



## BioSAXS and Complementary Techniques 2

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Beamline responsible BM29 BioSAXS at the ESRF



STREAMLINE

**Introduction**

**High Resolution Techniques**

**Dynamics - SAXS**

**Case Studies – LLPS**

**Case Studies – Antibodies**



## SUPRAMOLECULAR STRUCTURE

- 2 x Cryo-EM Titan Krios (operated by ESRF, IBS, EMBL & ILL)
- Electron microscopy platform (operated by IBS)
- SAXS/SANS (operated by ESRF & ILL)

## Protein Crystallisation

- HT Crystallisation (operated by EMBL)
- HT Membrane Protein Crystallisation (operated by IBS)

## Sample Characterisation

- Cellular imaging (operated by IBS)
- Mass Spectrometry (operated by IBS)
- Molecular Biophysics platform (operated by IBS)
- AUC, BLI, CD, DLS, Fluorimeter, ITC, MST, MP, PAOL, SEC-MALLS, SPR

## Protein Expression

- Cell Free (operated by IBS)
- Deuteration Lab (operated by ILL)
- ESPRIT (operated by IBS)
- Eukaryotic Expression Facility (operated by EMB)

## 3-D STRUCTURE ANALYSIS

- 6 x ESRF Structural Biology Beamlines (operated by ESRF)
- FIP2 Beamline (BM07) (operated by IBS)
- High Field NMR (operated by IBS)
- Neutron Diffraction instruments (operated by ILL)
- D19, LADI-III & DALI
- The in crystallo Optical Spectroscopy Laboratory (iCOS)



<https://www.psb-grenoble.eu/>



<https://pscm-grenoble.eu/>

Optical microscopy

Optical Spectroscopy

Rheology

Solid interfaces

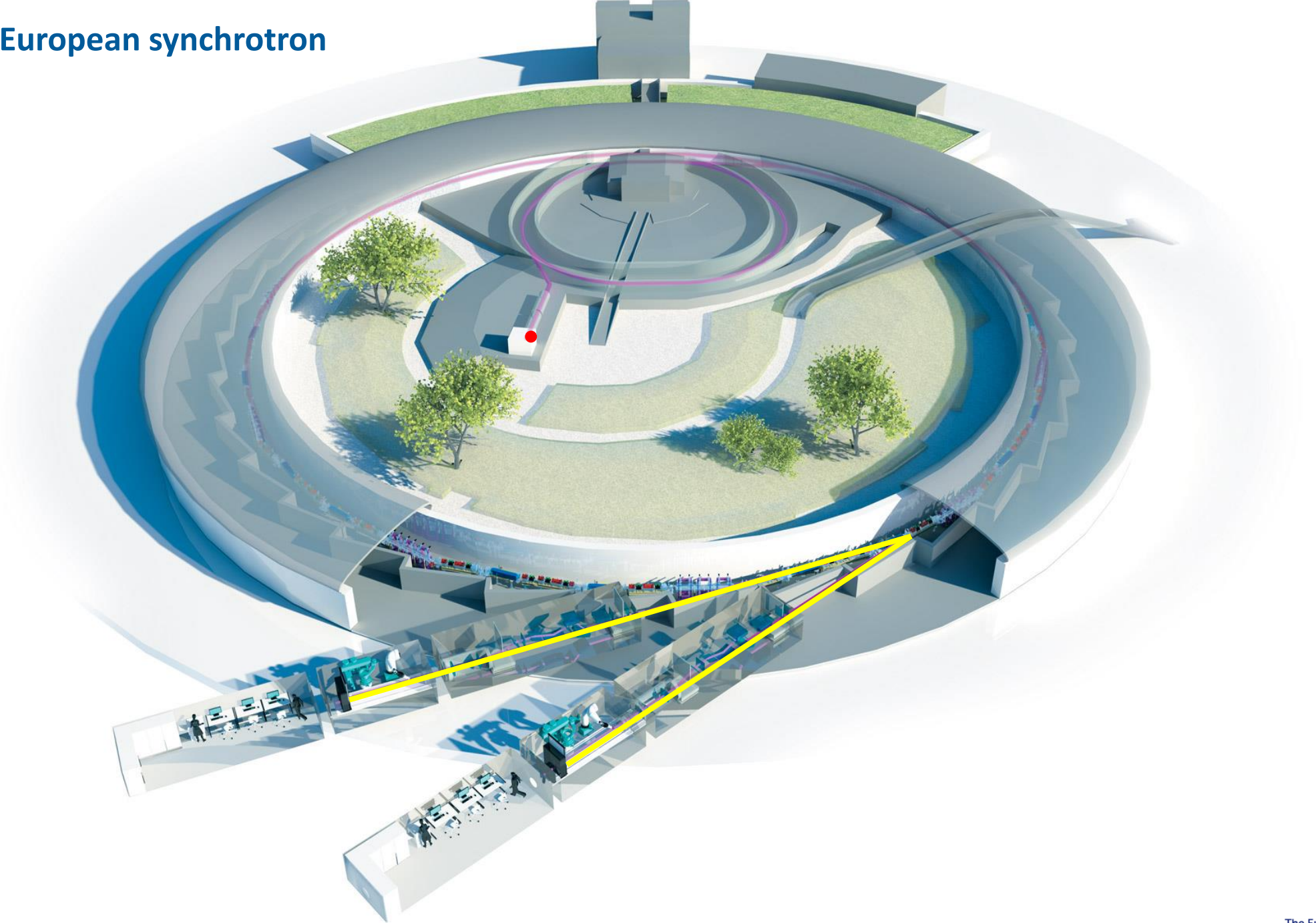
Calorimetry, Volumetry, and Thermal analysis

Liquid interfaces

Light Scattering

Chemical Laboratories for sample preparation

# ESRF: The European synchrotron



# ESRF: established by an inter-governmental convention signed by 13 member states



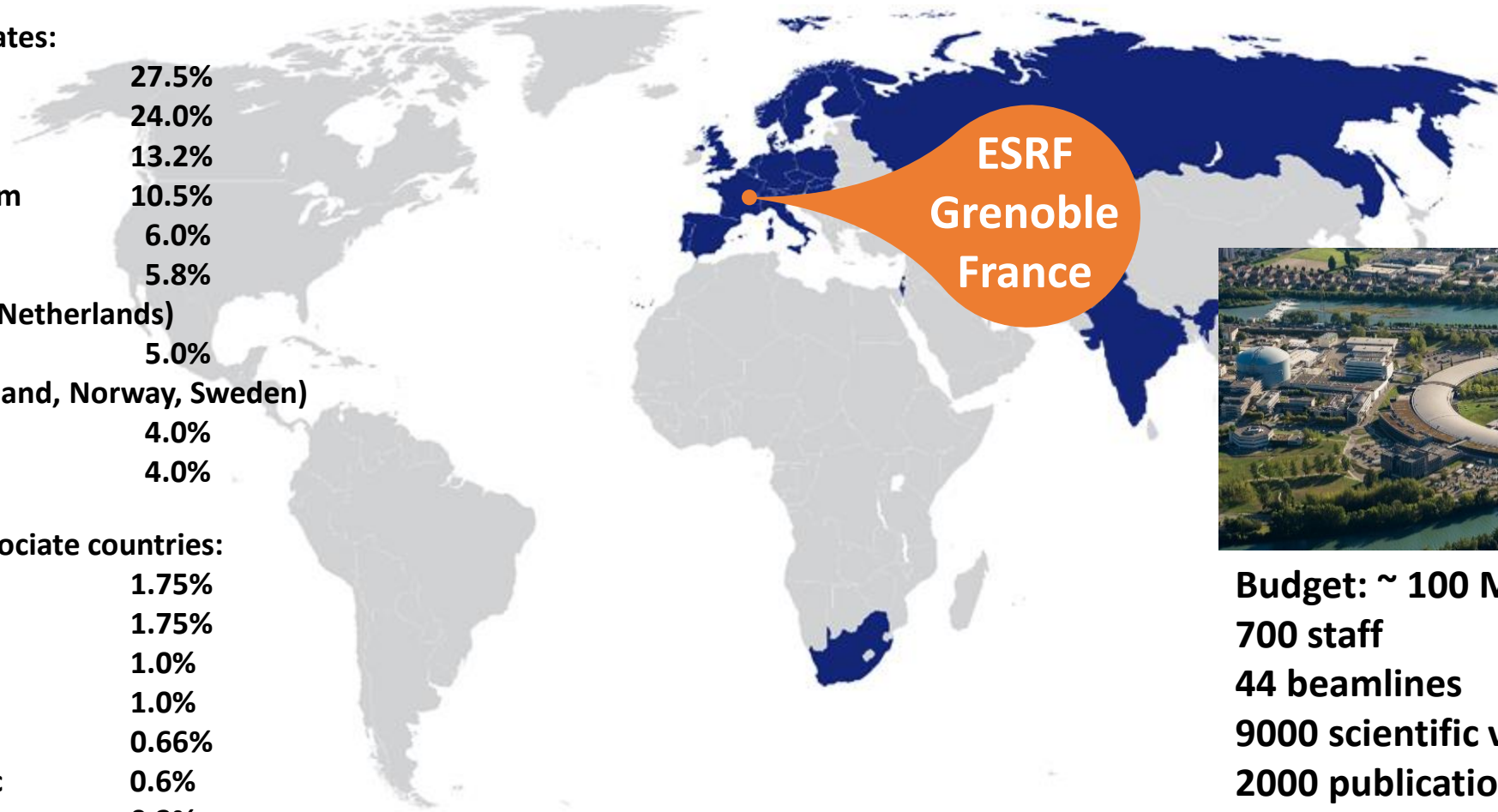
## 21 PARTNER COUNTRIES

### 13 Member states:

France	27.5%
Germany	24.0%
Italy	13.2%
United Kingdom	10.5%
Russia	6.0%
Benesync (Belgium, The Netherlands)	5.8%
Nordsync (Denmark, Finland, Norway, Sweden)	5.0%
Spain	4.0%
Switzerland	4.0%

### 8 Scientific associate countries:

Israel	1.75%
Austria	1.75%
Poland	1.0%
Portugal	1.0%
India	0.66%
Czech Republic	0.6%
South Africa	0.3%
Hungary	0.25%



**Budget: ~ 100 M€ per year**  
**700 staff**  
**44 beamlines**  
**9000 scientific visits per year**  
**2000 publications per year**  
**30% of research is with industry**



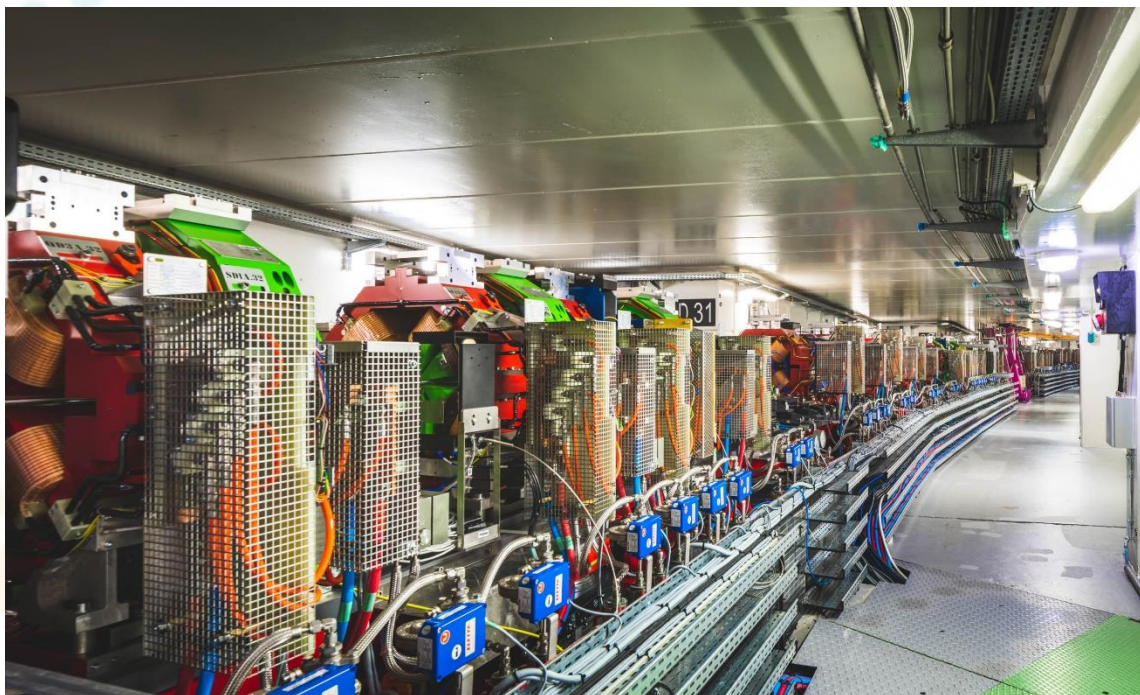
**ESRF-EBS**  
Extremely Brilliant Source

- opened to users 25/08/2020
- Investment of 150 M EUR
- Fourth generation source + new beamlines + data infrastructure

3<sup>rd</sup> generation - emittance H/V ~ 1000

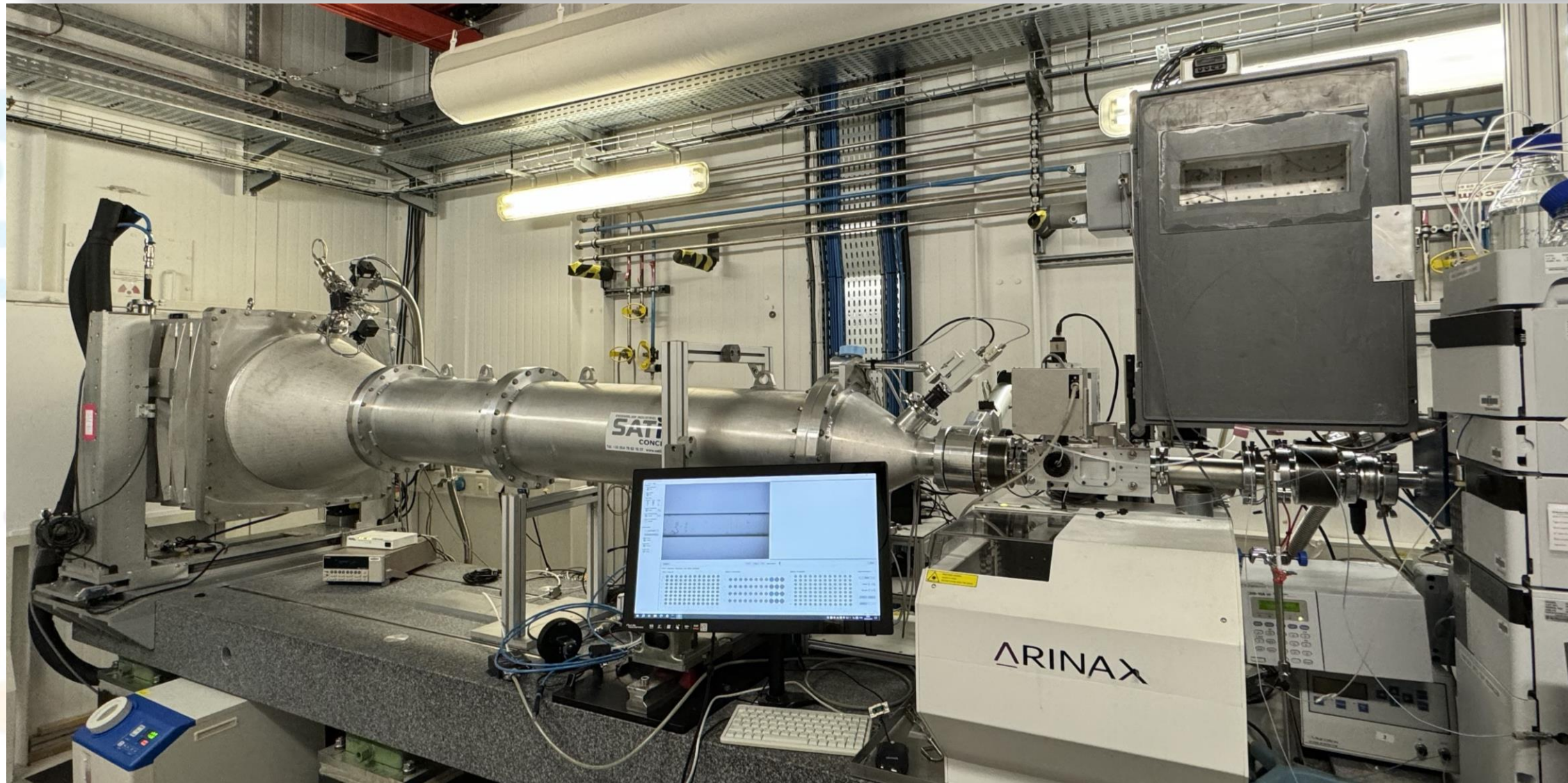


4<sup>th</sup> generation – H/V ~ 1



- Start – 10<sup>th</sup> Dec 2018
- 1<sup>st</sup> Electrons in Storage ring – 28<sup>th</sup> Nov 2019
- Commissioning – 14<sup>th</sup> Mar 2020
- COVID lockdown – 17<sup>th</sup> Mar 2020
- Actual Commissioning start – 15<sup>th</sup> June 2020
- 1<sup>st</sup> User – 3<sup>rd</sup> Sep 2020 (mail-in under lockdown conditions)







