Workshop on Advanced Data Collection with Multi-Axis Goniometry
MADaC 2015

Synchrotron SOLEIL - L'orme des Merisiers
Saint-Aubin, France
12th-14th of November 2015

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ABSTRACTS BOOKLET

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Summary

Workshop on Advanced Data Collection with Multi-Axis Goniometry

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Synchrotron SOLEIL - L'orme des Merisiers

Saint-Aubin, France

12th-14th of November 2015

Welcome

Multi-axis goniometry, more simplistically known as kappa goniometry, is being installed at a growing number of macromolecular crystallography end-stations worldwide. However, the great potential of the technique when approaching challenging structure determination remains to be fully exploited. This workshop, second of its kind, brings together experimenters and developers experts in the field in order to identify current bottlenecks and to explore possible solutions using aspects of kappa goniometry optimised for data collection strategies and processing for facilitating phase retrieval of difficult samples.

In image to the first workshop, both lectures and practicals will be given. Data collection practicals will be performed at beam line PROXIMA-1 of the Synchrotron SOLEIL.

Bienvenue

La goniométrie à axes multiples, mieux connue sous le nom de goniométrie kappa, est mondialement utilisée sur un nombre croissant de lignes de lumière dédiées à la cristallographie des macromolécules. L'énorme potentiel de cette technique dans le cadre de déterminations problématiques de structures cristallines reste à exploiter dans sa profondeur. Pour sa deuxième édition, ce workshop réunit des expertises dans ce domaine afin de tenter d'identifier les problèmes bloquants et trouver des solutions qui feront appel à la goniométrie kappa optimisée pour l'établissement de stratégies de collectes de données et pour faciliter le phasage d'échantillons problématiques.

A l'instar du premier workshop, il sera composé aussi bien de conférences invitées que de travaux pratiques.

Les TP sur l'acquisition de données auront lieu sur la ligne PROXIMA-1, du Synchrotron SOLEIL.

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Workshop on Advanced Data Collection with Multi-Axis Goniometry
MADaC 2015

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Programme

Thursday, November 12th

13:00 - 13:50 Registration & Welcome coffee

13:50 - 14:00 Welcome word, Jean Daillant, Director, Synchrotron SOLEIL

SESSION I
Multi-axis goniometry and strategies

Chairperson: Leonard CHAVAS

14:00 - 14:40 Optimising and driving Synchrotron experiments as a third party
Gérard Bricogne - *Global Phasing Ltd., Cambridge, UK*

14:40 - 15:20 A Better data collection strategy: You can always use a lower dose
Meitian Wang - *Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland*

15:20 - 16:00 Weak anomalous signal scaling: On extracting the needle from the haystack
Nicolas Foos - *Synchrotron SOLEIL, Gif-sur-Yvette, France*

16:00 - 16:30 Coffee break

SESSION II
Kappa-goniometry at synchrotrons and further developments

Chairperson: Martin SAVKO

16:30 - 17:00 Native SAD: The magic bullet for experimental phasing?
Vincent Olieric - *Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland*

17:00 - 17:30
Gleb Bourenkov

17:30 - 18:00 Multi-axis goniometry for MX at Diamond Light Source
Ralph Flaig - *Diamond Light Source, Didcot, UK*

19:00 - 20:30 Dinner

Friday, November 13th

SESSION III
Data processing for advanced data collection protocols

Chairperson: Pierre LEGRAND

09:00 - 09:30 Inside the black box of data processing
Harry Powell - *MRC Laboratory of Molecular Biology, Cambridge, UK*

09:30 - 10:00 Processing multi-axis, multi-wavelength and interleaved datasets with autoPROC
Clemens Vonrhein - *Global Phasing Ltd., Cambridge, UK*

10:00 - 10:30 Diffraction experiments of arbitrary geometry
Tim Grüne - *Swiss Light Source, Paul Scherrer Institute, Villigen, Switzerland*

10:30 - 11:00 coffee break

SESSION IV
Case studies

Chairperson: Serena SIRIGU

11:00 - 11:30 The use of a mini-kappa goniometer head for diffraction experiments at the ESRF
Andrew McCarthy – *ESRF, Grenoble, France*

11:30 - 12:00 The use of multi-axis goniometers for phasing of glycoprotein crystals
Thomas Krey - *Institut Pasteur and CNRS UMR 3569,Paris, France*

