Plugging into actin's architectonic socket

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Structures for three modular elements of actin-binding proteins provide hints of the deeper architectural principles governing the cortex of the eukaryotic cell.

Emil Fischer was warned in the prime of his career to avoid the protein problem because any chemical entity isolated from cells could only be a rudely torn fragment of the 'living fabric'. It is impossible to know where we would be if he hadn't perservered in his quest to determine the chemical structure of proteins, but a century later we seem to be on the verge of understanding the living fabric that was so daunting to Fischer's contemporaries. What has made this breakthrough possible is the discovery of common modular structures in cellular signalling and in docking domains of cytoskeletal cross-linking proteins[1,2], such as the calponin homology domain[3], a repeating structural domain of ABP-120[4], and the F-actin-binding headpiece of villin[5], structures of which are reported in this issue of *Nature Structural Biology*. The stuff of the cytoplasm is no ordinary material and may represent a class of excitable media not yet imagined by the nano-technologists. Actin is the wondrous substance underlying the amazing balletic movements seen in the periphery of activated cells in the form of 'ruffling' lamellae and 'waving' filopodia (Fig. 1). Carrying their own packets of free energy (in the form of ATP molecules) actin monomers serve as sources of ready-made tensile units for constructing the ensembles of actin filaments found in the submembranous layer of eukaryotic cells that we have previously termed the 'sensory cortex of the cell'[6].

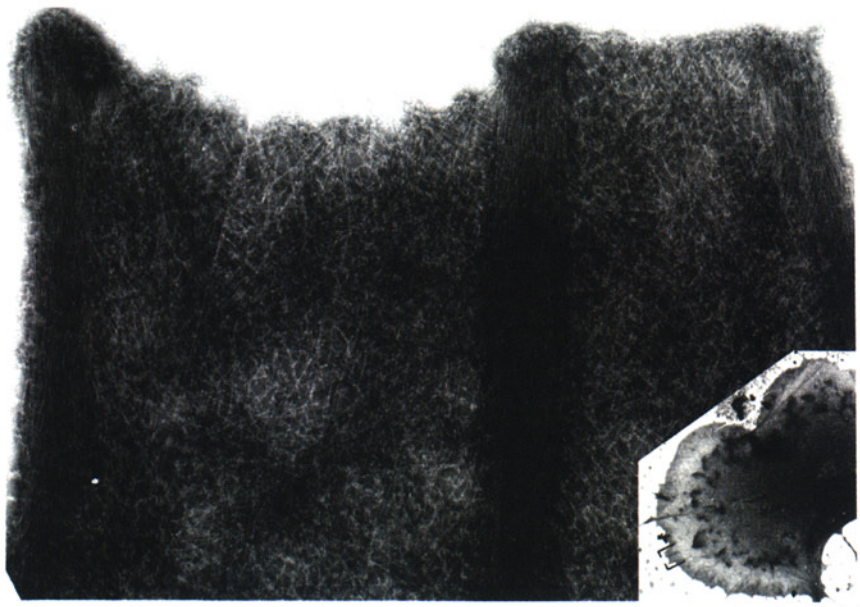

Fig. 1 An intermediate dense microfilament arrangement visualized from a PDGF-stimulated glial cell treated with a detergent-fixative mixture and negatively stained[21]. Image kindly provided by Dr. Anna-Stina Höglund.

The cytoskeleton is reckoned to be a 'tensegrity' structure[7], a type of extended construction in which forces are held in balance, maintaining an overall static appearance that belies the explosive potential within. Looking at a Ken Snelson tensegrity structure (Fig. 2*a*) it is easy to imagine the catastrophic result of 'snipping' one of the cables connecting the rigid stainless steel tubes in the organized matrix of stiff and tensile elements. Eukaryotic cells apparently use similar tricks: gelsolin molecules sever actin filaments, converting 'stiff gels' into pourable syrup, in response to calcium[8]; α-actinin and other actin filament cross-linkers serve as stiff struts linked to the extracellular world through transmembrane proteins[9]; thymosin and profilin sequester actin monomers in unpolymerized complexes[10] (Fig. 2*b*), preparing them for another cycle of construction. Nature's evolutionary process has evidently gone one step further than Snelson (but who is still going strong!) by hitting upon the idea of recycling tensegrity elements.