- sample transfer: 5 to 200  $\mu\text{L}$ .
- cycle time (load-clean)  $\sim$  60 s.
- Samples stored in 96 well plates or PCR tubes.
- Thermo-regulation:
  - storage: 4 – 40° C,
  - exposure cell: 4 – 60° C

BSXCuBE 3 Acquisition Result Beamline Setup System Log mx415 Help Signout

Safety shutter: DISABLE Energy: 12.500 keV Transmission: 0.00 % Ring Current: 0

Data path: /data/bm29/inhouse/bsxcube-test/

Sample Changer Experiment Name: SCTestname Load Parameters Save

Sample: Buffer:

Plate 1: 96 Deep Well Plate Plate 2: 4 x (8 + 3) Block Plate 3: 96 well Plate

Selected Well: 1:A:1 Sample Name: s1 Buffer Name: --Choose--

Concentration (mg/ml): 1 Volume µl: 50

SEU Temp. °C: 4 Storage Temp. °C: 4

Comment:

Add to Sample Table

Clear Table Optimisation Collaps Parameters: Parameters: Number of sample's 3

Name	Buffer	Plate	Row	Column	C (mg/mL)	Flow	Extra Flow t(s)	Volume (µl)	SEU Temp. °C	Storage Temp. °C	Energy	Viscosity	Number of Frames	Exposure(s)	Transmission %	Buffer mode	Recuperation	Comment
1 sample	bn2	3	D	2	1		5	60	50	6	12.5	low	7	1	100	Before & A		c
2 sample	bn1	2	B	2	0		5	67	58	66	17	low	7	1	8	After		c
3 sample	bn	1	A	1	1		5	60	50	6	12.5	low	7	1	100	Before		c

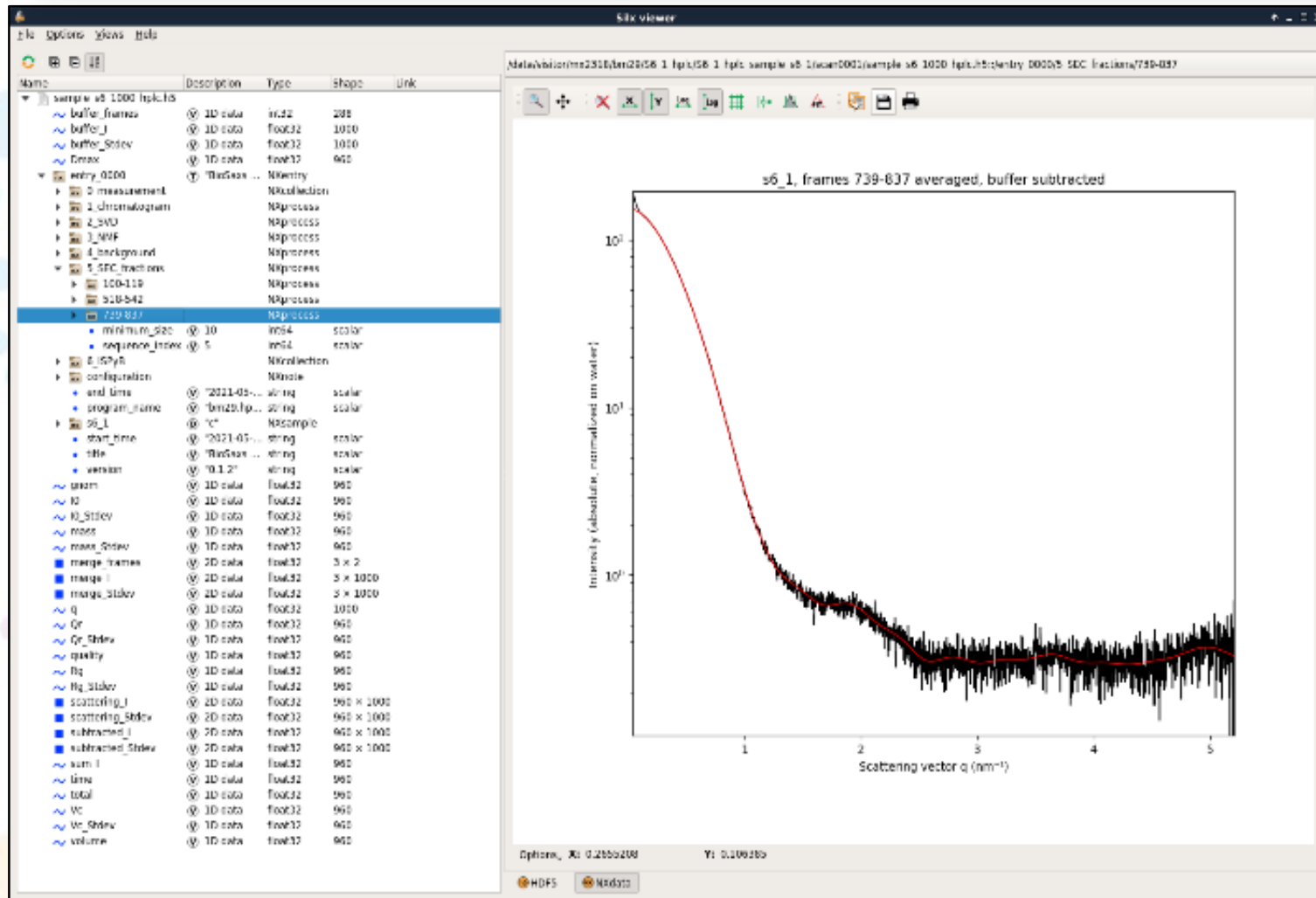
Run Now Add to Queue

Simplified 3 step process;

1. Select your sample position
2. Name your sample, concentration, etc.
3. Select "Run" or "Add to Queue"

The screenshot displays the BSXCuBE 3 web interface for HPLC control. At the top, there are status indicators for Front End (Wait for permission), Safety shutter (Fault state), Fast shutter (Closed), Energy (12.500 keV), and Transmission (0.00%). Below this, the HPLC section shows the experiment name 'myhplc' and a 10x10 grid of vial positions. Vial 1 is selected. The 'Auto Sample' is currently 'Set OFF'. To the right, 'Sample Parameters' are configured: Sample Name 'sample\_z', Vial Number '1', Injection Volume '50', Number of Frame '240', Column Elution T '4', Exposure Time '1', and various wavelength and flow rate settings. A table at the bottom lists the sample parameters for the selected vial.

Sample Name	Flow R. (mL/min)	Vial Number	Injection Volume (µl)	λ1 (nm)	λ2 (nm)	λ3 (nm)	λ4 (nm)	Concentration	Exposure Temp °C	Exposure Time (s)	Number of Frames	Column Elution (min)	Comment
z	1	1	50	260	280	350	450	1	20	2	240	4	c



.H5 files encompass opensource data  
reduction pipeline,  
FreeSAS by Jerome Kieffer

Kieffer, J et al,. (2022). J. Synchrotron Rad. 29, 1318-1328.

<https://github.com/kif/freesas/blob/build-deb>

ExiSAXS Extended ISPyB for SAXS

Version: 5.29.32  
 Released: 20230124

Home Shipment Prepare Experiment Data Explorer Manager Help

Select

search macromolecule

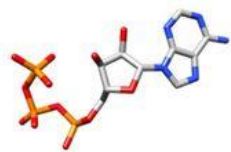
Log out MX2320@tully

Run	Sample	Frames (Average/Total)	Guinier			Gnom			Porod		Scattering	Kratky	Density	Guinier	Advanced
			Rg	Points	I0	Rg	Total	D <sub>max</sub>	Volume	MM Vol. est.					
#24	POC_MES	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#23	POC_LD_M25 0.660 mg/ml 20.0 C 50.0 µl	9 / 10	3.352 nm	20 - 52 (32)	48.0 ± 6.31e-1	4.430 nm	-503.929	20.722 nm	40.226 nm <sup>3</sup>	20.1 - 26.8 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#22	POC_MES	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#20	POC_LD_M10 0.260 mg/ml 20.0 C 50.0 µl	4 / 10	4.373 nm	15 - 23 (8)	18.5 ± 1.06e+0	4.528 nm	-553.627	18.082 nm	47.937 nm <sup>3</sup>	24.0 - 32.0 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#19	POC_MES	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#18	POC_LD_M25 0.660 mg/ml 20.0 C 50.0 µl	5 / 10	3.006 nm	21 - 74 (53)	44.0 ± 1.02e+0	3.230 nm	-575.362	11.718 nm	39.222 nm <sup>3</sup>	19.6 - 26.1 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#16	POC_MES	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#15	POC_MES	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#14	POC_DD_M10 0.260 mg/ml 20.0 C 50.0 µl	7 / 10	3.199 nm	22 - 63 (41)	16.1 ± 3.59e-1	3.246 nm	-437.592	11.865 nm	38.196 nm <sup>3</sup>	19.1 - 25.5 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#13	POC_MES	7 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#12	POC_Ac	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#11	POC_LD_A25 0.660 mg/ml 20.0 C 50.0 µl	4 / 10	3.362 nm	19 - 66 (47)	35.8 ± 5.26e-1	3.537 nm	-508.920	12.310 nm	42.728 nm <sup>3</sup>	21.4 - 28.5 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#10	POC_Ac	5 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#9	POC_Ac	5 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#8	POC_LD_A10 0.260 mg/ml 20.0 C 50.0 µl	8 / 10	3.269 nm	0 - 67 (67)	11.2 ± 3.23e-1	3.319 nm	-485.500	11.845 nm	44.941 nm <sup>3</sup>	22.5 - 30.0 ic					<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body
#7	POC_Ac	10 / 10													<input checked="" type="checkbox"/> Data Reduction <input checked="" type="checkbox"/> Ab Initio <input checked="" type="checkbox"/> Fit <input checked="" type="checkbox"/> Superposition <input checked="" type="checkbox"/> Rigor Body

Experiment  
 Nov 1, 2021 2:41:20 PM  
 Alina\_P\_CT\_sc  
 Samples: 15 of 15  
 Averages: 15 of 15  
 Subtractions: 5 of 5  
 SAMPLE CHANGER  
 Nov 1, 2021 2:11:29 PM  
 Alina\_P\_BMN\_sc  
 Samples: 15 of 15  
 Averages: 15 of 15  
 Subtractions: 5 of 5  
 SAMPLE CHANGER  
 Nov 1, 2021 1:53:52 PM  
 Alina\_P\_BM\_sc  
 Samples: 15 of 15  
 Averages: 15 of 15  
 Subtractions: 5 of 5  
 SAMPLE CHANGER  
 Nov 1, 2021 1:30:08 PM  
 Alina\_P\_AAN\_sc  
 Samples: 15 of 15  
 Averages: 15 of 15  
 Subtractions: 5 of 5  
 SAMPLE CHANGER  
 Nov 1, 2021 1:08:59 PM  
 Alina\_P\_AA\_sc  
 Samples: 24 of 24  
 Averages: 24 of 24  
 Subtractions: 8 of 8  
 SAMPLE CHANGER  
 Nov 1, 2021 12:15:11 PM  
 Alina\_NT\_2\_sc  
 Samples: 24 of 24  
 Averages: 24 of 24  
 Subtractions: 8 of 8  
 SAMPLE CHANGER  
 Nov 1, 2021 11:40:08 AM  
 Alina\_NT\_L\_sc  
 Samples: 24 of 24  
 Averages: 24 of 24  
 Subtractions: 8 of 8  
 SAMPLE CHANGER  
 Nov 1, 2021 11:17:24 AM  
 Alina\_POC\_sc  
 Samples: 24 of 24  
 Averages: 24 of 24  
 Subtractions: 8 of 8  
 SAMPLE CHANGER  
 Nov 1, 2021 9:52:33 AM

View

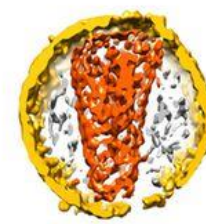
small molecules



proteins and protein complexes



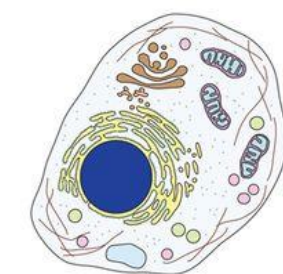
viruses and vesicles



Prokaryotic cells and organelles



Eukaryotic cells



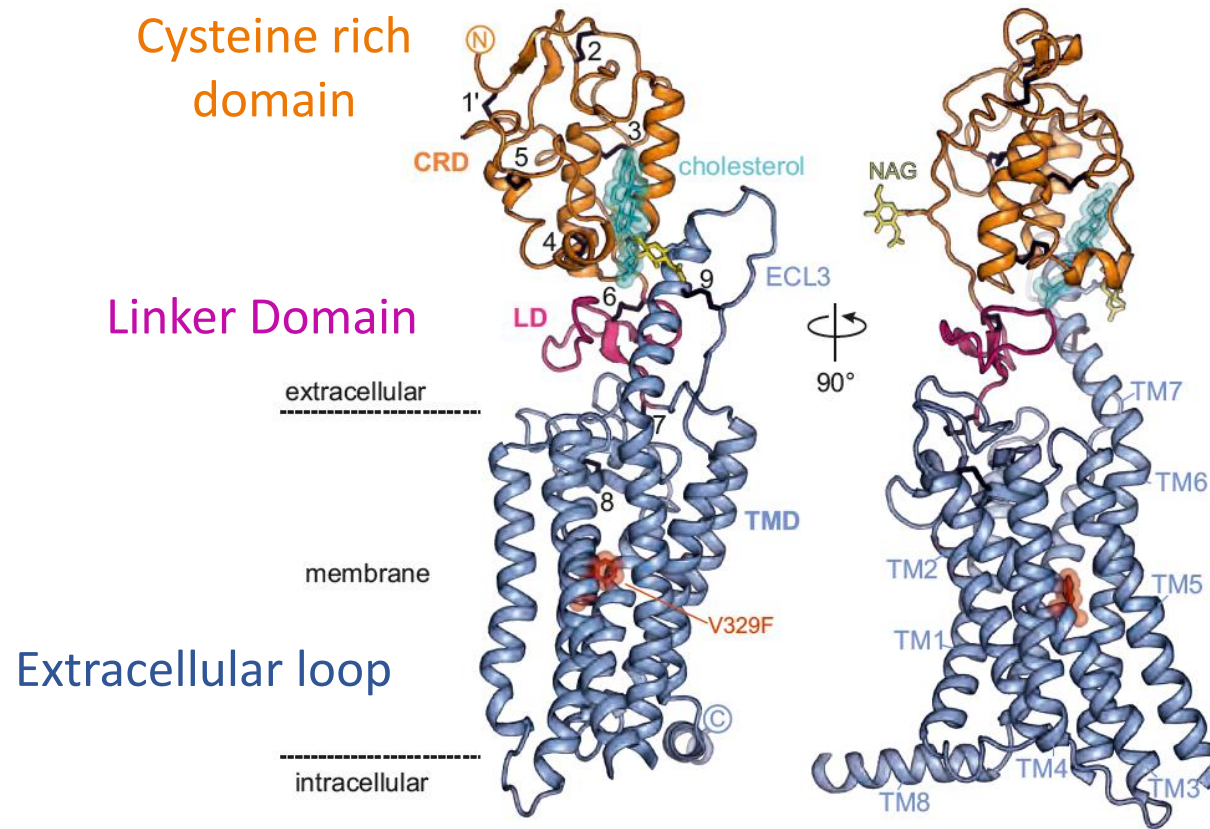
NMR Spectroscopy

X-ray Crystallography

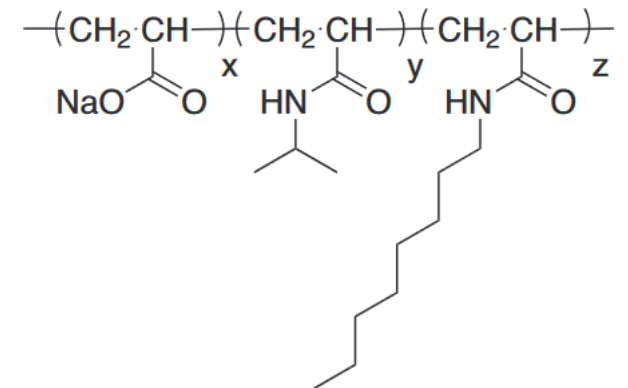
Single Particle Cryo-EM

Adapted from J. Hutchings Biochem Soc Trans (2018) 46 (4): 807–816. <https://doi.org/10.1042/BST20170351>

