Elsewhere in biology

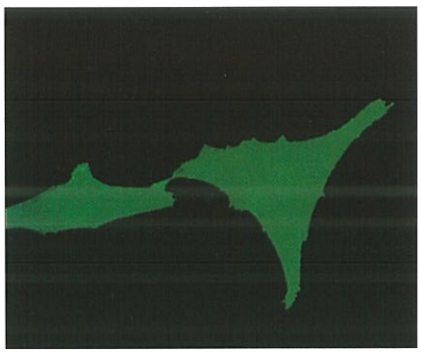
A selection of interesting papers published last month in *Chemistry & Biology*'s sister journals, *Current Biology*, *Folding & Design* and *Structure*, chosen and summarized by the staff of *Chemistry & Biology*.

Chemistry & Biology July 1998, 5:R171–R175

Translocation of PDK-1 to the plasma membrane is important in allowing PDK-1 to activate protein kinase B.

Karen E Anderson, John Coadwell, Len R Stephens and Phillip T Hawkins (1998). *Curr. Biol.* **8**, 684–691.

Protein kinase B (PKB) is involved in the regulation of apoptosis, protein synthesis and glycogen metabolism in mammalian cells. Phosphoinositide-dependent protein kinase (PDK-1) activates PKB in



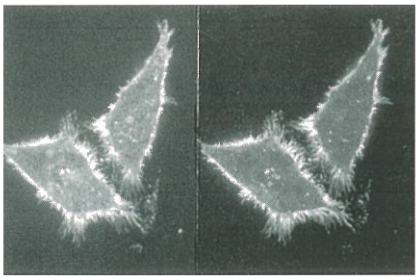
a manner dependent on phosphatidylinositol 3,4,5-trisphosphate (PtdIns(3,4,5)P_3), which is also needed for the translocation of PKB to the plasma membrane. It has been proposed that the amount of PKB activated is determined exclusively as a result of its translocation, and that a constitutively active pool of membrane-associated PDK-1 simply phosphorylates all the PKB made available. The effects of membrane localisation of PDK-1 on PKB activation have been investigated. Ectopically expressed PDK-1 translocated to the plasma membrane in response to platelet-derived growth factor (PDGF) and translocation was sensitive to wortmannin, an inhibitor of phosphoinositide 3-kinase. Translocation of PDK-1 also occurred upon its co-expression with constitutively active phosphoinositide 3-kinase, but not with an inactive form. Overexpression of PDK-1 enhanced the ability of PDGF to activate PKB. PDK-1 disrupted in the pleckstrin homology (PH) domain, which did not translocate to the membrane, did not increase PKB activity in response to PDGF, whereas membrane-targeted PDK-1 activated PKB to the extent that it could not be activated further by PDGF. In response to PDGF, binding of PtdIns(3,4,5)P_3 and/or PtdIns(3,4)P_2 to the PH domain of PDK-1 causes its translocation to the plasma membrane where it co-localises with PKB, significantly contributing to the scale of PKB activation.

19 May 1998, Brief communication, *Current Biology*.

Identification and functional analysis of the ezrin-binding site in the hyaluronan receptor, CD44.

James W Legg and Clare M Isacke (1998). *Curr. Biol.* **8**, 705–708.

ERM (ezrin, radixin and moesin) proteins function as linkers between the actin cytoskeleton and the plasma membrane. These proteins are also highly regulatable, making them ideal candidates to mediate important physiological events such as adhesion and membrane morphology and to control formation and breakdown of membrane–cytoskeletal junctions. Recently, a direct interaction *in vitro* has been demonstrated between ERM proteins and the hyaluronan receptor, CD44. The ezrin-binding site of CD44 has been mapped to two clusters of basic amino acids in a membrane-proximal region within the receptor's cytoplasmic domain. To investigate the functional importance of this interaction *in vivo*, a number of mutations within full-length CD44 were created and expressed in human melanoma cells. The authors demonstrate that mutations within the ezrin-binding site do not disrupt the plasma membrane localization of CD44



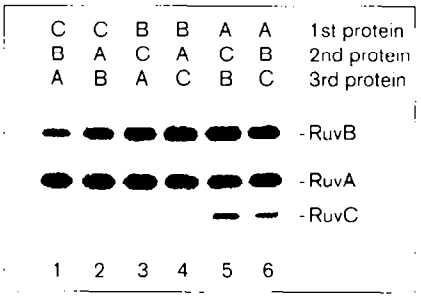
and, in addition, that this region is not required to mediate efficient hyaluronan binding. The studies suggest that ERM proteins mediate the outside–in, rather than inside–out, signalling of adhesion receptors.

