Bikitaite has an H-bonded H_2O chain along [010] termed 'one-dimensional ice'. The molecules are ordered, whereby one H atom per molecule is unbonded and the other is H-bonded. With increasing temperature, the H-bonding weakens continuously until the chain 'breaks' and isolated H_2O molecules are present.

Melanophlogite has quasi-free N_2, CO_2 and CH_4 molecules, but no H_2O, which partition between the $[5^{12}]$ and $[5^{12}6^2]$ cages. The molecules are orientationally disordered in the cavities and they have only weak dispersion interactions with the SiO_2-crystal framework. However, the incorporation of molecules is necessary to allow the SiO_2 framework to condense.

Cordierite and beryl contain quasi-free CO_2 molecules, as well as H_2O, in small cavities. The CO_2 molecules are orientated parallel to the crystallographic *x*-axis. The H_2O molecules have their H-H vector parallel to the *z*-axis in alkali-free crystals and are dynamically disordered about the *z*-axis. They show weak H-bonding with their frameworks.

Keywords: nanopores, H-bonding, vibrational spectroscopy

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TEM and XPS Evidences of O/F Ordering in NbO_2F

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NbO_2F is a member of a relatively small group of $MO_{3-x}F_x$ (M = Nb, Ta, Ti, Mo, W) oxyfluorides with the cubic ReO_3 structure type. They have been of interest in recent years as host compounds for Li-insertion in battery electrolytes [1] and also because of a pressure-induced phase transition from cubic ($Pm3m$) to rhombohedral ($R3c$) [2]. The average structure consists of corner-sharing $M(O,F)_6$ octahedra, as in undistorted ABO_3 perovskite, with the A sites being empty. Oxyfluorides are reported to exhibit a statistical distribution of O and F, generally attributed to their similar ionic radii (1.35 and 1.285Å, respectively). Bond-length/bond-strength calculations, however, suggest there should be a strong driving force for ordering. Evidence for one-dimensional O/F ordered columns along ⟨*001*⟩, but with no lateral correlation from one column to the next has already been recognized from transmission electron diffraction [4]. In the present work, coupled HRTEM and electron diffraction revealed the presence of characteristic transverse planes of diffuse intensity running through G±⟨*hk*⅓⟩* regions (*i.e.* existence of O/F-ordering), and XPS confirmed the existence of two distinct atomic positions for O and F and just one for Nb. These data are consistent with three-dimensional O/F ordering within NbO_2F structure.

[1] Permér L., Lundberg M., *J. Solid State Chem.*, 1989, **81**, 21. [2] Carlson S., et al., *Acta Cryst. B*, 2000, **56**, 189. [3] Vogt T., et al., *J. Solid State Chem.*, 1999, **144**, 228. [4] Brink F.J., et al., *J. Solid State Chem.*, 2002, **166**, 73.

Keywords: O/F ordering, electron diffraction, XPS

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Crystal Structures and Topology of New and Rare Arsenates

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The crystal chemical phenomena (e.g. polyhedral stacking variations, microtwinning etc.) which accompany the formation of real structures are considered on the basis of the results of structural study of a large group of new and rare natural arsenates (pushcharovskite, tillmannsite, zdenekite and mahnertite). The use of synchrotron radiation allowed to perform the crystal structure investigation of these four new and rare minerals and to reveal their structural peculiarities and topology.

The structure of pushcharovskite is characterized by some structural disorder and contains heteropolyhedral sheets formed by Cu-polyhedra and As -tetrahedra and linked by hydrogen bonds.

The main peculiarity of tillmannsite structure is connected with unique tetrahedral clusters $(Ag_3Hg)^{3+}$ revealed in this mineral in which mercury is characterized by the low-valence state.

Mahnertite and zdenekite have close chemical composition but different symmetry. These minerals contain a new type of mixed polyhedral sheets, which are characterized by the different mode of stacking.

