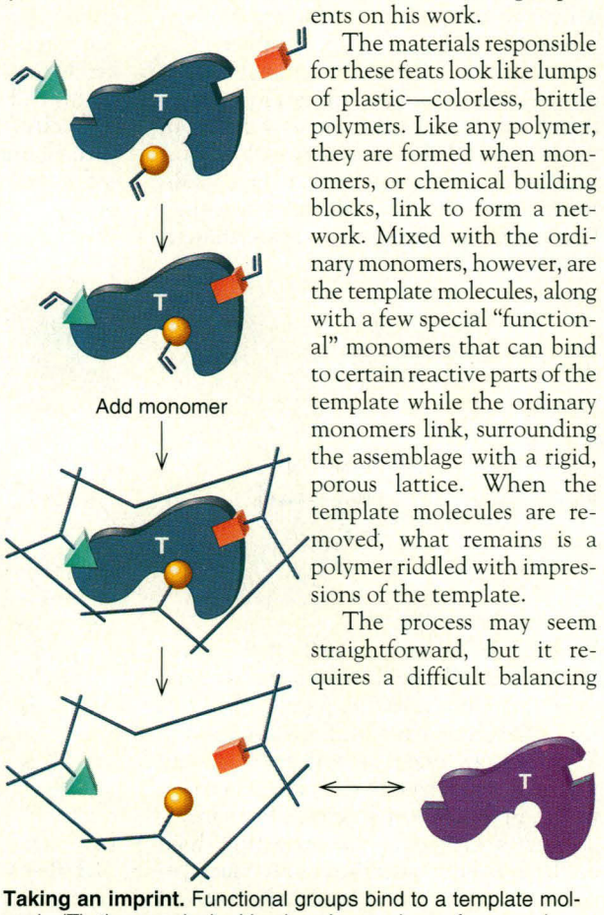
Molecular Imprints Make a Mark

By recording the shapes of template molecules in a matrix of polymer, chemists are creating new kinds of chemical sieves, sensors, and even catalysts

Sometimes in science, a good idea starts out bad. When Linus Pauling offered an explanation for the immune system's eerie ability to come up with a seemingly infinite variety of specific antibodies 40 years ago, he got it wrong. He speculated that the body sends out a fleet of building blocks that mold themselves around an invader like a cast around a template. Actually, the immune system relies on a plethora of ready-made antibodies. But a small group of chemists is now trying to exploit Pauling's template-and-cast strategy to create artificial equivalents of antibodies that can recognize and isolate molecules with equally exquisite selectivity.

The strategy has widespread practical appeal: Just as the body needs special agents to home in on specific molecules, chemists and biologists need to be able to separate chosen molecules from mixtures or trigger specific molecules to react. For now researchers have to rely on trial-and-error to find molecules—sometimes natural antibodies—that just happen to prefer the desired target over other components of a mix. But in the early 1970s, investigators including Günter Wulff of the University of Düsseldorf raised the possibility of custom-making a cast, or "imprint," of a desired target molecule. Since then, chemists have struggled with the excruciatingly delicate task of getting the right building blocks to assemble themselves around the template, then release it so that the cast can recognize other molecules. "The idea is conceptually beautiful, but in execution it's very difficult," says chemist Warren Ford of the University of Oklahoma. Adds Klaus Mosbach of the University of Lund, "For 20 years this has been a dream."

In the last few years, though, Mosbach and a few other dogged researchers have produced polymer imprints that recognize and selectively bind to, say, the left-handed version of a drug while shunning the right-handed version and others that can catalyze the breakdown of specific molecules. And just this year, they have made imprints that can pluck a specific protein out of a mixture of similar molecules. "People are finally starting to realize what this could be used for," says Mosbach, who has taken out a string of patents on his work.



SOURCE: SHEA ILLUSTRATION: K. SUTLIFF

Taking an imprint. Functional groups bind to a template molecule (T), then are locked in place by a polymer framework.

The materials responsible for these feats look like lumps of plastic—colorless, brittle polymers. Like any polymer, they are formed when monomers, or chemical building blocks, link to form a network. Mixed with the ordinary monomers, however, are the template molecules, along with a few special "functional" monomers that can bind to certain reactive parts of the template while the ordinary monomers link, surrounding the assemblage with a rigid, porous lattice. When the template molecules are removed, what remains is a polymer riddled with impressions of the template.

The process may seem straightforward, but it requires a difficult balancing act to bring it off, say chemists. "What you are trying to do is orchestrate a large number of interactions," says Frances Arnold of Caltech. "You are asking the whole thing to self-assemble." At the same time, the functional monomers shouldn't bind the template so tightly that it can't be released from the mold. Her own strategy is to equip the functional monomers with certain metal atoms, such as copper, that can form complexes with specific parts of a target organic compound. When it's time to release the template molecules, the bonds can be weakened by adding a reducing agent, which changes the copper's bonding properties.

