organic crystals due to their softness causing them to crack.

Therefore we have developed and tested different methods to obtain the required quality and low roughness surfaces of soft organic crystals. All polishing methods are based on a high speed air bearing which can be equipped with different tools. The crystal is mounted on a piezo driven x-,y-,z- stage and can be moved with respect to the polishing device.

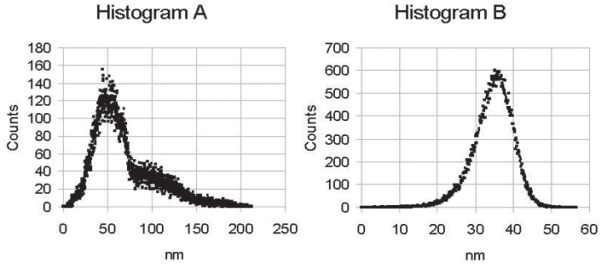
Beside different conventional polishing heads containing diamond grains of different sizes a custom made dimaond cutting tool was used. It consists of a monocrystalline diamond knife which is rotating perpendicular to the crystal surface.

The crystal surfaces generated with the different methods were characterized by atomic force microscopy (AFM). We used tapping mode imaging to reduce the forces on the crystal during the scanning process. The best quality surfaces possess a root-mean-square (rms) roughness of 5.3 nm for a 5 μm x 5 μm area. The rms roughness for a 80 μm x 80 μm area which corresponds to about 75 % of the whole crystal surface is about 14.8 nm.

We also cutted crystals using a commercial microtome suitable for electron microscopy sample preparation. Surfaces with a rms roughness of 4.7 nm for a 50 μm x 50 μm area and 2.9 nm for a 5 μm x 5 μm area could be achieved.

One big advantage of the air bearing cutting device compared to the microtome is the fast and easy processing of a large amount of crystals with high quality surfaces suitable for crystallographic and related experiments.

The figure below shows the histograms of height levels of a crystal polished by a conventional polishing head (A) and a crystal polished by the diamond knife tool (B).



[1] A. Meents et al, *J. Appl. Cryst.* **2009**, *42*, 901-905.

Keywords: organometallic, AFM

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Compositional distribution of isomorphic crystals during spontaneous crystallization

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A non-trivial bimodal distribution of crystals in the isomorphic composition was revealed at spontaneous mass precipitation in aqeuos solutions. Crystal ensembles of the series $(Pb,Ba)(NO_3)_2$, $(Co,Ni)(NH_4)_2(SO_4)\cdot 6H_2O$, $(Co,Ni)K_2(SO_4)\cdot 6H_2O$, $(NH_4,K)H_2PO_4$, $(Al,Cr)K(SO_4)_2\cdot 12H_2O$, and K(Cl,Br) were precipitated during 10–15 s in solutions of various isomorphic ratios at the supercoolings 10 °C and 20 °C. Precipitates were dried by filter paper after the solution decantation.

The isomorphic ratios in individuals of each the series were determined for random samplings of 100–120 individuals and some control samplings up to 400–500 crystals. X-ray fluorescence was used for $(Co,Ni)(NH_4)_2(SO_4)_2\cdot 6H_2O$ and $(Al,Cr)K(SO_4)_2\cdot 12H_2O$. Synchrotron-based XRF (ANKA FLT beamline, Karlsruhe) was used for the $(Pb,Ba)(NO_3)_2$ and $(Co,Ni)(NH_4)_2(SO_4)_2\cdot 6H_2O$ samplings [4]. Microprobe analyser ABT-55 Link AN 100000/85S was used for $(Co,Ni)(NH_4)_2(SO_4)_2\cdot 6H_2O$, $(Co,Ni)K_2(SO_4)_2\cdot 6H_2O$, and $(Al,Cr)K(SO_4)_2\cdot 12H_2O$ [4]. XRPD was used for the probes of K(Cl,Br), sieved to the fractions <10, 10–30, 30–50, 50–100 and >100 microns. Isomorphic compositions of crystal ensembles $(NH_4,K)H_2PO_4$ and K(Cl,Br) were also estimated by means of X-ray micro-tomography (SkyScan 1172).