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Keywords: arsenates, crystal structures, topology

MS08 MEMBRANEPROTEIN STRUCTURE AND FUNCTION

Chairpersons: Jacqueline M. Gulbis, Michael R. Garavito

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Does the Crystal Structure of Ammonium Transporter Tell us its Function?

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Ammonium is one of the most important nitrogen sources for bacteria, fungi and plants but it is toxic to animals. The ammonium transport proteins (Mep/Amt/Rh) are present in all domains of life, but the chemical identity of their substrate was uncertain. We have solved the structure of wild type AmtB from *E. coli* in two crystal forms at 1.8 and 2.1 Å resolution, respectively. Substrate transport occurs through a narrow, mainly hydrophobic pore located at the centre of each monomer of the trimeric AmtB. At the periplasmic entry, a binding site for NH_4^+ is observed. Two phenylalanine side chains (F107 and F215) block access into the pore from the periplasmic side. Further into the pore, the side chains of two highly conserved histidine residues (H168 and H318) bridged by a H-bond, lie adjacent with their edges pointing into the cavity. These histidine residues may facilitate the deprotonation of an ammonium ion entering the pore. Adiabatic free energy calculations support that an electrostatic barrier between H168 and H318 hinders the permeation of cations but not that of the uncharged NH_3. The structural data and energetic considerations strongly indicate that the Mep/Amt/Rh proteins are ammonia gas channels [1]. Interestingly at the cytoplasmic exit of the pore, two different conformational states are observed which might be related to the inactivation mechanism by its regulatory partner.

[1] Zheng L., Kostrewa D., Berneche S., Winkler F.K., Li X.D., *Proc. Natl. Acad. Sci. USA*, 2004, **101**,17090.

Keywords: ammonia transport, conformational change, X-ray structure

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Two OMPs: Autotranslocation and LPS-Deacylation

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The outer membrane proteins (OMPs) of Gram-negative bacteria are often involved in pathogenesis. Their exposure to the extracellular environment make them potential targets for antimicrobial treatments. We present recent crystal structures of two outer membrane proteins, NalP [1] and PagL.

NalP belongs to the autotransporter family. Autotransporters secrete their own N-terminal passenger domain with their C-terminal translocator domain. The crystal structure of the translocator domain of NalP from *Neisseria meningitidis* reveals an N-terminal helix inside

the pore of the β-barrel. This structure supports the model of passenger domain transport through the narrow β-barrel pore and is inconsistent with a model for transport through a central channel formed by an oligomer of translocator domains.

PagL is an enzyme that removes the 3-*O*-acyl chain of LPS, resulting in less toxic LPS. The crystal structure of PagL from *Pseudomonas aeruginosa* reveals a Ser-His-Glu catalytic triad. The surface hydrophobicity and the positions of aromatic residues, strongly suggest an unusual tilt of the β-barrel axis with respect to the membrane. We have modeled a substrate to the active site of PagL, providing insight into the specificity of PagL towards its substrate.

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Keywords: membrane protein structures, membrane channel transport, hydrolase

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X-ray Structures of Phospholipids in Bovine Heart Cytochrome *c* Oxidase

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Cytochrome c oxidase is the terminal oxidase of cell respiration which reduces molecular oxygen to water coupled with proton pumping. The structural and functional investigations of this enzyme have been greatly stimulated by the X-ray structural determinations of the bovine and bacterial enzymes in 1995. However, the composition and conformations of phospholipids have been yet to be determined. Phosphatidylethanolamines(PE), phosphatidylglycerols(PG), phosphatidylcholines(PC) and cardiolipins(CL) were identified in the crystalline bovine heart cytochrome c oxidase preparation by chemical analysis, showing unique fatty acid structures for each phospholipid except PC. All these phospholipids were detectable in the X-ray structure of bovine heart cytochrome c oxidase in the oxidized state at 1.8 Å resolution. The head groups of these phospholipids were located at either one of the surfaces of the transmembrane protein region facing the protein regions protruding to the aqueous phases. A CL located in the intermembrane side bridged the two monomers in the dimeric structure to stabilize the dimer state. The temperature factors of the three head groups of phospholipids (2PG and 1PE) as well as their tail portions in subunit III are unusually low as those of the phospholipids found in X-ray structures of proteins determined thus far, suggesting some physiologically important functions of subunit III, such as the O_2 storage.

