Hdj1 by 50–90%. These data demonstrate that Hdj1 has two peptide-binding sites and both sites are essential for the cochaperone activity. In the initial stages of the refolding reaction, one protomer of the Hdj1 homodimer binds through site 2 to the hydrophobic polypeptide. The other protomer binds through site 1 to the Hsp70 C-terminal region.

**Keywords: heat shock protein, molecular chaperone**

## MS22.P26

*Acta Cryst.* (2011) A**67,** C350

**The crystal structure of the autoinhibitory domain of talin**

Sheng Ye, Xianqiang Song, Rongguang Zhang. *Institute of Biophysics, Chinese Academy of Sciences, Beijing, 100101 (China).* E-mail: yesheng@moon.ibp.ac.cn

Talin is a large cytoskeletal protein essential for activating integrin adhesion receptors and coupling them to actin cytoskeleton. In the cytosol, talin adopts a default autoinhibited conformation where a major integrin binding site on the N-terminal talin head (talin-H) is masked by a C-terminal rod segment (talin-RS). Here we report the high resolution crystal structure of talin-RS. In combination with previous mutagenesis data, the structure provides insight into how talin-RS inhibits the integrin binding on talin-H. Structural comparison of the crystal structure and a previously reported NMR structure will also be presented. In addition, the crystal structure of talin-RS shows its unexpected capability of tetramerization. Our results shed light upon the mechanism of the talin autoinhibition in regulating integrin activation and signaling during diverse cell adhesion and migration processes.

**Kewywords: talin, autoinhibition, integrin**

## MS22.P27

*Acta Cryst.* (2011) A**67,** C350

**Reduced nDsbD sheds light on protein-protein interactions in disulfide cascades**

Emmanuel Saridakis,[a] Despoina A.I. Mavridou,[b] Paraskevi Kritsiligkou,[b] Alan D. Goddard,[b] Julie M. Stevens,[b] Stuart J. Ferguson,[b] Christina Redfield[b] [a]*Institute of Physical Chemistry, N.C.S.R. "Demokritos", Athens, (Greece).* [b]*Department of Biochemistry, University of Oxford, (United Kingdom).* E-mail: esaridak@chem.demokritos.gr

Bacterial growth and pathogenicity depend on the correct formation of disulfide bridges, a process controlled by the Dsb system in the periplasm of Gram negative bacteria. Proteins with a thioredoxin fold play a central role in this process. A general feature of thiol:disulfide exchange reactions is the need to avoid a long-lived product complex between protein partners.

We have solved the structure of the N-terminal domain of the transmembrane reductant conductor DsbD (nDsbD) of *E.coli* in its reduced form, to a resolution of 1.8 Å (PDB code 3PFU) [1]. This structure complements NMR, surface plasmon resonance, mutagenesis and *in vivo* experiments, as well as already published structures of the oxidised form of nDsbD [2], of the oxidised and reduced forms of the C-terminal domain of DsbD (cDsbD) [3] and of the covalent complex of nDsbD with cDsbD [4], in investigating the interaction between these two soluble domains of the protein [1]. The oxidation-state-dependent affinity of this interaction is explained in terms of subtle differences in structure and electrostatics. nDsbD is a fairly rigid oxidoreductase with a unique immunoglobulin-like fold which ensures that the active-site cysteines are only accessible after specific protein-protein interactions with its thioredoxin-like partners. Small conformational differences in the active-site and in the protective cap-loop region of reduced and oxidised nDsbD affect the way it interacts with its partners. The oxidation-state-dependent affinities between the two domains ensure that nDsbD and cDsbD are not trapped in non-productive complexes that would block electron transfer from the cytoplasm to the periplasm.

Our observations have wider implications for the interactions of the ubiquitous thioredoxin-like proteins with their substrates and are of general importance for oxidative protein-folding pathways in all organisms.

[1] D.A.I. Mavridou, E. Saridakis, P. Kritsiligkou, A.D. Goddard, J.M. Stevens, S.J. Ferguson, C. Redfield, *Journal of Biological Chemistry* (in press). [2] C.W. Goulding, M.R. Sawaya, A. Parseghian, V. Lim, D. Eisenberg, D. Missiakas ,*Biochemistry* **2002,** *41*, 6920-6927. [3] C.U. Stirnimann, A. Rozhkova, U. Grauschopf, R.A. Bockmann, R. Glockshuber, G. Capitani and M. G. Grutter, *Journal of Molecular Biology* **2006,** *358*, 829-845. [4] A. Rozhkova, C. U. Stirnimann, P. Frei, U. Grauschopf, R. Brunisholz, M.G. Grutter, G. Capitani and R. Glockshuber, *EMBO Journal* **2004,** *23*, 1709-1719.

**Keywords: thiol:disulfide oxidoreductases, oxidative folding**

## MS22.P28

*Acta Cryst.* (2011) A**67,** C350

**Crystal structure of acap1 involved in endocytic recycing**

Xiaoyun Pang,[a] Kai Zhang,[a] Qiangjun Zhou,[a] Jun Ma,[a] Ming Bai,[b] Victor W. Hsu,[b] Fei Sun[a] [a]*National Laboratory of Biomacromolecules, Institute of Biophysics (IBP), Chinese Academy of Sciences, Beijing 100101, China.* [b]*Division of Rheumatology, Immunology and Allergy, Brigham and Women's Hospital, and Department of Medicine, Harvard Medical School, Boston, Massachusetts 02115, (USA).* E-mail: pangxy@moon.ibp.ac.cn

Endocytic recycling is critical for many cellular events, including nutrient uptake, cell polarity, cell motility and signal transduction, but little is known about how cargo proteins are sorted, and even cargo sorting has been predicted to be unlikely in the endocytic recycling pathway. ACAP1 is Arf6 GTPase-activating Protein, controlling the return of Arf6 to the inactive GDP-bound state. ACAP1 is not only as key negative regulator of Arf6, but also as its effector by being core components of clathrin coat complexes. ACAP1 is reported as an adaptor of a novel clathrin coat complex for endocytic recycling in key physiological settings, the recycling of TfR from the recycling endosome, stimulation-dependent recycling of integrin and insulin-stimulated recycling of glucose transporter type 4 (Glut4). The molecular mechanism of cargo sorting remains to be known.

The crystal structures of N-portion and C-portion of ACAP1 have been determined by x-ray crystallography, and give help to elucidate the mechanism of its interaction with membrane and cargo protiens in Endocytic recycling pathway.

[1] T.R. Jackson, F.D. Brown, et al. *J Cell Biol* **2000,** *151(3),* 627-638. [2] J. Li, P.J. Peters, et al. *J Cell Biol* **2007,** *178(3),* 453-464.

**Keywords: crystal, clathrin, endocytic recycing**

## MS22.P29

*Acta Cryst.* (2011) A**67,** C350-C351

**APEH-mediated down-regulation of proteasome by potential anticancer molecules**