Macromolecular characterisation and crystallisation at LP3

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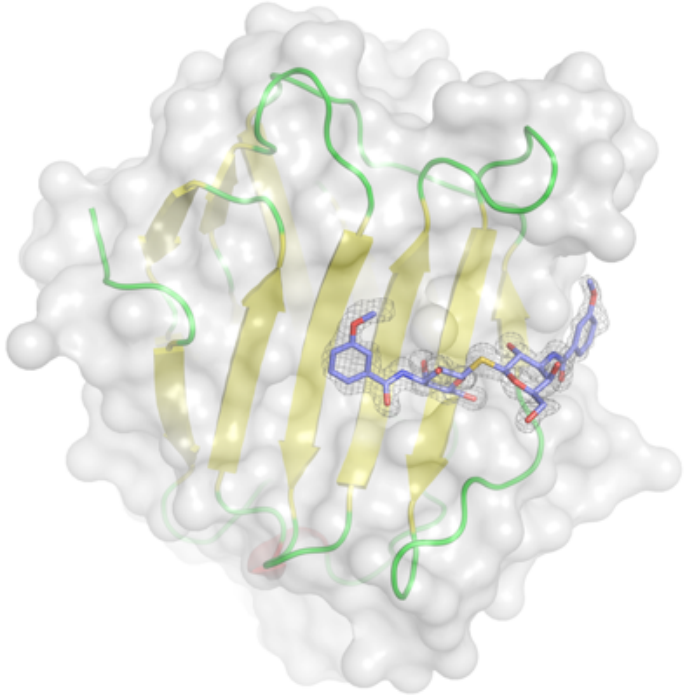

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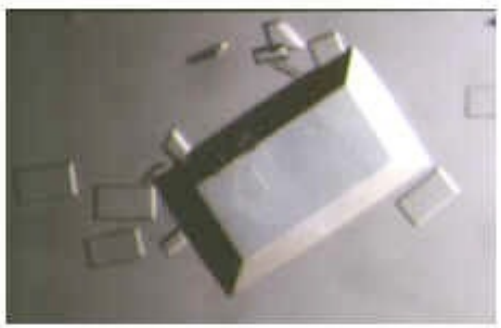
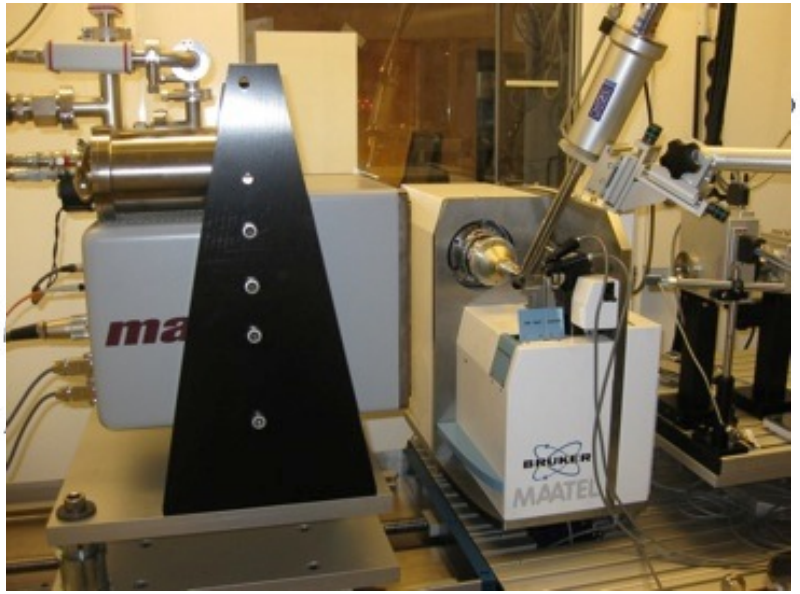
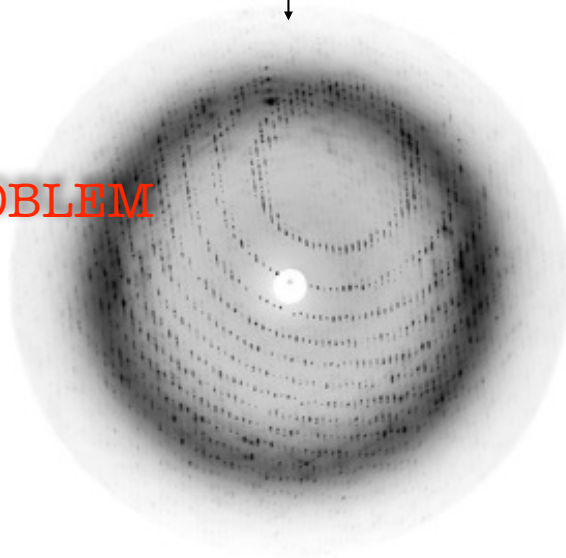
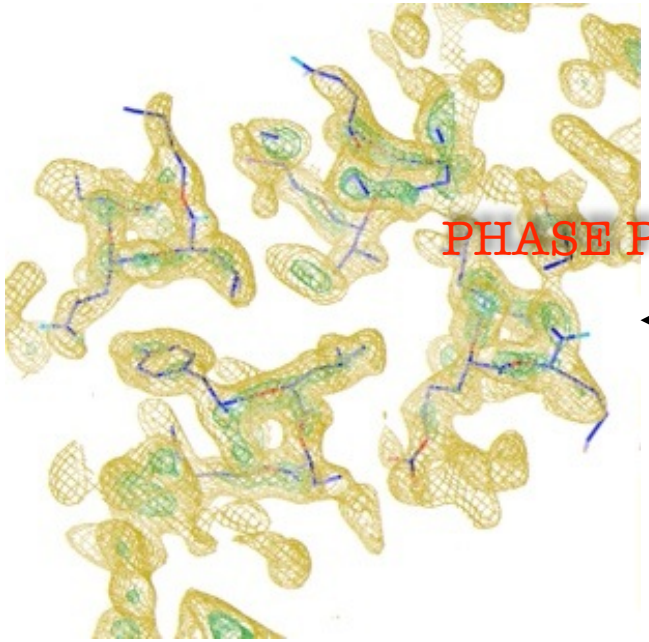
Crystallography 101

