundred peptide binders to a particular drug target, chemists can guess which small chemicals might bind most avidly to the same target.

An affinity for separations

Dyax was formed when the research outfit Protein Engineering Corp. (Cambridge, Massachusetts) merged with the pharmaceutical separations company Biotage, Inc. (Charlottesville, Virginia). The new entity remains very active in devising purification methods, a step which is often the most significant cost in the production of pharmaceuticals. Phage display is an ideal way to find an affinity ligand that makes purification more efficient. Standard chromatography can involve multiple steps with different types of columns, says Tom Ransohoff, Vice-president of Bioseparations. "What you're looking for is that point in hydrophobicity-, charge-, and size-space where your protein is located. It's very much an empirical, trial-and-error process, whereas we make molecules that bind your product and nothing else."

Dyax makes everything from laboratory-scale (12-mm diameter) cartridges to production-scale chromatography columns. The latter can process hundreds or even thousands of liters of extract in a single production run, and ultimately produce hundreds of kilograms of protein. Developing a new ligand can take several months, so this is usually done only at the scale-up stage.

The results can be an extraordinarily good fit to the customer's demands. "The beauty of phage display is that you can feed all the information in and make a system that suits your needs," says Ladner. "It means that you can do more than people ever thought you could do with monoclonal antibodies," the affinity agents most often used before the advent of phage display. As it is essentially an *in vitro* process, phage display can be done under harsh conditions including low or high pH, and the binding protein can be selected to bind at one pH, and not at another. The dissociation constant can be specified by varying the target concentration, and competitors that may interfere with binding *in vivo* can be added to the *in vitro* binding reaction. Targets can be self-antigens, toxic, or essential proteins.

The scaffold and the residues to be varied can be chosen depending on the target. The shape of the target may suggest that a flat, protruding, or helical segment would work best. This is an inexact science, however, and given the low cost of screening Dyax will often screen new targets with existing libraries.

Displaying an image

The location of a protein in the body can be a marker of disease. For example, peptides or small proteins that bind to hNE can be linked to radioisotopes and used to diagnose inflammatory bowel disease and pulmonary diseases involving inflammation. Animal trials of such agents are ongoing. Dyax is also searching for imaging agents that bind to blood clots. These could replace costly and invasive pulmonary angiograms.

Beyond Dyax, beyond phage

In partnerships, Dyax is using phage display in a wide variety of ways, from purification of vaccines (with Merck) or urokinase, an enzyme that may clear blocked catheters (with Argonex, Inc., Charlottesville, Virginia), to inhibition of intracellular signaling molecules (with Tularik, Inc., South San Francisco, California).

The type of phage used has also diversified, with M13, lambda, T4 and T7 all in use. The choice of phage depends on the insert size, and whether the researcher wants the protein to fold intracellularly (e.g., lambda) or extracellularly (e.g., M13). Only in the latter case will disulfides form properly.

Phage can be used to display the entire complement of an individual's antibodies; this complement can then be searched for a specific high affinity antibody. Reversing this situation, others have displayed epitopes from individuals with an infectious or autoimmune disease. These can then be probed with antibodies specific to the disease to identify the proteins responsible for the disease.

According to Ladner, Dyax's resources are limited, so large-scale trials of drugs in humans will require partnerships. "We're not Merck: we have to watch what we're doing," he says. For the same reason other possible applications, such as binding environmental toxins, are also on hold. But a combination of licensing, research collaborations, and separations contracts is more than enough to keep things busy for now.

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