

PAUL SCHERRER INSTITUT



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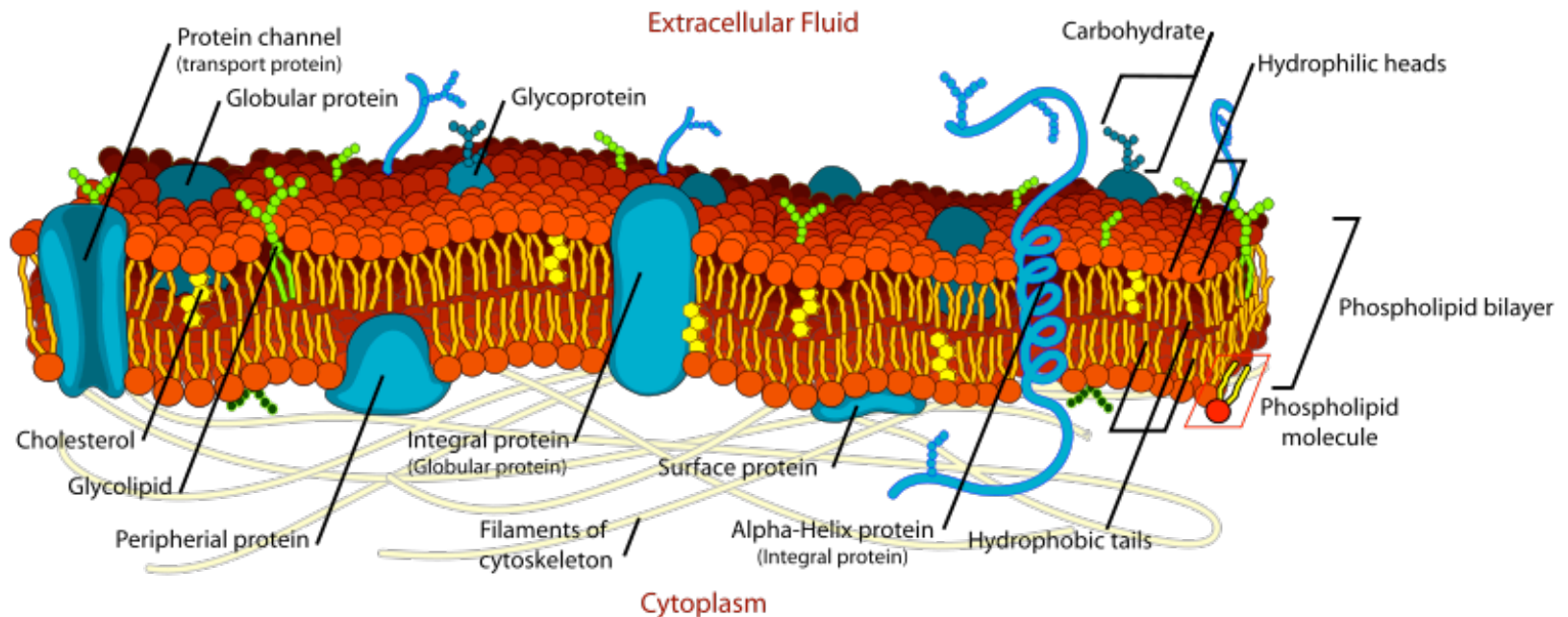
***In situ* serial crystallography for membrane protein structure determination**

21st September 2018, Empa Postdocs-II & PSI-FELLOW II-3i RETREAT 2018

Outline

- Structural determination of membrane protein by using lipid cubic phase method
- *In situ* method for delivering the sample for X-ray diffraction
- Successful cases from macromolecular crystallography beamlines in SLS

Structural determination of membrane protein by using lipid cubic phase method



MP → crystals → X-ray → MP structure

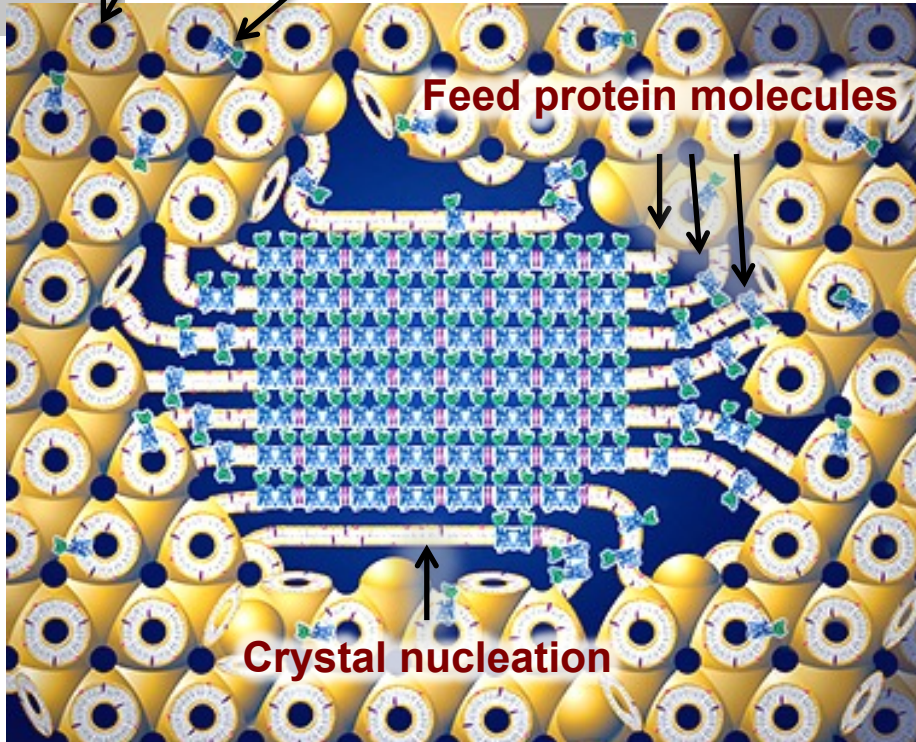
Lipid cubic phase (LCP)/*in meso* crystallization

