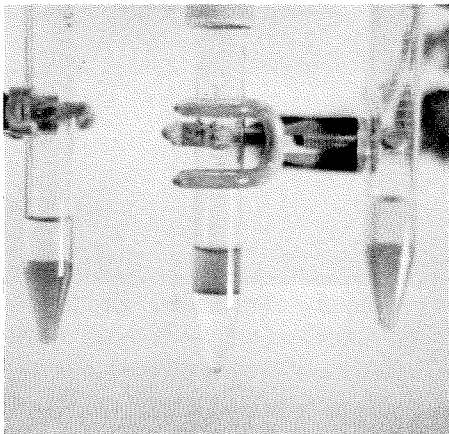


*Remove the
hook, and voilà!
Pure protein.*



Left: Copperless PEG and salt water—the protein (dark layer) stays down in the salt water. Center: PEG-copper and salt water—the protein moves up into the PEG layer. Right: PEG-copper and mild acid—the protein moves back out of the PEG layer.

Hooked on Proteins

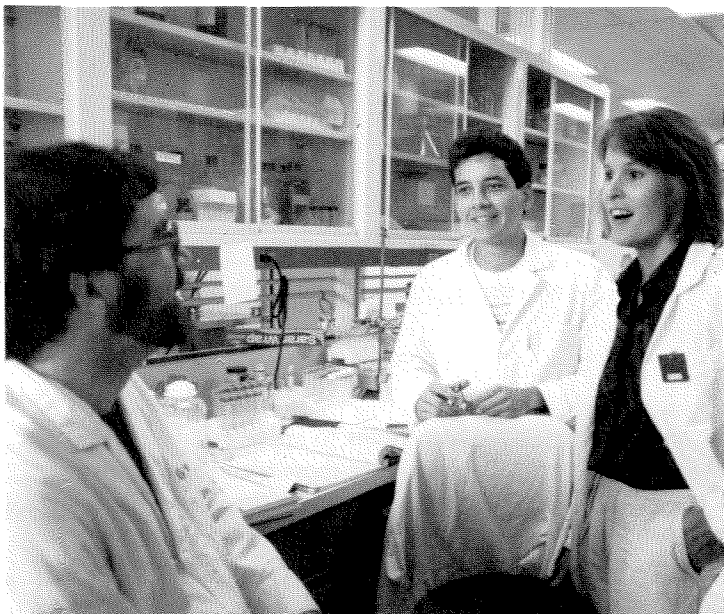
Picture a manufacturing plant making a hundred different models of industrial sewing machines, say, for export. The assembly line madly cranks out finished sewing machines, which pile up inside the doorless factory. Eventually a burst of sound blasts the factory's corrugated metal walls loose from their rivets, and a torrent of water washes the sewing machines out to sea in a tangle of smashed production equipment and bent structural steel. If you were a buyer of sewing machines with the zig-zag option, retrieving your consignment wouldn't be easy. Yet this is about what happens when genetically engineered cells that mass-produce a particular protein ship out their product. The cells are burst open (lysed), and the protein is extracted from a vat full of cellular wreckage including hundreds of different proteins. The purification process is usually messy, requiring a number of steps and a variety of nasty solvents.

Assistant Professor of Chemical Engineering Frances Arnold is working on better ways to separate out the protein. To catch the protein, Arnold has designed a "hook" attached to a water-soluble polymer molecule. Add the

polymer and some salt to the cell/protein solution, shake it once or twice to mix everything thoroughly, and let it sit for a moment. The polymer, with the protein in tow, bubbles up through the water to form a distinct, easy-to-separate layer on top. Once the polymer is safely in a separate container, remove the hook, and voilà! Pure protein.

The hook is actually a copper ion attached (chelated) to a derivative of polyethylene glycol (PEG). "The idea of using an affinity ligand attached to a polymer is not new," Arnold says. "What's new is that we use metals." The group began the work this past summer, when senior Ed Naranjo helped synthesize the PEG-copper polymer as a Summer Undergraduate Research Fellowship project.