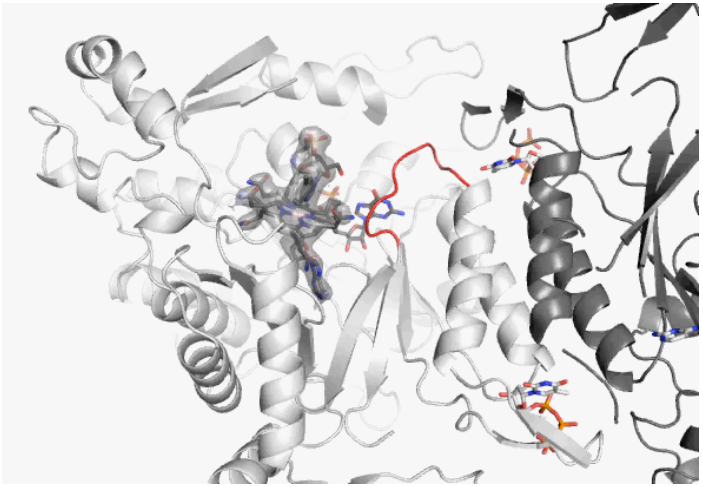






PHASE PROBLEM





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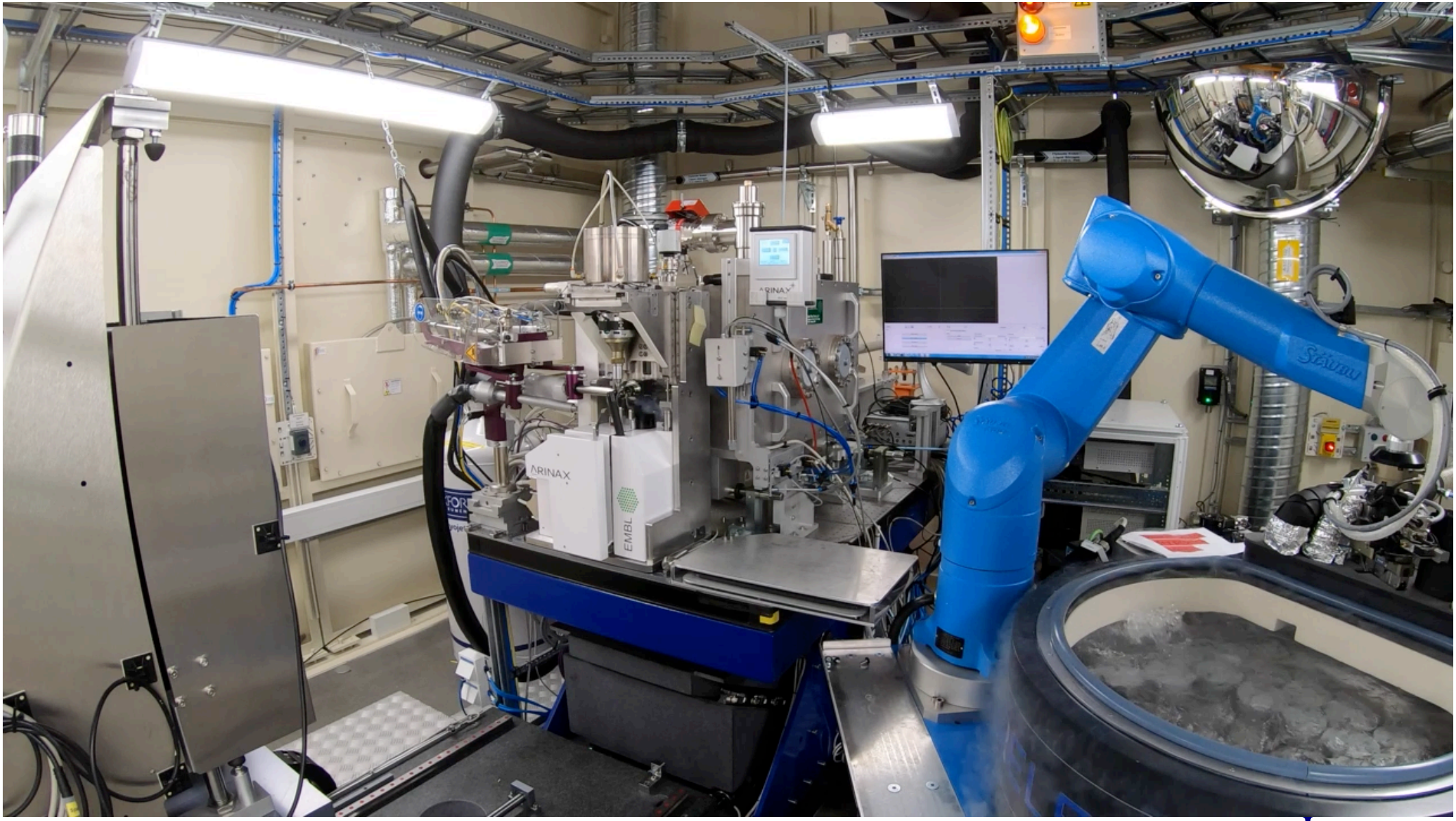
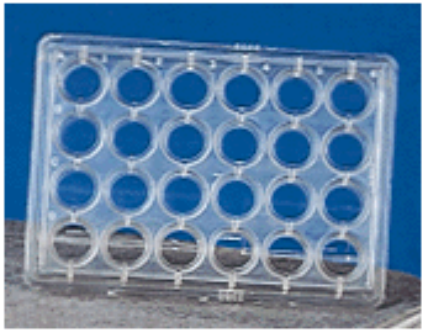
BioMAX at MAX IV: highly automated



movie by Uwe Müller, MAX IV

Crystallisation the old-fashioned way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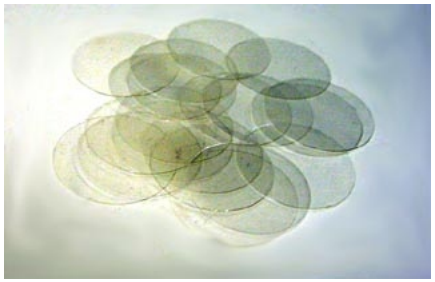
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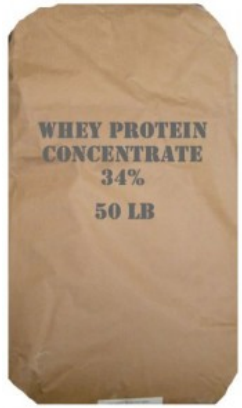

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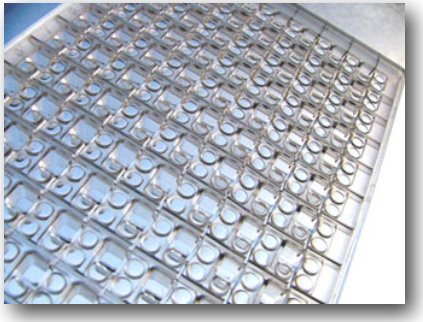
Crystallisation the modern way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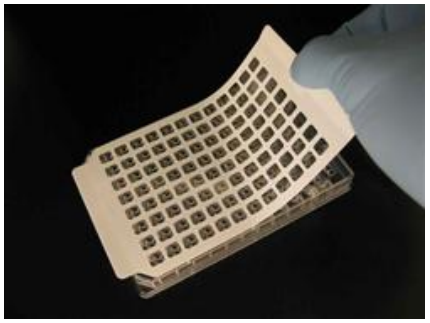

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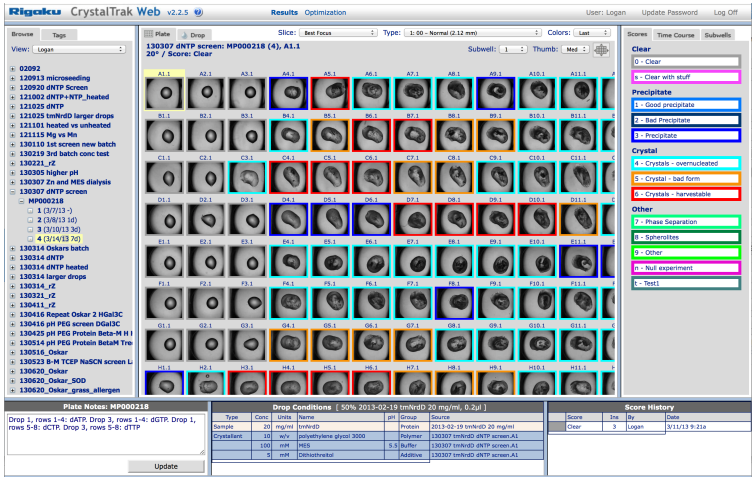

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- Robotic methods allow drops as small as 25 nl (0.025 µl), usually we use ~100 nl
- By hand one can only do ~1 µl, thus robotic methods consume 10–40 times less protein
- Robotics are expensive, so well-suited to core facilities

A systematic screen: PACT Premier

Figure 1

A PEG/pH (24)	B PEG/cation (24)
C PEG/anion (48)	

96-well plate

A **PEG/pH screen** 25 % w/v PEG 1500

						SPG system
						MIB system
						PCTP system
						MMT system
4	**5**	**6**	**7**	**8**	**9**	
pH of the buffer system						

B **PEG/cation screen** 20% w/v PEG 6000

						Acetate
						MES
						HEPES
						Tris
NaCl	**NH_4Cl**	**LiCl**	**$MgCl_2$**	**$CaCl_2$**	**$ZnCl_2$**	
0.2M of all, except for 0.01 M $ZnCl_2$.						

C **PEG/anion screen** 20% w/v PEG 3350

												No Buffer
												pH6.5
												pH 7.5
												pH 8.5
NaF	**NaBr**	**NaI**	**KSCN**	**$NaNO_3$**	**Na Formate**	**Na Acetate**	**Na_2SO_4**	**Na/K tartrate**	**Na/KPO_4**	**Na Citrate**	**Na Malonate**	
0.2M of each, except for 0.02 M Na/KPO_4.												0.1M Bis-tris propane

