lysozyme and truncated the N-terminal 19 residues. The structure was determined at the 3.1 Å resolution with a first-generation antihistamine, doxepin. The structure allows us to characterize its ligand-binding pocket in detail. Doxepin sits much deeper in the pocket than the antagonists in other aminergenic G protein coupled receptor (GPCR) structures and directly interacts with the highly conserved Trp428, a key residue in GPCR activation. Asp107, a strictly conserved residue in aminergic receptors, forms an anchor salt bridge with the amine moiety of doxepin. The antihistamine is also surrounded by highly conserved residues among aminergenic receptors including Ile115, Phe424 and Phe432. The well-conserved pocket and its mostly hydrophobic nature contribute to low selectivity of doxepin and other first-generation compounds causing considerable side effects. The pocket is associated with an anion-binding region occupied by a phosphate molecule.

Docking of various second-generation antihistamines reveals that the unique carboxyl-group present in this class of compounds interacts with Lys191 and/or Lys179, both of which form part of the anion-binding region and are not conserved in other aminergenic receptors.

The structural details of the antihistamine-binding pocket of H1R will be highly beneficial for guiding rational design of new antihistamines that do not penetrate the BBB while maintaining H1R selectivity.

Keywords: histamine, receptor, structure

MS.85.3

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Towards a structural understanding of drug and peptide transport within the proton dependent oligopeptide transporter (POT) family

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The proton dependent oligopeptide transporters (POTs) are a large family of integral membrane proteins that use the inwardly directed proton electrochemical gradient to transport small peptides, amino acids and nitrate across cellular membranes in both pro- and eukaryotic cells. Evolutionarily the POT family sits within the much larger Major Facilitator Superfamily (MFS), members of which contain a common structural motif of 12 transmembrane-spanning alpha-helical segments. The human genome contains four members of this family, two of which, PepT1 and PepT2 are responsible for the absorption of dietary peptides in the small intestine and peptide re-absorption in the kidney. Peptide transporters also contribute significantly to the oral bioavailability and pharmacokinetic properties of a number of important drug families, such as the beta-lactam antibiotics. To gain further insight into the molecular mechanism of drug and peptide transport, we determined the crystal structure of a prokaryotic member of the POT family, $PepT_{So}$, with similar substrate specificity and a high degree of sequence conservation to the mammalian PepT proteins [1]. The structure of $PepT_{So}$, together with our associated kinetic data, provides valuable new insights into mammalian peptide transport and provides the starting point for further structural and biochemical studies on this pharmaceutically important transporter family.

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Keywords: major facilitator superfamily, occluded state, peptide transport

MS.85.4

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Molecular basis of substrate-induced permeation by an amino acid antiporter

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Transporters of the amino acid, polyamine and organocation (APC) superfamily play essential roles in cell redox balance, cancer and aminoacidurias. The bacterial L-arginine/agmatine antiporter, AdiC, is the main APC structural paradigm and shares the "5+5 inverted repeat" fold found in other families like the Na+-coupled neurotransmitter transporters. The available AdiC crystal structures capture two states of its transport cycle [1-3]: the open-to-out apo and the outward-facing Arg+-bound occluded. However, the role of Arg+ during the transition between these two states remains unknown. Here, we show the crystal structure at 3.0 Å resolution of an Arg+-bound AdiC mutant (N101A) in the open-to-out conformation, completing the picture of the major conformational states during the transport cycle of the "5+5 inverted repeat" fold-transporters [4]. The N101A structure is an intermediate state between the previous known AdiC conformations. The Arg+-guanidinium group in the current structure presents high mobility and delocalization, hampering substrate occlusion and resulting in a low translocation rate. Further analysis supports that proper coordination of this group with residues Asn101 and Trp293 is required to transit to the occluded state, providing the first clues on the molecular mechanism of substrate-induced fit in a "5+5 inverted repeat" fold-transporter. The pseudo-symmetry found between repeats in AdiC, and in all fold-related transporters, restraints the conformational changes, in particular the transmembrane helices rearrangements, which occur during the transport cycle. In AdiC these movements take place away from the dimer interface, explaining the independent functioning of each subunit.

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Key words: AdiC, APC transporter, 5+5 inverted repeat fold

MS.85.5

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Crystal structure of the copper pump

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Copper ATPases, which belongs to type IB P-type ATPase, derive energy from ATP hydrolysis to maintain intercellular copper homeostasis. Mutations in the two human Copper ATPases ATP7A and ATP7B are responsible for the Menkes and Wilson diseases, respectively. Compared to the Type II P-Type ATPase SERCA1a, P1B-type ATPases have reduced number (6-8) of transmembrane helices and typically one or several heavy metal binding domains (HMBD) in either or both of their terminuses. The molecular understanding of this subfamily, e.g. how copper is delivered to the pumps and then transported through the enzyme and what role the HMBD plays, is significantly impaired by the lack of a high-resolution structure.