The compositional distributions are asymmetric with two modes. However the bimodality is not quite clear sometimes due to such samplings were selected without the finest fractions. The distributions are sensitive to the probe total composition and supercooling: the peaks move regularly to one of the end-member. The XRD measurements for the series $(Pb,Ba)(NO_3)_2$ and K(Cl,Br) displayed a nonlinear function of the composition on crystals sizes [3]. The compositional bimodality is coordinated with that of the surface nuclei observed by AFM on a crystal surface of the mixed potassium-rubidium diphthalate [1]. This is in accordance with a high dispersion of crystal composition measured on a surface of grown $(Pb,Ba)(NO_3)_2$-crystals [5].

The compositional bimodality can be understood by the developed concept of mixed crystal formation [1, 2]. It establishes nucleation of two kinds caused by kinetic factor and displayed by modified concentration diagrams.

Results obtained by different analytical methods show that spontaneous mass crystallisation as well as monocrystal growth of solid solutions lead to formation of crystals with rather complex compositional distributions. These data confirm substantially the concept of mixed crystal formation [1].

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[1] A.E. Glikin *Polymineral-metasomatic crystallogenesis* **2009**, *Springer NY*, 312. [2] L. Yu. Kryuchkova, A.E. Glikin, S.N. Kovalev, A.E. Voloshin *Proceedings of Russian Mineralogical Society* **2002**, *3*, 62-77. [3] A.E. Glikin, L. Yu. Kryuchkova, Yu. V. Plotkina, M. Yu. Sinai, P. Gille, J. Schneider, R. Stark *Proceedings of Russian Mineralogical Society* **2007**, *spessial issue "Crystallogenesis and Mineralogy", 7-35*. [4] L. Yu. Kryuchkova, A.E. Glikin, M. Yu. Sinai, A.A. Shiryaev *Proceedings of the16th BIWIC* **2009**, 232-238. [5] V.D. Franke, A.E. Glikin, L.Yu. Kryuchkova, E.V. Tabuns *Geology of Ore Deposits* **2007**, *49*, 7, 641-647.

Keywords: mass crystallization, isomorphism

MS35.P39

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A comparison towards counter-diffusion and sitting drop-vapor diffusion technique in improving resolution data of a novel organic solvent tolerant lipase

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Quality of diffraction data is important in order to generate a better electron density map which provides valuable, accurate information and enzyme functions in structure analysis afterward. To achieve the above objective, one way is to applied different kinds of crystallization techniques. In this study, we discussed about the improvement of crystal quality by counter-diffusion technique and sitting-drop-vapor diffusion technique.

A thermostable organic-solvent-tolerant Lipase 42 (L42) was successfully crystallized under the condition of 0.1M MES monohydrate, 0.1M NaH_2PO_4, 0.1M KH_2PO_4 and 2.0M NaCl using counter-diffusion and sitting-drop vapor diffusion technique. Remarkably after data processing, counter-diffusion technique shows an enhancement of resolution at 2.0Å [1] as compared to sitting-drop method with only 2.4Å. There are several advantageous of counter-diffusion technique; it promotes slower and mild diffusion of protein and precipitant.

As a conclusion, a better L42 lipase resolution was achieved by counter-diffusion technique. Thus, by obtaining a better model of protein, deeper understanding on protein- solvent interaction could be obtained

[1] M.S. Khusaini, R.N.Z.R.A RAhman, M.S.M. Ali, T.C. Leow, A.B. Salleh, M. Basri. *Acta Cryst.* **2011** *F67*, 401-403.

Keywords: counter diffusion, vapour diffusion, organic solvent tolerant lipase

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A component of the xanthomonadaceae T4SS Combines a VirB7 motif with a N0 Domain

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Type IV secretion systems (T4SS) are used by Gram-negative bacteria to translocate protein and DNA substrates across the cell envelope and into target cells. Translocation across the outer membrane is achieved via a ringed tetradecameric outer membrane complex made up of a small VirB7 lipoprotein (normally 30 to 45 residues in the mature form) and the C-terminal domains of the VirB9 and VirB10 subunits [1]. Several species from the genera of *Xanthomonas* phytopathogens possess an uncharacterized type IV secretion system with some distinguishing features, one of which is an unusually large VirB7 subunit (118 residues in the mature form).

