methylerythritol 4-phosphate (MEP) pathways. However, some steps of these pathways are catalysed by completely different enzymes in different strains. For example, the NADPH-dependent production of MEP from deoxyxylulose 5-phosphate (DXP) in the first committed step of the MEP pathway is catalysed by DXP reductoisomerase (DXR) in most bacteria, whereas an unrelated DXR-like (DRL) protein was recently found to catalyze the same reaction in some organisms. Because DRL is found instead of DXR in animal and human pathogens such as *Brucella* and *Bartonella*, DRL might be a new target for highly specific antibiotics against these DRL-harboring bacteria that would not affect beneficial bacteria that use DXR (like those present in the gut). Despite their catalytic similarity, DRL and DXR only show some sequence identity at the level of the NADPH-binding domain.

We have determined two X-ray crystal structures of the cattle pathogen *Brucella abortus* DRL (*Ba*DRL) enzyme: the apo state and a complex with the broad spectrum antibiotic fosmidomycin (FSM); solved to 1.5 and 1.8 Å resolution, respectively. Initial phases to solve the *Ba*DRL structure were obtained by the single-wavelength anomalous dispersion method of an osmium-derivative crystal belonging to the primitive orthorhombic space group $P2_12_12$. To improve the electron density a crystal averaging protocol was followed, combining the initial phases with data from a selenomethionine-labelled DRL crystal belonging to space group C2 and native data from a primitive triclinic (P1) crystal.

DRLs are dimers, with each polypeptide folding into three distinct domains starting with the NADPH-binding domain in resemblance to the structure of bacterial DXRs. Other than that, DRLs and DXRs show a very low structural relationship with a different disposition of the domains and topologically unrelated C-terminal domains that in DRL presents an unsuspected structural similarity with antifreeze proteins. The DRL active site, containing a catalytic magnesium ion, shows a unique arrangement, suggesting that the design of drugs that would selectively inhibit the DRL or the DXR enzymes should be feasible. DRL catalytic residues were assigned and found to be strictly conserved among the active DRL sequences and, most interestingly, structurally non-equivalent to the catalytic residues in DXRs. Single-residue mutations of some of these amino acids confirmed their importance in catalysis. The structure of DRL in complex with FSM explains in particular why, despite the structural differences, the inhibition mechanism remains essentially identical in the DRL and DXR family of enzymes.

Keywords: terpenoid, inhibition

MS01.P04

Acta Cryst. (2011) A**67,** C216

Structural study of enzymatic anti-baldwin ring closure in polyether formation

Kinya Hotta,[a] Xi Chen,[a] Robert S. Paton,[b] Atsushi Minami,[c] Hao Li,[a] Kunchithapadam Swaminathan,[a] Irimpan Mathews,[d] Kenji Watanabe,[e] Hideaki Oikawa,[c] Kendall N. Houk,[b] Chu-Young Kim[a] *[a]Department of Biological Sciences, National University of Singapore, 117543, Singapore. [b]Department of Chemistry and Biochemistry, University of California Los Angeles, Los Angeles, CA, 90095-1569, (USA). [c]Division of Chemistry, Hokkaido University, Hokkaido, 060-0810, (Japan). [d]Stanford Synchrotron Radiation Laboratory, SLAC National Accelerator Laboratory, Menlo Park, CA, 95124, (USA). [e]Division of Pharmaceutical Sciences, University of Shizuoka, Shizuoka, 422-8526, (Japan).* E-mail: dbshk@nus.edu.sg.

Despite recent advances in the understanding of polyether natural product biosynthesis, the enzymological mechanism of how these stereochemically complex compounds are generated remains elusive. In particular, many polyethers carry cyclic ether structures that are energetically disfavored to form, making them a chemically intriguing target of study. For example, it has been postulated [1] that the formation of a ladder polyether natural product brevetoxin B requires a series of 10 energetically disfavored, anti-Baldwin-type epoxide-opening ring closures to take place sequentially under a mild, aqueous condition. Recently, Jamison *et al*. demonstrated [2] that templated epoxide substrates in the presence of hydrogen-bonding water molecules undergo an epoxide ring opening cascade that leads to the formation of desired *trans-syn-trans* cyclic ether ladders with high stereoselectivity [3]. However, the question on the genesis of the initial templating cyclic ether units still remains unanswered.

