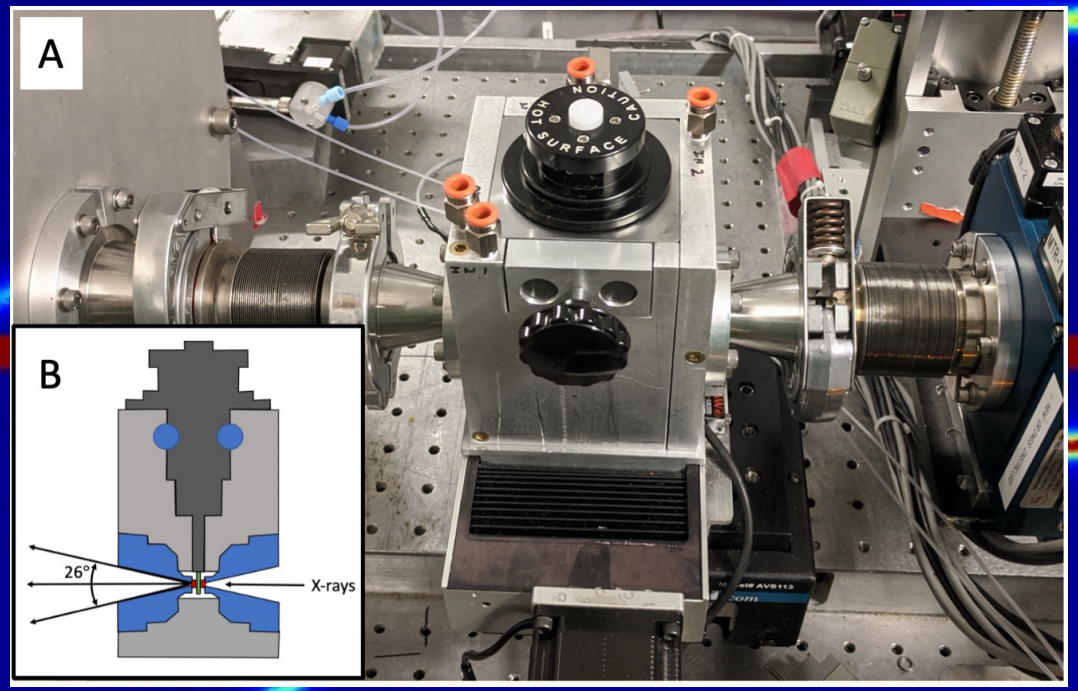
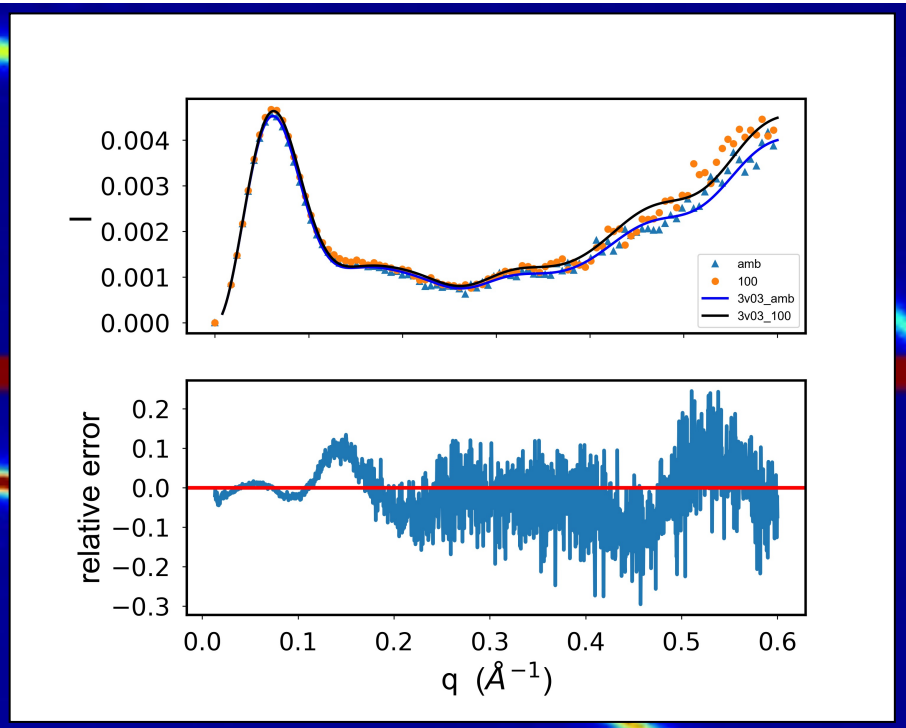
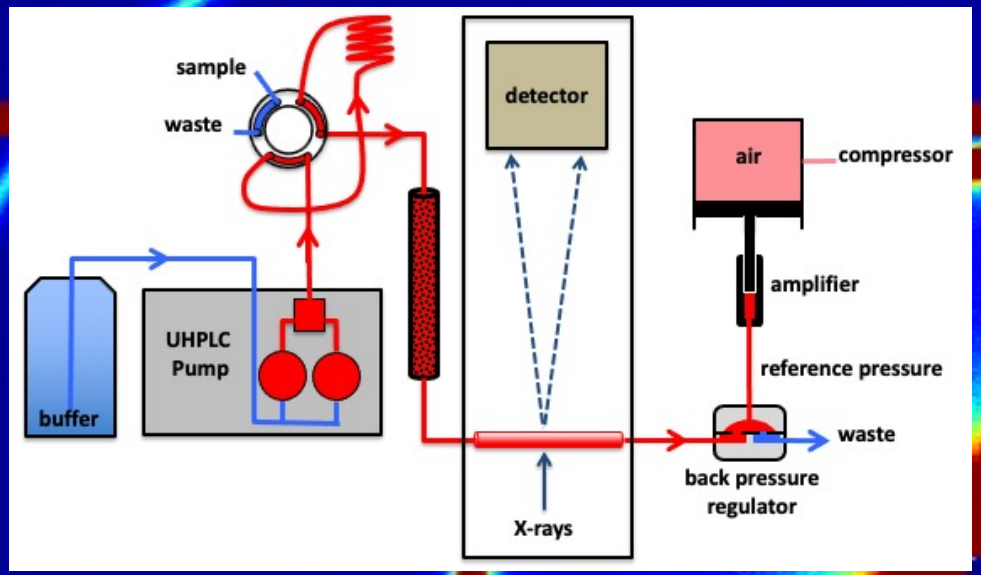
