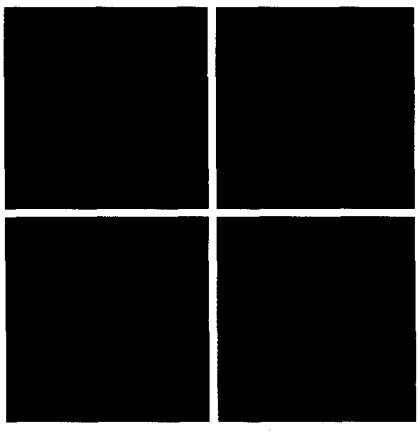
Elsewhere in biology

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

Chemistry & Biology February 1999, 6:R54–R57

☐ **Separate nuclear import pathways converge on the nucleoporin Nup153 and can be dissected with dominant-negative inhibitors.**
Sundeep Shah and Douglas J Forbes (1998). *Curr. Biol.* **8**, 1376–1386.

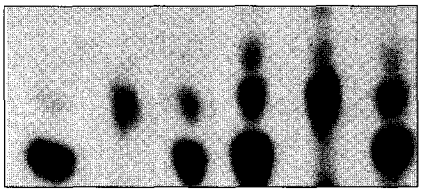
Proteins generally enter or exit the nucleus as cargo of one of a small family of import and export receptors. These receptors bear distant homology to importinβ, a subunit of the receptor for proteins with classical nuclear localisation sequences (NLSs). To understand the mechanism of nuclear



transport, the nuclear pore proteins that interact with the different transport receptors as they dock at the pore and translocate through it need to be identified. Two pathways of nuclear import were found to intersect at a single nucleoporin, Nup153, localized on the intranuclear side of the nuclear pore. Nup153 contains separate binding sites for importin α/β, which mediates classical NLS import, and for transportin, which mediates import of different nuclear proteins. Strikingly, a Nup153 fragment containing the importin β binding site acted as a dominant-negative inhibitor of NLS import, with no effect on transportin-mediated import. Conversely, a Nup153 fragment containing the transportin binding site acted as a strong dominant-negative inhibitor of transportin import, with no effect on classical NLS import. The interaction of transportin with Nup153 could be disrupted by a non-hydrolyzable form of GTP or by a GTPase-deficient mutant of Ran, and was not observed if transportin carried cargo. Neither Nup153 fragment affected binding of the export receptor Crm1 at the nuclear rim. Two nuclear import pathways, mediated by importin and transportin, converge on a single nucleoporin, Nup153. Dominant-negative fragments of Nup153 can now be used to distinguish different nuclear import pathways and, potentially, to dissect nuclear export.
8 December 1998, Research Paper, *Current Biology*.

☐ **Synergistic activation of SAPK1/JNK1 by two MAP kinase kinases *in vitro*.**
Sean Lawler, Yvonne Fleming, Michel Goedert and Philip Cohen (1998). *Curr. Biol.* **8**, 1387–1390.

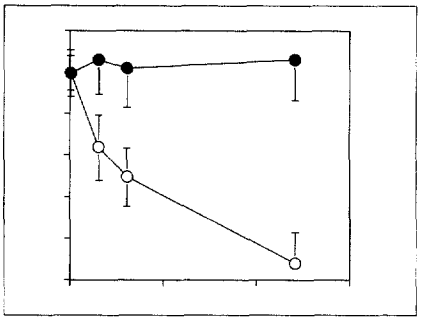
Mitogen-activated protein kinases (MAPKs) mediate many of the cellular effects of growth factors, cytokines and stress stimuli. Their activation requires the phosphorylation of a threonine and a tyrosine residue located in a Thr–X–Tyr motif (where X is any amino acid). This phosphorylation is catalysed by MAPK kinases (MKKs), which are all thought to be dual specificity enzymes that phosphorylate both the threonine and the tyrosine residue of the Thr–X–Tyr motif. Here, the authors report that the MAPK family member known as stress-activated protein kinase-1c (SAPK1c, also known as JNK1) is activated synergistically *in vitro* by MKK4; also called SKK1 and JNKK1) and MKK7 also called SKK4 and JNKK2). MKK4 was found to have a preference for the tyrosine residue, and MKK7 for the threonine residue, within the Thr–X–Tyr motif. These observations suggest that full activation of SAPK1c *in vivo* might sometimes require phosphorylation by two different MKKs, providing the potential for integrating the effects of different extracellular signals. It is also possible that other MAPK family members might be activated by two or more MKKs and that some MKKs may have gone undetected because they phosphorylate the tyrosine residue only, and therefore do not induce any



activation unless the threonine has first been phosphorylated by another MKK.
14 December 1998, Brief Communication, *Current Biology*.