If pure PEG is added to a protein solution containing a high concentration of a salt such as sodium sulfate, the PEG and salt instantly separate into two different phases. Proteins generally remain in the salt water, but if a few of the PEG molecules have copper ions attached to them, a protein like hemoglobin goes into the PEG phase by a factor of 200 to 1. And the separation is reversible—drop the pH of the solution from neutral to slightly acidic, and the protein returns to the saltwater layer. Merely dissolving ionic copper in the solution doesn't have the same effect,



From left: Wuenschell, Naranjo, and Arnold.

"When you're working on physical properties that nature hasn't paid much heed to, there's lots of room for improvement."

because the copper ions distribute themselves evenly between the two phases.

The protein's main copper-grabber is an amino acid called histidine. (Amino acids are the beads that, when strung together, make proteins. The amino acids interact with each other to give the protein its three-dimensional shape.) Histidine's structure consists of a ring of five atoms dangling from the amino-acid backbone. If the histidine is on the protein's surface, a nitrogen atom in the ring is positioned just right to stick to a passing metal atom. The attraction is just a passing fancy, though—the copper can be shoved aside by a hydrogen ion. So in a mildly acidic solution, where hydrogen ions abound, the histidines let go of the coppers, and the protein separates from the polymer.

Arnold's group has shown that the more histidines there are on the protein's surface, the more efficiently it is drawn into the polymer-copper layer. Hemoglobin—which has between 20 and 26 histidines per molecule, depending on the species from which it came—will migrate completely into the polymer layer. But only a small number of the molecules in a sample of yeast cytochrome c, which has only two histidines per molecule, will cross over into the polymer layer. "By adjusting the relative volumes of the PEG and saltwater phases," says Arnold, "we can recover

most of the hemoglobin—with a high degree of purity—from a mixture of cell debris and other proteins with fewer histidines."

But histidine is a fairly rare amino acid, and many useful proteins have no histidines on their surfaces at all. And while changing one or two amino acids in a molecule is no big deal, it would be impractical to try to make a protein with few histidines, like insulin, suddenly sprout 20 or 26 histidines on its surface. Arnold is investigating other, more efficient ways to make a protein's surface bind to metals. One method creates a metal-chelating site through protein engineering, substituting different amino acids at one or two positions in the molecule's original amino acid sequence. When the protein kinks into its natural shape, cleverly chosen substitutions will align to clutch a metal atom like a lobster claw pinching an unwary finger.

Robert Todd, a graduate student in Arnold's group, is constructing cytochrome c mutants containing different arrangements of histidines, aided by Professor of Organic Chemistry John Richards's group. "I think this kind of application of protein engineering is going to be very useful," says Arnold. "When you're trying to alter a protein's catalytic activity, its biological function, you're working against a very tight ceil-

ing. Nature has had five billion years to optimize function. But when you're working on physical properties that nature hasn't paid much heed to, there's lots of room for improvement. Furthermore, you're working on the protein's surface, far from its active site, where it's a lot easier to make one or two substitutions without disrupting the biological activity."

Arnold and Research Fellow Gerry Wuenschell, who helped develop the PEG-copper separation technique, have applied for a patent on it. "The technique uses very mild conditions," says Arnold. "And it's an extremely low-cost way to separate proteins. Polyethylene glycol is marvelously inexpensive, and salt, well . . . I hope that this technique will be used industrially. Purifying hemoglobin, for example—when you make blood substitutes from hemoglobin, you get a residue of virus particles and other contaminants as part of the process. And you don't want that going into the patient." Arnold plans to explore other separation methods using protein-metal interactions, while at the same time making this process more selective. This means designing new polymer chelates that will recognize amino acids other than histidine or that will pluck out a histidine only if it is surrounded by other specific amino acids. □—DS