A “shotgun” screen: JCSG+

Tube No.	Salt	Buffer	pH	Precipitant
1.1	0.2 M lithium sulfate	0.1 M sodium acetate	4.5	50 % v/v PEG 400
1.2	None	0.1 M sodium citrate	5.5	20 % w/v PEG 3000
1.3	0.2 M di-ammonium hydrogen citrate	None	-	20 % w/v PEG 3350
1.4	0.02 M calcium chloride	0.1 M sodium acetate	4.6	30 % v/v MPD
1.5	0.2 M magnesium formate	None	-	20 % w/v PEG 3350
1.6	0.2 M lithium sulfate	0.1 M phosphate/citrate	4.2	20 % w/v PEG 1000
1.7	None	0.1 M CHES	9.5	20 % w/v PEG 8000
1.8	0.2 M ammonium formate	None	-	20 % w/v PEG 3350
1.9	0.2 M ammonium chloride	None	-	20 % w/v PEG 3350
1.10	0.2 M potassium formate	None	-	20 % w/v PEG 3350
1.11	0.2 M ammonium dihydrogen phosphate	0.1 M Tris	8.5	50 % v/v MPD
1.12	0.2 M potassium nitrate	None	-	20 % w/v PEG 3350
1.13	None	0.1 M citrate	4.0	0.8 M ammonium sulfate
1.14	0.2 M sodium thiocyanate	None	-	20 % w/v PEG 3350
1.15	None	0.1 M Bicine	9.0	20 % w/v PEG 6000
1.16	None	0.1 M HEPES	7.5	10 % w/v PEG 8000/ 8 % v/v Ethylene glycol
1.17	None	0.1 M sodium cacodylate	6.5	40 % v/v MPD/ 5 % w/v PEG 8000
1.18	None	0.1 M phosphate/citrate	4.2	40 % v/v Ethanol/ 5 % w/v PEG 1000
1.19	None	0.1 M sodium acetate	4.6	8 % w/v PEG 4000
1.20	0.2 M magnesium chloride	0.1 M Tris	7.0	10 % w/v PEG 8000
1.21	None	0.1 M citrate	5.0	20 % w/v PEG 6000
1.22	0.2 M magnesium chloride	0.1 M sodium cacodylate	6.5	50 % v/v PEG 200
1.23	None	None	6.5	1.6 M tri-sodium citrate
1.24	0.2 M tri-potassium citrate	None	-	20 % w/v PEG 3350
1.25	0.2 M sodium chloride	0.1 M phosphate/citrate	4.2	20 % w/v PEG 8000
1.26	1.0 M lithium chloride	0.1 M Na citrate	4.0	20 % w/v PEG 6000
1.27	0.2 M ammonium nitrate	None	-	20 % w/v PEG 3350
1.28	None	0.1 M Na HEPES	7.0	10 % w/v PEG 6000
1.29	None	0.1 M Na HEPES	7.5	0.8 M sodium dihydrogen phosphate 0.8 M potassium dihydrogen phosphate
1.30	None	0.1 M phosphate/citrate	4.2	40 % v/v PEG 300
1.31	0.2 M zinc acetate	0.1 M sodium acetate	4.5	10 % w/v PEG 3000
1.32	None	0.1 M Tris	8.5	20 % v/v Ethanol
1.33	None	0.1 M Na/K phosphate	6.2	25 % v/v 1,2-propanediol 10 % v/v Glycerol
1.34	None	0.1 M Bicine	9.0	10 % w/v PEG 20,000/ 2% v/v Dioxane
1.35	None	0.1 M sodium acetate	4.6	2.0 M ammonium sulfate
1.36	None	None	-	10 % w/v PEG 1000/ 10 % w/v PEG 8000
1.37	None	None	-	24 % w/v PEG 1500/ 20 % v/v Glycerol
1.38	0.2 M magnesium chloride	0.1 M Na HEPES	7.5	30 % v/v PEG 400
1.39	0.2 M sodium chloride	0.1 M Na/K phosphate	6.2	50 % v/v PEG 200
1.40	0.2 M lithium sulfate	0.1 M sodium acetate	4.5	30 % w/v PEG 8000
1.41	None	0.1 M HEPES	7.5	70 % v/v MPD
1.42	0.2 M magnesium chloride	0.1 M Tris	8.5	20 % w/v PEG 8000
1.43	0.2 M lithium sulfate	0.1 M Tris	8.5	40 % v/v PEG 400
1.44	None	0.1 M Tris	8.0	40 % v/v MPD
1.45	0.17 M ammonium sulfate	None	-	25.5 % w/v PEG 4000/ 15 % v/v Glycerol
1.46	0.2 M calcium acetate	0.1 M sodium cacodylate	6.5	40 % v/v PEG 300
1.47	0.14 M calcium chloride	0.07 M sodium acetate	4.6	14 % v/v 2-propanol/ 30 % v/v Glycerol
1.48	0.04 M potassium dihydrogen phosphate	None	-	16 % w/v PEG 8000/ 20 % v/v Glycerol

Tube	Salt	Buffer	pH	Precipitant
2.1	None	0.1 M sodium cacodylate	6.5	1.0 M tri-sodium citrate
2.2	0.2 M sodium chloride	0.1 M sodium cacodylate	6.5	2.0 M ammonium sulfate
2.3	0.2 M sodium chloride	0.1 M HEPES	7.5	10 % v/v 2-propanol
2.4	0.2 M lithium sulfate	0.1 M Tris	8.5	1.26 M ammonium sulfate
2.5	None	0.1 M CAPS	10.5	40 % v/v MPD
2.6	0.2 M zinc acetate	0.1 M imidazole	8.0	20 % w/v PEG 3000
2.7	0.2 M zinc acetate	0.1 M sodium cacodylate	6.5	10 % v/v 2-propanol
2.8	None	0.1 M sodium acetate	4.5	1.0 M di-ammonium hydrogen phosphate
2.9	None	0.1 M MES	6.5	1.6 M magnesium sulfate
2.10	None	0.1 M Bicine	9.0	10 % w/v PEG 6000
2.11	0.16 M calcium acetate	0.08 M sodium cacodylate	6.5	14.4 % w/v PEG 8000/ 20 % v/v glycerol
2.12	None	0.1 M imidazole	8.0	10 % w/v PEG 8000
2.13	0.05 M caesium chloride	0.1 M MES	6.5	30 % v/v Jeffamine M-600
2.14	None	0.1 M Na Citrate	5.0	3.2 M ammonium sulfate
2.15	None	0.1 M Tris	8.0	20 % v/v MPD
2.16	None	0.1 M HEPES	7.5	20 % v/v Jeffamine M-600
2.17	0.2 M magnesium chloride	0.1 M Tris	8.5	50 % v/v ethylene glycol
2.18	None	0.1 M Bicine	9.0	10 % v/v MPD
2.19	None	None	7.0	0.8 M succinic acid
2.20	None	None	7.0	2.1 M DL-malic acid
2.21	None	None	7.0	2.4 M sodium malonate
2.22	1.1 M sodium malonate	0.1 M HEPES	7.0	0.5 % v/v Jeffamine ED-2001
2.23	1.0 M succinic acid	0.1 M HEPES	7.0	1 % w/v PEG 2000 MME
2.24	None	0.1 M HEPES	7.0	30 % v/v Jeffamine M-600
2.25	None	0.1 M HEPES	7.0	30 % v/v Jeffamine ED-2001
2.26	0.02 M magnesium chloride	0.1 M HEPES	7.5	22 % w/v polyacrylic acid 5100 sodium salt
2.27	0.01 M cobalt chloride	0.1 M Tris	8.5	20 % w/v polyvinylpyrrolidone K15
2.28	0.2 M tri-methylamine N-oxide	0.1 M Tris	8.5	20 % w/v PEG 2000 MME
2.29	0.005 M cobalt chloride 0.005 M cadmium chloride 0.005 M magnesium chloride 0.005 M nickel chloride	0.1 M HEPES	7.5	12 % w/v PEG 3350
2.30	0.2 M sodium malonate	None	7.0	20 % w/v PEG 3350
2.31	0.1 M succinic acid	None	7.0	15 % w/v PEG 3350
2.32	0.15 M DL - malic acid	None	7.0	20 % w/v PEG 3350
2.33	0.1 M potassium thiocyanate	None	-	30 % w/v PEG 2000 MME
2.34	0.15 M potassium bromide	None	-	30 % w/v PEG 2000 MME
2.35	None	0.1 M Bis Tris	5.5	2.0 M ammonium sulfate
2.36	None	0.1 M Bis Tris	5.5	3.0 M sodium chloride
2.37	None	0.1 M Bis Tris	5.5	0.3 M magnesium formate
2.38	1.0 M ammonium sulfate	0.1 M Bis Tris	5.5	1 % w/v PEG 3350
2.39	None	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.40	0.2 M calcium chloride	0.1 M Bis Tris	5.5	45 % v/v MPD
2.41	0.2 M ammonium acetate	0.1 M Bis Tris	5.5	45 % v/v MPD
2.42	0.1 M ammonium acetate	0.1 M Bis Tris	5.5	17 % w/v PEG 10000
2.43	0.2 M ammonium sulfate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.44	0.2 M sodium chloride	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.45	0.2 M lithium sulfate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.46	0.2 M ammonium acetate	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.47	0.2 M magnesium chloride	0.1 M Bis Tris	5.5	25 % w/v PEG 3350
2.48	0.2 M ammonium acetate	0.1 M HEPES	7.5	45 % v/v MPD