Other researchers link the functional groups to the templates with ionic or covalent bonds, then wash out the templates with various solvents. But whatever the strategy, says Arnold, investigators have succeeded in orchestrating the binding of functional monomers to only two or three sites on each template. The resulting imprints thus recognize two or three sites on their target molecules; antibodies, in contrast, bind their targets in about 10 places. This scarcity of binding sites can limit the specificity of some artificial imprints, concedes Mosbach.

But two or three binding sites is good enough, Wulff demonstrated in the late 1970s, for a molecular imprint to distinguish between two mirror-image, or chiral, forms of a target molecule. That's an ability with a particular appeal for the pharmaceutical industry. About 500 drugs on the market come in the form of molecules with chiral twins, only one of which may have the desired effect, says Mosbach. The presence of the inactive twin usually doesn't matter, but sometimes it can be devastating, as the drug thalidomide illustrated in the 1960s. Only one chiral form of this commonly prescribed morning-sickness medicine caused the birth defects that have made the drug notorious.

Separating chiral forms of a molecule is difficult even today; chemists in the pharmaceutical industry have to rely on trial and error to find separation media that prefer one handedness over the other. Molecular imprinting, by contrast, should allow chemists to custom-make their chiral sieves. So far, however, industry hasn't adopted the technique on a large scale.

Other types of applications are just starting to emerge from the labs, says Kenneth Shea of the University of California, Irvine. He is currently working on imprinted polymers that will go beyond recognizing and binding their targets to catalyzing chemical reactions. Nature's catalysts, enzymes, do their job by holding target molecules in a kind of chemical jig so that the bonds to be altered are in the correct position to encounter reactive chemical groups in the enzyme molecule. Shea thinks molecular imprints equipped with functional monomers that can attack specific bonds in the target molecule should be able to do the same job. "We want to use the technique not only to create a binding site but to position in space certain fundamental groups so they can react," says Shea.

Earlier this year, Shea demonstrated that the idea works, at least in a simple system: He reported that he has developed an imprinted polymer that can catalyze the removal of a hydrogen fluoride group from a complex molecule. If Shea can extend this success, he thinks his artificial enzymes should have a decided advantage over natural enzymes and the catalytic antibodies that biochemists have recently been developing. Explains Shea: "Because the [imprinted polymer] materials are hardy, they can be used at high temperature in organic solvents, unlike protein catalysts [enzymes and antibodies] that have to be in water and are rather sensitive."

While Shea and his colleagues say they are looking mainly at applications in chemistry, Mosbach says he and his group in Sweden are concentrating on opportunities in biomedicine. Last year in *Nature*, he and his colleagues described an imprinted polymer that can monitor levels of drugs such as Valium in the bloodstream—a task that now falls to immunoassays based on antibodies, he says. "Now, you don't need biological antibodies—you can use plastic antibodies. That makes life much easier," he says, explaining that polymer imprints should be cheaper to make than antibodies, which usually come from laboratory animals.

Antibodies and enzymes still have the upper hand in one key area, however: They can recognize proteins, the complex molecules that are the key actors in most of biology. Molecular imprinting, in contrast, hasn't been able to produce binding sites for proteins, because bulky protein templates can't slip in and out of the polymer network.

Now Mosbach is trying to erase that distinction with a variation of the molecular imprinting technique called surface imprinting. Instead of creating three-dimensional imprints within a mass of polymer, he forms shallow, two-dimensional molecular impressions in a thin polymer coating on the surface of a bead. Mosbach hasn't published his surface imprinting technique formally, though he described it briefly in the January *Trends in Biochemical Sciences*. But already he has some competition. Arnold says she, too, has developed a version of surface imprinting and has tested it on several different proteins.

To Oklahoma's Ford, surface imprinting sounds like the only promising way around the problem of getting proteins in and out of a polymer scaffolding. If it lives up to expectations, the technique may open the way to polymer-based biosensors and catalysts for proteins—and present enzymes and antibodies with their first serious rivals as biomedical tools. Evolution may have passed over the template-and-cast system for the immune system, but human beings, facing an equally daunting job of molecular recognition, may find it the method of choice.

–Faye Flam

CHEMISTRY

A Game of Molecular Tennis, Anyone?

The spherical geometry of buckminsterfullerene may have forged an unbreakable link between chemistry and soccer, but a group of chemists has now come up with another contender for the chemico-athletic crown: the molecular tennis ball. Like their athletic counterparts, these two molecular constructions have little in common other than their shape. While fullerenes form when many simple carbon units link up in the flame of an electric arc, the tennis balls take shape in solution, at room temperature, when two complex molecular components meet and stitch themselves together.

