

MS11. Hybrid approaches

Chairs: Guillermo Montoya, Jan Pieter Abrahams

MS11-O1 Two-dimensional membrane protein crystallography at X-FELs

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Compared to their three-dimensional counterparts, two-dimensional membrane protein crystals may offer the advantage of providing a more close-to-physiological environment for the protein molecules, in particular by reducing the risks of structural deformations due to non-native contacts. Moreover, quenching of possible structural motions, triggered by external stimuli, can be overcome. Unfortunately, the diffracting power of two-dimensional protein crystals is small, which makes radiation damage an insurmountable obstacle for X-ray diffraction at synchrotron sources. Free electron lasers now provide ultrashort and ultraintense X-ray pulses, which permit to acquire diffraction data before radiation damage has taken place. We report on the diffraction experiments performed at room temperature on bacteriorhodopsin two-dimensional crystals, using the submicrometer X-ray beam available at the CXI station of the LCLS free electron laser. The data demonstrate that the samples diffract to at least 4 Å of resolution. The results allow evaluating the potential and the limitations of two-dimensional crystallography at X-FEL, which emphasizes the role of the approach as a complementary technology to cryo electron microscopy.

Keywords: 2D crystals, membrane proteins, X-FEL, radiation damage

MS11-O2 Xen crystallography - choose your radiation

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Crystallographic structure determination can be carried out with X-rays, electrons, or neutrons as wave source. The number of 95,599 X-ray structures in the PDB [1] outnumbers 80 neutron structures and 47 electron structures by far. At present, however, the latter two technologies gain more and more interest, and this is for good reasons: they offer advantages over X-rays that make them complementary methods one should not forget to take into consideration when planning your experiment. In this talk I will relate to the specific choice of type of radiation with the presentation of two recent publications: The first one combines Molecular Dynamics with Anomalous X-ray dispersion in order to prove the K⁺ ions are transported through membrane channels by a "Knock-on" transport. This result knocks over a 60 year old dogma that assumed the co-transport of water molecules with K⁺ ions as shield to their electrostatic repulsion [2].

The second topic explains how to properly combine the results from X-ray data acquisition with neutron data. Common practise co-refines both data sets. I will argue that one should better treat separate experiments with separate refinement and explain how to combine both methods properly. Furthermore, hydrogen atoms, usually the focus of interest for macromolecular neutron data, should be refined rather than constrained. I will argue for separate refinement. Careful data interpretation is necessary at low data completeness often associated with neutron data, in order to soundly and reliably present your findings [3].

[1] www wwPDB.org, H.M. Berman, K. Henrick, H. Nakamura (2003) "Announcing the worldwide Protein Data Bank" *Nature Structural Biology* 10 (12): 98.

[2] D. A. Köpfer, C. Song, T. Gruene, G. M. Sheldrick, U. Zachariae, B. L. de Groot "Ion Permeation in K⁺ Channels Occurs by Direct Coulomb Knock-On" *Science*, 2014, 346 (6207) 352-355

[3] T Gruene, HW Hahn, AV Luebben, F Meilleur, GM Sheldrick "Refinement of Macromolecular Structures against Neutron Data with SHELXL-2013" *J. Appl. Cryst.* 2014, 47, 462-466

Keywords: X-ray diffraction and Molecular Dynamics, Hydrogen Positions from Neutron Data, Assessment of Data Quality