## Structural basis for Smoothened regulation by its extracellular domains

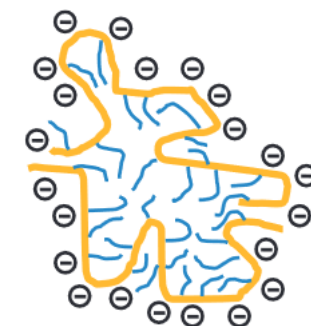


## Amphipol A8-35



X = 0.35, Y = 0.25, Z = 0.40

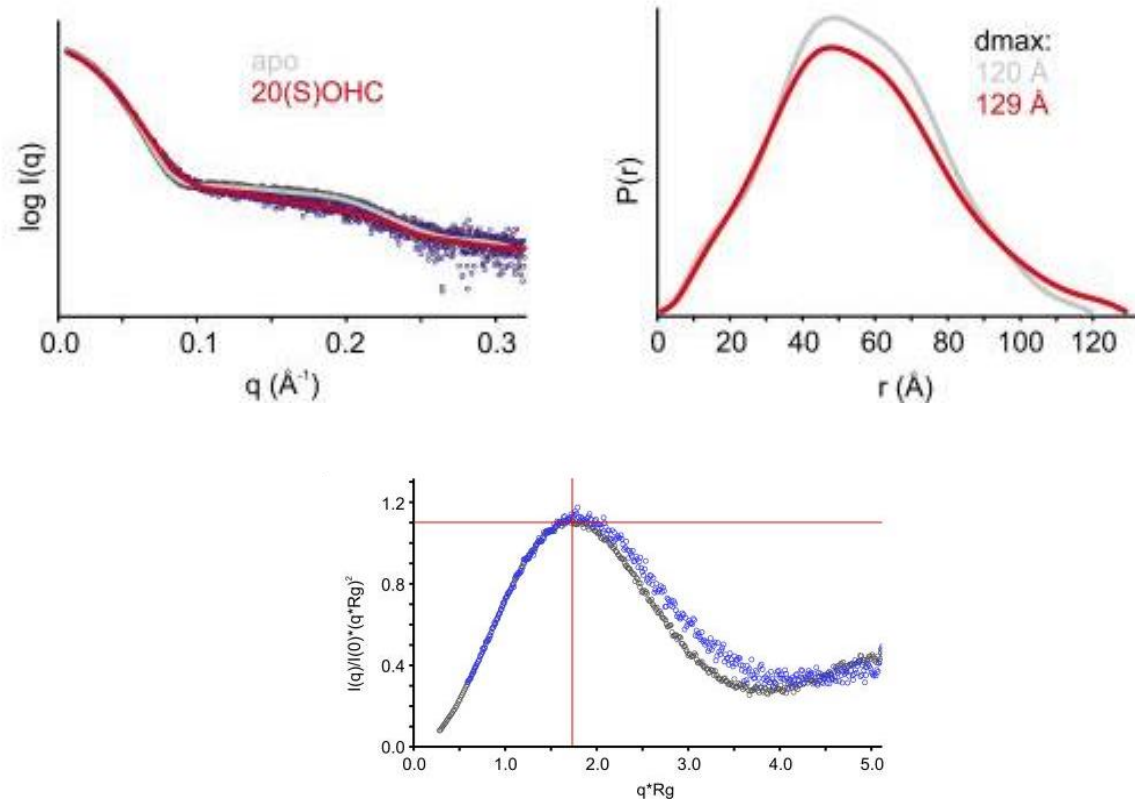
Figure 1A



Eamon F.X. Byrne, *Nature* vol. 535,7613 (2016): 517-522. doi:10.1038/nature18934

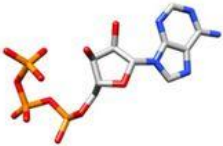


## Structural basis for Smoothened regulation by its extracellular domains



Eamon F.X. Byrne, *Nature* vol. 535,7613 (2016): 517-522. doi:10.1038/nature18934

small molecules



proteins and protein complexes



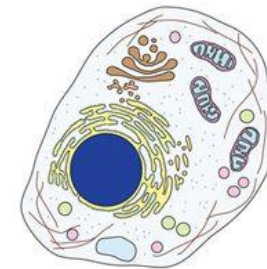
viruses and vesicles



Prokaryotic cells and organelles



Eukaryotic cells



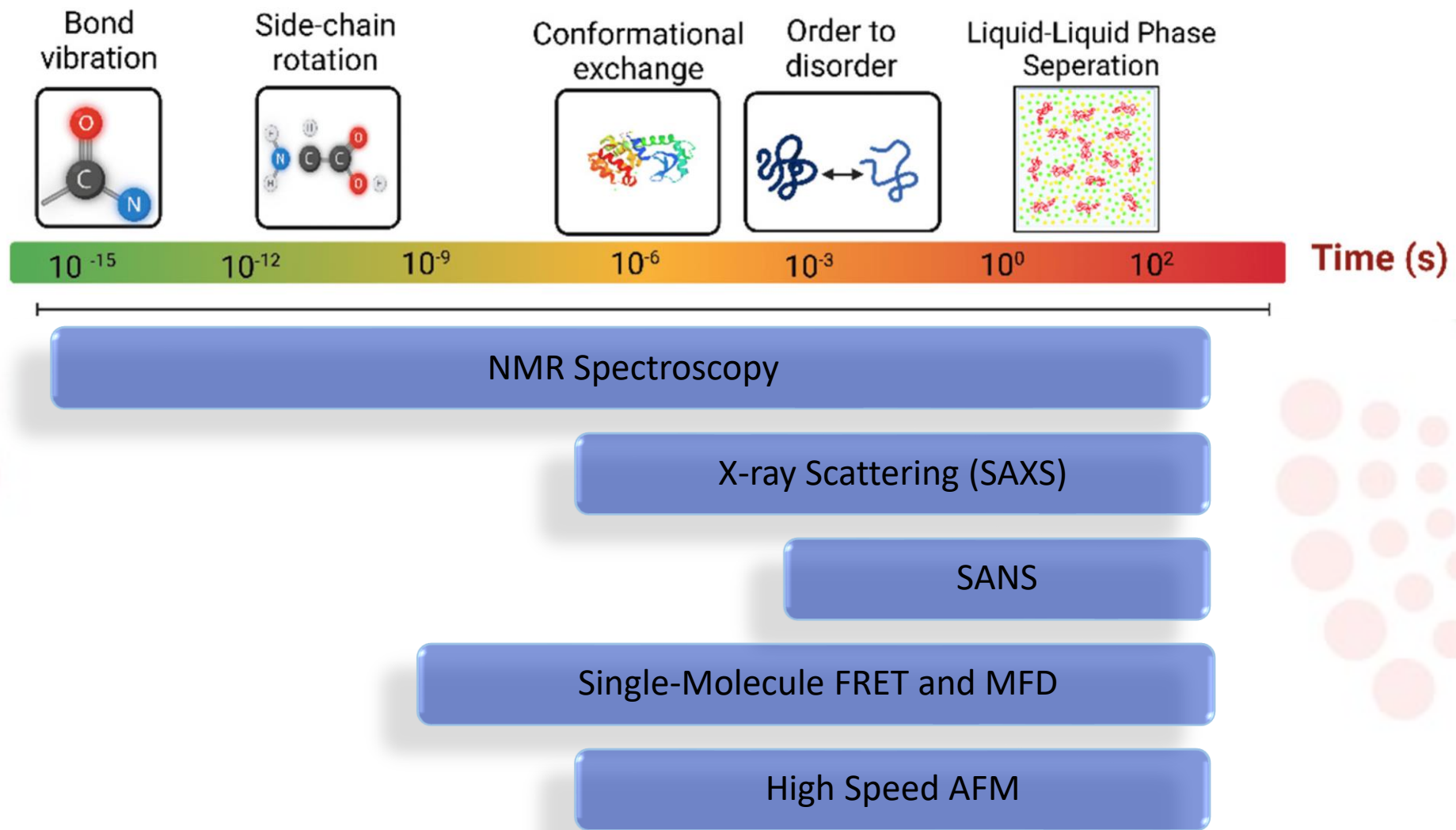
NMR Spectroscopy

X-ray Crystallography

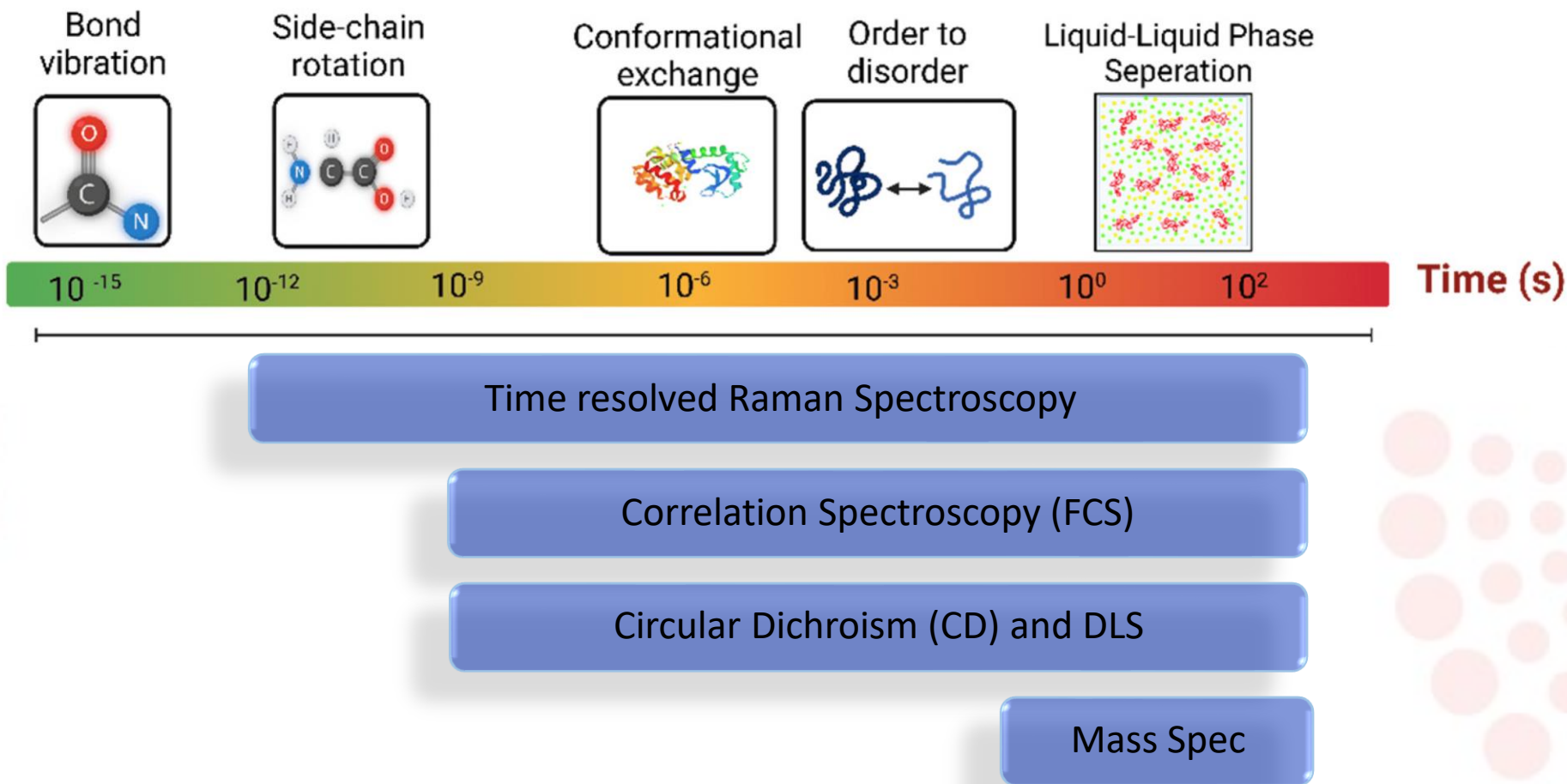
Single Particle Cryo-EM

So, why do we need BioSAXS?

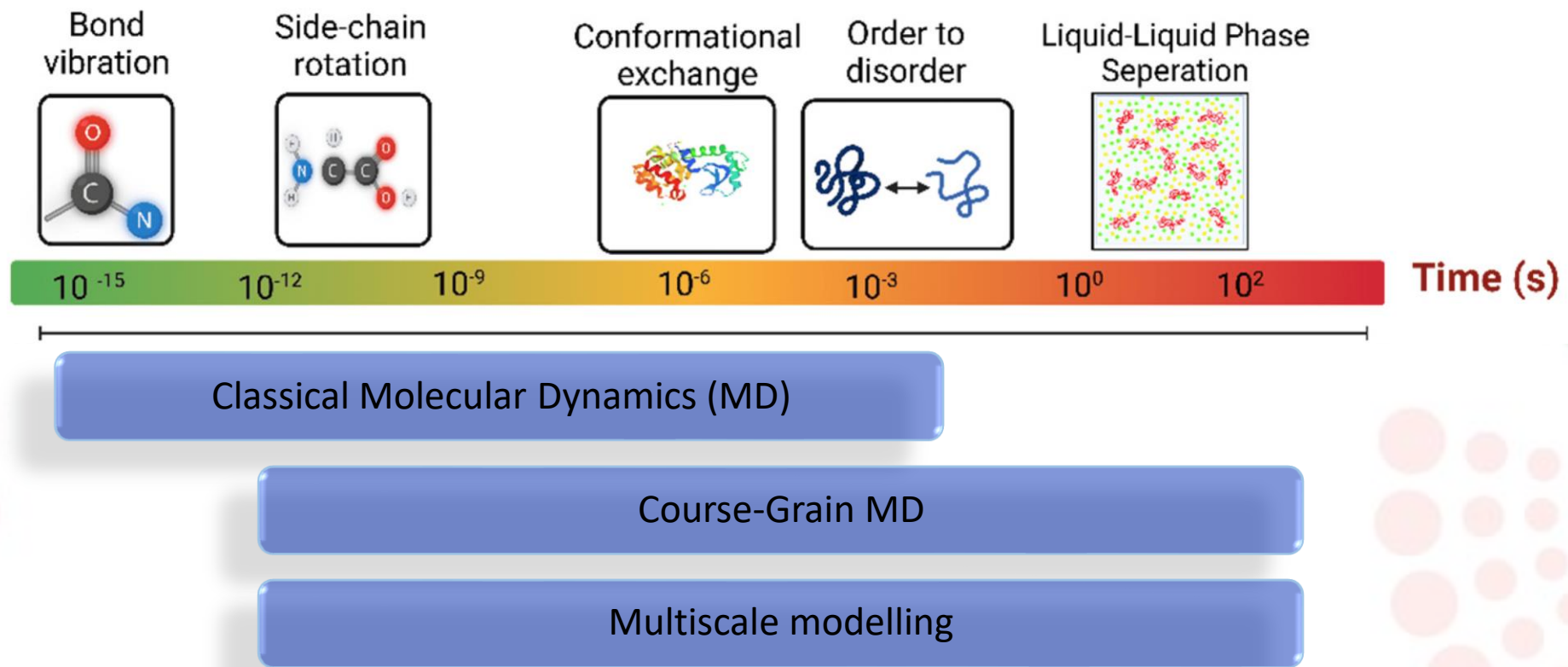
Adapted from J. Hutchings Biochem Soc Trans (2018) 46 (4): 807–816. <https://doi.org/10.1042/BST20170351>



