Crab nebula (a “plerion”) and hence would show evidence for continuing energy input. Now, Bietenholz et al. (1) have provided the direct evidence.

In a superb long-term radio mapping study of SN 1986J, using the full spatial resolution of intercontinental radio astronomy linking 20 telescopes through the Very Long Baseline Array (VLBA), Bietenholz et al. have imaged the appearance of a compact energetic source at the heart of the expanding, cooling remnant of the supernova. This provides the first direct evidence for a young neutron star (or perhaps black hole) associated with a supernova. It identifies our youngest known compact object for continuing study, and promises to start to fill the many lacunae in our understanding of the behavior of matter at extreme densities.

References and Notes

BIOSCHEMISTRY

De Novo Design of an Enzyme
Reinhard Sterner and Franz X. Schmid

Enzymes catalyze biological reactions under mild conditions with high specificities and rate enhancements of up to $10^{17}$-fold (1). This enormous catalytic power is the product of natural evolution, and biochemists have tried for more than a century to understand the underlying chemical principles. The salient test of our understanding of enzyme catalysis would be the design of an enzyme from scratch. On page 697 of this issue, Dwyer, Looger, and Hellinga (2) describe an important step toward achieving this goal. Using computer-based rational protein design, they turned the catalytically inert ribose-binding protein (RBP) into an enzyme that is highly active as a triose phosphate isomerase (TIM).

TIM is the prototype of the large family of ($\beta\alpha_8$)-barrel or TIM-barrel enzymes (3). It is active in glycolysis, catalyzing the interconversion between the ketose dihydroxyacetone phosphate (DHAP), and the aldose glyceraldehyde-3-phosphate (GAP). The secret of catalysis by TIM lies in the precise orientation of three critical amino acid residues (glutamate, histidine, and lysine) in its active site and in controlled movements of the protein chain during catalysis. The glutamate residue abstracts a proton from C1 of the substrate DHAP. The histidine then mediates the transfer of a proton from the C1 to the C2 oxygen within the forming cis-enediol intermediate, which in turn is stabilized by hydrogen bonding to the lysine residue. The intermediate then collapses to give GAP and regenerates the enzyme (4). The abstraction of the C1 proton is energetically highly unfavorable, and the enediol intermediate easily loses its phosphate by $\beta$-elimination. To avoid this side reaction, TIM uses a mobile loop as a lid to close the active site after the substrate is bound. Thus, the generation of TIM activity on a different protein scaffold is a demanding task.

Hellinga and his co-workers are pioneers in computational protein design. Recently, they succeeded in transforming RBP into a high-affinity receptor for non-natural ligands as diverse as serotonin and trinitrotoluene (5). Now, in going beyond binding and on to catalysis, they have again used RBP as the scaffold. The authors followed a three-step procedure. First, they defined geometries in which the three catalytically essential residues glutamate, histidine, and lysine are in a favorable orientation with respect to the enediol intermediate. Then they used a combinatorial search algorithm to find amino acid positions within the RBP-binding region that satisfy these geometrical constraints (6). Finally, they used their receptor-design algorithm to optimize the potential active site for the binding of the enediol intermediate (5).

In this way 14 RBP variants were designed, produced, and assayed for TIM activity. Remarkably, seven of these converted GAP into DHAP at a rate above background.

The most active RBP variant contained 10 amino acid substitutions, in addition to the catalytic residues glutamate, histidine, and lysine. This variant, which was much more labile than native RBP, was stabilized—again with computational design—by optimizing the residue layers surrounding the immediate binding surfaces (7). The stability of the resulting RBP variant, NovoTim1.2, approached that of native RBP. To further improve the catalytic activity, the authors turned to the completely different approach of directed evolution, which combines random mutagenesis with in vivo selection. The evolved subvariants of NovoTim1.2, which contained additional amino acid substitutions at the protein surface remote from the active site, catalyzed the TIM reaction at a rate $10^2$- to $10^6$-fold above background but with catalytic efficiency parameters $k_{cat}/K_M$ about...
three orders of magnitude below that for wild-type TIM. This reduced efficiency does not diminish the achievement, considering that native TIM is a kinetically perfect enzyme with a turnover that is limited only by the diffusion-determined rate at which substrate and enzyme encounter each other (4).

Attempts to achieve the de novo design of enzymes are not new (8). Antibodies with catalytic activities have been obtained by using analogs of transition states as antigens (9). Recently, Bolon and Mayo used computational design to transform the catalytically inert protein thioredoxin into an esterase (10). However, both the catalytic antibodies and the designed thioredoxin are much less active than the RBP variants transformed into TIM.

