Elsewhere in biology

Chosen and summarized by the staff of *Chemistry & Biology*

A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*.

Chemistry & Biology March1996, **3**:217–220

☐ **The Hin dimer interface is critical for Fis-mediated activation of the catalytic steps of site-specific DNA inversion.** Michael J. Haykinson, Lianna M. Johnson, Joyce Soong and Reid C. Johnson (1996). *Curr. Biol.* **6**, 163–177.

Hin is a member of an extended family of site-specific DNA recombinases, the DNA invertase/resolvase family. Hin dimers catalyze DNA inversion between two recombination sites; Hin activity requires the regulatory protein Fis, which associates with a *cis*-acting recombinational enhancer sequence. The authors show that the interface between the two Hin monomers is analogous to that in the related enzyme γδ resolvase, the structure of which has recently been solved, and that disruption of the interface by detergent increases the rate of the first chemical step of the inversion reaction, cleavage of the DNA strand. Disulfide-linked dimers of Hin are catalytically inactive, but if the crosslinkable Hin monomers are activated by Fis-bound enhancer before exposure to oxidizing agents they remain active. The authors conclude that the Fis-bound enhancer may trigger a conformational change in the Hin dimer that is important for concerted DNA cleavage within both recombination sites, and possibly also important for the subsequent exchange of DNA strands.
1 Feb 1996, Research Paper, *Current Biology*

☐ **Engineering green fluorescent protein for improved brightness, longer wavelengths, and fluorescence resonance energy transfer.** Roger Heim and Roger Y. Tsien (1996). *Curr. Biol.* **6**, 178–182.

The green fluorescent protein (GFP) of the jellyfish *Aequora victoria* is the first, and so far only, example of a cloned protein that generates strong visible fluorescence without the need for added cofactors. GFP variants would be very useful to track the fates of multiple proteins, or multiple cell lineages, simultaneously. The simplest way to shift the emission color of GFP is to substitute histidine or tryptophan for the tyrosine in the chromophore (Tyr66), but such blue-shifted point mutants are only dimly fluorescent. Here the authors describe additional substitutions, mainly in residues 145–163, that improve the visualization of the blue-shifted point mutants Tyr66His and Tyr66Trp, giving useable variants with excitation and emission maxima of about 381 and 445 nm, respectively. A second series of mutations starting with the red-shifted mutant Ser65Thr produced a GFP variant with an excitation peak of 504 and emission peak of 514 nm. Thus, at least three different colors of GFP can now be distinguished using appropriate filter sets. The authors further demonstrate that energy transfer between different colors of GFP can be used to detect protease activity.
1 Feb 1996, Research Paper, *Current Biology*

☐ **Double labelling of subcellular structures with organelle-targeted GFP mutants *in vivo*.** Rosario Rizzuto, Marisa Brini, Francesca De Giorgi, Raffaela Rossi, Roger Heim, Roger Y. Tsien and Tullio Pozzan (1996). *Curr. Biol.* **6**, 183–188.

The authors use the GFP mutants described above to label cellular organelles, by constructing two GFP chimeras that are targeted to mitochondria (using a signal derived from cytochrome c oxidase subunit VIII), and two GFP chimeras that are targeted to the nucleus (using the targeting information of the glucocorticoid receptor). Because the GFP mutants used are distinguishable in color, the nucleus and mitochondria can be visualized simultaneously in living cells. Similarly, two different variants of GFP were used to label the mitochondria of different cell types, and the events of cellular fusion, and the mitochondrial intermixing and/or fusion that follows, were directly monitored. The authors suggest that their results show that the direct study of protein–protein interactions and protein redistribution is now possible in living cells.
1 Feb 1996, Research Paper, *Current Biology*

☐ **Subcellular distribution of p21 and PCNA in normal and repair-deficient cells following DNA damage.** Rong Li, Gregory J. Hannon, David Beach and Bruce Stillman (1996). *Curr. Biol.* **6**, 189–199.