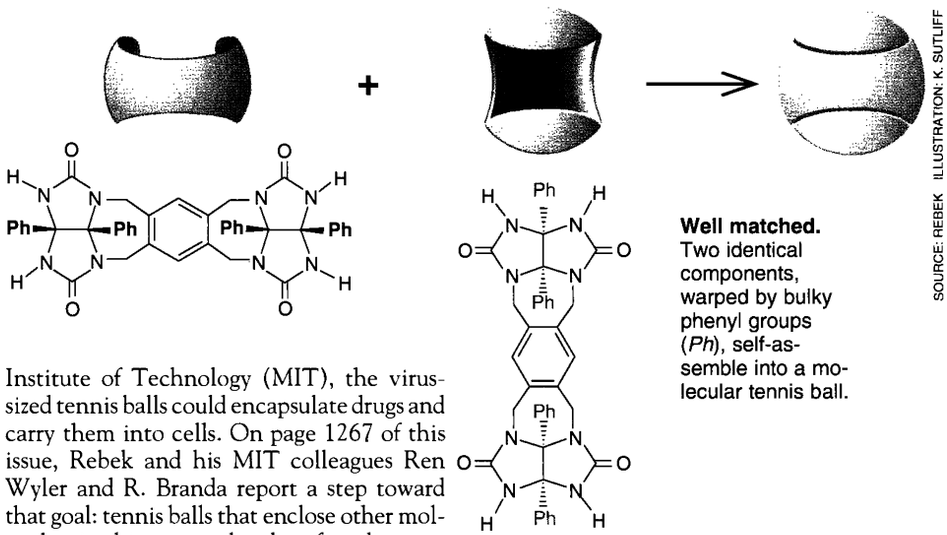
And while fullerenes are prized for the unique chemical and electronic properties of their carbon cages, the creators of these molecular tennis balls think they will be most valuable as containers for other compounds. Just as viruses slip into cells and release their DNA, says Julius Rebek of the Massachusetts Institute of Technology (MIT), the virus-sized tennis balls could encapsulate drugs and carry them into cells. On page 1267 of this issue, Rebek and his MIT colleagues Ren Wyler and R. Branda report a step toward that goal: tennis balls that enclose other molecules, in this case molecules of methane.

The achievement is attracting high marks from other chemists, not so much because of the promise of "artificial viruses" as because of the elegant molecular assembly technique adopted by the MIT group, who worked with Javier de Mendoza of Spain's Universidad Autónoma de Madrid. Instead of relying on extreme conditions or complicated chemical syntheses to form their cages, Rebek and his colleagues created components that would recognize each other and "self-assemble" in solution. That's the same approach the body uses to create complicated biomolecules, says Fraser Stoddart, an organic chemist at the University of Birmingham in the U.K., and the Rebek group's use of it, he says, "gets 5.9 from me for technical merit and 6.0 for artistic impression."

The trick to making the tennis balls self-assemble was to design identical pieces that, like the two segments of a tennis ball, can fit together in only one possible way: as a sphere. That meant shaping each piece so that its ends would be complementary to the middle of another piece, and curving it so that it can meet another piece in the proper embrace (see diagram). To create each curved component, the team reacted two molecules of diphenyl glycouril with one of durene tetrabromide. This resulted in a molecule with four bulky phenyl groups (C_2H_5) sticking out from one face. Phenyl groups prefer to stay apart, and the strain generated by the adjacent phenyl groups bends the molecule into the required half-tennis-ball shape.

Each of the resulting components is edged with alternating N-H and C=O groups. The researchers knew that when these groups are brought close together, weak hydrogen bonds form between the oxygen and hydrogen atoms. The shape of the molecules is such that when any two of them meet, each N-H group comes face to face with a C=O group. The result is eight bonds—the stitches that hold the tennis ball together. In work reported last year in *Angewandte Chemie*, the team used spectroscopy to confirm that their components had indeed assembled into supramolecular spheres. Since then, Carolyn Knobler of the University of California, Los Angeles, and William Davis of MIT have confirmed the structure by x-ray crystallography. "We really do [now] have proof of the shape," says Rebek.

They also know that the tennis balls can capture other substances. In this issue he and his colleagues report evidence from nuclear magnetic resonance spectroscopy showing that if they assemble the tennis balls in the presence of small molecules such as meth-



Well matched. Two identical components, warped by bulky phenyl groups (*Ph*), self-assemble into a molecular tennis ball.

SOURCE: REBEK ILLUSTRATION: K. SUTLIFF