



ELSEVIER

The latest in Engineering and design: the 2005 collection

Editorial overview

Sophie E Jackson and Lynne Regan

Current Opinion in Structural Biology 2005,
15:432–434

0959-440X/\$ – see front matter
© 2005 Elsevier Ltd. All rights reserved.

DOI 10.1016/j.sbi.2005.07.009

Sophie E Jackson

Cambridge University Chemical Laboratory,
Cambridge University, Lensfield Road,
Cambridge CB2 1EW, UK
e-mail: sej13@cam.ac.uk

Lynne Regan

Department of Chemistry, and Department of
Molecular Biophysics & Biochemistry, Yale
University, 266 Whitney Avenue, New Haven, CT
06520, USA
e-mail: lynne.regan@yale.edu

There have been many important advances and achievements in the field of Engineering and design during the past two years. This section captures some of the excitement and highlights in the areas of membrane proteins, protein–protein interactions (understanding the determinants of avidity and specificity), strategies for therapeutic interventions that target protein–protein interactions, the special case of repeat proteins and the design of novel bionanomaterials. In addition to rational design, strategies to exploit ‘the awesome power of genetic selection’ are expanding and maturing. We include reviews of the latest developments in selection and screening strategies, and the practical products that have been obtained from them.

Although our knowledge of membrane protein structure and function still lags behind that of globular proteins, the past few years have seen significant advances in this area. These can, in part, be attributed to a greater understanding not only of the proteins themselves but also of the lipid bilayers in which they sit. In her review, Booth describes studies of the biophysical properties of bilayers, such as stored curvature elastic stress, which are now known to play important roles in membrane protein insertion, folding, stability and function.

Lipid properties can now be rationally manipulated and exploited, not only to optimise yields of membrane protein reconstitution for structural studies but also to probe the relationship between lipid environment and membrane protein function. The review also describes novel studies in which mechanosensitive channels are used to investigate membrane properties and their effects on membrane protein function. Highlighted is the fact that biophysical measurements *in vitro* are now proving invaluable for describing mechanistic aspects of membrane protein insertion, integration and folding *in vivo*. The membrane protein field continues to advance by leaps and bounds, as illustrated by Booth’s discussions of membrane protein *de novo* design and redesign.

The molecular bases of increasing numbers of diseases are being elucidated. Many are associated with the malfunction, loss of function or gain of function of a specific protein. There are far too many examples to consider even listing here. The review by Ryan and Matthews, however, spotlights an interesting subset of diseases that result from the perturbation of specific protein–protein interactions. Their review discusses recent work on pathogen–host protein–protein interactions, which lead to viral or bacterial invasion of our cells, and highlights the common structural motifs that are involved — notably the β zipper. These regions are often unstructured in the parent protein and gain structure upon complex formation. This seems to be a common theme; other examples include the LIM proteins, which are linked to leukaemia and breast cancer.

This contrasts with huntingtin (htt), a polyglutamine repeat expansion protein that is linked to Huntington's disease. Htt is ubiquitously expressed with many interaction partners. It is thought that the disease state arises not only from the potential loss of interactions with normal binding partners but also through gain of function and new interactions between the polyglutamine repeat and other cellular proteins that are not normally htt-binding partners, thereby altering their function. Therapeutic strategies, including the use of therapeutic antibodies and peptide-based inhibitors, are discussed. With the current level of interest in mapping protein-protein interaction networks through physical and genetic approaches, many more cases in which the disruption of protein-protein interactions leads to a diseased state may be identified in the near future.