Lipid bilayer

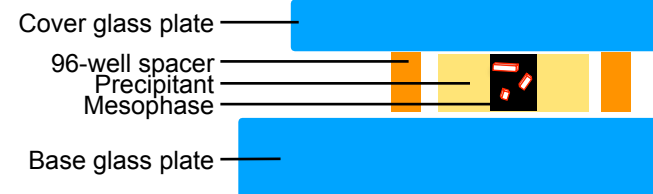
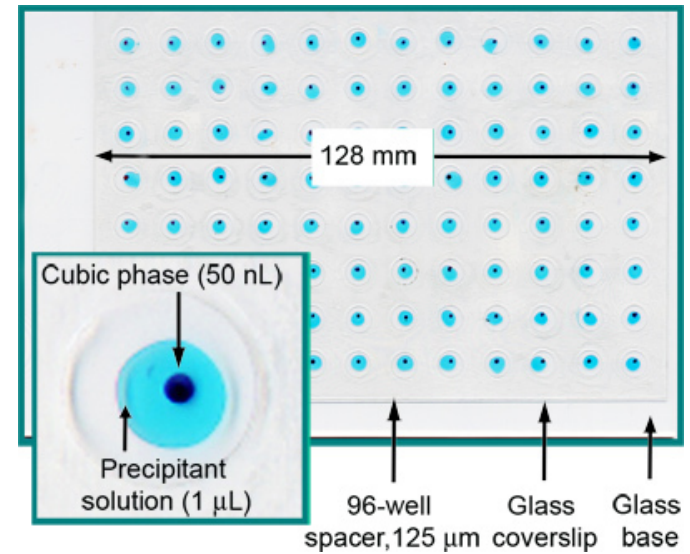
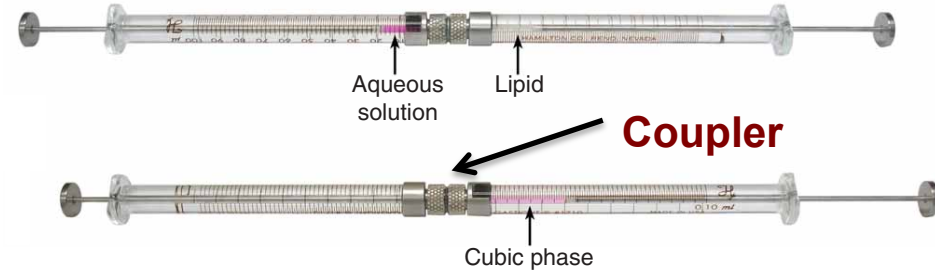
Membrane protein

Feed protein molecules

Crystal nucleation

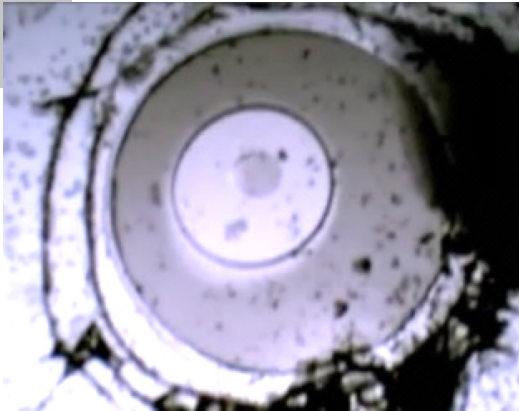


Mixing devices

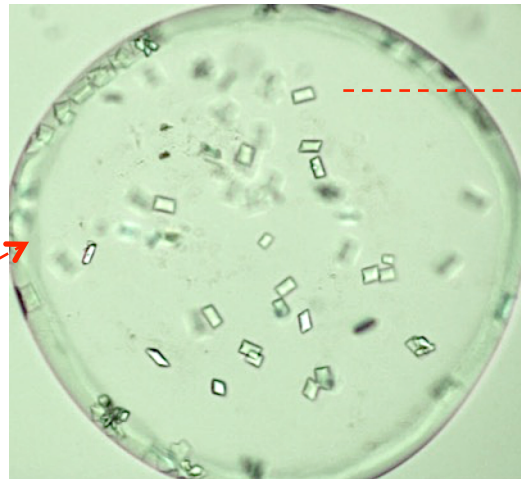


- Li, D., Boland, C., Walsh, K., & Caffrey, M. J. *Vis. Exp.* 67: e4000. 2012.
- Li, D., Boland, C., Aragão, D., Walsh, K., & Caffrey, M. J. *Vis. Exp.* 67: e4001. 2012.
- Caffrey, M., Porter, C. J. *Vis. Exp.* 45: e1712. 2010.
- Caffrey, M., Cherezov, V. *Nature Protocols.* 4:706-731. 2009.

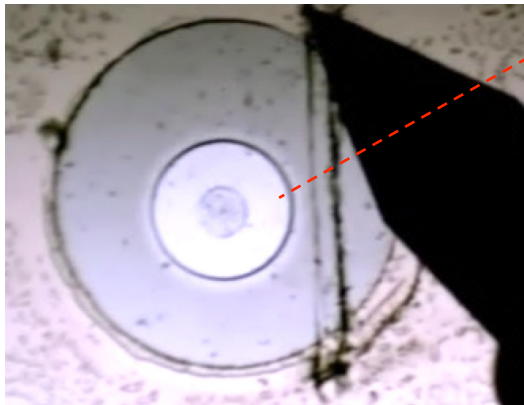
Sample preparation and crystals harvesting



