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 January 1997, **4**:81–85

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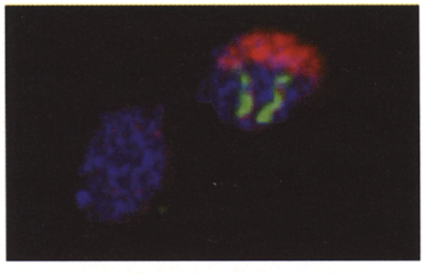
☐ **Protein phosphatase 2A regulates MPF activity and sister chromatid cohesion in budding yeast.** Jeremy Minshull, Aaron Straight, Adam D Rudner, Abby F Dernburg, Andrew Belmont and Andrew W Murray (1996). *Curr. Biol.* **6**,1609–1620.

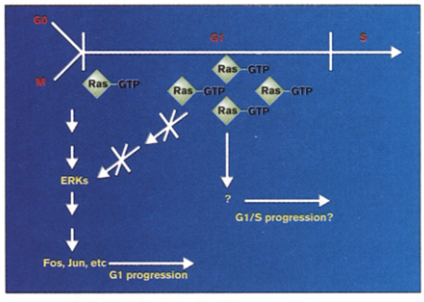
Mitosis is regulated by MPF (maturation promoting factor), the active form of Cdc2/28-cyclin B complexes. Increasing levels of cyclin B abundance and the loss of inhibitory phosphates from Cdc2/28 drives cells into mitosis, whereas cyclin B destruction inactivates MPF and drives cells out of mitosis. Cells with defective spindles are arrested in mitosis by the spindle-assembly checkpoint, which prevents the destruction of mitotic cyclins and the inactivation of MPF. Budding yeast *mad* mutants lack the spindle-assembly checkpoint: spindle depolymerization does not arrest such mutants in mitosis because they cannot stabilize cyclin B. A newly isolated mutant in the budding yeast *CDC55* gene, which encodes a protein phosphatase 2A (PP2A) regulatory subunit, shows a different checkpoint defect. In the presence of a defective spindle, these cells inactivate MPF and leave mitosis without inducing cyclin B destruction. This inactivation is due to inhibitory phosphorylation on Cdc28. Under these conditions, sister chromatid separation appears to be regulated by changes in MPF activity, and not by protein degradation. It is possible that this novel mitotic exit pathway is involved in adaptation to prolonged activation of the spindle-assembly checkpoint.



1 December 1996, Research Paper, *Current Biology*.

☐ **Cell cycle-dependent activation of Ras.** Stephen J Taylor and David Shalloway (1996). *Curr. Biol.* **6**, 1621–1627.

Ras proteins are essential in the transduction of signals from a wide range of cell-surface receptors to the nucleus. These signals may promote cellular proliferation or differentiation, depending on the cell background. It is well established that Ras is important in the transduction of mitogenic signals from activated growth-factor receptors, leading to cell-cycle entry. It is not yet clear, however, whether Ras controls signaling events during cell-cycle progression and, if so, at which point in the cell-cycle it is activated. The authors have developed a novel, functional assay for the detection of cellular activated Ras, and used it to show that Ras was activated in HeLa cells after release from mitosis, and in NIH 3T3 fibroblasts after serum-stimulated cell-cycle entry. In each case, Ras became maximally activated in mid-G1 phase. This activation was not associated with the recruitment of Grb2 by Shc, was dependent on gene transcription and protein synthesis, and was apparently independent of extracellular soluble ligands, suggesting that Ras may be regulated by the cell-cycle machinery as well as by receptor-mediated mechanisms. K-Ras activation also reached a maximum in mid-G1 phase in serum-stimulated fibroblasts. It has become clear, from this and other studies, that Ras uses multiple effectors to relay mitogenic signals to different cellular pathways. How these distinct Ras effector pathways interact to induce mitogenesis is not yet clear. The authors suggest that these pathways may be temporally coordinated by Ras in a cell-cycle dependent manner.