For many years, scientists have been trying to re-engineer proteins, particularly enzymes, for a multitude of practical applications (e.g. better laundry detergents). Limited success with rational approaches has led to the recent explosion of work adopting directed evolution strategies to engineer the desired properties into enzymes. Arnold and co-workers review recent work in this area. The relative merits and success of different approaches, including random mutagenesis, DNA shuffling, targeted mutagenesis, and homologous and non-homologous recombination, are all covered. Highlighted are some novel results that will influence future design strategies, for example, the number of mutations remote from active sites that are found to have beneficial effects on activity and the number of multiple mutations that are found to be beneficial even though the corresponding single mutations are not. These examples, and others, suggest that the most effective engineering strategies may be those that combine different techniques. This comprehensive review cites many examples of enzymes engineered for specific purposes, including enzymes with enhanced selectivity, stereospecificity and activity towards novel substrates (expanded substrate repertoires), and increased stability and solubility. In an interesting case, novel tRNA synthetases were designed/selected for their ability to specifically recognise non-natural amino acids. In this case, not only was positive selection used, but also negative selection was incorporated into the selection procedure to improve specificity. Extending these approaches, the review discusses how chemical and structural knowledge is increasingly being incorporated into design projects, and the success that has been achieved in this area.

Since it was first established some years ago that small peptides could self-assemble into filaments and fibrils, there has been an explosion of work that exploits this property to engineer new materials for a large number of uses. In their article, Fairman and Åkerfeldt review this fast-growing and exciting field. Work from the Fairman and Woolfson groups, using peptides that form coiled

coils, illustrates well the power of the technique. Fibres of different lengths, widths and morphologies can be made by subtly controlling the conditions; kinks and branches can be introduced using novel orthogonal chemistries, and peptides (and therefore fibrils) can be functionalised in many different ways, for example, by the introduction of tags that bind antibodies. In these cases, it is interesting that the two groups used complementary approaches; the Woolfson group focuses on electrostatic interactions in the coiled coils, whereas the Fairman group considers hydrophobic groups and packing with equal success. In addition to the all-helical coiled-coil structures, work on engineering predominantly β structures, such as amyloid and silk, is also discussed. A significant goal in this area is the introduction of 'smart behaviour', that is, materials that respond (preferably reversibly) to their environment. The conformational plasticity inherent in protein and peptide-based systems is ideal for this use. For example, silk, which is highly polymorphic in nature, changes conformation in response to wetting, redox and phosphorylation. Synthetic peptides are now being made that also have these desirable properties. Hydrogels and organogels are good examples whereby the transition between random coil, semi-flexible gels and more rigid gels can be controlled by solvent, pH, temperature, salt and, in some cases, concentration of peptide. These materials are now being investigated for potential use in tissue engineering, as scaffolds for the regeneration of cartilage and the promotion of nerve cell growth. The biocompatibility, immunogenicity and biodegradability of these novel materials, in conjunction with the fact that they can be modified or customised (e.g. tagged for interaction with specific cell types), makes them exciting and promising systems. In addition to potential applications in medicine, there is significant interest in these self-assembling peptide systems for use in patterning, miniaturised solar cells, and optical and electronic devices.

Although ubiquitous and comprising over 5% of proteins in metazoans, repeat proteins have, until recently, been little studied. The review by Main *et al.* highlights recent studies that have revealed fundamental aspects of the determinants of repeat protein folding and stability. The relative structural simplicity and modular nature of repeat proteins enables a more straightforward treatment of their physical properties than has proven possible for most globular proteins. Main *et al.* also highlight repeat protein function and present several examples of the re-engineering of these proteins to bind to different ligands.

It is a truism that, in order to perform a genetic selection or screen, there must be a means to link genotype to phenotype. Selection for activity in bacterial or other cells is an *in vivo* strategy with a long history, but the activities that can be readily selected are limited. The review by Leemhuis *et al.* presents alternative methods, such as

phage display, ribosome display and mRNA display, and discusses the pros and cons of each. Several of these approaches have been addressed in prior issues of *Current Opinion in Structural Biology*.

One innovative and exciting new approach that this review presents in detail, and that is now beginning to come into its own and be used in more applications, is *in vitro* compartmentalization (IVC). In IVC, the natural 'biological components' are kept to a minimum, and genotype and phenotype (the DNA encoding the protein

and the protein activity) are linked by co-encapsulation in a micelle or by co-attachment to streptavidin-coated beads. The examples shown reveal the power of this approach and hint at potential future applications.

One can identify common themes running through the different reviews in this section: the manifold importance of protein–protein interactions, the power of combining design and selection, and the overall success of numerous different design and engineering strategies that would have been unimaginable just a few years ago.