12:00 - 12:30 Experimental design in MX: A skill not to be ignored
Andrew Thompson - *Synchrotron SOLEIL, Gif-sur-Yvette, France*

12:30 - 14:00 Lunch

14:00 - 18:00 Practical applications

19:00 - 20:30 Dinner

Saturday, November 14th

09:00 - 12:30 Practical applications

12:30 - 14:00 Lunch

14:00 Departure

ABSTRACTS

Thursday, November 12th, 2015

SESSION I & II

MADaC - 2015

Thursday, November 12th

SESSION I
Multi-axis goniometry and strategies

Chairperson: Leonard CHAVAS

IT-01	Optimising and driving Synchrotron experiments as a third party *G. Bricogne*
IT-02	A Better data collection strategy: You can always use a lower dose *M. Wang*
IT-03	Weak anomalous signal scaling: On extracting the needle from the haystack *N. Foos*

SESSION II
Kappa-goniometry at synchrotrons and further developments

Chairperson: Martin SAVKO

IT-04	Native SAD: The magic bullet for experimental phasing? *V. Olieric*
IT-05	Gleb Bourenkov
IT-06	Multi-axis goniometry for MX at Diamond Light Source *R. Flaig*

Optimising and Driving Synchrotron Experiments as a Third Party

G. Bricogne, C. Flensburg, P. Keller,
W. Paciorek, & C. Vonrhein

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ABSTRACT

The ever-increasing speed of MX beamline instrumentation is leading to ever-stronger emphasis being placed on brevity of execution as the main design goal for data collection protocols, often to the exclusion of other criteria that would aim at achieving higher data quality. This can be counter-productive, especially, but not only, for phasing experiments.

Global Phasing, among others, has been interested in bucking that trend by creating combined capabilities for the fast design of optimal strategies and the direct supervision of their execution on an actual beamline. Our approach has been to aim for a full "third-party design and control" capability rather than for separate add-on programs that would need to be invoked by local software on each specific group of beamlines running under a given BCS (for Beamline Control Software).

To make such an integrated capability as transferable as possible across the huge diversity of beamline instruments and BCSs, finding the correct level of abstraction for all the components and processes involved is of paramount importance. Abstracting the processes requires focusing on the stage where description and action must dovetail.

For a strategy to achieve optimality, it needs to rely on complex sequences of measurements dictated by scientific criteria and imperatives; while in order to achieve "executability" it must be translatable into a sequence of instrumental actions available from the BCS. A common vocabulary is therefore required for the instructions in terms of which any complex strategy can be expressed, and on the basis of which the combined instrumental actions needed to execute these instructions can be requested from the BCS. We have defined such a common vocabulary and captured it into a data model for an Abstract Beamline Interface.

Progress made as part of a close collaboration with the Diamond Light Source (COL0044, on the I23 long-wavelength beamline) in implementing this paradigm into a highly adaptable workflow invoking new applications for optimal strategy design (especially, using multi-axis goniometers) will be presented, along with plans and existing collaborative links for disseminating this work to all European and several US synchrotrons.

It is expected that the uptake of this work on beamlines used in collecting diffraction data for structure-based drug discovery will enable the implementation of "Club Class" data collection on samples from which the highest-quality results are to be derived, along with the "Economy Class" data collection on samples from which only a binary answer (binds/doesn't bind) is needed.

A Better Data Collection Strategy: You Can Always Use a Lower Dose

M. Wang

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ABSTRACT

Typical data collection in macromolecular crystallography usually consists in measuring unique reflections with minimum multiplicity and maximum X-ray dose (10 – 20 MGy). This strategy, when employed for experimental phasing, is often suboptimal. Recent advances in beamline instrumentation (namely pixel array detectors and multi-axis goniometers) have enabled new data collection protocols, which distribute the total X-ray dose in multiple data sets and multiple crystal orientations. This method can reduce systematic measurement errors effectively and improve the quality achievable for the final data. I will describe such strategies and their applications for experimental phasing.

Weak Anomalous Signal Scaling : On Extracting the Needle from the Haystack

N. Foos(1), A.Thompson(1), P. Legrand(1)

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ABSTRACT

DECIPHERING AND OPTIMISING THE SCALING STEP IN DATA-REDUCTION

Currently 80 % of the structures in the wwwPDB are determined using synchrotron radiation sources, often on beamline with tuneable wavelength and advanced experimental setup. Even if the Molecular Replacement is still the most frequent method used to solve the phase problem, experimental phasing becomes more and more accessible. The simplest phasing experiment, applicable in most cases, would be single wavelength anomalous diffraction (SAD) with the native sulfur (S-SAD). Despite this good news, in practice S-SAD is still quite challenging and only few attempts are successful.

