Non-isomorphism: ancient enemy or blessing in disguise?

J.M. Holton[123]

[1]Biochemistry and Biophysics, University of California, San Francisco, [2]Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Lab, Berkeley, [3]Structural Molecular Biology Group, Stanford Synchrotron Radiation Lab, SLAC, Meno Park, CA, USA, **JMHolton@lbl.gov**

The problem of non-isomorphism has plagued macromolecular crystallography since the beginning [1, 2, 3], but if used properly it may prove instrumental in solving the phase problem by over-sampling the molecular transform [4] and studying structural flexibility. Changes in unit cell can be a tell-tale sign of non-isomorphism, but in general these are neither necessary nor sufficient to indicate incompatibility of the underlying structure factors. Why are they incompatible anyway? If the underlying protein structure is the same, then why aren't the structure factors? Simple rigid-body motions cannot be the whole story because these lead to steric clashes. The true underlying distortion of the molecule is most likely smoothly varying from one end of the unit cell to the other, and, of course, must also obey crystallographic symmetry. Here I present how periodic rubber-like distortions may be modelled using a collection of sine waves in space. This spatial distortion field (SDF) is similar in mathematical form to the Fourier synthesis of electron density from structure factors. The main differences are that the SDF is not a scalar field like electron density but a vector field describing changes in atomic position at every point in the unit cell. In adition, the number of terms in the SDF required to describe typical non-isomoprhism is relatively small: 3-5 orders are usually sufficient. After applying this SDF to a protein model the RMS deviation between coordinates is comparable to rigid-body alignment with the exception that there are no steric clashes. In addition, the SDF may be applied to electron density, allowing multi-crystal averaging across non-isomorphous crystal forms. This is essentially equivalent to evaluating the molecular transform between the Bragg spots. Structural flexibility inherent to function may also be excited by these rubber-like distortions, making SDFs a potentially useful tool for elucidating subtle changes by eliminating the "noise" of rubber-like non-isomorphous distortion.

References

[1] - B.S. Magdoff, F.H.C. Crick, Acta Cryst., **8** 461 (1955).
[2] - F.H.C. Crick, B.S. Magdoff, Acta Cryst., **9** 901 (1956).
[3] - C.C.F. Blake, R.H. Fenn, L.N. Johnson, D.F. Koenig, G.A. Mair, A.C.T. North, J.W.H. Oldham, D.C. Phillips, R.J. Poljak, V.R. Sarma, C.A. Vernon, in: M.G. Rossmann, E. Arnold (Eds.) International Tables for Crystallography **F**: Crystallography of biological macromolecules, Springer Netherlands, 2001. 745.
[4] - W.L. Bragg, M.F. Perutz, Acta Cryst, **5** 377 (1952).

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

Measuring energy-dependent photoelectron escape in microcrystals

S.L.S. Storm[1], A.D. Crawshaw[1], N.E. Devenish[1], R. Bolton[1,2], D.R. Hall[1], I. Tews[2], G. Evans[1]

[1]Diamond Light Source, Harwell Science & Innovation Campus, Didcot OX11 0DE, United Kingdom, [2]Department of Biological Sciences, Institute for Life Science, University of Southampton, Highfield Campus, Southampton SO17 1BJ, United Kingdom, **selina.storm@diamond.ac.uk**

With the increasing trend of using microcrystals and intense microbeams at synchrotron X-ray beamlines, radiation damage becomes a more pressing problem. Theoretical calculations by Nave and Hill [1] show that the photoelectrons primarily causing damage can escape microcrystals. This effect would become more pronounced with decreasing crystal size as well as at higher energies [2, 3]. To prove this effect, data from cryo-cooled lysozyme crystals of dimensions 5 x 3 x 3 μm^3 and 20 x 8 x 8 μm^3 mounted on cryo-transmission electron microscopy (TEM) grids were collected at 13.5 keV and 20.1 keV using a 2M CdTe Pilatus detector, which has similar quantum efficiency at both energies. Accurate absorbed doses were calculated with RADDOSE3D [4] through direct measurement of individual crystal sizes using scanning electron microscopy after the experiment and characterization of the X-ray microbeam. The data were processed with DIALS [5] and crystal lifetime was then quantified based on the $D_{1/2}$ metric. In this first systematic study, a longer crystal lifetime for smaller crystals was observed and crystal lifetime increased at higher X-ray energies supporting the theoretical predictions of photoelectron escape. The use of detector technologies specifically optimised for data collection at energies above 20 keV allows the theoretically predicted photoelectron escape to be quantified and exploited, guiding future microfocus beamline design choices.

