Structural biology
Paper alert

A selection of interesting papers that were published in the two months before our press date in major journals most likely to report significant results in structural biology.

Current Opinion in Structural Biology 2001, 11:263–270

Contents (chosen by)

- • of special interest
- •• of outstanding interest

Protein–nucleic acid interactions

Selected by Máire Convery*, Caitríona Dennis† and Siân Rowsell‡
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The leukemia-associated AML-1 (Runx1)-CBFα complex functions as a DNA-induced molecular clamp. Bravo J, Speck NA, Warren AJ: *Nat Struct Biol* 2001, 8:371-378.

AND

Structural analyses of DNA recognition by AML1/Runx-1 Runt domain and its allosteric control by CBFα. Tahirov TH, Inoue-Bungo T, Morii H, Fujikawa A, Sasaki M, Kimura K, Shiina M, Sato K, Kumasaka T, Yamamoto M *et al.*: *Cell* 2001, 104:755-767.

•• Significance: Two crystal structures of the core binding factor (CBF) transcription factor bound to DNA provide an insight into the regulation of DNA binding and show the consequences of mutations that cause human leukaemia.

Findings: Two papers describe the structure of a CBF bound to DNA. The CBF heterodimer consists of an AML1 Runt domain (CBFα) and CBFβ. The Runt domain is responsible for DNA binding and contacts the DNA recognition sequence through loop regions that bind the phosphates in both the major and minor grooves. Mutations causing leukaemia have been mapped to regions that are responsible for specific interactions with the DNA. The CBFβ domain has been shown stabilise the loop regions of the Runt domain (CBFα) that bind DNA, hence performing a regulatory role.

Structure of the RTP-DNA complex and the mechanism of polar replication fork arrest. Wilce JA, Vivian JP, Hastings AF, Otting G, Folmer RHA, Duggin IG, Wake RG, Wilce MCJ: *Nat Struct Biol* 2001, 8:206-210.

• Significance: Structural studies on the replication terminator protein–DNA complex provide insights into the molecular mechanism of polar replication fork arrest.

Findings: The crystal structure of the replication terminator protein (RTP) of *B. subtilis* bound to its cognate *Ter* DNA site sequence has been determined at 2.5 Å resolution. The authors have also studied the structural and dynamic features of the interaction using solution-state NMR techniques. A novel winged helix interaction is revealed in which the 'wings' do not form intimate interactions with the DNA, which is, unexpectedly, undistorted B-form. A model for the complete termination complex, in which two RTP dimers are bound cooperatively to *Ter* DNA, is proposed.

A mechanism for initiating RNA-dependent RNA polymerisation. Butcher SJ, Grimes JM, Makeyev EV, Bamford DH, Stuart DI: *Nature* 2001, 410:235-240.

•• Significance: The first structures of a viral RNA polymerase that allow the mechanism of self-initiation to be postulated.

Findings: The double-stranded RNA bacteriophage ϕ6 polymerase has an unusually rigid but typical hand-like architecture of all polymerases. It has two elaborations from the standard fold that result in the molecule being roughly spherical and that it unexpectedly shares with the polymerase from hepatitis C virus. Co-crystal structures with oligonucleotide and/or NTPs, and/or magnesium and manganese allow the mechanisms of unravelling the double-stranded RNA and selective initiation on the template strand to be elucidated.

Folding and binding

Selected by Tanja Kortemme, Sehat Nauli and Alex Watters
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Prediction and confirmation of a site critical for effector regulation of RGS domain activity. Sowa ME, He W, Slep KC, Kercher MA, Lichtarge O, Wensel TG: *Nat Struct Biol* 2001, 8:234-237.

•• Significance: A new method called 'Evolutionary Trace' is shown to aid the sequence- and structure-based identification of a regulatory protein–protein interaction site.

Findings: In a two-step procedure, members of a protein family are first classified on the basis of their sequence identities and, second, specific residues are found that are invariant within a class, but vary among different classes. The authors propose that these so-called 'trace residues' are likely to be important for functional specificity. Mutational targeting of trace residues experimentally confirms a predicted binding site and identifies key residues in the regulation of G-protein signalling.

Nanohedra: using symmetry to design self assembling protein cages, layers, crystals, and filaments. Padilla JE, Colovos C, Yeates TO: *Proc Natl Acad Sci USA* 2001, 98:2217-2221.

•• Significance: A novel strategy for building nanoparticles using the concept of symmetry is described and shown to work. (See also alert by Gilardi, in this issue, pp 266.)

Findings: The authors fused a dimeric and trimeric protein. Rules of symmetry dictate the types of nanostructures that would be observed when this basic construct oligomerises. The authors show by analytical ultracentrifugation, dynamic light

scattering and electron microscopy that the designed nanostructure is formed.

Functional proteins from a random-sequence library. Keefe AD, Szostak JW: *Nature* 2001, 410:715-718.
• Significance: The fraction of random polypeptides possessing binding or catalytic properties is of great importance in understanding the origins of proteins and for *de novo* protein design. This fraction may be less 1 in 10^8, which is the limit of conventional phage display techniques.
Findings: To gain as accurate a measurement as possible, the authors used mRNA display, with a library size of 10^{12}, to search for ATP binders in a library of 80-residue polypeptides. Initial selection rounds produced four, nonrelated ATP binders. Subsequent rounds of PCR mutagenesis of the library and further selection yielded a library entirely composed of polypeptides related to one of the original four ATP binders. One variant showed ATP binding with a $K_d < 1\mu M$. From these results, the authors estimate that 1 in 10^{10} random polypeptides has the ability to bind ATP.