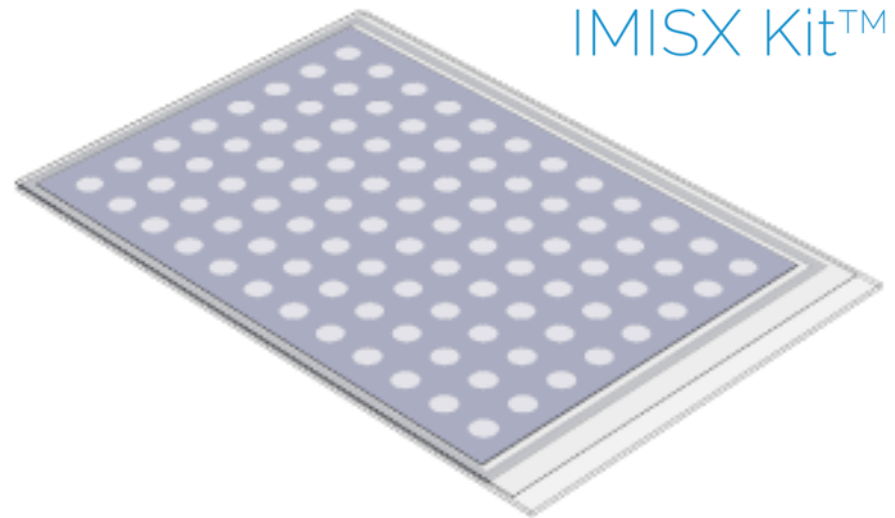
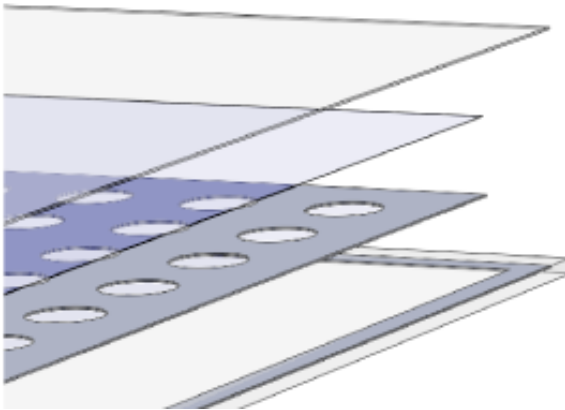
Lipidic cubic phase bolus



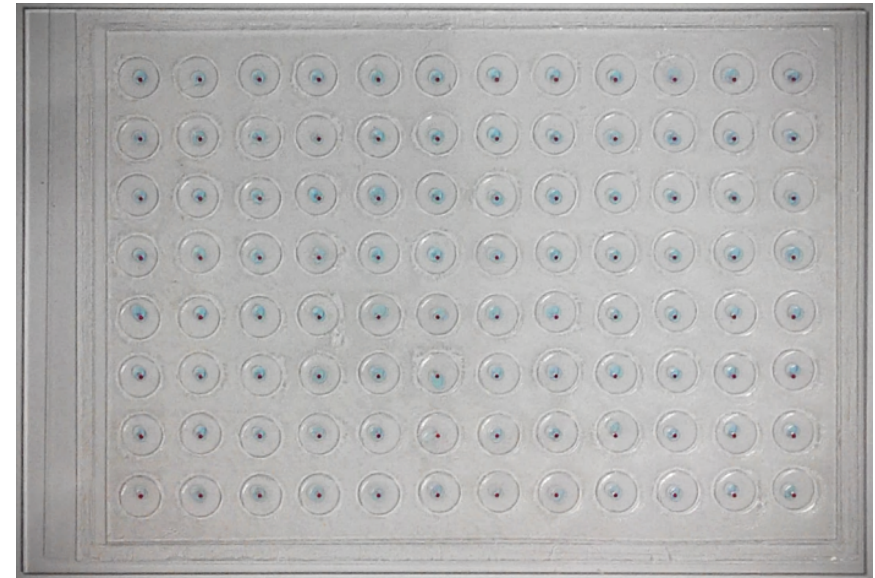
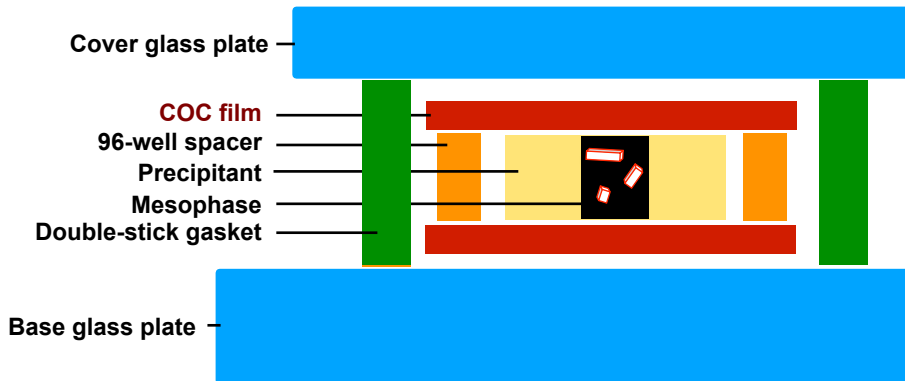
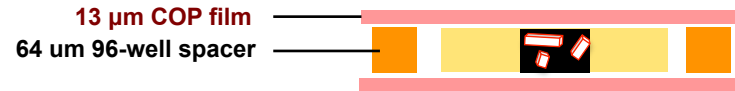
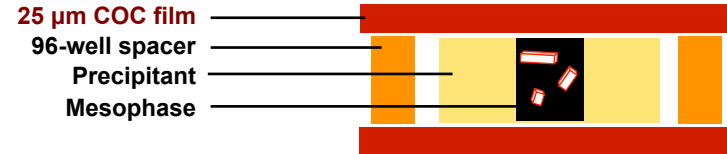
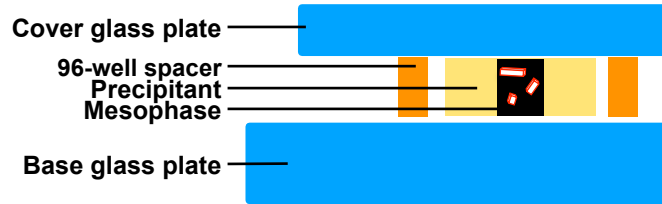
Harvest single crystal in one loop and do it many times