The p21 protein binds to the cyclin-dependent kinases (Cdks) and the proliferating cell nuclear antigen (PCNA). In mammalian cells, DNA damage leads to increased levels of p53, stimulating expression of p21 and leading to inhibition of Cdks and delay of cell-cycle progression. *p21* gene expression can also be induced in a p53-independent manner by stimulating quiescent cells with serum. The authors show that serum induction of p21 led to only transient increases in p21 levels, whereas DNA damage by UV irradiation led to a sustained high level of p21 that is tightly associated with nuclear structures. Cells deficient in nucleotide excision–repair showed a distinct pattern of detergent-insoluble p21 that co-localized with PCNA. This is consistent with previous results that the direct interaction between p21 and PCNA blocks PCNA's function in DNA replication, but not in DNA repair; the co-localization of p21 and PCNA in the nuclei of normal and repair-deficient cells indicates that p21 and PCNA interact during post-damage events.
1 Feb 1996, Research Paper, *Current Biology*

☐ **A new family of regulators of G-protein-coupled receptors?** David P. Siderovski, Andrew Hessel, Stephen Chung, Tak W. Mak and Michael Tyers (1996). *Curr. Biol.* **6**, 211–212.

Continuous stimulation of G-protein coupled receptors often leads to their desensitization, which is mediated in part by the actions of G-protein-coupled receptor kinases, which phosphorylate agonist-occupied receptors, and the arrestins, which subsequently bind to the receptors. The authors argue, based on the distribution of newly identified sequence motifs and a genetic experiment in yeast, that a third group of proteins, called the G0S8/Sst2p family, may also desensitize G-protein-coupled receptors.
1 Feb 1996, Brief Communication, *Current Biology*

☐ **Mutational effects on inclusion body formation in the periplasmic expression of the immunoglobulin V_L domain REI.** Winnie Chan, Larry R. Helms, Ian Brooks, Grace Lee, Sarah Ngola, Dean McNulty, Beverley Maleeff, Preston Hensley and Ronald Wetzel (1996). *Folding & Design* **1**, 77–89.

Inclusion body formation in bacteria is an important example of protein misassembly. Previous studies of mutational effects in other systems imply that it is the stability of a folding intermediate, not that of the native state, that is important in the formation of inclusion bodies. The authors examine a series of mutants of the light-chain variable domain of the REI antibody, V_L, and find that although all of these mutants contain the correct intradomain disulfide bond, they are all found in inclusion bodies to varying degrees. Inclusion body formation does not correlate with monomer/dimer equilibrium constants, but does correlate with the thermodynamic stability of the protein. Thus, at least for this protein, the intermediate important for inclusion body formation is a late equilibrium folding intermediate, not the transient intermediates that appear to be involved in other systems. This system may prove to be a useful model for light-chain amyloidosis and other immunoglobulin deposition diseases.
7 Feb 1996*, Research Paper, *Folding & Design*

☐ **Conformational switching in designed peptides: the helix/sheet transition.** Robert Cerpa, Fred E. Cohen and Irwin D. Kuntz (1996). *Folding & Design* **1**, 91–101.

The structure adopted by peptides and proteins depends not only on the primary sequence, but also on conditions such as solvent polarity or method of sample preparation. The authors examine the effect of solution conditions on the folded conformation of two peptides, one of which contains the amino acid *p*-phenylazo-L-phenylalanine, which isomerizes upon irradiation. Spectroscopic analysis indicates that the two peptides switch between α-helix and β-sheet secondary structures; the conformation is dependent on solution conditions including pH, NaCl concentration, temperature and peptide concentration. Conditions encouraging aggregation favor the β-sheet conformation, whereas those discouraging aggregation favor helix. The peptide containing the *p*-phenylazo-L-phenylalanine residue switches between α-helix and β-sheet upon irradiation. The dependence of conformation on solution conditions has important implications for *de novo* protein design.
8 Feb 1996*, Research Paper, *Folding & Design*

☐ **Solution conformation of an immunogenic peptide derived from the principal neutralizing determinant of the HIV-2 envelope glycoprotein.** A. Patricia Campbell, Brian D. Sykes, Erling Norrby, Nuria Assa-Munt and H. Jane Dyson (1996). *Folding & Design* **1**, 157–165.

The major determinants involved in induction of immune protection in HIV infections have been extensively examined in HIV-1. These studies determined that the dominant antigenic site is located within a disulfide-linked loop in the V3 region of envelope glycoprotein gp120. The homologous V3 region of gp125 in HIV-2 is also important in induction of neutralizing antibodies. If common structural features could be found between the V3 loops of HIV-1 and HIV-2, this might aid in the development of generally effective HIV vaccines. The authors describe an NMR study of a sequence derived from the V3 loop of HIV-2 and show that it contains a β-turn that is similar to that found in the V3 loop of HIV-1.
11 Mar 1996*, Research Paper, *Folding & Design*

☐ **Crystal structures of various maltooligosaccharides bound to maltoporin reveal a specific sugar translocation pathway.** R. Dutzler, Y.-F. Wang, P.J. Rizkallah, J.P. Rosenbusch and T. Schirmer (1996). *Structure* **3**, 127–134.

