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*.

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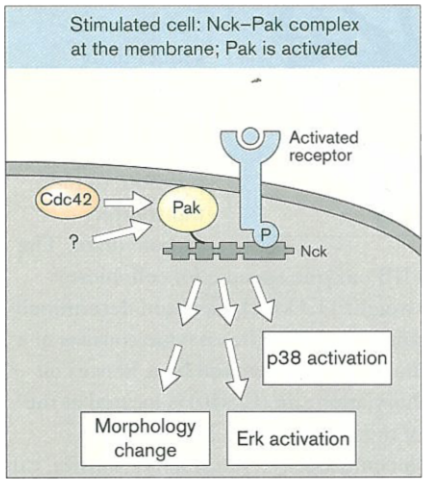
Chemistry & Biology March 1997, **4**:233–236

☐ **Activation of Pak by membrane localization mediated by an SH3 domain from the adaptor protein Nck.** Wange Lu, Steve Katz, Ruchika Gupta and Bruce J Mayer (1997). *Curr. Biol.* **7**, 85–94.

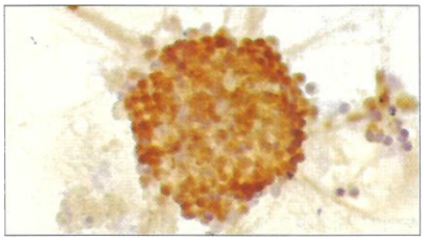
Src homology 2 and 3 (SH2 and SH3) domains are found in a wide variety of proteins implicated in signaling and frequently mediate protein–protein interactions in signal transduction. The adaptor protein Nck consists of three SH3 domains followed by one SH2 domain. Although Nck is presumed to bind to tyrosine-phosphorylated proteins using its SH2 domain, and to bind to downstream effector proteins using its SH3 domain, little is known about its biological function. The Pak family of serine/threonine kinases are known to be activated by binding to the GTP-bound form of Cdc42 or Rac1, which are small GTPases of the Rho family that are involved in regulating the organization of the actin cytoskeleton. The authors show that Nck can mediate the relocalization and subsequent activation of the Pak1 kinase. Nck associates with Pak *in vivo*, using the second of its three SH3 domains, and the localization of this individual Nck SH3 domain, or of Pak kinase itself, to the membrane results in activation of Pak and stimulation of downstream mitogen activated protein kinase cascades. Activation of downstream signaling by the membrane-localized Nck SH3 domain is blocked by a kinase-inactive mutant form of Pak1. Thus, localization of Pak1 to the membrane is sufficient for its activation. The results imply that the Nck adaptor protein might link changes in tyrosine phosphorylation of cellular proteins to the Cdc42/Pak signaling pathway.



16 January 1997*, Research Paper, *Current Biology*

☐ **CD4-independent association between HIV-1 gp120 and CXCR4: functional chemokine receptors are expressed in human neurons.** Joseph Hesselgesser, Meredith Halks-Miller, Virginia DelVecchio, Stephen C Peiper, James Hoxie, Dennis L Kolson, Dennis Taub and Richard Horuk (1997). *Curr. Biol.* **7**, 112–121.

Chemokines are a family of proteins that attract and activate immune cells by interacting with specific receptors on the surface of their targets. It has been shown that specific chemokines can inhibit HIV-1 infection and that chemokine receptors can function as coreceptors, in combination with CD4, for HIV-1 invasion. The authors have shown previously that certain chemokine receptors are expressed on subsets of neurons in various regions of the adult central nervous system.



Using a combination of immunohistochemical staining and receptor-binding studies, the authors show that hNT cells (differentiated human neurons derived from the cell line NTera 2) express the functional chemokine receptors CXCR2, CXCR4, CCR1 and CCR5. Specific chemokines bind with high affinity to hNT neurons. They also show that the envelope glycoprotein from the T-cell-tropic human immunodeficiency virus 1 (HIV-1) strain IIIB is a CD4-independent, dose-dependent inhibitor of the binding of stromal cell-derived factor 1 to its receptor, CXCR4. These data support the recent findings that members of the chemokine receptor family, including CCR5 and CXCR4, function as coreceptors in combination with CD4 for HIV-1 invasion, and provide evidence for the direct, CD4-independent association of the viral envelope protein of the HIV-1 strain IIIB with the chemokine receptor CXCR4.