25 May 1998, Brief communication, *Current Biology*.

Formation of RuvABC–Holliday junction complexes *in vitro*.

Adelina A Davies and Stephen C West (1998). *Curr. Biol.* **8**, 725-727.

In *Escherichia coli*, the RuvA, RuvB and RuvC proteins are required for the late stages of homologous recombination and DNA repair. RuvA and RuvB form a complex that interacts with Holliday junctions — crossed DNA structures that are recombination intermediates — and promotes branch migration; RuvC is a junction-specific endonuclease that resolves Holliday junctions and completes the recombination process. Because genetic and biochemical experiments suggest that the processes

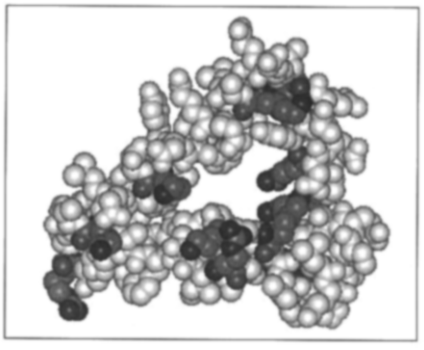


of branch migration and resolution are linked, coimmunoprecipitation experiments were carried out to determine whether the three Ruv proteins interact to form a functional complex (RuvABC). Using a synthetic Holliday junction, a multisubunit complex containing the junction and RuvA, RuvB and RuvC was detected. In

the absence of RuvB, RuvAC–junction complexes were observed. The identification of a RuvABC–junction complex provides direct evidence that the RuvABC proteins interact at the Holliday junction.
25 May 1998, Brief communication, *Current Biology*.

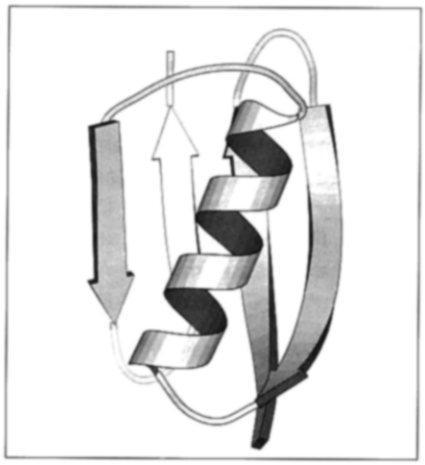
Fold and function predictions for *Mycoplasma genitalium* proteins.
Leszek Rychlewski, Baohong Zhang and Adam Godzik (1998). *Fold. Des.* **3**, 229–238.

Uncharacterized proteins from newly sequenced genomes provide perfect targets for fold and function prediction. For 38% of the entire genome of *Mycoplasma genitalium*, sequence similarity to a protein with a known structure can be recognized using a new sequence alignment algorithm. When comparing genomes of *M. genitalium* and *Escherichia coli*, > 80% of *M. genitalium* proteins have a significant sequence similarity to a protein in *E. coli* and there are > 40 examples that have not been recognized before. For all cases of proteins with significant profile similarities, there are strong analogies in their functions, if the functions of both proteins are known. The results presented here and other recent results strongly support the argument that such proteins are actually homologous. Assuming this homology allows one to make tentative functional assignments for > 50 previously uncharacterized proteins, including such intriguing cases as the putative β-lactam antibiotic resistance protein in *M. genitalium*. Using a new profile-to-profile alignment algorithm, the three-dimensional fold can be predicted for almost 40% of proteins from a genome of the small bacterium *M. genitalium*, and tentative function can be assigned to almost 80% of the entire genome. Some predictions lead to new insights about known functions or point to hitherto unexpected features of *M. genitalium*.
15 June 1998, Research Paper, *Folding & Design*.



