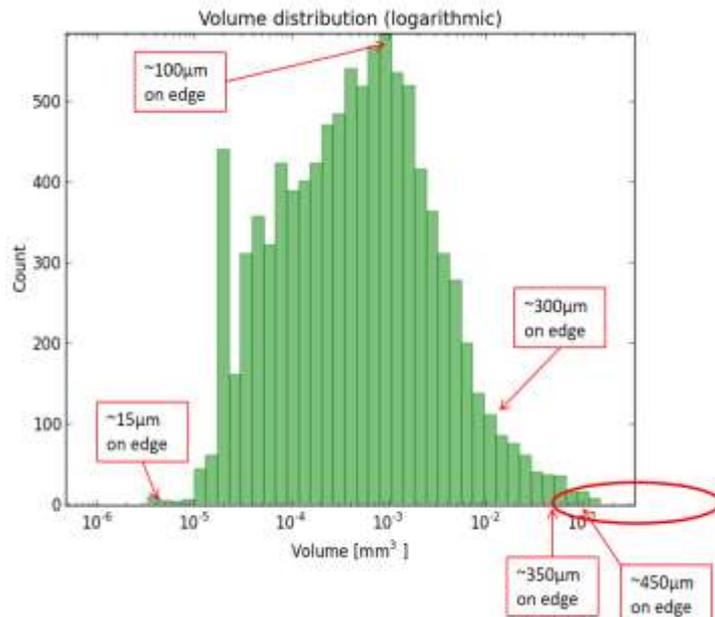


Background – neutron crystallography can provide crucially important information on biological structure that cannot be obtained any other way – eg protonation states, redox systems, drug-protein interactions, hydration details.

The problem – despite pivotal advances (instrumentation, perdeuteration), requirements for **protein crystal volume** heavily restrict access to the range of biological problems for neutron crystallography.

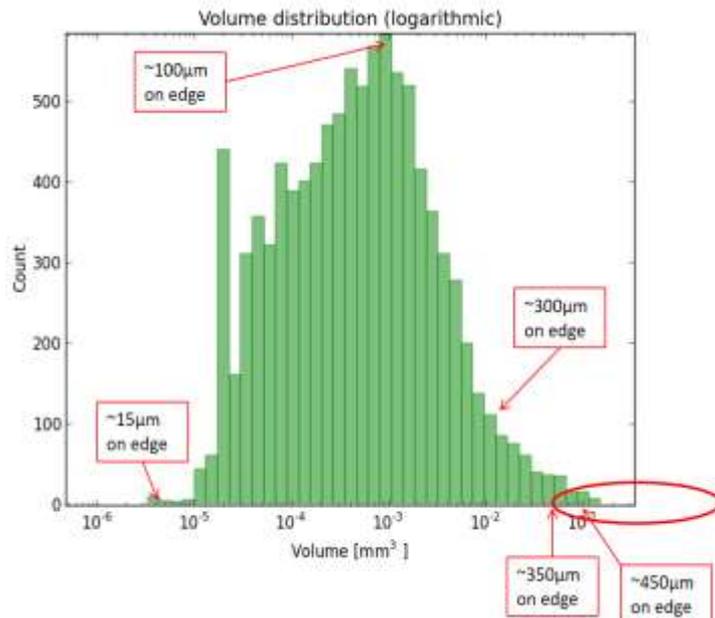


- **Ashley Jordan** and Trevor Forsyth: ILL Life Sciences Group, Grenoble
- **Marialucia Longo** and Tobias Schrader: Juelich
- **Zoe Fisher**, ESS, Lund

MASSIF @ESRF data showing crystal volumes processed by synchrotron X-ray analyses (courtesy M. Bowler)

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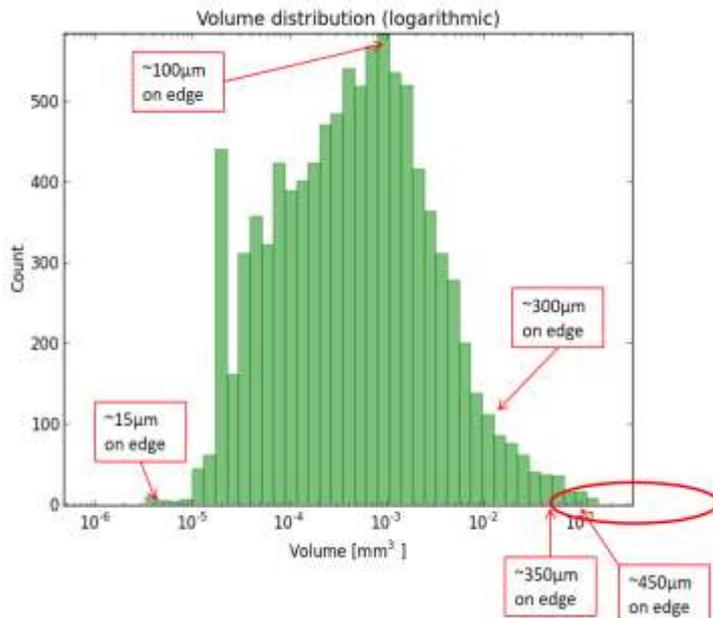


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Data from MASSIF @ESRF showing range of crystal volumes processed by synchrotron X-ray analyses

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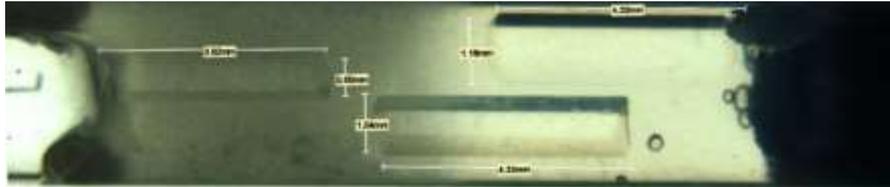


- **Ashley Jordan** and **Sciences Group,**
- **Marialucia Longo**
- **Zoe Fisher,** ESS,

