Fortifying a protein through family ties

The knowledge that proteins in the same family often share similar structures has provided researchers a way of tinkering with a natural blood-clot dissolver in hopes of making it last longer in the body. If animal studies confirm the longevity of the new compound, an altered form of tissue plasminogen activator (tPA), the finding could boost tPA's effectiveness in treating heart attacks and stroke.

Prolonging the clot buster's survival may require chemically manipulating it so it no longer binds to its chief natural inhibitor. But researchers attempting to alter tPA's structure face a challenge: No one knows what the compound looks like. Biochemist Joseph F. Sambrook grabbed the next best alternative – another protein in the same family whose structure is already known. Sambrook chose the digestive enzyme trypsin because it shares many of the same amino acid sequences and, like tPA, interacts with a natural inhibitor.

Working at the University of Texas Southwestern Medical Center in Dallas, Sambrook's team compared the pattern and location of amino acids on trypsin that bind to the enzyme's inhibitor in cows with the pattern of amino acids that make up tPA. That comparison revealed an unexplained loop of seven amino acids on tPA's surface, adjacent to the corresponding site where trypsin attaches to its inhibitor.

"The location of the extra sequence was very provocative," says study coauthor Edwin L. Madison.

Through genetic manipulation, the investigators then created a form of tPA that lacked the loop. Comparative *in vitro* experiments showed that this alteration prevented tPA's natural inhibitor from binding to the protein and inactivating it. At the same time, the altered tPA retained 95 percent of its ability to dissolve blood clots.

Whether the new form of tPA can actually improve anticlotting therapy in humans remains unclear. Robert Kamen of the Genetics Institute in Cambridge, Mass., which manufactures one bioengineered version of tPA, suggests that factors other than the drug's interaction with an inhibitor may be more important in determining its longevity in the body.

Sambrook told SCIENCE NEWS he has begun testing the altered tPA's effectiveness in rats and dogs. In addition, he says his laboratory has modified other key portions of the tPA molecule, including the site at which liver cells ordinarily attach during the body's early attempt to clear the compound from the bloodstream.

After examining a similar receptor site on the enzyme urokinase – an even closer relative of tPA whose structure remains undetermined – the Dallas team inserted on the tPA surface a clump of sugar molecules that "covers the receptor site like an umbrella," preventing the liver cells from attaching, he says.

A tPA molecule that combines both alterations may hold the most promise for prolonged action in the human bloodstream, Sambrook suggests.

In the meantime, the findings provide further evidence of evolutionary links between proteins, according to Sambrook and Dagmar Ringe, a chemist at the Massachusetts Institute of Technology in Cambridge. Moreover, says Ringe, studies that use the structure of one protein to visualize and alter the function of another suggest the possibility of more dramatic remodeling of proteins. The practice of redesigning proteins by moving whole segments from one protein to another may someday become commonplace, she says.

"Protein engineers, with their predilection for conservative replacements, may be too timid. ... It may be possible to make multifunctional enzymes by grafting widely differing structures into the middle, as well as the ends, of other stable proteins," Ringe writes in a commentary accompanying Sambrook's results in the June 29 NATURE. In such a scheme, researchers could take a protein whose structure is commonly associated with one function, such as digestion, and alter it to perform quite another task, such as antibody protection. This, she says, "raises the spectacle of a bizarre, man-made biochemical bestiary in which wolves with fleece pursue sheep with fangs." – *R. Cowen*

Discovering the colorful New World of tin

Like Old World explorers setting off for uncharted lands brimming with material riches, organic chemists have been probing carbon's look-alike atoms – largely silicon, but most recently tin. As a starring element in such things as protein, diamond, Velcro, DNA, pencils, plastic wrap, stickum-paper, nylon and ice cream, carbon has earned celebrity status among atoms. But silicon has gained in prestige, and new research is raising the status of tin.

All three elements share the same column in the periodic table and exhibit many similar chemical behaviors. Though scientists are still uncovering the fundamentals of silicon and tin chemistry, they already have learned how to string silicon atoms into polysilanes – silicon-based polymers good for making fibers, hard coatings and other materials. "It's as though we've discovered a new continent," says silicon-chemistry pioneer Robert West at the University of Wisconsin-Madison. "Because of its analogy to carbon, and yet difference, silicon compounds are quite exciting to work with."

The same goes for tin compounds, says chemist Lawrence R. Sita. At Carnegie Mellon University in Pittsburgh, he and Richard D. Bickerstaff have made a tin version of a pinwheel-shaped molecule called propellane, a chemical structure known only in theory until chemists assembled a real one out of carbon and hydrogen in 1982. Sita views the tin version of propellane and the lessons learned in its making as a Rosetta stone that may help open the field of organo-tin chemistry.

"We would like to uncover the [type of] general rules for structure and bonding for tin compounds that we already have in organic [carbon-based] chemistry," Sita told SCIENCE NEWS. The Pittsburgh chemists will report their findings in a forthcoming JOURNAL OF THE AMERICAN CHEMICAL SOCIETY. "It's new and quite exciting," West says.

Five tin atoms form the hub of the new propellane. Two of them, called bridgehead atoms, bind to other tin atoms in the hub. The other three hub atoms bind both to the bridgehead atoms and to two bulky hydrocarbon constituents that form the blades of the pinwheel and prevent the structure from buckling and snapping.

"The most surprising feature of the molecule is its color," Sita says. The intense blue-violet hue can signify unusual optical and electronic properties, suggesting that the compound might serve as a basis for useful new materials. Now the researchers are trying to use transition metals such as cobalt for linking tin propellane molecules. "We'd like to use the tin propellane as the structural unit to form a rigid [molecular] rod," Sita says. In a solid phase, he reasons, such molecules would tend to order themselves along one axis and exhibit magnetic behavior due to a sufficient number of unpaired electrons, yielding a moldable polymer with magnetic properties.

In the May 10 JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, he and Bickerstaff report making the first organo-tin compounds having two fused squares of tin atoms. Solutions of the compounds are "thermochromic," starting out colorless at frigid liquid-nitrogen temperatures and then turning yellow, orange and finally deep orange-red when they reach room temperature. "In tin chemistry the rule of thumb is 'if it's colored it's important,'" Sita says. The next step, he adds, is to develop sturdy tin-based polymers by linking the fused squares into ladder-like structures. "We anticipate a new vista of novel materials." – *I. Amato*