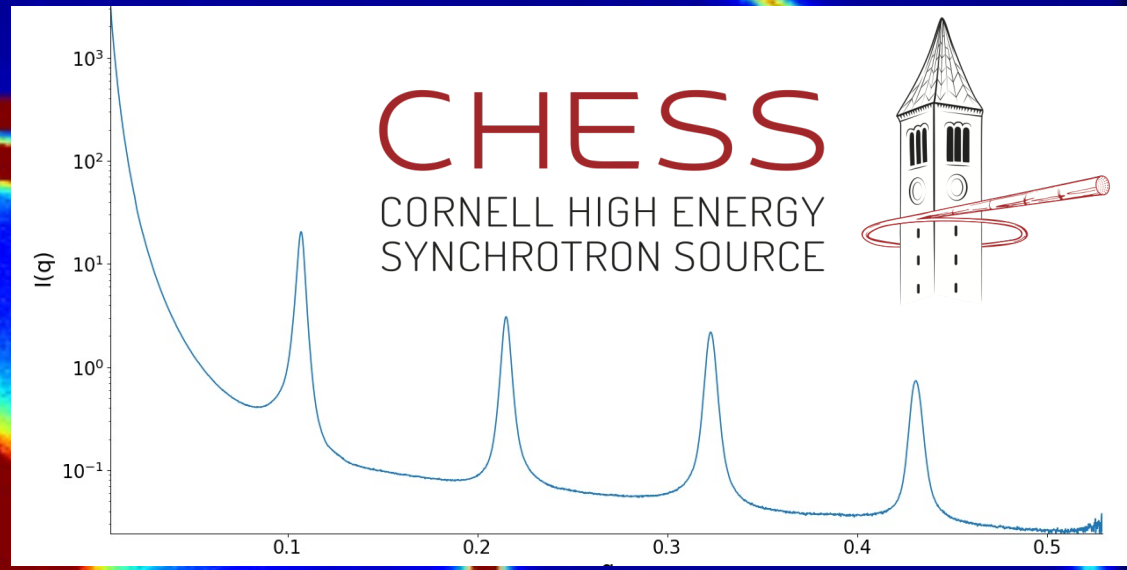
HP-Bio: High Pressure BioSAXS for Deep Life and Extreme Biophysics