Due to the small contribution of anomalous scatterers over the total scatterers, which leads to a relatively low ΔF /F ratio (where ΔF is the difference in intensity between Bijvoet pairs), the challenge for this method is the proper determination of the very weak anomalous signal (except for DLS-I23 beamline, which can go to very long wavelengths and hence increase the ΔF signal).

The work presented here is focused on trying to improve scaling steps during the data reduction process for this specific case. Based on already solved S-SAD structures, we attempted to determine the criteria which give the best chance of phasing success. For that purpose, we developed tools to automatically test and validate or reject different scaling settings using the XDS program(3). We observed that the best results for the phase determination was not always well correlated with the metrics currently useded to assess the quality and strength of the anomalous signal.

This observation led us to propose that, in the case of low anomalous signal, it is necessary to decrease the number of scaling parameters. For example, we show that it is best to ignore Bijvoet differences in the scaling procedure (FRIEDEL'S_LAW=TRUE). This scaling schema leads to a decrease in the statistics indicators of quality but allow the calculation of better electron density maps.

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Native SAD:
The Magic Bullet for Experimental Phasing?

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ABSTRACT

Native SAD uses the weak anomalous scattering of light elements naturally present in the target macromolecule (P, S, Cl, K and Ca). Its success relies on very accurate measurement of the reflection intensities, which is limited by both random and systematic errors, as well as radiation damage, and crystal quality. A data collection strategy [1], which can yield high quality anomalous data, was developed at beamline X06DA-PXIII at the Swiss Light Source. It consists in collecting multiple low-dose data sets on only one crystal entity in multiple orientations. The method benefits from very stable X-ray source and optics, a high-precision multi-axis PRIGo goniometer [2] and a readout noise-free PILATUS detector calibrated for low energies.

I will describe the key features of our anomalous data collection strategy and its application for native SAD phasing. I will present real-life examples, including challenging cases where our low-dose multi-orientation strategy was combined with the multi-crystal merging approach [3]. Finally, I will present first results on a fully automated and fast native SAD data collection protocol using both SmarGon (SmarAct GmbH), the commercial multi-axis goniometer based on PRIGo, and the new hybrid photon counting detector EIGER 16M (Dectris, Ltd.).

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ABSTRACT

Gleb Bourenkov

Multi-axis Goniometry for MX at Diamond Light Source

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ABSTRACT

Diamond Light Source [1] is the UK third generation synchrotron located south of Oxford and currently operates five beamlines for macromolecular crystallography (MX) and soon seven beamlines will serve the MX user community with state of the art facilities [2]. All MX beamlines provide tools for standard data collection but given the increasing complexity and associated challenges with bigger macromolecular complexes, membrane proteins and smaller crystals, different approaches are often required to get the best possible data out of these samples. Therefore, the different beamlines have started to specialize in order to address specific aspects of data collection to allow for successful structure solution. In this presentation I will focus on the implementation and use of multi-axis goniometers which are currently installed on three of the operational beamlines. There are many use cases where the application of multi-axis goniometry will be beneficial but there is some challenge involved to make users aware of the possibilities that the various tools provide and how to translate these into user-friendly procedures. An outlook on future plans will also be given.

REFERENCES

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2. http://www.diamond.ac.uk/Mx.html

ABSTRACTS

Friday, November 13th, 2015

SESSIONS III & IV

MADaC - 2015

Friday, November 13th

SESSION III
Data processing for advanced data collection protocols

Chairperson: Pierre LEGRAND

IT-07	Inside the black box of data processing *H. Powell*
IT-08	Processing multi-axis, multi-wavelength and interleaved datasets with autoPROC *C. Vonrhein*
IT-09	Diffraction experiments of arbitrary geometry *T. Grüne*

SESSION IV
Case studies

Chairperson: Serena SIRIGU

IT-10	The use of a mini-kappa goniometer head for diffraction experiments at the ESRF *A. McCarthy*
IT-11	The use of multi-axis goniometers for phasing of glycoprotein crystals *T. Krey*
IT-12	Experimental design in MX: A skill not to be ignored *A. Thompson*

Inside the Black Box of Data Processing

H. Powell

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ABSTRACT

Diffraction data collected on synchrotron beamlines is increasingly being processed *via* automated pipelines and new users have little idea of the steps involved in going from images to a file containing indexed, integrated and scaled reflections. I will take a look inside the "black box" and outline the methods involved in indexing, refinement of crystal and detector parameters, and of integration itself. While focussing on the methods implemented in Mosflm and its user interface iMosflm, the methods used in other programs will be discussed as well. The importance of the various statistics derived from scaling and merging of integrated intensities will be discussed.