Here, we report the crystal structure of Lsd19, an epoxide hydrolase responsible for the biosynthesis of ionophore polyether lasalocid A, in complex with substrate and product analogs. This is the first atomic structure of a natural enzyme capable of catalyzing the disfavored epoxide-opening cyclic ether formation. The unique catalytic ability of Lsd19 was analyzed through a combination of sequence analysis, crystal structure determination, and computational study. Our structural and computational studies indicate that an intricate pre-organization of the binding pocket which favorably stabilizes the transition state structure for the disfavored cyclization allows Lsd19 to violate Baldwin's rule for ring closure without the use of additional cofactors or modifications to the epoxidated substrate to steer the course of reaction pathway. Furthermore, amino acid sequence alignment and homology-based structure modeling of different ionophore polyether epoxide hydrolases has provided insight into the general mode of cyclic ether formation in polyether biosynthesis. Our study has significantly advanced the efforts toward understanding the chemical principle behind nature's solution for synthesizing highly complex polyether natural products, and provided a fundamental understanding of how an enzyme can overturn the natural course of a chemical transformation to deliver a reaction product that is otherwise difficult to obtain.

[1] K. Nakanishi, *Toxicon* **1985**, *23*, 473-479. [2] I. Vilotijevic, T.F. Jamison, *Science* **2007**, *317*, 1189-1192. [3] C.J. Morten, J.A. Byers, T.F. Jamison, *J. Am. Chem. Soc.* **2011**, *133*, 1902–1908.

Keywords: anti-Baldwin, polyether, biosynthesis

MS01.P05

Acta Cryst. (2011) A**67,** C216-C217

A pentavalent G-actin:RPEL assembly required for regulation of MRTF-A subcellular localisation

Stéphane Mouilleron,[a] Carola A. Langer,[b] Sebastian Guettler,[b] Neil Q. McDonald,[a] Richard Treisman,[b] *[a]Structural Biology and [b]Transcription groups, Cancer Research UK London Research Institute, Lincoln's Inn Fields Laboratories, 44 Lincoln's Inn Fields, London (UK). E-mail:stephane.mouilleron@cancer.org.uk*

Serum response factor transcriptional activity is controlled through interactions with regulatory cofactors such as the coactivator MRTF-A (Myocardin-Related Transcription Factor A). MRTF-A transcriptional activity and subcellular localization are regulated in vivo by changes in cellular actin dynamics, which alter its interaction with G-actin. The G-actin-sensing mechanism of MRTF-A resides in its N-terminal domain, which consists of three tandem RPEL repeats. We previously described the first molecular insights into RPEL function obtained from structures of two independent $RPEL^{MRTF\text{-}A}$ peptide:G-actin complexes [1]. Regulation of MRTF-A requires all three actin-binding RPEL motifs in the MRTF-A regulatory domain, but multimeric G-actin•MRTF-A complexes have not been characterized. We describe here a pentavalent and trivalent G-actin•RPEL domain assemblies. In the pentavalent complex actins bind each RPEL motif, and the spacer sequences between them; in contrast, the trivalent complex lacks the C-terminal

spacer- and RPEL-actins, which bind weakly in solution. Cytoplasmic MRTF-A localization in resting fibroblasts requires spacer-actin binding. The bipartite importin {alpha-beta} binding site is buried in the pentameric assembly, explaining how elevated G-actin concentrations prevent MRTF-A nuclear import. The structures show how actin loads onto the RPEL domain, and reveal a molecular mechanism by which actin can control the activity of one of its ligands.

[1] S. Mouilleron, S. Guettler, C.A. Langer, R. Treisman, N.Q. McDonald, *EMBO J* **2008,** *27,* 3198.

Keywords: actin, structure, MRTF-A

MS01.P06

Acta Cryst. (2011) A**67,** C217

The Molybdenum Storage Protein – a special kind of metallo-protein

Juliane Poppe,[a] Ron Huelskoetter,[a,b] Bjoern Kowalewski,[b] Klaus Schneider[b] and Ulrich Ermler,[a] [a]*Dept. of Molecular Membrane Biology, Max-Planck-Institute of Biophysics, Frankfurt/Main (Germany).* [b]*Faculty of Chemistry, University of Bielefeld, Bielefeld (Germany).* E-mail: Juliane.Poppe@mpibp-frankfurt.mpg.de

The diazotrophic soil bacterium *Azotobacter vinelandii* utilizes a FeMo-cofactor containing nitrogenase to accomplish nitrogen fixation. As nitrogenase is required in large amounts, the cell extracts a lot of molybdenum (Mo) from the environment and stores it in a special Molybdenum Storage Protein (MoSto)[1] as polyoxometalate clusters[2]. MoSto crystallizes in a needle-like shape. Best crystals diffract to 1.6Å resolution. An X-ray crystal analysis of the Mo-loaded MoSto revealed different types of Mo-oxide based clusters some being covalently bound while others are not. These clusters are synthesized in an ATP-dependent process whose mechanism is not yet known. *In-vitro* experiments showed that it is possible to entirely deplete MoSto from its metal clusters and subsequently reload it again. The depletion is a pH-driven triphasic process which can be varied with temperature and time of incubation [1]. MoSto can load up to 120 atoms per protein molecule. Further research is necessary to determine the mechanism how the clusters are built from monomolybdates and how their selective release from MoSto is organized.