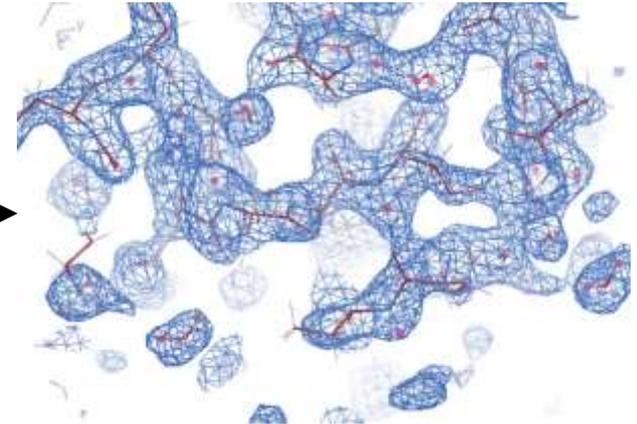


Data from MASSIF @ESRF showing range of crystal volumes processed by synchrotron X-ray analyses

Enhanced crystal growth in high magnetic fields



XDS

**D19**

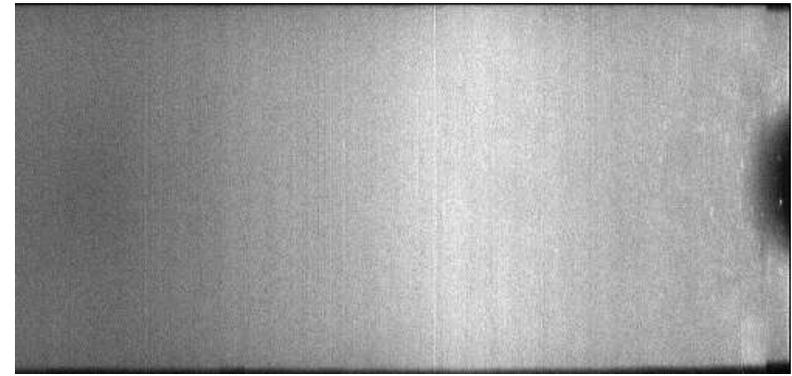
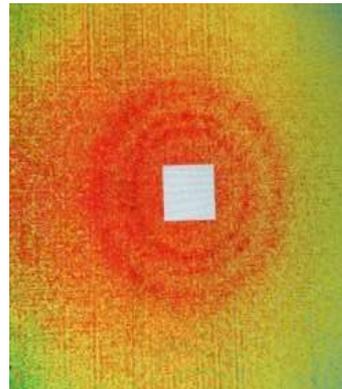
omega scan (0.07° /step)
 $P2_12_12_1$ ($a= 30.64$ $b=57.02$
 $c=74.53$)

**Estelle with Kay
Diederichs** (Konstanz).
Problems of RETREAT
resolved – at least for
high res. protein
structures.

Neutron structure
refinement: high quality
neutron scattering
length density maps.
1.9A data at 93%
completeness

Microcrystallite alignment

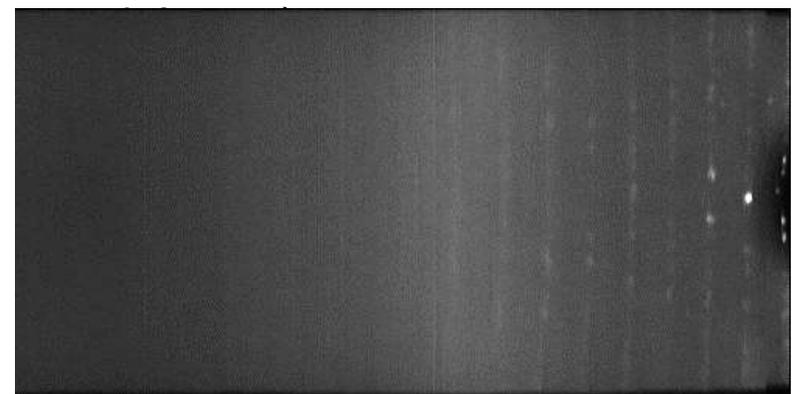
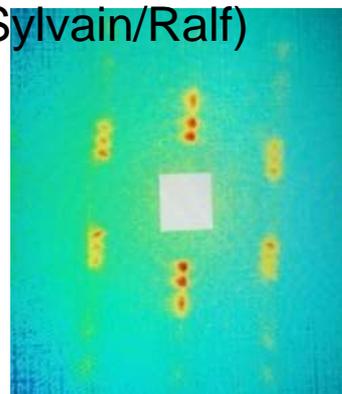
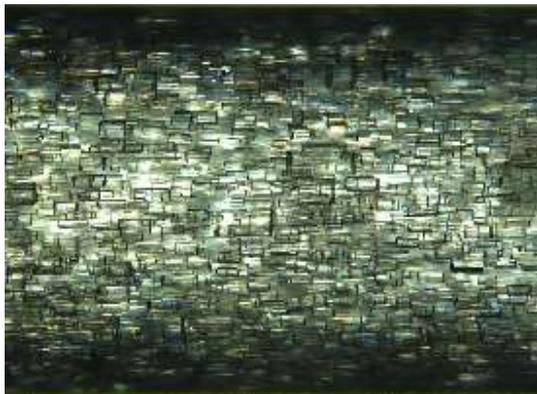
Unaligned