References

[1] - Nave & Hill, J. Synchrotron Rad. 12, 299-303 (2005).
[2] - J.A. Cowan & C. Nave, *J. Synchrotron Radiat.* 15, 458-462 52008).
[3] - Sanishvili et al. (2011), PNAS 108(15), 6127-6132.
[4] - Zeldin et al. (2013), J. Appl. Cryst. 46, 1225-1230.
[5] - Winter et al. (2018), Acta Cryst. D74, 85-97.

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

X-ray data collection and structure solving of Dps protein by multiple-crystal macromolecular crystallography methods

V. Kovalenko[1], N. Loiko[1,2], E. Tereshkin[1], K. Tereshkina[1] , A. Popov[3], Y. Krupyanskii[1]

[1]Semenov Institute of Chemical Physics RAS, Moscow, Russia, [2]Federal research center for fundamental prin ciples of biotechnology of RAS, Moscow, Russia, [3]ESRF, Grenoble, France, **yuriifkru@gmail.com**

In recent years, the increasing brightness of X-ray beamline for macromolecular crystallography has made it possible to obtained appropriative diffraction data for further procession by standard crystallographic software tools from crystals of very small size. To obtain bright enough diffraction spots we should deliver more radiation dose to the crystal, which leads to the critical radiation damage before we able to collect enough data to solve the crystal structure. However, if a few degrees of oscillation data per crystal are available, diffraction images can be processed by standard crystallographic software, and when the resulting partial datasets were checked for high level of isomorphism, them could be merged to produce the final complete data set.

In present work the process of diffraction data collection from Escherichia coli bacteria Dps protein crystals 3-7 micron sized was described. The study of influence on final data set of various data collection parameters such as exposure time and diffraction wedge wideness per one crystal were carried out. Here, to achieve the best result in selecting the isomorphs partial datasets for merging hierarchical cluster analysis was applied. This method use distance between data sets a and b calculated from correlation coefficient (cc(a,b)) between common intensities of these sets ($d(a,b) = \sqrt{(1 - cc(a,b)^2)}$) as a metric for non-isomorphism. The calculations of these distance performed by ccCluster program [1].

Final diffraction data set consist of 256 monocrystal diffraction data, overall 450 monocrystals diffraction data was processed by XDS software [2]. The highest resolution of obtained structure is 2.2 Å, R-factor free value for this resolution equal 0.2598. Structure was deposited in PDB with unique four letter code as 6QVX.

References

[1] - G. Santoni, U. Zander, C. Mueller-Dieckmann, G. Leonard, and A. Popov, Hierarchical clustering for multiple-crystal macromolecular crystallography experiments: the ccCluster program, Journal of applied crystallography, **50,** 2017 p. 1844-1851.

[2] - W. Kabsch, XDS, Acta Crystallographica Section D, **66,** 2010, p. 125-132.

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

Theory and methods in microcrystallography of biological macromolecules

M. Cianci

Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche, 60131, Ancona, Italy, **m.cianci@unvpm.it**

During the last decade, crystallography of microcrystals has become the method of choice for a large number of projects in structural biology. Still today, attempts to collect data from microcrystals of 5–20 μm at their longest dimension, require a dedicated strategy and multi-crystal data collection. While most of the crystal structures can be solved by molecular replacement, in many cases still experimental phasing from microcrystals is needed.