In situ method for delivering the sample for X-ray diffraction

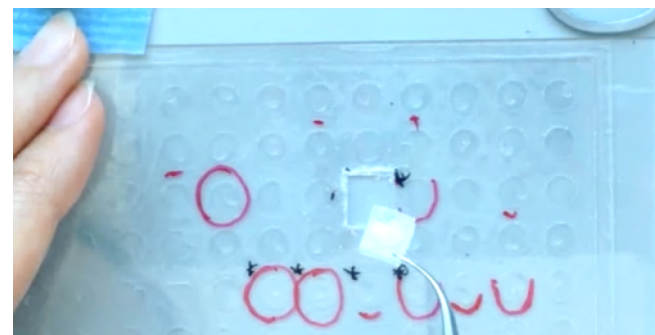
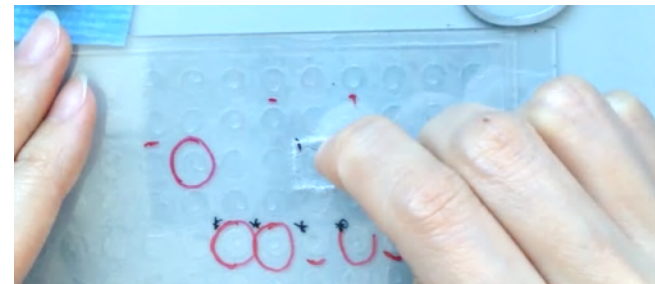
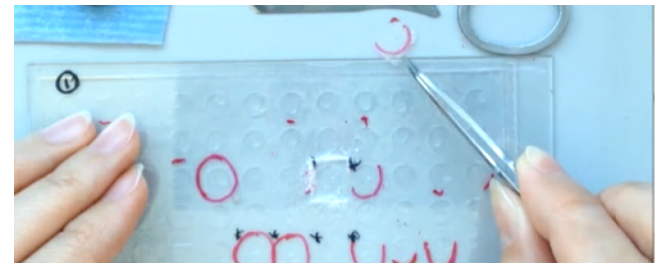
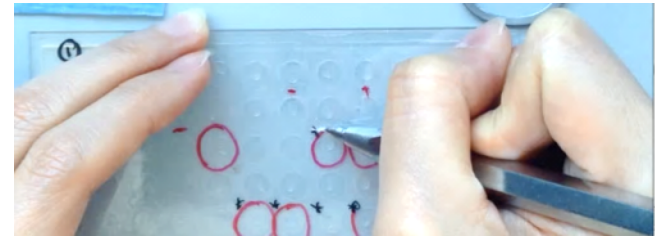
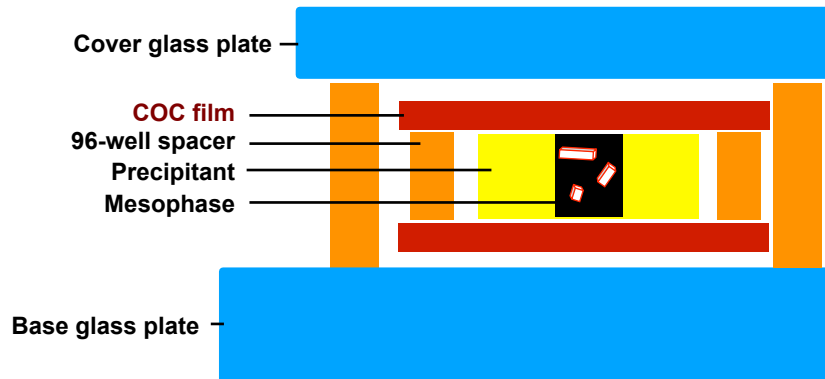


In meso in situ crystallization plates (IMISX)



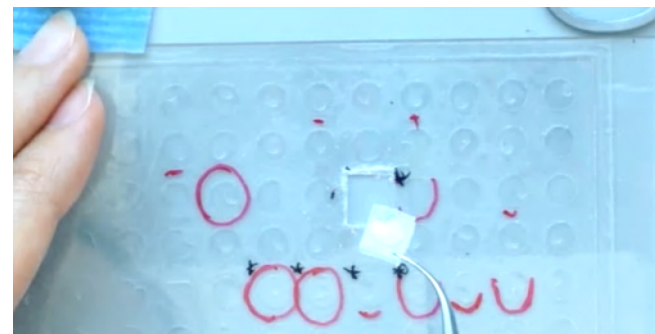
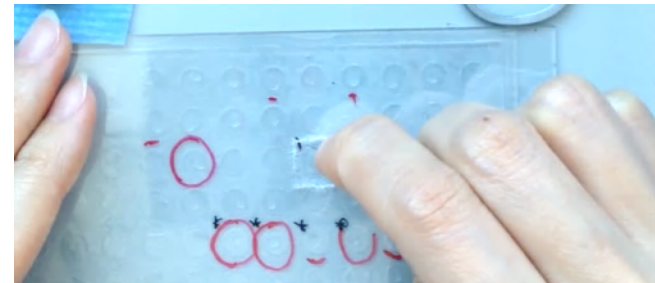
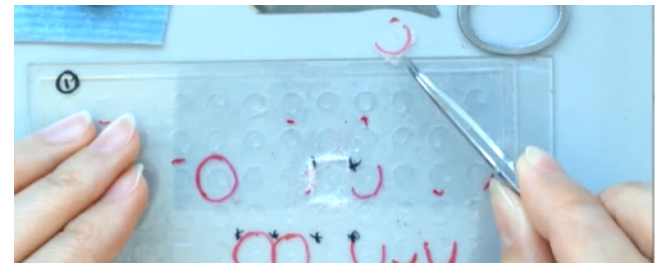
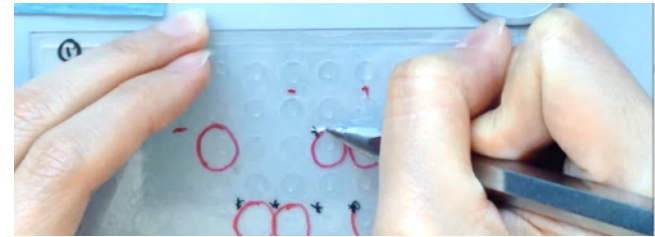
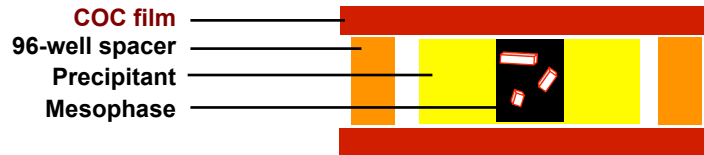
How to harvest the crystals from the IMISX plate