Macromolecular assemblages

Selected by Andreas Engel
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The bacterial conjugation protein TrwB resembles ring helicases and F_1-ATPase. Gomis-Rüth FX, Moncallian G, Perez-Luque R, Gonzalez A, Cabezon E, de la Cruz F, Coll M: *Nature* 2001, 409:637-641.
•• Significance: DNA passes from one cell to another through highly specific pores – a process of fundamental importance for horizontal gene transfer. The structure of TrwB, which forms the hexameric pore of the *E. coli* conjugative system R388, has been solved by X-ray crystallography. Strikingly, this DNA translocator has amazing similarity in terms of dimensions, shape, fold and nucleotide-binding domain (NBD) to helicases and to the fusion machine NFS, as well as to the F_1-ATP synthase. It transpires that many ATP-driven nanomachines share structural features without having any sequence homology.
Findings: TrwB is an integral membrane protein comprising 507 residues. A soluble 437-residue variant has been crystallised and solved to 2.4 Å. TrwB forms hexamers that exhibit a central channel with a narrowing at the distal end of the membrane-attachment site. The hexamer has a diameter of 110 Å and a height of 90 Å. The NDB contains a Walker A and a Walker B motif, and shares strong structural homology with F_1-ATP synthase. Topological searches revealed the structural homology of the NBD with those of RecA and several helicases. The structural homology is highest with the β subunit of the F_1-ATP synthase in the occupied conformation. The transmembrane region of TrwB was modelled based on two transmembrane helices of the photosynthetic reaction centre.

Structure of the bacterial flagellar protofilament and implications for a switch for supercoiling. Samatey FA, Imada K, Nagashima S, Vonderviszt F, Kumasaka T, Yamamoto M, Namba K: *Nature* 2001, 410:331-337.
•• Significance: Bacteria survive by swimming in a biased random walk towards nutrients. This trick is achieved by a concerted 0.8 Å conformational change in the subunits of the flagellar filament upon a change in its rotation sense, thus switching between tumbling and swimming. This tiny conformational change and its effect on the flagellum structure have been elucidated by X-ray analysis and modelling experiments.
Findings: F41, a 41K fragment of *Salmonella* flagellin, was crystallised into thin crystals that allowed the F41 structure to be solved at 2 Å. The complex α/β protein contains three major domains and includes a novel fold termed β folium. Fibre diffraction analyses have shown that the difference between repeats of the L and R type filament conformations is only 0.8 Å. Docking the F41 structure onto a low-resolution filament density map from cryo-electron microscopy and fibre diffraction provided an atomic model of one protofilament. Stretching this protofilament in 0.1 Å increments and subsequent energy minimisation revealed a conformational switch of the β hairpin of domain 1, which is involved in axial interactions within the filament. The structure provides a deep insight into the working of the flagellum and allows the effect of point mutations to be understood.

Bacterial Na^+-ATP synthase has an undecameric rotor. Stahlberg H, Müller DJ, Suda K, Fotiadis D, Engel A, Meier T, Matthey U, Dimroth P: *EMBO Rep* 2001, 2:229-233.
• Significance: Biochemical analyses have provided solid evidence that the proton : ATP ratio in the F_1F_o-ATP synthase is about 4, fostering the model of a dodecameric rotor in the F_o domain. Recent findings showed, however, that the rotor is decameric in yeast mitochondria and tetradecameric in chloroplast. The observation of undecameric bacterial rotors by electron crystallography and atomic force microscopy (AFM) completes the catalogue of nondodecameric rotors in prokaryotes and eukaryotes. These results now stimulate further studies on the precise mechanism of converting the energy of the electrochemical gradient into mechanical rotation.
Findings: 2D crystals prepared by reconstitution of isolated rotors from *Ilyobacter tartaricus* in the presence of lipids yielded cryo-electron microscopic projection maps at a resolution of 6.9 Å. The P1 unit cell housed two rotors in opposite orientations that exhibited 11 petals each and opposite vorticity. AFM revealed rings protruding differently from the membrane, suggesting the rotors are packed in an upwards and downwards orientation. The lower rings had 11 protrusions that could be discerned in the unprocessed topographs.

Theory and simulation

Selected by Andras Fiser
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Automated multiple structure alignment and detection of a common substructural motif. Leibowitz N, Fligelman ZY, Nussinov R, Wolfson HJ: *Proteins* 2001, 43:235-245.
•• Significance: Although multiple structural alignment is a crucial tool to analyse structural variations in similar proteins, only a very few attempts have been made so far to set up algorithms to systematically address the problem.
Findings: An automated method is presented, based on the geometric hashing paradigm, to perform multiple structural alignment of proteins and to simultaneously detect common substructural motifs. The method considers all the structures at the same time and does not depend on sequence order, nor does it require input other than the coordinate files.

Protein docking using continuum electrostatics and geometric fit. Mandell JG, Roberts VA, Pique ME, Kotlovyi V, Mitchell JC, Nelson E, Tsigelny I, Ten Eyck LF: *Protein Eng* 2001, 14:105-113.
• Significance: Solvent effects are often a major missing aspect of various structure modelling approaches. A new feature of the presented docking algorithm is that it provides a

physicochemically more realistic and accurate approach by incorporating solvent effect in its potential function.
Findings: A larger cluster of correct answers was produced using the new potential function to dock a wide variety of protein–protein interactions. Another observation is that favourable free-energy clusters are good indicators of binding sites.

Prion protein; evolution caught en route. Tompa P, Tusnady GE, Cserzo M, Simon I: *Proc Natl Acad Sci USA* 2001, 98:4431-4436.
• Significance: In the intensively studied subject of the prion proteins, a new theory is provided about the possible integral membrane origin of these molecules.
Findings: A rigorous theoretical testing and analysis is presented that illustrates that there is a very high probability that prion proteins contain three transmembrane segments. This suggests that prion, originally an integral membrane protein, was expelled into the extracellular space as a result of certain mutations. Therefore, the unique structural ambiguity of prions is due to the fact that, in evolutionary terms, these molecules are proceeding to become soluble proteins. The elapsed time was insufficient to create a 'minimally frustrated' sequence with a single energy minima and a single unique conformation.