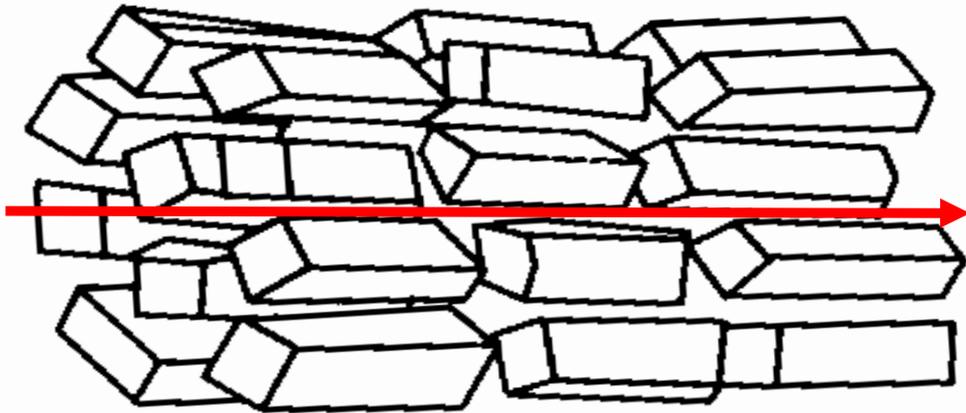
SANS data (D11)
(Sylvain/Ralf)

High-angle data (D19) (Estelle)

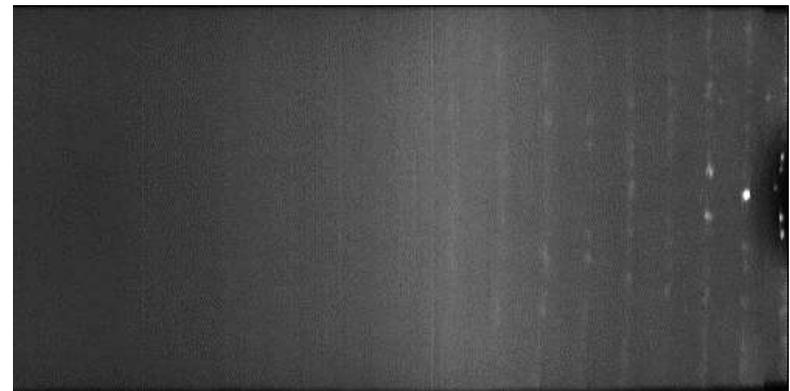
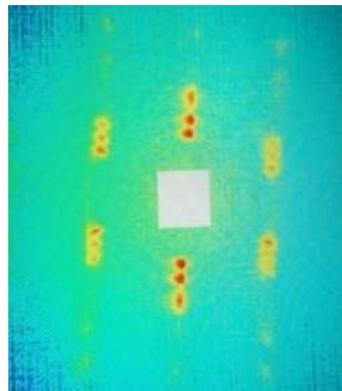
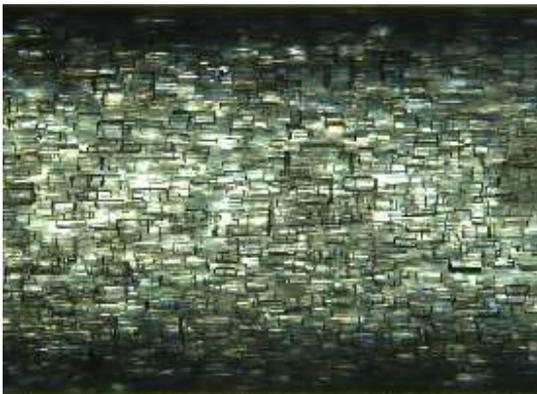
Magnetically aligned



Microcrystallite alignment – opening up the MX histogram

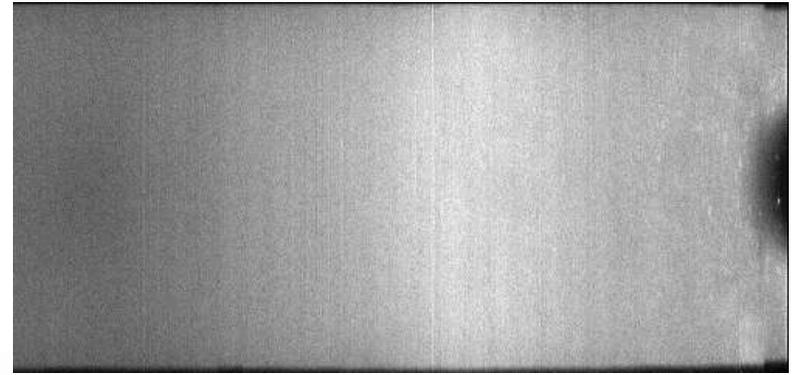
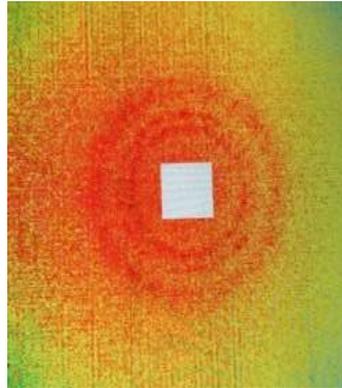


- Access to much wider range of proteins in MX histogram
- Loss of information from cylindrical averaging far less than might be expected
- Data largely 2D in character - rapid data collection (1-2 days)
- No phasing problem since X-ray structures are almost always available