De novo determination of macromolecular structures requires accurate measurement of structure factors and thereby estimation of the phases from the crystals of the given specimen. The anomalous signal from naturally occurring (S, P, Ca, etc) or incorporated (Se, Hg, etc) anomalous scatterers, can be harnessed with a Single-wavelength Anomalous Dispersion (SAD) experiment. Today, the properties of new generation synchrotrons, or new long-wavelength tunable beamlines for microcrystals [1,2], optimization of the X-ray scanning routines, data collection and processing flows [3], new algorithms for data merging [4,5], allow to collect, in just few hours, a full data set with anomalous signal by merging data from more than hundred micro crystals collected thus enabling X-ray diffraction data collection and phasing in microcrystallography (Fig. 1) [5,6]. Moreover, by conducting an extensive survey of 115 PDB sulphur SAD depositions and testing the statistical distribution that these represented, a useful predictor for aiding experimental success using sulphur SAD was developed [7]. So, we will present the current state-of-art of theory and methods in our hands for data collection and phasing in microcrystallography of biological macromolecules and wishes for the future.

References

[1] - M. Cianci et al., P13, the EMBL macromolecular crystallography beamline at the low-emittance PETRA III ring for high- and low-energy phasing with variable beam focusing. J. Sync. Rad. **24**, 323-332 (2017).
[2] - M. Cianci et al., Data collection with a tailored X-ray beam size at 2.69 Å wavelength (4.6 keV): sulfur SAD phasing of Cdc23Nterm. Acta Cryst. Section D, Biol. Cryst. **72**, 403-412 (2016).
[3] - U. Zander et al., MeshAndCollect: an automated multi-crystal data-collection workflow for synchrotron macromolecular crystallography beamlines. Acta Cryst. Section D, Biol. Cryst **71**, 2328-2343 (2015).
[4] - U. Zander et al., Merging of synchrotron serial crystallographic data by a genetic algorithm. Acta Cryst. Section D, Biol. Cryst., **72**, 1026-1035 (2016).
[5] - N Nicolas, M Cianci, M Nanao. Choosing your (Friedel) mates wisely: Grouping data sets to improve anomalous signal. Acta Crystallographica, Structural Biology Section, D**75**, 200-210 (2019).
[6] - M. Cianci, M.Nanao and T.R. Schneider. Long-wavelength Mesh&Collect native SAD phasing from microcrystals. Acta Crystallographica, Structural Biology Section, D**75**, 192-199 (2019).
[7] - A. Olczak and M. Cianci. The signal-to-noise ratio in SAD experiments. Cryst. Reviews, **24**, 73-101 (2017).

Synergy between synchrotrons and free electron lasers in studying ion transport with serial crystallography

P. Nogly

Institute of Molecular Biology and Biophysics, Department of Biology, ETH Zurich, Switzerland,
przemyslaw.nogly@mol.biol.ethz.ch

Ion pumping microbial rhodopsins are integral membrane proteins employing a common 7-transmembrane helices architecture to transport different ion types. The specific residue composition impacts the protein dynamics and transport mechanism.

Rhodopsins utilize retinal chromophore to harvest light energy for protein activation, which makes them an ideal target for pump-probe experiments. We employ serial crystallography to capture structural intermediates in “real-time” and at non-cryogenic temperatures. I will present a combination of the X-ray Free Electron Laser and more accessible synchrotron data, which provide complementary insights into protein dynamics and ion transport.

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

Free-jet sample injection for synchrotron serial data collection

R.B. Doak

Max-Planck-Institut für medizinische Forschung, 69120 Heidelberg, Germany,
bruce.doak@mpimf-heidelberg.mpg.de