Nucleic acids

Selected by Gary Parkinson
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Structural and thermodynamic studies on mutant RNA motifs that impair the specificity between a viral replicase and its promoter. Kim C-H, Tinoco I: *J Mol Biol* 2001, 307:827-839.
• Significance: Solution structures of four mutant triloop RNA motifs from brome mosaic virus (BMV) genomic RNA were determined in an attempt to elucidate the role of the 'clamped base motif' in replicase recognition. The 3′-end region of BMV genomic RNA forms a tRNA-like structure, with one critical structural element forming a stem-loop C motif that is necessary and sufficient for the binding of RNA replicase. The four stem-loop C triloop mutants destroy enzymatic recognition.
Findings: There are no effects on the secondary structure, sugar pucker or base pairing of the stem helix region resulting from modification of the triloop region. Two of the mutant sequences, 5′-GUA-3′ and 5′-UUA-3′, reveal increased flexibility at position 6, with U6 modelled in two distinct conformations. The flexibility observed in position 6 and the loss of enzymatic recognition is consistent with the clamped adenine motif model. The locking of A6 in a solvent-accessible orientation is the key structural element in the stem-loop C motif.

Solution structure of an A-tract DNA bend. MacDonald D, Herbert K, Zhang X, Polgruto T, Lu P: *J Mol Biol* 2001, 306:1081-1098.
• Significance: DNA duplex sequences that contain runs of four to six adenine bases exhibit a significant curvature that can be observed by their anomalous gel retardation. It has previously been shown that the use of NMR with residual dipolar couplings can be used to determine the overall curvature of double-stranded DNA. This technique has been applied by the authors in determining the solution structure of a dodecamer containing an A-tract, d(GGCAAAAAACGG/d(CCGTTTTTTGCC).
Findings: The structure reveals an overall helix axis bend of 19°, with 14° of the bending occurring in the GC regions flanking the A-tracts. This is consistent with the solution and gel electrophoresis experiments, and with the junction model proposed by Crothers and colleagues (HS Koo, HM Wu, DM Crothers: *Nature* 1986, 320:501-506). The A-tract reveals an intrinsic curve of 5°. There is a significant propeller twist of 16° in the A•T base pairs, resulting in bifurcated hydrogen bonds. The 5′ bend consists of 3° tilt and 8° roll occurring between base pairs C•G3 and A•T4, whereas the larger 3′ bend of 10° occurs over two base steps (AT9–CG10 and CG10–GC11).

Solution structure of the A loop of 23S ribosomal RNA. Puglisi JD, Blanchard SC: *Proc Natl Acad Sci USA* 2001, 98:3720-3725.
• Significance: The authors present the solution structure of a modified and unmodified A-loop RNA and compare it with the A-loop observed in the 50S ribosomal subunit crystal structure. The A-loop is highly conserved, containing a 2′-*O*-methyl ribose modification at position U2552, and is an essential RNA component of the ribosome peptidyl transferase centre.
Findings: The solution structure of the A-loop contains nucleotides 2548–2560 of *E. coli* 23S rRNA, with and without the 2′-*O*-methyl modification. The A-loop adopts a compact fold, with noncanonical base pairing between C2556 and U2552 that modulates the loop architecture. The solution and crystal structures differ substantially, implying a structural rearrangement of the A-loop during docking into the peptidyl transferase centre.

Structural basis of the enhanced stability of a mutant ribozyme domain and a detailed view of RNA-solvent interactions. Juneau K, Podell E, Harrington DJ, Cech TR: *Structure* 2001, 9:221-231.
• Significance: The use of *in vitro* evolution made possible the identification of a highly stable isomorphous mutant RNA that readily crystallised and diffracted to a higher resolution (2.25 Å versus 2.8 Å), thus providing new details of metal ion–RNA interactions. Although the crystal structure of P4–P6 had been previously solved, these various mutations convey structural stability that can be rationalised by the crystal structures.
Findings: Single-site mutants of P4–P6, a 160-nucleotide domain of the self-splicing *Tetrahymena thermophila* intron, were isolated by successive rounds of *in vitro* selection and amplification. The C209 deletion mutant allows the adjacent bulged adenine to enter the P4 helix and form an A•G base pair, attenuating the conformational flexibility of the helix. Diffraction quality was improved (2.25 Å versus 2.8 Å), allowing the assignment of 27 magnesium ions and a detailed view of the water structure on the inside of the folded RNA domain.

Detection of alkali metal ions in DNA crystals using state-of-the-art X-ray diffraction experiments. Tereshko V, Wilds CJ, Minasov G, Prakash TP, Maier MA, Howard A, Wawrzak Z, Manoharan M, Egli M: *Nucleic Acids Res* 2001, 29:1208-1215.
• Significance: The authors demonstrate that the replacement of Na^+ by K^+, Rb^+ or Cs^+, in conjunction with the precise measurement of anomalous differences in intensity, provides a method for detecting metal-ion-binding sites in nucleic acid crystals.
Findings: Two DNA sequences were selected for their well-characterised crystallographic properties – a Z-DNA-forming hexamer of sequence CGCGCG and an A-DNA-forming decamer of sequence GCGTATACGC that was 2′-*O*-modified at the T6 position. Crystals were grown in various salt conditions and diffracted at the Advanced Photon Source at several wavelengths. Crystals

that contained Ba^+, Cs^+ or Rb^+ grown under medium to high salt conditions could be solved using single anomalous diffraction (SAD). Once solved, anomalous difference Fourier and double-difference maps can be used to reveal other metal ions sites that scatter with a moderate anomalous signal. These sites can be further analysed on the basis of salt concentration and occupancy. The location of possible Na^+ can only be inferred by their replacement with metal ions with higher binding affinities, which scatter with a strong anomalous signal.

Sequences and topology

Selected by Richard Copley
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MH1 domain of smad is a degraded homing endonuclease. Grishin NV: *J Mol Biol* 2001, 307:31-37.