[1] J. Schemberg *et al*., *ChemBioChem* **2008**, *9,* 595-602. [2] D. Fenske *et al*., *ChemBioChem* **2005**, *6,* 405-413

Keywords: molybdate, cluster, bacterial

MS01.P07

Acta Cryst. (2011) A**67,** C217

Features critical for membrane binding revealed by DivIVA crystal structure

Maria A. Oliva,[a,b] Sven Halbedel,[c] Stefan Freund,[a] Thomas Leonardl,[a,d] Dimitri Veprintsev,[a] Leendert Hamoen,[c] Jan Löwe,[a] [a]*Structural Studies Division, Medical Research Council-Laboratory of Molecular Biology, Cambridge (UK).* [b]*Departamento Físico-Química, Centro de Investigaciones Biológicas, CSIC-Madrid (Spain).* [c]*Center for Bacterial Cell Biology, Institute for Cell and Molecular Biosciences. Newcastle University (UK).* [d]*Laboratory of Molecular Biology, NIDDK. National Institutes of Health, 9000 Rockville Pike, Bethesda (USA).* E-mail: marian@cib.csic.es

DivIVA is a highly conserved protein in Gram-positive bacteria that localizes at the poles and division sites, presumably through direct sensing of membrane curvature. The correct localization of the protein is essential for its biological function, which involve both the correct placement of the septum at midcell and also the segregation of the DNA into the pre-spore during sporulation. DivIVA deletion causes filamentous growth in *Bacillus subtilis*, whereas overexpression causes hyphal branching in *Streptomyces coelicolor*. The mechanism of subcellular DivIVA targeting and function remain unknown. We present data that demonstrates that DivIVA binds directly to bacteria membrane and explains how this protein shows a multifunctional behavior.

We prove that DivIVA is a two independent domains protein linked by a flexible tag and both domains together are essential to fulfill protein localization and function. The crystal structure determination shows that both domains share a parallel coiled-coil folding. Surprisingly the N-terminal domain also includes crossed and intertwined loops between the two coiled-coil molecules, which is completely new and is involved in protein targeting in combination with the presence of a key hydrophobic residue surrounded by positively charged residues. To clarify N-terminal domain function in DivIVA membrane targeting we have used an *in vivo* and *in vitro* approach, which shows Phe17 and its chemical context in DivIVA structure, is essential for membrane binding. We propose that the hydrophobic residues insert into the membrane and that the positively charged residues bind to the membrane surface. Also low-resolution crystal structure of the C-terminal domain displays a curved tetramer made from two parallel coiled-coils, which is the lowest oligomerization state of DivIVA showed by AUC. The N-terminal and C-terminal parts have been merged into a model of the full length, 30nm long DivIVA protein. Our experiments suggest that full-length DivIVA is an elongated tetramer with membrane targeting residues at both ends, which increase the protein ability to bind to membranes and other proteins.

Keywords: bacterial cell division, peripheral membrane protein, membrane curvature

MS01.P08

Acta Cryst. (2011) A**67,** C217-C218

Structural basis for complement Factor I control and its disease associated sequence polymorphisms

Pietro Roversi,[a] Steven Johnson,[a] Joseph J.E. Caesar,[a] Florence McLean,[a] Kirstin J. Leath,[a] Stefanos A. Tsiftsoglou,[b] B. Paul Morgan,[c] Claire L. Harris,[c] Robert B. Sim,[b] and Susan M. Lea,[a] [a]*Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, England, (UK);* [b]*Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, England, (UK);* [c]*Department of Infection, Immunity and Biochemistry, School of Medicine, Cardiff University, Heath Park, Cardiff CF14 4XN,Wales, (UK).* E-mail: Pietro.roversi@path.ox.ac.uk

The complement system is a key component of innate immune defence, coordinating inflammation with innate and adaptive responses, both in infection and in homeostasis. Complement regulation is critical for prevention and control of disease.

We have determined the crystal structure of the complement regulatory enzyme human Factor I (fI). Factor I is seen to be in a proteolytically inactive form, demonstrating that it circulates in a zymogen-like state, despite being fully processed to the mature sequence.

Mapping of functional data from mutants of fI onto the structure suggests that this inactive form is maintained by the non-catalytic heavy chain allosterically modulating activity of the light chain.

Once the ternary complex of fI, a cofactor and a substrate is formed,