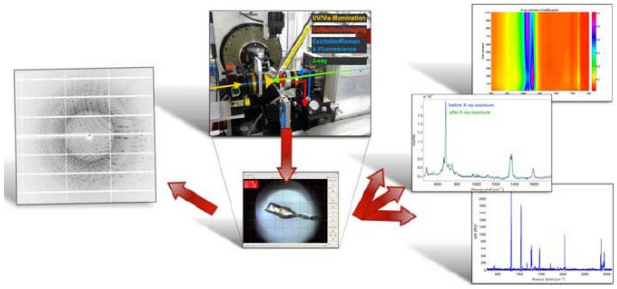

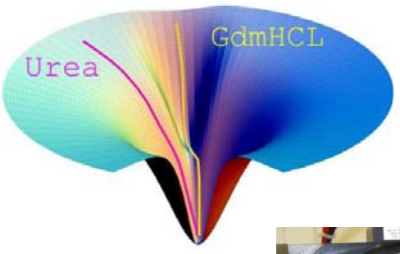

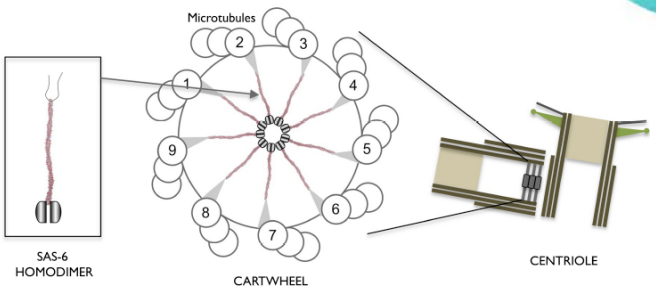

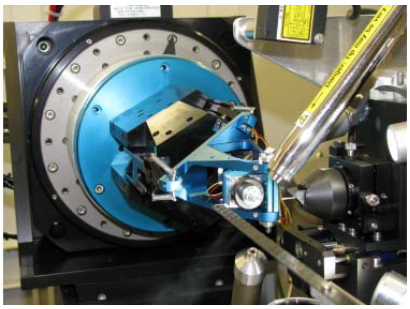

SLS Symposium on

Biophysics

Tuesday, June 7, 2011

10:00 to 12:15, WBGB/019

10:00 *In crystallo* optical Spectroscopy at Beamline X10SA
Florian Dworkowski, G. Pompidor, V. Thominet, C. Schulze-Briese, M. R. Fuchs

10:30 Current developments on S-SAD/P-SAD phasing methods and the multi-axes goniometer PRIGO at the PX beamlines
Sandro Waltersperger, G. Peng, C. Pradervand, W. Glettig, C. Schultze-Briese, B.C. Wang, P. Dumas, E. Ennifar, V. Olieric and M. Wang

11:00 Coffee

11:15 Towards quantifying protein-protein interactions using synchrotron-based oxidative footprinting
Saša Bjelić, L. Malmström, R Aebersold, M. Steinmetz

11:45 **Structural basis of the nine-fold symmetry of centrioles**
D. Kitagawa, I. Vakonakis, N. Olieric, M. Hilbert, D. Keller, Vincent Olieric, M. Bortfeld, M.C. Erat, I. Flückiger, P. Gönczy, M.O. Steinmetz

In crystallo optical Spectroscopy at Beamline X10SA.