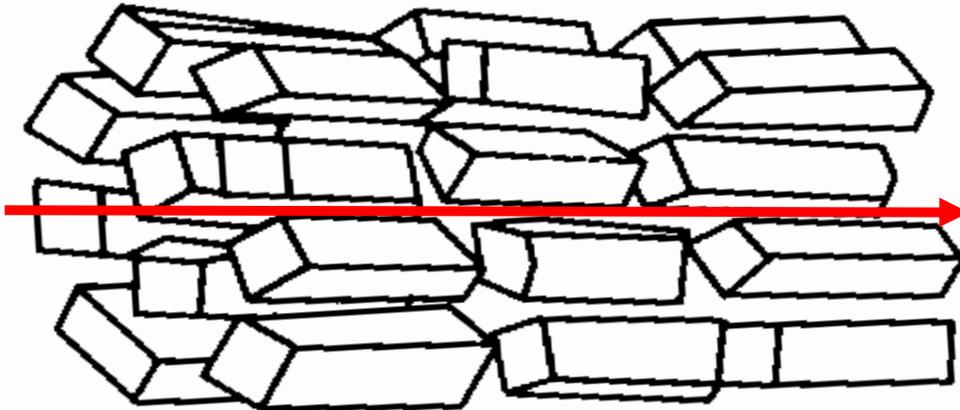


Microcrystallite alignment

Unaligned

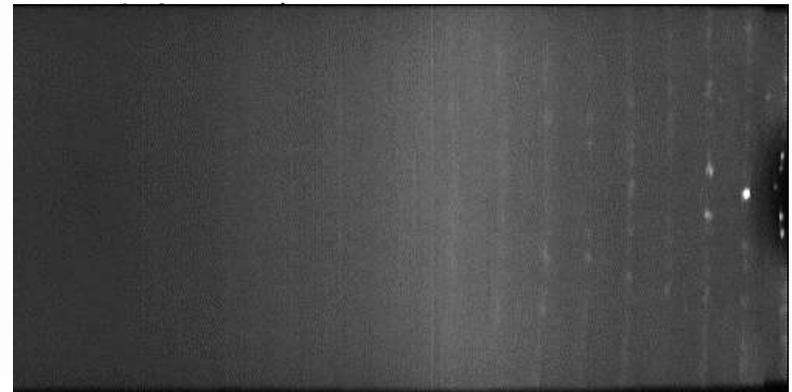


Magnetically aligned



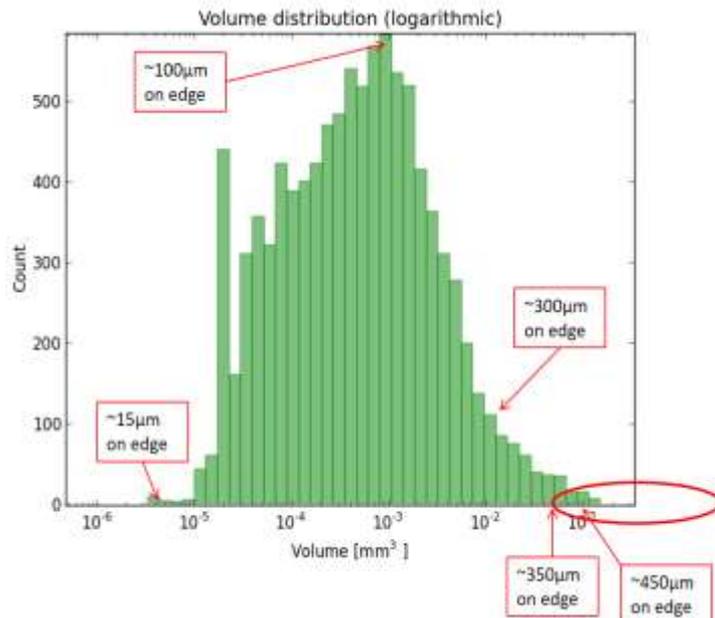
Cylindrical averaging of crystallites about a principal axis – fibre ordering

High-angle data (D19) (Estelle



Background – neutron crystallography can provide crucially important information on biological structure that cannot be obtained any other way – eg protonation states, redox systems, drug-protein interactions, hydration details.

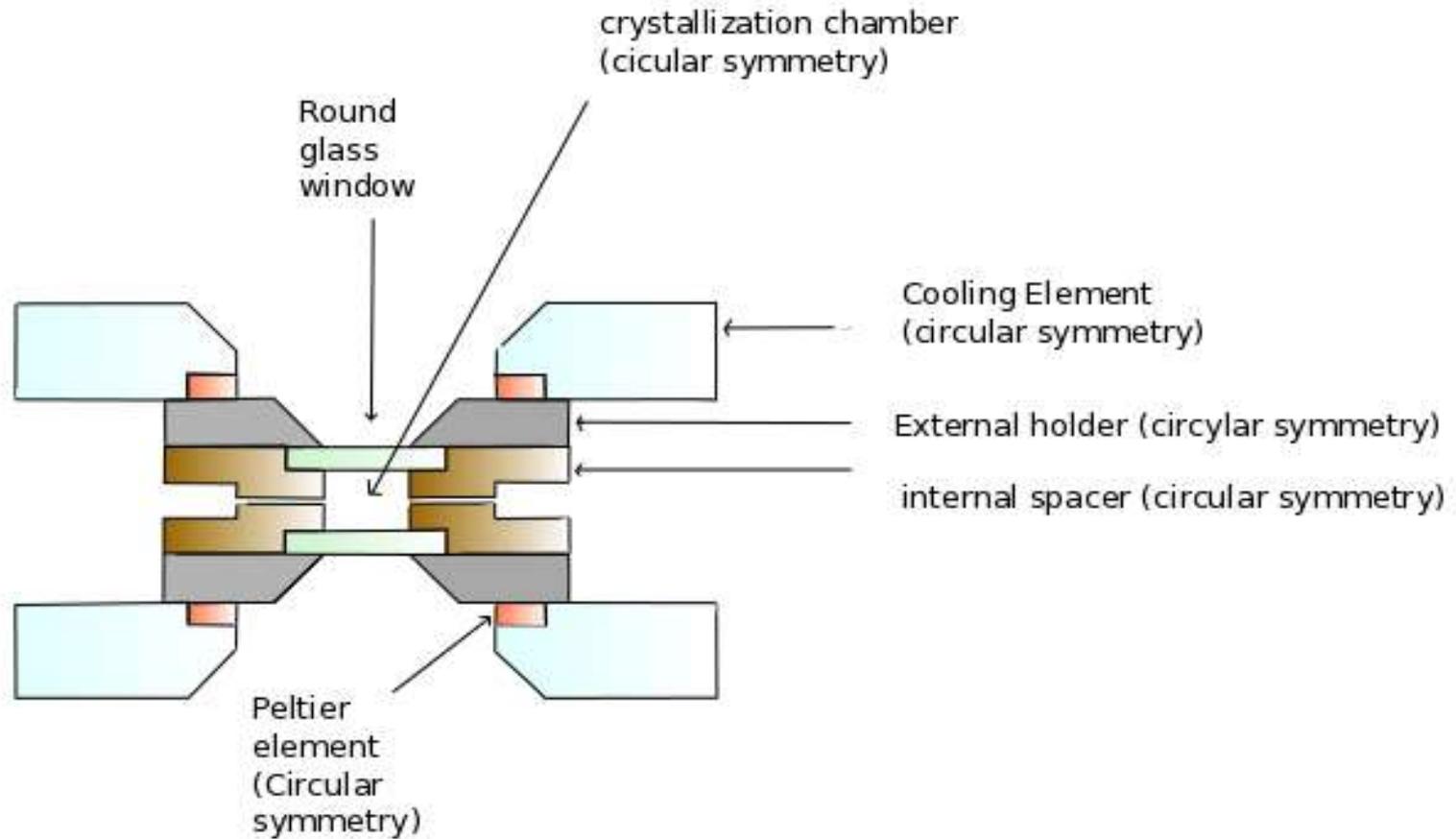
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Data from MASSIF @ESRF showing range of crystal volumes processed by synchrotron X-ray analyses

