# Elsewhere in biology

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

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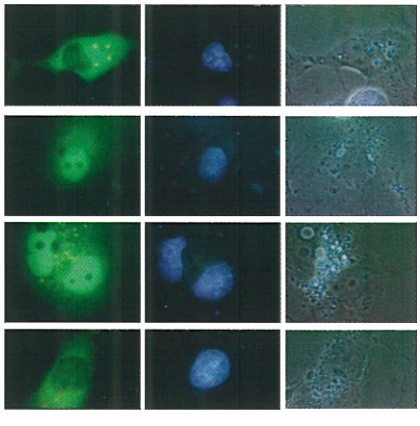
☐ **Probing the role of homomeric and heteromeric receptor interactions in TGF-β signaling using small molecule dimerizers.**
Brent R Stockwell and Stuart L Schreiber (1998). *Curr. Biol.* **8**, 761–770.

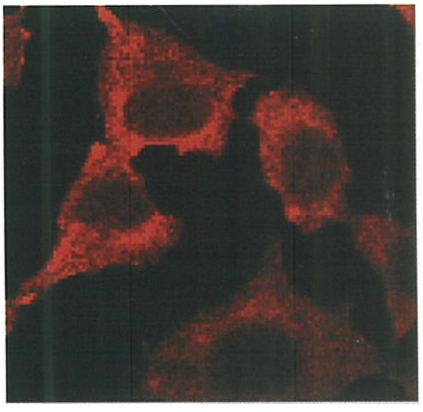
Transforming growth factor β (TGF-β) arrests many cell types in the G1 phase of the cell cycle and up-regulates plasminogen activator inhibitor 1 (PAI-1). The type I (TGF-βRI) and II (TGF-βRII) TGF-β receptors mediate these and other effects of TGF-β on target cells. TGF-β initially binds to TGF-βRII and subsequently TGF-βRI



is recruited to form a heteromeric complex. TGF-βRI phosphorylates the downstream effectors Smad2 and Smad3, leading to their translocation into the nucleus. The authors explored the role of receptor oligomerization in TGF-β signaling by constructing fusion proteins containing receptor cytoplasmic tails linked to binding domains for small-molecule dimerizers. They showed that non-toxic, small molecules designed to oligomerize cytoplasmic tails of TGF-β receptors at the plasma membrane can activate TGF-β signaling. TGF-β normally signals through two receptors that are both necessary for signaling in the small-molecule system described in this paper, but a dimerizer activates signaling through a single type of receptor that is sufficient to induce TGF-β signaling. The methods of activating TGF-β signaling described in this paper could be extended to signaling pathways of other TGF-β superfamily members such as activin and the bone morphogenetic proteins.
4 June 1998, Research Paper, *Current Biology*.

☐ **The Kit receptor promotes cell survival via activation of PI 3-kinase and subsequent Akt-mediated phosphorylation of Bad on Ser136.**
Peter Blume-Jensen, Ralf Janknecht and Tony Hunter (1998). *Curr. Biol.* **8**, 779–782.

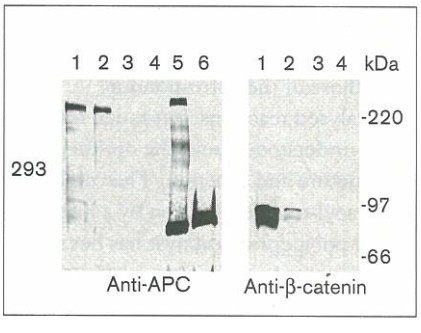
The *c-kit*-encoded receptor protein tyrosine kinase for stem cell factor (Kit/SCF-R) is essential for the development of cells within the hematopoietic, melanogenic and gametogenic lineages. SCF stimulation induces activation of phosphatidyl-inositol (PI) 3-kinase, which is required for SCF-induced mitogenesis and cell survival, and for activation of the serine/threonine protein kinase Akt. Using Kit/SCF-R mutants, the authors found that, in response to SCF, Akt became activated and mediated phosphorylation of Bad, a pro-apoptotic molecule, in a PI-3-kinase-dependent manner. Phosphorylation of Bad was restricted to Ser112 and Ser136 *in vivo*, but only the Akt phosphorylation site Ser136 was essential for SCF-promoted cell survival. Furthermore, Bad and Akt interacted and colocalized in intact cells. A Kit/SCF-R gain-of-function mutant that has increased mitogenic and PI 3-kinase activation potential, due to the absence of the two protein kinase C



negative feedback phosphorylation sites, enhanced both Akt activation and Bad phosphorylation and also resulted in increased cell survival. Such a mechanism could account for how deregulated PI 3-kinase activity and naturally occurring gain-of-function point mutants of Kit/SCF-R lead to cellular transformation and fatal malignancies in man.
8 June 1998, Brief Communcation, *Current Biology*.