☐ **Phosphatidylinositol-4,5-bisphosphate is required for endocytic coated vesicle formation.**
Matthias Jost, Fiona Simpson, Jennifer M Kavran, Mark A Lemmon and Sandra L Schmid (1998). *Curr. Biol.* **8**, 1399–1402.

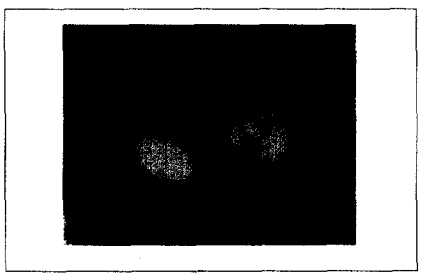
Receptor-mediated endocytosis via clathrin-coated vesicles has been extensively studied and, although many of the protein players have been identified, much remains unknown about the regulation of coat assembly and the mechanisms that drive vesicle formation. Some components of the endocytic machinery interact with inositol polyphosphates and inositol lipids *in vitro*, implying a role for phosphatidylinositols *in vivo*. No direct evidence for the involvement of phosphatidylinositols in clathrin-mediated endocytosis exists to date, however. Using well-characterized PH domains as high-affinity and high-specificity probes in combination with a perforated cell assay that reconstitutes



coated vesicle formation, the authors provide the first direct evidence that PtdIns(4,5)P_2 is required for both early and late events in endocytic-coated vesicle formation.
14 December 1998, Brief Communication, *Current Biology*.

☐ **Nuclear import of Ran is mediated by the transport factor NTF2.**
Alicia Smith, Amy Brownawell and Ian G Macara (1998). *Curr. Biol.* **8**, 1403–1406.

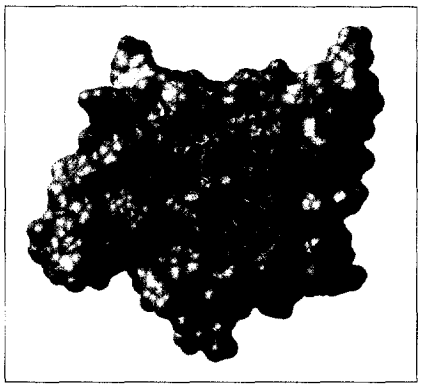
A concentration gradient of the GTP-bound form of the GTPase Ran across nuclear pores is essential for the transport of many proteins and nucleic acids between the nuclear and cytoplasmic compartments of eukaryotic cells. The mechanisms responsible for the dynamics and maintenance of this Ran gradient have been unclear. The authors now show that Ran shuttles between the nucleosol and cytosol, and that cytosolic Ran accumulates rapidly in the nucleus in a saturable manner that is dependent on temperature and on the guanine-nucleotide exchange factor RCC1. The authors conclude that NTF2 functions as a transport receptor



for Ran, permitting rapid entry into the nucleus where GTP–GDP exchange mediated by RCC1 converts Ran into its GTP-bound state. The Ran–GTP can associate with nuclear Ran-binding proteins, thereby creating a Ran gradient across nuclear pores.
14 December 1998, Brief Communication, *Current Biology*.

☐ **How and why phosphotyrosine-containing peptides bind to the SH2 and PTB domains.**
Yingyao Zhou and Ruben Abagyan (1998). *Fold. Des.* **3**, 513–522.