Calponins represent an important family of regulatory proteins in smooth muscle cells. As in other instances in biology, where domains have been spliced during evolution into proteins unrelated except for their functional reliance on a particular domain (SH2, SH3, Pleckstrin homology domains and others)[1,11], the unique N-terminal domain of calponin is found in other proteins[2], notably α-actinin, filamin, dystrophin, neuronal protein, Ras-GAP and the related human protein Vav. The crystal structure of a calponin homology (CH) domain reported in this issue[3] is one of a pair of actin-binding modules found at the end of spectrin, a large multi-segmented protein that confers strength and elasticity to the red blood cell. The CH-domain from spectrin is homologous to the actin-binding portion of α-actinin, suggesting that they recognize the same site on the surface of the actin filament. Remarkably, a chimera of gelsolin in which segment 2 (out of 6) is replaced with the CH-domain of α-actinin retains actin filament severing activity[12]. This suggests that a common binding 'socket' on actin filaments can engage modular elements presented by a variety of cytoplasmic proteins.

David DeRosier and colleagues have produced three-dimensional reconstructions from electron micrographs of frozen-hydrated actin filaments decorated with the CH-domains of α-actinin[13] (Fig. 3*a*). By substracting the electron density of an actin filament they obtained a 'bell-shaped' image (Fig. 3*b*), which was interpreted as enclosing two CH-domains based on its resemblance to the bi-ellipsoidal 'V-shaped' density seen at the end of α-actinin in electron micrographs. However, it is clear from the crystal structure of the CH-domain that the 'bell' is only large enough to contain one CH-domain (see Fig. 3*c*,*d*).

The modelling of the CH-domain–actin interaction is constrained not only by the bell-shaped density, but by chemical cross-linking data that identify regions of the actin and actin-binding protein sequences that are near each