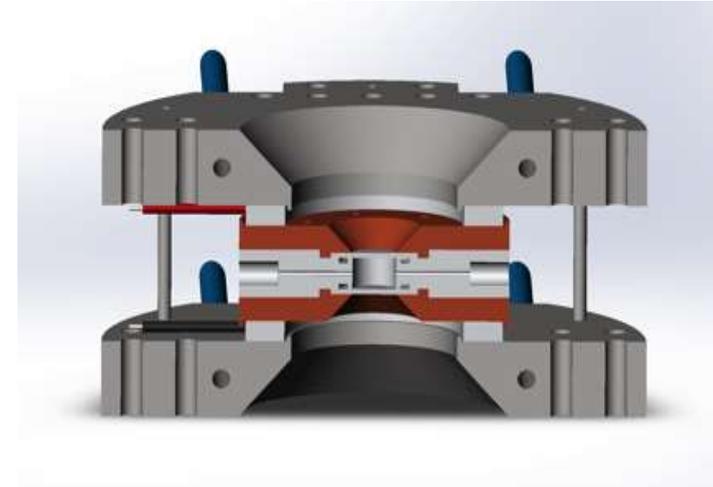
Introduction



Introduction

Circular symmetry:

- Uniform and isotropic transmission of the heat into the crystallization chamber.



Central angular opening on the crystallization chamber:

- Possibility to apply characterization spectroscopic technique as well.

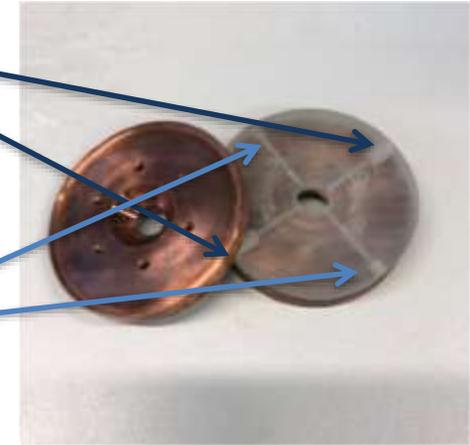
2. Batch configuration: internal spacer



Stainless steel version
of the internal spacer
for batch crystallization

In and out-put capillary

Holes for pt100
temperature sensors



3D printed version of
the internal spacer for
batch crystallization

Possibility to easily have new exchangeable internal spacers

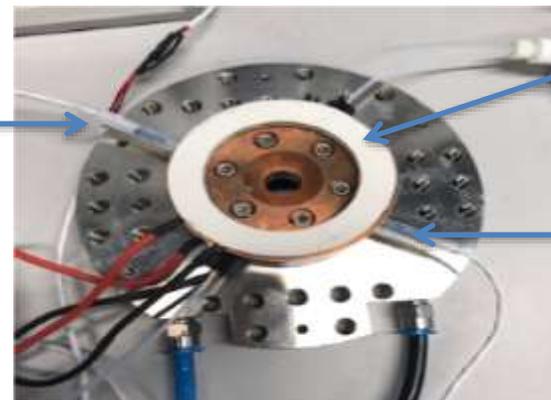
Batch configuration: Filling step



- 1 Avoid bubbles inside the crystallization chamber (filling from the top before closing does not help!).
- 2 Avoid breaking of the windows during the closing of the chamber (close enough to seal but not too much)!