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Processing Multi-axis, Multi-wavelength and Interleaved Datasets with autoPROC

C. Vonrhein, C. Flensburg, P. Keller, W. Paciorek, A. Sharff & G. Bricogne

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ABSTRACT

autoPROC[1] is a set of tools and programs to automate the whole range of steps involved in data processing: (1) analysis of collections of images and image headers, (2) indexing of diffraction images, (3) determination of accurate cell parameters, (4) integration of a series of images, (5) creation of files of intensities and amplitudes in various formats (MTZ, Scalepack), (6) automatic determination of most likely space group symmetry, (7) analysis of anomalous signal and (8) automatic determination of a sensible high-resolution limit. It contains special features for processing multi-sweep datasets, including (a) consistent indexing across sweeps, ensuring identical indexing for sweeps measured on the same crystal, by taking multi-axis goniostat settings into account, (b) internal scaling and merging of each sweep separately and (if applicable) (c) joint scaling of all sweeps simultaneously and merging of related sweeps after that scaling step.

autoPROC allows a detailed and general description of the underlying hardware, including more common multi-axis goniostats such as Kappa and Eulerian systems. It enables the detection of multiple lattices and ice-rings, also providing some initial treatment of those rather common artefacts at the stage of indexing, integration and scaling/merging (to avoid contamination of the final dataset). The computation of the positions and shapes of reflections expected to appear on each image is performed in simcal_predict[6] and visualised using GPX2[7], giving the user accurate and detailed feedback on the integration and parameter refinement process.

Detailed information allowing a user to understand each step, as well as providing help for further decision making, is presented as text and in graphical form. Recent developments have focused on the presentation of these results in HTML.

The present processing functionalities rely in part on third-party programs like XDS/XSCALE[2], POINTLESS[3] and AIMLESS[4] as well as on tools from the CCP4[5] suite of programs.

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6. Partly supported by collaboration contract COL0044 between Global Phasing and the Diamond Light Source.
7. Initially developed by Wolfgang Brehm (while in the group of Kay Diederichs at University of Konstanz/DE) during an internship with Global Phasing during the Summer of 2014 with generous support by the MX group (particularly PX-III) of the Swiss Light Source.

Diffraction Experiments of Arbitrary Geometry

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ABSTRACT

Single crystal diffraction is a powerful technique to determine the atomic structure of molecules. Multi–axis goniometers enable the collection of data with a high degree of multiplicity. In contrast to continued rotation about the same axis, “real” multiplicity reduces systematic errors. Inhouse data often reach a very high quality and the determination of the chirality of small organic compounds with only light atoms has become routine[1]. Similarly, high–quality data from macromolecular structures improve the success rate of advanced phasing techniques like Sulphur SAD.

The program *XDS* is well–known for the integration and scaling of single crystal diffraction data[2]. It is very fast and can be used non–interactively. These are two of the reasons why *XDS* is popular at synchrotron beamlines and used in various pipelines like *autoPRO*[3] and *xia2*[4]. *XDS* can process virtually all types of crystallographic diffraction data with only very few requirements: the data must stem from a single crystal, collected with monochromatic radiation with the rotation method, and the detector must be built up from planar modules. Some of the more complicated scenarios include the pseudo–cylindrical detector P12M at the long–wavelength beamline I23 at the Diamond synchrotron[5] and a dynamically changing shadow from the diamond Anvil cell used for high–ressure crystallography.

This talk will describe the coordinate systems used within *XDS*. The respective parameters from the *XDS* input file will be highlighted and also how to determine their values from a given setup. The concepts have been implemented in the program *sfrmtools* that facilitates the use of *XDS* with data frames in the Siemens Frame Format used by Bruker AXS. I will illustrate the theoretical description with some challenging data sets collected with four circle goniometers at home sources, with the κ–goniometer the the BESSY Synchrotron[6] and our latest results to use *XDS* for electron diffraction data collected with a modified electron microscope[7].

