of these experiments in a manner that is compatible with the current high throughput crystallisation (HTPX) robotics available at the EMBL Hamburg outstation. The additional opportunities for crystal detection using ANS-based fluorescence are also now available for external users who wish to submit their samples to the EMBL HTPX.

[1] Groves, M.R., Muller, I.B., Kreplin, X. & Muller-Dieckmann, *J. Acta Crystallogr D Biol Crystallogr.* **2007** Apr; 63 (Pt 4): 526-535

Keywords: crystallization; UV fluorescence; image analysis

FA1-MS09-P06

Dehydration-Induced Phase Transition in D-xylose Isomerase. Francesco Gramiccia[a], Céline Besnard[b], Yves Pellegrinelli[a], Sebastian Basso[a], Marc Schiltz[a]. [a]*Laboratoire de Cristallographie, EPFL, Lausanne.* [b]*Laboratoire de Cristallographie,UNIGE, Geneva.*
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D-xylose isomerase, which causes the isomerization of glucose to fructose, has a large market in the food industry because of its application in the production of high-fructose corn syrup. In order to fully understand and control the activity of the protein a good knowledge of the structural response of the protein to changes in the environmental conditions is necessary. Since proteins function in aqueous media and nearly half of the volume of protein crystals is occupied by water, protein-water interactions are of great interest.
We have now identified a dehydration-induced phase transition in D-xylose isomerase from streptomyces rubiginosus. The transition, characterized using both powder and single crystal diffraction, occurs at room temperature for relative humidity around eighty percent. After the transition, the crystal recovers its crystalline state and diffracting power. The symmetry is reduced from space-group I222 to its subgroup $P2_12_12$ but the effects of this symmetry break on the structure are subtle. The decrease of the unit-cell volume by more than 15 percent produces more pronounced and interesting structural rearrangements in the crystal.

[1] Gummow R.J., Liles D.C., *Mat. Res. Bull*, **1993**, 28, 1293. [2] Grirrane, A.; Pastor, A.; Galindo, A.; Ienco, A.; Mealli, C. *Chemm. Commun.* **2003**, 512.

Keywords: protein X-ray crystallography; phase transitions and structure; isomerases

FA1-MS09-P07

Crystallization and Structure of Two Bovine Insulin Derivatives. Biserka Prugovečki[a], Ivica Đilović[a], Dubravka Matković-Čalogović[a]. [a]*Department of Chemistry, Faculty of Science, University of Zagreb, Zagreb, Croatia.*
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Insulin is a polypeptide hormone, produced by the β-cells in the pancreas, that regulates carbohydrate metabolism and it also takes part in the metabolism of fat and proteins. Insulin is structured as two polypeptide chains, chain A consists of 21 and chain B of 30 amino acids. Bovine insulin differs in sequence from human insulin at residues A8, A10 and B30. There are three forms of insulin hexamers. These forms of insulin are used in therapeutc preparations for the control of diabetes.
The crystal structure of T_6 bovine insulin was described by Smith et al in 2005 [1]. It has been known for many years that native insulin is in the T_3R_3 form in the crystals grown in high chloride ion concentrations [2].
This work presents crystallization of two bovine insulin derivatives: in high iodine and bromine concentrations. Structural investigations on bovine bromo- and iodo-derivatives show that these halogen atoms are bound to the zinc ions. Crystals were grown by the hanging drop vapour diffusion crystallisation method. Single crystal diffraction data were collected on laboratory instrument at 100 K. The investigated insulin derivatives belong to the R3 rhombohedral space group with cell parameters a = 79.73 Å, c = 36.34 Å and a = 79.25 Å, c = 36.97 Å for the bromo- and iodo-derivative, respectively. Conformation of the insulin molecule and coordination of Zn-ions will be discussed.

[1] Smith, G. D., Pangborn, W. A., Blessing, R. H. *Acta Cryst*., **2005**, D61, 1476. [2] Kaarsholm, N. C., Ko H. C., Dunn M. F. *Biochemistry*, **1989**, 28, 4427.

Keywords: insulin; protein structures; X-ray structure analysis

FA1-MS09-P08

***Arabdiopsis* SET-domain Proteins with Different Histone Methyltransferase Activity.** Mohummad Aminur Rahman[a], Silje Veie Sandvik[a], Tage Thorstensen[a], William Davies[a], Reidunn B. Aalene[a]. [a]*Department of Molecular Biosciences, University of Oslo PO box 1041 Blindern, N-0316, Oslo, Norway.*
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Proteins containing a conserved SET domain catalyze the methylation of lysine residues on histone tails, and influences chromatin structure. The SET-domain proteins of Arabidopsis can be grouped in the same evolutionarily conserved subclasses as found in Drosophila and mammals [1], i.e. E(Z), TRX, ASH1 and Su(VAR)3-9, that have specificities for different lysine substrates. Different SET domain proteins may also differ en product specificity, i.e. they can add one, two or three methyl groups on a given lysine residue. The structures of more than ten SET domains have been solved [2], but they do not cover all substrate and product specificities. We aim at solving the structure of the Arabidopsis SUVR4, for which a novel HMTase specificity was demonstrated in that monomethylated histone H3K9 is its preferred substrate in vitro [3], and for ASHH1 and ASHH2 of the ASH1 class. For the latter two Arabidopsis proteins HMTase activity against H3 has been demonstrated. Although the specific target lysine(s) and product specificity

have not been identified yet, ASHH1 and ASHH2 have been suggested to add monomethyl and trimethyl to H3 K36, respectively, [4]. In order to determine the protein structures, SET domain proteins (ASHH1, SUVR4) were overexpressed in *E. coli* BL21 cells and purified by Maltose affinity chromatography. After the first step purification, the protein production was relatively high; ~ 50 mg of protein from 1.5 L induced cells. The proteins were further purified by using Ion-exchange chromatography and Size-exclusion chromatography and we are at the moment ready to start crystallographic process to determine the structures.