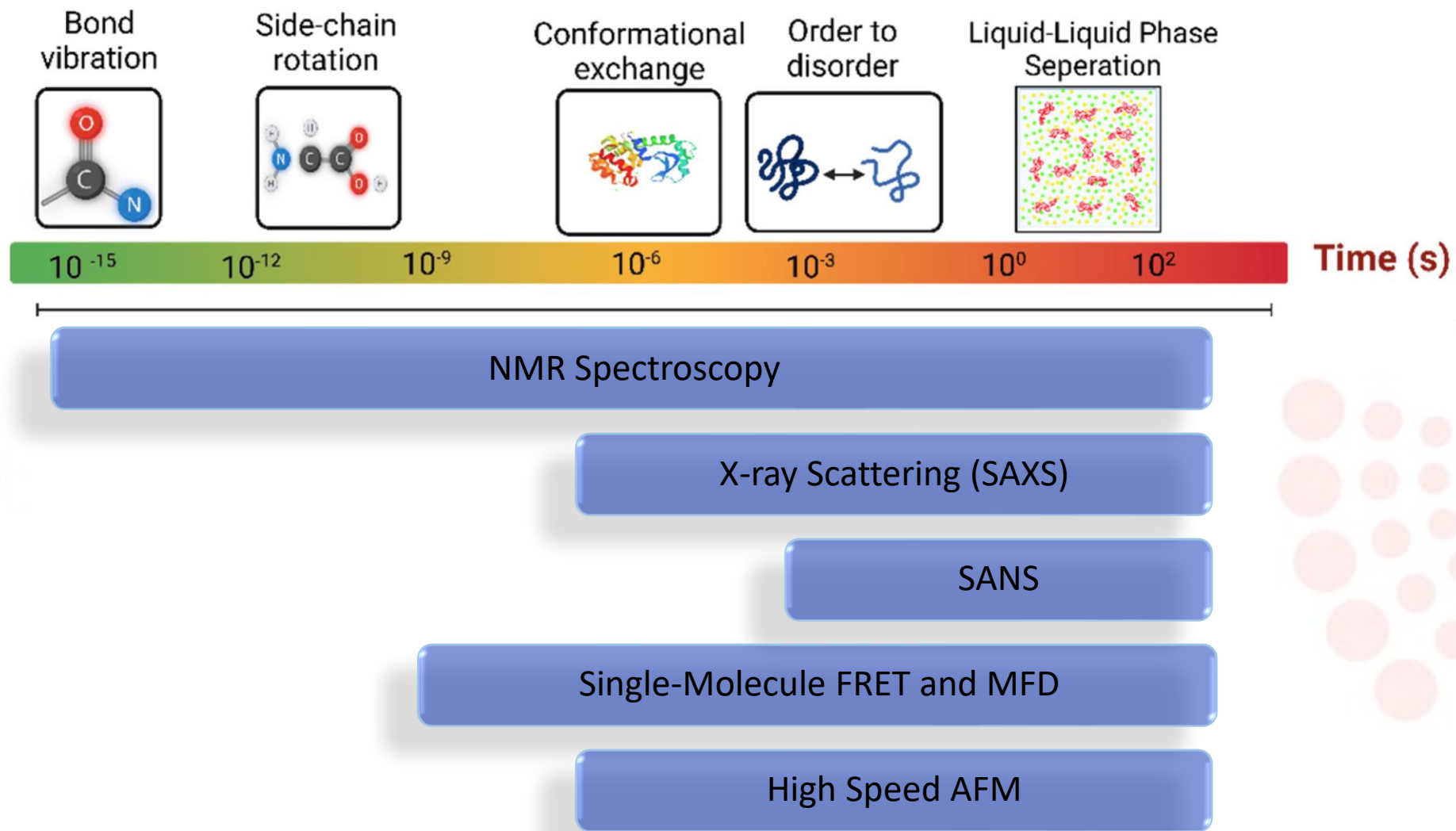
Adapted from Evans R, Biomolecules. 2023; 13(1):124



Adapted from Evans R, Biomolecules. 2023; 13(1):124



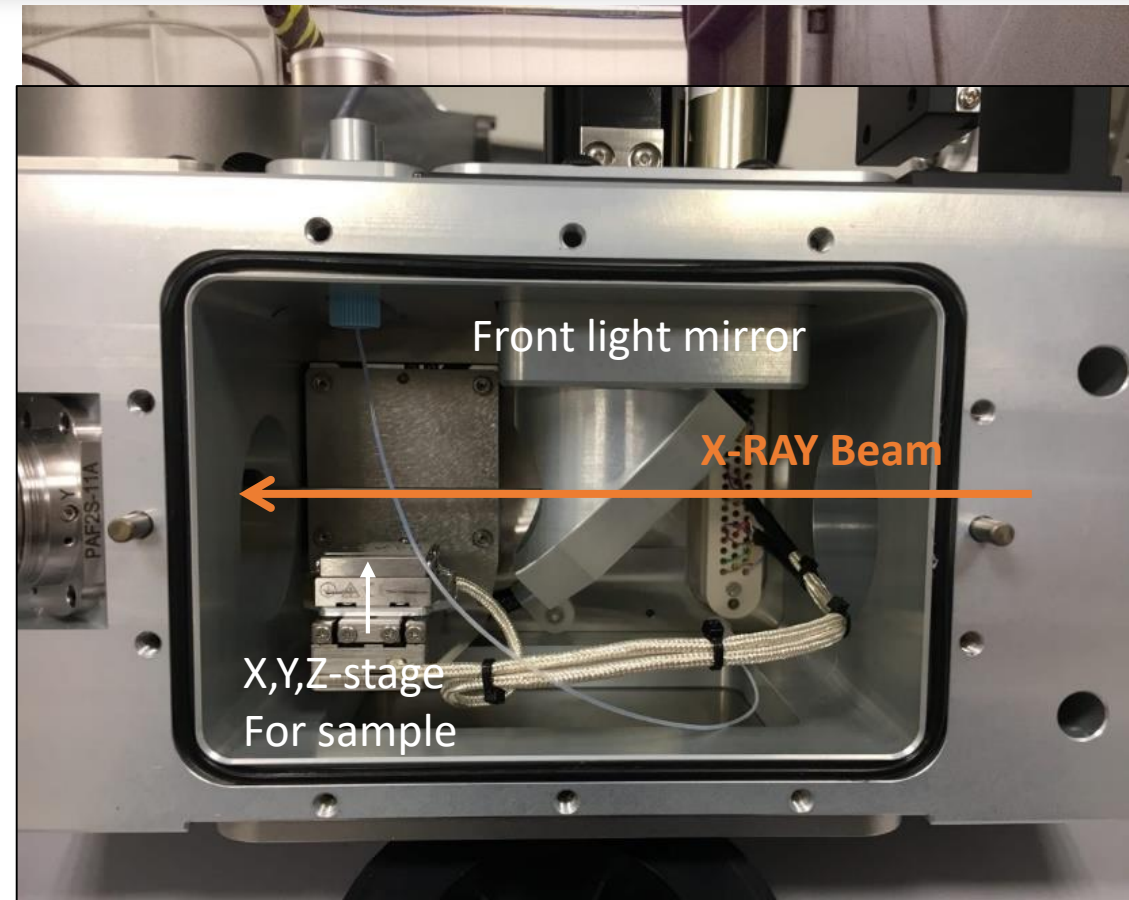
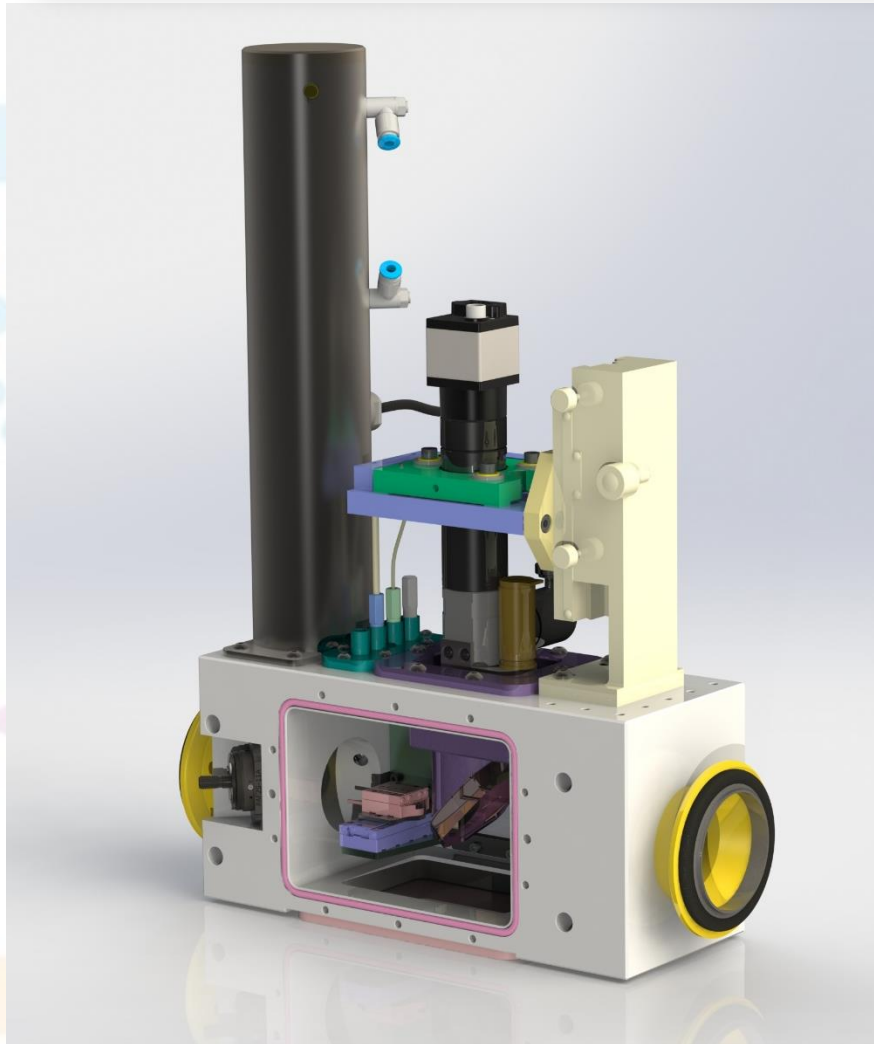
Adapted from Evans R, Biomolecules. 2023; 13(1):124



Adapted from Evans R, Biomolecules. 2023; 13(1):124

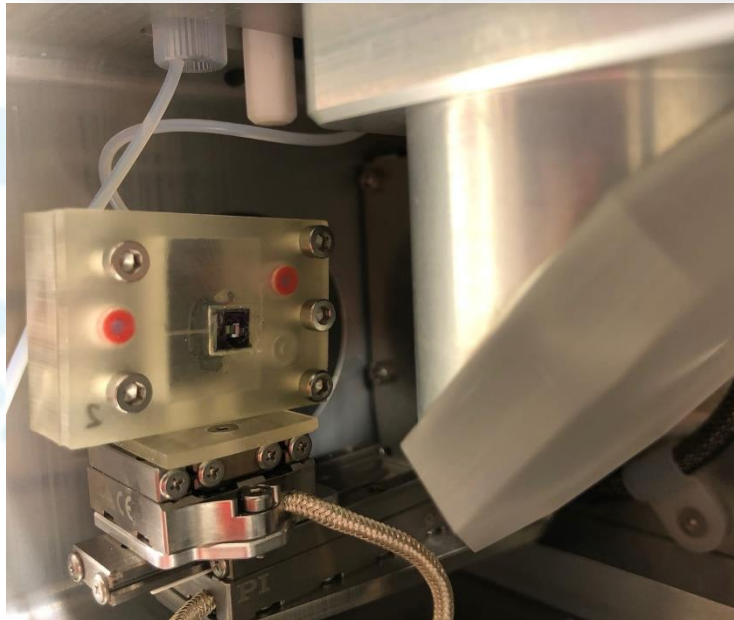


- Thermo-regulation:
  - storage: 4 – 40 °C,
  - exposure cell: 4 – 60 °C
  - Allows temperature ramps
  
- Concentration series
- pH series
- Time series
  
- (e.g. amyloid fibrillation)



- SEU developed at ESRF-BM29 in collaboration with EMBL Grenoble and EMBL Hamburg





Fixed Chip Holder

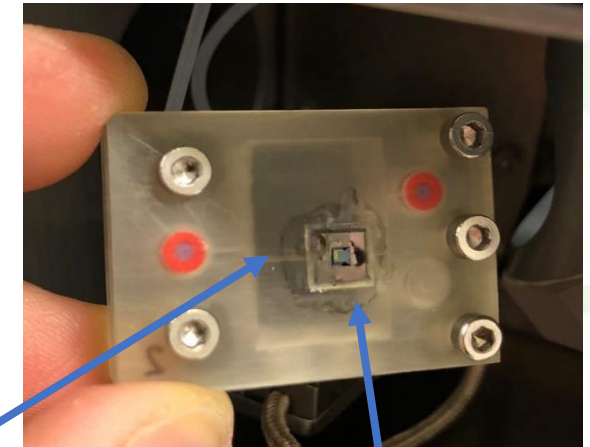
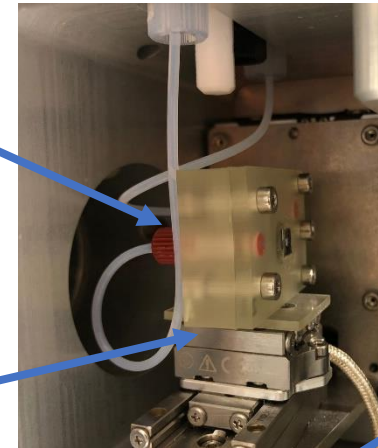
X-ray windows materials

- 100  $\mu\text{m}$  thick COC film
- 1  $\mu\text{m}$  thick Silicon Nitride

## Standardized Chip Design

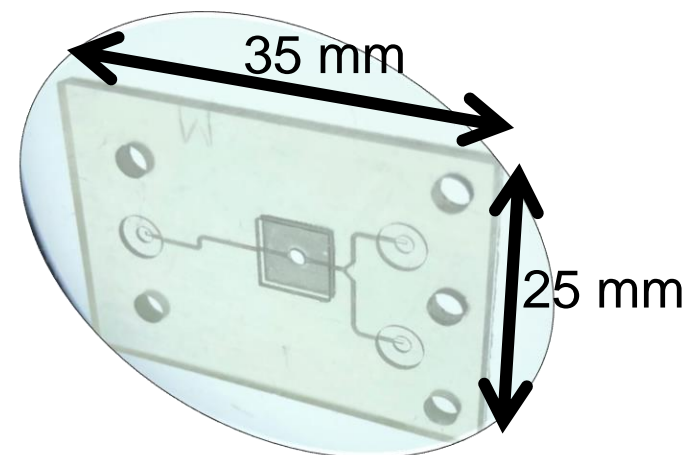
Standard fluid connectors

Sliding chip fixation



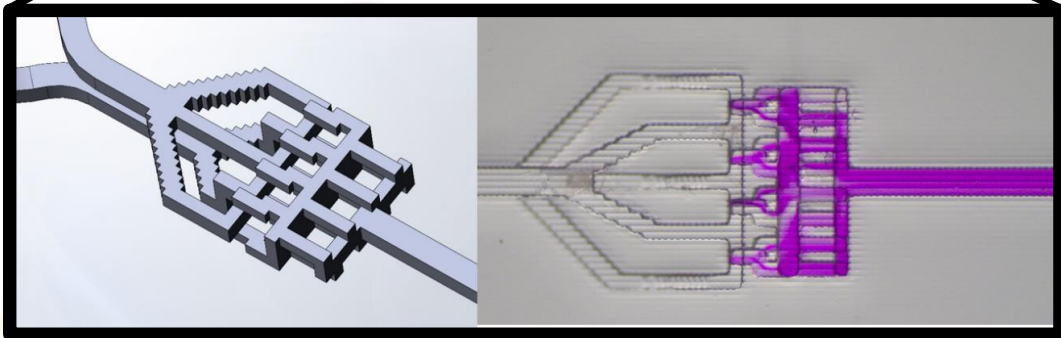
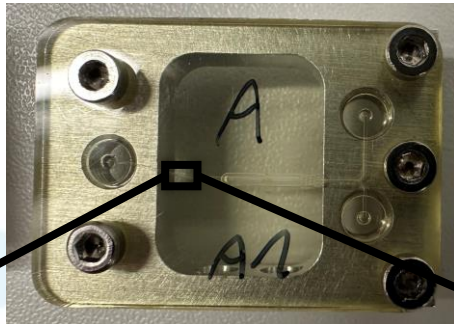
Microfluidic region