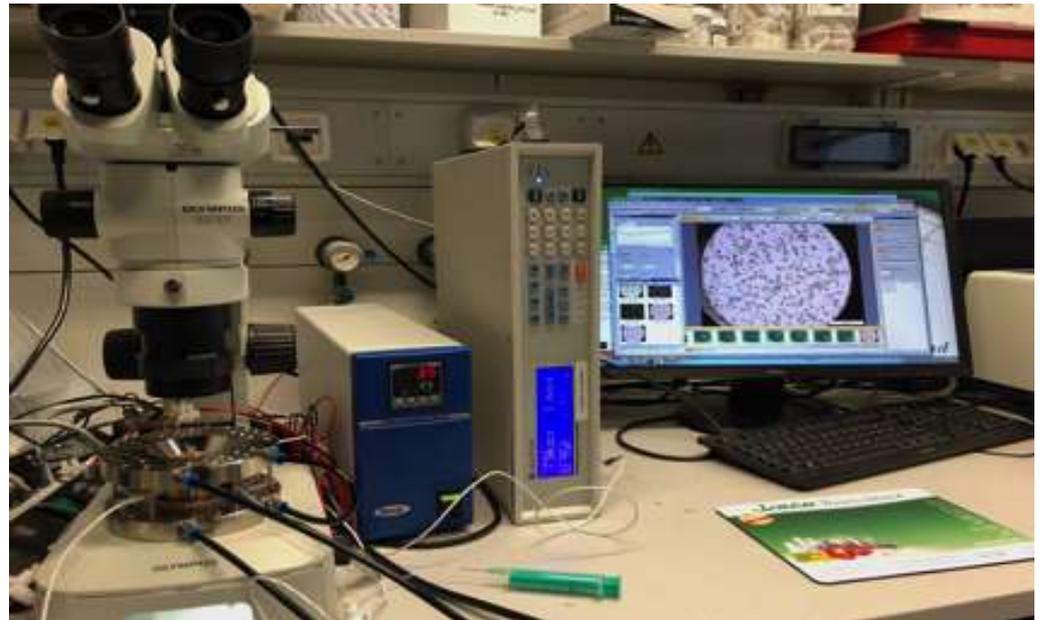
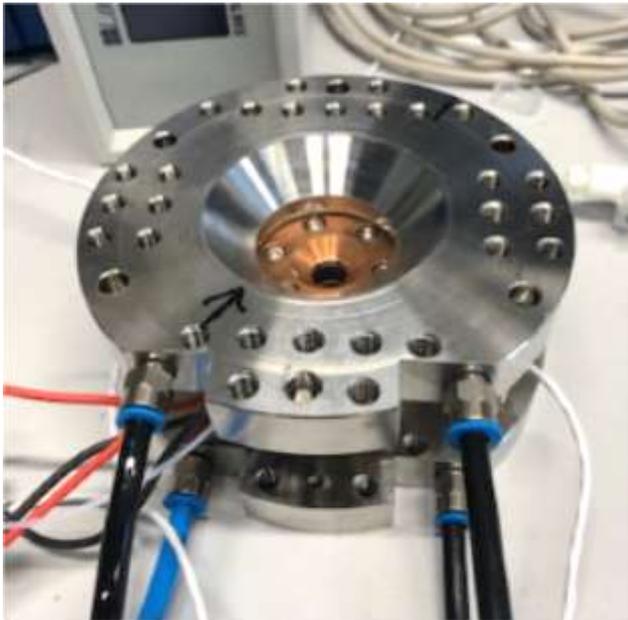
Pt100
(T sensor)



Peltier Element

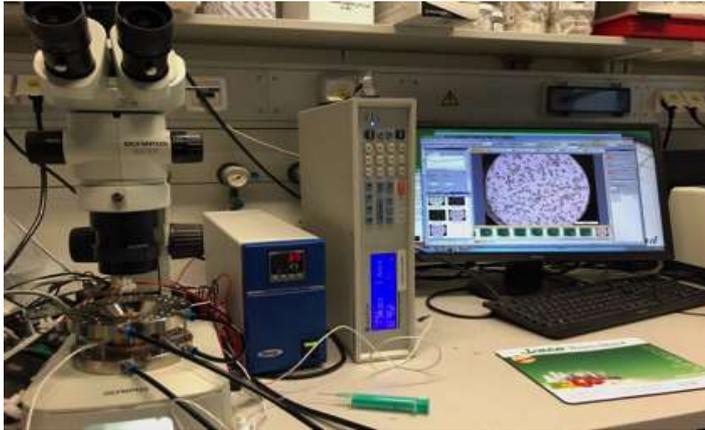
Pt100
(T controller)

Batch configuration

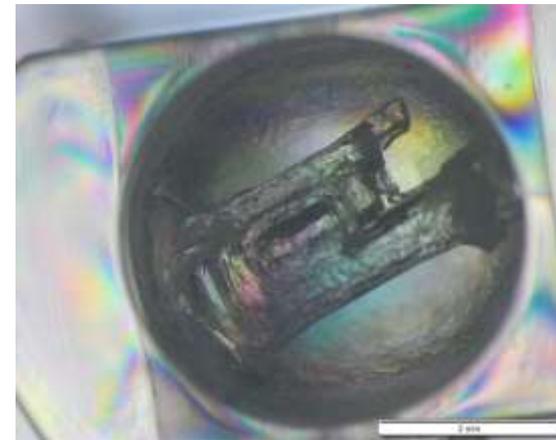


Scientific Summary slide

- First successful test of batch/vapour diffusion crystallization apparatus



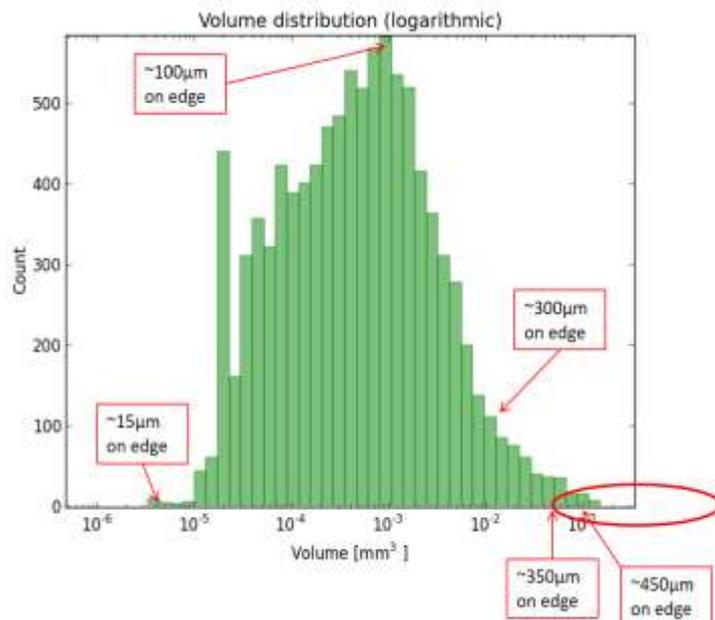
- First successful crystallization of a relevant protein: Streptavidin with Biotin – tested on Biodiff



Computer control of crystallization apparatus will allow to offer it to users of BIODIFF

Background – neutron crystallography can provide crucially important information on biological structure that cannot be obtained any other way – eg protonation states, redox systems, drug-protein interactions, hydration details.