We will report a 3.2Å crystal structure of a L. pneumophila copper pump that displays high sequence homology with other IB copper pumps, including human ATP7A and ATP7B. We have caught the protein in the copper-free E2-P state, and its over-all fold is similar to the corresponding state of SERCA1a. The two extra transmembrane helices (Ma and Mb) in the N-terminus are positioned adjacent to the equivalents to TM1 and TM2 of SERCA1. Furthermore, Mb is kinked and assists in the formation of a groove at the membrane interface, which we believe is crucial for copper entrance. A conserved aspartate-methionine pair near the groove and a conserved glutamate on M2 suggests how copper enter and exit the pump, respectively. Side-chain orientations of the residues that form the ion binding sites explain how the high affinity (femtomolar range) copper coordination is distorted and allow copper release. We suggest a complete mechanism for the ion transport though the pump.

We have not been able unambiguously assign where the HMBD is positioned, but our data suggests two possible locations which may explain the suggested dual role of HMBD as a self-regulator and a transient copper-deliverer. Our structure also provides a framework to understand a range of missense mutations associated with the Menkes and Wilson diseases.

Keywords: crystallography, P-type ATPase, copper pump

MS.86.1

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Challenges for refinement at low resolution

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X-ray diffraction plays a pivotal role in understanding of biological systems by revealing atomic structures of proteins, nucleic acids, and their complexes, with much recent interest in very large assemblies. Since crystals of such large assemblies often diffract weakly (resolution worse than 4 Å), we need methods that work at such low resolution. In macromolecular assemblies, some of the components may be known at high resolution, while others are unknown: current refinement methods fail as they require a high-resolution starting structure for the entire complex. Determining the structure of such complexes, which are often of key biological importance, should be possible in principle as the number of independent diffraction intensities at a resolution better than 5 Å generally exceeds the number of degrees of freedom. We recently introduced a new method, termed DEN (Deformable Elastic Network), that adds specific information from known homologous structures but allows global and local deformations of these homology models. Our approach uses the observation that local protein structure tends to be conserved as sequence and function evolve. Cross-validation with R_{free} determines the optimum deformation and influence of the homology model. For test cases at 3.5 – 5 Å resolution with known structures at high resolution, our method gives significant improvements over conventional refinement in the model as monitored by coordinate accuracy, the definition of secondary structure, and the quality of electron density maps.

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Keywords: homology model, model accuracy, macromolecular assemblies

MS.86.2

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Lessons of diffraction resolution and the crustacyanin structures

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The coloration of the lobster shell, famously known from its colour change on cooking, derives from a complicated mix of astaxanthin carotenoid molecules and several proteins in complex. Firstly a structure of one of the two gene-groups of proteins was solved, apocrustacyanin A1, using protein crystallography with softer X-rays (wavelength 2Å) and optimised xenon anomalous scattering, then refined at 1.4Å resolution [1]. This was then used to achieve a molecular replacement solution of the β-crustacyanin dimer complex at 3.2Å in spite of a very high solvent content of ~80% [2]. Crystals of the α-crustacyanin complex of eight β-crustacyanins of molecular weight 320 kDa are available but diffracted poorly thus far [3]. Rigid body fitting of the β-crustacyanin dimer to negative stain electron microscopy (EM) single particle images along with SAXS data of α-crustacyanin have very recently yielded a 30Å structure [4]. At present the molecular tuning parameters causing the 100nm bathochromic shift of the β-crustacyanin are at least known from our work and have already stimulated considerable further research in theoretical and carotenoid chemistry. The further 50 nm bathochromic shift for the α-crustacyanin versus the β-crustacyanin optimally requires higher resolution eg from cryoEM or an X-ray crystal structure of α-crustacyanin. In parallel, several relevant carotenoids have been investigated by chemical crystallography at 0.8Å resolution along with their colours in solution and the crystalline state by UV/Vis spectroscopy [5,6]. In current work (Chayen, Govada, Helliwell and Tanley to be published) experiments involving a micro-beam (ie about 10 microns diameter) scanned across an α-crustacyanin crystal at Diamond Light Source is underway to search for the best ordered portion.

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Keywords: microscopy, SAXS, crystallography

MS.86.3

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Studying membrane fusion at molecular resolution

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