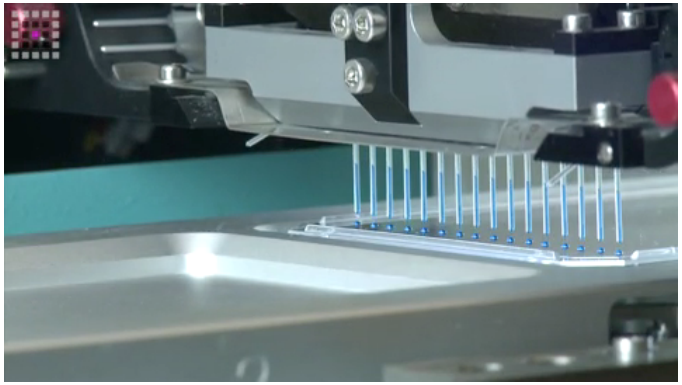
JCSG+ is essentially a collection of conditions that worked well for (mostly prokaryotic) proteins in the Joint Structural Genomics Consortium (JCSG; www.jcsg.org), who have solved > 1500 crystal structures

High-throughput crystallisation at LP3

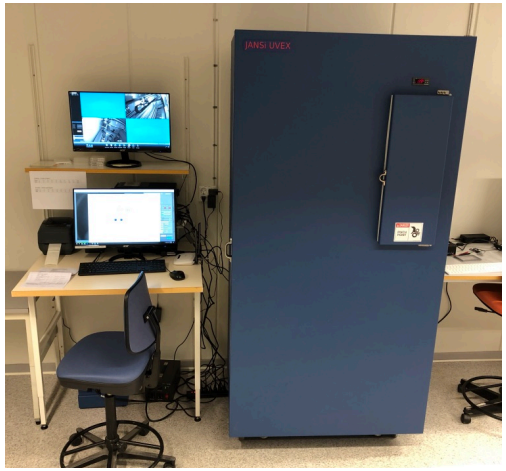
Tecan Freedom Evo 150
reformatting of crystallisation
screens

TTP Labtech dragonfly
optimisation screens

Rigaku Minstrel HT UV
imaging unit

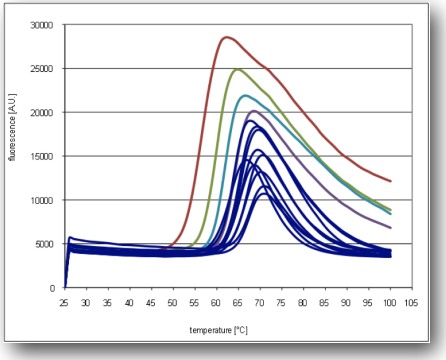


TTP Labtech mosquito LCP
nanolitre dispensing

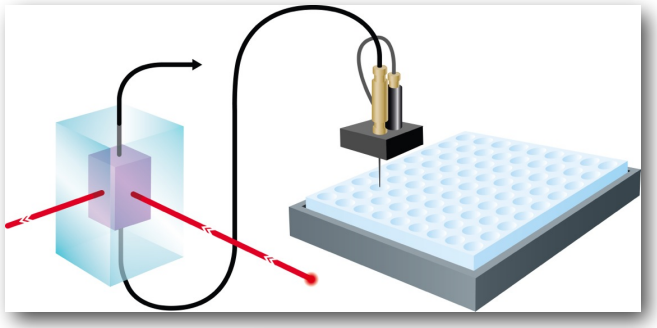


Jansi Uvex p256
imaging unit

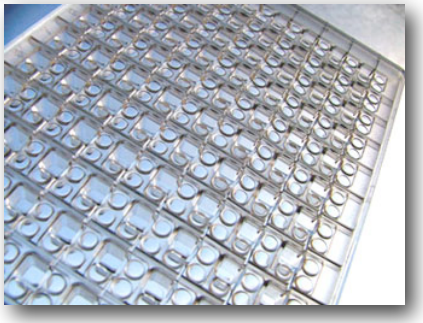
Efficient protein characterisation and crystallisation



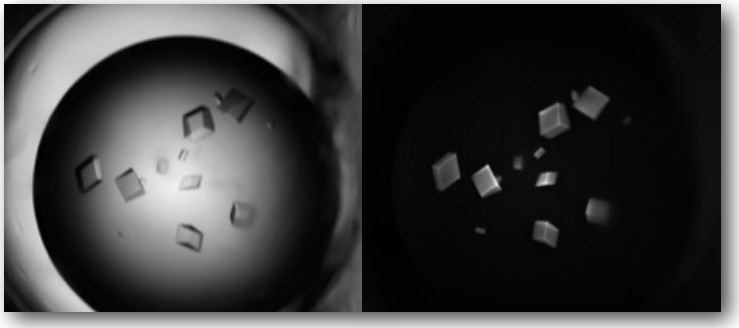
Thermofluor (DSF) enables identification of maximally stabilising conditions for crystallisation



Dynamic light scattering enables analysis of effects of buffers and additives on aggregation



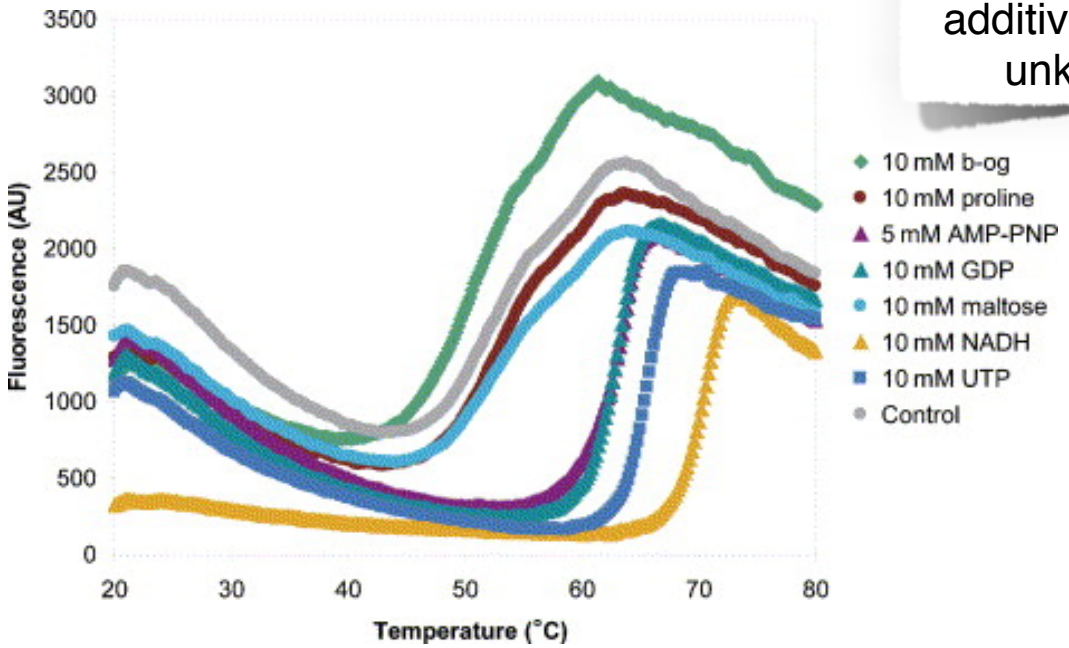
nanolitre dispensing robot enables low-volume, high density crystallisation trials in 96-well plates, with 1-3 protein drops per well, with minimal sample consumption



UV imaging enables unambiguous identification of protein crystals
Images stored in web-accessible database

Improving crystallisability: DSF

- Purity and homogeneity of a protein sample is critical for crystallisation
- Application of orthogonal biophysical characterisation methods can be very helpful
- Stability of protein towards unfolding is somewhat correlated to its ability to crystallise
- Unfolding can be studied by the *exposure of hydrophobic surface* upon heating, using a fluorescent dye as a probe - **differential scanning fluorimetry (DSF)**
- Additives that significantly increase the stability will also increase the probability of obtaining crystals



Effect of 7 different additives on a protein of unknown function

Thermofluor-based high-throughput stability optimization of proteins for structural studies

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[c] *Hauptman–Woodward Institute, Buffalo, NY 14203, USA*
[d] *AstraZeneca, R&D Mölndal, SE-431 83 Mölndal, Sweden*

