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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

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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

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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

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Studying membrane fusion at molecular resolution

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MS

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Membrane fusion is controlled by, and relies on, the activity of fusogenic proteins that lower the energy barrier of the process and drive membrane bilayer rearrangements. Although high-resolution structures are available for a number of fusion proteins, functional understanding of the fusion process requires studying these proteins directly on membrane bilayers. To this end, the molecular interactions of fusogenic proteins and their conformational changes occur during fusion are probed using cryo electron tomography (cryo-ET). Cryo-ET allows for the 3D visualisation of macromolecular complexes *in-situ* in their native hydrated environment that is achieved by flash freezing of the specimen.

Two distinct fusion processes are examined, namely viral fusion and developmental fusion. The model system for viral fusion in this study is the herpes simplex virus 1 (HSV-1) that requires four glycoproteins– gB, gD and gH/L – for entry. We have determined the structure of trimeric gB, presented on liposomes, using cryo-ET and applying 3D averaging methods. The gB crystal structure was further fitted into the EM map. The structure revealed that gB was inserted into liposomes via its fusion loops and this insertion induced a distinctive curvature of the outer leaflet of the target membrane. We suggest that this re-shaping of the outer leaflet membrane constitutes a key step in viral and cellular membrane fusion.

The model system for developmental fusion is the eukaryotic developmental cell-cell fusion proteins AFF-1 and EFF-1 of *C. elegans* (CeFF) that were recently described. We have characterized the structure of CeFF proteins presented on pseudotype vesicular stomatitis virus using cryo-ET. Side views of individual spikes were apparent in central sections of the tomograms and suggested CeFF induced membrane bending. Higher order assemblies in the form of penta- or hexa- meric "flower" shaped could be observed in slices through the tomograms oriented peripheral to the pseudotyped virus particles. It is plausible that these supercomplex arrays may have a critical function in bending and deforming plasma membranes to mediate fusion and might constitute the minimal fusion machinery.

Keywords: electron, tomography, fusion

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Low resolution electron crystallography challenges in organic and inorganic crystals with transmission electron microscope (TEM)

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Despite its success in solving crystal structures, X-Ray diffraction has serious limitations to deal with structures of nm size level. After the discovery of precession electron diffraction (PED) in TEM several nanocrystal structure determinations have been obtained so far [1]. In contrast with macromolecules obtained X-Ray resolution data (range close to 0.1 nm),TEM obtained precession electron diffraction data may have diffraction resolution up to 0.02 nm in oriented zone axis (ZA) patterns.Although the alternative to solve crystal structures from TEM collected data looks promising specially for small molecules via 3D automatic diffraction tomography techniques[2],the problem is that structure solution from reflections collected from either oriented ZA PED patterns or from automatic diffraction tomography techniques from the same nanocrystal is limited by the "missing cone" problem; such data cannot be recovered because of the TEM tilting stage limitations (tilt range usually from -45° to + 45°). Besides this limitation, is possible to elaborate strategies (particularly good for beam sensitive organic crystals) where one collects either manually or automatically several hundreds of electron diffraction patterns through a fast scanning in PED mode of a whole area (eg 5x5 μm) containing several nanocrystals.As a result of the fast rate scanning, all collected PED patterns can be recorded without using cryo-techniques; in addition, use of PED will reveal many possible "perfectly oriented ZA patterns" for those crystals that were accidentally close (few degrees)to perfect ZA orientations [3]. Collecting such data set may contain many ZA reflections without "missing cone" data limitations (Fig.1).Such data set from randomly oriented ZA electron diffraction patterns are usually adequate to calculate ab-intio crystal cell parameters [3] and for ab-initio solving crystal structures of small organic or inorganic crystals.

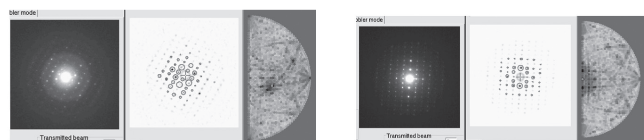

Fig 1 : (left) : [211] ZA PED pattern of penicillin G potassium and its corresponding orientation within the stereographic projection (right) penicillin G PED pattern [001] ZA.Samples from C.Giacovazzo , Bari University.

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Keywords: electron crystallography, precession electron diffraction

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Low resolution neutron crystallography of biological macromolecules

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Neutron crystallography provides direct experimental information on hydrogen/deuterium atom positions, solvent structure and arrangement in macromolecular complexes [1-3]. Crystallography has long moved from the point when these were parameters seen as a surplus in the light of other experimental challenges. Beyond atomic resolution structures, where protonation states must be assumed even with X-ray data resolution better than 0.7Å [4] and solvent effects easily missed, lies a true grasp of function in the context of large macromolecular assemblies and specific biological environments.

Neutron crystallography was historically seen as a time consuming,