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The use of a Mini-kappa Goniometer Head for Diffraction Experiments at the ESRF

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ABSTRACT

Most macromolecular crystallography (MX) synchrotron based diffraction experiments use single-axis diffractometers. This markedly contrasts with small molecule crystallography, where the majority of diffraction data are collected using multi-axis goniometers. A novel miniaturised κ-goniometer head, the MK3, was developed by the EMBL-ESRF in Grenoble to allow the reorientation of macromolecular crystals (1). It is available on the majority of the structural biology beamlines at the ESRF, as well as elsewhere. The MK3 is fully controllable via MxCuBEv2 and to facilitate its use for advanced data collection strategies a number of protocols using a customized Passerelle-EDM based workflow engine called the *Beamline Expert System* have been developed (2). Here, I will present the current implementation of the MK3 on the MX-beamlines at the ESRF and how it can be used for advanced data collections. I will also present some recent results, illustrating how the alignment of macromolecular crystals can improve the success rate of diffraction experiments compared to data obtained from randomly aligned crystals.

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The Use of Multi-axis Goniometers for Phasing of Glycoprotein Crystals

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ABSTRACT

Soluble ectodomains of viral and cellular glycoproteins involved in membrane fusion can be challenging targets for crystallographers. These proteins are multi-domain proteins that are stabilized by a complex pattern of disulfide bridges and often highly glycosylated. In most cases these proteins are produced in eukaryotic expression systems like mammalian or insect cells. If crystallization is successful, crystals often diffract only to moderate resolution and can be difficult to reproduce due to heterogeneity in glycosylation and batch-to-batch variations.

In many cases the phase problem cannot be solved by molecular replacement. Production of selenomethionine (SeMet)-labelled protein is generally possible in eukaryotic expression systems, but a trade-off between the required SeMet concentration in the medium and the protein yield and SeMet incorporation level is observed to various extents in different expression systems. Therefore in many cases the production of derivative crystals for experimental phasing requires classical heavy atom screening.

Four case studies, in which we determined the crystal structure of glycoproteins involved in membrane fusion by experimental phasing will be presented. A particular importance will be given to the question, how the use of multi-axis goniometers and - tightly related to this - low-dose exposure data collection strategies can contribute to this procedure.

Experimental Design in MX a skill not to be ignored

A. Thompson
and the PROXIMA 1 beamline team, past and present.
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ABSTRACT

In the “age of the dinosaurs”, when I collected my first X-ray data set from a protein crystal, the crystal was mounted, in a sealed but humidified environment, inside a capillary stuck to a goniometer head by plasticine and kept close to room temperature by a cooled nitrogen gas stream. Data recorded on X-ray sensitive film took a long time to develop (and even more to scan and analyse!), the onset of radiation damage was rapid and hence translation of the sample to “fresh parts of the crystal” a routine strategy. Data collection strategies were designed to measure all reflections with minimum sample exposure, and if you had collected 16 degrees of oscillation data in 30 minutes you were “doing very well”. Crystals were changed regularly and the orientation of the fresh crystal chosen, using eye, ruler and International Tables volume A, to complete data already collected. It was impossible to collect data without looking at images and planning ahead. The questions posed from this “prehistoric era” will seem spookily familiar to modern crystallographers, even though the context was different.

Modern software tools give rapid feedback (post analysis, such as the various integration programs, parallelized and rapid) and advice (pre analysis, based on a subset of images and prior knowledge of beamline parameters) on both quality and completeness of data, and highly advanced software tools for structure solution have inbuilt intelligence, helping us avoid pitfalls for the unwary. With the help of huge advances in technology (detectors, nano-positioning…), beamlines have made huge strides in controlling the quality and alignment of X-ray beams, the goniometers that support crystals and the detection and quality of data. Nonetheless, as Z. Dauter remarked in his seminal paper on Data Collection Strategies (*Acta Cryst* ,1999, **D55** : 1703 - 1717), data collection is the last, crucial, and often very difficult or impossible to repeat, step in the chain of structure solution. When you have spent months or years optimizing your crystals, it is worth investing some thought before collecting the data.

This talk will present some indications as to the questions to ask when planning an experiment, as well as some of the reasons why I believe that these questions are still important.