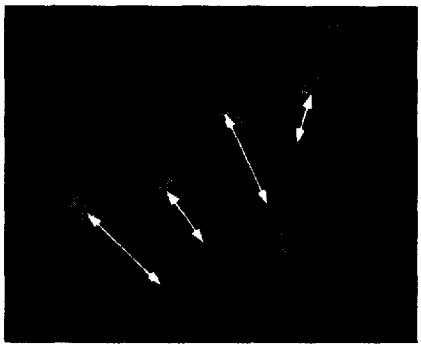
Specific recognition of phosphotyrosine-containing protein segments by Src homology 2 (SH2) and phosphotyrosine-binding (PTB) domains plays an important role in intracellular signal transduction. Although many SH2/PTB-domain-containing receptor–peptide complex structures have been solved, little has been done to study the problem computationally. Prediction of the binding geometry and the binding constant of any peptide–protein pair is an extremely important problem. A procedure to predict binding energies of



phosphotyrosine-containing peptides with SH2/PTB domains was developed. The average deviation between experimentally measured binding energies and theoretical evaluations was 1.8 kcal/mol. Binding states of unphosphorylated peptides were also predicted reasonably well. *Ab initio* predictions of binding geometry of fully flexible peptides correctly identified conformations of two pentapeptides and a hexapeptide complexed with a v-Src SH2 domain receptor. The binding energies of phosphotyrosine-containing complexes can be effectively predicted using the procedure developed here. It was also possible to predict the bound conformations of flexible short peptides correctly from random starting conformations.
11 December 1998, Research Paper, *Folding & Design*.

☐ **Solution conformation of a parallel DNA triple helix with 5′ and 3′ triplex–duplex junctions.**
Juan Luis Asensio, Tom Brown and Andrew N Lane (1998). *Structure* **7**, 1–11.

Polypurine·polypyrimidine sequences of DNA can form parallel triple helices via Hoogsteen hydrogen bonds with a third DNA strand that is complementary to the purine strand. The triplex prevents transcription and could therefore potentially be used to regulate specific genes. Structure determination of triplex–duplex junctions can help us to

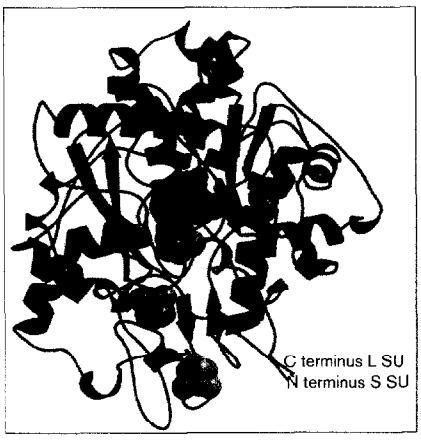


understand the structural basis of specificity, and aid in the design of optimal antigene oligonucleotides. The solution structures of the junction triplexes d(*GAGAGA*CGTA)–X(TACG-*TCTCTC*)–X–(*CTCTCT*) and d(*CTCT-CT*)–X–(*TCTCTC*AGTC)–X–(GACT*G-AGAGA*) (where X is bis(octylphosphate) and nucleotides in the triplex regions are in italics) have been solved. The structure is characterised by significant changes in the conformation of the purine residues, asymmetry of the 5′ and 3′ junctions, and variations in groove widths associated with the positive charge of the protonated cytosine residues in the third strand. The observed sequence dependence of the triplex structure, and the distortions of the DNA at the 5′ and 3′ termini has implications for the design of optimal

triplex-forming sequences, both in terms of the terminal bases and the importance of including positive charges in the third strand. Thus, triplex-stabilising ligands might be designed that can discriminate between TA·T-rich and CG·C$^+$-rich sequences that depend not only on charge, but also on local groove widths. This could improve the stabilisation and specificity of antigene triplex formation.
24 December 1998, Research Paper, *Structure*.

☐ ***Desulfovibrio desulfuricans* iron hydrogenase: the structure shows unusual coordination to an active site Fe binuclear center.**
Yvain Nicolet, Claudine Piras, Pierre Legrand, Claude E Hatchikian and Juan C Fontecilla-Camps (1999). *Structure* **7**, 13–23.

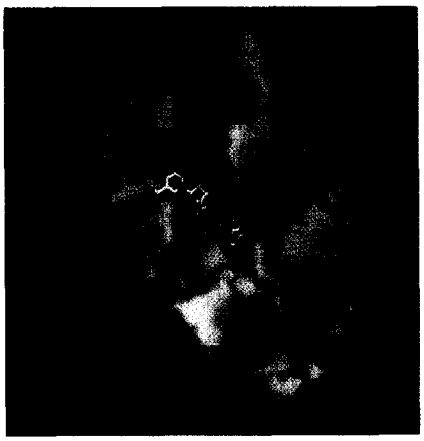
Many microorganisms have the ability to either oxidize molecular hydrogen to generate reducing power or to produce hydrogen in order to remove low-potential electrons. These reactions are catalyzed by two unrelated enzymes: the Ni–Fe hydrogenases and the Fe-only hydrogenases. The structure of the heterodimeric Fe-only hydrogenase from *Desulfovibrio desulfuricans* — the first for this class of enzymes is reported. With the exception of a ferredoxin-like