Thursday April 29th

Time	Session	Presenter
1:00pm - 1:30pm	Why High pressure?	Cathy Royer (RPI)
1:30pm - 2:00pm	Why SAXS?	Nozomi Ando (Cornell)
2:00pm - 2:45pm	SAXS fundamentals	Thomas
2:45pm - 3:00pm	Break	
3:00pm - 3:30pm	Sample preparation, validation	Watkins
3:30pm-4:15pm	Advanced analysis overview	Meisburger
4:15pm-4:45pm	High Pressure SAXS	Richard Gillilan (CHESS)

Friday April 30th

Time	Session	Presenter
9am- 12pm	SAXS Data Processing Tutorial Tutorial 1 Tutorial 2 Tutorial 3 Example Data (Link to Dropbox)	(Gillilan, Hopkins, Skou, Ando Lab)
1pm – 5pm	Remote HP SAXS Data Acquisition Session I.	

Saturday, May 1st

Time	Session	Presenter
9am - 1pm	Remote HP SAXS Data Acquisition Session II	
2pm – 6pm	Data Analysis Help Sessions	

Importance of high-pressure biology and growing research community

- Biophysics
  - ✓ Folding
  - ✓ Protein-protein interactions
  - ✓ Catalysis/biochemistry
- High pressure processing (HPP)
  - ✓ Food science
  - ✓ Pharma
- Deep life
  - ✓ Strategies for adaptation
  - ✓ Limits of life/astrobiology
  - ✓ Rules of life
  - ✓ Novel tools for Mol. Bio.
  - ✓ Evolutionary history

Annu. Rev. Biophys. 2021. 50:X–X
https://doi.org/10.1146/annurev-biophys-100120-072804

The Molecular Basis for Life in Extreme Environments

Nozomi Ando,[1,2] Blanca Barquera,[3] Doug Bartlett,[4] Eric Boyd,[5] Audrey A. Burnim,[1] Amanda S. Byer,[1] Daniel Colman,[5] Richard E. Gillilan,[6] Martin Gruebele,[7,8,9] George Makhatadze,[3] Catherine A. Royer,[3] Everett Shock,[10] A. Joshua Wand,[11,12,13] and Maxwell B. Watkins[2]

Thinking about Pressure

Unit	MPa	atm*	bar	psi
MPa	1	9.8692 …	10	145.03
atm		1	1.01325	14.695 …
bar			1	14.503 …

Soda can
0.3 MPa (3 atm)

lab gas cylinder
20 MPa (200 atm)

