SLS Symposium on

Diffraction and Macromolecular Crystallography

Tuesday, May 8, 2018

10:00 to 12:15, SPECIAL ROOM: WHGA/001/Auditorium

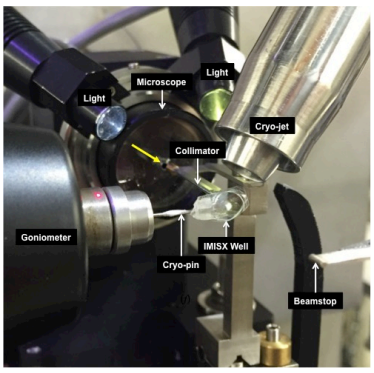
10:00 Fragment screen targeting tubulin
Tobias Mühlethaler, M. E. Sharpe, A. Douangamath, A. E. Prota, and M. O. Steinmetz

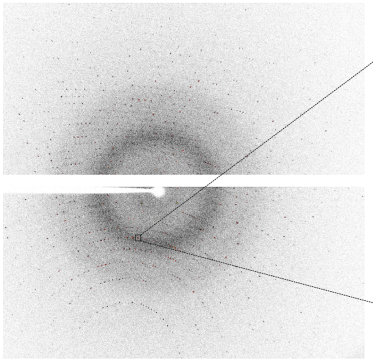
10:30 Advanced data collection strategies for experimental phasing at the SLS
Shibom Basu, V. Olieric, C-Y Huang, J. A. Wojdyla, J. W. Kaminski, E. Panepucci, and M. Wang

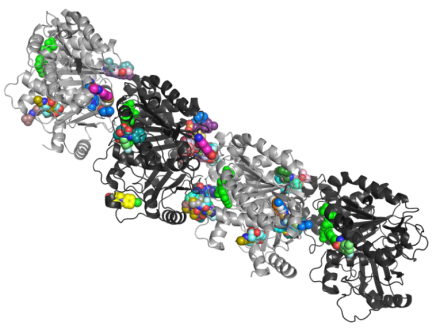
11:00 Coffee break

11:15 In meso in situ serial crystallography (IMISX) of membrane proteins using automatic data collection program at Swiss Light Source (SLS) macromolecular crystallography (MX) beamline
Chia-Ying Huang, V. Olieric, N. Howe, E. Panepucci, S. Basu, J. Wokdyla, J. Kaminski, R. Warshamanage, M. Caffrey, and M. Wang

11:45 Development of JUNGFRAU Detector for Macromolecular Crystallography
Filip Leonarski, S. Redford, A. Mozzanica, O. Bunk, B. Schmitt, and M. Wang







Fragment screen targeting tubulin

Tobias Mühlethaler[1], May E. Sharpe[2], Alice Douangamath[3], Andrea E. Prota[1], Michel O. Steinmetz[1,4]

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Microtubule filaments are a major component of the cell cytoskeleton and are involved in various activities like cell division, cell motility, and intracellular transport. They are formed from the protein tubulin that can undergo cycles of polymerization and depolymerization. To perform their various functions, microtubules must be highly dynamic. The so called dynamic instability of microtubules is naturally regulated by a large number of accessory proteins but can also be affected by microtubule-targeting drugs. These drugs are used to treat cancer as they disrupt the process of cell division of the highly proliferating tumor cells.

So far, six different binding sites have been identified on tubulin. While the structures as well as the modes of action between the drugs targeting these sites vary they share one common trait: they are large and complex molecules. Hence, they are difficult and expensive to synthesize as well as not orally bioavailable lowering patient comfort. To overcome this limitation new starting points for drug development have to be found. X-ray-based fragment screening represents such an option as fragments are of low molecular weight and are able to target smaller, yet unidentified binding pockets on tubulin.

Here, we present our findings from our X-ray-based fragment screening campaign at the XChem facility at Diamond using our well established tubulin crystal system. We were able to identify 59 fragments binding to four known as well as additional six newly identified binding sites. Overall, we found five common binding motifs targeting different pockets on tubulin showing promising selectivity. Taken together, we now have various small molecule scaffolds as starting points to develop either new microtubule-targeting agents or biochemical tools for the better understanding of microtubule dynamics.

