Elsewhere in Biology

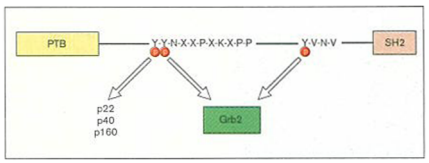
Chosen and summarized by the staff of *Chemistry & Biology*

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

Chemistry & Biology December 1996, **3**:1047–1050

☐ **The Shc adaptor protein is highly phosphorylated at conserved, twin tyrosine residues (Y239/240) that mediate protein–protein interactions.** Peter van der Geer, Sandra Wiley, Gerald D Gish and Tony Pawson (1996). *Curr. Biol.* **6**, 1435–1444.

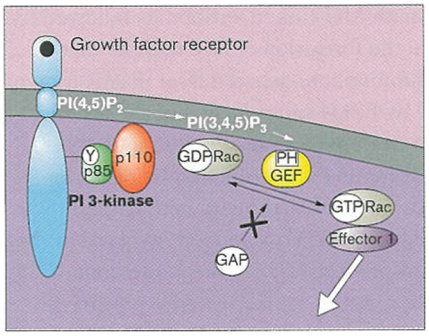
One of the first steps in signal transduction is the activation of protein-tyrosine kinases in the cytoplasmic tails of cell-surface receptors. Phosphorylated tyrosine residues in activated receptors or docking proteins act as binding sites for the Src homology 2 (SH2) or phosphotyrosine-binding (PTB) domains of cytoplasmic signaling proteins. Shc is an adaptor protein that contains both PTB and SH2 domains and becomes phosphorylated on tyrosine in response to many different extracellular stimuli. Only a single Shc phosphorylation site, the tyrosine at position 317 (Y317) was previously known; Y317 has been implicated in Grb2 binding and activation of the Ras pathway. Two major novel Shc tyrosine phosphorylation sites, Y239 and Y240, are identified here. Y239 and Y240 are coordinately phosphorylated by the Src protein-tyrosine kinase *in vitro*, and in response to epidermal growth factor stimulation or in *v-src*-transformed cells *in vivo*. They appear to be important in the association of Shc with Grb2, and are highly conserved in evolution. The identification of two novel Shc phosphorylation sites indicates that Shc has the potential to interact with multiple downstream effectors. The authors propose that distinct Shc phosphorylation isomers form different signaling complexes and therefore activate separate downstream signaling cascades.



1 November 1996, Research Paper, *Current Biology*

☐ **Phosphatidylinositol 3-kinase signals activate a selective subset of Rac/Rho-dependent effector pathways.** Karin Reif, Catherine D Nobes, George Thomas, Alan Hall and Doreen A Cantrell (1996). *Curr. Biol.* **6**, 1445–1455.

Phosphatidylinositol 3′-hydroxyl kinase (PI 3-kinase) is activated by many growth factor receptors and is thought to exert its cellular functions by elevating phosphatidylinositol (3,4,5)-triphosphate levels in the cell. It is required for growth-factor induced changes of the actin cytoskeleton that

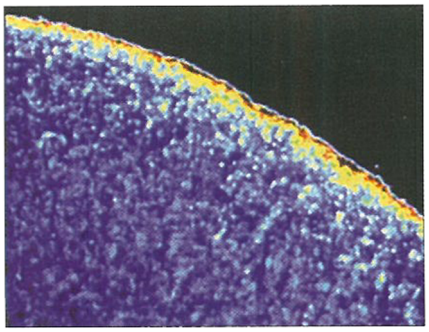


are mediated by the GTPases Rac and Rho. Recently, it has become clear that Rac and Rho are involved in regulating gene transcription. The authors use membrane targeting of the p110 catalytic subunit, but not the p85 regulatory subunit, of PI 3-kinase to generate a constitutively active enzyme. Expression of this active PI 3-kinase induced actin reorganization in the form of Rac-mediated lamellipodia and focal complexes, and Rho-mediated stress fibres and focal adhesions. Expression of active PI 3-kinase did not, however, induce the Ras/Rac/Rho signaling pathways that regulate gene transcription controlled by the *c-fos* promoter, the *c-fos* serum response element or the transcription factors Elk-1 and AP-1. The results demonstrate that PI 3-kinase induces a selective subset of cellular responses, but is not sufficient to stimulate the full repertoire of Rac- or Rho-mediated responses.