domain, the structure represents a novel protein fold. The conformation of the subunits can be explained by the evolutionary changes that have transformed monomeric cytoplasmic enzymes into dimeric periplasmic enzymes. Plausible electron- and proton-transfer pathways and a putative channel for the access of hydrogen to the active site have been identified. The unrelated active sites of Ni–Fe and Fe-only hydrogenases have several common features: coordination of diatomic ligands to an Fe ion; a vacant coordination site on one of the metal ions representing a possible substrate-binding site; a thiolate-bridged binuclear center; and plausible proton- and electron-transfer pathways and substrate channels. The diatomic coordination to Fe ions makes them low spin and favors low redox states, which might be required for catalysis. The paucity of protein ligands to this center suggests that it was imported from the inorganic world as an already functional unit.
24 December 1998, Research Paper, *Structure*.

☐ **Structure of the adenylation domain of an NAD$^+$-dependent DNA ligase.**
Martin R Singleton, Kjell Håkansson, David J Timson and Dale B Wigley (1999). *Structure* **7**, 35–45.

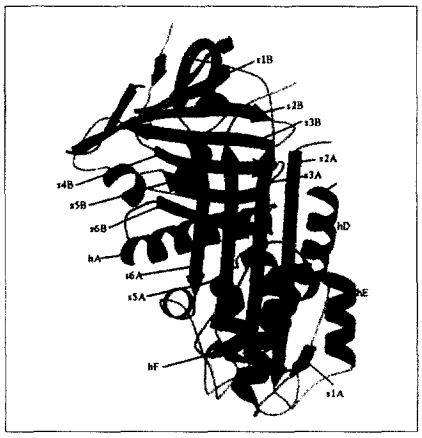
DNA ligases catalyse phosphodiester-bond formation between adjacent bases in nicked DNA, thereby sealing the nick. A key step in the catalytic mechanism is the formation of an adenylated DNA intermediate. The adenyl group is derived from either ATP (in eucaryotes and archaea) or NAD$^+$ (in bacteria). This difference in cofactor specificity suggests that DNA ligase might be a useful antibiotic target. The crystal structure of the adenylation domain of the NAD$^+$-dependent DNA



ligase from *Bacillus stearothermophilus* has been determined. Despite a complete lack of detectable sequence similarity, the fold of the central core of this domain shares homology with the equivalent region of ATP-dependent DNA ligases, providing strong evidence for the location of the NAD$^+$-binding site. Comparison of the structure of the NAD$^+$-dependent DNA ligase with that of ATP-dependent ligases and mRNA-capping enzymes demonstrates the manifold utilisation of a conserved nucleotidyltransferase domain within this family of enzymes. Although this conserved core domain retains a common mode of nucleotide binding and activation, it is the additional domains at the amino terminus and/or the carboxyl terminus that provide the alternative specificities and functionalities in the different members of this enzyme superfamily.
24 December 1998, Research Paper, *Structure*.

☐ **The crystal structure of plasminogen activator inhibitor 2 at 2.0 Å resolution: implications for serpin function.**
Stephen J Harrop, Lucy Jankova, Murray Coles, Daniel Jardine, Jason S Whittaker, Alison R Gould, Andreas Meister, Garry C King, Bridget C Mabbutt and Paul MG Curmi (1999). *Structure* **7**, 43–54.

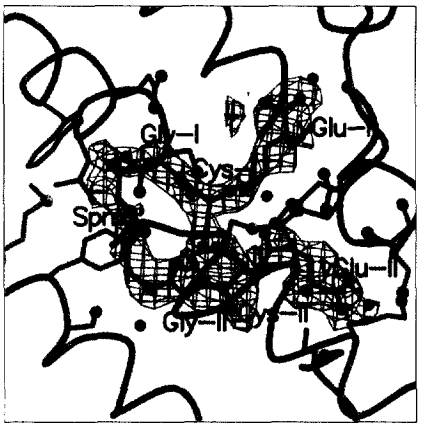
Plasminogen activator inhibitor 2 (PAI-2) is a member of the serpin family of protease inhibitors that function via a dramatic structural change from a native, stressed state to a relaxed form. This transition is mediated by a segment of the serpin termed the reactive centre loop (RCL); the RCL is cleaved on interaction with the protease and becomes inserted into β sheet A of the serpin. Major questions remain as to what factors facilitate this transition and how they relate to protease inhibition. The crystal structure of a mutant form of human PAI-2 in the stressed state has been determined. The RCL is completely disordered in the structure. An examination of polar residues that are highly conserved across all serpins identifies functionally important regions.