Open the glass plate with glass cutter and retrieve the IMISX well with blade

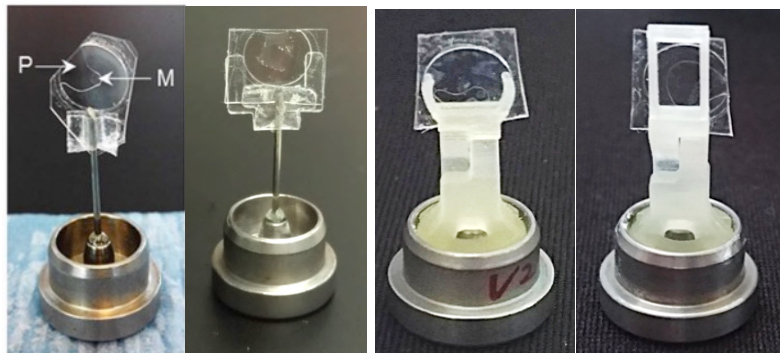


How to harvest the crystals from the IMISX plate

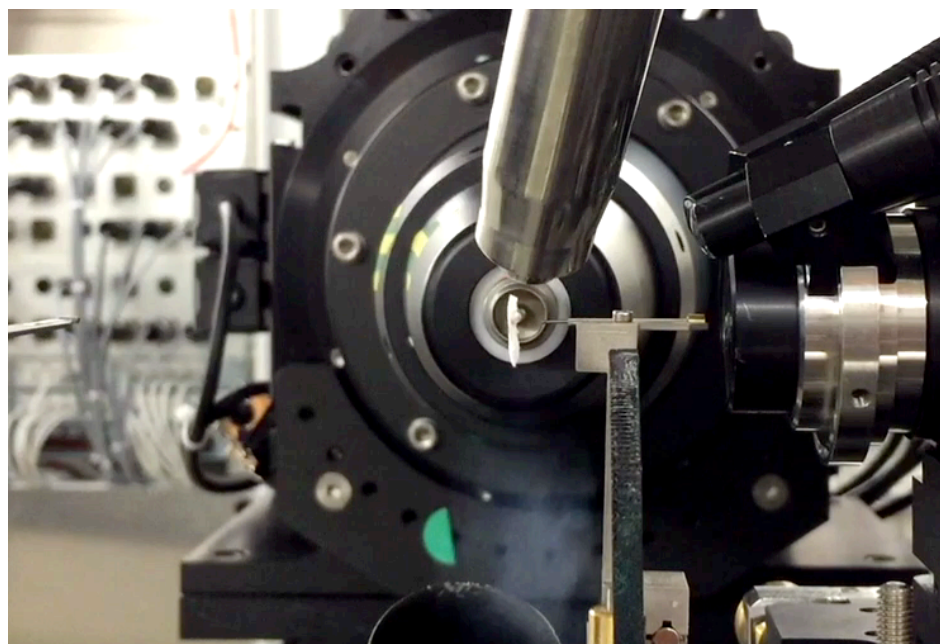
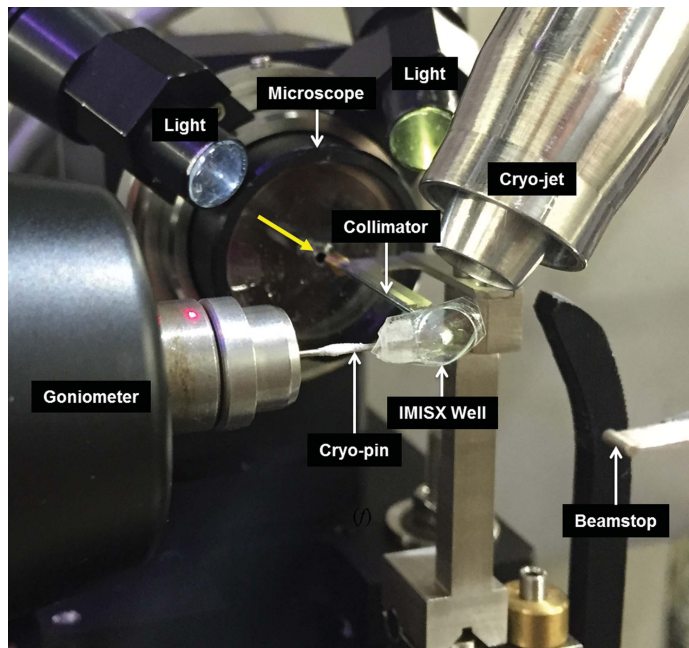
Open the glass plate with glass cutter and retrieve the IMISX well with blade