1 November 1996, Research Paper, *Current Biology*

☐ **Chromophore-assisted laser inactivation of a repulsive axonal guidance molecule.** BK Müller, DG Jay and F Bonhoeffer (1996). *Curr. Biol.* **6**, 1497–1502

The axons of retinal ganglion neurons form a precise topographic map in the optic tectum in the midbrain, and the guidance of retinal axons by directional cues in the tectum is crucial in this process. Temporal, but not nasal, retinal axons avoid posterior tectal membranes and grow on anterior membranes as a result of repellent guidance activities that are linked by glycosylphosphatidylinositol (GPI) anchors to the posterior membranes. A putative GPI-anchored repulsive guidance molecule with a molecular weight of 33 kDa has previously been characterized. Indirect results from experiments *in vitro* support the hypothesis that this 33 kDa molecule guides temporal retinal axons. To assess whether the 33 kDa protein is involved in axon guidance *in vitro*, the authors raised monoclonal antibodies against molecules that had been removed from tectal membranes by

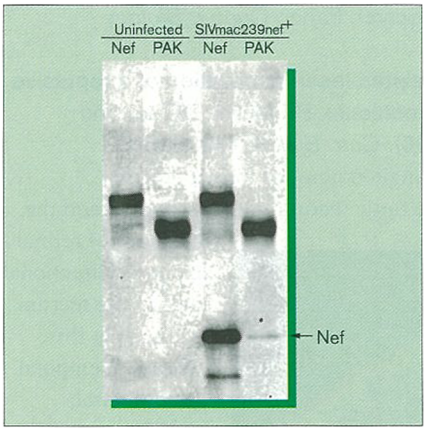


treatment with phosphatidylinositol-specific phospholipase C, which cleaves GPI anchors. A monoclonal antibody, F3D4, recognized the putative guidance molecule. F3D4 was next covalently linked to the chromophore malachite green (MG); incubation of antigens with MG-linked antibodies followed by laser irradiation leads to the formation of free radicals that destroy the antigen. Chromophore-assisted laser inactivation of the antigen recognized by F3D4 caused a loss of the repellent activity from posterior tectal membranes *in vitro*, so that temporal retinal fibers were no longer repelled by posterior tectal membranes. Thus, the F3D4 antigen, here named repulsive guidance molecule (RGM) is indeed involved in the guidance of retinal axons *in vitro*. *In vivo*, the expression of RGM increases from the anterior to the posterior pole of the optic tectum. Retinal axons are thus probably guided by gradients of repulsive guidance molecules, of which there appear to be several.

1 November 1996, Research Paper, *Current Biology*

☐ **Activation of PAK by HIV and SIV Nef: importance for AIDS in rhesus macaques.** Earl T Sawai, Imran H Khan, Phillip M Montbriand, B. Matija Peterlin, Cecilia Cheng-Mayer and Paul A Luciw (1996). *Curr. Biol.* **6**, 1519–1527.

The primate lentiviruses, human immunodeficiency virus types 1 and 2 (HIV-1 and HIV-2) and simian immunodeficiency virus (SIV), encode a conserved accessory gene product, Nef. *In vivo*, Nef is important for the maintenance of high virus loads and progression to AIDS in SIV-infected adult rhesus macaques. In tissue culture cells, Nef interacts with a cellular serine kinase, designated Nef-associated kinase.

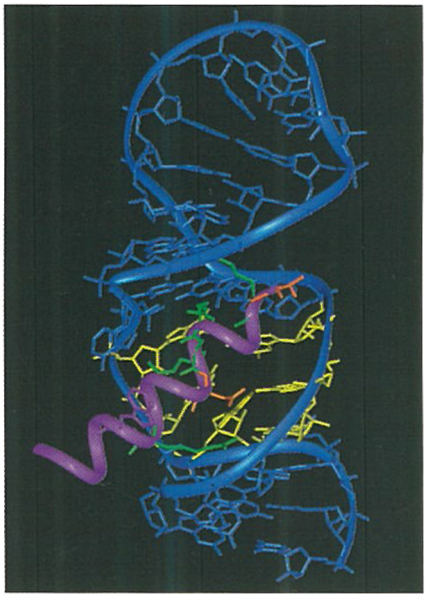


This study identifies the Nef-associated kinase as a member of the p21-activated kinase (PAK) family of kinases and investigates the role of the Nef-associated kinase *in vivo*. Point mutants in Nef were constructed that do not associate with or activate the PAK-related kinase, and these mutants were inserted into SIV that was used to infect macaques. Virus recovered from infected macaques was found to have reverted to prototype Nef function and sequence very soon after infection. Reversion of the kinase-negative mutant to a kinase-positive genotype preceded the development of high virus loads and disease progression. Thus, there is a strong selective pressure *in vivo* for the interaction between Nef and the PAK-related kinase.

