A Continuing Series

The Reign of Trial and Error Draws to a Close

In the 1990s, drugs, polymers, and catalysts will be designed from a deep knowledge of molecular structure and function

SCIENCE IN THE '90s

ALL IT TAKES is a few keystrokes—and there on the computer screen, looking as solid as an object you could hold in your hands, is an animated enzyme in vivid color. Type a command and it begins to rotate, turning this way and that to show a deep indentation—the enzyme's active site—from any perspective you desire. Type in another command and the imaginary enzyme begins to vibrate, much as the real enzyme does when it is pummeled by water molecules inside the cell. Type in yet another command and a second molecule approaches: a hypothetical new drug that can only be effective if it fits into the active site as snugly as a key in a lock. Will it? The visions on the screen will soon reveal the answer.

Welcome to the world of "rational" molecular design, a phrase that refers to a revolutionary proposition: that chemists, biochemists, and materials scientists are no longer condemned to the endlessly repetitive trial-and-error methods of the past. Instead of blindly screening hundreds or thousands of compounds to find one with the desired properties, these scientists can now bring high-powered computers to bear on the problem, along with a steadily accumulating body of knowledge about how molecules behave. They can even begin to tailor their creations to carry out precisely the function desired, whether it be targeting the AIDS virus or getting the household laundry clean.

The payoff, moreover, could be huge. Pharmaceutical companies typically screen some 10,000 compounds for every drug that finally gets approved by the Food and Drug Administration; using rational design to streamline that process could save millions of dollars. The materials produced by catalysts are incorporated into end products worth more than $1 trillion; using rational design to improve the catalysts could have a ripple effect that is economically gargantuan.

One vivid example of what rational design is all about comes from the National Institutes of Health where Ira Pastan and his colleagues have been making major strides toward the ancient goal of a "magic bullet": a molecule that would home in on a single type of cell—a tumor cell, say—and destroy it.

To avoid having to go out and look for such a bullet at random, says Pastan's associate Mark Wellingham, the idea is to it build piece by piece. For a homing device, the NIH group turned to the antibody molecules, each of which carries a pair of recognition sites capable of sensing cell surface proteins with remarkable precision. Just within the past year or so, several groups have shown how to snip off these recognition sites and mount them on a much simpler protein framework, where the sites stay stable and active. These investigators have also been able to encode the amino acid sequences of the new proteins in stretches of artificial DNA, so that the free-floating recognition sites can be produced in bulk by genetic engineering.

With the homing device in hand, says Wellingham, the next step is to attach it to something lethal. The NIH group uses a fragment of a bacterial toxin, which they combine with a variety of homing devices using the same genetic engineering technique. The result is an arsenal of exceedingly accurate magic bullets—perhaps the most striking being one that specifically kills cells in tissue culture that have been infected with the AIDS virus.

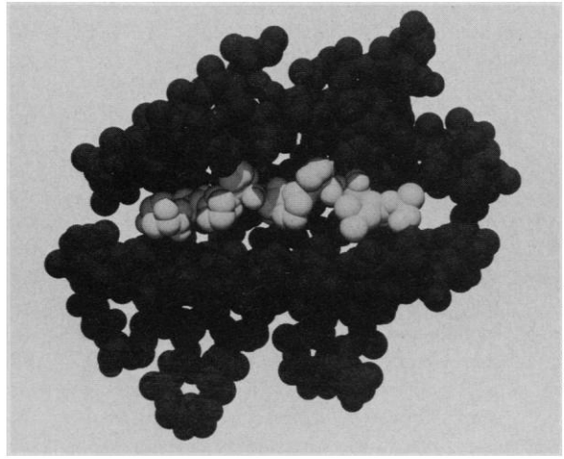
"It's safe to say that we're a long way from any clinical application," says Wellingham, who notes that the biomedical literature is full of therapies that looked great in tissue culture but were useless in the body. Nonetheless, it's an intriguing start.

In a similar vein, rational design can also help make proteins more stable. Theory has it that natural proteins are only as stable as they have to be to do their job in the environs of the cell; evolution has no incentive to push them any further. If true, this is good news for the drug designers and chemists who are trying to adapt natural enzymes to industrial uses, because it means that a few judicious modifications might enable these molecules to survive in a far greater range of chemical environments than they now can.

Happily, the theory does appear to be true. A striking verification has recently come from Brian Matthews at the University of Oregon. Matthews and his colleagues have been working with a certain enzyme produced by a bacterial virus known as the T4 phage. Known as a lysozyme, the enzyme eats through the cell wall and allows the phage to gain entry to the bacterium. The enzyme doesn't have any known application, but as a model system it has the advantage of being relatively simple and very well characterized.

Knowing the structure, Matthews and his colleague modified it by substituting amino acids all along the protein chain to alter its flexibility. They substituted other amino acids to stabilize protein domains known as alpha helices. And they introduced a variety of disulfide bonds to make the protein as a whole more rigid. The result is an enzyme that retains its activity, yet is far more stable than the natural variety.

As these examples suggest, computers are not essential to rational design. If the insight is there, a lot of the design can proceed at the conceptual level. Nevertheless, the advent of high-powered scientific workstations has made a tremendous difference in researchers' ability to cope with the



Du Pont CRDD Protein Crystallography

Lock and key. *The computer shows a foreign protein (light) binding to an immune system molecule known as MHC (dark).*

complexity of their problems.

"Nowadays you have this vast amount of three-dimensional structural data coming in from enzymes, receptors, and other macromolecules," says biochemist Michael Clare of the G. D. Searle Pharmaceutical Company. "The complexity of these larger systems is such that you *have* to build your model with computer graphics. There's no way you could build a real ball-and-stick model and manipulate it."