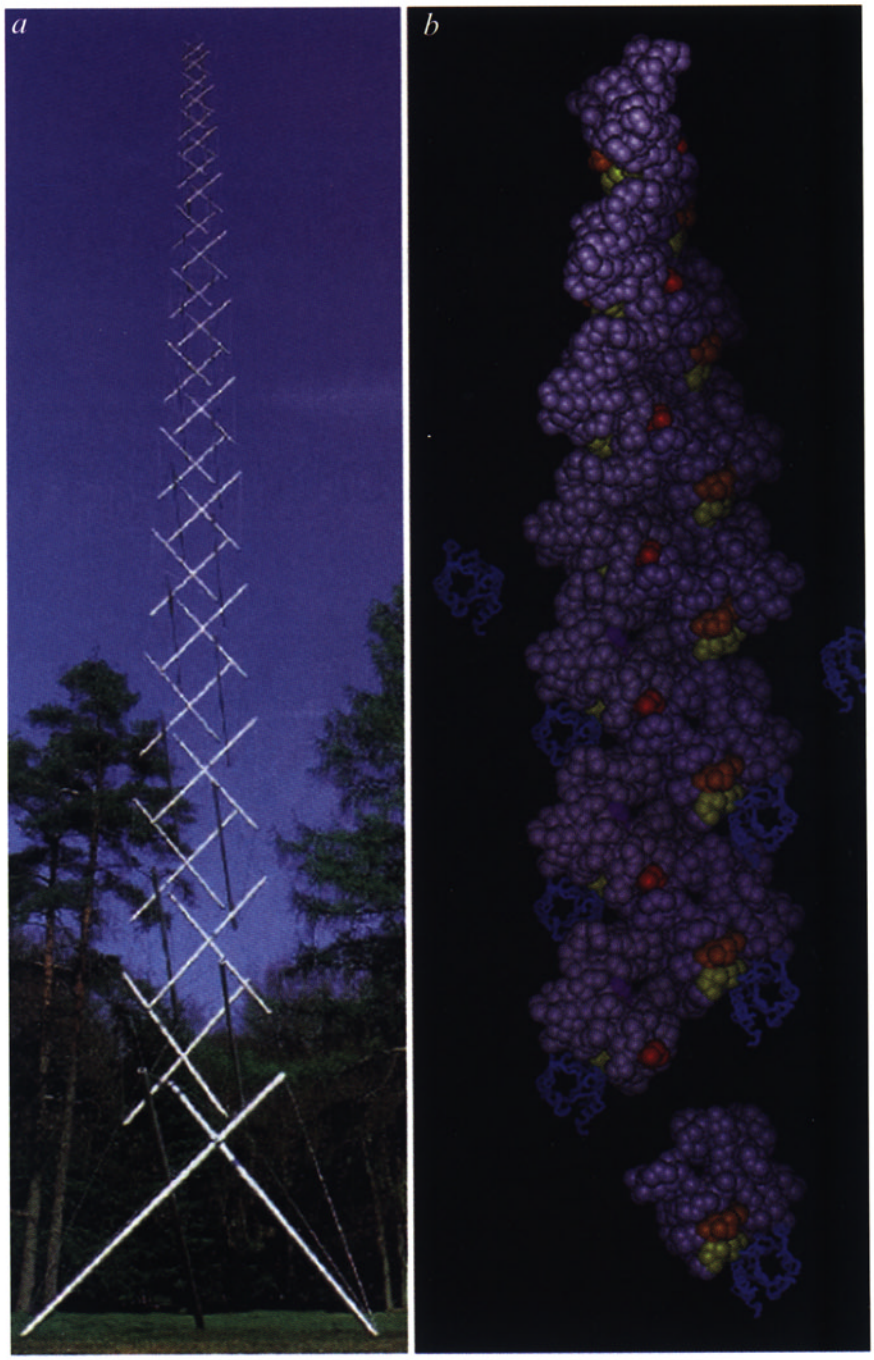

Fig. 2 *a*, *Needle Tower II* by Kenneth Snelson and *b*, ribbon-to-helix polymerization model of actin (gunmetal with decorations) with profilin (blue ribbons)[15]: red, actin N terminus; yellow, actin C-terminal region (360–375); gold, actin residues 105–120; purple, actin-bound ATP. All subsequent figures will follow this scheme. Image of *Needle Tower II* generously provided by Kenneth Snelson.

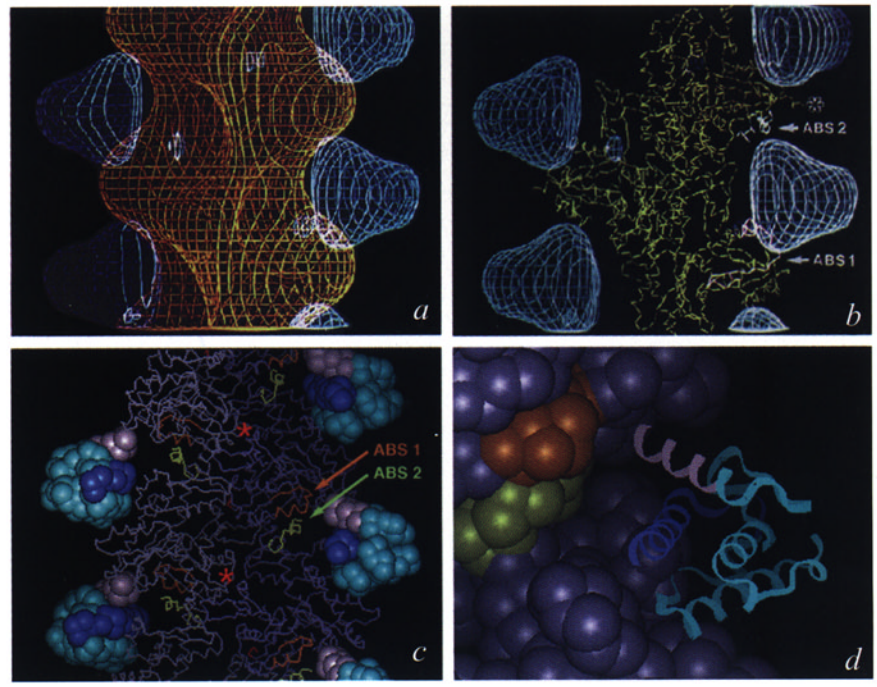

Fig. 3 *a*, Electron density of α-actinin (αA1-2) bound to actin from a cryoelectron micrograph (reproduced with permission from McGough)[13]; *b*, a fit to the electron density using the Heidelberg F-actin model[14] (reproduced with permission from McGough[13]); *c*, a fit to the electron density using the spectrin CH-domain structure[3] and the ribbon-to-helix F-actin model[15]; and *d*, a closeup of the contact sites implicated thorugh zero length cross-linking[22] and peptide mapping[23]. ABS1 and ABS2 label actin peptides cross-linked to α-actinin. Asterisks in (*b*) and (*c*) label the actin N terminus on the filament models. CH domain coordinates kindly provided by Dr. K.D. Carugo.

other in the three-dimensional complex, which allows discrimination between differing models. Docking of the two proteins requires an atomic model for the actin filament. Assuming that only one CH-domain 'fits the bell', a comparison of CH-domain–actin interfaces based on two current atomic filament models[14,15] (Fig. 3*b*,*c*) shows that juxtaposition of the cross-linked regions is straightforward.