1 November 1996, Research Paper, *Current Biology*

☐ **A docking and modelling strategy for peptide–RNA complexes: applications to BIV Tat–TAR and HIV Rev–RBE.** Jayashree Srinivasan, Fabrice Leclerc, Wei Xu, Andrew D Ellington and Robert Cedergren (1996). *Folding & Design* **1**, 463–472.

Despite the great interest in the interaction between RNAs and proteins, no general protocol for modeling these complexes is presently available. The authors report a general strategy for docking and modeling RNA–protein complexes. The docking procedure involves minimizing electrostatic and van der Waals interaction energies of conformationally rigid structures during docking. After docking, libraries of amino acid sidechain conformations are searched to obtain the best interactions between the peptide and the RNA. The structure of a bovine immunodeficiency virus (BIV) Tat peptide bound to BIV TAR RNA has been reproduced using this method, and a model for the structure of the arginine-rich HIV-1 Rev peptide ($Rev_{34–50}$) interacting with the Rev-binding element (RBE) has also been developed. The resulting model of the $Rev_{34–50}$-RBE complex predicts that although no single arginine sidechain is responsible for complex formation, residues Arg2, Arg5 and Arg11 are more important for binding than the other arginine residues in the peptide.



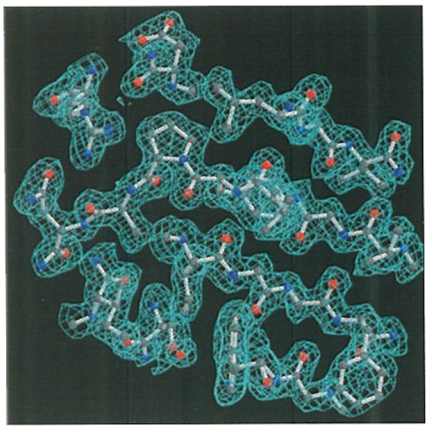
22 November 1996, Research Paper, *Folding & Design*

☐ **The structure of the C-terminal domain of methionine synthase: presenting *S*-adenosylmethionine for reductive methylation of B_{12}.** Melinda M Dixon, Sha Huang, Rowena G Matthews and Martha Ludwig (1996). *Structure* **4**, 1263–1275.

In both mammalian and microbial species, B_{12}-dependent methionine synthase catalyzes methyl transfer from methyltetrahydrofolate (CH_3-H_4folate) to homocysteine. The B_{12} (cobalamin) cofactor is essential for this reaction, accepting the methyl group from CH_3-H_4folate to form methylcob(III)alamin, then donating the methyl group to

**Folding & Design* is published using a continuous publication system. The date given is the date that the paper appeared on the internet. You can access the World Wide Web site for all Current Biology Ltd journals (including *Folding & Design*) via BioMedNet at http://BioMedNet.com.

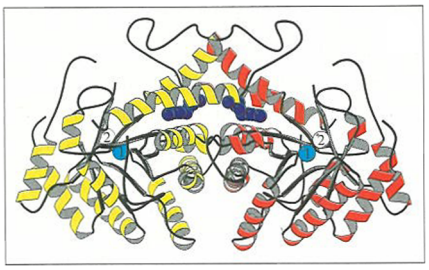
homocysteine to generate methionine and cob(I)alamin. Occasionally the highly reactive cob(I)alamin intermediate is oxidized to the catalytically inactive cob(II)alamin form. Reactivation to sustain enzyme activity is achieved by a reductive methylation, requiring *S*-adenosylmethionine (AdoMet) as the methyl donor and, in *Escherichia coli*, flavodoxin as an electron donor. The intact system is controlled and organized so that AdoMet, rather than CH_3-H_4folate, is the methyl donor in the reactivation reaction. The authors report the crystal structure of the 38 kDa carboxy-terminal fragment (residues 901–1227) of *E. coli* methionine synthase that comprises the AdoMet-binding site, which is essential for reactivation. The structure is a C-shaped single domain whose central feature is a bent antiparallel β sheet; the fold has not been observed before. AdoMet binds near the center of the inner surface of the domain and is held in place by both sidechain and backbone interactions. The conformation of bound AdoMet, and the interactions that determine its binding, differ from those found in other AdoMet-dependent enzymes. The sequence Arg-x-x-x-Gly-Tyr is critical for the binding of AdoMet to methionine synthase. The position of bound AdoMet suggests that large areas of the carboxy-terminal and cobalamin-binding fragments must come into contact to transfer the methyl group of AdoMet to cobalamin. The catalytic and activation cycles may be turned off and on by alternating physical separation and approach of the reactants.