But by using molecular graphics to visualize the situation, Clare adds, and using a battery of algorithms to calculate conformational energies, molecular orbitals, and the like, "you can figure out what's important before you go off and make thousands of analogs for testing."

This argument has not been lost on the pharmaceutical industry: the companies have been enthusiastic pioneers in rational design techniques for more than a decade. A typical drug company today has a large staff of researchers doing molecular modeling full time, backed up by several dozen synthetic organic chemists ready to test their concoctions in the laboratory. The goal—still a ways off—is to reduce the number of variations required from thousands to hundreds, or even dozens. "About half of our sales are to the pharmaceutical industry," says Richard Cramer, chief scientist of one of the major software vendors in this field, Tripos Associates in St. Louis. "There is much more unhappiness at the cost of repeated cut and try there."

Meanwhile, a similar motivation is being felt in materials science and catalysis research, where trial-and-error techniques have reigned supreme for generations. In Mountain View, California, for example, a consulting and research firm known as Catalytica, Incorporated, has spent the past 5 years spreading the gospel of what it calls "assisted" catalysis design.

"Suppose I say, 'Here's a type of catalyst that I think might solve my problem,' " says Catalytica president James Cusumano. What is the optimum structure?

Among the compounds that Cusumano is referring to are the molecularly engineered layered structure (MELS) catalysts, one of several new materials Catalytica has been working on in recent years. The MELS family may one day eliminate the need for caustic sulfuric acid and hydrogen fluoride in a variety of important industrial reactions, says Cusumano.

If they do accomplish this feat, it would be by means of their unique sandwich structure. At the molecular level the catalyst consists of slices of "bread," each one a crystalline layer of zirconium, oxygen, and phosphorous atoms, surrounding a "filling" of organic molecules sticking out to either side of the phosphorous atoms. These organic side chains are what do the work, catalyzing the reactant molecules that diffuse in between the layers.

So the first step in designing a MELS catalyst is to choose the right mix of side groups, says Cusumano. Computers are a big help: "Using theoretical quantum chemistry, we've developed a program that gives a reasonably good predictive value" about how various side groups will react. "This saves a lot of experiments," he adds.

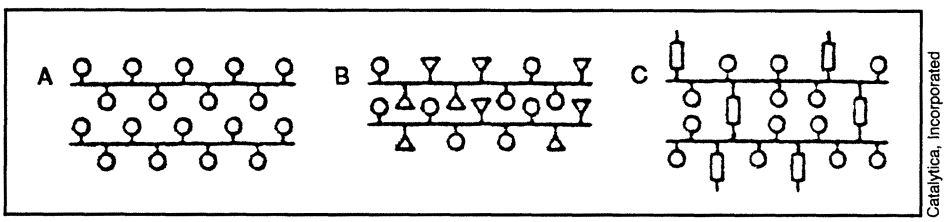
Once the computer investigations have produced a candidate structure, says Cusumano, the action moves to the laboratory. There the structure is synthesized and tested by a battery of analytical techniques such as solid-state nuclear magnetic resonance and x-ray crystallography. "These are automated systems that can spit out data at a tremendous rate," he says. "What is the size of the sandwich? Are there any crystal defects? Are there any components of the reactants left after the catalyst is used? And how does all this relate to the side groups? In short—do I really have what I think I have?"

Armed with this data, the researchers can go back, refine their designs, and start the loop again. You could hardly call the process easy, Cusumano says, "but compare it to making hundreds of MELS catalysts and screening them. The ability to bring this array of technologies to bear means we can develop a commercial catalyst from scratch far faster than we could 10 years ago." Indeed, he says, Catalytica expects to have a number of MELS catalysts in commercial use within 2 years.

If there is one thing every practitioner of rational design can agree upon, it's that there's still a long way to go before trial and error are eliminated from the design process completely—if they ever are. "The theory and the practice just aren't there yet," says Tripos' Cramer.

Fortunately, however, there is also widespread agreement that the theory and the practice of rational design should improve enormously in the 1990s. Not only will the science continue to improve, but that knowledge will be complemented by further growth in the power and flexibility of the computers.

In the next 10 years, according to biochemist Anton J. Hopfinger of the University of Illinois, Chicago, "I expect to see much more in the way of large-scale simulation. Molecular dynamics calculations will allow us to follow chemical assembly in time and space—even as a function of temperature."



Molecular sandwich. *Catalytica's layered MELS catalysts can incorporate a wide variety of side groups. They can be all the same (A) or mixed (B). They can even act as spacers (C).*

"The technology is going to be everywhere, on every desktop," agrees Tripos' Cramer, who also expects to see substantial improvements in the algorithms for modeling. Among them are "Monte Carlo" methods for calculating molecular shapes and wave functions by statistical techniques; molecular dynamics algorithms that treat the structures as mechanical systems moving according to interatomic forces; and molecular orbital calculations that model the making and breaking of chemical bonds at the quantum level.

Indeed, Cramer is optimistic that the next decade will see a major breakthrough in one of science's hottest fields: protein folding. A reliable calculation of how proteins fold in three dimensions, starting from nothing more than knowledge of their amino acid sequence, has proved to be an enormously difficult problem, he says, in part because any given sequence can potentially assume an astronomical number of shapes. But if the problem can be solved, the solutions would provide an equally large benefit to molecular biologists, allowing them to understand the structure and function of proteins that are essentially impossible to crystallize and analyze by traditional x-ray techniques. To Cramer, this is nothing less than the conquest of the Mount Everest of computational chemistry.

■ M. MITCHELL WALDROP