• Significance: The author demonstrates that a transcription factor important in metazoan development has evolved from an endonuclease

Findings: A distant structural similarity, indicative of homology, between I-*Ppo*I endonuclease and the MH1 domain of Smads, which are a crucial component of TGF-β signalling cascades, is reported. The similarity between the two proteins is not obviously apparent, but loose structural similarity, together with conservation both of key functional residues and of the mode of DNA binding, presents a persuasive argument for homology. This example illustrates how specialised proteins crucial for normal metazoan development can evolve from ancient components present in all the kingdoms of life.

Evolution of function in protein superfamilies, from a structural perspective. Todd AE, Orengo CA, Thornton JM: *J Mol Biol* 2001, 307:1113-1143.

•• Significance: A detailed survey of the relationship between protein folds, sequences and the evolution of function.

Findings: By performing detailed analyses of 31 protein superfamilies, the authors have described a number of important trends in the evolution of enzymatic function. Conservation of catalytic mechanism appears to be one of the dominant themes in enzyme evolution. Substrate specificity, on the other hand, is not maintained across large phylogenetic distances. Enzyme EC numbers are not particularly well conserved within superfamilies and so are less suited for evolutionary analysis. One-third of superfamilies exhibit catalytic residue 'migration', that is, within homologous proteins, catalytic residues appear to move to different locations in the overall structure. The paper is rich in examples that clearly illustrate the concepts being described.

Regulatory potential, phyletic distribution and evolution of ancient, intracellular small-molecule-binding domains. Anantharaman V, Koonin EV, Aravind L: *J Mol Biol* 2001, 307:1271-1292

• Significance: A thorough analysis of the occurrence of widespread small-molecule-binding domains (SMBDs).

Findings: The authors have performed exhaustive searches to characterise the distribution and domain architectures of proteins containing any of 21 SMBDs, as well as identifying an additional three new candidate domains of this class; they consider it unlikely that many more such domains will be identified. In the majority of cases, SMBDs do not share folds with enzymes that process similar substrates. The authors demonstrate that there is an excess of SMBD-containing domain architectures that are not shared between different organisms, showing that there is considerable plasticity in the evolution of proteins containing these domains (183 in a single lineage versus 55 in two lineages). When domain architectures are conserved, it is usually within the context of metabolic enzymes.

Engineering and design

Selected by Gianfranco Gilardi
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Oxidation of polychlorinated benzenes by genetically engineered CYP101 (cytochrome P450cam). Jones JP, O'Hare EJ, Wong L-L: *Eur J Biochem* 2001, 268:1460-1467.

•• Significance: CYP101 has been modified through protein engineering to oxidise an important class of harmful environmental contaminants, the polychlorinated benzenes (PCBs).

Findings: The active site of CYP101 was modified by site-directed mutagenesis to allow the enzyme to utilise various PCBs as substrates. The active site was made more hydrophobic to encourage binding of the nonpolar PCBs and more crowded to force the PCBs closer to the haem cofactor. This redesign was achieved by making the mutants F87W-Y96F, F87W-Y96F-F98W and F87W-Y96F-V247L. All the mutants were found to have a higher activity than the wild-type towards the various PCB compounds tested; the F87W-Y96F-F98W mutant showed a reasonably high turnover rate towards 1,3-DCB (143 nmol of product per nmol of CYP101 per min) and 1,3,5-TCB (119 nmol of product per nmol of CYP101 per min). The turnover of these substrates by this mutant was also highly coupled (greater than 90%) to NADH consumption. This high coupling is important if the mutant enzyme is to be engineered into a host for the breakdown of PCBs.

Nanohedra: using symmetry to design self assembling protein cages, layers, crystals, and filaments. Padilla JE, Colovos C, Yeates TO: *Proc Natl Acad Sci USA* 2001, 98:2217-2221.

• Significance: Two self-assembling protein structures, a cage and a filament, were designed using a set of rules of symmetry that can be used to build several types of assembly. (See also alert by Kortemme *et al.*, in this issue, pp 263.)

Findings: A set of rules of symmetry was designed and used to generate a set of theoretical models of self-assembling proteins. These models were screened and two of the best, a cage and a filament, were built. The symmetric cage was built by fusing one monomer of trimeric bromoperoxidase to one monomer of dimeric M1 matrix protein. The two oligomerisation domains were fused together using a short nine-residue helix linker. By applying a sample of purified protein solution to a support slide, the occurrence of a 15 nm self-assembling cage was confirmed by EM. The cage was also confirmed using dynamic light scattering and equilibrium sedimentation to show it was the right size. In a similar fashion, a filament was built by fusing one monomer of dimeric M1 matrix protein to one monomer of dimeric carboxyesterase using a five-residue α-helical linker. The filament was also confirmed by EM and was found to form long bundles and networks. Further development of these self-assembling proteins could have a wide variety of applications and could be used to provide insights into how self-assembly occurs in nature.

De novo design and characterisation of copper centers in synthetic four-helix-bundle proteins. Schnepf R, Hörth P, Bill E, Weighardt K, Hildebrandt P, Haehnel W: *J Am Chem Soc* 2001, 123:2186-2195.

•• Significance: This paper reports the creation of a *de novo* designed copper protein by integrating a rational design,

template-assisted synthetic protein (TASP) assembly and a combinatorial approach. A *de novo* copper-binding site was selected from a library of variants of a four-helix bundle scaffold on the basis of spectral properties. This is the first time a copper site has been designed in a four-helix bundle.

Findings: A library of 96 protein variants was fabricated by the modular assembly of four-helix bundles on a dodecapeptide template. The design allowed the incorporation of histidine, cysteine or aspartic acid residues in various combinations around a potential copper-binding site. The proteins with the best copper-binding spectral properties (based on the S–Cu) were selected and further characterised. Three variants, Mop5, Mop6 and Mop7, with absorption maxima at 410 nm, 403 nm and 379 nm, respectively, were selected. Circular dichroism experiments confirmed the α-helical structure predicted by the design, which was not significantly perturbed by copper binding. Resonance Raman spectroscopy was consistent with S–Cu stretching (for Mop5, excitation at 413 nm lead to a prominent band at 326.5 cm^{-1}) and EPR data were also consistent with values observed for natural copper proteins. The proteins were highly susceptible to cysteine oxidation, probably due to accessibility to solvent.