[1] Baumbusch L.O., Thorstensen T., Krauss V., Fischer A., Naumann K., Assalkhou R., Schulz I., Reuter G., Aalen R.B. *Nucleic Acids Res.*, **2001**, 29, 4319-4333. [2] Qian C., Zhou M.-M. C*ell. Mol. Life Sci.* 63 **2006**, 2755–2763. [3] Thorstensen T., Fischer A., Grini P.E., Sandvik S.V., Johnsen S.S., Reuter G., Aalen R.B. *Nucleic Acids Research*, **2006**, 34, 5461-5470. [4] Zhao Z., Yu Y, Meyer D., Wu C., Shen W.H., *Nat. Cell Biol.*, **2005**, 7, 1156-1160.

Keywords: histone; lysine; methylation

FA1-MS09-P09

Protein Crystallisation Screening by the Counter-diffusion Technique. Luis A. Gonzalez-Ramirez[a], Alfonso G. Caballero[a], D. Choquesillo-Lazarte[a], J. M. Garcia-Ruiz[a]. [a]*Laboratorio de Estudios Cristalograficos, IACT (CSIC-U. Granada), Granada. Spain.*
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Crystallisation remains the rate-limiting step in protein structural determination due to the large number of variables that must be systematically adjusted for optimal crystal formation. Typical parameters which influence crystal growth include the temperature, the concentration, purity and nature of the protein in question as well as the concentration and nature of other chemical components normally used such as buffers, additives and precipitating agents [1].
Over the last 20 years, numerous efforts in order to make crystallization conditions more tractable have led to a number of novel crystallisation conditions screenings and crystallisation strategies [2, 3]. A particularly useful method to grow protein crystals is the counter-diffusion. This technique is based on the diffusion of both precipitating agent and protein in opposite directions thereby generating a supersaturation wave across the growth chamber. This supersaturation wave effectively screens different crystallisation conditions of progressively lower supersaturation values while travelling along the crystal growth chamber [4].
This work describes the screening of crystallisation conditions for 40 proteins using the counter-diffusion technique. The experimental set up involves the use of very thin capillaries (ø = 0.1 mm) within the Granada Crystallisation Box (GCB) [5], commercially available from Triana Science & Technology. One of the many advantages of using such thin capillaries is that it greatly reduces the amount of protein solution needed for each single screening experiment to as little as 300 nanolitres.
We have analysed the effect of the pH in combination with the most frequently used precipitating agents, namely, three polyethylene glycols of varying molecular weight (PEG-400, PEG-4000, PEG-8000) plus ammonium sulphate on the crystallisation of 40 proteins. Additionally, we have tested whether the use of a mixture of the three PEGs has the same effect on the crystallisation that any of them separately. The results of the different screened crystallisation conditions in terms of the success of crystallisation and crystal quality (as determined by X-ray diffraction) are discussed.

[1] McPherson A., *Methods in Enzymology*, **1985**, 114, 112. [2] Jankarik J., Kim S.H., *J. Appl. Cryst.*, **1991**, 24, 409. [3] Kimber M.S., *Proteins: Structure, Function, and Genetics*, 2003, 51, 562. [4] Garcia-Ruiz J.M., *Methods in Enzymology*, **2003**, 368, 130. [5] Garcia-Ruiz J.M., Gonzalez-Ramirez L.A., Gavira J., Otalora F., *Acta Cryst.*, **2002**, D58, 1638.

Keywords: protein crystallisation; crystallisation techniques; screening; counter-diffusion

FA1-MS09-P10

Protein Crystallization: Robotics, Procedures and Developments. Fabrice Gorrec[a], Olga Perisic[a], Katharine Michie[a], Gebhard Schertler[a], Jan Löwe[a]. [a]*MRC Laboratory of Molecular Biology (LMB), Cambridge, UK.*
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LMB scientists can undertake initial crystallization experiments and also crystal condition refinements for each new protein sample using automated protocols [1]. The LMB protein crystallization facility is high-throughput and includes various robots (at room temperature and at 4°C). The protocols are straightforward and setting up plates is easy. This enables LMB scientists to operate independently. This is crucial when targets are difficult to crystallize and many rounds of screens are required to test new constructs, like for the cell division protein FtsQ [2]. The standard initial screening protocol is comprised of 17 MRC sitting-drop plates pre-filled with a wide variety of commercially available screen kits using the Genesis workstation (Tecan). Process is fast and requires only small volume of protein. The 17 plates are set up within an hour using 272 µl of sample (for 100 nl drops of the target protein and mother liquor) on the ScreenMaker (Innovadyne) or the Mosquito (TTP LabTech). About 10,000 plates a year are set up for initial screening alone. Refinement, custom matrices and scale-up screens are made in any type of plate. A refinement screen in MRC plate is made in three minutes on the Sciclone i1000 workstation (Caliper) with a custom-made in house SBS footprint reservoir (commercialized by Swissci) containing stock solutions.
New methods and tools are continuously developed and integrated into our crystallization facility. For example, The MRC multi-wavelength imaging system allows assessment of crystals regardless of clarity of the drops. The LMB screen database [3] is a Web based tool to perform basic data mining about the initial screens. To further aid users in the optimization of difficult crystallization conditions,