1 December 1996, Research Paper, *Current Biology*

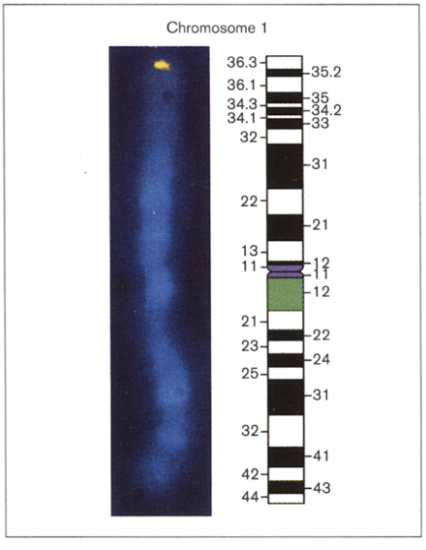
☐ **Lithium inhibits glycogen synthase kinase-3 activity and mimics wingless signalling in intact cells.** Vuk Stambolic, Laurent Ruel and James R Woodgett (1996). *Curr. Biol.* **6**, 1664–1668.

Exposing eukaryotic cells to lithium ions (Li^+) during development has marked effects on cell fate and organization. The phenotypic consequences of Li^+ treatment on *Xenopus* embryos and sporulating *Dictyostelium* are similar to the effects of inhibition or disruption, respectively, of a highly conserved protein serine/threonine kinase, glycogen synthase kinase-3 (GSK-3). In *Drosophila*, the GSK-3 homolog is encoded by *zw3*^{sgg}, a segment-polarity gene involved in embryogenesis; in higher eukaryotes, GSK-3 is part of the Wingless signaling pathway. GSK-3 has been suggested to be important in signal transduction pathways downstream of phosphoinositide 3-kinase and mitogen-activated protein kinases. The authors investigated the effect of Li^+ on the activity of the GSK-3 family. At physiological doses, Li^+ reversibly inhibits the activity of human GSK-3β and *Drosophila* Zw3^{Sgg}, but not that of other protein kinases. Treatment of cells with Li^+ inhibits GSK-3-dependent phosphorylation of the microtubule-associated protein Tau, and in *Drosophila* S2 cells and rat PC12 cells this treatment mimics Wingless signaling, inducing the accumulation of cytoplasmic Armadillo/β-catenin. This

reveals a possible molecular mechanism of the effect of Li^+ on development and differentiation.
1 December 1996, Research Paper, *Current Biology*

☐ **Apo-3, a new member of the tumor necrosis factor receptor family, contains a death domain and activates apoptosis and NF-κB.** Scot A. Marsters, James P Sheridan, Christopher J Donahue, Robert M Pitti, Christa L Gray, Audrey D Goddard, Kenneth D Bauer and Avi Ashkenazi (1996). *Curr. Biol.* **6**, 1669–1676.

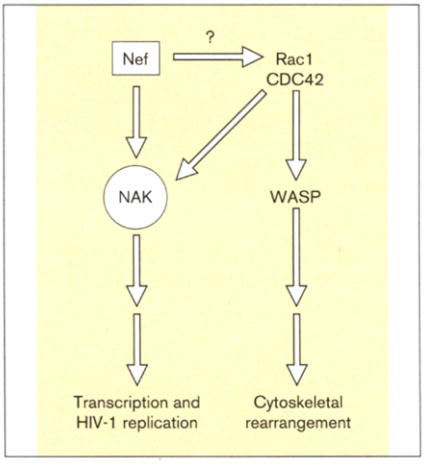
Two receptors that contain the so-called 'death domain' have been described to date: tumor necrosis factor receptor 1 (TNFR1) and Fas/Apo-1 (CD95). Both belong to the TNFR gene family. The death domain of TNFR1 mediates the activation of programmed cell death (apoptosis) and of the transcription factor NF-κB, whereas the death domain of CD95 only appears to activate apoptosis. The authors have identified an additional member of the TNFR family, a transmembrane protein of ~47 kDa that is similar to members of the TNFR family in its extracellular, cysteine-rich domains and in having a cytoplasmic death domain. The *Apo-3* gene mapped to human chromosome 1p36.3, a location that is suspected to contain a tumor suppressor gene. *Apo-3* mRNA was detected in several human tissues, consistent with the possibility that this receptor may regulate multiple signaling functions. Several lines of evidence suggest that Apo-3 is a third member of the TNFR family that activates apoptosis, and suggest that Apo-3, TNFR1 and CD95 engage a common apoptotic cell-death machinery. Apo-3 did not specifically bind to the Apo-2 ligand, suggesting the existence of a distinct ligand for Apo-3. Apo-3 mRNA is expressed in various tissues, consistent with the possibility that this receptor may regulate multiple signaling functions.



