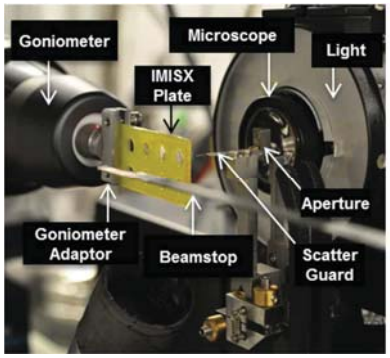


SWISS LIGHT SOURCE SLS

SLS Symposium on

Macromolecular Crystallography

Tuesday, February 9th, 2016

10:00 to 12:15, WBGB/019

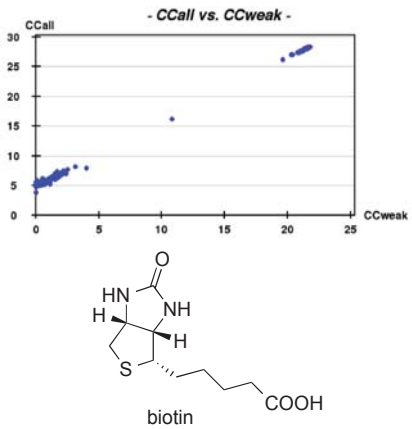
10:00 Dataset merging in serial crystallography
R. Warshamanage, V. Olieric, M. Wang and K. Diederichs

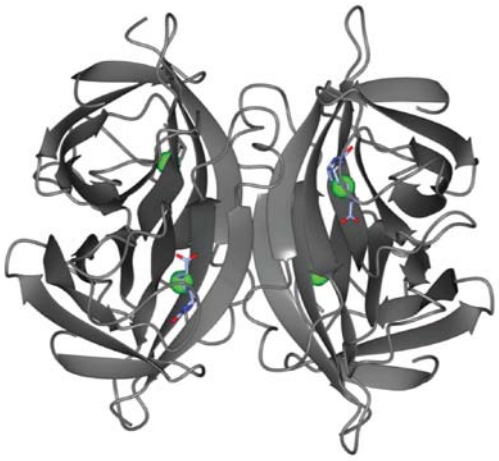
10:30 Finer data collection with EIGER detector
Arnau Casanas, Marcus Mueller, Clemens Schulze-briese and Meitian Wang

11:00 Coffee

11:15 Recent Advances in Synchrotron Data for Experimental Phasing by Anomalous Dispersion
Aaron D. Finke

11:45 Serial crystallography with XFEL and synchrotron sources
Tobias Weinert





Dataset merging in serial crystallography

R. Warshamanage[1], V. Olieric[1], M. Wang[1], K. Diederichs[2]

[1]*Swiss Light Source, Paul Scherrer Institute, CH 5232 Villigen, Switzerland*

[2]*Fachbereich Biologie, Universität Konstanz, D-78457 Konstanz, Germany*

rangana.warshamanage@psi.ch

In the synchrotron serial crystallography experiments the sub-micron crystals in the *meso*-phase are sandwiched between thin synthetic cyclic olefin copolymer plates [1]. These tiny crystals are then measured *in situ* with a synchrotron X-ray microbeam as shown in figure 1. Small rotations of crystals are achieved by turning the whole plate mounted on the goniometer thereby allowing to record full intensities of reflections. In this geometry the completeness of each individual dataset is not only dependent on the diffracting power of the crystal but also on the rotation angle, which in turn depends on the crystal morphology and the location of the crystal in the *meso*-phase. A full dataset is recovered by merging many partial datasets, but prior to merging them the isomorphous datasets should be identified. Our approach on identifying isomorphous datasets is based on the correlation coefficient between individual datasets [2]. The exact dataset-selection procedure and our recent results will be presented.

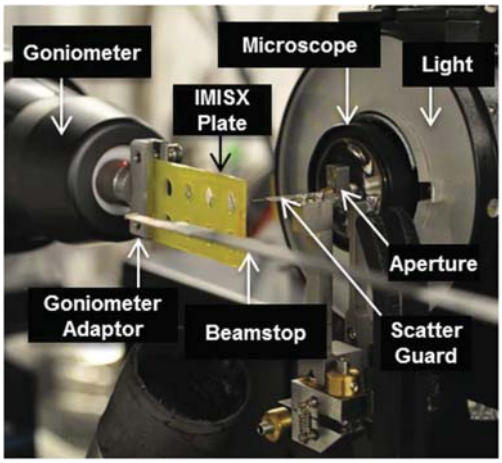


Figure 1: A view of a section of an IMISX plate in the goniometer positioned for SX data collection on beamline PXII (X10SA) at SLS.

Reference:

1. Huang, C.-Y., Olieric, V., Ma, P., Panepucci, E., Diederichs, K., Wang, M. & Caffery, M. (2015). *Acta. Cryst.*, **D71**, 1238-1256
2. Huang, C.-Y., Olieric, V., Ma, P., Howe, N., Vogeley, L., Liu, X., Warshamanage, R., Weinert, T., Panepucci, E., Kobilka, B., Diederichs, K., Wang, M. & Caffery, M. (2016). *Acta. Cryst.*, **D72**, 93-112