Design, synthesis, and characterisation of a novel hemato-protein. Xu Z, Farid RS: *Protein Sci* 2001, 10:236-249.

• Significance: The authors describe a synthetic haem-binding protein designed completely *de novo* using a rational design approach involving an in-house protein design program: CORE. This allows the fabrication of a four-helix bundle based on a helix with 18 unique positions, rather than on the heptad repeat of other designs, which the authors claim is not truly representative of α-helical proteins.

Findings: Two synthetic parallel four-helix bundle proteins are presented. Each consists of two pairs of identical α-helix-forming peptides linked at the N terminus by an S–S bond. The first protein (6H7H) has eight histidine residues, allowing the binding of up to four *bis*-ligated haems (two perpendicular pairs); the second (6H7S) has a single haem. For the former, it was found that the first two haems bound cooperatively with estimated K_D values of 80 ± 10 nM and 18 ± 2 nM, respectively, based on fitting to a linear combination of component difference spectra. The absorbance maximum of the dihaem protein was found to be 415.5 nm. The authors were unable to estimate as precisely the dissociation constants for the third and fourth haems, but they were able to demonstrate that they were significantly higher. The 6H7S bundle bound a single haem (through unknown ligation) with an estimated K_D of 12 ± 5 nm. The designed proteins were found to be highly thermostable (apo-6H7H only 40% unfolded at 95ºC). For dihaem 6H7H, two electrochemical potentials of –91 mV and –133 mV were found and attributed to the two haems, although there was no predicted electronic interaction. For the four-haem-bound peptide, the redox potentials were found to be –195 mV and –110 mV.

Structure-based chimeric enzymes as an alternative to directed enzyme evolution: phytase as a test case. Jermutus L, Tessier M, Pasamontes L, van Loon PGM, Lehman M: *J Biotechnol* 2001, 85:4-24.

• Significance: This paper reports a comparison of the use of selected point mutations with the fabrication of a hybrid enzyme to improve the thermostability of the phytase enzyme.

Findings: A sequence comparison of the phytase enzyme from *Aspergillus terreus* and its relatively more thermostable homologue from *Aspergillus niger* was made, which provided the choice of point mutations to improve the thermostability of the former. Four mutants were constructed and characterised (M1: E41A, D42G; M2: H61E; M3: A68S, A72E, A73E 77N; M2/3: combination of M2 and M3). Despite increasing the propensity to form salt bridges and hydrogen bonds, these mutations were found to make no significant increase in thermostability, as measured by enzyme activity and fluorescence spectroscopy. Three hybrid proteins were made in which entire secondary structure elements (one helix, one β strand or both) from the *A. niger* enzyme were transplanted into the *A. terreus* phytase. It was found that, after replacing one helix (residues 66–82), levels of thermostability similar to *A. niger* were found for the *A. terreus* phytase. The mid-point of thermal denaturation (T_m) measured by fluorescence emission moved from 52.0ºC to 54.3ºC, and the T_m measured by residual activity increased from 49.5ºC to 52.5ºC. The authors suggest creating hybrid enzymes as an alternative to screening large populations of mutants as a method for the introduction of desired properties to an enzyme.

Lipids

Selected by Richard Newman
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Structural mechanism of endosome docking by the FYVE domain. Kutateladze T, Overduin M: *Science* 2001, 291:1793-1796.

•• Significance: The recruitment of trafficking and signalling proteins to membranes containing phosphatidylinositol 3-phosphate [PtdIns(3)P] is mediated by FYVE domains. The FYVE domain of EEA1 (early endosome antigen 1 protein) is targeted to endosomes in several stages and is recruited to membranes through the insertion of a conserved hydrophobic loop into the lipid bilayer. There follows simultaneous ligation of PtdIns(3)P and insertion into the membrane. These actions enhance affinity for endosomes. The structural plasticity of the FYVE domain implicates it as a key player in dynamically integrating Rab5 and syntaxins into the endosomal membrane fusion complex.

Findings: The NMR solution structure of the FYVE domain of EEA1 in the free state was compared with structures of the domain complexed with PtdIns(3)P and mixed micelles. The multistep binding mechanism involved nonspecific insertion of a hydrophobic loop into the lipid bilayer, thus positioning and activating the binding pocket. Ligation of PtdIns(3)P then induced a global structural change, drawing the protein termini over the bound phosphoinositide by extension of a hinge. Specific recognition of the 3-phosphate was determined indirectly and directly by two clusters of conserved arginines.

Structures of beta-ketoacyl-acyl carrier protein synthase I complexed with fatty acids elucidate its catalytic machinery. Gotthardt-Olsen J, Kadziola A, von Wettstein-Knowles P, Siggaard-Anderson M, Larsen S: *Structure* 2001, 9:233-243.

•• Significance: Carbon–carbon bond formation is essential in biochemistry and the reaction catalysed by β-ketoacyl synthase (KAS) enzymes, known as the Claisen condensation, is utilised by enzymes with an α-β-α-β-α or thiolase fold. The enzyme β-ketoacyl-acyl carrier protein synthase (KAS) I is vital for the synthesis of the saturated (C16) and unsaturated (C16:1 and C18:1) fatty acids that characterise the membrane lipids of *E. coli*. The presence of double bonds in membranes increases their fluidity, allowing them to cope with decreases in temperature. The authors have revealed the structural basis of the ability of KAS I to initiate the synthesis of unsaturated fatty acids by

solving the structure of KAS I complexed with C10 and C12 fatty acids.
Findings: Using X-ray crystallography to solve the structures of the above-mentioned complexes, the authors propose that the catalytic mechanism of KAS I is based on a Cys-His-His triad in the active site. Transfer of the fatty acid onto the active site cysteine is assisted by a nucleophilic elbow and oxyanion hole. Both histidines are required for decarboxylation; one donating a hydrogen bond to the malonyl thioester oxo group and the other abstracting a proton from the leaving group. Thiolase fold enzymes are important in the biotechnology industry. The transformation of KAS genes into plants is being tried to enable the production of new oils in crops, and fatty acid synthesis can be specifically targeted in pathogenic bacteria causing tuberculosis and in malaria-causing protozoans. Structure-based drug design utilising knowledge of the structures of the KAS I complexes should prove relevant to these investigations.

