

# Unnatural Amino Acid Could Prove Boon for Protein Therapeutics

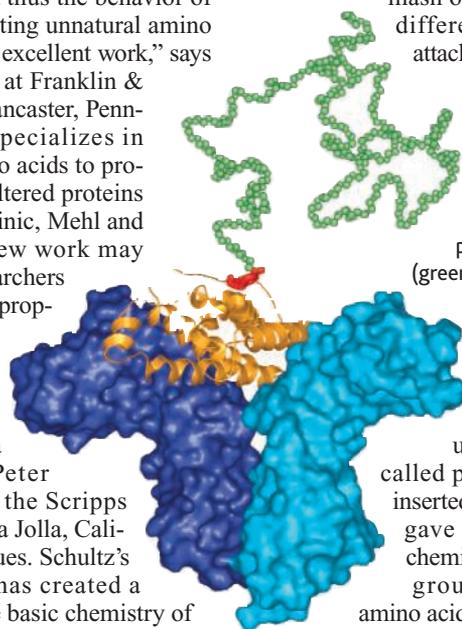
Protein-based therapeutics are a bright spot for drug companies in troubled times. Their annual market is expected to surpass \$50 billion by 2010. But proteins can suffer from problems not found with conventional small-molecule drugs. Some trigger immune reactions, and proteases and other compounds inside the body can quickly chop them up and clear them out. Cloaking protein drugs with a polymer called polyethylene glycol (PEG) can help hide them from the immune system. But it also makes some protein drugs less reliable because proteins sporting different numbers of PEGs may behave differently inside the body.

At the meeting, researchers from a California biotech company showed that they could precisely control the number of PEGs on each molecule, and thus the behavior of protein drugs, by inserting unnatural amino acid into proteins. "It's excellent work," says Ryan Mehl, a chemist at Franklin & Marshall College in Lancaster, Pennsylvania, who also specializes in adding unnatural amino acids to proteins. Although such altered proteins are still far from the clinic, Mehl and others say that the new work may eventually enable researchers to tailor the medicinal properties of proteins much as they do with small-drug molecules today.

The new technique builds on more than a decade of work by Peter Schultz, a chemist at the Scripps Research Institute in La Jolla, California, and his colleagues. Schultz's team over the years has created a scheme for altering the basic chemistry of proteins (*Science*, 14 July 2000, p. 232). Virtually all organisms construct their proteins from 20 amino acids, although scores more exist. Those 20 amino acids are encoded by the four nucleic acids that make up DNA. Those nucleic acids form three-letter "codons" that signal which amino acid should be inserted in a growing protein chain. Schultz's team pioneered an approach to hijack one of those codons to have it insert a non-natural amino acid instead. So far Schultz's lab has doctored proteins from *Escherichia coli* and other organisms to include some 50 different amino acids, which provide different chemical handles

the researchers can use to alter the chemistry of the proteins.

In 2003, Schultz helped set up a new San Diego biotech company called Ambrx to take advantage of the new technology. At the meeting, Ho Sung Cho, Ambrx's director of molecular technology and process development, reported that he and his colleagues have made rapid strides in making precise modifications to human growth hormone (hGH), a protein used widely to promote growth in undersized children. The current hGH, Cho and others note, is made by linking copies of PEG to some of the 11 lysine amino acids on the protein. Researchers have found that hGH with about four PEGs per molecule strikes the best balance of safety and effectiveness. In practice, however, drugmakers end up with a mish-mash of hGH molecules with different numbers of PEGs attached to different sites.



**Hookup.** Unnatural amino acid (red) added to human growth hormone (gold) helps researchers attach protective polymers (green).

To get around this variability, Cho's team made 20 different versions of hGH, each of which had an unnatural amino acid called p-acetylphenylalanine inserted at a different site. That gave the analogs a unique chemical handle called a keto group, which standard amino acids lack. The researchers then linked a single PEG to each keto group and tested the compounds in cell cultures containing mouse and rat cells. Some of the variations destroyed hGH's effectiveness, but many did not. When the group tested six promising analogs in mice, all worked and lasted longer in the animals than the commercial version of the drug did. A single injection of the best variant, for example, showed the same efficacy after 1 week as daily injections of the commercial hGH. If the result holds up in people, it could not only reduce the number of injections needed for hGH patients but also give drug companies a new way to tailor-make protein-based drugs.

**SAN DIEGO, CALIFORNIA** In this biotech hotbed, biological chemistry was front and center at the 229th national meeting of the American Chemical Society from 12–16 March.

## Nanofibers Seed Blood Vessels

Researchers have made heady progress in regenerating tissues such as cartilage and skin, which either don't require an extensive blood supply or are thin enough to tap into nearby blood vessels. They've had far more trouble regenerating thick tissues such as heart muscle that require a blood supply throughout. But nanotechnology may soon provide some help.

At the ACS meeting, chemist Sam Stupp of Northwestern University in Evanston, Illinois, reported that his team has developed a novel variety of self-assembling nanofibers that strongly promote the growth of new blood vessels both in cell cultures and preliminary animal tests. "It's preliminary, but I thought it was the most interesting talk I heard at the meeting," says Harvard University chemist George Whitesides. "It could be the beginning of something genuinely important."

Stupp and his Northwestern colleagues have been perfecting their self-assembling nanofibers for years. A year and a half ago, the group reported making nanofibers that promote the regrowth of nerve cells in rats (*Science*, 3 October 2003, p. 47). Before that, the team had used their nanofibers to promote the growth of hydroxyapatite crystals that form a primary component of bone (*Science*, 23 November 2001, p. 1635).

In each case, the group starts by crafting two-part molecules called peptide amphiphiles (PAs) that contain oily hydrocarbon chains linked to water-friendly peptide groups. In water the hydrocarbons naturally clump together, but negative charges on the peptides repel one another and keep the molecules apart. By sprinkling positive ions into the solution, however, the researchers can counter the peptides' mutual repulsion, allowing the oily hydrocarbon tails to pack together into nanofibers with the peptides facing outward.

In the new study, Stupp—working with graduate student Kanya Rajangam, and John Lomasney, a pathologist at Northwestern University School of Medicine in Chicago—searched for peptides to promote the growth of blood vessels, a process called angiogenesis. Other researchers had discov-

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