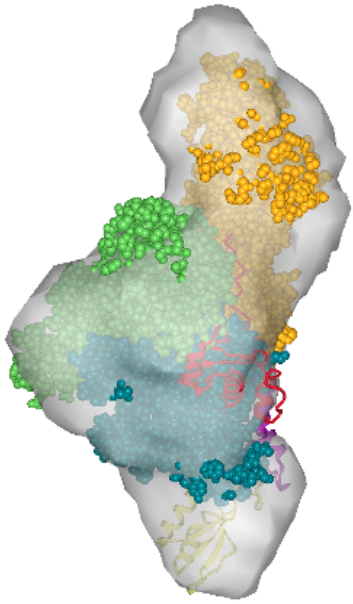

Figure 1. Efl1-Sdo1 complex model fitted into SAXS map. SAXS map obtained from ab-initio model [DAMMIF: Franke, D. and Svergun, D.I. (2009) *J. Appl. Cryst.*, 42, 342-346]. Complex model obtained by a simutaneous docking into SAXS map [Sculptor: Birmans *et al* (2011). *J. Struct Biol*. 173 428-435]

Keywords: Shwachman-Diamond Syndrome, Elongation factor-like 1, ribosomopathy, biosaxs

MS2 Development of new types of sample preparation (both XFEL & synchrotrons)

Chairs: Jörg Standfuss, Gwyndaf Evans

MS2-P1 Structure Determination of Membrane (and Soluble) Proteins Using In Meso In Situ Serial X-ray Crystallography at Room and Cryogenic Temperatures

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The lipid cubic phase continues to grow in popularity as a medium in which to generate crystals of membrane and soluble proteins for high-resolution X-ray structure determination (**1, 2**). To date, the PDB includes 313 records with 105 unique structures attributed to the *in meso* method (**2**). The most successful *in meso* protocol uses glass sandwich crystallization plates, but they are challenging to harvest crystals from. Here, we present a novel *in meso in situ* serial crystallography (IMISX) method which employs a thin cyclic olefin copolymer (COC) windowed plate for *in situ* data collection (**3**). The bolus of mesophase in which crystals grow on the plate contains tens to hundreds of crystals that are clearly visible with an in-line microscope at macromolecular crystallography synchrotron beamlines at both room and cryogenic temperatures. The data acquisition software DA+ GUI at the PX I (X10SA) and PXII (X06SA) beamlines at the Swiss Light Source (SLS) provides a semi-automated 'select and collect' protocol for serial crystallographic data collection with IMISX samples. The method has been demonstrated with β_2AR, AlgE, $PepT_{St}$ and DgkA as model membrane proteins and with lysozyme and insulin as test soluble proteins at room and/or at cryogenic temperatures (**3, 4**). Structures were solved by molecular replacement or by experimental phasing using bromine and native sulfur SAD methods to

resolutions ranging from 1.5 to 2.8 Å with micrometer-sized crystals and nano-gram to micro-gram quantities of protein. The IMISX and IMISXcryo method work with readily available, inexpensive materials and are compatible with high-throughput *in situ* serial data collection at macromolecular crystallography synchrotron beamlines.

1. Caffrey, M., Cherezov, V. (2009) Nature Protocols. 4:706-731.

2. Caffrey, M. (2015) Acta Cryst. F71, 3-18.

3. Huang, C.-Y. et al. (2015) Acta Cryst. D71, 1238-1256.

4. Huang, C.-Y. et al. (2016) Acta Cryst. D72, 93-112.

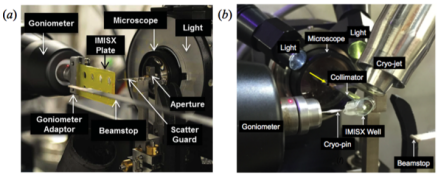

Figure 1. Experimental setup for IMISX data collection at 293 K (*a*) and 100 K (*b*)

Keywords: AlgE, β2-adrenergic receptor, bromine SAD, DgkA, experimental phasing, GPCR, in meso, in situ, insulin, lipid cubic phase, mesophase, membrane protein, PepTSt, serial crystallography, sulfur SAD

MS2-P2 Microfluidic devices for fast time-resolved studies

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Microfluidics enable the precise control of liquid volumes on the nanoliter scale within micron-sized channels. These very well defined flow conditions make this technology predestined for fundamental time-resolved investigations of biomacromolecules at microfocused X-ray sources.[1] This approach has many advantages compared to more traditional set-ups in terms of sample consumption, accuracy of measurements and the ability to access time-scales down to 10s of μs for time-resolved (TR) studies.

Microfluidic devices can be manufactured using a number of different polymers, such as PDMS, COCs or polyimide films (Kapton®), each having different optical and mechanical properties and varying ease of manufacture. With the advent of more powerful and brighter X-ray sources, Kapton and COCs have received great interest as materials for X-ray compatible devices due to their low-background and high stability. Alternatively, microfluidic liquid jet devices offer a free-flowing sample stream for XFELs and highly brilliant synchrotron sources.[2]

The precise control of reaction conditions have been demonstrated for the synthesis of nanoparticles[3] as well as in time-resolved structural studies of biomacromolecules using X-ray scattering[1b] and diffraction[5] as well as spectroscopy.[6] For TR studies, the reaction processes can be initiated by light activation (down to femtoseconds) or rapid mixing, where the time-resolution is only dependent on the diffusion rates of small molecules through the sample thickness (<1μm in laminar flow conditions, >10s μs).[7]

Our group specializes in the design, manufacture and implementation of microfluidic devices, providing sample environments that address specific experimental condition requirements. Alongside this tailored approach, and in collaboration with local synchrotron facilities, we are making standardized microfluidic set-ups for TR biomacromolecular structural studies for general users.

1.(a) Toft, K. N.; *et al.*; *Analytical Chemistry* **2008,** *80* (10), 3648-3654; (b) Pollack, L. *Biopolymers* **2011,** *95* (8), 543-549.

2.Trebbin, M.; Kruger, K.; DePonte, D.; Roth, S. V.; Chapman, H. N.; Forster, S. *Lab on a Chip* **2014,** *14* (10), 1733-1745.

3.Lazarus, L. L.; *et al.*; *Lab on a Chip* **2010,** *10* (24), 3377-3379.

4.Rahman, M. T.; *et al.*; *RSC Advances* **2013,** *3* (9), 2897-2900.

5.Chapman, H. N.; *et al.*;. *Nature* **2011,** *470* (7332), 73-77.

6.Park, H. Y.; *et al.*; *PNAS* **2008,** *105* (2), 542-547.

7.Levantino, M.; *et al.*; *Curr Opin Struct Biol* **2015,** *35*, 41-8.

Keywords: Microfluidics, time-resolved, SAXS