X-ray region



~ 5  $\mu\text{L}$  Channels  
 ~ 100 x 100  $\mu\text{m}$

Designed by Anton Popov with help from PSCM and Peter Van Den Linden



**BSXCuBE 3** Acquisition result

Front End	Safety shutter	Fast shutter	Energy	Wavelength	Transmission
Open	Closed	Closed	12.500 keV	0.99 Å	100.00 %

Loop Scan

Piezo Stages

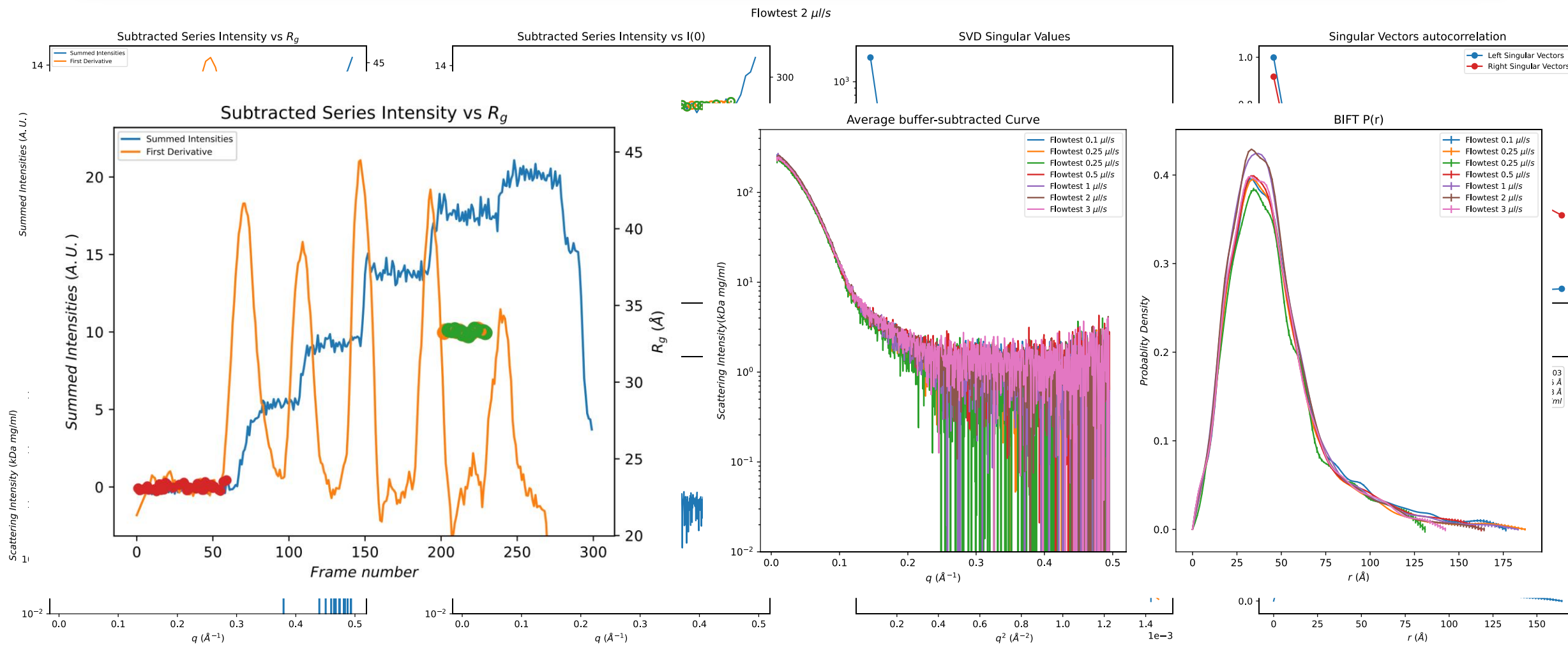
Position (Z, Y)

Position (X)

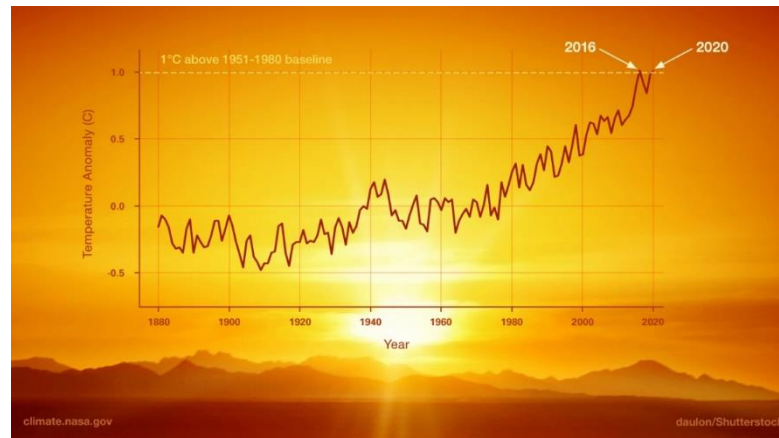
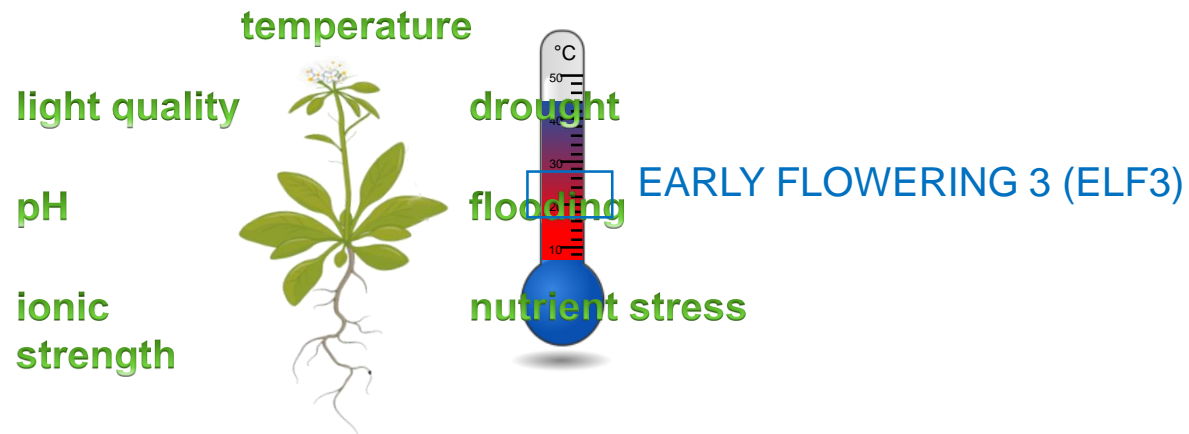
Pump Control

<input type="button" value="initialize"/> <input type="button" value="enable"/> <input type="button" value="stop"/> <input type="button" value="state"/> <input type="button" value="info"/> <input type="button" value="close"/> <input type="button" value="home"/> <input type="button" value="home_neg_lim"/> <input type="button" value="switch_valve"/>	<input type="text" value="0"/> <input type="button" value="Aspirate Pump A"/> <input type="text" value="0"/> <input type="button" value="Dose Pump A"/>	<input type="button" value="initialize"/> <input type="button" value="enable"/> <input type="button" value="stop"/> <input type="button" value="state"/> <input type="button" value="info"/> <input type="button" value="close"/> <input type="button" value="home"/> <input type="button" value="home_neg_lim"/> <input type="button" value="switch_valve"/>	<input type="text" value="0"/> <input type="button" value="Aspirate Pump B"/> <input type="text" value="0"/> <input type="button" value="Dose Pump B"/>
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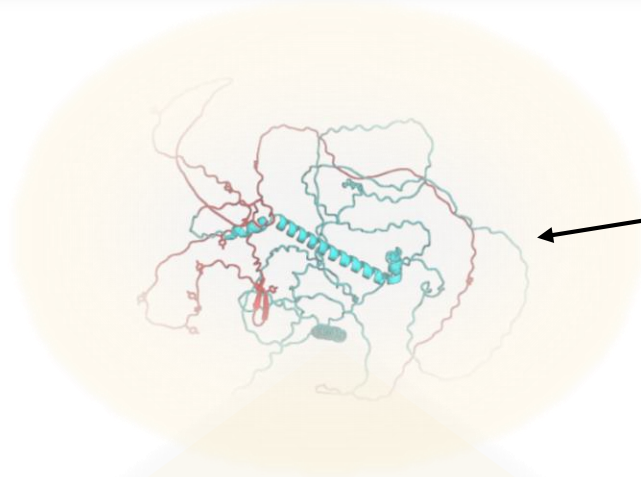
Designed by Bastion Schubert and Antonio Calio with help from PSCM and Peter Van Den Linden



BSA buffer titration, 50 frames

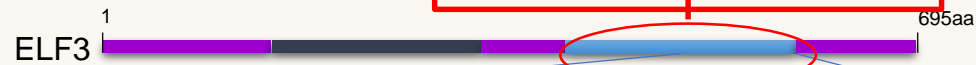






Barbed Wire

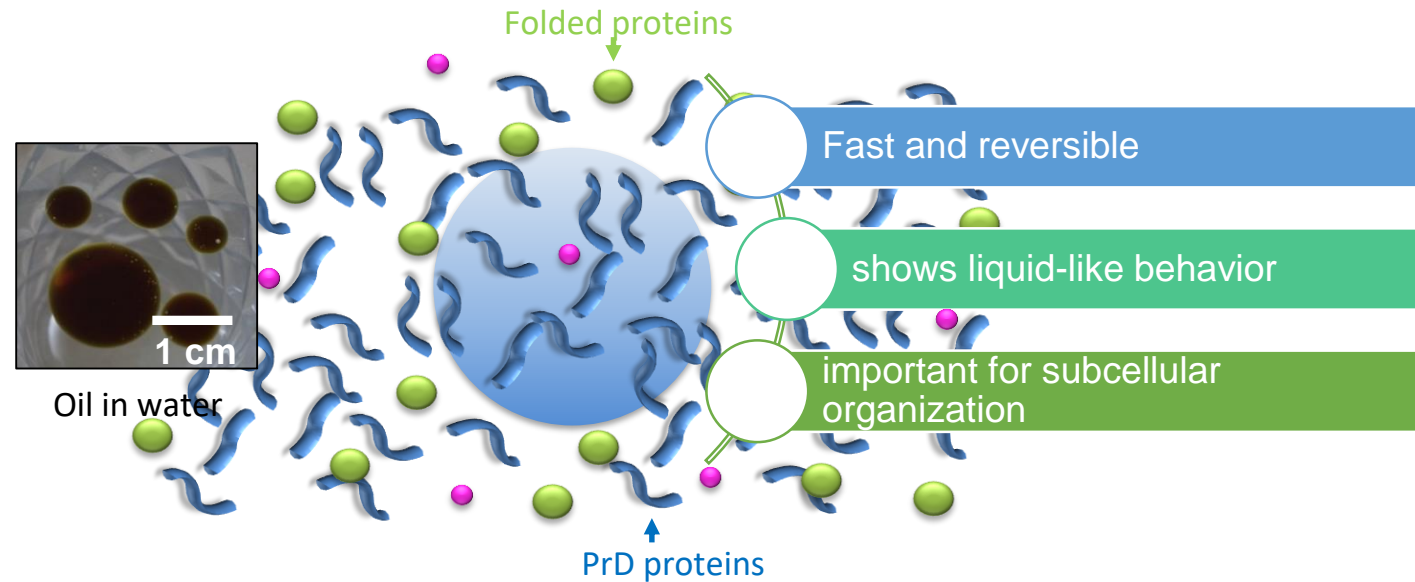
**Prion-like domain (PrD)**



...PPGNGYFPPYGMMPTIMNPY CSS **QQQQQQQ**CPNEQMNQFGHPGNLQNT **QQQQQRSDNEPAPQQQQQ**PTKSYPRARKSRQGS TGSSPSGPQ

- ELF3 is 700 amino acids with little secondary structure
- ELF3 is predicted to undergo phase separation. Can LLPS act as a mechanism for temperature sensing?

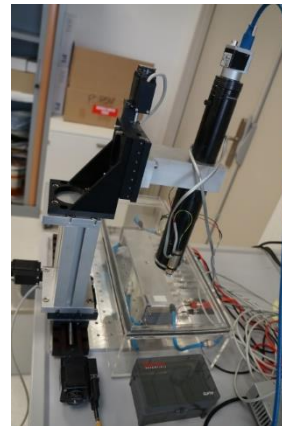
## Liquid-liquid phase separation (LLPS)



Hypothesis: LLPS is a key factor for thermosensing by ELF3 in plants



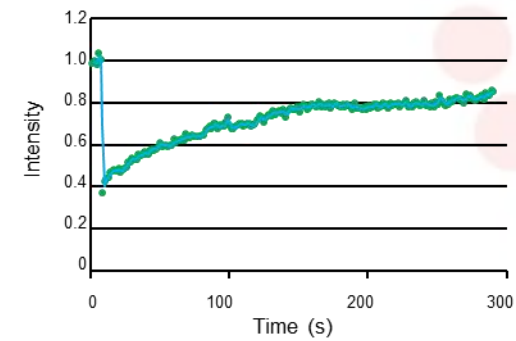
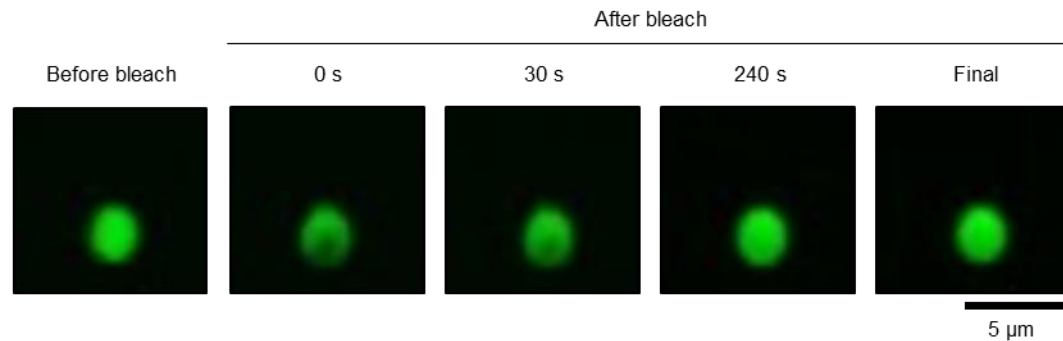
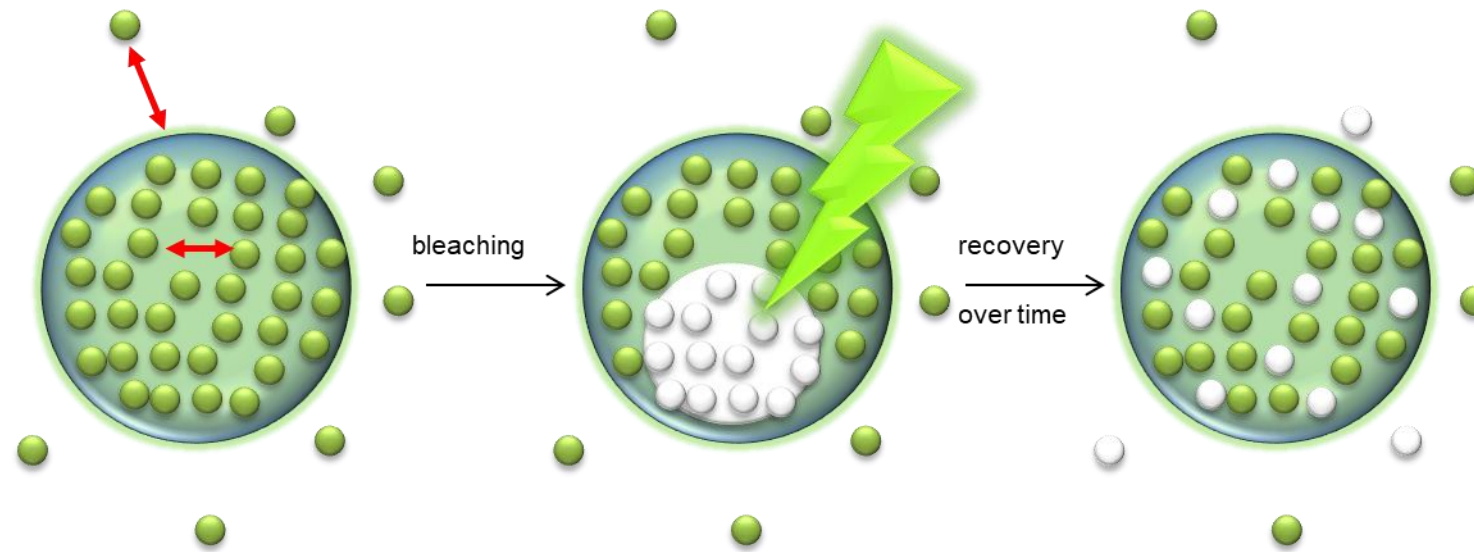
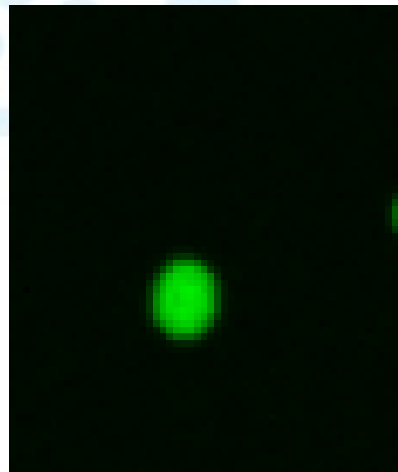
4 °C to 27 °C



