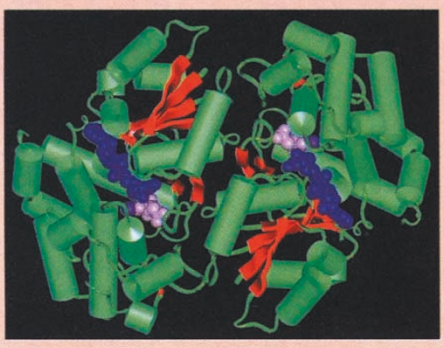
Multitalented enzyme yields secrets

Dehydroquinate synthase (DHQS), for many years an enzymological enigma, has yielded some of its secrets in a new study with implications for both antibiotic development and industrial biotechnology. Catalyzing a key reaction in the shikimate pathway, through which many organisms synthesize aromatic compounds, DHQS appears to carry out several distinct reaction steps in a single active site. Researchers had hypothesized that many of the steps might occur spontaneously, with the enzyme present as a passive bystander. In a recent issue of *Nature* (**394**:299–301, 1998), scientists in the United Kingdom and United States report that the three-dimensional structure of DHQS overturns this model, showing that the enzyme could in fact play an active role in each reaction. "The complexity of the reaction mechanism is somewhat unique, and also the fact that without a structure, you're really left completely in the dark as to how a single enzyme can do so many things," explains Katherine Brown, a senior author on the paper. While modifying and accelerating the multistep enzyme may eventually prove useful in industrial applications, more immediate goals include the rational design of compounds that inhibit it. Because DHQS is found in many fungi, plants, pathogenic bacteria and protozoans, but not in mammals, it is an attractive target for developing broad-spectrum antibiotics.



Fullerenes hotwired to DNA

A new approach for preparing molecular assemblies at the nanometer scale may one day allow the construction of microtransistors and miniaturized devices. By exploiting electrostatic interactions with the phosphate groups on DNA's backbone, University of South Carolina researchers, led by James Tour, have shown that it is possible to organize cationic derivatives of fullerenes—C_{60} aromatic carbon compounds shaped like soccer balls—into molecular assemblies using phiX174 DNA as a template (*Angew. Chem. Int. Ed.* **37**:1528–1531, 1998). The complexes, about 1 micron long, can be obtained by a rapid single-step method and are easily imaged by electron microscopy without the need for heavy-metal staining. "Although I expect that the use of fullerenes will now be explored in the [DNA] imaging field, our immediate interests lie in the potential of this strategy to synthesize nanoarchitectures," Tour explains. As well as trying to covalently link the fullerene units to obtain more rigid polyfullerenes (and thus dispense with the need for a DNA scaffold), his team is looking at semi-conducting properties of these complexes. "Coupling that to the use of higher order DNA structures (e.g., stars or branches) as template routes could result in the synthesis of diverse molecular electronic devices, like transistors," he suggests.

Synthetic hormone created

Researchers at Ligand Pharmaceuticals (San Diego, CA) and SmithKline Beecham (King of Prussia, PA) have discovered the first nonpeptide small molecule capable of mimicking the action of a protein hormone in vivo, possibly opening the way to the development of compounds with greater pharmaceutical utility than biologically produced peptides (*Science* **281**:257–259, 1998). Using an assay for granulocyte colony-stimulating factor (G-CSF) receptor activation, the scientists identified the molecule from a panel of synthetic compounds produced at SmithKline. The highly symmetrical chemical is believed to act in a manner similar to G-CSF, which induces dimerization of its receptor, initiates proliferation of neutrophils, and is often administered to patients suffering from neutropenia. Unlike the hormone, though, the new compound is species-specific and appears to work only on mouse cells. "The mouse and human receptors are 66% identical. As you might imagine, we're pursuing the development of a number of human assays," explains Peter Lamb, Associate Director of Transcription Research at Ligand. Lamb adds that the result is an important proof of concept, as the two companies are also searching for small molecules that may act on the erythropoietin, thrombopoietin, and leptin receptors.

Research News Briefs written by Alexander Castellino, Alan Dove, LeeAnn Leshko, and Regina Raz.

Guiding antiviral therapy

Using oligonucleotides to single out sequences for degradation by a cellular RNA-degrading enzyme, RNase P, scientists at Yale University (New Haven, CT) have successfully blocked influenza virus replication in mouse cells. Deborah Plehn-Dujowich and Sidney Altman first designed and tested in vitro oligonucleotides—termed external guide sequences (EGSs)—that would bind mRNAs encoded by the viral polymerase and nucleocapsid. These oligonucleotides were targeted to three sites in the viral polymerase and two in the nucleocapsid that were accessible to degradation by RNase T1. Sequences encoding the EGSs were then cloned separately into a retroviral vector and stably transfected in C127 mouse cells. Compared with control clones containing no EGSs, viral protein and particle production was inhibited up to 80% when EGS containing clones were infected with the influenza virus. This inhibition reached 90–100% when the infected clones contained EGSs against the mRNAs of both polymerase and nucleocapsid. According to Altman, the methodology is not affected by the mutational variation in the targeted flu genes. "The goal of the experiments was to test the general utility of the EGS technology," he says. The findings are reported in *PNAS* (**95**:7327–7332, 1998).

New force for ligand binding

Ligand-binding forces may be measured directly at the single-molecule level thanks to a strategy developed by researchers in Switzerland from the Paul Scherrer Institute (PSI; Villgen), the University of Zurich, the University of Basel, and Novartis (Basel). The scientists engineered single-chain variable fragments (scFvs) for directed immobilization on an ultraflat gold surface. By immobilizing the antifluorescein scFv antibody at a low density, the researchers were able to select an individual protein using atomic force microscopy (AFM) imaging and then measure the antigen binding force of the selected molecule using an AFM tip coated with covalently immobilized fluorescein antigen. The group performed more than 250 binding-unbinding cycles on the same molecule without losing binding activity. "This is the first time that imaging and force measurements were made within the same experiment addressing an individual molecule," says Louis Tiefenauer, lead author of the study. The group's results (*PNAS* **95**:7402–7405, 1998), demonstrate that this technique can clearly discriminate unbinding forces of closely related mutant molecules from wild type. Tiefenauer believes that "further development (of this strategy) could prove useful for ligand screening."