***F. Dworkowski*[1], *G. Pompidor*[1], *V. Thominet*[1], *C. Schulze-Briese*[1,2], *M. R. Fuchs*[1]**

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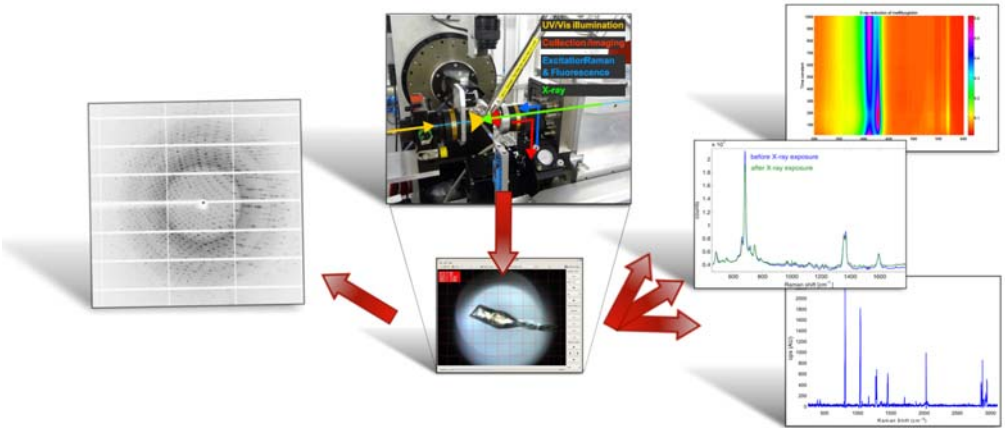
X-ray diffraction based structure determination of biological macromolecules is one of the fundamental tools of a structural biologist. However, this method is limited to elucidate the atomic structure of a macromolecule, but does not yield any information about its electronic structure. By combining optical spectroscopy with X-ray diffraction, it becomes possible to investigate not only atomic but also vibrational and electronic parameters of the molecule of interest, yielding important complementary data. This data can be utilized, for example, to verify the redox state of a metal center contained in the protein, observe the amount of radiation damage the sample is subjected to during diffraction measurements, confirm correct binding of ligands or even selectively trap enzyme reaction intermediates in the crystal [1].

This approach, however, is only useful when both methods are applied to the same single protein crystal. At the SLSpectroLab at beamline X10SA we thus integrated a multimode, on-axis, on-line micro-spectrophotometer into the high resolution X-ray diffraction end station [2]. This setup allows for closely interweaved acquisition of diffraction on spectroscopic data without compromising sample integrity. Currently we support measurement of UV-Visible absorption and fluorescent spectroscopic data as well as resonant and non-resonant Raman scattering.

Here we show recent improvement of the instrument and the capabilities it offers on the example of monitoring specific photo-reduction of disulphide bonds in protein crystals during diffraction measurements.



References

1. Pearson, A.R. and R.L. Owen, *Combining X-ray crystallography and single-crystal spectroscopy to probe enzyme mechanisms.* Biochemical Society Transactions, 2009. **037**(2): p. 378-381.
2. Owen, R.L., et al., *A new on-axis multimode spectrometer for the macromolecular crystallography beamlines of the Swiss Light Source.* J Synchrotron Radiat, 2009. **16**(Pt 2): p. 173-82.

Current developments on S-SAD/P-SAD phasing methods and the multi-axes goniometer PRIGO at the PX beamlines

Sandro Waltersperger[1)], Guanya Peng[1)], Claude Pradervand[1)], Wayne Glettig[5)], Clemens Schultze-Briese[2)], B.C. Wang[3)], Philippe Dumas[4)], Eric Ennifar[4)], Vincent Olieric[1)] and Meitian Wang[1)]

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5) CSEM, Neuchâtel, Switzerland

The usage of the small but significant anomalous signal of intrinsic sulfur atoms of proteins can offer distinct advantages over derivatization based phasing methods and has become very popular during the past years (S-SAD). The same applies for the structure determination of nucleic acids but using phosphorous atoms. Studies on data-redundancy, data-quality, X-ray wavelength and resolution cut-offs have been performed to date and state the current limitations of this method. The requirement of highly redundant data usually collected with relatively low-energy X-rays entails strong absorption effects and radiation damage, thereby causing inaccuracy in data processing, scaling and phasing.

We aim to further develop S-SAD and P-SAD protocols for crystals showing mid-range diffraction between 2.3 and 2.8 Å resolution. Promising results applying optimized measurement protocols have been obtained and will be presented as well as new hardware beamline developments. These invoce the novel multi-axes goniometer PRIGO (Parallel Robotics Inspired Goniometer, Figure 1) that allows to collect data with true redundancy and Bijvoet pairs collected at the same image (to minimize radiation damage effects) and the PILATUS detector to reduce the signal-to-noise ratio to detect very small intensities.

These optimized measurement protocols will be very useful for general users at macromolecular crystallography beamlines to deal with challenging S-SAD/P-SAD datasets.