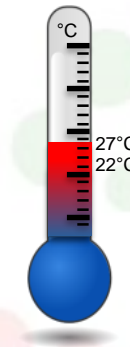
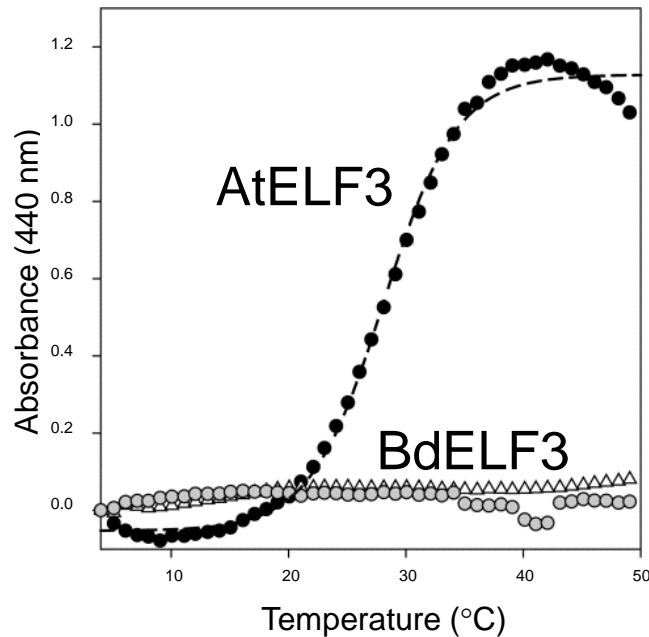
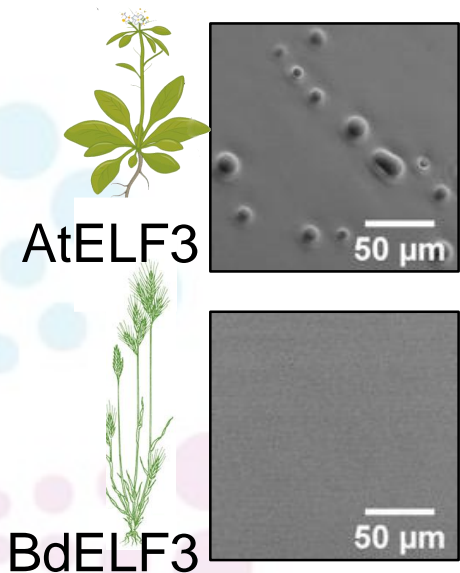
4 °C to 27 °C to 4 °C

Hutin et al., 2023 PNAS (in press)  
 Popov et al., 2023 (in preparation)

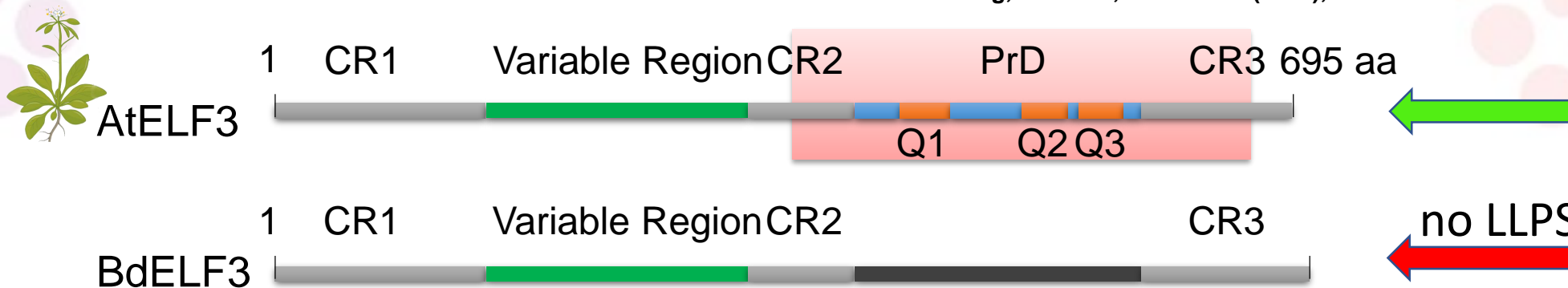




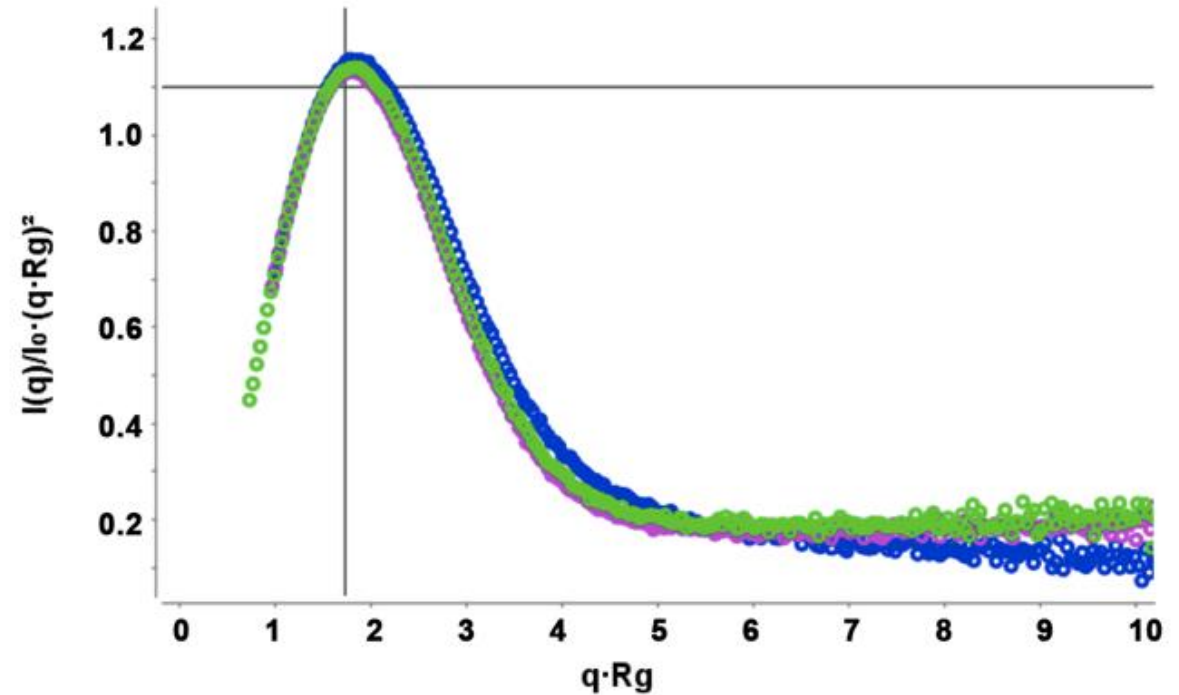
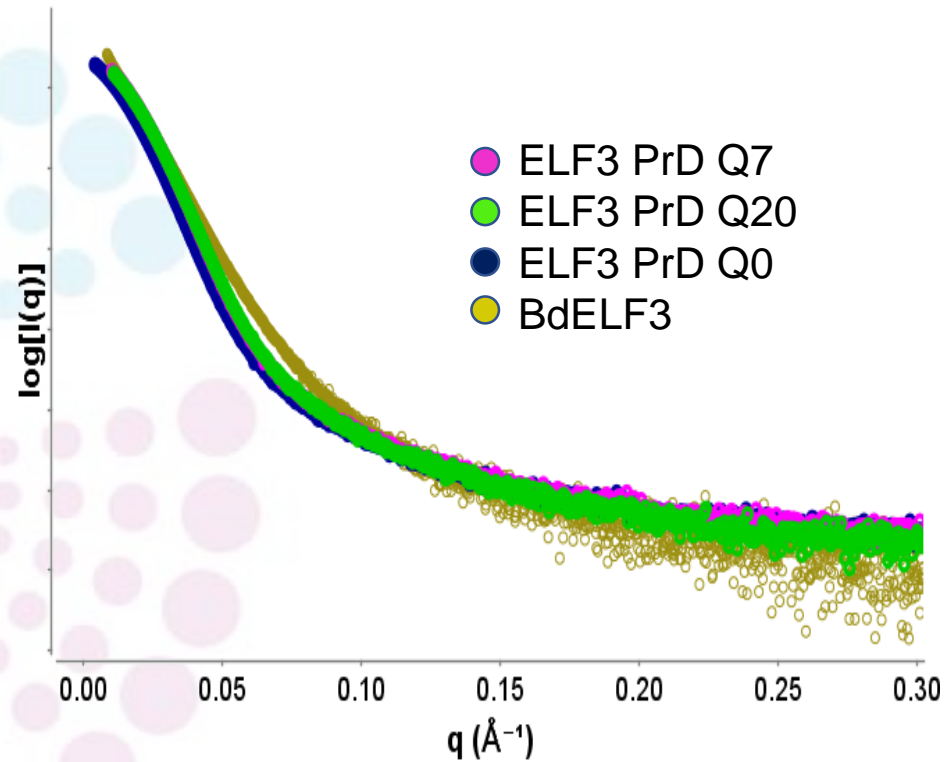
Jung, Barbosa, Hutin et al., 2020 Nature



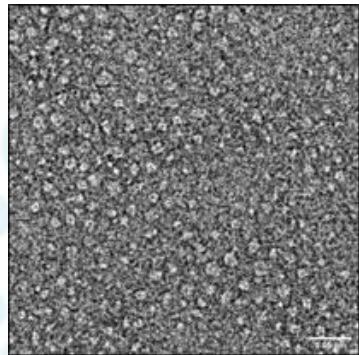
Jung, Barbosa, Hutin et al. (2020), *Nature*



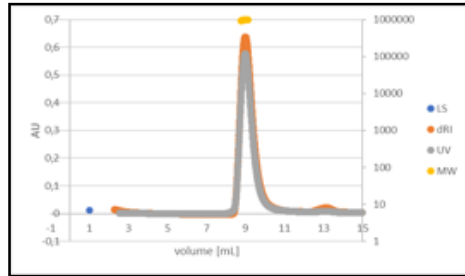
How can we structurally  
characterise ELF3 in the dilute and  
condensed phase?



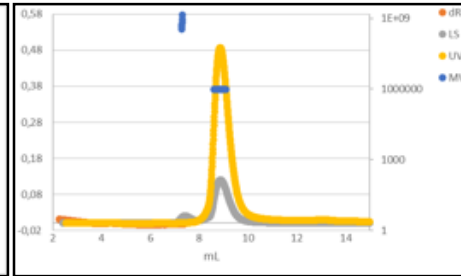
Scattering curves and Kratky plots for different ELF3-PrD constructs from Arabidopsis and Brachypodium in the dilute phase- proteins appear as an multimeric species of an globular shape



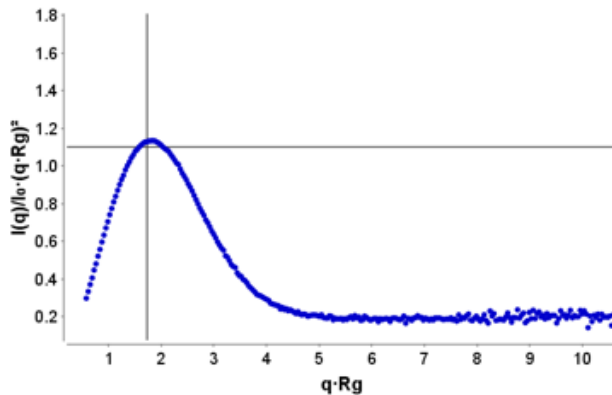
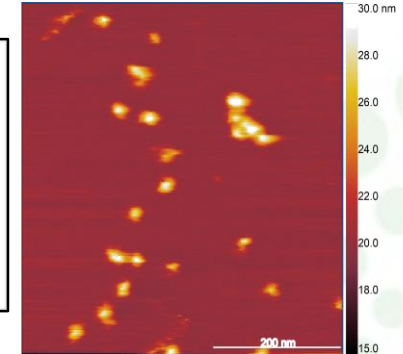
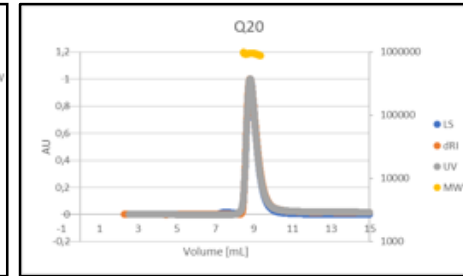
976.2 kDa