Seminal Serial Femtosecond Crystallography (SFX) measurements were carried out at the LCLS X-ray Free-Electron Laser (XFEL) in Dec-2009, one decade ago. It was obvious even then that XFEL experimentation would suffer from a severe limit on the possible number of XFEL endstations. In contrast, there is no sparsity of synchrotron endstations. Less than four years later, our group therefore initiated a collaboration with the PXII beamline of the Swiss Light Source (SLS) to adapt serial crystallography for synchrotron use. Instrumentation and techniques were developed during six SLS beamtimes between Oct-2013 and May-2014, with our existing rod-mount MPI XFEL High Viscosity Extrusion (HVE) injector simply bolted directly onto the PXII goniometer. A paper describing this very first Synchrotron Serial Crystallography (SSX) was submitted in Jun-2014 and published in due course [1]. An HVE injector head is much more massive than a sample loop, however, and so basically incompatible with the delicate fine motion drives of a synchrotron goniometer. As an interesting side note, this is *not* the case for a miniature low viscosity sample injector (a "GDVN" injector) we designed and fabricated at the same time, building it into a conventional SPINE button for universal and standard goniometer mounting. In Oct-2013 synchrotron GDVN was well ahead of its time, but will come into its own if high intensity synchrotrons eventually permit X-ray exposures of one microsecond duration or less. We later transitioned our SLS HVE experiments to our block-mount MPI injectors, attached via a miniature manual XYZ stage to the weight-tolerant spindle platform of the goniometer. This was a suitably robust arrangement, but not optimal. An alternative scheme is to mount the HVE injector on a separate, stout, remotely-controllable XYZ stage. This was the approach taken as our SSX technology was ported to the ESRF ID30A beamline in Jun-2016 and it remains a favoured approach in current SSX experiments, mostly since an existing injector can be employed with little or no redesign or modification. Nevertheless an HVE injector can certainly be mounted directly onto a synchrotron goniometer head, provided the weight and torque limitations of the head are carefully taken into account during the design. Constraints are also imposed by the X-ray collimator and beam stop, by the optical monitoring microscope and illumination, by the orientation of the goniometer axis (specifically, whether horizontal or vertical), and by the need to collect extruded sample. Our most recent MPI SSX injector, designed in this manner, has now been installed and is available for use at the BioMAX endstation of the MAX IX synchrotron. Apart from allowing the injector nozzle to be positioned by use of the goniometer drives, such a "bolt-on" injector permits any synchrotron endstation having the same goniometer fixture to immediately be converted to SSX use. This SSX injector development will be presented and the future of HVE injection at high brilliance synchrotrons discussed. The author is the leading international expert on design and use of free-jet sample injectors, both low and high viscosity and for both XFELs and synchrotrons.

References

[1] - S. Botha, K Nass, T.R.M. Barends, W. Kabsch, B. Latz, F. Dworkowski, L. Foucar, E. Panepucci, M. Wang, R.L. Shoeman, I. Schlichting, and R. B. Doak, Acta Cryst. D71, 387-397 (2015).

Data analysis methods for two-dimensional serial femtosecond crystallography: paving the way to the time-resolved study of large-scale movements in membrane proteins

C.M. Casadei[1], K. Nass[1], A. Barty[2], M. Hunter[3], X. Li[1], C. Padeste[1], M. Coleman[3], M. Frank[3], B.F. Pedrini[1]

[1]Paul Scherrer Institut, 5232 Villigen PSI, Switzerland. [2]Center for Free Electron Laser Science, DESY, Notkestrasse 85, 22607 Hamburg, Germany. [3]Lawrence Livermore National Laboratory, 7000 East Avenue, Livermore, CA 94550, USA, **Cecilia.Casadei@psi.ch**

Serial diffraction images can be recorded from radiation-sensitive membrane protein two-dimensional (2D) crystals using ultra-short and ultra-bright free electron laser X-ray pulses focused to the sub-μm and a low background environment. The interest in this exotic and demanding data collection mode resides in that membrane proteins arranged periodically in a monolayer maintain their physiological dynamics.

A dedicated processing pipeline was developed for the analysis of serial femtosecond crystallography (SFX) data from 2D crystals. 2D-SFX data present common features with well established methods, in particular serial crystallography from three-dimensional crystals and 2D electron diffraction. Yet there are intrinsic differences with each of these techniques, requiring the development of customized code. On one hand, unlike diffraction intensities from 3D crystals, 2D-SFX intensities are continuous in the out-of-plane direction of reciprocal space. On the other hand, the need of merging techniques that account for indexing ambiguity in serial images complicates the analysis with respect to single-crystal methods [1]. Our processing method deals with such peculiarities and includes an algorithm that allows to extend the resolution limit of the usable data by improving the signal to noise ratio of the measured intensities, which is inherently poor due to the weak scattering power of monolayers [2].

References

[1] - C.M. Casadei *et al.*, IUCrJ **6**, 34-45 (2019).
[2] - C.M. Casadei *et al.*, IUCrJ **5**, 103-117 (2018).