The new work by the Hellinga lab (2) exemplifies the enormous power of computational biology, and illustrates how this approach can be combined with directed evolution. The latter is well suited to identify beneficial mutations far from the active site. Such mutations are difficult to find by computation but important for the fine-tuning of catalysis (11).

Recently, in equally exciting work, Baker and co-workers designed from scratch a small protein with a new folding topology and verified its structure by x-ray crystallography (12). With the important contributions of these two studies as a foundation, the design of tailored catalytic activities on artificial proteins seems now to be within reach. Such an achievement would be a milestone on the path to synthetic biology, with enormous potential for applications in medicine and biotechnology.

**References**


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**MATERIAL SCIENCE**

**Spinning Continuous Fibers for Nanotechnology**

Yuris Dzenis

Nano
tubes of carbon and other ma
terials are arguably the most fascinat
ing materials playing an important role in nanotechnology today. Their unique mechanical, electronic, and other properties are expected to result in revolutionary new materials and devices. However, these nanomaterials, produced mostly by synthetic bottom-up methods, are discontinuous objects, and this leads to difficulties with their alignment, assembly, and processing into applications. Partly because of this, and despite considerable effort, a viable carbon nanotube–reinforced super
anocomposite is yet to be demonstrated. Advanced continuous fibers produced a revolution in the field of structural materials and composites in the last few decades as a result of their high strength, stiffness, and continuity, which, in turn, meant processing and alignment that were economically feasible. Fiber mechanical properties are known to substantially improve with a decrease in the fiber diameter. Hence, there is a considerable interest in the development of advanced continuous fibers with nanoscale diameters. However, conventional mechanical fiber spinning techniques cannot produce fibers with diameters smaller than about 2 μm robustly. Most commercial fibers are several times that diameter, owing to the trade-offs between the technological and economic factors.

Electrospinning technology enables production of continuous polymer nano
fibers from polymer solutions or melts in high electric fields. When the electric force on induced charges on the polymer liquid overcomes surface tension, a thin polymer jet is ejected. The charged jet is elongated and accelerated by the electric field, undergoes a variety of instabilities, dries, and is deposited on a substrate as a random nanofiber mat. The first patent on the process was awarded in 1934; however, outside of the filter industry, there was little interest in the electrospinning or electrospray nanofibers, until the mid-1990s (1). Since that time, the process attracted rapidly growing interest triggered by potential applications of nanofibers in the nanotechnology. The publication rate has nearly doubled annually, reaching about 200 articles in 2003. Over a hundred synthetic and natural polymers were electro
spun into fibers with diameters ranging from a few nanometers to micrometers (see the figure, panel A).

The main advantage of this top-down nanomanufacturing process is its relatively low cost compared to that of most bottom-up methods. The resulting nanofiber samples are often uniform and do not require expensive purification (panels B and C). Unlike submicrometer-diameter whiskers, inorganic nanorods, carbon nanotubes, and nanowires, the electrospray nanofibers are continuous. As a result, this process has unique potential for cost-effective electro
temperature control of fiber placement and integrated manufacturing of two- and three-dimensional nanofiber assemblies. In addition, the nanofiber continuity may alleviate, at least in part, concerns about the properties of small particles (2). Nanofibers are expected to possess high axial strength combined with extreme flexibility. The nanofiber assemblies may feature very high open porosity coupled with remarkable specific surface area. Yet, these assemblies would possess excellent structural mechanical properties. Uses of nanofibers in composites, protective clothing, catalysis, electronics, biomedicine (including tissue engineering, implants, membranes, and drug delivery), filtration, agriculture, and other areas are presently being developed. Clearly, there is a growing interest in the process, but the results reported to date are centered mostly on the empirical production and the proposed uses of polymer nanofibers. At the same time, thorough understanding of the mechanisms of jet formation and motion is needed for the development of robust methods of process control. Analysis of the electrospinning process is complicated by electro
temperature coupling, nonlinear rheology, and unusual jet instabilities. Some progress was recently made on modeling of jet initi
tion (3, 4). Steady-state spinning was modeled in the nonlinear rheologic regime important for polymer jets (5, 6). Experimental observations and modeling of bending (or whipping) instability (7, 8) produced a major breakthrough in process analysis. It substantially improved our understanding of the jet motion and removed an early controversy in the electrospinning studies over the interpretation of long–ex-

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