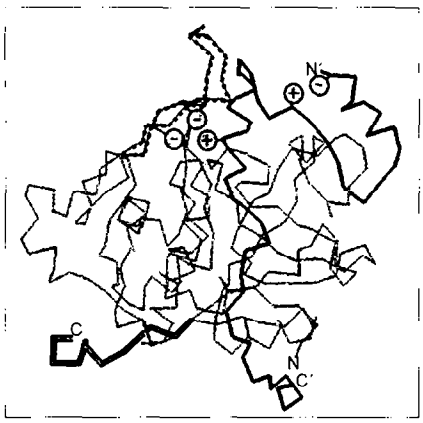
A strategy for detecting the conservation of folding-nucleus residues in protein superfamilies.
Stephen W Michnick and Eugene Shakhnovich (1998). *Fold. Des.* **3**, 239–251.

Nucleation-growth theory predicts that fast-folding peptide sequences fold to their native structure via structures in a transition-state ensemble that shares a small number of native contacts (the folding nucleus). Experimental and theoretical studies of proteins suggest that residues participating in folding nuclei are conserved among homologs. The authors attempted to determine if this is true in proteins with highly diverged sequences but identical folds (superfamilies), and describe a strategy based on comparisons of residue conservation in natural superfamily sequences with simulated sequences for the same proteins. The basic assumptions of the strategy were that natural sequences will conserve residues needed for folding and stability plus function, the simulated sequences contain no functional conservation and nucleus residues make native contacts with each other. On the basis of these assumptions, seven potential nucleus residues in ubiquitin superfamily members were identified . Non-nucleus conserved residues were also identified; these are proposed to be involved in stabilizing native interactions. The authors found that all superfamily members conserved the same potential nucleus residue positions, except those for which the structural topology is significantly different. These results suggest that the conservation of the nucleus of a specific fold can be predicted by comparing designed simulated sequences with natural highly diverged sequences that fold to the same structure. Such a strategy could be used to help plan protein folding and design experiments, to identify new superfamily members, and to subdivide superfamilies further into classes having a similar folding mechanism.
15 June 1998, Research Paper, *Folding & Design*.



Non-homology knowledge-based prediction of the papain prosegment folding pattern: a description of plausible folding and activation mechanisms.
Alberta Jaqueline Padilla-Zúñiga and Arturo Rojo-Domínguez (1998). *Fold. Des.* **3**, 271–284.

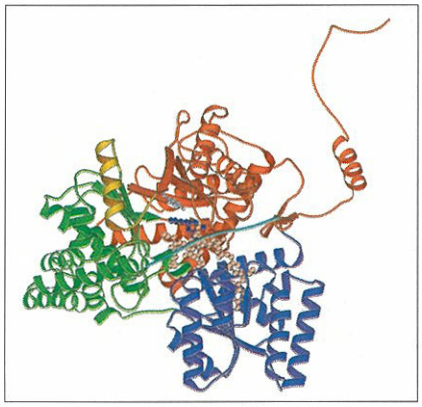
A detailed knowledge of three-dimensional conformations is necessary in order to understand the close relationship between protein structure and function. Among current methodologies, homology modeling is an important tool for obtaining reliable geometries and it provides a direct alternative to X-ray or nuclear magnetic resonance (NMR) techniques. In contrast, predictive methods with no three-dimensional template (non-homology) still require further validation and systematization. A non-homology knowledge-based strategy for the structural prediction of the proregion of a cysteine proteinase zymogen is presented. This method analyzes individual sequences and multiple alignments of homologous sequences, making use of different published



algorithms and incorporating all available structure-related information to obtain improved predictions. This strategy yielded acceptable secondary structure and general three-dimensional assignments when compared with crystallographic data from homologous proteins. The authors discuss the successes and failures of the strategy as a contribution to non-homology prediction development. In addition, on the basis of the information analyzed and generated in this work, plausible folding and activation mechanisms for thiol-proteinase precursors that attempt to shed light on the molecular basis of prosegment functions are proposed.
15 June 1998, Research Paper, *Folding & Design*.

Conformational changes on substrate binding to methylmalonyl CoA mutase and new insights into the free radical mechanism.
Filippo Mancia and Philip R Evans (1998). *Structure* **6**, 711–720.