IMISX data collection at cryogenic temperature

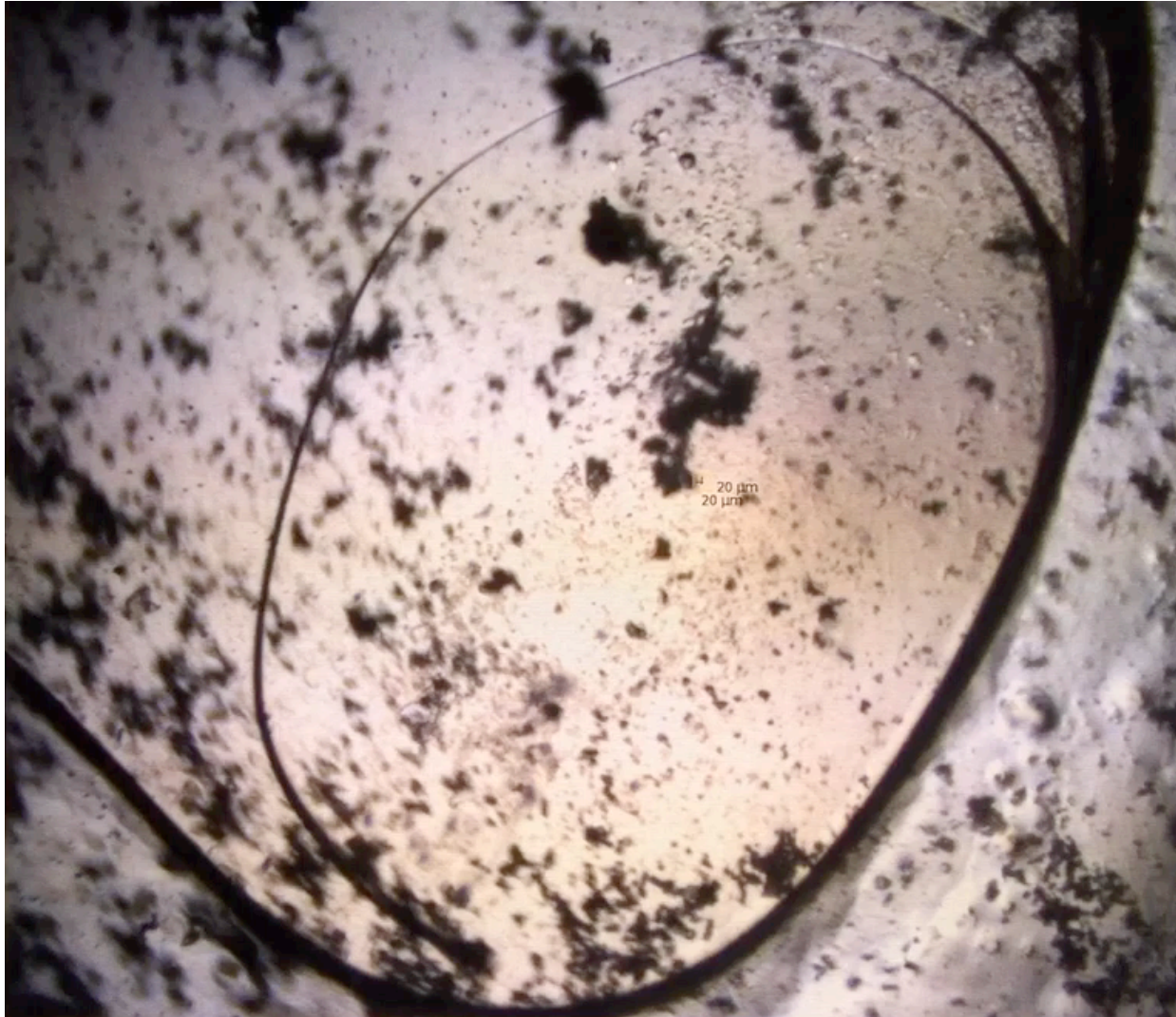


Data collection at 100K



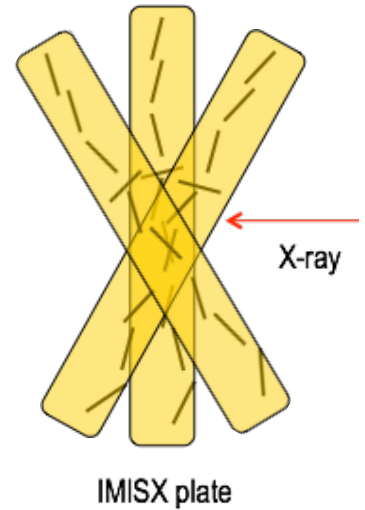
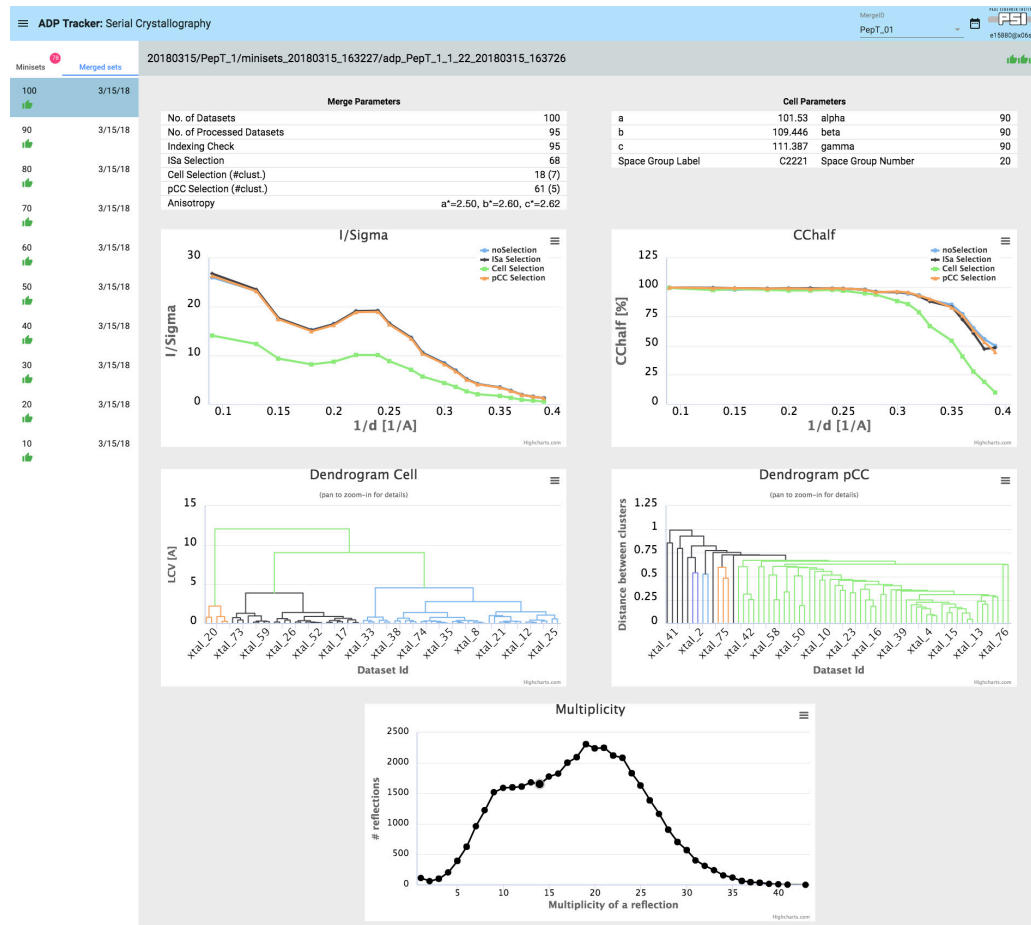
X-rays
←
± 45 degs