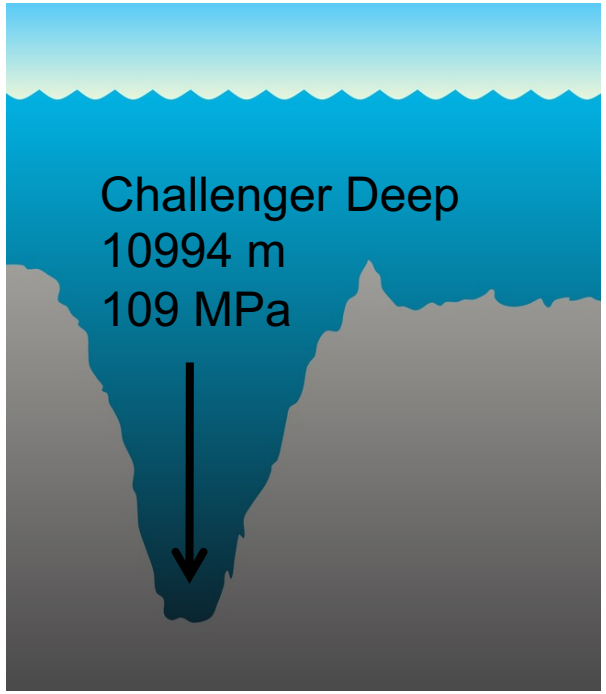
PEEK tubing bursts
48 MPa (480 atm)

Common metal
pipes burst
55-120 Mpa
(550-1200 atm)

*"standard atmosphere" (atm) - defined by CGPM in 1954
1 torr (symbol = Torr) = 1/760 atm (CGPM, 1954)
"standard pressure" in STP = 1 bar (IUPAC in 1982)

Thinking about Pressure

Unit	MPa	atm	bar	psi
MPa	1	9.8692	10	145.04
atm		1	1	14.7
bar			1	14.5
psi				1



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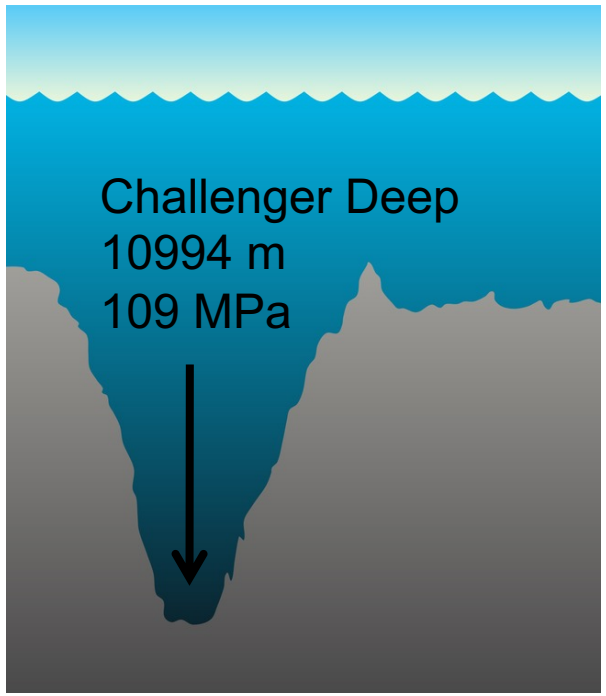
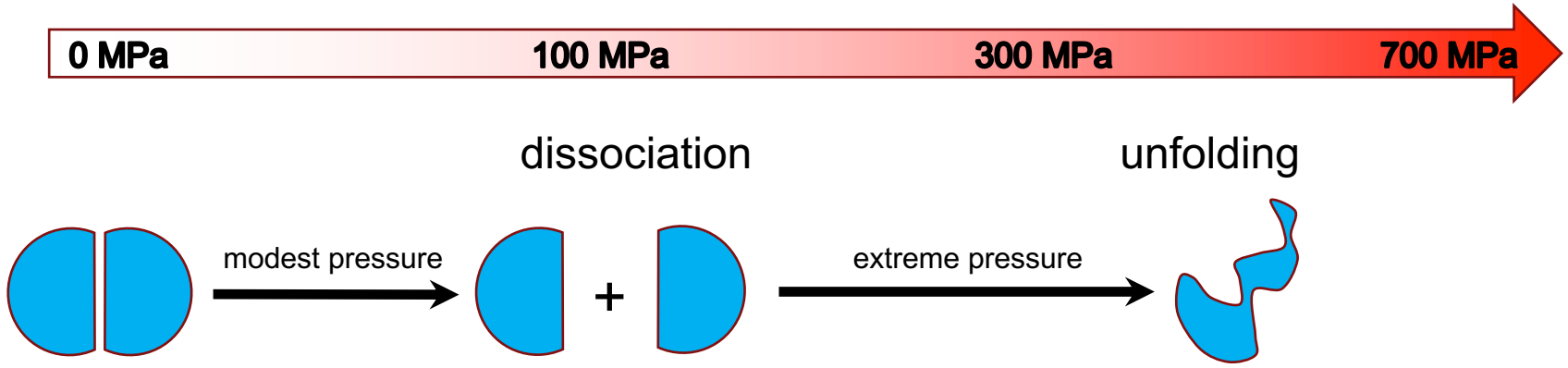