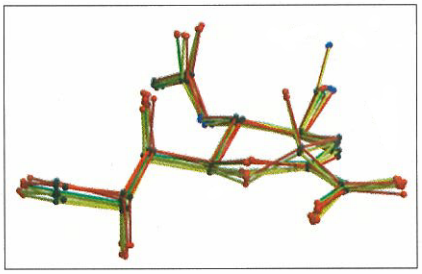
Methylmalonyl CoA mutase catalyses the interconversion of succinyl CoA and methylmalonyl CoA via a free radical mechanism. The enzyme belongs to a family of enzymes that catalyse intramolecular rearrangement reactions in which a group and a hydrogen atom on adjacent carbons are exchanged. These enzymes use the cofactor adenosylcobalamin (coenzyme B_{12}) that breaks to form an adenosyl radical, thus initiating the reaction. Determination of the structure of substrate-free methylmalonyl CoA mutase was initiated to provide further insight into the mechanism of radical formation. Two structures of methylmalonyl CoA mutase from *Propionibacterium shermanii* are reported here. The first structure is of the enzyme in a nonproductive complex with CoA, which serves as a model for the substrate-free conformation of the enzyme, as it is very similar to the second structure derived from a truly substrate-free crystal. The true substrate-free structure also shows the adenosyl group bound to the cobalt atom. Comparison of this structure with that of the previously reported complex of the enzyme with a substrate analogue



shows that major conformational changes occur upon substrate binding. The substrate-binding site of the enzyme is located within a $(\beta\alpha)_8$ TIM-barrel domain. In the absence of substrate, this TIM-barrel domain is split apart and the active site is accessible to solvent. When substrate binds, the barrel closes up with the substrate along its axis and the active site becomes completely buried. The closure of the active-site cavity upon substrate binding displaces the adenosyl group of the cofactor from the central cobalt atom into the active-site cavity, which triggers formation of the free radical that initiates the rearrangement reaction. The TIM-barrel domain is substantially different from all others yet reported: in its unliganded form it is broken open, exposing the small hydrophilic sidechains which fill the centre. The typical barrel structure is only formed when substrate is bound.
15 June 1998, Research Paper, *Structure*.

Drug design against a shifting target: a structural basis for resistance to inhibitors in a variant of influenza virus neuraminidase.
Joseph N Varghese, Paul W Smith, Steven L Sollis, Tony J Blick, Anjali Sahasrabudhe, Jennifer L McKimm-Breschkin and Peter M Colman (1998). *Structure* **6**, 735–746.

Inhibitors of the influenza virus neuraminidase have been shown to be effective antiviral agents in humans. Several studies have reported the selection of novel influenza strains when the virus is cultured with neuraminidase inhibitors *in vitro*. These resistant viruses have mutations either in the neuraminidase or in the viral haemagglutinin. Inhibitors in which the glycerol sidechain at position 6 of 2-deoxy-2,3-dehydro-*N*-acetylneuraminic acid (Neu5Ac2en) has been replaced by carboxamide-linked hydrophobic substituents have recently been reported and shown to select neuraminidase variants. This study sought to clarify the structural and functional consequences of replacing the glycerol sidechain of the

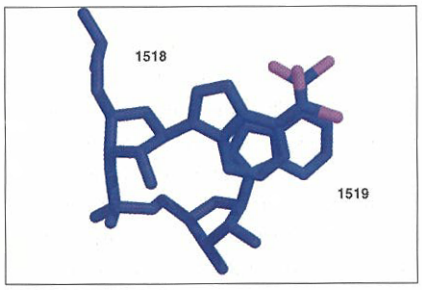


inhibitor with other chemical constituents. The neuraminidase variant Arg292→Lys is modified in one of three arginine residues that encircle the carboxylate group of the substrate. The structure of this variant in complex with the carboxamide inhibitor used for its selection, and with other Neu5Ac2en analogues, is reported. The structural consequences of the mutation correlate with altered inhibitory activity of the compounds compared with wild-type neuraminidase. The Arg292→Lys variant of influenza neuraminidase affects the binding of substrate by modification of the interaction with the substrate

carboxylate, which might be one of the structural correlates of the reduced enzyme activity of the variant. Inhibitors that have replacements for the glycerol at position 6 are further affected in the Arg292→Lys variant because of structural changes in the binding site that apparently raise the energy barrier for the conformational change in the enzyme required to accommodate such inhibitors. These results provide evidence that a general strategy for drug design when the target has a high mutation frequency is to design the inhibitor to be as closely related as possible to the natural ligands of the target.
15 June 1998, Research Paper, *Structure.*

The structure of a methylated tetraloop in 16S ribosomal RNA.
Jason P Rife and Peter B Moore (1998). *Structure* **6**, 747-756.

