not compatible with any stable crystalline state. A helpful term in description of the controlled crystallization process is "***dominating adhesion mode" (DAM)***, i.e. the most probable adhesion realized between two macromolecules under the given conditions, usually but not necessary, the highest affinity adhesion mode. Preferences of adhesion modes can be regulated e.g. by a selective mutation of protein surface but also much more easily by changing pH, buffer composition, anions, additives, precipitants etc. Any chemicals adhering temporarily to the specific places on the protein surface and blocking efficiently unwanted adhesion modes between proteins are called "***protein surface shielding agents"(PSSA)***. Because of complex effects of components used in the crystallization solution, many different low-efficiency PSSA's have already been intuitively used long time in crystallization screens under the concepts of additives, precipitants, anions. In the case that DAM leads to formation of a single crystal we receive a unique ***group of crystal contact areas (GCCA)*** related directly to the specific crystal properties (space group, water contents, diffraction quality, twinning, etc.). It is evident that well diffracting crystals are formed only when a single adhesion mode dominates but a reverse statement need not be true. Because of the possible strain induced by deposition of macromolecules in the growing molecular cluster, the contact areas realized in the crystal are not necessarily identical with the contact areas in solution. However, the contact areas of the dominating adhesion mode should be very similar in both states. Hydrophilic polymers are generally very efficient PSSAs, because they protect a much larger area on protein surface than their low-molecular weight equivalents upon binding to protein surface. Thus the polymers have generally better propensity to eliminate unwanted adhesion modes between protein molecules forming a crystal.

Practical part: We made an experimental scan of an efficiency of a number of water soluble polymers and co-polymers as novel ***protein surface shielding agents*** in the crystallization process. Sixteen novel water soluble polymers were tested for their efficiency in protein crystallization. Six of them were selected as precipitants to two new polymer crystallization screens (POLYA, POLYB) /2/. The screens were independently tested in six laboratories on 30 different globular proteins. Supported by GA AV IAA500500701.

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Keywords: polymers; co-polymers; protein crystallization; protein surface shielding agents; molecular adhesion

FA1-MS09-P04

Expression, Purification and Crystallization of Human Bile Acid-coA: Amino Acid N-Acyltransferase (BAAT).

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Bile acid-coenzyme A:Amino Acid N-Acyltransferase (BAAT) is the sole enzyme responsible for conjugation of primary and secondary bile acids to taurine and glycine [1].

The human cDNA BAAT sequence was cloned into the pET15 vector and its expresssion was tested in several *E.coli* strains. Because of the toxicity associated with the overexpression of BAAT in several strains, BL21 (DE3) C41 was selected to express the recombinant protein. Only with this specific strain, a reasonable amount of the protein was obtained in the soluble fraction.

The purification steps involved Nickel-sepharose chromatography followed by reverse IMAC after histidine-tag cleavage. Size-exclusion and hydrophobic interaction chromatography steps were also included to increase the purity of the sample. After these steps the protein shows as a single band in both SDS and native PAGE, The purified enzyme was assessed for activity and was found to be able to catalyze the conjugation of taurine to CoA-fatty acids [2].

The presence of non physiological inter- and/or intramolecular disulfide bounds between the three cysteine residues present in the protein sequence as a source of sample microhetetogenity was also examined. Three cysteine mutants (C235A, C372A and C373A, and the triple mutant without cysteines) were constructed and tested following the same protocols used for the wild type protein and no significant differences in the behaviour of the four species were found.

Crystallization trials with the apo- and holo-wild type enzyme, as well as with the three mutants, are in progress but up to now only microcrystals not adequate for X-ray diffraction studies have been obtained.

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Keywords: BAAT; bile acids; conjugation

FA1-MS09-P05

Novel Crystallisation Plate Imaging System Using ANS-Based Fluorescence at the EMBL Hamburg High-Throughput Crystallisation Facility.

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We have previously demonstrated that the addition of low concentrations (μM-nM) of a nonspecific fluorescent dye (1,8-ANS) does not significantly hinder crystal growth in a set of trial proteins [1]. The resulting fluorescence images collected on a high-resolution fluorescence microscope show that these protein crystals are strongly contrasted against other common crystallisation drop phenomena, such as precipitate and phase separation. However, the experiments were performed on a fluorescence microscope and no specialised equipment currently exists for the visualisation of ANS-based fluorescence (excited at 365 nm). Here we present a novel and cost-effective device for visualisation

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.

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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.

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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