1 December, Research Paper, *Current Biology*

■ **CDC42 and Rac1 are implicated in the activation of the Nef-associated kinase and replication of HIV-1.** Xiaobin Lu, Xiaoning Wu, Ana Plemenitas, Haifeng Yu, Earl T Sawai, Arie Abo and B Matija Peterlin (1996). *Curr. Biol.* **6** 1677–1684.

The so-called negative factor (Nef) of human and simian immunodeficiency viruses (HIV-1, HIV-2 and SIV) is required for high levels of viremia and progression to AIDS. Nef causes cellular activation, increased viral infectivity and decreased expression of CD4 on the cell surface. It has been shown that Nef associates with a cellular serine kinase (NAK) activity and, more recently, that NAK has structural and functional similarity to p21-activated kinases (PAKs). The authors show that Nef activates NAK via the small GTPases CDC42 and Rac1. Dominant-negative PAK (PAKR) and dominant-negative GTPases (CDC42Hs-N17 and Rac1-N17) block the ability of Nef to associate with and activate NAK. Furthermore, constitutively active small GTPases potentiate the effects of Nef. PAKR, CDC42Hs-N17 and Rac1-N17 also decrease levels of HIV-1 production to those of virus from which the *nef* gene is deleted. By activating NAK via small GTPases and their downstream effectors, Nef interacts with regulatory pathways required for cell growth, cytoskeletal rearrangement and endocytosis. Thus, NAK could participate in the budding of new virions, the modification of viral proteins and the increased endocytosis of surface molecules such as CD4. Blocking the activity of these GTPases could lead to new therapeutic interventions against AIDS.



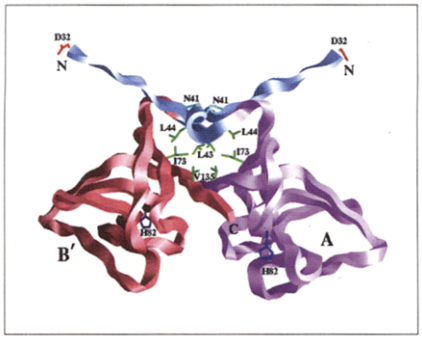
1 December 1996, Research Paper, *Current Biology*

☐ **The UmuD′ protein filament and its potential role in damage-induced mutagenesis.** Thomas S Peat, Ekaterina G Frank, John P McDonald, Arthur S Levine, Roger Woodgate and Wayne A Hendrickson (1996). *Structure* **4**, 1401–1412.

Damage induced 'SOS mutagenesis' may occur transiently as part of the global SOS response to DNA damage in bacteria. The UmuD protein is produced in an inactive form but converted to the active form, UmuD′, by a RecA-mediated self-cleavage reaction. UmuD′, together with UmuC and activated RecA (RecA*), allows the DNA polymerase III holoenzyme to replicate DNA across chemical and UV induced lesions. The efficiency of this reaction depends on several intricate protein–protein interactions. Recent X-ray crystallographic analysis shows that in addition to forming molecular dimers, the amino- and carboxy-terminal tails of UmuD′ extend from a globular β structure to associate and produce crystallized filaments. The authors find that these filaments appear to relate to biological activity. Higher order oligomers are found in solution with UmuD′, but not with

■ See also the Minireview on pages 13–15 by Trono and Wang.

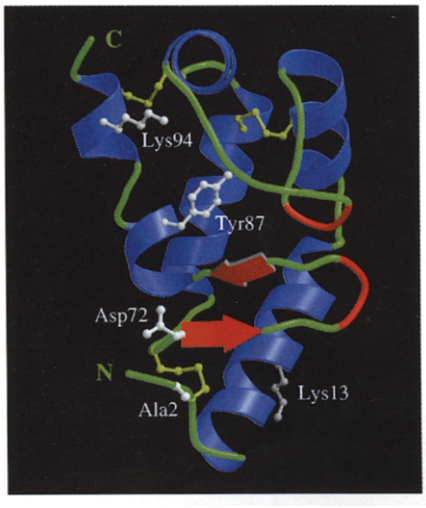
UmuD nor with a mutant of UmuD′ lacking the extended amino terminus. Deletion of the amino terminus of UmuD′ does not affect its ability to form molecular dimers but does severely compromise its ability to interact with a RecA-DNA filament and to participate in mutagenesis. Mutations in the carboxy terminus of UmuD′ result in both gain and loss of function for mutagenesis. The activation of UmuD to UmuD′ appears to cause a large conformational change in the protein that allows it to form oligomers in solution at physiologically relevant concentrations. Properties of these oligomers are consistent with the filament structures seen in crystals of UmuD′.