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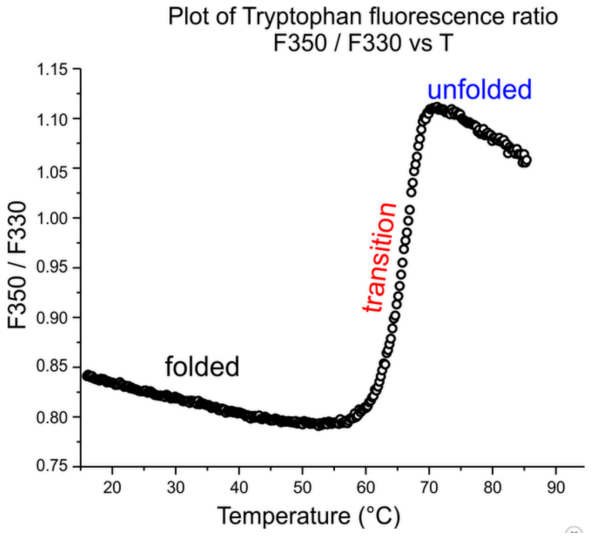
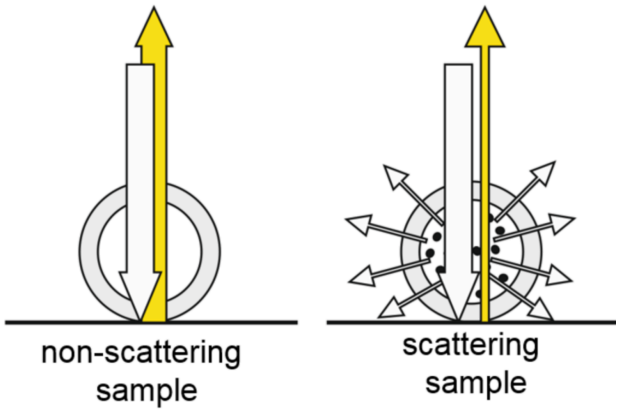
nanoDSF

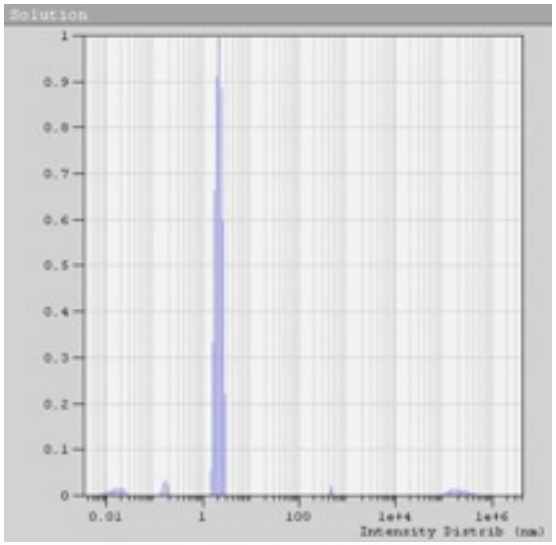
- Prometheus NT.48 from Nanotemper
- Allows extremely accurate measurement of unfolding curves using the intrinsic fluorescence of Trp and Tyr
- **Independent of reporter dye**
- Back-reflection technology allows simultaneous detection of aggregation
- Disadvantage: done in capillaries



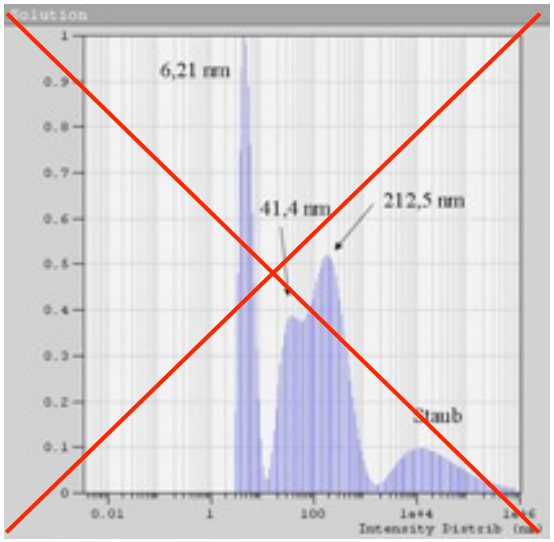


Improving crystallisability: DLS

- Generally, only samples that have a reasonably narrow, monomodal size distribution will crystallise
- This can be studied using **dynamic light scattering (DLS)**



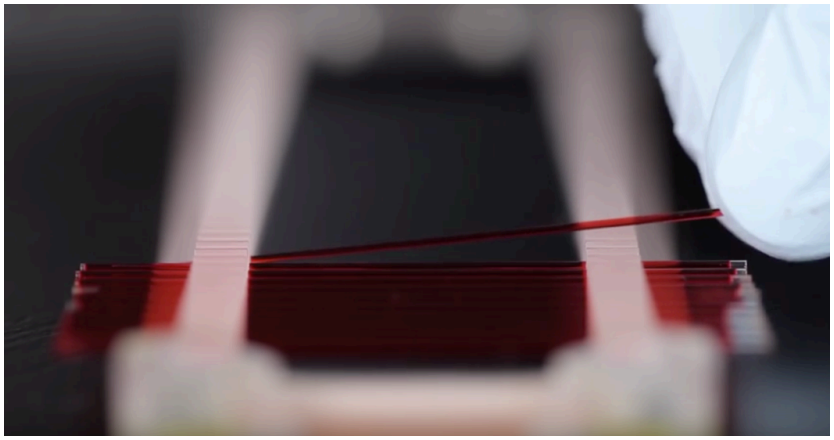
monodisperse protein solution



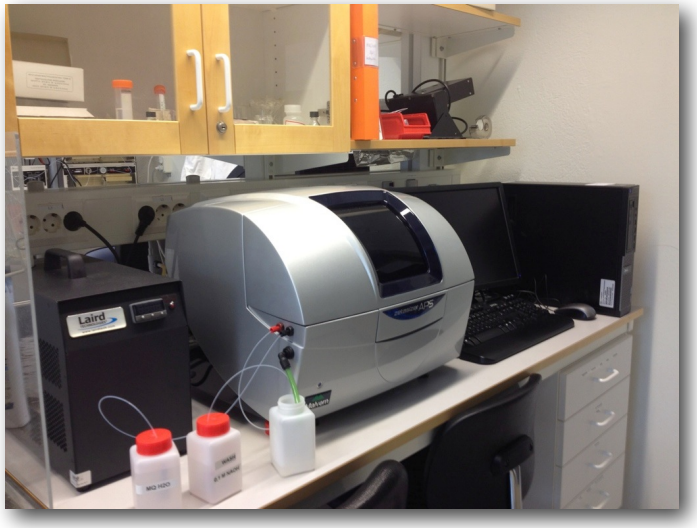
polydisperse protein solution

Protein characterisation at LP3

NanoDSF: Nanotemper Prometheus
uses intrinsic Trp/Tyr fluorescence instead of an extrinsic dye



https://www.youtube.com/watch?v=AIugRtTe3rw



Dynamic light scattering: Malvern Zetasizer APS

Automated visualisation of the experiments

http://www.youtube.com/watch?v=L4JNO6TOX2U

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The facility today

Facility director: Derek Logan
Research engineers: Maria Gourdon (**maria.gourdon@biol.lu.se**)
Céleste Sele (**celeste.sele@biol.lu.se**)

screen	manufacturer
PACT Premier	Molecular Dimensions
JCSG+	Molecular Dimensions
ProPlex	Molecular Dimensions
Structure screen 1 + 2	Molecular Dimensions
Morpheus	Molecular Dimensions
MemStart & MemSys	Molecular Dimensions
Memgold	Molecular Dimensions
Stura Footprint / MacroSol	Molecular Dimensions
Crystal Screen 1 + 2	Hampton Research
PEG-Ion Screen 1	Hampton Research
PEG-Ion Screen 2	Hampton Research
Crystal Screen Cryo	Hampton Research