Water Depth
10 meters = 1 bar

Density +4.5%
(1 kbar, 5°C)

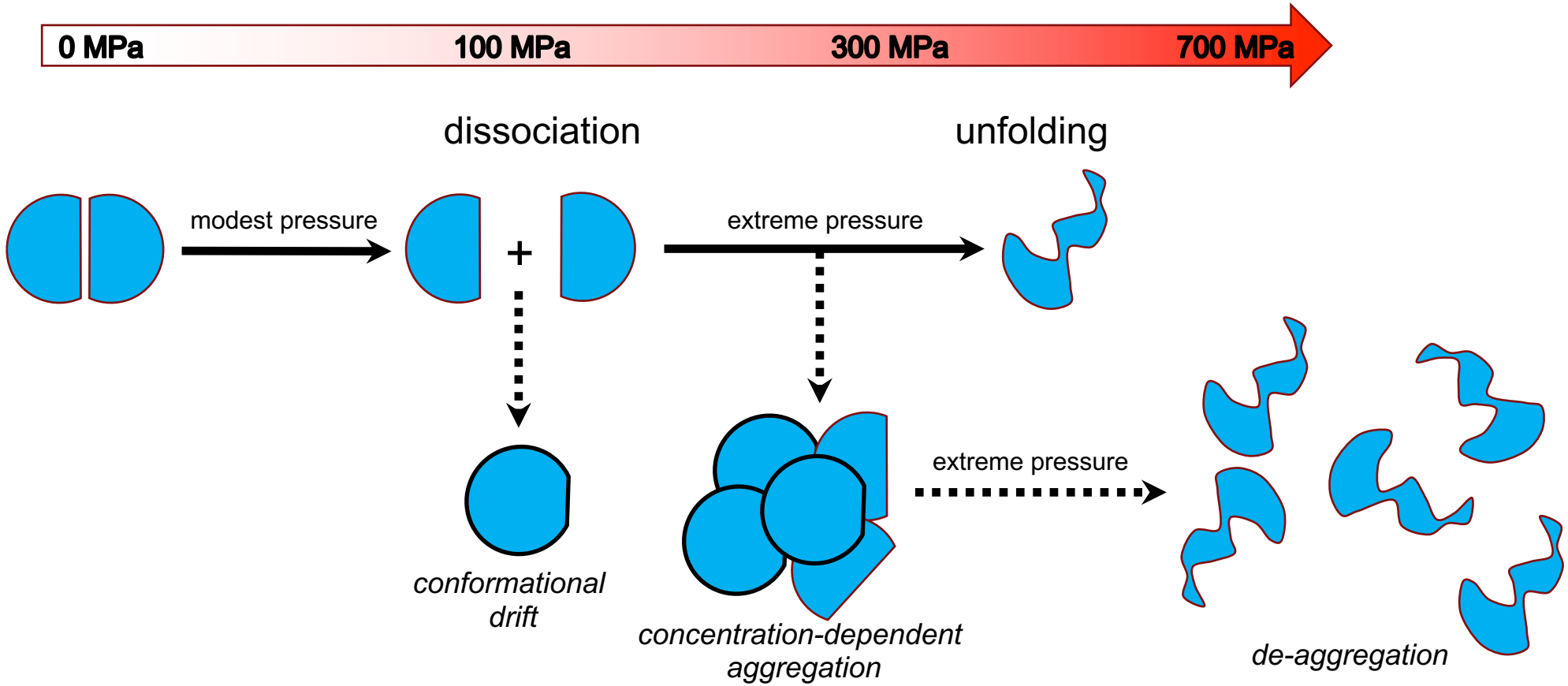
Viscosity -5%
(1 kbar, 5°C)