967.6 kDa



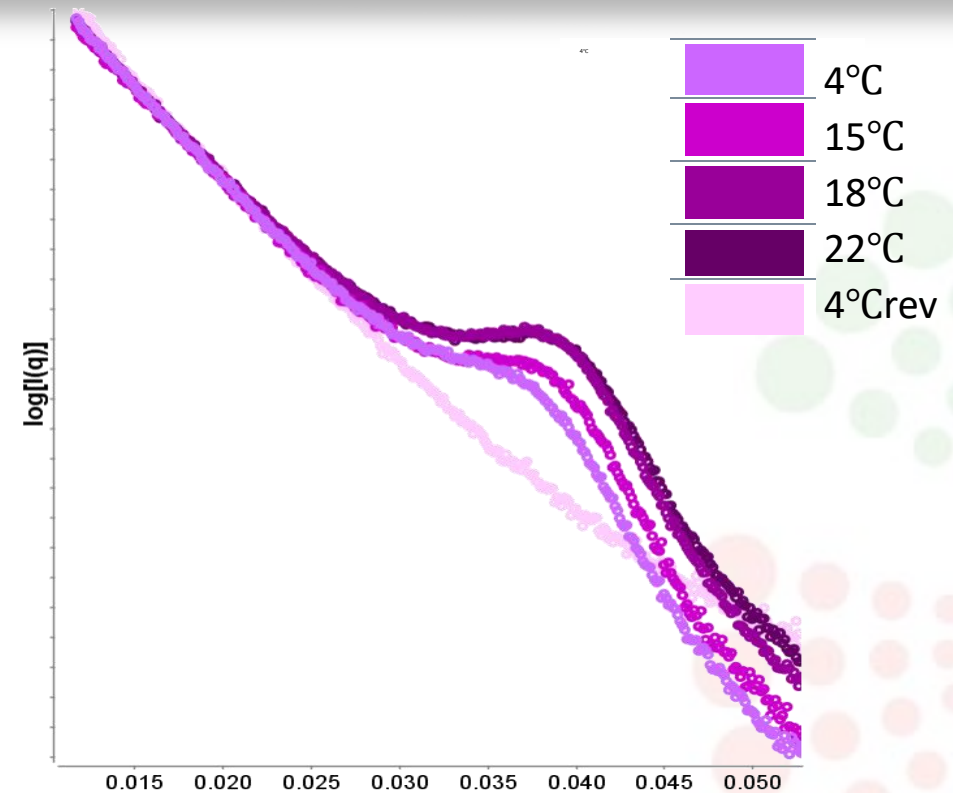
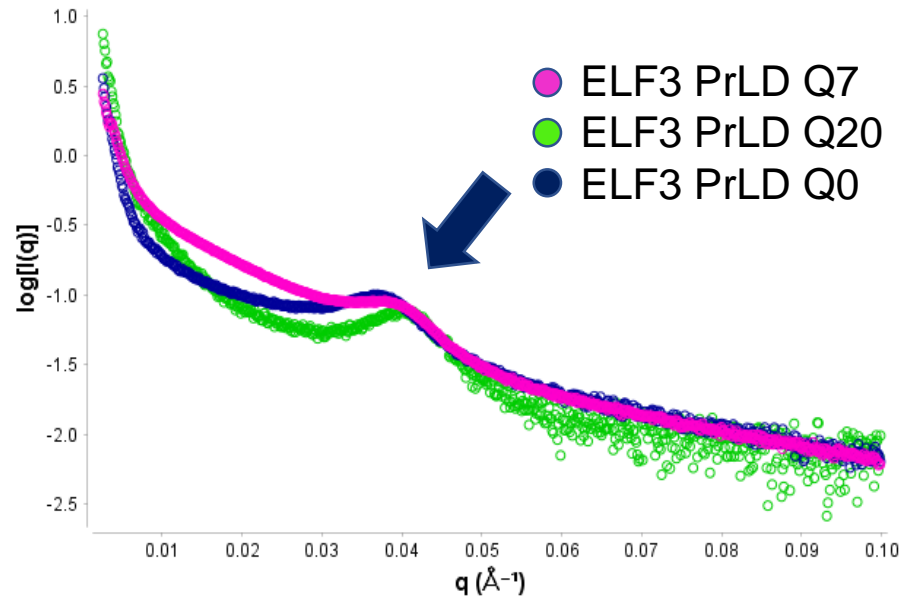
936.2 kDa



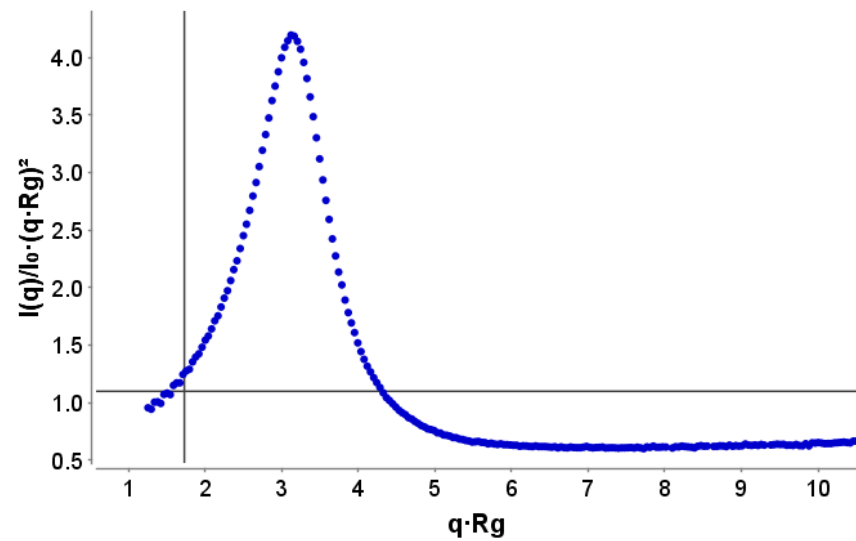
Dilute Phase

SAXS, Electron Microscopy and Size Exclusion  
Multiangle Laser Light Scattering and AFM  
demonstrate formation of a large ~30-mer of ELF3  
PrD

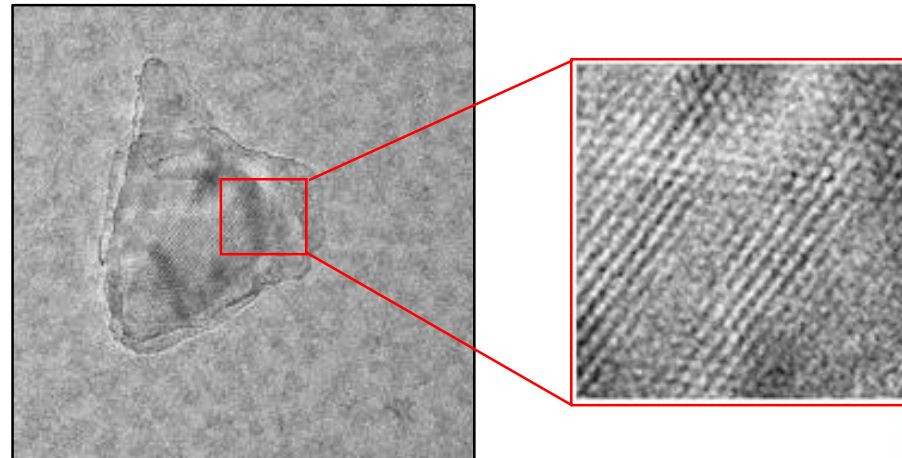
Fundamental unit of the PrD – homogeneous,  
globular oligomer of an intrinsically disordered  
polypeptide

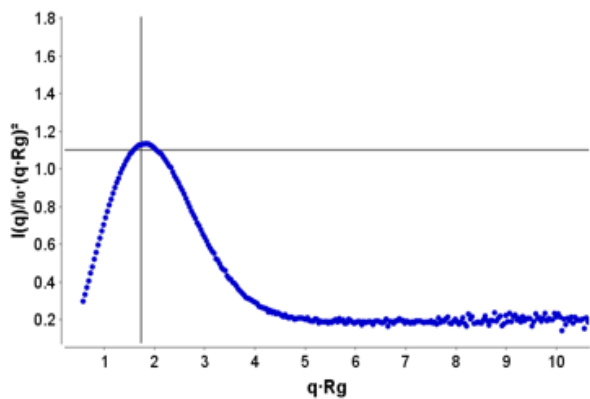
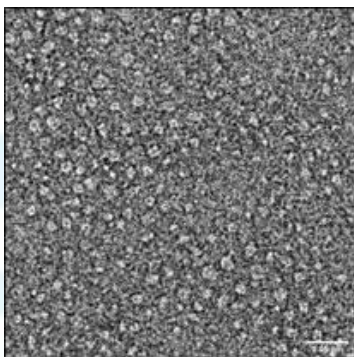


- Condensed phase exhibits structure factor peak in scattering curve  
=> long range ordering
- Appearance of peak is temperature dependent

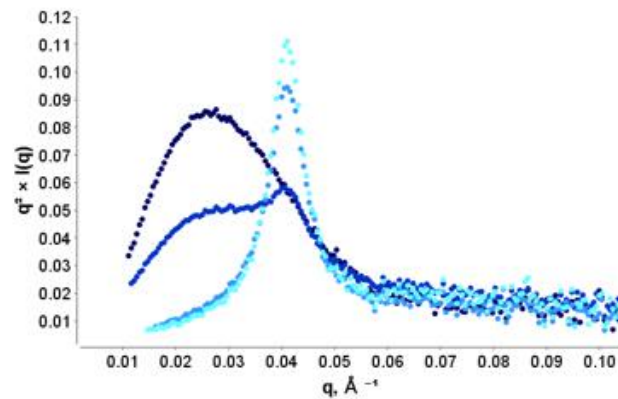
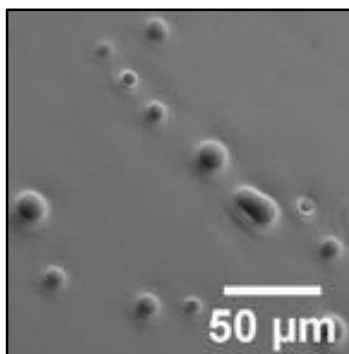


Hydrogel phase

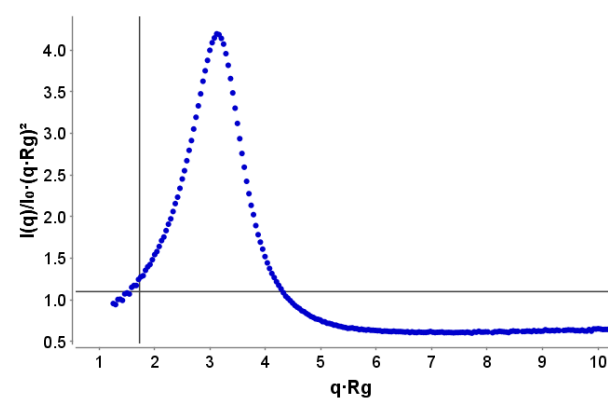
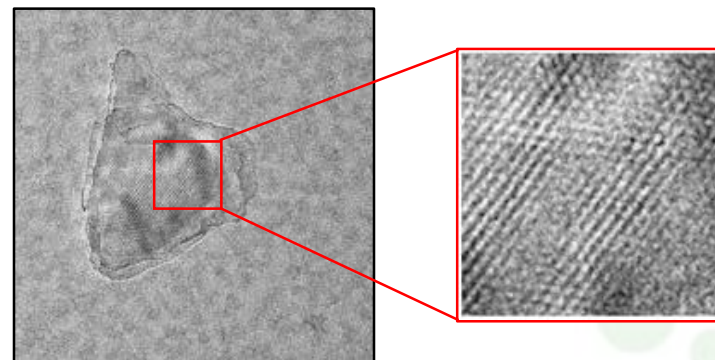




Dilute Phase



Condensate liquid phase

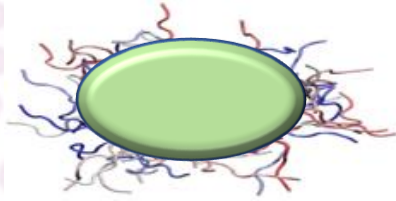


Hydrogel phase

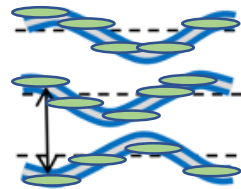


$$I(q) = sc1 \cdot P(q) \cdot S(q) + sc2 \cdot I_{chain} + back + \frac{AP}{q^4}$$

Revolution  
ellipsoid form  
factor



Structure factor



The parameters  $sc1$  and  $sc2$  are scale factors.

1)      2)      3)

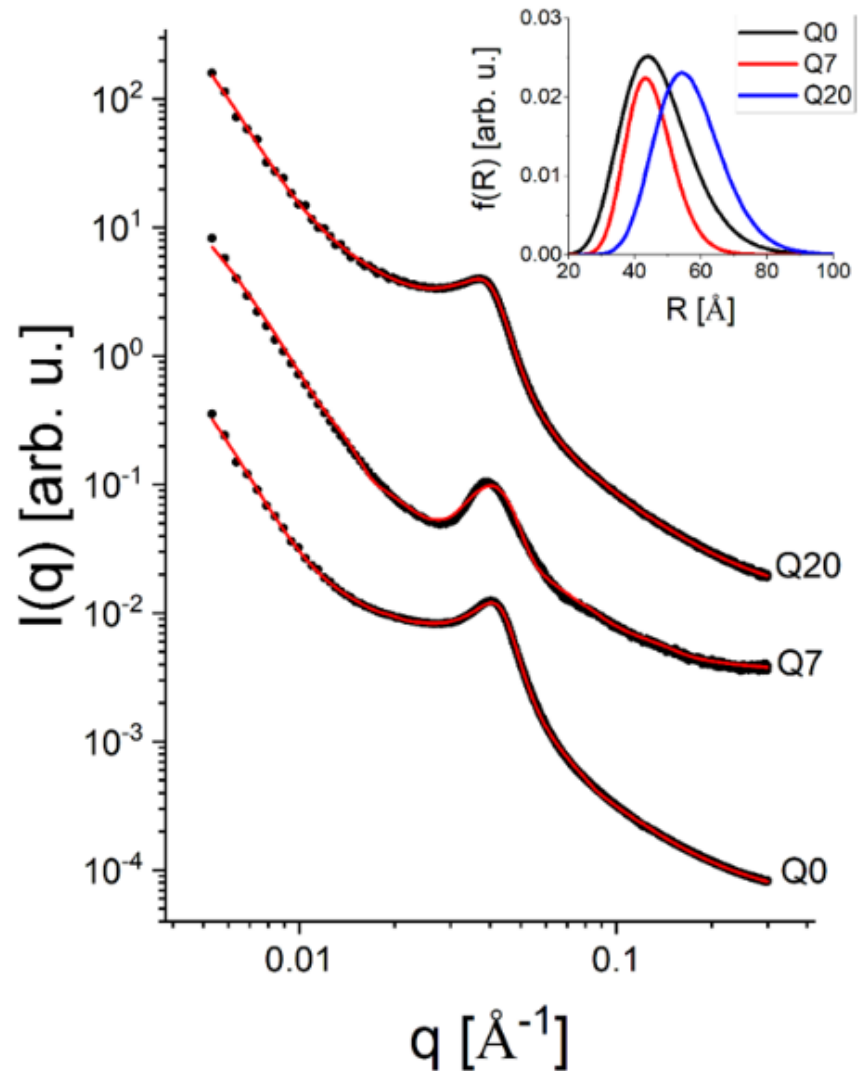
Overall (“total”) background considering the contribution of:

1) the chains in the disordered proteins to the overall scattering, modeled by a Gaussian chain like scattering

$$I_{chain}(q) = \frac{2[\exp(q^2 R_G^2) - 1 + q^2 R_G^2]}{(q^2 R_G^2)^2}$$

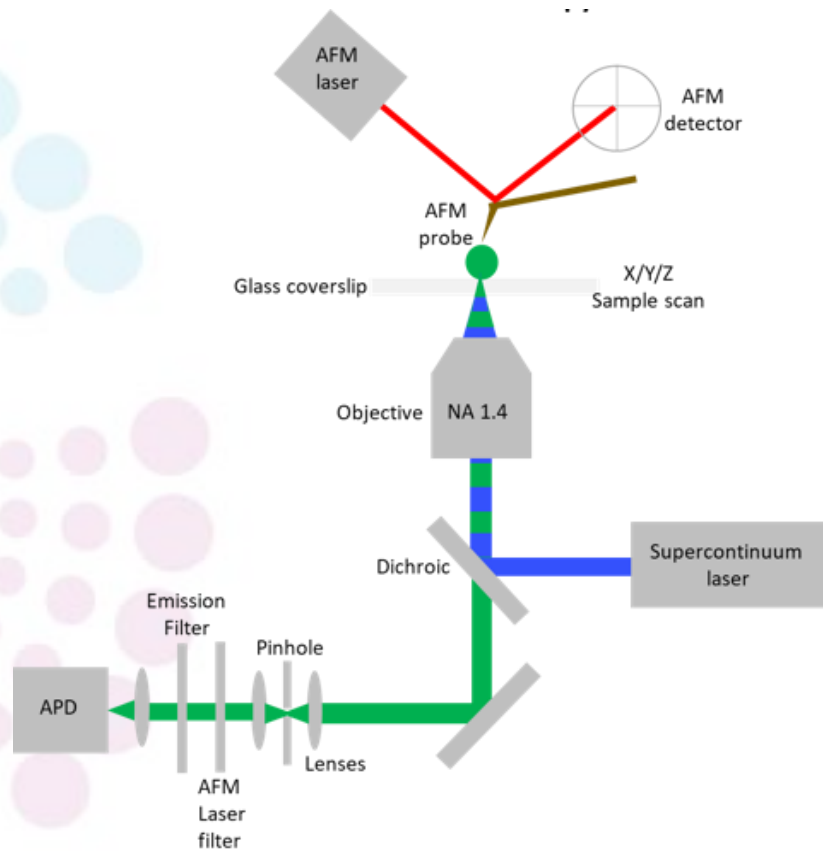
2) The diffuse background, a constant term;

3) The possible presence of large aggregates.