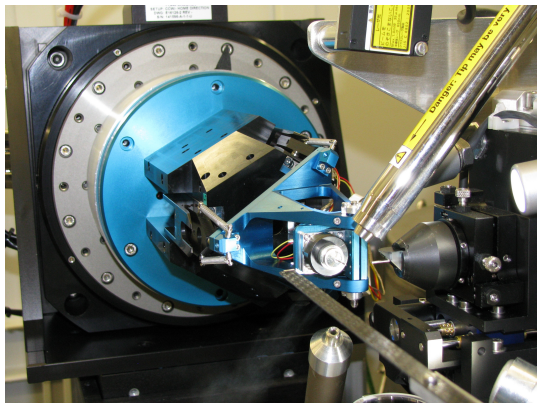

Figure 1: The multi-axes goniometer PRIGO installed at X06DA

TOWARDS QUANTIFYING PROTEIN-PROTEIN INTERACTIONS USING SYNCHROTRON-BASED OXIDATIVE FOOTPRINTING

S. Bjelić[1], L. Malmström[2],, R Aebersold[2] ,M. Steinmetz[1]
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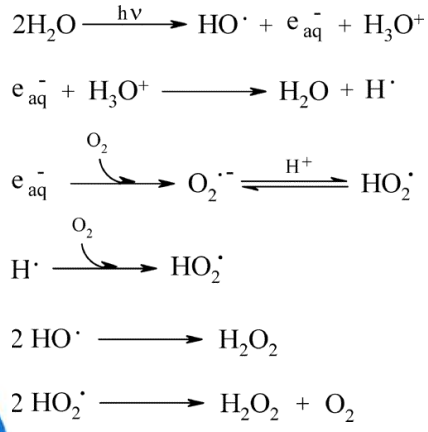
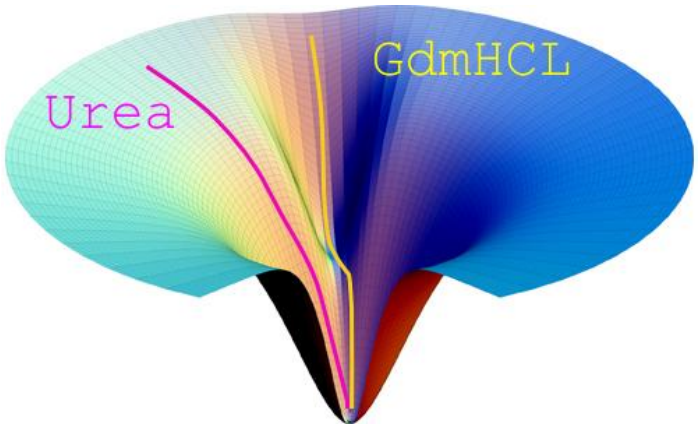
[2]Department of Biology, ETHZ, Zurich

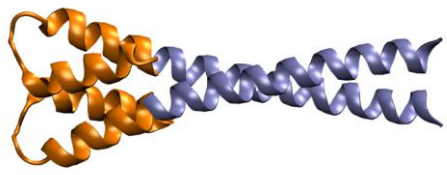
Protein-protein interactions are central in all of biology but are difficult to study quantitatively under native conditions. Synchrotron-based oxidative footprinting is a proxy for measuring solvent exposure and splits water into hydroxyl-radicals that oxidize the solvent-accessible amino acids. Changes in solvent-exposure can hence be detected by measuring the relative level of oxidation, which in turn make it possible to derive quantitative information about the protein-protein interaction.

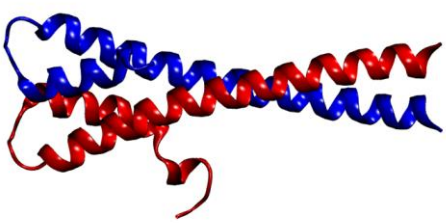
We used synchrotron-based oxidative footprinting to oxidize solvent exposed amino acids of a homo-dimer, EB1c, in a dose-dependent manner. The samples were digested using trypsin and the resulting peptides were analyzed using Selected Reaction Monitoring (SRM), a targeted proteomics technology.





