MS3-P2 Two-dimensional protein crystallography at FELs

Cecilia M. Casadei[1], Bill Pedrini[1]

1. Paul Scherrer Institute

email: cecilia.casadei@psi.ch

Membrane proteins are involved in a number of crucial and diverse biological functions ranging from cross-membrane transportation to signal reception. The structural characterization of these macromolecules is hindered by the difficulty in producing well diffracting three-dimensional crystals, limiting the range of application of conventional X-ray crystallography in these systems.

Membrane proteins arrange favorably in periodic monolayers in a lipidic environment. This configuration presents the advantage that molecules are found in a close-to-physiological arrangement. In addition, the use of ultrashort free electron laser (FEL) X-ray pulses allows to largely outrun radiation damage phenomena in biological samples at ambient temperature.

Two-dimensional (2D) protein crystals of bacteriorhodopsin proved to give rise to diffraction spots to resolution of 4Å at the Linac Coherent Light Source FEL, where the variable geometrical configuration of the experiment opens up to the possibility of collecting diffraction intensities along reciprocal space Bragg rods (B. Pedrini *et al., Phil. Trans. R. Soc. B* **369**, 2014). The main bottleneck resides to date in 2D data processing and reduction due to the absence of dedicated software in conventional X-ray crystallography software suites.

The most recent advancements in 2D crystallography data treatment will be presented with particular focus on the opportunities and challenges of using intensities collected along reciprocal space rods.

Keywords: 2D-crystallography, membrane proteins, FEL

MS3-P3 FIP-BM30A at the ESRF: an automated beamline for protein crystallography with unique features

Jean-Luc Ferrer[1], Yoann Sallaz-Damaz[1], Xavier Vernede[1], Michel Pirocchi[1], Christophe Berzin[1], Monika Budayova-Spano[1], Pascale Israel-Gouy[1], Franck Borel[1], David Cobessi[1]

1. Institut de Biologie Structurale, Grenoble, France

email: jean-luc.ferrer@ibs.fr

FIP-BM30A is an automated beamline for protein crystallography at the ESRF. But it is also a very flexible beamline, that can host a large variety of experiments, such as on-line absorption spectroscopy, humidity controlled, micro-ship *in situ* diffraction, Etc.

From the seminal work accomplished on beamline FIP-BM30A (ESRF) in 2000' to the present developments, robot based systems significantly changed the crystallography experiment strategy. They open possibilities for new strategies, give a high flexibility to the experimental setup, and make automation and remote control much easier. The robotized platform in operation on FIP-BM30A, named G-Rob, plays as a fully integrated, multi-purpose automated and remotely controlled diffractometer. G-Rob integrates several functions: classical sample changer; goniometer for frozen samples or capillaries [1], including frozen sample transfer from a storage Dewar; crystallization trays handling for *in situ* screening and data collection on crystallization plates and microchips [2]; beam monitoring; on line crystal fluorescence/absorption; crystal harvesting; Etc. Thanks to its tool changer, the robot arm can go automatically from one application to another. G-Rob can be easily upgraded with new functions. As an example of this flexibility, the G-Rob system on FIP-BM30A was recently expended with a new functionality: a dual gripper for SPINE-format sample holder, that makes possible the sample dismounting/mounting operation in a single trajectory, reducing this way the cycle time for sample exchange by a factor ~2.

Among the last results obtained with G-Rob are: (i) Automated structure resolution at room temperature (*in situ*), for the analysis of protein dynamic; (ii) Automated structural screening for the fragment based drug design strategy. New functions are also under development, such as the remote controlled robotized crystal harvesting [3].

Another, but important, feature of beamline FIP-BM30A is a new web-based user interface, named WIFIP. With this interface, several users can share the control of the experiment, from sample handling to data reduction, through a web browser, on the beamline or from the lab or home. Web-based technology makes the access easy, and the communication very fluid, even with a limited bandwidth.

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