15 December 1996, Research Paper, *Structure*

☐ **Crystal structure of a fungal elicitor secreted by *Phytophthora cryptogea*, a member of a novel class of plant necrotic proteins.** Guillaume Boissy, Eric de La Fortelle, Richard Kahn, Jean-Claude Huet, Gérard Bricogne, Jean-Claude Pernollet and Simone Brunie (1996). *Structure* **4**, 1429–1439.

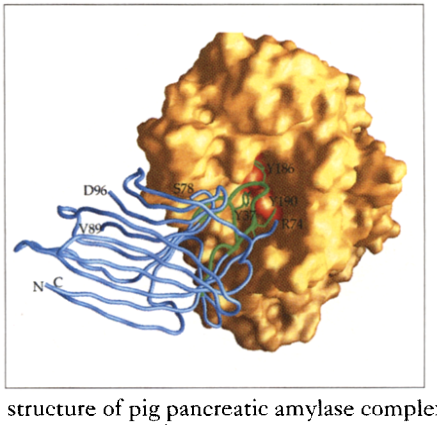
Elicitins are a novel class of plant necrotic proteins secreted by *Phytophthora* and *Pythium fungi*, parasites of many economically important crops. These proteins induce leaf necrosis in infected plants and elicit an incompatible hypersensitive-like reaction, leading to the development of a systemic acquired resistance against a range of fungal and bacterial plant pathogens. The crystal structure determination of β-cryptogein (CRY), secreted by *Phytophthora cryptogea*, was determined using the multiwave-length anomalous diffraction technique and refined to 2.2 Å resolution. The overall structure has a novel fold consisting of six helices and a beak-like motif, whose sequence is highly conserved within the family, composed of an antiparallel two-stranded β sheet and an Ω loop. This motif is assumed to be a major recognition site for a putative receptor and/or ligand. Two other distinct binding sites seem to be correlated to the level of necrotic activity of elicitins. The determination of the crystal structure of a member of the elicitin family may make it possible to separate the activity that causes leaf necrosis from that inducing systemic acquired resistance to pathogens, making it feasible to engineer a non-toxic elicitin that only elicits plant defences. Such studies should aid the development of non-toxic agricultural pest control.



15 December 1996, Research Paper, *Structure*

☐ **Substrate mimicry in the active center of a mammalian amylase: structural analysis of an enzyme–inhibitor complex.** Coralie Bompard-Gilles, Patrice Rousseau, Pierre Rougé and Françoise Payan (1996). *Structure* **4**, 1441–1452.

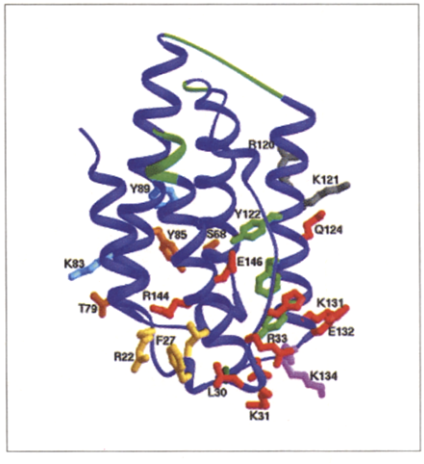
α-Amylases catalyze the hydrolysis of glycosidic linkages in starch and other related polysaccharides. The α-amylase inhibitor (α-AI) from the bean *Phaseolus vulgaris* belongs to a family of plant defence proteins and is a potent inhibitor of mammalian α-amylases. The structure of pig pancreatic α-amylase (PPA), complexed with either a carbohydrate inhibitor (acarbose) or a proteinaccous inhibitor (Tendamistat), is known, but the catalytic mechanism is poorly understood. The crystal structure of pig pancreatic amylase complexed with α-AI was refined to 1.85 Å resolution. In complex with PPA, α-AI has the typical dimer structure common to legume lectins. Two hairpin loops extending out from the jellyroll fold of a monomer interact directly with the active-site region of the enzyme molecule, and the inhibitor molecule fills the whole substrate-docking region of PPA, mimicking the enzyme–acarbose ligand interactions and preventing enzyme residues from moving into their ligand-induced functional positions. The inhibitor also destroys the water channel leading from the 'flexible loop' to the heart of the active-site depression. α-AI inhibits the activity of insect, plant and mammalian α-amylases. Understanding the mode of action of these proteins at the molecular level would be of interest for several reasons. Carbohydrate, especially starch, normally represents the majority of the human diet. Cleavage of starch by α-amylases is the first step in the enzymatic degradation of polysaccharides, and inhibition of α-amylases reduces post-prandial glucose peaks, which is of particular importance in patients with diabetes. These studies may also help in the design of transgenic plants, expressing either native or modified α-AI genes, that would be resistant to some predatory insects.