Here, we report the NMR and X-ray structures of the VirB7 subunit from *Xanthomonas citri* subsp. citri ($VirB7_{XAC2622}$) and its interaction with VirB9 [2]. The solution structure showed that $VirB7_{XAC2622}$ has an unfolded N-terminus and a unique C-terminal domain whose topology is strikingly similar to that of N0 domains found in proteins from different systems involved in transport across the bacterial outer membrane. We submitted the globular N0 domain of $VirB7_{XAC2622}$ to crystallization trials, that resulted in large plates which belong to space group $C222_1$ and diffracted up to 1.0 Å. Molecular replacement was performed using the solution structure of the globular domain as the search model, resulting, after refinement, in a model with R_{work} of 13.0% and R_{free} of 15.2%.

NMR solution studies showed that residues 27-41 of the disordered flexible N-terminal region of $VirB7_{XAC2622}$ interacts specifically with the VirB9 C-terminal domain, resulting in a significant reduction in the conformational freedom of both regions. We show that $VirB7_{XAC2622}$ oligomerizes through interactions involving conserved residues in the N0 domain and residues 42-49 within the flexible N-terminal region and that these homotropic interactions can persist in the presence of heterotropic interactions with VirB9. Finally, we propose that $VirB7_{XAC2622}$ oligomerization is compatible with the core complex structure in a manner such that the N0 domains form an extra layer on the perimeter of the tetradecameric ring.

[1] V. Chandran, R. Fronzes, S. Duquerroy, N. Cronin, J. Navaza, G. Waksman, *Nature* **2009**, *462*, 1011-1015. [2] D.P. Souza, M.O. Andrade, C.E. Alvarez-Martinez, G.M. Arantes, C.S. Farah, R.K. Salinas, *PLoS Pathogens* **2011**, *Accepted for publication*.

Keywords: structural biology, protein NMR, type IV secretion system

MS36.P02

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The structure of a bacterial esterase essential for NO-stress response. Rafael M Couñago,

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Nitrosative stress is an important factor in host-pathogen interactions. *Neisseria gonorrhoeae* (ng) and *N. meningitidis* (nm) are closely related human pathogens that can pose significant health problems. Both bacteria display a series of mechanisms to cope with oxidative and nitrosative stress, which can come from the bacteria's own metabolism and the host's microenvironment, including the host's innate immune response. Previous work [1] has identified Esterase D (EstD) as essential for protection of *N. gonorrhoea* against nitrosative stress in vitro and for its intracellular survival in human primary cervical epithelial cells. EstD is a carboxylic ester hydrolase and is part of the NmlR regulon, which is upregulated during nitrosative stress and present in a number of pathogenic bacteria.

Here we present the structure of EstD from *N. meningiditis* (97% sequence identity to ngEstD) at 1.3 Å resolution. The enzyme displays the characteristic α/β hydrolase family fold and a catalytic triad formed by Ser, Asp and His residues. The identity of active site residues was further confirmed by mutagenesis studies. The structure of mnEstD also reveals that the "gate-keeper" Cys-54 residue is found at the enzyme's active site entrance. In the eukaryotic counterparts of Neisserial EstDs, oxidation of this residue by glutathione has been shown to modulate the enzyme's activity. Likewise, we show that wild-type mnEstD can be reversibly inhibited via oxidation of Cys-54 by glutathione and that an engineered Ala-54 mnEstD mutant is insensitive to glutathione challenge. Although common in eukaryotes, modulation of protein function by glutathione oxidation has been shown for only a handful of proteins in prokaryotes.

Ongoing experiments in *Neisseria* are to complement our biochemical and structural analysis of EstD and will provide a better understanding of how EstD's function protects these pathogens against nitrosative stress and the host's innate immune response.

[1] Potter et al., *J. Infect. Diseases* **2009**, *200*, 273-278.

Keywords: bacterial, esterase, crystallography

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Crystal structure of Clostridium perfringens Delta toxin and a model of its pore-form

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