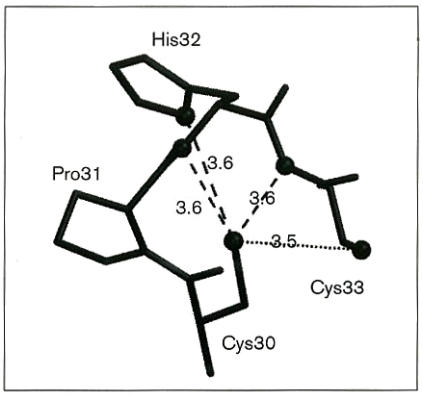
Ribosomal RNAs contain many modified nucleotides. The functions of these nucleotides are poorly understood and few of them are strongly conserved. The final stem loop in 16S-like rRNAs is an exception in both regards. In both prokaryotes and eukaryotes, the tetranucleotide loop that caps the 3′-terminal stem contains two N^6,N^6-dimethyladenosine residues. The sequence and pattern of methylation are conserved within the loop, and there is evidence that these methylated nucleotides play an important role in



subunit association and the initiation of protein synthesis. Because of the integral role that helix 45 plays in ribosome function, it is important to know what consequences these methylated nucleotides have on its structure. The solution structure of a 14-nucleotide analog of the terminal stem loop of bacterial 16S rRNA, which contains N^2-methylguanosine as well as two N^6,N^6-dimethyladenosines, has been solved. The methylation of the 16S rRNA stem loop completely alters its conformation, which would otherwise be a GNRA tetraloop. It is likely that the conformation of this loop is crucial for its function, having implications for its interaction with ribosomal subunits and its role in the initiation of protein synthesis.
15 June 1998, Research Paper, *Structure.*

Crystal structures of reduced and oxidized DsbA: investigation of domain motion and thiolate stabilization.
Luke W Guddat, James CA Bardwell and Jennifer L Martin (1998). *Structure* **6**, 757-767.

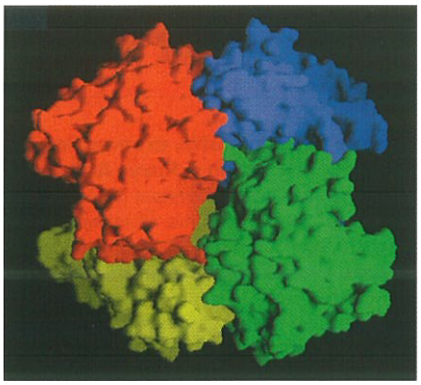
Redox proteins that incorporate a thioredoxin fold have diverse properties and functions. The bacterial protein-folding factor DsbA is the most oxidizing of the thioredoxin family. DsbA catalyzes disulfide-bond formation during the folding of secreted proteins. The extremely oxidizing nature of DsbA has been proposed to result from either domain motion or stabilizing active-site interactions in the reduced form. In the domain motion model, hinge bending between the two domains of DsbA occurs as a result of redox-related conformational changes. The authors have determined the crystal structures of reduced and oxidized DsbA in the same crystal form and at the same pH (5.6). The crystal structure of a lower pH form of oxidized DsbA has also been determined (pH 5.0). These new crystal structures of DsbA, and the previously determined structure of oxidized DsbA at pH 6.5, provide the foundation for analysis of structural changes that occur upon reduction of the active-site disulfide bond.The redox proteins that incorporate a thioredoxin fold have diverse properties and functions. The structures of reduced and oxidized DsbA reveal that hinge bending motions do occur between the two domains. These motions are independent of redox state, however, and therefore do not contribute



to the energetic differences between the two redox states. Instead, the observed domain motion is proposed to be a consequence of substrate binding. Furthermore, DsbA's highly oxidizing nature is a result of hydrogen bond, electrostatic and helix-dipole interactions that favour the thiolate over the disulfide at the active site.
15 June 1998, Research Paper, *Structure.*

Lactate dehydrogenase from the hyperthermophilic bacterium *Thermotoga maritima*: the crystal structure at 2.1 Å resolution reveals strategies for intrinsic protein stabilization.
Günter Auerbach, Ralf Ostendorp, Lars Prade, Ingo Korndörfer, Thomas Dams, Robert Huber and Rainer Jaenicke (1998). *Structure* **6**, 761-781.