The technological advances in this project will allow studying protein-protein interactions quantitatively as well as protein motions in a high-throughput mode as the method is highly automated using robotics. Sample demand is 2-3 order smaller compared to X-ray or NMR methods. It works in minimally perturbed systems, as no chemicals besides water are needed and the proteins are measured in solution.





Structural basis of the nine-fold symmetry of centrioles

Daiju Kitagawa[1,5], Ioannis Vakonakis[2,4,5], Natacha Olieric[3,5], Manuel Hilbert[2,3,5], Debora Keller[1], Vincent Olieric[2], Miriam Bortfeld[3], Michèle C. Erat[4], Isabelle Flückiger[1], Pierre Gönczy[1,5], Michel O. Steinmetz[3,5]

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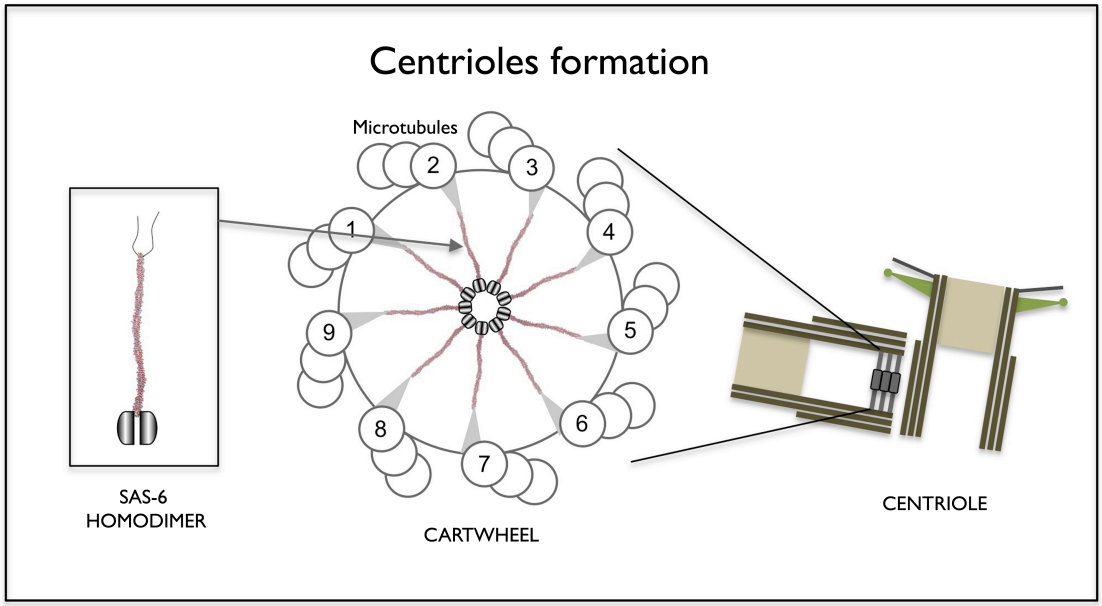
The centriole and the related basal body is an ancient organelle characterized by a universal nine-fold radial symmetry and which is critical for generating cilia, flagella and centrosomes. The mechanisms directing centriole formation are not understood and represent a fundamental open question in biology since its discovery almost fifty years ago with the advent of electron microscopy. Here, we demonstrate that the centriolar protein SAS-6 forms rod-shaped homodimers that interact through their N-terminal domains to form oligomers. We establish that such oligomerization is essential for centriole formation in *C. elegans* and human cells. We further generate a structural model of the related protein Bld12p from *C. reinhardtii*, in which nine homodimers assemble into a ring from which nine coiled-coil rod domains radiate outwards. Moreover, we demonstrate that recombinant Bld12p self-assembles into structures akin to the central hub of the cartwheel, which serves as a scaffold for centriole formation. Overall, our findings establish a structural basis for the universal nine-fold symmetry of centrioles [1].



Reference:

[1] Kitagawa D., Vakonakis I., Olieric N., Hilbert M., Keller D., Olieric V., Bortfeld M., Erat M.C., Flückiger I., Gönczy P., Steinmetz M.O. (2011) Structural basis of the nine-fold symmetry of centrioles. ***Cell***, 144(3): 364-75 (Must read article in Faculty of 1000)