A vexing question in the field of actin-binding proteins is how gelsolin severs actin filaments. Gelsolin segment 2 can be replaced by the CH-domain from α-actinin in chimeras possessing severing activity[10], so that it must bind in a CH-domain binding site. Furthermore, successful docking of gelsolin requires that segments 1 and 4 bind neighbouring monomers across the filament axis. When segments 1 and 4 were positioned onto the Heidelberg model[14], using the crystal structure of the actin–gelsolin segment 1 as a guide, 'steric clashes' with subdomain-2 of actin on the same strand occurred[16]. Although this gave rise to a reasonable model for filament severing, the authors recognized that it did not explain the requirement for segment-2 binding. Now that the binding site on F-actin of the CH-domain has been revealed by electron microscopy[13], and the structure of the CH-domain has been determined[3], it is possible to suggest tentative models for the placement of gelsolin segment-2 on F-actin (Fig. 4).

Plugging into the 'architectonic socket' might initiate a flow of momentum along actin filaments and through the extended structures that contribute to cortical tension. In Snelson's tensegrity structures, gravity pulling on the heavy steel rods generates tension in the connecting cables. In the cytoplasm, gravity is negligible in comparison with the forces imparted to macromolecules by solvent molecules populating the tail of the brownian momentum distribution. By contrast, when an actin subunit undergoes a ribbon-to-helix transition—as when a socket-bound profilin molecule is released near the barbed end of a growine actin filament (Fig. 2*b*). Each subunit rotates by 13° and shortens by 0.83nm, accompanied by the hydrolysis of one molecule of ATP (8×10^{-20} joules), a force of 100 pN is generated[15], about two orders of magnitude higher than average brownian forces at physiological temperatures. This calculation suggests that forces generated within actin filaments are strong enough to overcome the random buffeting by solvent molecules. In fact, any enzyme that can plug into the actin 'power-grid' through a socket-binding protein would experience a mechanical jolt propelling the reaction vectorially over the activation barrier. Thus, the clocking of biochemical processes in eukaryotic cells need not follow laws of the Arrhenius type, and reaction rates measured in solution might be inapplicable for explaining cellular phenomena.

A set of actin-binding proteins comprising actin depolymerization factor (ADF), cofilin, villin headpiece, and dematin, distinct from those possessing CH-domains, are believed to bind at a common site on actin filaments[17]. Like myosin, these proteins have lysine-rich (KKEK) sequences that may also engage the N terminus of actin from an anchorage point in the socket. In the NMR structure of the 35-residue villin headpiece[5], the hydrophobic and charged residues implicated in F-actin binding are found clustered on one face of the subdomain, consistent with binding in the socket.

The discovery that calponin homology domains are found in both signalling and actin-binding proteins has enabled the deeper architectural principles of 'the living fabric' to be glimpsed. Modular units plugging into a tensile network of actin filaments can redirect chemomechanical energy along signal transduction and motile pathways. In α-actinin and ABP-120, CH-domains are held apart by stiff linkers, analogous to Snelson's stainless steel tubes (Fig. 2*a*). These linkers too are constructed of modular elements, both to confer stiffness and to provide docking sites, such as SH3 domains to poly-proline-presenting proteins such as VASP. In ABP-120[4], the repeating segments have an immunoglobulin-like fold, a structure particularly well-suited for building up rigid rod-like elements, as exemplified by the structures of cadherin[18] and CD4[19] molecules.