21 January 1997, Research Paper, *Current Biology*

☐ **CD22 is a negative regulator of B-cell receptor signalling.** Lars Nitschke, Rita Carsetti, Bettina Ocker, Georges Köhler and Marinus C Lamers (1997). *Curr. Biol.* **7**, 133–143.

Antibody responses are triggered by binding of antigen to the B-cell antigen receptor (BCR). The strength of the resulting signal determines the outcome of the response, which may vary from the induction of tolerance to the antigen, to the production of specific high-affinity antibodies. Additional

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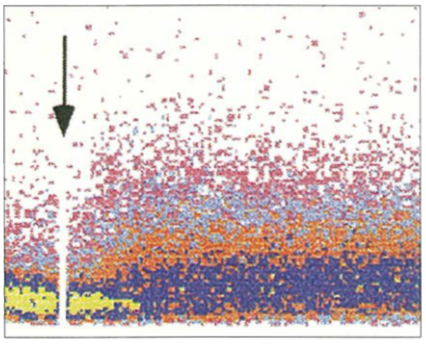
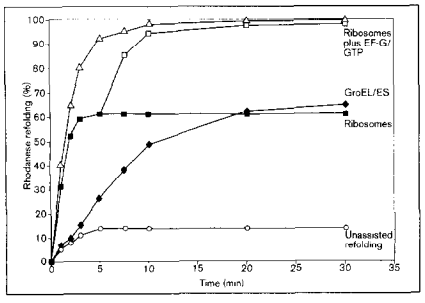
cell-surface proteins, such as the transmembrane protein CD22, can assist the BCR in its function. The cytoplasmic tail of CD22 contains three immunoreceptor tyrosine-based inhibitory motifs, which are phosphorylated upon BCR-crosslinking and can bind the tyrosine phosphatase SHP-1, a putative negative regulator of signaling from the BCR. To assess the role of CD22 *in vivo*, the authors have generated $CD22^{-/-}$mice by targeted gene inactivation. These mice have normal numbers of peripheral B cells, but the phenotype of these cells is more mature than that seen in the wildtype. Recirculating B cells are absent from the bone marrow but the distribution of the two B-cell subtypes, B-1 and B-2, is normal.



After BCR-cross-linking *in vitro*, splenic $CD22^{-/-}$B cells show increased Ca^{2+} influx and a lower survival caused by an increased induction of apoptosis. In contrast, there is an increased proliferative response to the B-cell mitogen lipopolysaccharide (LPS) and a shorter average lifespan in the B-cell compartment. Furthermore, T-cell independent immune responses are impaired, whereas T-cell dependent responses are normal. The authors suggest that the absence of CD22 expression lowers the signaling threshold for BCR-cross-linking, changing the fate of the B cell. In their model, engagement of the receptor without T-cell help leads to increased induction of apoptosis, explaining the shorter lifespan of $CD22^{-/-}$B cells and the low response to T-cell independent antigens.
21 January 1997, Research Paper, *Current Biology*

☐ **Ribosomes and ribosomal RNA as chaperones for folding of proteins.** Wieslaw Kudlicki, Ashley Coffman, Gisela Kramer and Boyd Hardesty (1997). *Fold. Des.* **2**, 101–108.

