Engineering and design

Editorial overview

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Introduction

In this second year of Engineering and Design, we review recent protein engineering work in the context of industrial, pharmaceutical and materials applications. Also reviewed are approaches that use nucleic acids both as a means of directing molecular design and engineering catalysts. Many of the topics reflect areas that have been actively investigated for many years, but have only recently been impacted by recombinant DNA technology. Most of the topics covered are interdisciplinary in nature and attempt to achieve some novel functionality in a designed molecule for a practical objective.

Pantoliano (pp 559–568) begins with a discussion of recent work in the area of ultrastable protein design. The work focuses on bacterial serine proteases, which are well understood mechanistically and structurally. Serine proteases have been the subject of numerous studies aimed at modifying protein stability in a variety of hostile environments, both for practical applications in laundry detergents and as catalysts for organic synthesis. The work makes clear that the current strategies for protein stabilization involving the formation of disulfide bonds, salt links, hydrophobic residue substitutions, etc. do not always produce expected results. In some situations, stabilizing modifications can produce equivalent results in both aqueous and non-aqueous environments, whereas in other situations, stabilization is achieved in one solvent environment (e.g. non-aqueous organic solvents) but not another. One of the most interesting results to emerge from this work is the additivity of effects of individual mutations on protein stability.

Nilsson and coworkers (pp 569–575) describe a variety of applications of fusion proteins created by gene splicing. These include enhancements in recombinant protein folding and affinity purification, as well the formation of fused or linked functional domains for drug targeting, immunology, and protein epitope display. What is remarkable about these studies is the apparent ease with which protein domains can be spliced together with relatively infrequent interference in refolding interconnected domains. Indeed, in some cases, considerable stabilization and solubilization is afforded to domains fused to other molecules. Fusion proteins are clearly useful tools for biotechnologists and protein engineers alike.

The greater understanding of the results of protein engineering depends on determining the three-dimensional structure of the protein molecule or a homologue. Three-dimensional structures are obtained through methods of protein X-ray crystallography, or for smaller proteins, by multidimensional NMR spectroscopy. Forest and Schutt (pp 576–581) review current protein engineering work aimed at introducing heavy-atom-binding sites into protein molecules to facilitate phasing of X-ray diffraction data, and engineering molecular surface interactions to control crystal formation. Active work in this area continues to amplify the impact of recombinant DNA technology on structural studies of proteins and nucleic acids.

Cappello (pp 582–586) outlines the progress and potential applications of synthetic biomaterials for prosthetic devices such as sutures, tendons, vascular grafts and cartilage. These studies have motivated several studies of hybrid polymeric materials that incorporate specific sequences that stimulate cell attachment. The elastin and elastin copolymer systems that are generated using conventional synthetic methods are among the best understood in terms of structure–function relationships. The lessons learned from these and other synthetic polymers may be extended to polymers produced by recombinant methods. This budding technology should provide clinicians with a variety of devices to aid in tissue transplant and repair.

In addition to biomaterials applications, there has been expanding activity in engineering protein molecules as components of molecular electronics devices, as reviewed by Sligar and Salemme (pp 587–592). Several site-directed substitution experiments have been described recently that aim to modify the spectroscopic, electronic or self-organizing properties of photoactive or redox-active proteins. Whereas some of these are simply illustrative of basic strategies that might be useful for molecular electronic applications, others specifically focus on central issues of molecular electronics device fabrication and function. These include the relative stabilization of spectroscopic states in molecules such as bacteriorhodopsin for applications in photo-optical memory devices, and

Abbreviation

CDR—complementarity-determining region.

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the immobilization of oriented heme-containing proteins on electrode surfaces for biosensor applications.

Antibody engineering (Presta, pp 593–596) continues to be an area of intense activity, owing both to its practical applications in therapeutics and drug targeting (also discussed by Nilsson and coworkers, (pp 569–575), as well as potential applications as catalysts. In the former area, Presta describes substantial progress that has been made in 'humanizing' mouse monoclonal antibodies using protein engineering methods. In most cases, simple grafting of antigen-binding loops, or complementarity-determining regions (CDRs), onto a human framework is insufficient to recover the binding affinity inherent in the mouse antibody. By introducing suitable mutations into the human framework, however, one can better accommodate the grafted CDRs and, in most cases, recover nearly full binding capacity. Thus, a combination of CDR grafts and framework adjustments using structure-based design principles makes humanization a reliable and practical alternative to the engineering of natural human antibodies. Alternatively, the advent of human antibodies produced by transplanting the human immune system into mice or selecting antibodies from human phage display libraries (see also Wells and Lowman, pp 597–604) does not seem far off.

The past two years have seen tremendous progress in the development of general schemes for sorting and selecting novel and improved binding properties for peptides and proteins, as reviewed by Wells and Lowman (pp 597–604). In new biological schemes, collectively called phage display, 10^7–10^8 different proteins or peptides are displayed on individual filamentous phage particles and separated by affinity chromatography for binding to a target receptor, antibody, hapten, enzyme or substrate. In new chemical strategies, huge libraries of soluble or immobilized peptides can be screened for binding to a target protein. Both approaches and their combinations harbinger a new era of protein, peptide and drug design, where the requirements for detailed understanding of binding principles are more relaxed because of the ease with which diverse sets of molecules may be generated, screened and remutated for optimal binding properties. These methods, however, are most efficiently applied in the context of a detailed structure because mutagenesis can then be focused to those regions of the molecule where it counts the most. Moreover, these studies are likely to generate an enormous amount of new information linking structure with function, which will surely help to clarify the rules for rational protein and drug design.

In the final review in this section, Cech (pp 605–609) reviews recent work in ribozyme engineering. Ribozymes are structured RNA molecules that can catalyze a variety of reactions. These have been most extensively studied in extensions of their natural catalytic role, which is the cleavage of RNA or DNA. Ribozyme engineering is a rapidly growing field that fuses aspects of enzyme engineering, nucleic acid antisense technology, and polymerase chain reaction amplification technology. Therapeutic applications of ribozymes are particular interesting, as they might provide general methods for specifically controlling viral replication or protein expression.

In summary, the tools offered by recombinant DNA technology and conventional chemical synthesis are acting synergistically to drive a comprehensive new technology of molecular engineering. As outlined here, this technology promises wide practical applications in areas of engineered therapeutics, biomaterials and molecular electronics.

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