Membrane proteins

Selected by Irmgard Sinning
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The voltage-sensitive sodium channel is a bell-shaped molecule with several cavities. Sato C, Ueno Y, Asai K, Takahashi K, Sato M, Engel A, Fujiyoshi Y: *Nature* 2001, 409:1047-1051.
• Significance: The low-resolution structure of the voltage-sensitive sodium channel reveals complex internal cavities and pores. Homologous voltage-sensitive calcium and tetrameric potassium channels may possess related structures.
Findings: The structure of the voltage-sensitive sodium channel from the eel *Electrophorus electricus* has been determined by single-particle image analysis at 19 Å resolution. The channel is bell-shaped, with a height of about 135 Å and a side length of about 100 Å at the bottom and 65 Å at the top. Antibody labelling was used to test the tentative domain assignments. The C terminus of the channel is flexible and the wide, square-shaped end of the channel is located in the cytoplasm. Large internal cavities on the cytoplasmic and extracellular sides connect to four narrow, peripheral, low-density regions in the transmembrane domain. These unique cavities may be involved in voltage sensing and gating.

Three-dimensional structure of a voltage-gated potassium channel at 2.5 nm resolution. Sokolova O, Kolmakova-Partensky L, Grigorieff N: *Structure* 2001, 9:215-220.
• Significance: The low-resolution structure of a full-length voltage-gated potassium channel (Shaker channel from *Drosophila)* confirms the 'hanging gondola' model of the extracellular tetramerisation (T1) domain. If T1 is involved in gating, it must be an indirect effect via the thin connectors that separate the T1 domain from the transmembrane domain.
Findings: The structure of the entire Shaker channel was determined at 25 Å resolution by single-particle image analysis and shows the expected fourfold symmetry. The channel has a molecular mass of about 400 kDa and comprises two distinct domains of about 100 and 300 kDa linked by thin connectors about 2 nm long. Interpretation of this low-resolution data was possible using the crystal structures of the isolated T1 domain and of the KcsA channel, which are both tetramers. The KcsA channel was placed in the larger domain (about 10 × 6 nm), which was therefore proposed to be the transmembrane part. The connectors border the sides of putative windows between the two domains that seem to be about 3 × 2 nm in size.

Carbohydrates and glycoconjugates

Selected by Sabine L Flitsch* and Philip AS Lowden†
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Automated solid-phase synthesis of oligosaccharides. Plante OJ, Palmacci ER, Seeberger PH: *Science* 2001, 291:1523-1527.
• Significance: This is the first report of automated solid-phase oligosaccharide synthesis having been applied to some selected oligosaccharide targets.
Findings: A number of significant advances have recently been made in enabling solid-phase synthesis of oligosaccharides. In this report, such solid-phase methodology was used for the automated synthesis of a number of targets: two phytoalexin elicitor β-glucans (hexasaccharide and dodecasaccharide), a trisaccharide and an α-1,2-linked decamannoside. Coupling methods employed for forming glycosidic linkages involved either trichloroacetimidates or glycosyl phosphates.

Construction of glyco-clusters by self-organisation of site-specific glycosylated oligonucleotides and their cooperative amplification of lectin-recognition. Matsuura K, Hibino M, Yamada Y, Kobayashi K: *J Am Chem Soc* 2001, 123:357-358.
• Significance: This paper describes a new strategy for the construction of defined periodic glycoclusters, which should be useful tools in studying polyvalent interactions between sugars and other biomolecules, such as lectins.
Findings: Site-specifically galactosylated oligonucleotide 20-mers were synthesised and hybridised with a half-sliding complementary oligonucleotide to generate gapped heteroduplexes that provide rigid scaffolds to display the sugars in defined orientations. It was shown that the galactosyl clusters were cooperatively recognised by a galactose-specific lectin.

Genetic remodelling of protein glycosylation *in vivo* induces autoimmune disease. Chui D, Sellakumar G, Green RS, Sutton-Smith M, McQuistan T, Marek KW, Morris HR, Dell A, Marth JD: *Proc Natl Acad Sci USA* 2001, 98:1142-1147.
• Significance: Distinct changes in protein glycosylation have been reported in various autoimmune syndromes. This paper reports, for the first time, evidence that autoimmune disease is caused by a genetic defect in the pathway of *N*-glycosylation.
Findings: Mammalian α-mannosidase II resides in the Golgi apparatus and catalyses the trimming of two mannose residues from hybrid *N*-linked oligosaccharides, regulating the branching pattern of *N*-glycans. It is shown that alterations in *N*-glycan branching caused by mutation of the α-mannosidase II gene result in a systemic autoimmune disease similar to human lupus erythematosus.

Biophysical methods

Selected by Steve Matthews
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Analysis of enzyme kinetics using electrospray ionization mass spectrometry and multiple reaction monitoring: fucosyltransferase V. Norris AJ, Whitelegge JP, Faull KF, Toyokuni T: *Biochemistry* 2001, 40:3774-3779.
• Significance: Mass spectrometry (MS) is well established in the characterisation of biological molecules. This is, in part, because of its ability to analyse gas-phase ions of thermally unstable species of a wide range of molecular weights. Also,

MS allows the direct analysis of mixtures without the need for radioactive or chromogenic labelling. This is particularly attractive for the analysis of enzyme kinetics, which conventionally requires either radiolabelled or chromogenic substrates. An alternative approach comprising ESI-MS (electrospray ionisation-MS) coupled to flow injection analysis (FIA) and multiple reaction monitoring (MRM) has been successfully applied to the analysis of enzyme kinetics.
Findings: -1,3-Fucosyltransferase V (Fuc-T V) was chosen to demonstrate this technique. Fuc-T V catalyses the transfer of L-fucopyranose (Fuc) from GDP-Fuc to type II (Galβ1–4GlcNAcβ1–R) acceptors. Reactions were performed in amine-containing buffers and diluted in a suitable solvent, and then directly analysed without purification. Both decreased mass resolution and MRM, in the tandem mass spectrometric mode, were used to enhance the sensitivity and selectivity of detection. The approach allowed the simultaneous monitoring of multiple processes, including substrate consumption, product formation and the intensity of an internal standard. The results showed that data from ESI-MS coupled to MRM compared well with conventional radioactivity-based assays.

