1275-1283. [4] R. H. Schwall, L. Y. Chang, P. J. Godowski, D. W. Kahn, K. J. Hillan, K. D. Bauer, T. F. Zioncheck, *J Cell Biol* **1996**, *133,* 709-718.

Keywords: cancer, protein-protein interaction, drug

MS22.P23

Acta Cryst. (2011) A**67,** C349

The crystal structure of APPL2 BAR-PH domain

Yujie CHEN,[a] Bin CHEN,[b] Aimin XU,[b] Quan HAO,[a] [a]*Department of Physiology, The University of Hong Kong,* [b]*Department of Medicine, The University of Hong Kong.* E-mail: qhao@hku.hk

APPL (Adaptor protein containing PH domain, PTB domain and leucine zipper motif) is an adaptor protein with two isoforms APPL1 and APPL2. As a scaffold protein, the interaction of APPL with Akt2 [1], small GTPases [2], FSHR, and AdipoR [3] are associated with the roles in cell proliferation, cell cycle control, metabolism/insulin sensing [4], [5], relaying signal from endosome and transcription by activating AMPK related signaling pathway [6]. As a protein communication hub, APPL provdes a mechanism for spatial regulation of signaling transmission and processing. However, it has also been shown that APPL2 plays opposing functions from APPL1 and there have been emerging evidences that BAR-PH domain of APPL1 and APPL2 can interact with each other to form heterodimer *in vivo*.

The structure of APPL2 BAR-PH domain has been solved to resolution of 3.2 Å using X-ray crystallography. The dimer exhibits a canonical four-helical fold of conformation, just like the shape of crescent or banana, which is implicated in the assocication with curved membranes. The difference between APPL1 BP domain and APPL2 BP domain lays in the curvature of the crescents. The curvature radius increases to ~70 Å, in sharp contrast with ~55 Å of APPL1 BP dimer's [7]. It has been proposed that curvature radius of BAR family protein corresponds to specific type of endosome to which it binds. Also, the protein surface shows different electrostatic property. Thus it is reasonable to conclude that the structure of APPL2 BAR-PH domain may be implicated in alteration of endosome assocation specificity, also in the change of the profile of spatial regulation of signaling transmission.

[1] Mitsuuchi, et al. *Oncogene* **1999,** *18 (35),* 4891-4898. [2] C.A. Nechamen, R.M. Thomas, J.A. Dias, *Mol. Cell. Endocrinol* **2007,** 260–262, 93–99. [3] X .Mao, C.K. Kikani, et al. . *Nat. Cell Biol.* **2006,** *8,* 516–523. [4] S. Deepa, L.Q. Dong. *Am J Physiol Endocrinol Metab,* **2009,** *296(1)*, E22-36. [5] B. Chandrasekar et al. *J Biol Chem* **2008,** *283(36)*, 24889-24898. [6] S. Rashid et al. *J Biol Chem* **2009,** *284(27)*. [7] J. Li, X. Mao, L.Q. Dong, F. Liu, L. Tong. *Structure* **2007,** *15(5)*, 525-33.

Keywords: APPL2, BAR-PH domain, crystal structure

MS22.P24

Acta Cryst. (2011) A**67,** C349

Regulation of an *Arabidopsis Thaliana* potassium channel

Antonio Chaves Sanjuán, Maira Diaz Vergara, Martín Martínez Ripoll, Armando Albert de la Cruz, Maria José Sánchez Barrena. *Departament of Crystallography and Structural Biology, Instituto de Química Física "Rocasolano", CSIC, Madrid. (Spain).* E-mail: xantonio@iqfr.csic.es

Under abiotic stress, plant cells use calcium signaling pathways to activate certain ion channels and give a correct response. Potassium (K+) is an essential ion for plant development. Plants living under low K+ conditions often adapt their K+ uptake through a recently discovered calcium signaling pathway which mobilizes potassium channel in roots. Under K+ deficiency, a calcium sensor activates a kinase that in turn phosphorylates and activates the K+ channel. When K+ levels are restored, a phosphatase dephosphorylates and inactivates the channel.

We are carrying out structural studies (crystallographic and ITC experiments) with this K+ channel and its binding partners to understand at molecular level how K+ uptake is regulated under stress conditions.

Keywords: potassium channel, abiotic stress

MS22.P25

Acta Cryst. (2011) A**67,** C349-C350

Structure-based mutagenesis studies of human type II Hsp40, Hdj1

Hironori Suzuki, Shuji Noguchi, Yoshinori Satow, Toshiyuki Shimizu. *Graduate School of Pharmaceutical Sciences, University of Tokyo (Japan).* E-mail: hi-suzuki@mol.f.u-tokyo.ac.jp

Hsp40 is a co-chaperone of Hsp70 that correctly folds polypeptides in non-native forms. Hsp40 is divided into two domains: the N-terminal DnaJ-like domain and the C-terminal domain. During protein refolding, DnaJ-like domain of about 8 kDa stimulates the ATPase activity of Hsp70. C-terminal domain of 21 kDa interacts with non-native polypeptides and also with the C-terminal region of Hsp70, and delivers the polypeptides to Hsp70. In this study aiming at elucidation of co-chaperone mechanism of Hsp40, we determined the crystal structures of the C-terminal domain of human type II Hsp40, Hdj1, complxed with the C-terminal octapeptide of human Hsp70, GPTIEEVD. Furthermore, we carried out mutagenesis studies on Hdj1 to identify the surface regions responsible for the cochaperone activity.

The crystal structure of the C-terminal domain of Hdj1 in complex with the octapeptide was refined at 1.9 Å resolution. Based on the refined structure, we designed Hdj1 single-point mutants at the peptide-binding sites. The Hdj1 mutants were expressed in *E. coli* and purified with cation-exchange chromatography. Cochaperone activities of wild-type Hdj1 and its mutants were measured using a luciferase refolding assay. Furthermore, the crystal structures of the mutants were determined.

C-terminal domain of Hdj1 is a twisted, horseshoe-shaped homodimer. The protomer consists of 11 β-strands and 3 α-helices and is folded into elongated globular domains I and II, and a C-terminal helix region related to dimerization. The octapeptides are located in two distinct sites of domain I, sites 1 and 2, of each protomer. In the site 1, the octapeptide forms an anti-parallel β-sheet with β2 strand of domain I. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 181, Lys 182 and Lys 184 of Hdj1. The Ile side-chain of the octapeptide fits into the hydrophobic concaved surface. The site 1, therefore, is attributed to the recognition site toward Hsp70. The octapeptide in the site 2 forms an anti-parallel β-sheet with β4 strand of domain I. This site is located just behind the site 1. The negatively-charged side-chains of the octapeptide form salt bridges with the side-chains of Lys 213, Lys 217 and Lys 306 of Hdj1. The side-chain of Pro and Ile of the octapeptide are situated at the hydrophobic region which is flat and wider than the concave of the site 1. This region is suited for binding of the non-native polypeptides with hydrophobic side-chains bulkier than Ile. Hence, it is conceivable that the site 2 is the binding site toward non-native polypeptides. The point mutants at site 1 or site 2 are confirmed by determination of the structure that only one of the sites is be disrupted while the other site remains unchanged. These mutants reduced the cochaperone activity of

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