15 November 1996, Research Paper, *Structure*

☐ **The crystal structure of a class II fructose-1,6-bisphosphate aldolase shows a novel binuclear metal-binding active site embedded in a familiar fold.** Serena J Cooper, Gordon A Leonard, Sean M McSweeney, Andrew W Thompson, James H Naismith, Seema Qamar, Andrew Plater, Alan Berry and William N Hunter (1996). *Structure* **4**, 1303–1315.

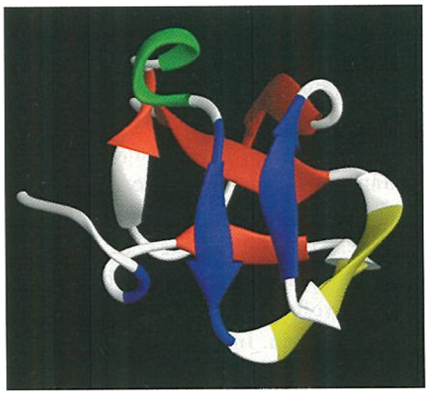
Aldolases catalyze a variety of condensation and cleavage reactions, with exquisite control on the stereochemistry. These enzymes are attractive catalysts for synthetic chemistry. There are two classes of aldolase: class I aldolases use Schiff's base formation with an active-site lysine to form the reaction intermediate, while class II enzymes require a divalent metal ion (usually zinc). Structures are available for class I FBP-aldolases but there is a paucity of detail on the class II enzymes. A dissection of structure–activity relationships in these enzymes may assist the construction of designed aldolases for use as biocatalysts in synthetic chemistry. The structure of the dimeric class II FBP-aldolase from *Escherichia coli* has been determined using data to 2.5 Å resolution. The fold is an $(\alpha/\beta)_8$ barrel. The active centre, at the carboxy-terminal end of the barrel, contains a novel bimetallic-binding site with two metal ions 6.2 Å apart. One ion, the identity of which is not certain, is buried; it is not clear whether it is involved in stabilization of the structure or in catalysis. The other metal ion is zinc and is positioned at the surface of the barrel to participate in catalysis. Comparison of the structure with a class II fuculose aldolase suggests that these enzymes may share a common mechanism. Nevertheless, the class II enzymes fall into two categories when subunit size and fold, quaternary structure and metal-ion binding sites are considered.



15 November 1996, Research Paper, *Structure*

☐ **Refined solution structure of type III antifreeze protein: hydrophobic groups may be involved in the energetics of the protein–ice interaction.** Frank D Sönnichsen, Carl I DeLuca, Peter L Davies and Brian D Sykes (1996). *Structure* **4**, 1325–1337.

Antifreeze proteins are found in certain fish inhabiting polar sea water. They depress the freezing points of blood and body fluids below that of the surrounding sea water by binding to and inhibiting the growth of seed ice crystals. The proteins are believed to bind irreversibly to growing ice crystals in such a way as to change the curvature of the ice–water interface, leading to freezing point depression, but the mechanism of high-affinity ice binding is not yet fully understood. The solution structure of the type III antifreeze protein was determined by multidimensional NMR spectroscopy. Twenty-two structures converged and display a root mean square difference from the mean of 0.26 Å for backbone atoms and 0.62 Å for all non-hydrogen atoms. The protein exhibits a compact fold with a relatively large hydrophobic core, several short and irregular β sheets and one helical turn. The ice-binding site, which encompasses parts of the carboxy-terminal sheet and a loop, is planar and relatively nonpolar. The polar

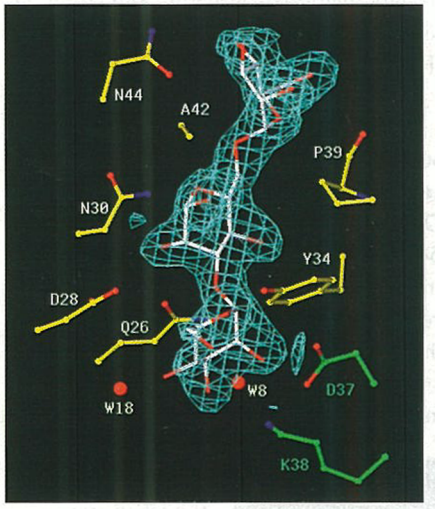


sidechain atoms of the putative ice-binding residues Gln9, Asn14, Thr15, Thr18 and Gln44 are spaced so as to match the spacing of water molecules in the prism planes ($\{10\bar{1}0\}$) of the hexagonal ice crystal, in agreement with the adsorption-inhibition mechanism of action. The sidechain conformations limit the number and strength of possible protein–ice hydrogen bonds, however. This suggests that other entropic and enthalpic contributions, such as those arising from hydrophobic groups, could be involved in the high-affinity adsorption of ice by the protein.