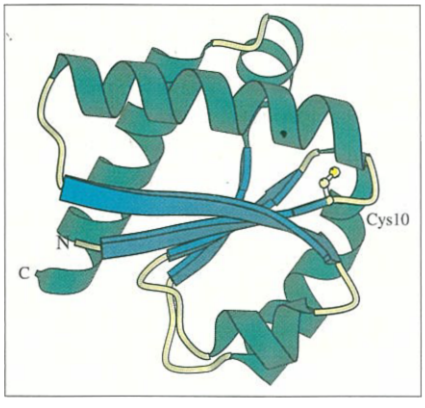
Provocative recent reports indicate that the large subunits of either prokaryotic or eukaryotic ribosomes have the capacity to promote the refolding of denatured enzymes. In this paper, large subunits of salt-washed *Escherichia coli* ribosomes have been shown to promote the refolding of denatured rhodanese. The ability of the ribosomes to carry out renaturation is a property of the 50S ribosomal subunit, specifically the 23S rRNA. The refolding and release of enzymatically active rhodanese leaves the ribosomes in an inactive state or conformation for subsequent rounds of refolding. Inactive ribosomes can then be activated by elongation factor G (EF-G) plus GTP or by cleavage of their 23S rRNA by α-sarcin. Activation by either mechanism is strongly inhibited by the EF-G·GDP·fusidic acid complex.



4 February 1997, Research Paper, *Folding & Design*

☐ **The structure of an energy-coupling protein from bacteria, $IIB^{cellobiose}$, reveals similarity to eukaryotic protein tyrosine phosphatases.** Rob LM van Montfort, Tjaard Pijning, Kor H Kalk, Jonathan Reizer, Milton H Saier Jr, Marjolein MGM Thunnissen, George T Robillard and Bauke W Dijkstra (1997). *Structure* **5**, 217–225.

The bacterial phosphoenolpyruvate-dependent phosphotransferase system (PTS) mediates the energy-driven uptake of carbohydrates and their concomitant phosphorylation; it is also intimately involved in the regulation of a variety of metabolic and transcriptional processes in the bacterium. The multiprotein PTS consists of a membrane channel and at least four cytoplasmic proteins or protein domains that sequentially transfer a phosphoryl group from phosphoenol-pyruvate to the transported carbohydrate. The crystal structure of the IIB enzyme specific for cellobiose, $IIB^{cellobiose}$ (molecular weight 11.4 kDa), has been determined (1.8 Å resolution; R factor of 18.7%). The enzyme consists of a single four-stranded parallel β sheet flanked by α helices on both sides. The phosphorylation site (Cys10) is located at the carboxy-terminal end of the first β strand. No positively charged residues, which could assist in phosphoryl transfer, can be found in or near the active site. A comparison between $IIB^{cellobiose}$ and the structurally similar low molecular weight protein tyrosine phosphatases provides insight into the mechanism of the phosphoryltransfer reactions in which $IIB^{cellobiose}$ is involved. The differences in tertiary structure and active-site composition between $IIB^{cellobiose}$ and the glucose-specific $IIB^{glucose}$ give a structural explanation for why the carbohydrate-specific components of different families cannot complement each other.

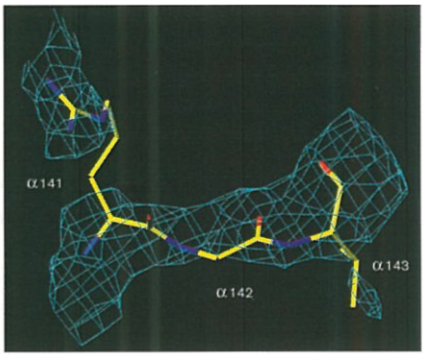


15 February 1997, Research Article, *Structure*

☐ **Structures of a hemoglobin-based blood substitute: insights into the function of allosteric proteins.** Kenneth S Kroeger and Craig E Kundrot (1997). *Structure* **5**, 227–237.

Potential blood substitutes can be based on hemoglobin, but two problems arise with acellular hemoglobin-based blood