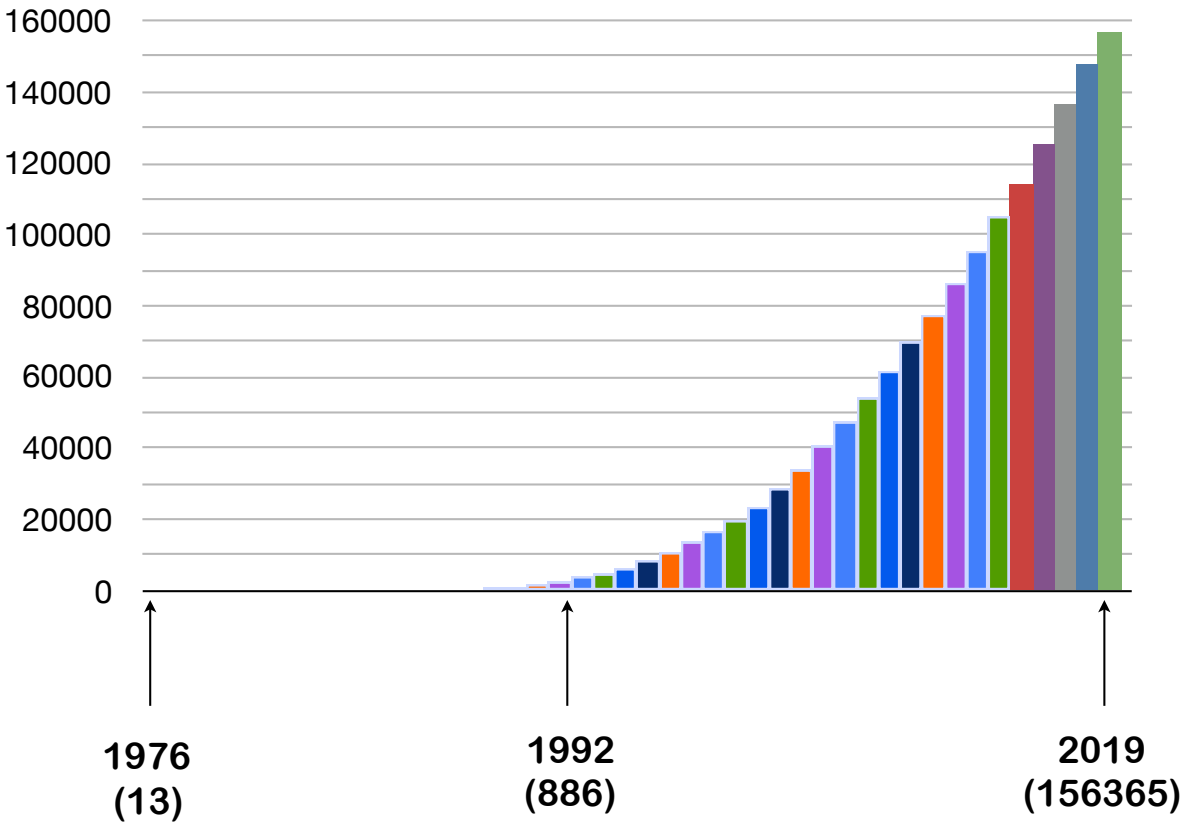
What is needed: 15 microlitres of pure, monodisperse protein (at 1-10 mg/ml) for a 96-condition screen

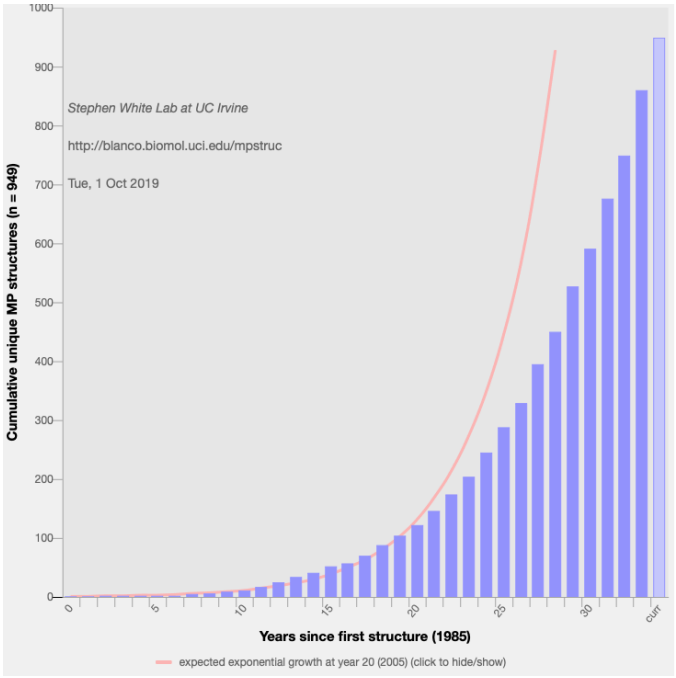
(much less for biophysical characterisations)

screen	manufacturer
Additive Screen	Hampton Research
Detergent Screen	Hampton Research

we also have buffer screens used in DSF and DLS studies of protein stability

New developments: membrane protein crystallisation



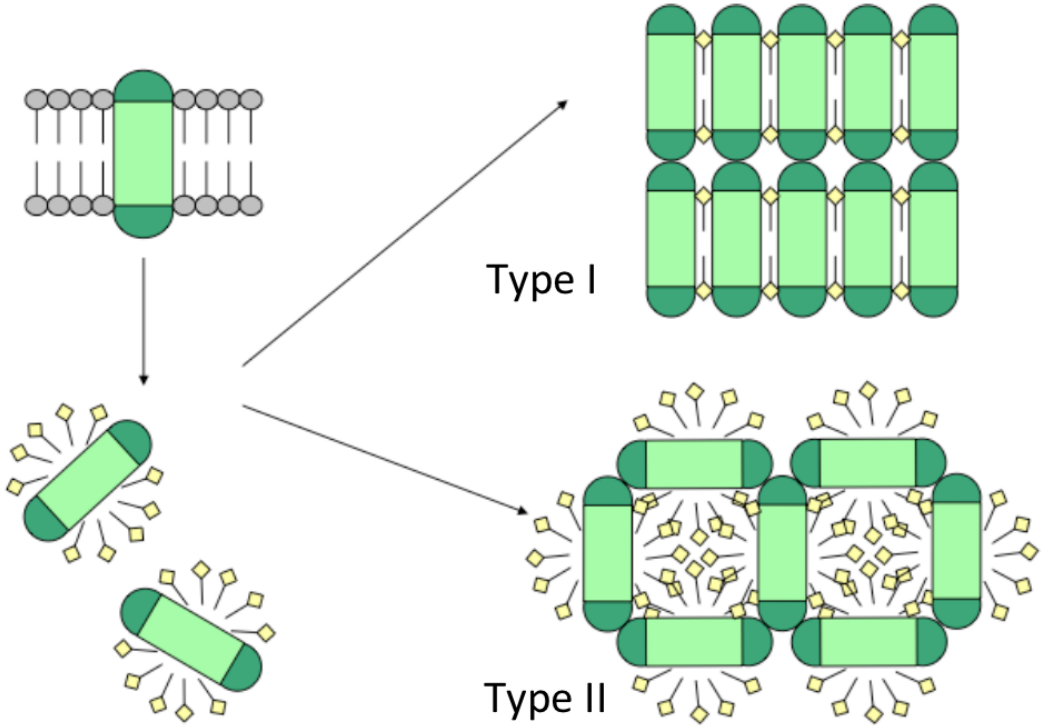


structures deposited in the Protein Data Bank (www.pdb.org)
156365 structures of ~55 000 unique proteins
(at 90% sequence identity)

Total membrane protein structures as of 1/10/2019
2848 structures from 949 unique membrane proteins

Difficulties in membrane protein crystallisation

Detergent micelle hinders crystal contacts!

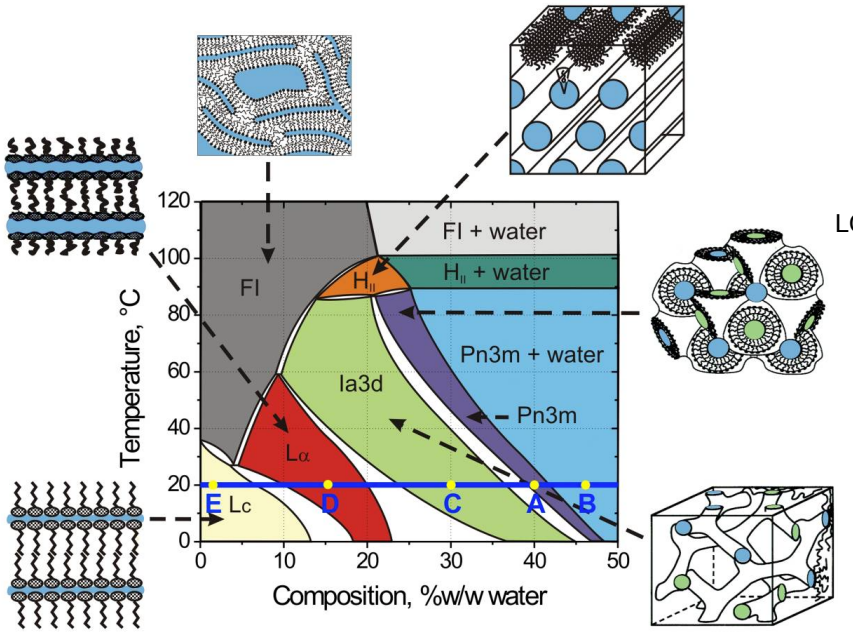


Membrane protein crystals

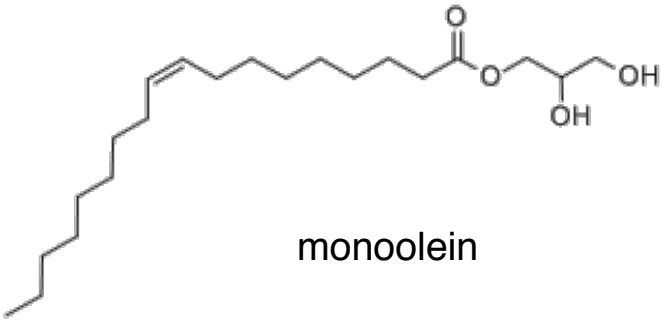
- Most often Type II
- Weak crystal contacts
- High solvent content
- High diffraction variability between crystals
- Uneven diffraction (anisotropic)

