Benjamin Podbilewicz,[b] Kay Grünewald,[a,b] [a]*Oxford Particle Imaging Centre, Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, OX3 7BN, (UK).* [b]*Department of Molecular Structural Biology, Max Planck Institute of Biochemistry, Martinsried, 82152, (Germany).* [c]*Institute of Biotechnology, University of Helsinki, Helsinki, 00014, (Finland).* [d]*Department of Biology, Technion - Israel Institute of Technology, Haifa 32000, (Israel).* [e]*Department of Microbiology, School of Dental Medicine, and Department of Pathobiology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104-6002, (USA).* E-mail: tzviya@strubi.ox.ac.uk

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

MS.86.4

Acta Cryst. (2011) A**67,** C188

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

StavrosNicolopoulos,[a] EdgarRauch,[b] D.Georgieva, J.P.Abrahams,[c] [a]*NanoMEGAS SPRL, Blvd Edmond Machtens 79, B-1080 (BrusselsBelgium).* [b]*SIMAP/GPM2 laboratory CNRS-Grenoble INP, BP 46101 rue de la Physique, 38402 Saint Martin d'Heres, (France).* [c]*Department of Biophysical and Structural Chemistry, LeidenUniversity, (The Netherlands).* E-mail: info@nanomegas.com

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.

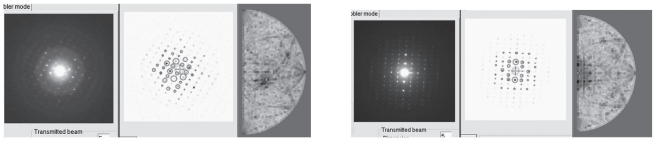


Fig 1 : (left) : [211] ZA PED pattern of penicillin G potassium and its corresponding orientation within the stereographic projection (right) penicillin G PED pattern [001] ZA.Samples from C.Giacovazzo , Bari University.

[1] *Ultramicroscopy, Elcryst2005 Proceedings* **2007**, *107,* 6-7. Editors S. Nicolopoulos, T. Weirich. [2] E. Mugnaioli, T. Gorelik, U. Kolb, *Ultramicroscopy* **2009**, *109*, 758-765 [3] D. Gueorguieva, PhD Thesis, Leiden Univ. **2008** *Electron crystallography of three dimensional protein crystals.*

Keywords: electron crystallography, precession electron diffraction

MS.86.5

Acta Cryst. (2011) A**67,** C188-C189

Low resolution neutron crystallography of biological macromolecules

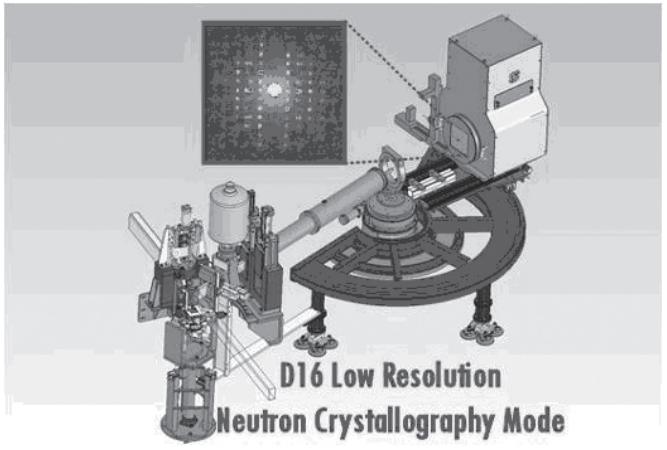
Susana C.M. Teixeira,[a,b] J. Allibon,[b] S. Mason,[b] T. Forsyth,[a,b] B. Demé,[b] M. Johnson,[b] [a]*EPSAM, Keele University, Keele, Staffordshire ST5 5BG, (U*K). [b]*Institut Laue Langevin, 6 Rue Jules Horowitz, 38042 Grenoble cedex 9, (France).* E-mail: teixeira@ill.fr

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,

complex technique requiring prohibitive crystal volumes and long data collection times. The technique came of age as a result of developments in many areas from sample preparation to instrumentation [5], alongside developments in complementary structural methods.

The particular case of low resolution neutron crystallography will be presented in the context of the upgrading of the monochromatic D16 neutron beamline at the Institut Laue Langevin (ILL). Similar experiments were previously carried out on the DB21 beamline at the ILL, where the neutron flux and detector sensitivity were poorer. A low resolution crystallography mode is under commissioning on D16 where users will use cold neutrons to collect neutron diffraction data on crystals of 0.01mm^3 or smaller. The role of the technique in the modern structural biology scenario will be demonstrated: typical applications and sample environment will be described, namely for membrane proteins where contrast variation is used to study areas in contact with the membrane *in vivo*. Obtaining suitable crystals of membrane proteins is still a challenge and information on the packing of detergent and membrane proteins in the crystal will be a significant contribution to filling an historical gap between crystallography and membrane proteins.