15 December 1996, Research Paper, *Structure*

☐ **Zinc-mediated dimer of human interferon-α_{2b} revealed by X-ray crystallography.** Ramaswamy Radhakrishnan, Leigh J Walter, Alan Hruza, Paul Reichert, Paul P Trotta, Tattanahalli L Nagabhushan and Mark R Walter (1996). *Structure* 4, 1453–1463.

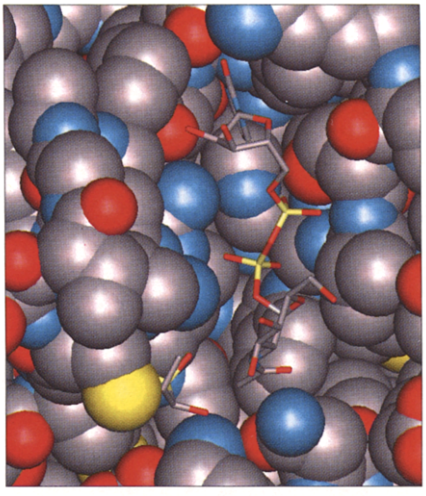
The human interferon (huIFN-α) family displays broad-spectrum antiviral, antiproliferative and immunomodulatory activities on a variety of cell types. No crystal structures of IFN-α's have previously been reported, and previous structural models of these proteins have been based on the structure of murine IFN-β (muIFN-β). The authors report the crystal structure of recombinant human interferon-α_{2b} (huIFN-α_{2b}) at 2.9 Å resolution. HuIFN-α_{2b} is found in the crystal as a noncovalent dimer; extensive interactions at the dimer interface are mediated by a zinc ion, a mode of subunit association not previously observed in cytokines. HuIFN-α_{2b} displays considerable structural similarity with muIFN-β, interleukin-10 and interferon-γ, which also bind related class 2 cytokine receptors. The authors have used structural comparisons and previously reported structure–activity data to identify protein surfaces that appear to be important in receptor activation. The biological role of the dimer is currently unknown. The structure of huIFN-α_{2b} provides an accurate model for analysis of the >15 related type I interferon molecules, and a basis for further studies aimed at understanding receptor–ligand interactions for this biologically important family of molecules.



15 December 1996, Research Paper, *Structure*

☐ **Structure of UDP-*N*-acetylglucosamine enolpyruvyl transferase, an enzyme essential for the synthesis of bacterial peptidoglycan, complexed with substrate UDP-*N*-acetylglucosamine and the drug fosfomycin.** Tadeusz Skarzynski, Anil Mistry, Alan Wonacott, Susan E Hutchinson, Valerie A Kelly and Kenneth Duncan (1996). *Structure* 4, 1465-1474.

UDP-*N*-acetylglucosamine enolpyruvyl transferase (MurA), catalyses the first committed step of bacterial cell wall biosynthesis and is a target for the antibiotic fosfomycin. The only other known enolpyruvyl transferase is 5-enolpyruvyl-shikimate-3-phosphate (EPSP) synthase, an enzyme involved in the shikimic acid pathway and the target for the herbicide glyphosate. Inhibitors of enolypyruvyl transferases are of biotechnological interest as MurA and EPSP synthase are found exclusively in plants and microbes. The crystal structure