Grid scan and spots evaluation



20 x 20 μm beamsize,
50 x 50 grids,
100 Hz

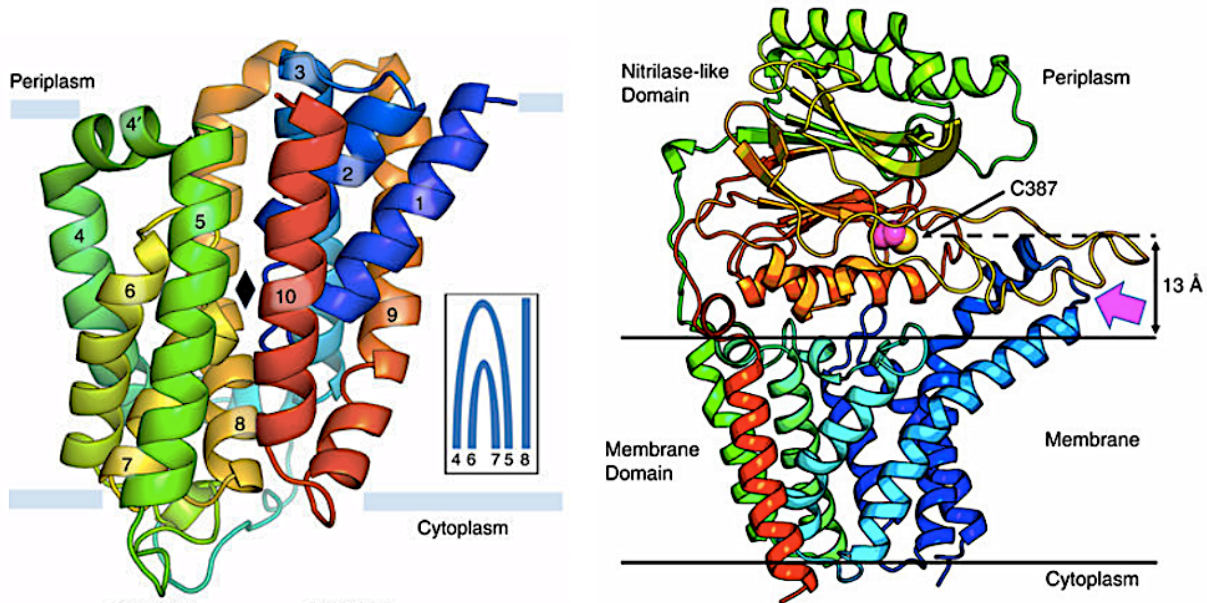
ADP and ADM



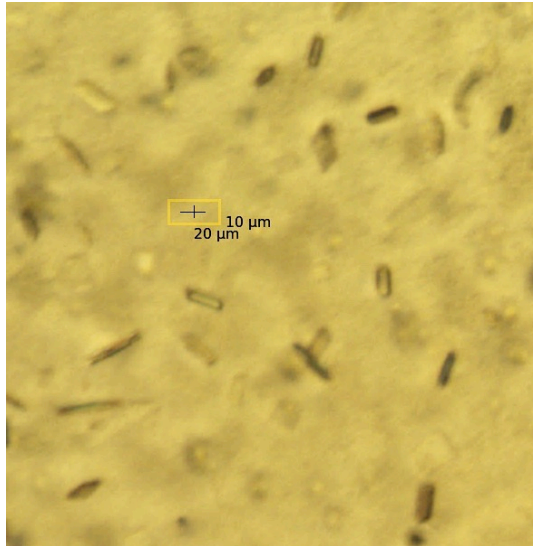
Feedback to data processing

- Wojdyla *et al.*, *J Synchr. Rad.* (2018) 25. 293-303.
- Basu *et al.*, *J Synchr. Rad.* (2018) submitted.