Pressure-induced structural changes



“For the above-described cases, in which dissociation leads to substantial changes in conformation, the pressure perturbation appears as the only feasible way to access the "dissociated/denatured" state that is in equilibrium with the native state, without changing the chemical composition of the medium, or the total energy of the system, at concentrations that make accurate observations possible.” – Silva JL, Weber G. 1993. Pressure Stability of Proteins. *Annu. Rev. Phys. Chem.* 44(1):89–113

Pressure-induced structural changes



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Equilibrium is shifted by pressure according to how much the volume changes

$$K_p = \left(\frac{[A][B]}{[AB]} \right)_{pressure = p}$$

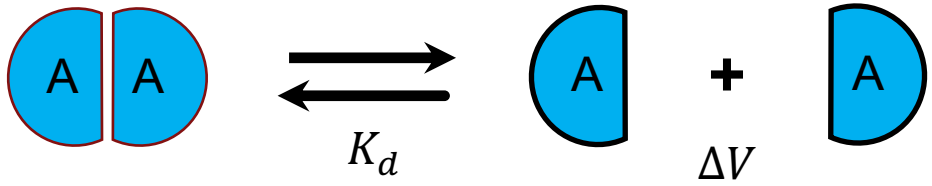
P (MPa)	K_p/K_1
100	3.4
200	11.6
300	39.2
400	133.2

R = 8.314 x 10^{-2} ideal gas constant (L x bar x K^{-1} x mol $^{-1}$)
T = 295 K
P = 2000 bar
Suppose ΔV = - 30 cm^3/mol

$$\frac{K_p}{K_1} = e^{-\frac{\Delta V}{RT}(p-1)} = e^{0.03\times1999/(0.083\times295)} = 11.6$$

* Winter R. Annual Review of Biophysics. 2019;48(1):441-63.

Pressure-induced dissociation and conformational drift



$$K_d = \frac{[A]^2}{[AA]}$$

$$\ln\left[\frac{\alpha}{1-\alpha}\right] = p\left(\frac{\Delta V}{RT}\right) + \ln\left(\frac{K_d}{4C}\right) \longrightarrow K_d, \Delta V$$