☐ **Sequence-based design of a peptide probe for the APC tumor suppressor protein.**
Victoria Allen Sharma, Jennifer Logan, David S King, Ray White and Tom Albers (1998). *Curr. Biol.* **8**, 823–830.

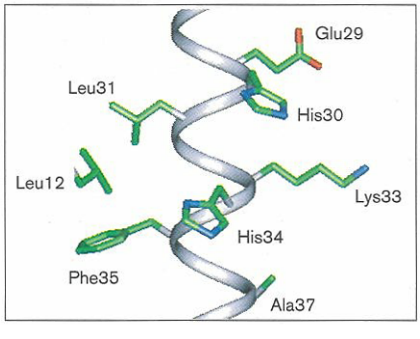
Finding new molecular probes targeted for proteins is limited by the absence of generally applicable recognition rules, because, although proteins form specific associations, predictive rules for protein pairing are generally unknown. Here, the authors describe amino-acid sequence patterns capable of mediating specific pairing of a widespread protein motif: the parallel, dimeric, α-helical coiled coil. The pairing rules were



tested by designing a 54-residue peptide (anti-APCp 1) that is predicted to dimerize preferentially with a coiled-coil sequence from the adenomatous polyposis coli (APC) tumor suppressor protein. Biochemical experiments showed that the anti-APC peptide preferentially forms a heterodimeric coiled coil with mutant and full-length APC proteins. The specificity of the designed peptide is sufficient to support several applications that commonly use antibodies. The observed specificity of anti-APCp 1 validates the pairing rules used as the basis for the probe design, and it suggests that residues in the core positions of coiled coils help impart pairing selectivity.
18 June 1998, Research Paper, *Current Biology*.

☐ **Site-selective control of the reactivity of surface-exposed histidine residues in designed four-helix-bundle catalysts.**
Kerstin S Broo, Lars Brive, Richard S Sott and Lars Baltzer (1998). *Fold. Des.* **3**, 303–312.

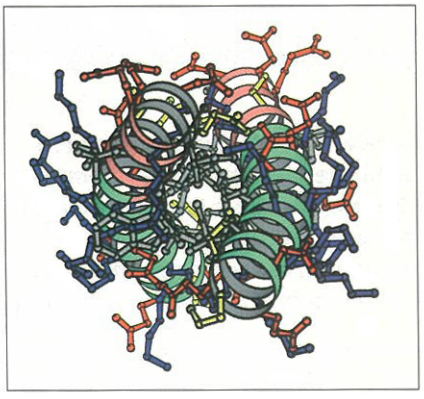
The *de novo* design of proteins provides insight into protein folding and opens pathways towards the construction of new proteins. Designed and folded polypeptide catalysts have, so far, been reported that exhibit catalytic efficiencies




of more than three orders of magnitude over those of the corresponding uncatalysed reactions, but much remains to be understood about the optimisation of structure and function. The catalysis of an acyl-transfer reaction by a histidine-based polypeptide catalyst has been demonstrated previously with a catalytic efficiency of more than three orders of magnitude. Histidine, the most versatile amino acid in catalysis, has a $pK_a$ value that is close to 7 and it can therefore function as a nucleophilic catalyst, a general-acid catalyst and a general-base catalyst in aqueous solution around neutral pH. Histidine can also bind anions in its protonated form and cations in its unprotonated form. Thus, the tailoring of the reactivity of histidine can be expected to be of great interest in a number of catalysed reactions. Here, the authors systematically vary the environment of histidine residues in designed helix–loop–helix motifs to modulate histidine $pK_a$ values and reactivities. 25 helix–loop–helix motifs were designed in which surface-exposed histidine residues were flanked by neutral, negatively charged and positively charged groups and the histidine's proximity to the hydrophobic core was varied. The 57 histidine $pK_a$ values were determined using $^1H$ NMR spectroscopy and found to be in the interval 5.2–7.2 with changes ranging from a decrease of 1.3 $pK_a$ units to an increase of 0.7 $pK_a$ units compared with the $pK_a$ for an unperturbed histidine residue. The principles described in this paper should be useful in the engineering of novel catalysts.
30 June 1998, Research Paper, *Folding & Design*.

☐ **Structural basis of the oligomerization of hepatitis delta antigen.**
Harmon J Zuccola, James E Rozzelle, Stanley M Lemon, Bruce W Erickson and James M Hogle (1998). *Structure* **6**, 821–830.