Successful cases from macromolecular crystallography beamlines in SLS

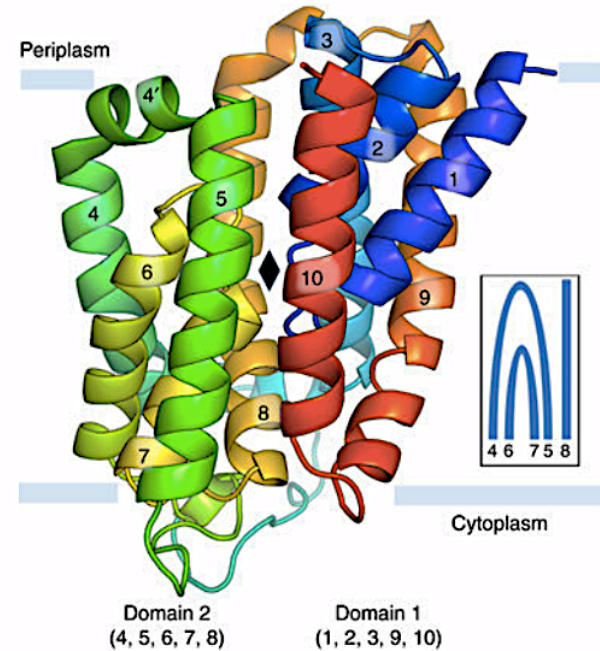


Application of the IMISX on enzyme for peptidoglycan biosynthesis, first de novo structure solved by IMISX method

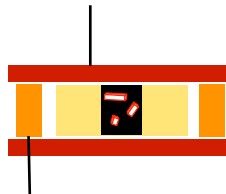


The enzyme involved in the processing of carrier lipids required for the synthesis of bacterial cell walls → important target for antibiotic design

(2018) Nat Commun 9: 1078, doi:10.1038/s41467-018-03477-5

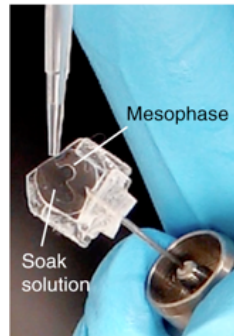


25 μm COC film



140 μm 96-well spacer

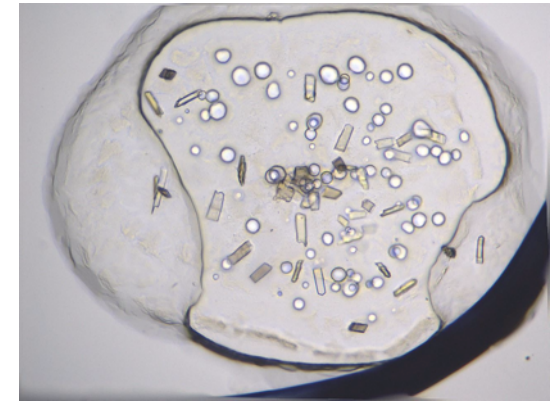
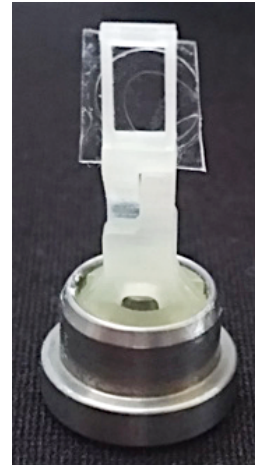
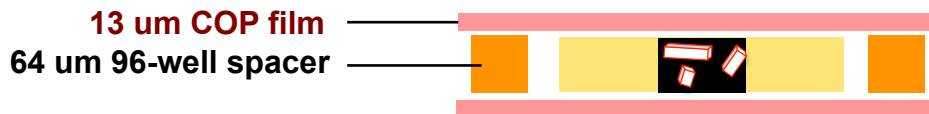
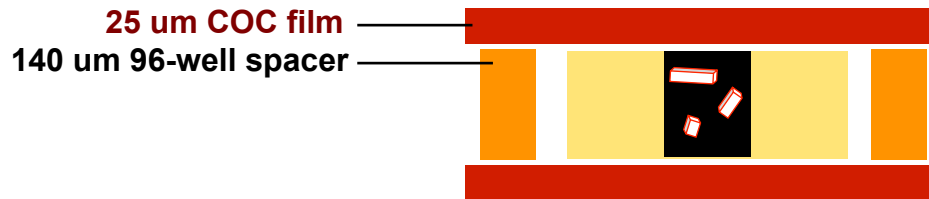
Add HA-solution



Phasing the structure

Continually developing thinner material for IMISX plate

- G protein–coupled receptors (GPCRs)
- Thinner material.



Conclusion

IMISX, IMISX-experimental phasing and automated data collection

- No crystal harvesting
- Ligand- and HA-soaking capabilities
- Reduced background with thin material (COC, COP, SiN)
- Automated data collection at both room and cryogenic temperature
- Real-time data processing/selection

| Beamline | PXI (X06SA) | PXII (X10SA) | PXIII (X06DA) |
|-----------------------------------------------------|------------------------------------------------------------------------|--------------------------------------------------------------|------------------------------|
| Source | U19 | U19 | 2.9T Superbend |
| Energy range | 6.0 – 17.5 keV | 6.5 – 20.0 keV | 5.5 – 17.5 keV |
| Flux, phs/s (12.4 keV, focused beam) | $2 \times 10^{11} \leftrightarrow 2 \times 10^{12}$ | 2×10^{12} | 5×10^{11} |
| Beamsize, μm^2 (with focusing, slits) | $2 \times 1 \leftrightarrow 100 \times 100$ (fast beam size change) | 50×10 $30 \times 10, 20 \times 10, 10 \times 10$ | $80 \times 45 \mu\text{m}^2$ |
| Goniometer | Micro-diffractometer (SmarGon coming) | | Multi-axis PRIGo |
| Detector | EIGER 16M | PILATUS 6M | PILATUS 2M |
| Data collection time | 2 – 3 minutes | | |
| Sample changer | IRELEC CATS | | |

My thanks go to

- **Paul Scherrer Institut/Swiss Light Source**

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