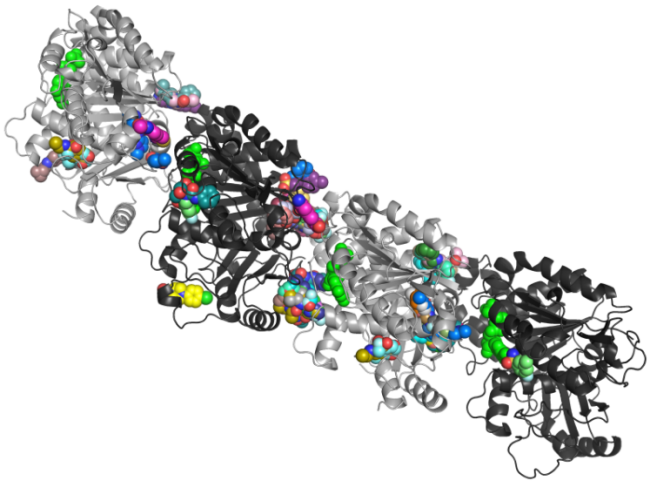

Figure 1: Fragment hits (in spheres representation) bound to a complex consisting of two tubulin subunits (black and grey ribbons).

Advanced data collection strategies for experimental phasing at the SLS

Shibom Basu[1], Vincent Olieric[1], Chia-Ying Huang[1], Justyna Aleksandra Wojdyla[1], Jakub W. Kaminski[1], Ezequiel Panepucci[1], and Meitian Wang[1]
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Phasing is the last hurdle in *de novo* structure determination using macromolecular X-crystallography (MX). Single Anomalous Dispersion (SAD), the most popular choice of phasing methods, requires either soaking of heavy elements or protein expression with selenomethionine, which is often a time-consuming and difficult process. Thereby, a SAD experiment exploiting the anomalous signals from low Z elements (mainly, S, P, Cl, Ca) natively present in the biomolecules – called native-SAD method – appears as a 'magic-wand' for *de novo* structure determination. At the SLS, we developed data collection strategies for both conventional crystals (50 -100 μm) and small crystals (< 30 μm).

At beamline X06DA, we implemented a multi-orientation high multiplicity low-dose data collection strategy for conventional crystals [1,2]. Using a multi-axis goniometer (PRIGo), we applied the method successfully on more than 100 cases, including 43 *de novo* structures, over the last 3 years. Key to the success of this strategy is high multiplicity, which may mean hours of measurement in unfavorable cases of low resolution, low symmetry, or high dose sensitivity. To address such cases, we developed an automated and fast strategy using the high precision multi-axis goniometer SmarGon, together with the fast noise-free EIGER16M detector. At an unprecedented speed of 60°/sec and in a fully automated manner, we applied our strategy on 10 different real-life targets, including one human membrane protein, large complexes, and a large triclinic structures. Native-SAD experiments on all these structures were performed in time ranging from 30 s to maximally 7 min. Clearly, the rapidity and simplicity of this strategy, which is widely applicable at most MX beamlines, promote native-SAD as the primary choice for experimental phasing.

For micron-sized crystals (< 30 μm) – typically mounted on mesh, loop or fixed target chip – small wedges (10 - 20°) can be collected using serial synchrotron crystallographic (SSX) approach [3]. SSX strategy facilitates data collection from small and weakly diffracting membrane proteins crystals in particular. For such cases, we developed *in meso in situ serial crystallography* (IMISX) [3,4], which enables diffraction measurement *in situ*, *i.e.* without crystals harvesting. In order to meet the growing demands from SSX users and increase the throughput, a complete automation from crystal identification, data collection, to analysis, scaling and merging of hundreds of datasets has been developed. The software suite, which runs in parallel to data collection, now routinely supports SSX measurement for both academic and industrial users.

Reference:

[1] Weinert *et al.*, (2015) *Nat. Methods*, **12**, 131-133
[2] Olieric *et al.*, (2016) *Acta Cryst.* **D72**, 421-429
[3] Huang *et al.*, (2015) *Acta Cryst.* **D71**, 1238-1256
[4] Huang *et al.*, (2016) *Acta Cryst.* **D72**, 93 - 112

In meso in situ serial crystallography (IMISX) of membrane proteins using automatic data collection program at Swiss Light Source (SLS) macromolecular crystallography (MX) beamline

Chia-Ying Huang[1], Vincent Olieric[1], Nicole Howe[2], Ezequiel Panepucci[1], Shibom Basu[1], Justyna Wokdyla[1], Jakub Kaminski[1], Rangana Warshamanage[1], Martin Caffrey[2], Meitian Wang[1]