A buried polar cluster beneath β sheet A (the so-called 'shutter' region) is found to stabilise both the stressed and relaxed forms via a rearrangement of hydrogen bonds. A statistical analysis of interstrand interactions indicated that the shutter region can be used to discriminate between inhibitory and noninhibitory serpins. This analysis implied that insertion of the RCL into β sheet A up to residue P8 is important for protease inhibition and hence the structure of the complex formed between the serpin and the target protease.
24 December 1998, Research Paper, *Structure*.

☐ **Crystal structure of *Trypanosoma cruzi* trypanothione reductase in complex with trypanothione, and the structure-based discovery of new natural product inhibitors.**
Charles S Bond, Yihong Zhang, Matthew Berriman, Mark L Cunningham, Alan H Fairlamb and William N Hunter (1999). *Structure* **7**, 81–89.

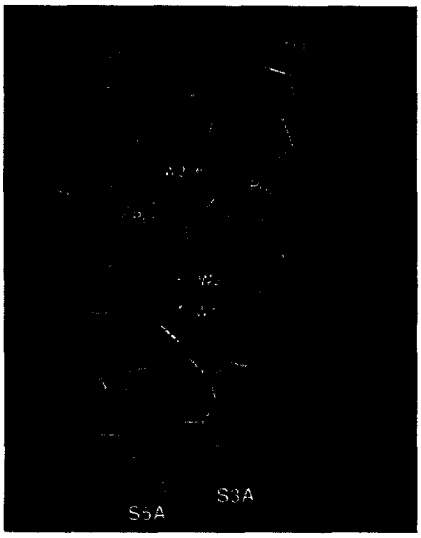
Trypanothione reductase (TR) helps to maintain an intracellular reducing environment in trypanosomatids, a group of protozoan parasites that afflict humans and livestock in tropical areas. TR has been validated as a chemotherapeutic target by molecular genetics methods. To assist the development of new therapeutics, we have characterised the structure of TR from the pathogen *Trypanosoma cruzi* complexed with the substrate trypanothione and used the structure to guide database searches and molecular modelling studies. The TR–trypanothione-disulfide structure has been determined to 2.4 Å resolution. The chemical interactions involved in enzyme recognition and binding of substrate can be inferred from this structure. Comparisons with the related mammalian enzyme, glutathione reductase, explain why each enzyme is so specific for its own substrate. A CH•••O hydrogen bond can occur between the active-site histidine and a carbonyl of the substrate. This interaction contributes to enzyme



specificity and mechanism by producing an electronic induced fit when substrate binds. Database searches and molecular modelling using the substrate as a template and the active site as receptor have identified a class of cyclic-polyamine natural products that are novel TR inhibitors. These could be used as novel lead compounds in the search for improved medicines to treat a range of parasitic infections.
4 January 1999, Research Paper, *Structure*

☐ **The structure of active serpin 1K from *Manduca sexta*.**
Jinping Li, Zhulun Wang, Bertram Canagarajah, Haobo Jiang, Michael Kanost and Elizabeth J Goldsmith (1999). *Structure* **7**, 103–109.

The reactive center loops (RCL) of serpins undergo large conformational changes triggered by the interaction with their target protease. Available crystallographic data suggest that the



serpin RCL is polymorphic, but the relevance of the observed conformations to the competent active structure and the conformational changes that occur on binding target protease has remained obscure. New high-resolution data on an active serpin, serpin 1K from the moth hornworm *Manduca sexta*, provide insights into how active serpins are stabilized and how conformational changes are induced by protease binding. By comparison with other active serpins, especially α1-antitrypsin, a model is proposed in which interaction with the target protease near P1 leads to conformational changes in β sheet A of the serpin.
4 January 1999, Research Paper, *Structure*