"borrowed" from Susanna Horsefield

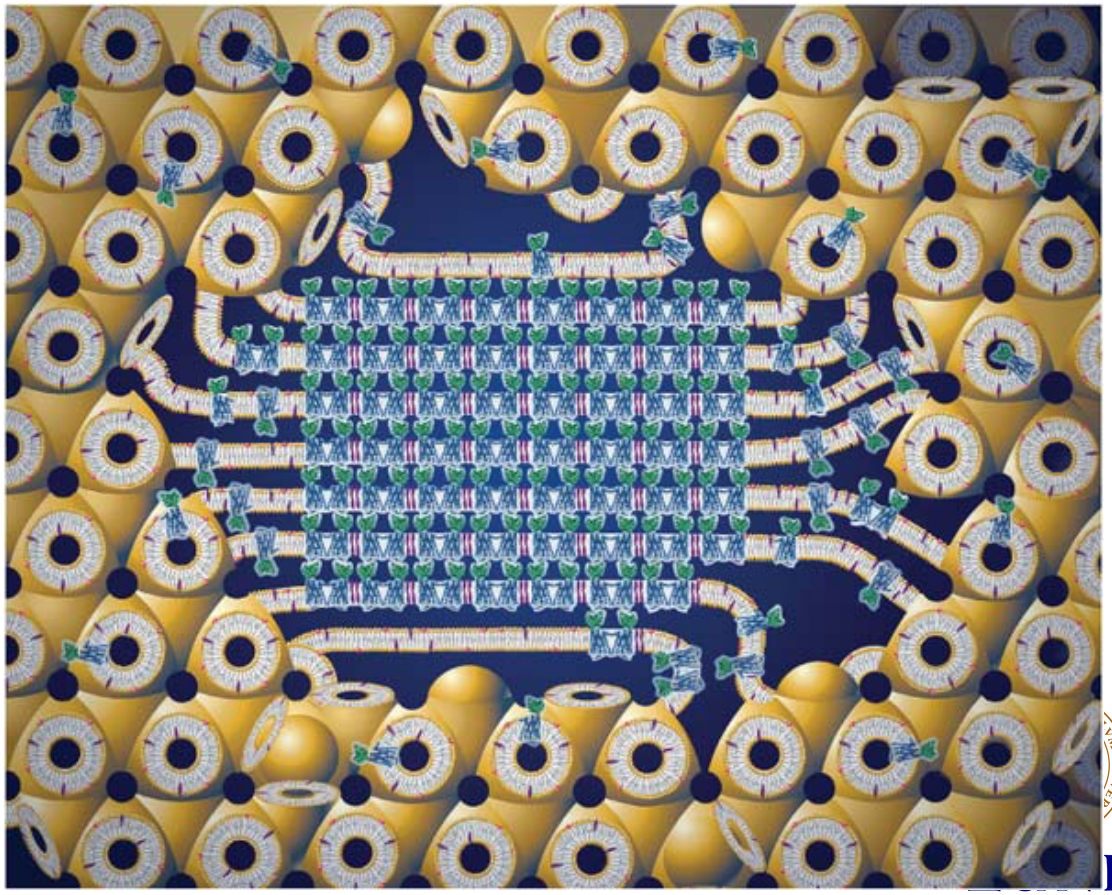
Lipidic cubic phase

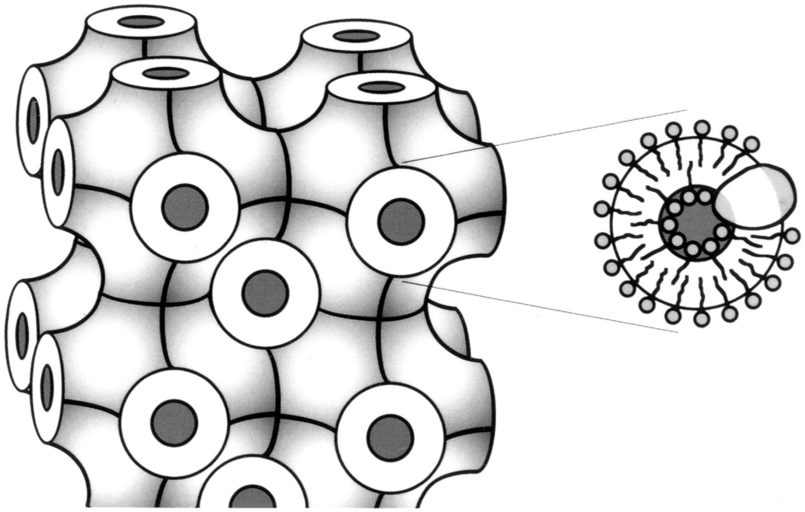


LCP



monoolein

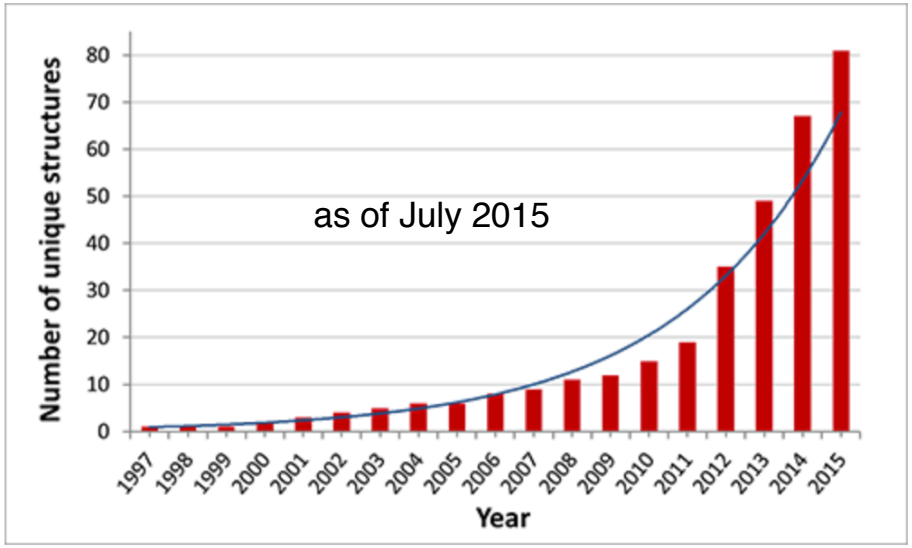


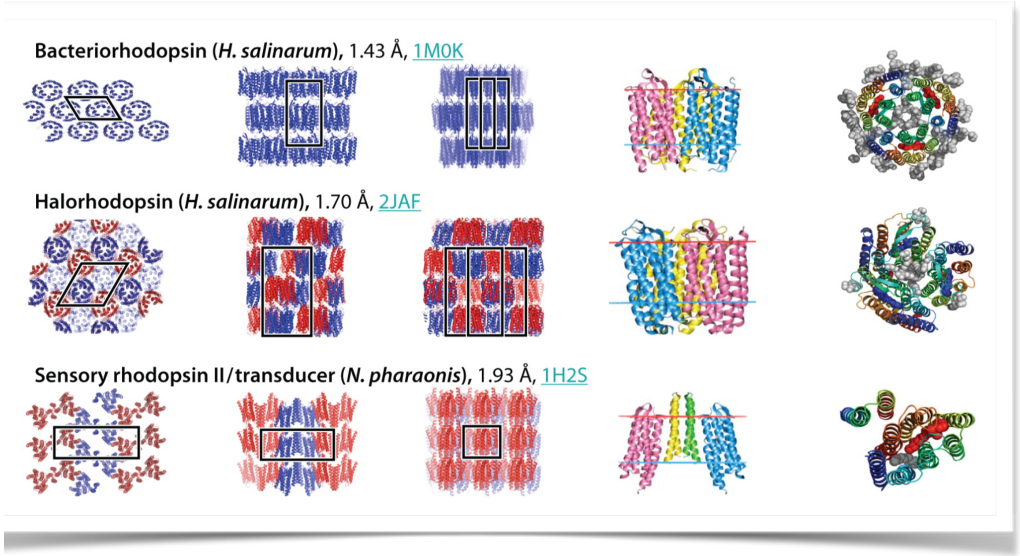


No. of LCP structures is growing exponentially

Structures obtained by *in meso* crystallization (including crystallization in lipidic cubic and sponge phases).