In smooth muscle cells, calponin regulates both sarcomeric and cortical tension. Is it possible that the vigorous motile activity accompanying signal transduction through G-protein coupled reactions in eukaryotic cells is a manifestation of this socket-mediated chemomechanical process? The small G-proteins Cdc42, Rac and Rho regulate the formation of cell surface-associated filopodia, lamellipodia, and stress fibres respectively[20] in response to extracellular signals with a subsequent increase in motile

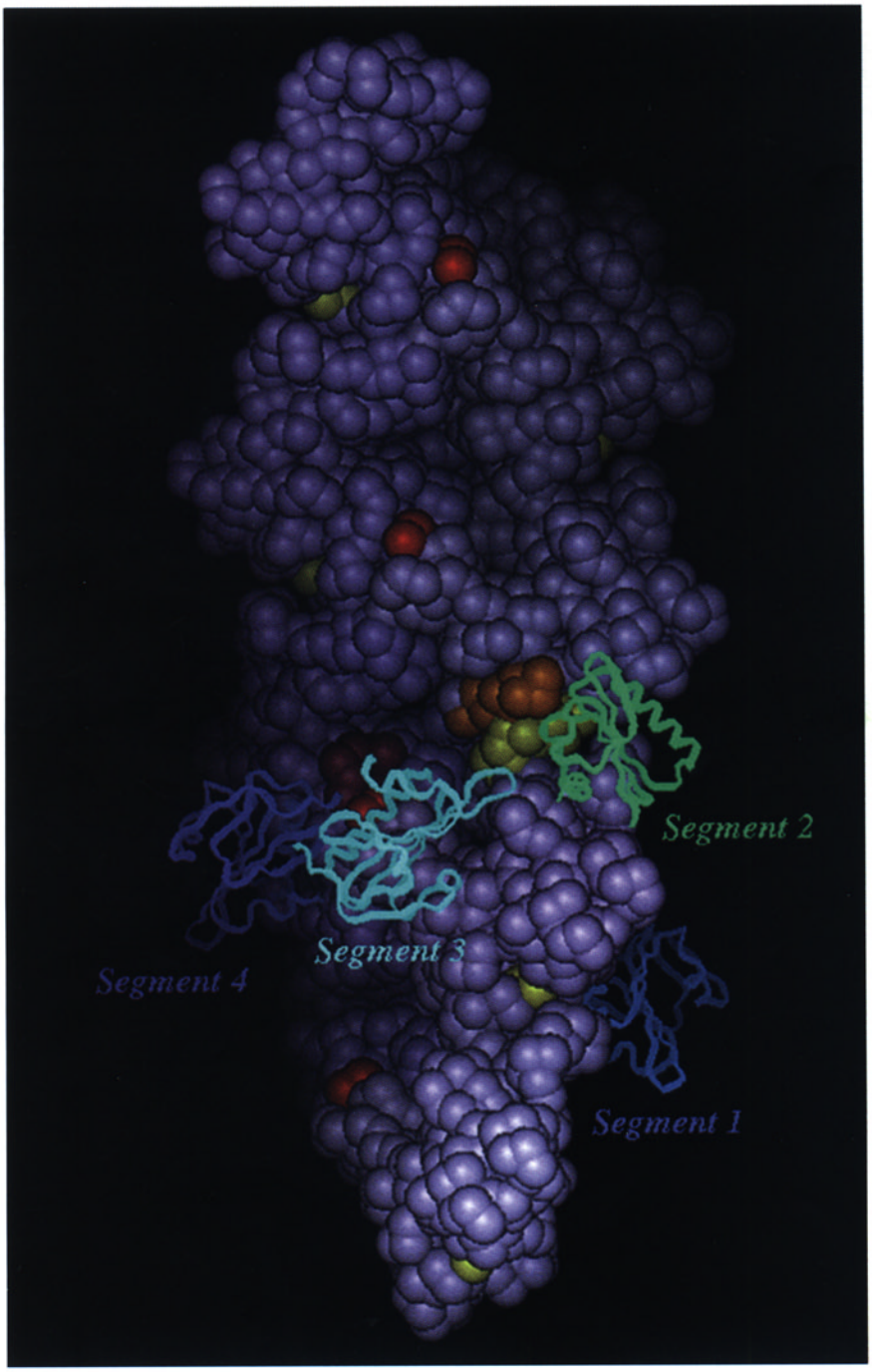

Fig. 4 A model of gelsolin segments 1 through 4 binding to the ribbon-to-helix F-actin model[15]. Severin segment 2 from the NMR structure of ref. 24 has been used to represent gelsolin segment 2. Segment 1 has been shown to interact at the actin N terminus[25] (shown in red) and residues 18–28[26] shown here on a subunit related by helical symmetry to the one binding segment 1 on the backside in cranberry. Actin–gelsolin segment 1 coordinates kindly provided by Dr. Allan Weeds.

activity. RasGAP and Vav are guanine-nucleotide-exchange factors for these proteins and Vav becomes oncogenic when its N-terminal CH actin-binding domain is deleted[2]. Apparently, meddling with the cell's chemomechanical circuits results in the kind of uncontrolled motions in the living fabric that characterize the transformed phenotype.