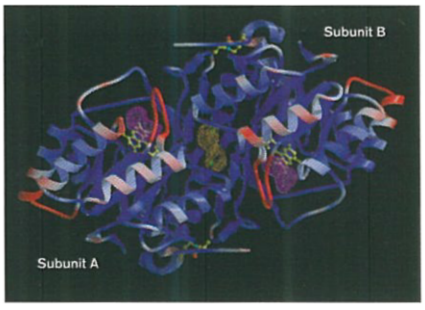
substitutes: the oxygen affinity of purified human hemoglobin is too high for it to deliver oxygen to tissues, and hemoglobin tetramers dissociate into αβ dimers that can cause kidney damage. In this paper, the structure of the blood substitute rHb1.1 is described in two forms: the deoxy-rHb1.1 (2.0 Å resolution) and cyanomet-rHb1.1 (2.6 Å resolution). Deoxy-rHb1.1 adopts the classic 'T state' quaternary structure, but cyanomet-rHb1.1 adopts a novel quaternary structure, the B state. The most striking feature of the tertiary structures is that rHb1.1 has a reduced oxygen affinity as a result of breaking a charged hydrogen bond involving LysβI08 in the T→B state transition. In addition, dimerization is prevented by the insertion of a glycine residue between the sequences of the normal α chains to produce one covalently continuous di-α-chain. The structural change reduces the oxygen affinity of rHb1.1 by changing the relative stability of the deoxy and ligand-bound states. It also highlights the importance of small conformational changes in allosteric proteins, even in their most rigid domains. Three ligand-bound quaternary structures of hemoglobin (R, Y/R2 and B) have now been described whereas only one quaternary structure has been observed for deoxyhemoglobin (T). The structural degeneracy of the high oxygen affinity form of hemoglobin is an important reminder that allosteric proteins may have multiple quaternary structures that are functionally very similar. This degeneracy of quaternary structures has important implications for the regulation of allosteric proteins, because different quaternary structures may be stabilized by different allosteric effectors.



15 March 1997, Research Article, *Structure*

☐ **The crystal structure of the flavin containing enzyme dihydroorotate dehydrogenase A from *Lactococcus lactis*.** Paul Rowland, Finn S Nielsen, Kaj Frank Jensen and Sine Larsen. *Structure* **5**, 239–252.

Dihydroorotate dehydrogenase (DHOD) is a flavin mononucleotide containing enzyme which catalyzes the oxidation of (*S*)-dihydroorotate to orotate. There is currently great interest in investigating inhibitors of DHOD as potential therapeutic agents for treating diseases involving aberrant cell proliferation. DHOD has recently been implicated as a possible target of the novel immunosuppressive agent leflunomide, which has shown promise in human clinical trials for rheumatoid arthritis and in blocking rejection after allograft and xenograft. Brequinar sodium is another immunosuppressive agent known to work by inhibiting mitochondrial DHOD, and consequently the production of UMP in *de novo* pyrimidine biosynthesis. DHOD from *Lactococcus lactis* contains two genes encoding different

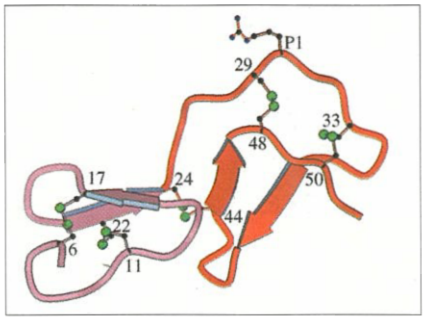


functional enzymes with only 30% identity: DHODA, an efficient dimer, and DHODB. Sequence alignments have identified three different families among the DHODs: the two enzymes from *L. lactis* belong to two of the families, whereas the enzyme from *E. coli* is a representative of the third. This paper describes the first three-dimensional structure of a DHOD, the crystal structure of DHODA from *L. lactis* (2.0 Å resolution). This structure has allowed the function of many of the conserved residues in DHODs to be identified, many of which are associated with binding the flavin group. In addition, important differences in some of the active-site residues which vary across the distinct DHOD families, imply significant mechanistic differences. The substrate cavity, although buried, is located beneath a highly conserved loop which is much less ordered than the rest of the protein and may be important in giving access to the cavity. The location of the conserved residues surrounding this cavity suggests the potential orientation of the substrate.

15 February 1997, Research Article, *Structure*

☐ **A new structural class of serine protease inhibitors revealed by the structure of the hirustasin–kallikrein complex.** Peer RE Mittl, Stefania Di Marco, Gabriele Fendrich, Gabriele Pohlig, Jutta Heim, Christian Sommerhoff, Hans Fritz, John P Priestle and Markus G Grütter (1997). *Structure* **5**, 253–264.

