Engineering and design

Editorial overview

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Biomolecular engineering is an emerging field that integrates the structural and physical properties of natural macromolecules to optimize biological function and even to create new macromolecules not found in nature. This rapidly developing area, reviewed in this section, joins disciplines ranging from molecular biology to protein and synthetic polymer chemistry.

The tools of molecular biology are the most frequently used to probe natural proteins and produce new ones *de novo*. As reviewed by Zoller (pp 605–610), the past few years have for the most part been a period of method refinement, with few fundamentally new methods reported for DNA synthesis or site-specific mutatgenesis. Nevertheless, improvements have occurred in the high-level expression of active proteins in bacteria and mammalian cells. These developments in protein production have been crucial in allowing detailed functional and structural characterization. In addition, the technology of phage display, in which mutant proteins can be presented on the surface of filamentous phage particles and selected by affinity chromatography *in vitro*, raises the possibility of rapid and generic screening of random epitope libraries for optimized or new binding properties.

A significant proportion of protein-engineering experiments involve the introduction of point mutations into well characterized enzymes in order to investigate the roles of specific residues in catalytic mechanisms (Atkins and Sligar, pp 611–616) and substrate specificities (Wilson and Agard, pp 617–623). In many respects, these experiments are descendants of traditional chemical-modification studies. Unlike chemical modification, however, one can propagate a mutant protein, and the site of modification as well as the extent of modification (barring misincorporation) can be assured. Limitations of the diversity of substitution to the naturally occurring amino acids potentially can be overcome by site-specific misincorporation, semi-synthetic methods and the introduction of thiols to effect specific chemical modifications. Early results from mutated enzymes were often rationalized as simple electrostatic or van der Waals effects. The more recent studies reviewed in this section reflect a more mature understanding of the complex interrelationships among non-covalent forces that govern binding and much of catalysis.

Protein engineering has been applied to the improvement or production of new catalytic activities based on natural enzyme mechanisms. In the case of catalytic antibodies, reviewed by Lewis and Hilvert (pp 624–629), new catalysts are created using the adaptive ability of the immune system to generate high-affinity binding sites for transition-state (TS) analogs. An impressive range of catalytic antibodies for specific reactions has been produced thanks to the ingenuity of enzymologists and synthetic organic chemists in creating TS analogs. Indeed, some reactions are catalysed by antibodies for which there are (apparently) no natural counterparts. Although this approach has rarely produced catalysts that rival natural enzymes in catalytic efficiency, it is only a matter of time before iterative protein-engineering methods supported by X-ray structures of TS-analog–antibody complexes, or clever biological selections or phage display technology improve these catalysts.

The *de novo* synthesis of proteins and protein-like hybrid molecules aims to produce new structures based upon design principles derived for known protein structures. As reviewed by Sander (pp 630–637), one approach to *de novo* design is to assemble multiple copies of a natural subdomain sequence to produce repetitive protein structures. Another approach is to 'minimalize' simple protein motifs such as the four-helix bundle and antiparallel β-sandwiches. However, many of the physical properties of these molecules more closely resemble those of a molten globule, an intermediate state in protein folding that possesses much native-like secondary structure with disordered side chains in the hydrophobic core. Overall, these exciting experiments in *de novo* design indicate that secondary structure plus a non-specific hydrophobic core are sufficient for stable folding but appear insufficient for a fixed structure with a uniquely packed interior. This may be one reason why these proteins have resisted attempts to crystallize them. As more specific interior packing arrangements and tertiary constraints are designed *de novo*, it appears that engineered proteins with more native-like properties will soon be obtained.

Biopolymers have many desirable physical properties that have not been duplicated in conventional organic polymers, as reviewed by Tirrell, Fournier and Mason

Abbreviation
TS—transition-state.

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(pp 638–641). Moreover, the precise control over polymer length and sequence afforded by recombinant DNA technology offers the potential to produce a new generation of polymer-based materials. These could, for example, incorporate the functional properties of globular proteins into polymeric arrays that could be fabricated for material applications. So far, this work has focussed on the synthesis and expression of canonical silk and elastomer polymer and copolymer sequences for fiber and biomechanical applications. Other efforts aim to control regular chain folding in a repetitive polymer in order to facilitate spontaneous formation of two-dimensional sheets.

Biopolymers, organized both as linear arrays and two-dimensional lattices, can provide the structural building blocks required for the assembly of designed molecular composites. More ambitious objectives involve the production of engineered nanostructures that might in principle incorporate several engineered biomolecules in a functional assembly. Silicon-based semiconductors, which organize devices as superpositions of two-dimensional arrays, inspire similar designs for biopolymers. Kornberg and Darst (pp 642–646) review what may be a general approach to forming a biotinylated phospholipid which forms a two-dimensional membrane array. Additional ordered arrays of biotinylated macromolecules can be built upon this by allowing them to bind to the tethered streptavidin. Originally, this system provided a means for organizing large macromolecular assemblies into two-dimensional arrays that could facilitate structural analysis by electron-diffraction studies. It now appears feasible to connect the specific binding offered by these two-dimensional arrays to the prototype biosensor reviewed by McConnell *et al.* (pp 647–652). Such devices that marry the specificity of proteins to solid-state electronic substrates may have wide-ranging applications as biosensors or electronic devices.

One ultimate objective in 'nanofabrication' involves the production of macromolecular assembles in which interactions among the elements are uniquely determined (as in multicomponent complexes such as ribosomes). The use of one- or two-dimensional lattices as frameworks is limited to the generation of symmetric assemblies. Seeman (pp 653–661) describes studies to create stable models of DNA recombination junctions. These studies have necessitated the development of computational methods that allow the design of DNA lattices and polyhedra with specific connections. That this approach can succeed has been demonstrated through the synthesis and production of a DNA cube. This work suggests a strategy for ultimately engineering asymmetric molecular assemblies.

The field of biomolecular engineering is in its infancy. Its development has been accelerated by virtue of the synergy between the synthetic tools of chemistry and recombinant DNA technology, and the increasing understanding of the relationships between macromolecular structure and function.

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