We imagine that actin-binding proteins can act as chemomechanical coupling agents in processes such as receptor-linked signal transduction. In the usual theories of enzyme kinetics, it is assumed that binding of substrate lifts the free energy of the enzyme into an activated state and random brownian motion 'kicks the protein' with sufficient force to bring about the required transitions. In our view, free energy of activation can be obtained mechanically by attaching to actin filaments through CH-domains with the actin-rich cortex serving as a 'tension bath' analogous to the thermodynamic 'heat bath', but capable of a much more vigorous and directed scrubbing action.

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1. Cohen, G.B., Ren, R., & Baltimore, D. *Cell* **80**, 237–248 (1995).
2. Castresana, J. & Saraste, M. *FEBS Letters* **374**, 149–151 (1995).
3. Carugo, K.D., Banuelos, S. & Saraste, M. *Nature Struct. Biol.* **4**, 175–179 (1997).
4. Fucini, P., Renner, C., Herberhold, C., Noegel, A.A. & Holak, T.A. *Nature Struct. Biol.* **4**, 223–230 (1997).
5. McKnight, C.J., Matsudaira, P.T. & Kim, P.S. *Nature Struct. Biol.* **4**, 180–184 (1997).
6. Schutt, C.E. & Lindberg, U. *Curr. Biol.* **1**, 257–258 (1991).
7. Ingber, D.E. *J. Cell Sci.* **104**, 613–627 (1993).
8. McLaughlin, P.J. & Weeds, A.G. *Ann. Rev. Biophys. Biomol. Struct.* **24**, 643–675 (1995).
9. Schwartz, M.A. & Ingber, D.E. *Mol.Biol.Cell* **5**, 389–393 (1994).
10. Pantaloni, D. & Carlier, M.-F. *Cell* **75**, 1007–1014 (1993).
11. Dolittle, R.F., *Ann. Rev. Biochem.* **64**, 287–314.
12. Way, M., Pope, B. & Weeds, A.G. *J.Cell Biol.* **119**, 835–842 (1992).
13. McGough, A., Way, M., & DeRosier, D. *J.Cell Biol.* **126**, 433–443 (1994).
14. Lorenz, M. Popp, D. & Holmes, K.C. *J.Mol.Biol.* **234**, 826–836 (1993).
15. Schutt, C., Rozycki, M., Chik, J. & Lindberg, U. *Biophys. J.* **68**, 12s–18s (1995).
16. McLaughlin, P.J., Gooch, J.T., Mannherz, H.-G. & Weeds, A.G., *Nature* **364**, 685–692 (1993).
17. Pope, B.,Way, M., Matsudaira, P.T. & Weeds, A. *FEBS Lett.* **338**, 58–62 (1994).
18. Shapiro, L., Fannon, P.D., Kwong, A., Thompson, M.S., Lehman, G., Grubel, J.-F., Legrand,J., Als-Nielsen, D., Colman, R. & Hendrickson, W.A. *Nature* **374**, 327–337 (1995)
19. Wang, J., Yan, Y., Garrett, J., Liu, J., Rodgers, D.W., Garlick, R.L., Garlick, G., Tarr, E., Husain, Y., Reinherz, E.L. & Harrison, S.C. *Nature 348*, 411–418 (1990)
20. Nobes, C. D. & Hall, A. *Cell* **81**, 53–62 (1995)
21. Karlsson, R., Lassing, I., Hoglund, A.-S. & Lindberg, U. *J. Cell. Physiol.* **121**, 96–113 (1984).
22. Mimura, N., & Asano, A. *J. Biol. Chem.* **262**, 4717–4723 (1987)
23. Lebart, M.-C., Méjean, C., Roustan, C., Benyamin, Y. *J. Biol. Chem.* **268**, 5642–5648 (1993)
24. Schnuchel, A., Wiltscheck, R., Eichinger, L., Schleicher, M., Holak, T.A. *J. Mol. Biol.* **247**, 21–27 (1995)
25. Sutoh, K., Yin, H., *Biochemistry* **28**, 5269–5275 (1989)
26. Feinberg, J., Benyamin, Y., Roustan, C. *Biochem. Biophys. Res. Commun.* **209**, 426–432 (1995)