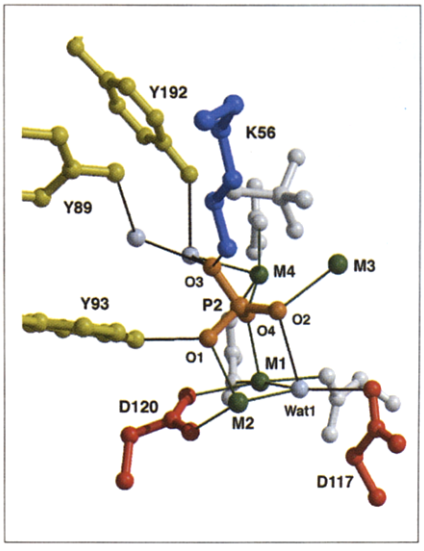


of *Escherichia coli* MurA complexed with UDP-*N*-acetylglucosamine (UDP-GlcNAc) and fosfomycin was determined at 1.8 Å resolution. The overall protein architecture is similar to that of EPSP synthase. The catalytic site is situated in a deep cavity between the two domains; the fosfomycin molecule is covalently bound to the cysteine residue Cys115, which is essential for catalysis, and UDP-GlcNAc makes several hydrogen-bonding interactions with residues from both domains. These results should aid the design of inhibitors which would interfere with enzyme-catalyzed reactions in the early stage of the bacterial cell wall biosynthesis. Furthermore, this structure provides a model for predicting active-site residues in EPSP synthase that may be involved in catalysis and substrate binding.

15 December 1996, Research Paper, *Structure*

☐ **The structural basis for pyrophosphatase catalysis.** Pirkko Heikinheimo, Jukka Lehtonen, Alexander Baykov, Reijo Lahti, Barry S Cooperman and Adrian Goldman (1996). *Structure* 4, 1491–1508.

Soluble inorganic pyrophosphatase (PPase), an essential enzyme central to phosphorus metabolism, catalyzes the hydrolysis of the phosphoanhydride bond in inorganic pyrophosphate. Catalysis requires divalent metal ions which affect the apparent pK_as of the essential general acid and base on the enzyme, and the pK_a of the substrate. Three to five metal ions are required for maximal activity, depending on pH and enzyme source. A detailed understanding of catalysis by this enzyme would aid in understanding the nature of biological mechanisms of phosphoryl transfer, and the role of divalent cations. The authors report the first two high-resolution structures of yeast PPase, at 2.2 and 2.0 Å resolution with R factors of around 17%. One structure contains the two activating metal ions; the other also contains the product, $(MnP_i)_2$. The latter structure shows an extensive network of hydrogen bond and metal ion interactions that account for virtually every lone pair on the product phosphates. It also shows that a water molecule/hydroxide ion bridges two metal ions and a phosphate bound to four Mn^{2+} ions. The structure-based model of the PPase mechanism posits that the nucleophile is the hydroxide ion. This aspect of the mechanism is formally analogous to the 'two-metal ion' mechanism of alkaline phosphatase, exonucleases and polymerases. A third metal ion coordinates another water molecule that is probably the required general acid. Extensive



Lewis acid coordination and hydrogen bonds provide charge shielding of the electrophile and lower the p*K*a of the leaving group. This 'three-metal ion' mechanism differs from that of other phosphoryl transfer enzymes, perhaps indicating a very early origin for enzyme-catalyzed phosphoanhydride hydrolysis: the authors suggest that an early form of this enzyme must have been present as organisms moved from the 'RNA world' to the 'DNA world'.
15 December 1996, Research Paper, *Structure*

☐ **Crystal structure of vancomycin.** Martina Schäfer, Thomas R Schneider and George M Sheldrick (1996). *Structure* 4, 1509–1515.

Vancomycin and other related glycopeptide antibiotics are clinically very important because they often represent the last line of defence against bacteria that have developed resistance to antibiotics. Vancomycin is believed to act by binding nascent cell wall mucopeptides terminating in the sequence D-Ala-D-Ala, weakening the resulting cell wall. Extensive NMR and other studies have shown that the formation of asymmetric antibiotic dimers is important in peptide binding. Despite intensive efforts the crystal structure of vancomycin has been extremely difficult to obtain; high-resolution data were not available, and the structure was too large to be solved by conventional 'direct methods'. Using low-temperature synchrotron X-ray data combined with new *ab initio* techniques for solving the crystallographic phase problem, the crystal structure of vancomycin has been determined at atomic resolution. The structure confirms that vancomycin exists as an asymmetric dimer. The dimer conformation allows the docking of two D-Ala-D-Ala peptides in opposite directions; these presumably would be attached to different glycopeptide strands. In the crystal, one of the binding pockets is occupied by an acetate ion that mimics the C terminus of the nascent cell wall peptide; the other is closed by the asparagine sidechain, which occupies the place of a ligand. The occupied binding pocket exhibits high flexibility but the closed binding pocket is relatively rigid. The authors propose that the asparagine sidechain may hold the binding pocket in a suitable conformation for peptide docking, swinging out of the way when the peptide enters the binding pocket.
15 December 1996, Research Paper, *Structure*