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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.

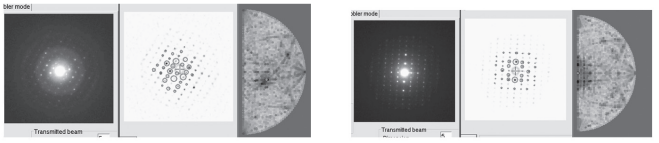

Fig 1 : (left) : [211] ZA PED pattern of penicillin G potassium and its corresponding orientation within the stereographic projection (right) penicillin G PED pattern [001] ZA.Samples from C.Giacovazzo , Bari University.

[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.*

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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,