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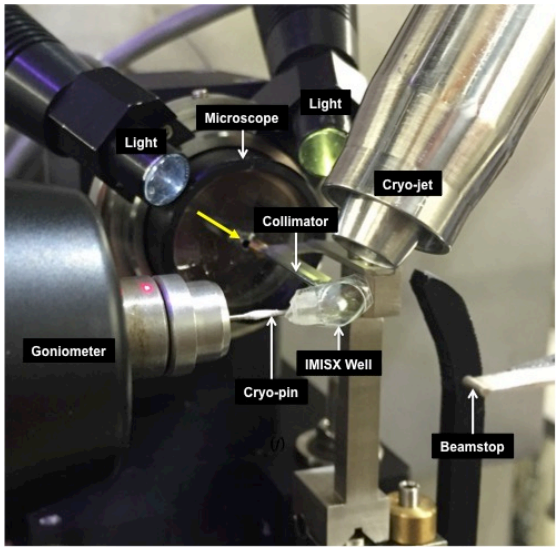
Membrane proteins perform essential roles in signal and energy transduction, metabolism and transport, and contribute to the structural integrity of cells. In order to understand how membrane proteins work at a molecular level, high-resolution three-dimensional structural information is needed. Lipid cubic phase (LCP) provide a closer analogue of native membranes to support crystallization and resulted in crystals with better packing and higher diffraction resolution (1). However, the glass plates designed for crystallization trial of LCP crystals are challenging to harvest crystals from. To response this challenge and allow the screening of diffraction quality crystals in LCP with minimal perturbation, we have recently developed the IMISX method for serial crystallography data collection of membrane proteins (2, 3). With the method, hundreds of tiny crystals in LCP can be collected *in situ* in a serial and high-throughput manner at beamline X06SA at the Swiss Light Source by using the variable beam size, fast detector, and dedicated data collection software (4). The method has been validated with a wide range of membrane proteins including GPCR and applied to solve a *De Novo* structure (2, 3, 5). The IMISX method works with inexpensive materials and can be adopted at most macromolecular crystallography synchrotron beamlines.



References

[1] Caffrey, M., and Cherezov, V. (2009) Nat. Protoc. 4:706-731. [2] Huang, C.-Y. *et al.,* (2015) Acta Cryst. D71, 1238-1256. [3] Huang, C.-Y. *et al.,* (2016) Acta Cryst. D72, 93-112. [4] Wojdyla, J. A. *et al.,* (2018) J. Synchrotron Rad. 25: 293-303. [5] Ghachi *et al.,* (2018) Nat. Commun. 9:1078.

Development of JUNGFRAU Detector for Macromolecular Crystallography

Filip Leonarski, Sophie Redford, Aldo Mozzanica, Oliver Bunk, Bernd Schmitt, Meitian Wang

Swiss Light Source, Paul Scherrer Institut

Photon-counting detectors have made a revolutionary impact on synchrotron macromolecular crystallography (MX) in the last decade and changed MX data collection methods and strategies fundamentally. However, in the emerging fields of MX like low-energy phasing and high flux serial crystallography, even the current state-of-the-art detectors are not adequate. Here we describe the application of a direct detection charge integrating pixel-array detector (JUNGFRAU) for such emerging MX techniques. The JUNGFRAU achieves both single-photon sensitivity and high dynamic range without count-rate limitation by a dynamic gain switching technology. The improvement over the previous generation of detectors will be presented using one of the most challenging phasing methods in MX with both test and real-life examples. The gain in data accuracy and acquisition speed makes JUNGFRAU very suitable for next generation MX beamlines at diffraction-limited storage rings.

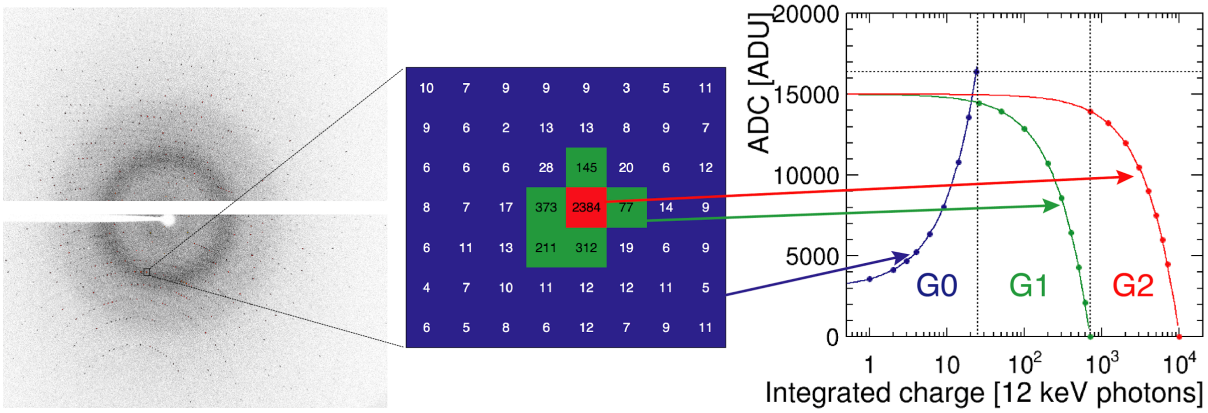


Figure 1 A diffraction pattern measured with a JUNGFRAU 1Mpixel detector and an illustration of the extended dynamic range with three gain levels (G0, G1 and G2).