L(+)-Lactate dehydrogenase (LDH) catalyzes the last step in anaerobic glycolysis, the conversion of pyruvate to lactate, with the concomitant oxidation of NADH. Extensive physicochemical and structural investigations of LDHs from both mesophilic and thermophilic organisms have been undertaken in order to study the temperature adaptation of proteins. In this study the authors have determined the high-resolution crystal structure of LDH from the hyperthermophilic bacterium *Thermotoga maritima* (TmLDH), the most thermostable LDH to be isolated so far. It was hoped that the structure of TmLDH would serve as a model system to reveal strategies of protein stabilization at temperatures near the



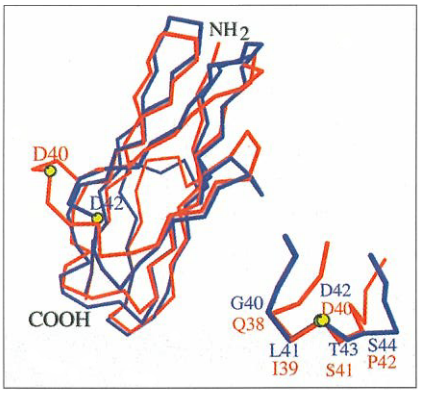
boiling point of water. The structure has been determined as a quaternary complex with the cofactor NADH, the allosteric activator fructose-1,6-bisphosphate, and the substrate analog oxamate. Structural comparisons with five LDHs from mesophilic and moderately thermophilic organisms and with other ultrastable enzymes from *T. maritima* reveal possible strategies of protein thermostabilization. There is a strong conservation of the three-dimensional fold and the catalytic mechanism. Going from lower to higher physiological temperatures a variety of structural differences can be observed: an increased number of intrasubunit ion pairs; a decrease of the ratio of hydrophobic to charged surface area; an increased secondary structure content including an additional unique 'thermohelix' in TmLDH; more tightly bound intersubunit contacts mainly based on hydrophobic interactions; and a decrease in both the number and the total volume of internal cavities. Similar strategies for thermal adaptation can be observed in other enzymes from *T. maritima*.

15 June 1998, Research Paper, *Structure*.

The structure of immunoglobulin superfamily domains 1 and 2 of MAdCAM-1 reveals novel features important for integrin recognition.

Kemin Tan, Jose M Casasnovas, Jinhuan Liu, Michael J Briskin, Timothy A Springer and Jia-huai Wang (1998). *Structure* **6**, 793–801.

Mucosal addressin cell adhesion molecule 1 (MAdCAM-1) is a cell adhesion molecule that is expressed on the endothelium in mucosa, and guides the specific homing of lymphocytes into mucosal tissues. MAdCAM-1 belongs to a subclass of the immunoglobulin superfamily (IgSF), the members of which are ligands for integrins. Human MAdCAM-1 has a unique dual function compared to other members in the same subclass in that it binds both the integrin $\alpha4\beta7$, through its two IgSF domains, and a selectin expressed on leukocytes, via carbohydrate sidechains. The structure determination of the two IgSF domains and comparison to the amino-terminal two-domain structures of vascular cell adhesion molecule 1 (VCAM-1) and intercellular adhesion molecules (ICAM-1 and ICAM-2) permit the the



molecular basis of the interactions between integrins and their preferred ligands to be assessed. The crystal structure of a fragment containing the two IgSF domains of human MAdCAM-1 has been determined and reveals two separate integrin-recognition motifs. The structure comparison of MAdCAM-1 to other members of the same IgSF subclass reveals some interesting features. Firstly, MAdCAM-1, like VCAM-1, has the key integrin-binding residue located on the protruding CD loop of domain 1 and binds to an integrin that lacks an I domain. This is in contrast to ICAM-1 and ICAM-2 where the key residue is located at the end of the C strand on a flat surface and which bind to integrins that contain I domains. Secondly, architectural differences in the CD loops of MAdCAM-1 and VCAM-1 cause an 8 Å shift in position of the critical aspartate residue, and may partly determine their binding preference for different integrins. Finally, the unusual charge distribution of the two-domain fragment of MAdCAM-1 is predicted to orient the molecule optimally for integrin binding on the top of its long mucin-like stalk.

15 June 1998, Research Paper, *Structure*.