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

Towards a protein-based crystal host system

J. Sprenger[1,2], O. Yevanov[3], J. Carey[4], A. Schulz[5], F. Drouard[1], C. Lawson[6], H. Chapman[3], C. von Wackenfeldt[7], S. Linse[2] and L. Lo Leggio[1]

[1]Department of Chemistry, University of Copenhagen, Denmark, [2]Department of Biochemistry and Structural Biology, Lund University, Sweden, [3]Center for Free-Electron Laser Science, DESY, Hamburg, Germany, [4]Chemistry Department, Princeton University, Princeton, New Jersy, USA, [5]Department of Plant and Environmental Sciences, University of Copenhagen, Denmark, [6]Institute for Quantitative Biomedicine, Rutgers University, New Jersey,USA, [7]Department of Biology and LP3, Lund University, Sweden,
Jasp@chem.ku.dk

X-ray crystallography is a powerful tool in structural biology as most protein structures today have been determined using this method. With the aim to allow structural determination from X-ray diffraction also of proteins excluded from conventional crystallography - as they do not crystallize - we recently started to develop a crystal host (HOSTAL) method. This approach is similar to the crystal sponge methods for structure determination of small molecules [1] but purely protein based using protein crystal with large large solvent channels into which small to mid-size guest proteins can be incorporated via soaking (Figure 1).

With help of confocal microscopy we could show that small guest proteins such as fluorophore-labeled calmodulin can enter the solvent channels of a host crystal made of domain swapped TrpR protein [2] and occupy ~40 % of the host's solvent channels (manuscript in review). The analysis of the X-ray diffraction data indicates differences in the solvent channel electron density for the host with guest compared to the host alone. However, the guest structures could not yet be solved by conventional methods likely due insufficient crystallographic order of the guest. Present work makes use of different ways to order the guest proteins e.g. by promoting specific host-guest interactions. Aside single crystal also serial crystallography approaches are followed to enable the use of microcrystals to lower the guest diffusion times into the host. For the better interpretation of weak signals from diffraction of this semi-ordered crystal system we are furthermore trying to make use of the diffuse scattering signal as described in [3] to solve the guest structure.

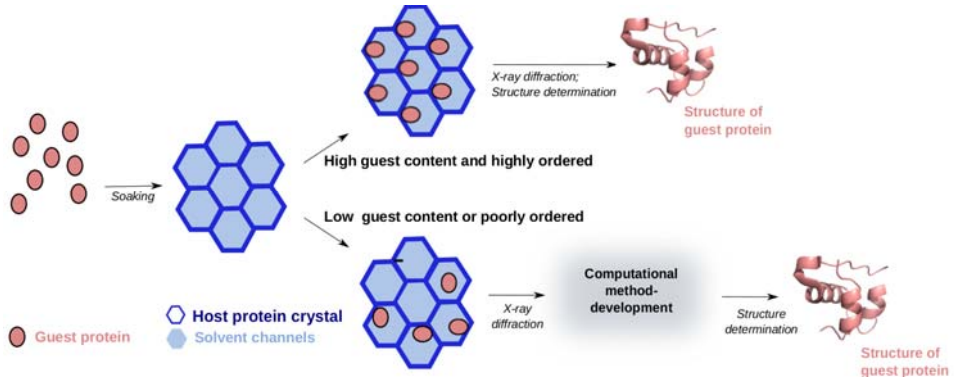

Figure 1: Schematic representation of the crystal host (HOSTAL) system

References

[1] - D. Fujita et al, Nature Communication, **3**, 1093 (2012).
[2] - C.L. Lawson et al, Structure **12**, 1099-110 (2012).
[3] - K. Ayyer et al, Nature. **530**(7589):202-6 (2016).