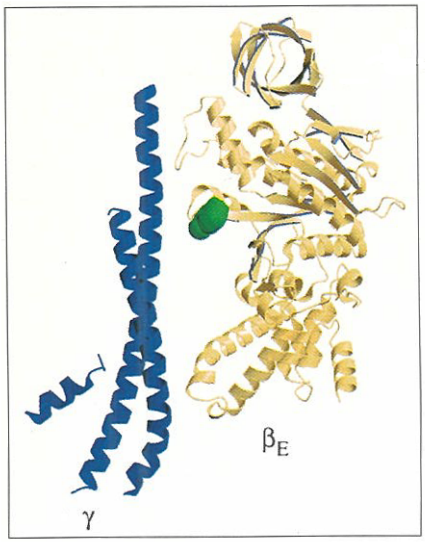
The hepatitis D virus (HDV) is a small satellite virus of hepatitis B virus (HBV). Coinfection with HBV and HDV causes severe liver disease in humans. The small 195 amino-acid form of the hepatitis delta antigen (HDAg) functions as a trans activator of HDV replication. A larger form of the protein containing a 19 amino-acid carboxy-terminal extension inhibits viral replication. Both of these functions are mediated, in part, by a stretch of amino acids predicted to form a coiled coil (residues 13–48) common to both forms.



It is believed that HDAg forms dimers and higher ordered structures through this coiled-coil region. The high-resolution crystal structure of a synthetic peptide corresponding to residues 12–60 of HDAg has been solved. The determination of the structure shows that HDAg dimerizes through an antiparallel coiled coil. Dimers associate further to form octamers through residues in the coiled-coil domain and residues carboxy-terminal to this region. The authors findings suggest that the structure of HDAg represents a previously unseen organization of a nucleocapsid protein and raise the possibility that the amino terminus could play a role in binding the viral RNA.
15 July 1998, Research Paper, *Structure*.

☐ **Bovine $F_1$-ATPase covalently inhibited with 4-chloro-7-nitrobenzofurazan: the structure provides further support for a rotary catalytic mechanism.**
George L Orriss, Andrew GW Leslie, Kerstin Braig and John E Walker (1998). *Structure* **6**, 831–837.

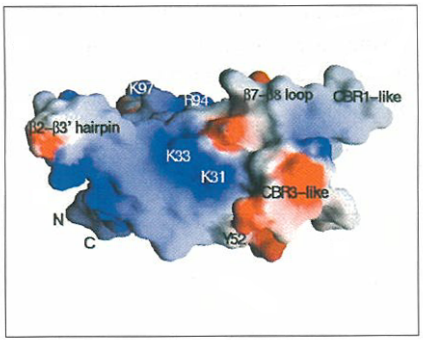
$F_1$-ATPase is the globular domain of $F_1F_0$-ATP synthase that catalyses the hydrolysis of ATP to ADP and phosphate. The crystal structure of bovine $F_1$-ATPase has been determined previously, and is comprised of five different subunits in the stoichiometry $\alpha_3\beta_3\gamma\delta\varepsilon$; the three catalytic β subunits alternate with the three α subunits around the centrally located single γ subunit. To understand more about the catalytic mechanism, $F_1$-ATPase was




inhibited by reaction with 4-chloro-7-nitrobenzofurazan (NBD-Cl) and the structure of the inhibited complex ($F_1$–NBD) determined using X-ray crystallography. The $F_1$–NBD inhibited state showed that NBD-Cl reacts with the phenolic oxygen of Tyr311 of the $\beta_E$ subunit, which contains no bound nucleotide. The inhibitor appears to prevent the $\beta_E$ subunit from being changed into a nucleotide-binding state, a change that would otherwise be brought about by the rotation of the $\gamma$ subunit in the active enzyme. The results presented here are consistent with a rotary catalytic mechanism of ATP synthesis and hydrolysis, which requires the sequential and concerted participation of all three catalytic sites. NBD-Cl inhibits the enzyme by preventing the modified subunit from adopting a conformation that is essential for catalysis to proceed.
15 July 1998, Research Paper, *Structure*.

☐ **Crystal structure of the C2 domain from protein kinase C-δ.**
H Pappa, J Murray-Rust, LV Dekker, PJ Parker and NQ McDonald (1998). *Structure* **6**, 885–894.