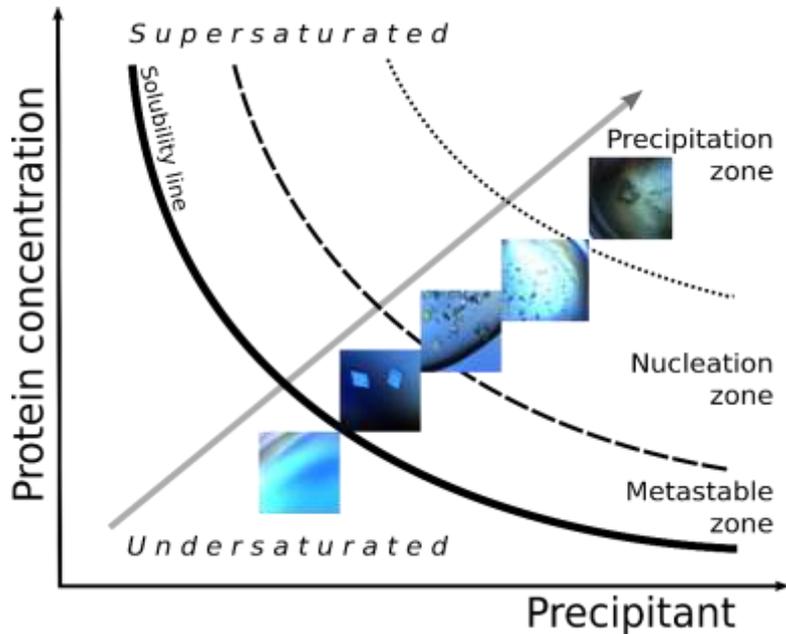
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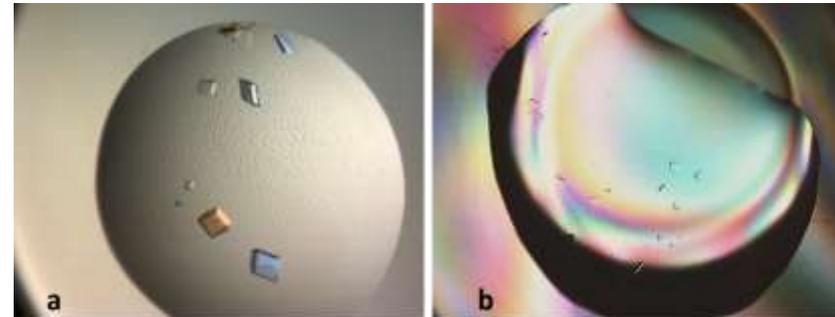
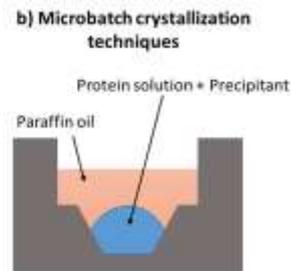
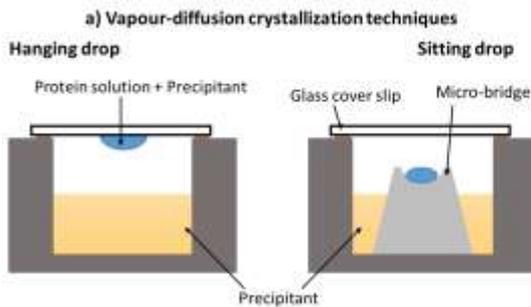
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Data from MASSIF @ESRF showing range of crystal volumes processed by synchrotron X-ray analyses

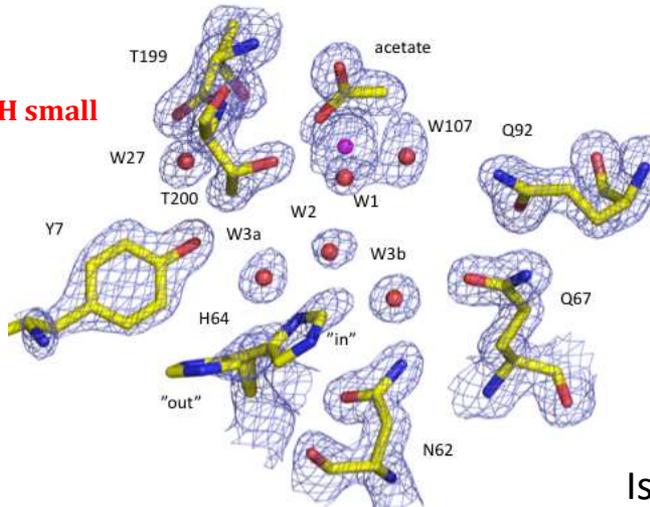
Phase diagram mapping – optimization, scale-up



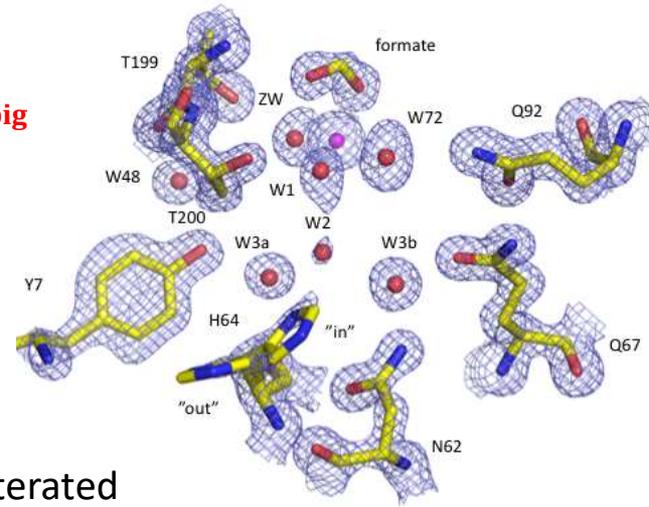
- Set up *phase diagram* for the “best” conditions and systematically explore pH, [protein], [ppt] in 6 μL drops
- HD & SD VD, batch (dialysis failed)
- *BEST: 25% PEG 3350, 0.2 M Na acetate, 100 mM Tris pH 8.5*
- Scale up to *24, 50, 150 μL*
- *Additives had to be adjusted* in largest volumes (!)