[1] S.C.M. Teixeira, G. Zaccai, et al., *Chemical Phys.* **2008**, *345*, 133-151. [2] M.P. Blakeley, S.C.M. Teixeira, I. Petit-Haertlein, I. Hazemann, A. Mitschler, M. Haertlein, E. Howard, A.D. Podjarny, *Acta Cryst.* **2010**, *D66*, 1198-1205. [3] M.P. Blakeley, P. Langan, N. Niimura, A. Podjarny, *Curr. Opin. Struct. Biol.* **2008**, *18(5)*, 593-600. [4] A. Podjarny, A. Mitschler, I. Hazemann, T. Petrova, F. Ruiz, M. Blakeley, M.T. Dauvergne, F. Meilleur, M. Van Zandt, S. Ginell, A. Joachimiak, D. Myles, *Acta Cryst.* **2004**, *A60*, s155. [5] T. Gutberlet, U. Heinemann, M. Steiner, *Acta Cryst.* **2001**, *D57*, 349-354.

Keywords: neutron, contrast-variation, crystallography

MS.87.1

Acta Cryst. (2011) A**67,** C189

Handedness of two-fold helices and chiral space-groups

Mikiji Miyata, Norimitsu Tohnai, Ichiro Hisaki, Toshiyuki Sasaki, *Departament of Material and Life Science, Graduate School of Engineering, Osaka University, Suita, Osaka 565-0871 (Japan).* E-mail: miyata@mls.eng.osaka-u.ac.jp

We have not so far discussed about right- or left-handedness of enantiomeric assemblies with two-fold helices. In fact, a two-fold helical axis with 180 degrees rotation and translation has only a symbol 2_1, while a distinguishable three-fold helical axis has two symbols; 3_1 for a right-handed helix and 3_2 for a left-handed helix. In daily life, one can use right- or left-handed stairs. When one walks upstairs, turns to the left on staircase landing, and further go upstairs, the stairs can be recognized to be right-handed. The reverse right-turn yields left-handed stairs.

We investigated supramolecular chirality for 1D assemblies of benzene molecules. Benzene molecules form right-handed 2_1 helical assembly with side-to-side contact in channels of inclusion crystals of cholic aicd [1]. It was confirmed that polymorphic crystals of benzene itself have 2_1 helical assemblies with two and three kinds of molecular contacts for $P2_1/c$ and *Pbca*, respectively. Moreover, we analyzed 1D hydrogen bonding networks of primary ammonium carboxylates. The networks have 2_1 helical connection among nitrogen and oxygen [2].

We termed such enantiomeric relation of the two-fold helices as supramolecular tilt chirality [3], [4]. This handedness definition has consistency with the helical discrimination for polymeric materials such as DNA and proteins. It is no doubt that a large amount of organic crystals consist of molecular assemblies with right- or left-handed helices, likewise biopolymers. The Cambridge Structural Database involves over 500,000 data till 2011. Among them, over seventy percent have space groups with two-fold helices. Moreover, it was found that supramolecular chirality lies in each step of hierarchical structures, such as helical molecular assemblies, bundles of the helices, complimentary helical assemblies of host and guest components.

In the crystallographic theory, the concept of both point groups and space groups in mathematics is described as assemblies of points, where materials are approximated as a single point. The theory based on one-point approximation is surely correct in the case of atoms and ions with spherical symmetry, but is not considered to be always correct in the case of molecules, particularly, organic molecules with various shapes. These molecules require multi-point approximation methods instead of the one-point method. Now we know that a two- and three-point approximation method enables us to discriminate right- or left-handedness of 2_1 helical assemblies.

Among 65 chiral space-groups, only eleven groups are split into the enantiomeric pairs, while the remaining 43 groups are not split into the pairs. This mainly comes from the crystallographic theory that two-fold rotations have no handedness. We checked handedness of space-groups involving 2_1-, 4_2-, 6_3-helices, two-, three-, four-, six-rotations as well as asymmetric $P1$ group with multipoint approximation methods. As a result, the 43 chiral space-groups were found to be split into the enantiomeric pairs.

[1] A. Tanaka, I. Hisaki, N. Tohnai, M. Miyata, *Chem. Asian J.* **2007**, *2*, 230-238. [2] T. Yuge. T. Sakai, N. Kai, I. Hisaki, M. Miyata, N. Tohnai, *Chem. Eur. J.* **2008**, *14*, 2984-2993. [3] M. Miyata, N. Tohnai, I. Hisaki, *Acc. Chem. Res.* **2007**, *40*, 694-702. [4] I. Hisaki, N. Tohnai, M. Miyata, *Chirality* **2008**, *20*, 330-336.

Keywords: organic crystal, helical structure, space groups

MS.87.2

Acta Cryst. (2011) A**67,** C189-C190

Molecular building block approach to chiral coordination polymers and noncovalent porous materials

Iwona Justyniak,[a] Tomasz Kaczorowski,[a] Daniel Prochowicz,[b] Janusz Lipkowski,[a] Janusz Lewiński,[a,b] [a]*Institute of Physical Chemistry, Polish Academy of Sciences, Kasprzaka 44/52, 01-224 Warsaw, (Poland).* [b]*Department of Chemistry, Warsaw University of Technology, Noakowskiego 3, 00-664 Warsaw, (Poland).* E-mail: ijustyniak@ichf.edu.pl

Among the vast library of building blocks simple achiral bipyridines are the most commonly used linkers for constructing metal-organic frameworks (MOFs), but their chiral derivatisation have encountered difficulties. Therefore, developing versatile strategies for facile generation of chiral bipyridine-type ligands is the key challenge for