Visualization of unwinding activity of duplex RNA by DbpA, a DEAD box helicase, at single-molecule resolution by atomic force microscopy. Henn A, Medalia O, Shi S-P, Steinberg M, Franceschi F, Sagi I: *Proc Natl Acad Sci USA* 2001, 98:5007-5012.
• Significance: Atomic force microscopy (AFM) is becoming increasingly important in structural biology. The technique has the potential to assay protein–nucleic acid interactions at the molecular level for individual complexes. Examination of the helicase activity of DbpA by AFM provides a means for directly visualising its unwinding catalysis at single-molecule resolution.
Findings: The *E. coli* protein DbpA possesses ATPase-specific activity toward the peptidyl transferase centre in 23S rRNA. Although its ATPase activity has been well characterised, its RNA helicase activity remains poorly understood. Biochemical assays and AFM show that DbpA exhibits ATP-stimulated unwinding activity of duplex RNA, regardless of its primary sequence. AFM images facilitate direct observation of the unwinding reaction of the helicase on long stretches of double-stranded RNA. Furthermore, the binding of DbpA to RNA in the absence of ATP and the formation of a Y-shaped intermediate in the presence of ATP were distinguishable.

Fluorescence characterization of the transcription bubble in elongation complexes of T7 RNA polymerase. Liu C, Martin CT: *J Mol Biol* 2001, 308:465-475.
• Significance: The stability of the enzyme–DNA–RNA complex during the elongation phase of transcription is essential to the production of full-length RNA transcripts. Transcription initiation in the T7 RNA polymerase system has been well characterised and, despite its small size, T7 RNA polymerase shows all the fundamental features characteristic of RNA polymerases. These include specific initiation, an early, unstable, abortive complex, and its conversion to a stably elongating ternary complex. Detailed studies of the elongation complex, including the size of the bubble and heteroduplex length, are essential for understanding the elongation mechanism. A novel fluorescent base analogue was used to probe stable but stalled elongation complexes. The highly fluorescent cytidine analogue pyrrolo-dC has excitation and emission maxima far from those of DNA and protein. It also pairs with guanine and shows reduced fluorescence in duplex DNA relative to single-stranded DNA, which can be used to monitor local DNA melting.
Findings: Placement of this new probe at specific positions in the nontemplate strand shows clearly that the elongation bubble extends about eight bases upstream of the pause site, whereas traditional 2-aminopurine probes show that the elongation bubble extends only about one nucleotide downstream of the last base incorporated. The positioning of the active site very close to the downstream edge of the bubble is consistent with previous studies and with similar studies of the promoter-bound pre-initiation complex. The results show clearly that the RNA–DNA hybrid can be no more than eight nucleotides in length and characterisation of different paused species suggests that these dimensions are not sequence or position dependent. Finally, the results confirm that the ternary complex is not stable with short lengths of transcript, but persists for a substantial time when paused in the middle or at the end of duplex DNA.

Proteins

Selected by Martin Noble
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Two-state allosteric behavior in a single-domain signaling protein. Volkman BF, Lipson D, Wemmer DE, Kern D: *Science* 2001, 291:2429-2433.
•• Significance: Regulatory processes of all kinds depend on the capacity of signalling systems to exist in two (or more) distinguishable states. Interconversion between these states can be the result of covalent modification or reversible binding processes, and has been understood in terms of two alternative models of allosteric effect. NMR analysis of protein structure and dynamics has been used to characterise the interconversion between two signalling states of the nitrogen regulatory protein C (NtrC).
Findings: Using three different relaxation techniques, the dynamics of NtrC were characterised in two naturally occurring states (phosphorylated and not phosphorylated), as well as in a mutant form of NtrC that partially mimics the phosphorylated state. A combination of the results from the three types of experiment allowed the characterisation of the structural rearrangements that occur over a very broad range of timescales and indicated that, before phosphorylation, the conformation of NtrC exchanges between two states, one of which resembles the active conformation. Thus, this protein demonstrably conforms to models of allosteric protein, whereby an effector (here phosphorylation) influences a pre-existing equilibrium of conformational states.

The influence of macromolecular crowding and macromolecular confinement on biochemical reactions in physiological media. Minton AP: *J Biol Chem* 2001, 276:10577-10580.
• Significance: The correlation of structure to *in vivo* function is often achieved through *in vitro* measurements of protein activities. Such measurements generally neglect the effect of molecular 'crowding', which is a consequence of the large number of molecular species that fill the cellular milieu, each of which might be present at a relatively low concentration.
Findings: This paper reviews the consequences of a cellular environment on the processes of binding and the activity of biological molecules. Amongst the findings is the observation that, in a crowded regime, equilibrium association constants can be perturbed by a factor of 10 or more. Reference is also made to

the potential effect of crowding on the kinetics of enzyme reactions, where the reaction cycle involves significant conformational changes. As well as provoking thought, this paper shows how the 'nonphysiological' environment of a protein crystal might have important characteristics in common with the inside of a cell.

Dynamic coupling between the SH2 and SH3 domains of c-Src and Hck underlies their inactivation by C-terminal tyrosine phosphorylation. Young MA, Gonfloni S, Superti-Furga G, Roux B, Kuriyan J: *Cell* 2001,105:115-126.