15 November 1996, Research Paper, *Structure*

☐ **The 2.0 Å structure of a cross-linked complex between snowdrop lectin and a branched mannopentaose: evidence for two unique binding modes.** Christine Schubert Wright and Gerko Hester (1996). *Structure* **4**, 1339–1352.

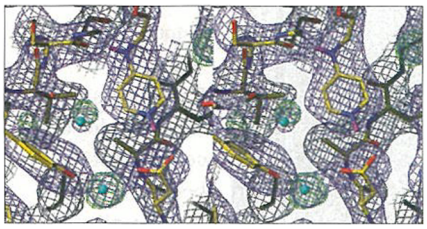
Galanthus nivalis agglutinin (GNA), a mannose-specific lectin from snowdrop bulbs, is a tetrameric member of the family of *Amaryllidaceae* lectins that exhibit antiviral activity towards HIV. Its subunits are composed of three pseudo-symmetrically related β-sheet domains, each with a conserved mannose-binding site. Crystal structures of monosaccharide and disaccharide complexes of GNA have revealed that all 12 binding sites of the tetramer are functional, and that the degree of occupancy is dependent on the availability of subsidiary interactions from neighboring subunits. The complex of GNA with a branched mannopentaose ((Man1,6-(1,3-Man)Man-1,6-(1,3-Man)Man) described here, simulates a more biologically relevant complex. Two unique mannopentaose binding modes co-exist in the complex. In one, the conserved monosaccharide-binding pocket in domain 1 (CRD1) is used to cross-link twofold related GNA dimers bridged by the outer 3,6 tri-Man arm. Inter-linked dimers assemble helically along the 4_1 crystal axis forming a pore-like structure. In the other, the complete 3,6 tri-Man arm binds to an extended binding region in domain 3 (CRD3) with subsites for each terminal Man and the internal Man positioned in the conserved monosaccharide pocket. This structure provides insights into the possible mechanisms of the cross-linking that is known to occur when lectins interact with specific multivalent cell surface receptors during events such as agglutination and mitogenic stimulation. As GNA has a large number of sites available for mannose binding, it can form lattice structures in several ways. High affinity mannose binding occurs only at the two domain sites located near dimer interfaces.



15 November 1996, Research Paper, *Structure*

☐ **Enzyme flexibility, solvent and 'weak' interactions characterize thrombin–ligand interactions: implications for drug design.** Richard A Engh, Hans Brandstetter, Gudrun Sucher, Andreas Eichinger, Ulrich Baumann, Wolfram Bode, Robert Huber, Thomas Poll, Rainer Rudolph and Wolfgang von der Saal (1996). *Structure* **4**, 1353–1362.

There have been intensive efforts to develop orally administrable antithrombotic drugs using information about the crystal structures of blood coagulation factors, including thrombin. Most of the low molecular weight thrombin inhibitors studied so far are based on arginine and benzamidine. To expand the database of information on thrombin–inhibitor binding, the authors studied two new classes of inhibitors. They report the structures of three new inhibitors complexed with thrombin, two based on 4-aminopyridine and one based on naphthamidine. Several geometry changes in the protein mainchain and sidechains were observed to accompany inhibitor binding. The two inhibitors based on 4-aminopyridine bind in notably different ways: one forms a water-mediated hydrogen bond to the active site Ser195, the other induces a rotation of the Ser214–Trp215 peptide plane that is unprecedented in thrombin structures. These binding modes also differ in their 'weak' interactions, including CH-O hydrogen bonds and interactions between water molecules and aromatic π-clouds. Induced-fit structural changes were also seen in the structure of the naphthamidine inhibitor complex. The authors point out that protein flexibility and variable water structures are essential elements in protein–ligand interactions, and that ligand design strategies that fail to take this into account may overlook or underestimate the potential of lead structures. Further, the significance of 'weak' interactions must be considered both in crystallographic refinement and in analysis of binding mechanisms.



15 November 1996, Research Paper, *Structure*