Passive transport of maltodextrins across the outer membrane of *E. coli* occurs through the channel formed by maltoporin. The channel is specific for maltooligosaccharides, which cannot pass through non-specific porins. The authors describe the crystal structures of the complexes of maltoporin with maltose, maltotriose or maltohexose. The maltoporin channel lining contains a hydrophobic path, composed of hydrophobic residues (the 'greasy slide'), which forms van der Waals contacts with the maltooligosaccharide. The 'slide' directs the ligand from the extracellular surface through a channel constriction to the periplasmic outlet. At the constriction, a network of polar amino acids forms hydrogen bonds with the substrate, explaining the stereospecificity of the channel.
15 Feb 1996, Research Paper, *Structure*

☐ **Perturbations in the surface structure of A22 Iraq foot-and-mouth disease virus accompanying coupled changes in host cell specificity.** Stephen Curry, Elizabeth Fry, Wendy Blakemore, Robin Abu-Ghazaleh, Terry Jackson, Andrew King, Susan Lea, John Newman, David Rowlands and David Stuart (1996). *Structure* **3**, 135–145.

Foot-and-mouth disease virus (FMDV) is an antigenically diverse picornavirus that infects cloven-hoofed animals.

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Antigenic variations in the A22 subtype of the virus arise when it is subjected to different growth conditions. Here, the structures of two variants of A22 FMDV are determined, to investigate the basis for antigenic variability. The capsid in FMDV is composed of four proteins, VP1–4. In both A22 variants, the major antigenic loop of VP1 is flexible, but its overall disposition is distinct from that observed in other FMDV serotypes. The different conformation of this loop causes an alteration in the fold of an adjacent loop from the VP3 protein. In one of the A22 variants, a single point mutation in VP2 appears to perturb the structure of the major antigenic loop in VP1. These results provide two examples of the propagation of structural perturbations across the surface of a virion.
15 Feb 1996, Research Paper, *Structure*

☐ **Adenylate kinase motions during catalysis: an energetic counterweight balancing substrate binding.** CW Müller, GJ Schlauderer, J Reinstein and GE Schulz (1996). *Structure* **3**, 147–156.

Nucleoside monophosphate (NMP) kinases catalyze the transfer of the γ phosphate of ATP to NMP. The crystal structures of several NMP kinases from different species have been solved previously, with or without bound substrate. Superposition of these structures indicates that there are large domain movements upon substrate binding. To date, however, structures of the same enzyme, with and without bound substrate, have not been available to allow direct comparison of the two states. Here, the structure of *E. coli* adenylate kinase in the absence of substrate is solved and compared to the previously solved structure of the substrate-bound enzyme. Upon substrate binding, the enzyme increases its chain mobility in a region remote from the active center. The region 'resolidifies' when the products are released, causing an increase in internal binding energy. This increase may be necessary to counterbalance the loss of energy caused by substrate binding, because the phosphoryl transfer reaction does not provide any energy.
15 Feb 1996, Research Paper, *Structure*

☐ **Crystal structures of murine polyomavirus in complex with straight-chain and branched-chain sialyloligosaccharide receptor fragments.** Thilo Stehle and Stephen C Harrison (1996). *Structure* **3**, 183–194.

Murine polyomavirus binds to straight-chain receptors, terminating in (α2,3)-linked sialic acid, on the surface of susceptible cells. Some polyomavirus strains also bind to branched-chain oligosaccharides with a second, (α2,6)-linked sialic acid moiety. The difference in the strains has been linked to mutation of a single residue (position 91) in the major capsid protein, VP1. Strains with Glu91 bind only straight-chain receptors, whereas strains with Gly91 bind both straight- and branched-chain receptors. Here, the structure of polyomavirus with the Gly91 mutation has been determined in a complex with model compounds for both straight- and branched-chain receptors. The sialic acid-(α2,3)-galactose moiety, which is common to both compounds, makes specific contacts with VP1 that are identical to those made with VP1 of strains containing Glu91. The additional (α2,6)-linked sialic acid moiety of the branched-chain receptor fragment fits into a surface pocket, but does not form hydrogen bonds to VP1. In either case, there are relatively few hydrogen bonds between glycan and protein. It is the nature of the linkage itself that is the principal determinant of specificity, not the position of particular hydroxyl groups.
15 Feb 1996, Research Paper, *Structure*