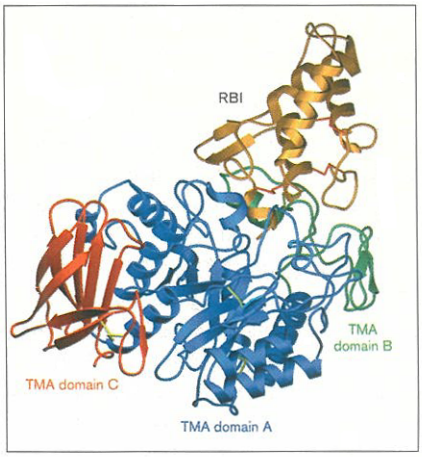
The protein kinase C (PKC) family of lipid-dependent serine/threonine kinases plays a central role in many intracellular eukaryotic signalling events. Members of the novel (δ, ε, η, θ) subclass of PKC isotypes lack the $Ca^{2+}$ dependence of the conventional PKC isotypes and have an amino-terminal C2 domain. Biochemical data suggest that this domain serves to translocate novel PKC family members to the plasma membrane and might influence binding of PKC activators. In this paper, the determination of the crystal structure of PKC-δ C2 domain indicates an unusual variant of the C2 fold. Structural elements unique to this




C2 domain include a helix and a protruding β hairpin, which might contribute basic sequences to a membrane-interaction site. The amino-terminal sequence of $Ca^{2+}$-independent novel PKCs defines a divergent example of a C2 structure similar to that of phospholipase C-δ. The $Ca^{2+}$-independent regulation of novel PKCs is explained by major structural and sequence differences resulting in three nonfunctional $Ca^{2+}$-binding loops. The observed structural variation and position of a tyrosine-phosphorylation site suggest the existence of distinct subclasses of C2-like domains which might have evolved distinct functional roles and mechanisms to interact with lipid membranes.
15 July 1998, Research Paper, *Structure*.

☐ **A novel strategy for inhibition of α-amylases: yellow meal worm α-amylase in complex with the *Ragi* bifunctional inhibitor at 2.5 Å resolution.**
Stefan Strobl, Klaus Maskos, Georg Wiegand, Robert Huber, F Xavier Gomis-Rüth and Rudi Glockshuber (1998). *Structure* **6**, 911–921.

α-Amylases catalyze the hydrolysis of α-D-(1,4)-glucan linkages in starch




components, glycogen and various other related carbohydrates. There is a wide range of industrial and medical applications for these enzymes and their inhibitors. The *Ragi* bifunctional α-amylase/trypsin inhibitor (RBI) is the prototype of the cereal inhibitor superfamily and is the only member of this family that inhibits both trypsin and α-amylases. The mode of inhibition of α-amylases by these inhibitors has so far been unknown. The authors have determined the crystal structure of yellow meal worm α-amylase (TMA) in complex with RBI, revealing a novel mechanism of inhibition of α-amylases from insect and mammalian sources by proteins from cereals. Because RBI inhibits two important digestive enzymes of animals, it constitutes an efficient plant defense protein and might be used to protect crop plants from predatory insects.
15 July 1998, Research Paper, *Structure*.

☐ **Cation binding to the integrin CD11b I domain and activation model assessment.**
Eric T Baldwin, Ronald W Sarver, Garold L Bryant Jr, Kimberly A Curry, Michael B Fairbanks, Barry C Finzel, Robert L Garlick, Robert L Heinrikson, Nancy C Horton, Laura-Lee C Kelley, Ana M Mildner, Joseph B Moon, John E Mott, Veronica T Mutchler, Che-Shen C Tomich, Keith D Watenpaugh and Veronica H Wiley (1998). *Structure* **6**, 923–935.

The integrin family of cell-surface receptors mediate cell adhesion through interactions with the extracellular matrix or other cell-surface receptors. The α chain of some integrin heterodimers includes an inserted 'I domain' of about 200 amino acids that binds divalent metal ions and is essential for integrin function. It has been proposed that the I domain of the integrin CD11b adopts a unique 'active' conformation when bound to its counter receptor and that the lack of adhesion in the presence of $Ca^{2+}$ ion reflected the stabilization of an 'inactive' I-domain conformation. The authors set out to independently determine the structure of the CD11b I domain and to evaluate the structural effects of divalent ion binding to this protein. The authors have determined the X-ray structure of a new crystal form of the CD11b I domain in the absence of added metal ions using multiple isomorphous replacement. Metal ions were easily introduced into this crystal form allowing the assessment of the structural effects of divalent cation binding at the metal ion dependent adhesion site. The equilibrium binding constants for these ions were determined by titration calorimetry. The overall protein conformation and metal-ion coordination of the I domain is the same as that observed for all previously reported CD11α I-domain structures and a CD11β I-domain complex with $Mn^{2+}$. Addition of the cations $Mg^{2+}$, $Mn^{2+}$ and $Cd^{2+}$ to the metal-free I domain does not induce conformational changes in the crystalline environment. Moreover, the authors find that $Ca^{2+}$ binds poorly to the I domain, which serves to explain its failure to support adhesion. These data show that the active conformation described previously is likely to be a construct artifact and that the currently available data do not support a dramatic structural transition for the I domain during counter-receptor binding.

15 July 1998, Research Paper, *Structure*.