Finer data collection with EIGER detector

Arnau Casanas, Marcus Mueller, Clemens Schulze-briese, Meitian Wang

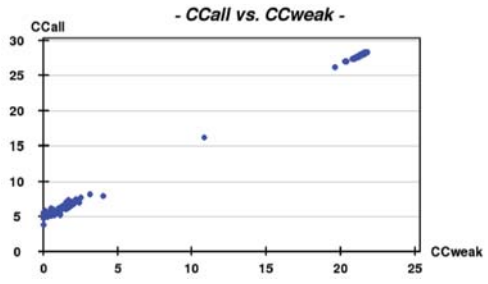
Single photon-counting devices developed in recent years have represented a major breakthrough in detector technology enabling noise-free detection and novel acquisition modes. The Dectris EIGER detector offers a pixel size of 75 × 75 μm^2, frame rates up to 3 kHz and a dead-time of 3.8 μs. An EIGER 1M prototype was tested at the Swiss Light Source beamline X10SA for its application in macromolecular crystallography. The combination of the fast frame-rate and the very short dead-time enables finer data collection plus the numerous advantages associated to higher rotation speeds. A careful analysis of the image summation of extremely fine phi-sliced images yields the best overall statistics/data and can also be used to optimize the dose by finding the best the balance between diffraction and radiation damage. Data collected on both test and challenging crystals will be presented. In addition, the latest results obtained with an EIGER 16M will also be reported.

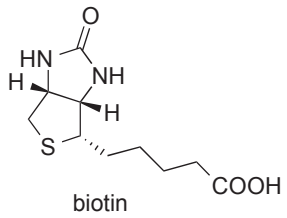
Recent Advances in Synchrotron Data for Experimental Phasing by Anomalous Dispersion

Aaron D. Finke

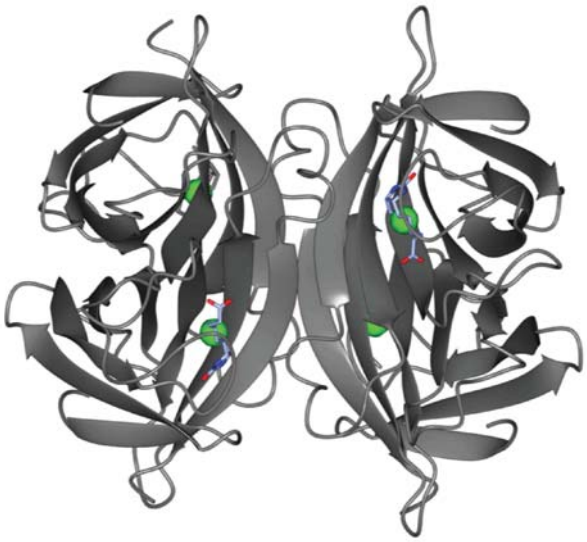
MX-Group, Swiss Light Source, Paul Scherrer Institute, Paul Scherrer Institute

Experimental phasing by single- or multiple-wavelength anomalous diffraction (SAD or MAD) is the most popular method of de novo macromolecular structure determination. Recent overhauls at third-generation synchrotron light sources have dramatically sped up phasing data collection protocols. In particular, the reintroduction of multi-axis goniometers has enabled two major advances in anomalous data collection: the ability to collect "true" high-multiplicity, low-dose datasets on a single crystal entity, maximizing the anomalous signal output while minimizing both systematic and random measurement errors;[1] and alignment of even-fold crystal symmetry axes along the spindle axis to collect Bijvoet pairs, thus eliminating time-dependent errors in anomalous signal such as beam inhomogeneity, detector errors, and radiation damage.[2] We present our results in applying both strategies to challenging problems in experimental phasing, for example, the native-SAD solution of the streptavidin:biotin complex, shown below.





biotin



References

1. Weinert, T.; Olieric, V.; *et al.* "Fast native-SAD phasing for routine macromolecular structure determination," *Nat. Methods* **2014**, *12,* 131–133.
2. Finke, A. D.; Panepucci, E.; Vonrhein, C.; Wang, M.; Bricogne, G.; Olieric, V. "Advanced Crystallographic Data Collection Protocols for Experimental Phasing," *Methods Mol. Biol.* **2016**, *1320*, 175–191.

Serial crystallography with XFEL and synchrotron sources

Tobias Weinert[1], Przemyslaw Nogly[1], Natacha Olieric[1], Kathrin Jäger[1], Daniel James[1], Dardan Gashi[1], Florian Dworkowski[2], Ezequiel Panepucci[2], James Leuenberger[2], Meitian Wang[2], Michel Steinmetz[1] and Jörg Standfuss[1]

[1] Laboratory of Biomolecular Research, Paul Scherrer Institut
[2] Swiss Light Source, Paul Scherrer Institut

Serial crystallography is still a young method[1]. Despite software[2,3] and experimental end stations still being in an early developmental stage, when compared to refined conventional crystallography research environment, recent advances point towards a bright future of the method beyond x-ray free electron lasers (XFELs). In a simple setup we performed serial crystallography experiments at SLS beamline X06SA, using a lipidic cubic phase injector[4]. Despite the preliminary nature of the data we were able to obtain a structure of Bacteriorhodopsin to about 3 Å resolution (Figure 1). The setup can currently be used for sample characterization prior to XFEL experiments, but will have additional use cases in future experiments. Recent XFEL experiments at LCLS and at SACLA allowed us to collect time resolved serial crystallography data for light activated proton pump Bacteriorhodopsin as well as the characterization of a protein ligand system that will be studied in by time resolved methods in the future.

1. Boutet *et al.* High-Resolution Protein Structure Determination by Serial Femtosecond Crystallography. *Science (80-.).* **74,** 10–13 (2011).
2. Barty *et al. Cheetah* : software for high-throughput reduction and analysis of serial femtosecond X-ray diffraction data. *J. Appl. Crystallogr.* **47,** 1118–1131 (2014).
3. White *et al.* CrystFEL: A software suite for snapshot serial crystallography. *J. Appl. Crystallogr.* **45,** 335–341 (2012).
4. Weierstall *et al.* Lipidic cubic phase injector facilitates membrane protein serial femtosecond crystallography. *Nat. Commun.* **5,** 3309 (2014).