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

An automated and universal approach for high-throughput serial crystallography of membrane proteins using *CrystalDirect* technology

S. Basu[1], R. Healey[2], A.-S. Humm[1], F. Dupeux[1], A. Pica[1], C. Leyrat[2], A. McCarthy[1], S. Granier[1], J.A. Márquez[1]

[1]European Molecular Biology Laboratory (EMBL), Grenoble-outstation, France, [2]Institut de Génomique Fonctionnelle (IGF), Montpellier, France, **shbasu@embl.fr**

Serial synchrotron crystallography (SSX) is considered as an attractive approach to determine structures of challenging membrane proteins, *especially*, when crystals are grown in Lipid Cubic Phase (LCP) [1]. However, transferring crystals from standard crystallization media to sample-delivery systems, compatible with SSX experiments can be difficult and often require reformulation of crystallization protocols. In addition to this, SSX typically consumes huge amounts of sample. In order to streamline sample delivery, reduce sample consumption and enable full automation of SSX experiments, we have developed a universally applicable approach from crystallization to structure determination – *CrystalDirect* for rapid SSX experiments on membrane proteins. We demonstrate that this technology [2] can be used for automated crystallization of two human transmembrane enzymes [4, 5] in Lipid Cubic Phase (LCP), which is otherwise an extremely tedious process [3]. Moreover, it can be used to streamline the preparation of samples for SSX experiments, either through automated crystal harvesting and cryo-cooling or by direct analysis through *in situ* diffraction experiments in CrystalDirect plates. This approach requires under 1 µg of sample and can be applied both to obtain cryogenic as well as room temperature structures in less than 20 mins. In this talk, various examples will be presented and the importance of full automation in SSX experiments will be discussed. The versatility and automation provided by the *CrystalDirect* technology, which can be easily adopted at any modern synchrotron, is expected to broaden SSX applicability by eliminating the need for complex experimental steps an increasing the throughput of SSX beamlines.

References

[1] - Mishin et al., Expert opin in Drug Discov., 2019.
[2] - Zander et al., Acta Cryst. D., 2016.
[3] - Caffrey, Acta Cryst. F., 2015.
[4] - Vasiliauskaité-brooks et al., Nat. Comm., 2018.
[5] - Vasiliauskaité-brooks et al., Nature, 2017.

Abstract submitted for contribution to the User-dedicated Microsymposium 3 (UDM3):

Serial crystallography at the SPB-SFX instrument at the European XFEL

A.R. Round[ab], R.J. Bean[a], J. Bielecki[a], H.J. Kirkwood[a], R. Letrun[a], B. Manning[a], L. Lopez Morillo[a], G. Mills[a], N. Reimers[a], T. Sato[a], J. Schulz[a], C. Signe Takem[a], M. Sikorski[a] and A.P. Mancuso[ac]

[a]European XFEL, Holzkoppel 4, 22869, Schenefeld Germany, [b]School of Chemical & Physical Sciences, Keele University, Keele, Staffordshire, ST5 5NH, UK, [c]Department of Chemistry and Physics, La Trobe Institute for Molecular Science, La Trobe University, Melbourne, Victoria, 3086, Australia,
adam.round@xfel.eu

The Single Particles, Cluster and Biomolecules and Serial Femtosecond Crystallography (SPB/SFX) instrument [1] is optimised for biological structure determination benefitting from the unique properties and capabilities of the European X-Ray Free-Electron Laser. Serial crystallography is used predominantly with high speed jets greater than 30 m/s to enable data collection at the matching MHz rate [2] provided by the XFEL. A wide variety of GDVN nozzles are required to cover the different needs of each experiment we operate with glass capillary, ceramic and 3D printed nozzles which allow for double flow focusing as well as mix and inject enabling time resolved studies of chemical reactions. In addition to this we have just commissioned an additional instrument operating at atmospheric pressure which can be of benefit for the jets. It also enables a new simplified viscous injector setup as well as fixed target data collection [3] enabling serial data collection with low sample consumption. This range of delivery methods will be further extended with the ongoing developments to improve capabilities and reduce sample consumption such as drop-on-demand using acoustic droplet injection and drop in oil delivery methods.

References

[1] - A.P. Mancuso et al, The Single Particles, Clusters and Biomolecules and Serial Femtosecond Crystallography instrument of the European XFEL: initial installation, J. Synchrotron Rad. 26, 660-676 (2019).
[2] - M.O. Wiedorn et al, Megahertz serial crystallography, Nature Communications volume 9, Article number: 4025 (2018).
[3] - P. Roedig et al, High-speed fixed-target serial virus crystallography, Nat Methods.14(8):805-810 (2017).