Total structures: 257 **Unique proteins: 81**

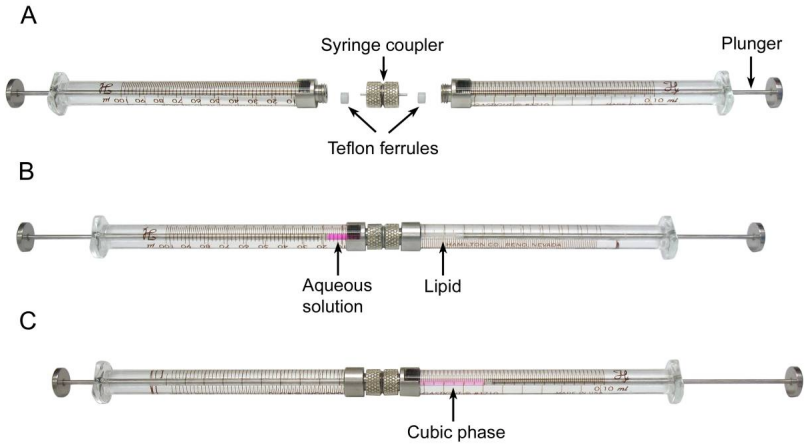




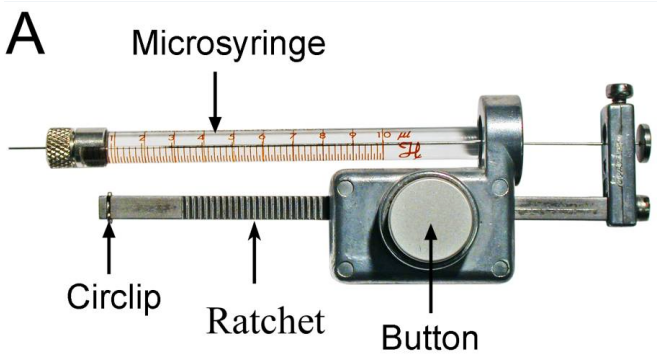
	Protein Family*	Total Structures	Unique Proteins	Best Resolution
1	Adiponectin receptors	2	1	2.40
2	Claudins	1	1	2.40
3	Enzymes	7	4	1.90
4	G Protein-Coupled Receptors	62	33	1.80
5	Heme-Copper Oxidases	16	2	1.80
6	Ion Channels	2	1	3.10
7	Microbial Rhodopsins	56	8	1.43
8	Outer Membrane Proteins	10	6	1.85
9	Peptides	4	1	1.08
10	Photosynthetic Proteins	10	3	1.86
11	Sec and Translocase Proteins	2	1	2.40
12	Transporters	28	9	1.50

http://cherezov.usc.edu/structures.htm

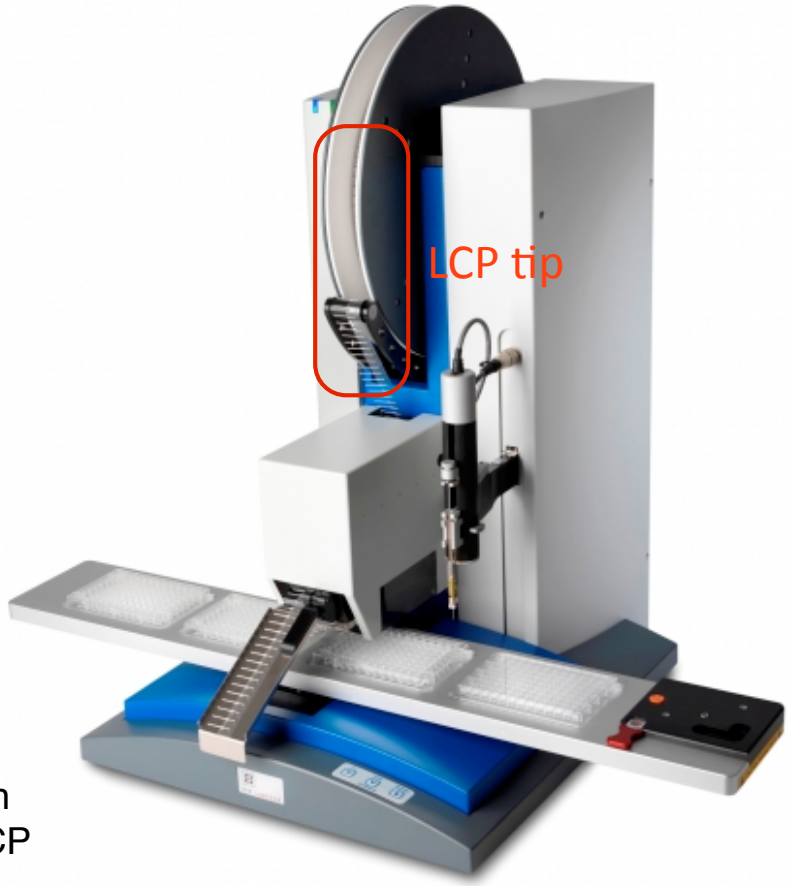
LCP is somewhat tricky to handle



Generate the cubic phase



Dispense the cubic phase

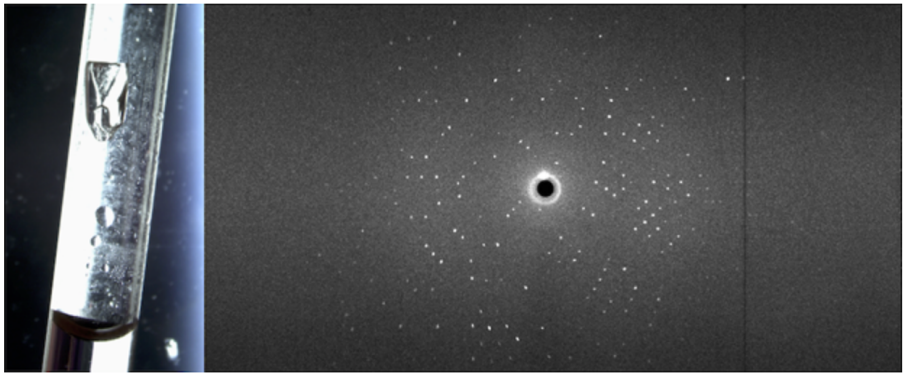


TTP Labtech
mosquito LCP

Example of synergies we can achieve

Larger Crystals a Step Forward for Bioscience and ESS User Program

AUG 31, 2015



Support Labs. The first successful user experiment at ESS demonstrates the importance of neutrons to the life sciences, and shows the way forward for ESS as a user facility.

LUND and GARCHING—The heavy machinery and rapid development of the European Spallation Source (ESS) construction site make it easy to see that the project is a reality on the ground. What is less visible is that ESS is already taking shape as a user facility. The first successful ESS user experiment began last fall in collaboration with researchers from the Lund University (LU) Department of

Above: Photograph (left) of a large, single crystal of X-2 L110F in complex with XXXG mounted in a quartz capillary undergoing H/D exchange (dimensions are ~1.3 x 1.2 x 1.0 mm; 1.6 mm3); and (right) a representative monochromatic neutron diffraction image collected using BIODIFF at FRM-II. IMAGES: ESS

PROTEINS
STRUCTURE • FUNCTION • BIOINFORMATICS

Carbohydrate binding module recognition of xyloglucan defined by polar contacts with branching xyloses and CH-π interactions

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Acta Cryst F
STRUCTURAL BIOLOGY COMMUNICATIONS
ISSN 2053-230X

Crystallization, neutron data collection, initial structure refinement and analysis of a xyloglucan heptamer bound to an engineered carbohydrate-binding module from xylanase

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Received 5 May 2015
Accepted 11 June 2015

Edited by J. Newman, Bio21 Collaborative Crystallization Centre, Australia

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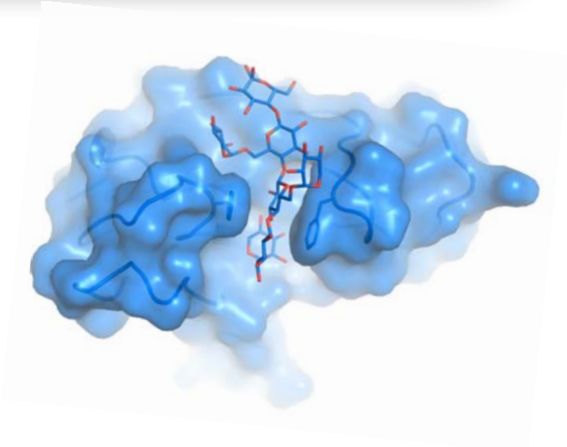
Zoë Fisher



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Neutron Crystallographic Studies Reveal Hydrogen Bond and Water-Mediated Interactions between a Carbohydrate-Binding Module and Its Bound Carbohydrate Ligand

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