Keywords: cytochrome oxidase, phospholipids protein interactions, X-ray crystallography of biological macromolecules

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Structure of Rhodopsin as a G Protein-Coupled Receptor

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In rhodopsin, light-induced isomerization of the 11-*cis*-retinal to its all-*trans* form initiates conformational changes within the transmembrane protein that enable the activated rhodopsin to bind and activate the heterotrimeric G protein transducin on the cytoplasmic surface. The structure of rhodopsin (PDB code 1GZM) was recently determined at 2.65Å resolution [1, 2] in a $P3_1$ crystal form where the packing retains an amphipathic molecular environment similar to that in a membrane, so the transducin-interaction segments from the cytoplasmic interface are likely to represent their native conformations. By docking this crystal structure into a cryo-EM map of 2D crystals [3] we consider the mechanistically important details in the membrane context.

The kinked transmembrane helices enfold the retinal in a tight binding pocket. In the membrane interior, ordered water molecules mediate key interactions. Glu113 with a water molecule hydrogen bonded between its main chain and side chain oxygen atoms forms a complex counterion for the protonated Schiff base linking the retinal to helix H7, which may then stabilise the salt bridge with the protonated Schiff and help to stabilise the dark state. Other water molecules extend the inter-helical hydrogen bonding networks, linking Trp265 in the retinal (ligand) binding pocket to the NPxxY motif on the cytoplasmic side, important for G protein-coupling, and the Glu113 counterion to the extracellular surface. Modulation of these networks is likely to be involved in the activation process.

The G protein interaction sites mapped to the cytoplasmic ends of H5 and H6 and a spiral extension of H5 are elevated above the bilayer. The conserved Glu134-Arg135 ion pair in H3 is sequestered in the walls of a surface cavity. The highest temperature factors in the cytoplasmic loops suggest that they are quite flexible when not interacting with G protein or regulatory proteins. A tightly bound detergent molecule wraps around the kink in H6, stabilizing the structure around the potential hinge in H6. These findings support an activation mechanism that involves pivoting movements of kinked helices, which, while maintaining hydrophobic contacts in the membrane interior, can be coupled to amplified translation of the helix ends near the membrane surfaces.

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Keywords: membrane protein structures, G protein-coupled receptors, ligand binding pocket

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Dephosphorylation of the Calcium Pump Coupled to Conterion Occlusion

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We have crystallized rabbit sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA1a) in complex with aluminium fluoride (E2-AlF) and solved its structure at 3.0 Å resolution using PHASER. We find the planar aluminium fluoride group to be located between the conserved Asp351 side chain and a water molecule positioned for hydrolysis. Further, we find the cation-conducting pathway to be in an occluded and protonated state. Further supported by biochemical data we conclude that the transition state of hydrolysis of the phosphoenzyme intermediate couples with occlusion of bound H+ counter-cations[1]. The mechanism is then similar to that of phosphorylation from ATP, which couples to the occlusion of Ca2+ ions[2]. A regulatory K+ site, identified by difference Fourier analysis using crystals prepared in $RbCl^3$, is observed to stabilize a helix cluster which positions the conserved TGES motif to activate the hydrolysis at the phosphorylation site. Initial identification of crystallization conditions was based on a screen of 48 combinations of PEG, salt, pH and alcohol additives, which we have found to be of general value for the crystallization of Ca2+-ATPase in various functional states.

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Keywords: Ca^{2+}-ATPase hydrolysis, reaction mechanisms, crystallization macromolecular