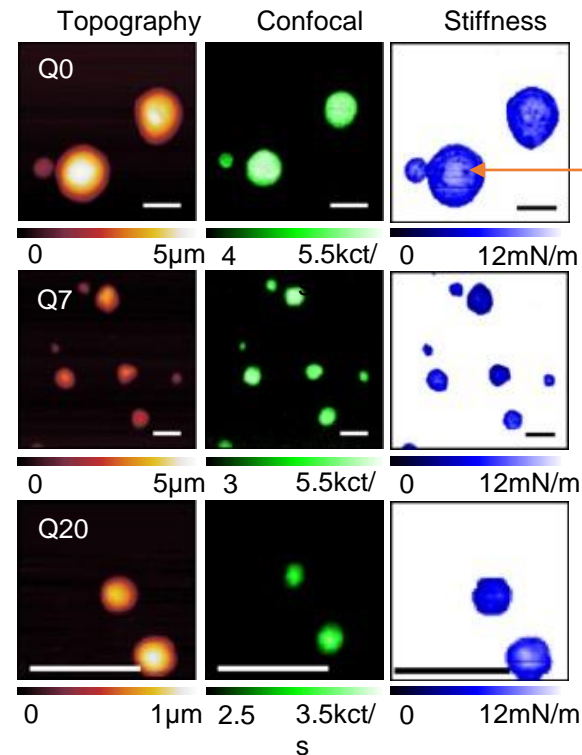


Parameter	Description	Q0	Q7	Q20
$a$ [Å]	Lattice factor or, in this case, lamellar periodicity.	146.3(3)	146.1(6)	<b>157.6(3)</b>
$\sigma_a$ [Å]	Quantifies the distortion relative to an ideal 2D-hexagonal lattice, being zero for an ideal lattice.	0.106(6)	0.120(9)	<b>0.070(6)</b>
$\Gamma$	Full width at half maximum (FWHM) of the peak, the same for all peaks in the SAXS curve.	0.0138(1)	0.0140(2)	<b>0.0156(2)</b>
$\eta$	Varying from 0 to 1, is the fraction of Lorentz function in the Pseudo-Voigt function.	0.21(4)	1.00(9)	<b>0.07(3)</b>
$c$	Constant ensuring that the product of form factor and structure factor fulfils the equation for the Porod invariant.	10(1)	10(8)	<b>10(1)</b>
$R$ [Å]	Radius of the particles.	47.5(3)	44.9(7)	<b>56.5(1)</b>
$\sigma_R$ [%]	Relative polydispersity of $R$ .	23(4)	16(4)	<b>18(1)</b>
$R_G$ [Å]	Radius of gyration related to the polymer-like scattering due to the disordered domains.	21(1)	32(9)	<b>122(8)</b>
$R_{Agg}$ [Å]	Radius of the aggregates.	375(11)	237(5)	<b>334(9)</b>
$\sigma_{R_{Agg}}$ [%]	Relative polydispersity of $R_{Agg}$ .	48(1)	60(1)	<b>50(1)</b>
$sc_1$	Scale factor	0.056(3)	0.009(7)	<b>0.037(1)</b>
$sc_2$	Scale factor	0.0013(1)	0.003(2)	<b>0.026(3)</b>
$sc_{Agg}$	Scale factor	0.92(1)	0.93(1)	<b>0.94(1)</b>
$back \times 10^{-6}$	<b>Constant incoherent scattering contribution.</b>	<b>158(1)</b>	<b>372(1)</b>	<b>86(1)</b>

## AFM – Confocal microscopy

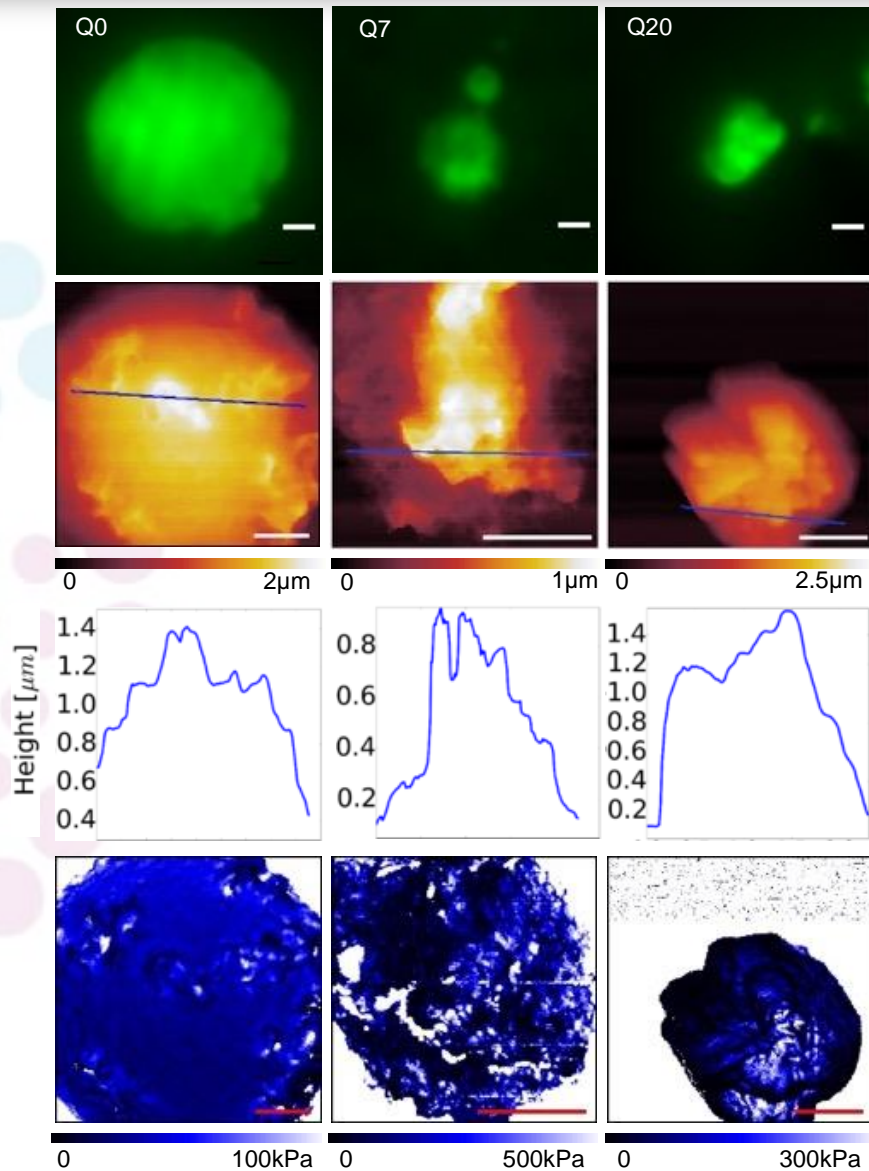


correlative microscopies- AFM and fluorescence



Stiffer microenvironments

Collaboration with Luca Costa, CBS Montpellier

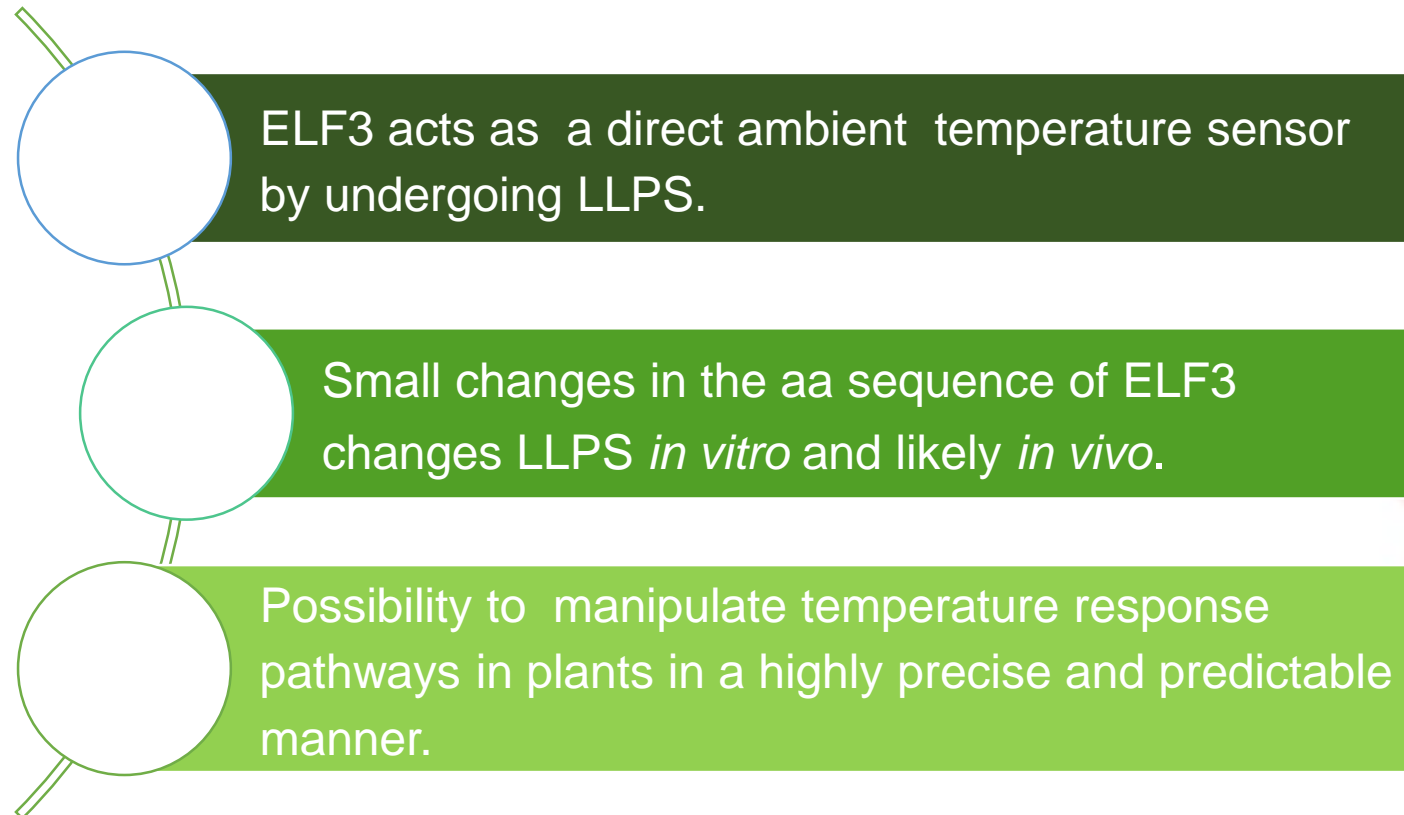


Wide-field fluorescence and AFM on same droplets

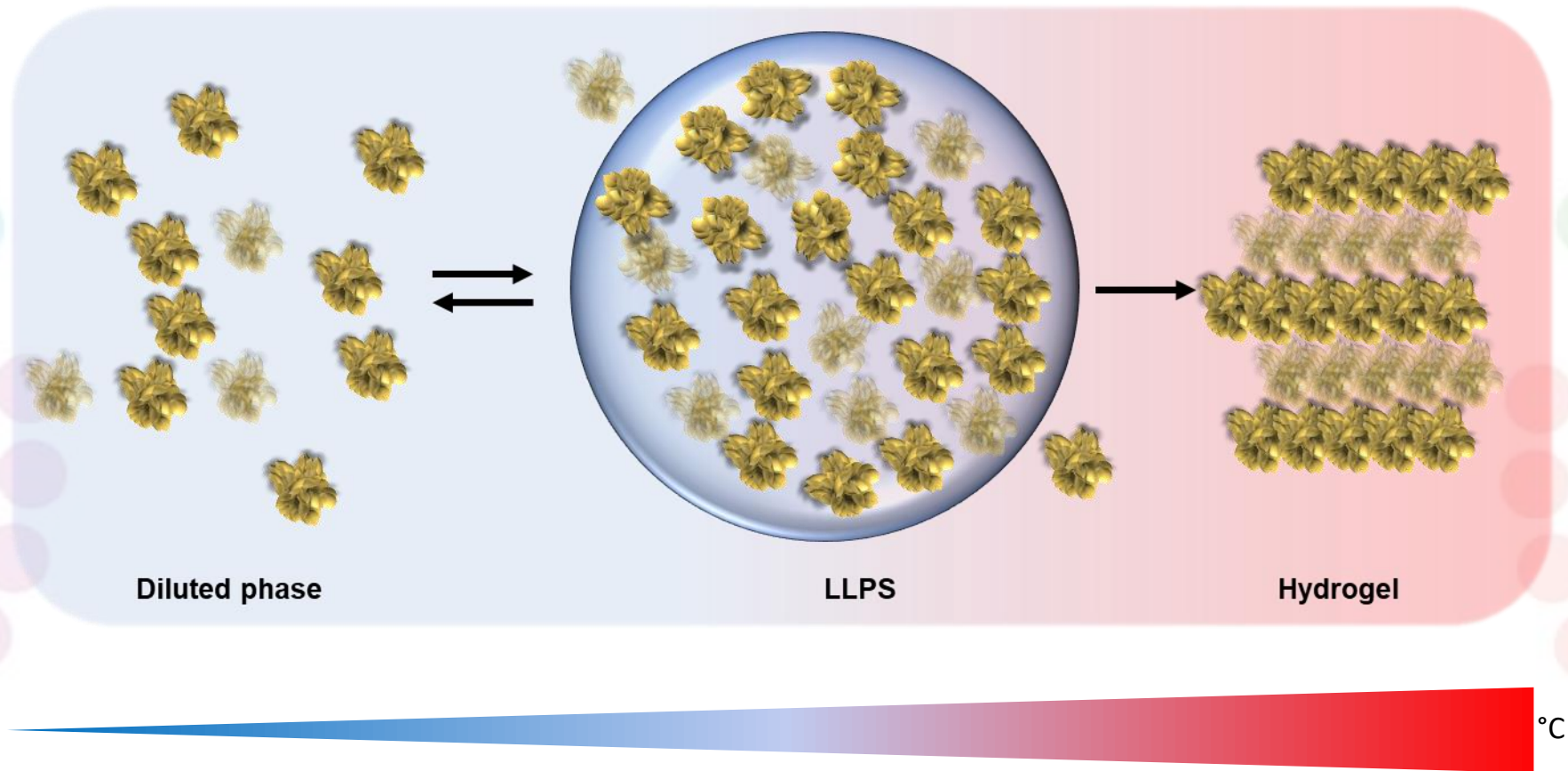
Height profiles- step-like pattern suggesting flat lamellar layers

Stiffness is variable within the droplet and due to the protein sequence microenvironments arise during aging

## Macromolecular condensates are dynamically structured



## Cartoon representation of LLPS formation



Petra Pernot

Hayden Fisher

Antonino Calio

Anton Popov

Data Analysis:

Jérôme Kieffer

Martha Brennich

BLISS:

Antonia Beteva

Marcus Oskarsson

BsXCube3:

Jean-Baptiste Florial

Marcus Oskarsson

Databases:

Alejandro De Maria Antolinos

Maxime Chaillet

Mecanics/Electronics:

Jonathan Gignes

John Surr

Fabien Dobias

Thierry Giraud

New Flight tube:

Pascal Theveneau

Detector:

Marie Ruat

Alejandro Homs

Exposure Units + SC:

Raphaël Cohen-Abrahams

Gergely Papp

Florent Cipriani

Andrew McCarthy

Clement Sorez

Franck Felisaz

LPCV, Grenoble, France

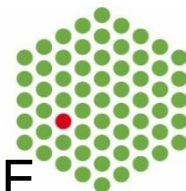
Chloe Zubieta

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