•• Significance: The authors applied forced molecular dynamics to obtain insight into the structure/function relationship of Src family tyrosine kinases. As well as demonstrating the power of simulations in obtaining information about dynamic processes from structures determined by X-ray crystallography, this paper highlights the role of a largely underinvestigated region of the Src family kinases.

Findings: By studying the correlated movements in simulations of c-Src and Hck, the authors have identified the SH3–SH2 linker as an important region, responsible for communicating the effect of C-terminal tail phosphorylation to the c-Src-kinase activation segment. This hypothesis has been tested by mutagenesis, which has shown that the substitution of glycines into the SH3–SH2 linker results in a constitutively active c-Src kinase.

Catalysis and regulation

Selected by Jon D Stewart
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Phosphite dehydrogenase: an unusual phosphoryl transfer reaction. Vrtis JM, White AK, Metcalf WW, van der Donk WA: *J Am Chem Soc* 2001, 123:2672-2673.

• Significance: Phosphite dehydrogenase is the first enzyme discovered to catalyse redox chemistry on inorganic phosphorus compounds. This study sheds the first light on the mechanism of this unusual enzyme.

Findings: Phosphite dehydrogenase isolated from *Pseudomonas stutzeri* WM88 catalyses the NAD^+-dependent oxidation of phosphite to phosphate and NADH. Direct transfer of the phosphorus-bound hydrogen to the nicotinamide was demonstrated by a deuterium-labelling experiment, which also revealed that phosphite reductase directed hydride transfer to the *Re*-face of NAD^+. The deuterium-labelled phosphite was also used to determine the isotope effect on hydride transfer (V_{max}^H/V_{max}^D = 2.1 ± 0.1 and $V_{max}^H/K_{M,phosphite}$ / $V_{max}^D/K_{M,phosphite}$ = 1.8 ± 0.3). These values indicate that hydride transfer is either partially or wholly rate limiting under steady-state conditions.

Activation of class III ribonucleotide reductase by thioredoxin. Padovani D, Mulliez E, Fontecave M: *J Biol Chem* 2001, 276:9587-9589.

•• Significance: A physiologically relevant mode of enzyme activation has finally been discovered for a ribonucleotide reductase that functions under anaerobic conditions.

Findings: It has long been known that formation of the glycyl radical of class III ribonucleotide reductases requires a reducing system (NADPH, flavodoxin and flavodoxin reductase), a 17.5 kDa iron–sulfur protein ([β]-protein), *S*-adenosylmethionine and a sulfhydryl reducing agent. Although the sulfhydryl reducing agent is absolutely essential, and dithiothreitol (DTT) is commonly used, its specific role was unknown. Here, it is demonstrated that the combination of micromolar levels of thioredoxin and thioredoxin reductase can substitute for millimolar concentrations of DTT. Moreover, the purpose of the sulfhydryl reducing agent is now known: it reduces a disulfide bond on the ribonucleotide reductase polypeptide; once this has occurred, the enzyme retains activity in the absence of sulfhydryl reducing agents. The possibility that this disulfide bond is an artefact of the protein isolation process is intriguing, particularly in light of the high concentrations of disulfide reducing agents found in the cytoplasm.

Crystallographic evidence for substrate-assisted catalysis in a bacterial β-hexosaminidase. Mark BL, Vocadlo DJ, Knapp S, Triggs-Raine BL, Withers SG, James MNG: *J Biol Chem* 2001, 276:10330-10337.

• Significance: The structure of a bacterial hexosaminidase establishes that retaining glycosidases of this class are able to operate in the absence of an enzymatic nucleophile because of neighbouring group participation by the substrate.

Findings: The X-ray crystal structure of the *Streptomyces plicatus* β-hexosaminidase was solved to 2.2 Å resolution. In contrast to the active sites of other retaining glycosidases, that of β-hexosaminidase lacks a carboxylate sidechain positioned to interact with the reactive anomeric carbon of the substrate by ion pairing or formation of a covalent intermediate. It was therefore proposed that the carbonyl oxygen of the 2-acetamido group of the substrate forms a covalent bond with the anomeric carbon, thereby forming a cyclic oxazolinium intermediate that is subsequently opened by the attack of water at the anomeric carbon, which forms the β-anomer of the sugar and regenerates the 2-acetamido moiety. This proposal was supported by a co-crystal structure of the enzyme complexed with an inhibitor that mimics the cyclic oxazolinium intermediate. The active site structure appears to shield the substrate so that a water molecule cannot attack the acetamido carbonyl.

pH-induced structural changes regulate histidine decarboxylase activity in ***Lactobacillus*** 30a. Schelp E, Worley S, Monzingo AF, Ernst S, Robertus JD: *J Mol Biol* 2001, 306:727-732.

• Significance: A novel mechanism of pH regulation of enzyme activity has been uncovered that involves a folding/unfolding transition of a protein α helix that makes up part of the substrate-binding pocket.

Findings: The structure of *Lactobacillus* histidine decarboxylase had been determined previously from crystals grown at pH 4.8, conditions under which the enzyme is highly active. This structure revealed that the homotrimeric enzyme contains three active sites located at the subunit interfaces and that helix B (residues 53–68) from an adjacent subunit provides important contacts with the bound substrate. In this work, crystals were grown at pH 8.0, a state in which the enzyme is essentially inactive. This structure showed no electron density for the helix B region, indicating that this segment of the protein was disordered at pH 8.0. The structural transition could be explained by the protonation behaviour of two acidic residues (Asp53 and Asp198), one of which occurs in helix B, whereas the other lies elsewhere in the active site. Under acidic conditions, in which the sidechains of both Asp53 and Asp198 are protonated, a hydrogen-bonded dimer can form; however, when both are deprotonated, electrostatic repulsion destabilises this region and leads to helix unwinding. This structural change is highly localised, and the remaining portions of the protein were essentially unchanged when structures of the pH 4.8 and pH 8.0 forms of the enzyme were compared.