H/H small

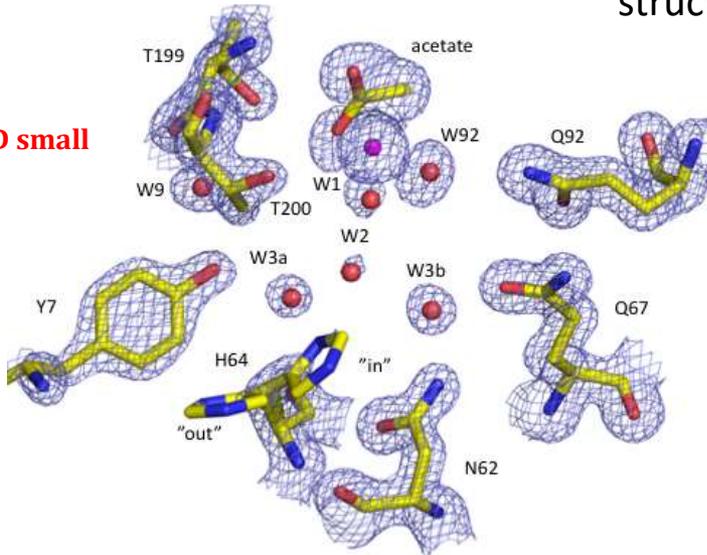


H/H big

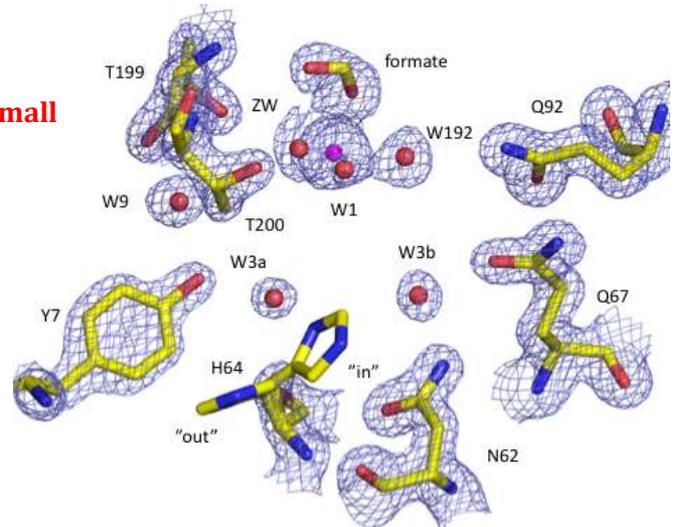


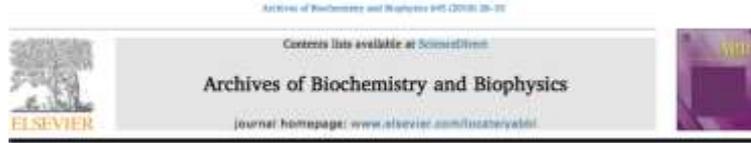
Isomorphism of deuterated and hydrogenated protein structures

H/D small



D/D small

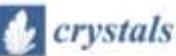




Deuteration of human carbonic anhydrase for neutron crystallography: Cell culture media, protein thermostability, and crystallization behavior

K. Koruza^a, B. Lafumat^a, Á. Végvári^b, W. Knecht^c, S.Z. Fisher^{a,c,*}

^aDepartment of Biology & Lund Protein Production Platform, Lund University, Högskolan 35, Lund SE205, Sweden
^bDepartment of Medical Biochemistry & Biophysics, Karolinska Institutet, Solnaströg. 2, Stockholm 17177, Sweden
^cScientific Activities Division, European Spallation Source ESRF, Tomtegatan 24, Lund SE1109, Sweden



Article

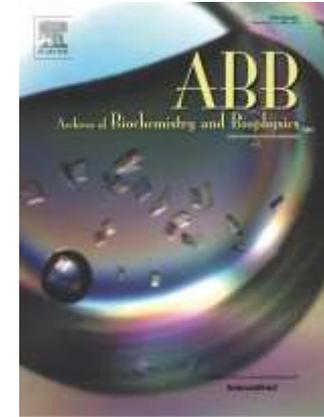
From Initial Hit to Crystal Optimization with Microseeding of Human Carbonic Anhydrase IX—A Case Study for Neutron Protein Crystallography

Katarína Koruza^{1,*}, Bénédicte Lafumat¹, Maria Nyblom¹, Wolfgang Knecht¹ and Zoë Fisher^{1,2,*}

Koruza, K., Lafumat, B., Nyblom, M., Mahon, B.P., Knecht, W., McKenna, R., Fisher, S.Z. (2019) "Structural comparison of protiated, H/D exchanged, and deuterated human carbonic anhydrase IX" – *submitted*

Jordan, A., Devos, J. Mossou, E., Bowler, M., Schrader T., Forsyth, V.T. Forsyth, The use of high magnetic fields for large protein crystal growth and microcrystal alignment in neutron crystallography. J. Appl. Cryst. (in prep)

Jordan, A., et al, Meth. Enzym. (in prep).



Journal of Structural Biology

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Using neutron crystallography to elucidate the basis of selective inhibition of carbonic anhydrase by saccharin and a derivative

Katarína Koruza^a, Brian P. Mahon^{a,1}, Matthew P. Blakeley^a, Andreas Ostermann^a, Tobias E. Schrader^a, Robert McKenna¹, Wolfgang Knecht^{a,1}, S. Zoë Fisher^{a,1}

Show more

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