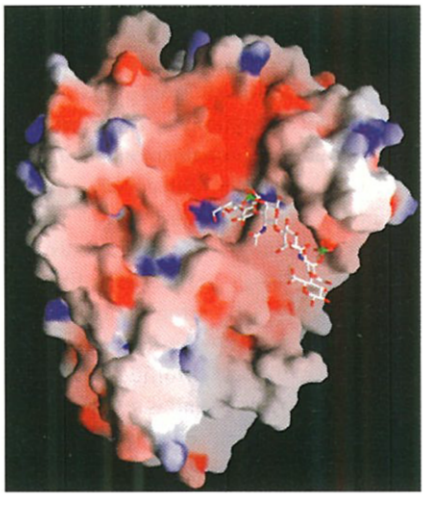
Hirustasin is a potent inhibitor of tissue kallikrein and a member of a family of serine protease inhibitors. Elevated levels of kallikrein have been found in human colon carcinoma cell lines and in human breast cancer cells. The crystal structure of the complex between tissue kallikrein and hirustasin was analyzed at 2.4 Å resolution. Hirustasin folds into a brick-like structure that is dominated by five disulfide bridges and is sparse in secondary structural elements. The cysteine residues, which are conserved in related inhibitors, are connected in an *abab cdecde* pattern that causes the polypeptide chain to fold into two similar motifs. The disulfide bridges maintain the tertiary structure and present the primary binding loop to the active site of the protease. The general structural topography and disulfide connectivity of hirustasin has not previously been described. The crystal structure of the



kallikrein–hirustasin complex reveals that hirustasin differs from other serine protease inhibitors in its conformation and its disulfide bond connectivity, making it the prototype for a new class of inhibitor. The disulfide pattern shows that the structure consists of two domains, but only the carboxy-terminal domain interacts with the protease. The disulfide pattern of the amino-terminal domain is related to the pattern found in other proteins. Kallikrein recognizes hirustasin by the formation of an antiparallel β sheet between the protease and the inhibitor. The P_1 arginine binds in a deep negatively charged pocket of the enzyme. An additional pocket at the periphery of the active site accommodates the sidechain of the P_4 valine.
15 February 1997, Research Article, *Structure*

☐ **Structure of a human lysosomal sulfatase**. Charles S Bond, Peter R Clements, Samantha J Ashby, Charles A Collyer, Stephen J Harrop, John J Hopwood and J Mitchell Guss (1997). *Structure* **5**, 277–289.

Sulfatases catalyze the hydrolysis of sulfuric acid esters on the pathways for the catabolism of glycosaminoglycans and glycolipids and in the synthesis of steroid hormones. Deficiencies of specific lysosomal sulfatases that are involved in the degradation of glycosaminoglycans lead to rare inherited clinical disorders termed mucopolysaccharidoses. Studies of multiple sulfatase deficiency have contributed to the identification of the location and the characterization of the sulfatase active site. To understand the catalytic mechanism of sulfatases, and ultimately the determinants of their substrate specificities, the authors determined the crystal structure of amino-acetyl-galactosamine-4-sulfatase (2.5 Å resolution) at both 123K and 273K. The structure has two domains, the larger of which belongs to the α/β class of proteins and contains the active site. Preliminary data is presented for crystals soaked in the monosaccharide amino-acetyl-galactosamine, the structure of which forms a product complex of the enzyme. The structure of amino-acetylgalactosamine-4-sulfatase reveals that residues conserved amongst the sulfatase family are involved in stabilizing the calcium ion and the sulfate ester in the active site. This suggests an archetypal fold for the family of sulfatases. A catalytic role is proposed for the post-translationally modified highly conserved cysteine residue. Despite a lack of any previously detectable sequence similarity to any protein of known structure, the large sulfatase domain that contains the active site closely resembles that of alkaline phosphatase: the calcium ion in the sulfatase can be superposed on one of the zinc ions in alkaline phosphatase and the sulfate ester of Cys91 can be superposed on the phosphate ion found in the active site of alkaline phosphatase.



15 February 1997, Research Article, *Structure*