α = extent of dissociation

Linear fit of pressure series gives volume change and dissociation constant

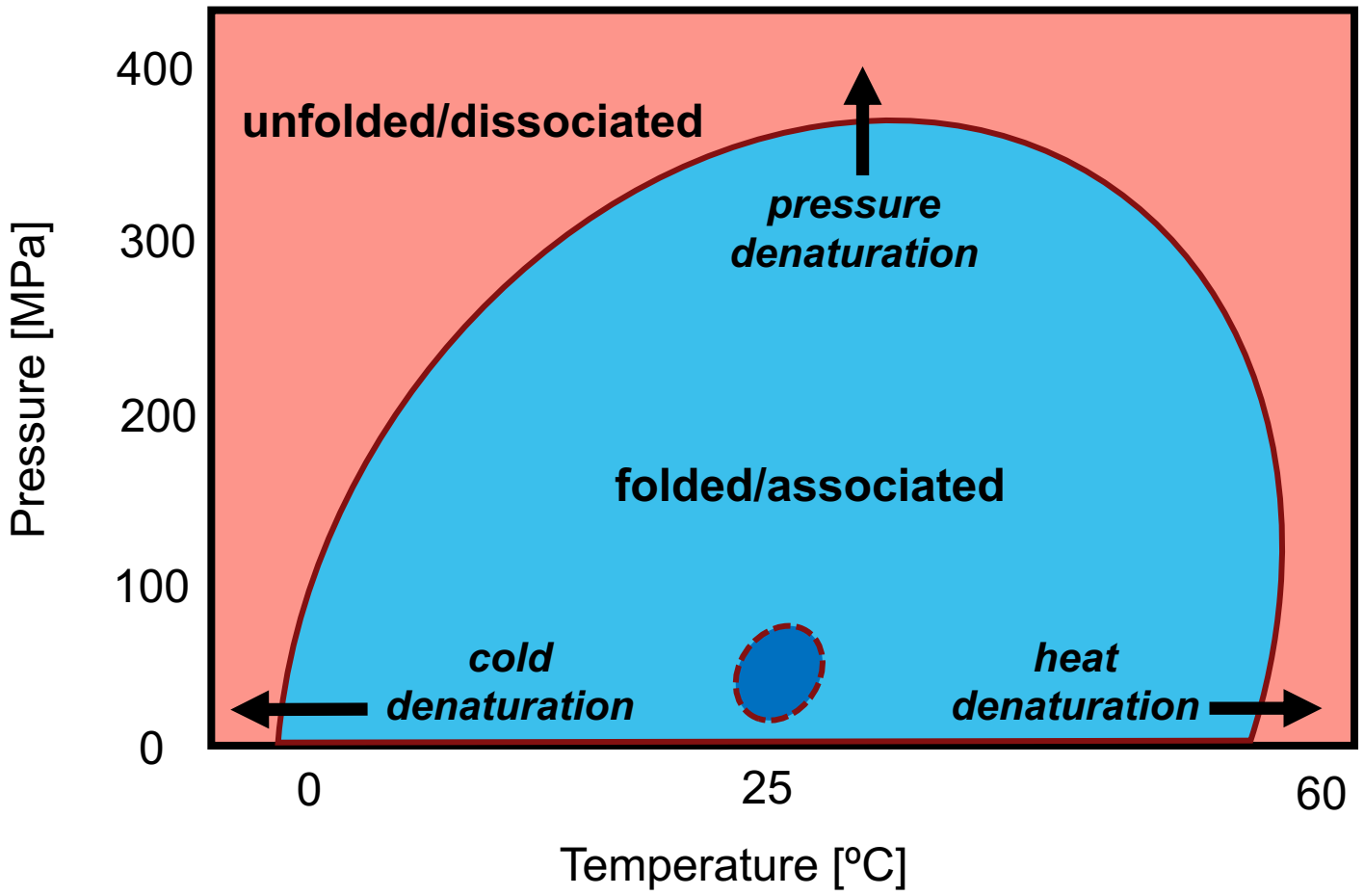
*Weber, G. *Protein interactions*. (Chapman and Hall, 1992).

$$\Delta p = \left(\frac{RT}{\Delta V_c}\right) \ln\left(\frac{C_2}{C_1}\right) \longrightarrow \Delta V_c$$

Botelho MG, Rietveld AWM, Ferreira ST. 2006. Long-Lived Conformational Isomerism of Protein Dimers: The Role of the Free Energy of Subunit Association. Biophysical Journal. 91(8):2826–32

- Conformational drift
- Conformational heterogeneity
- Pressure hysteresis
- Concentration independent
- “deterministic”

Thermodynamics of stability



Smeller, L. Pressure–temperature phase diagrams of biomolecules. *Biochimica et Biophysica Acta (BBA) - Protein Structure and Molecular Enzymology* **1595**, 11–29 (2002).

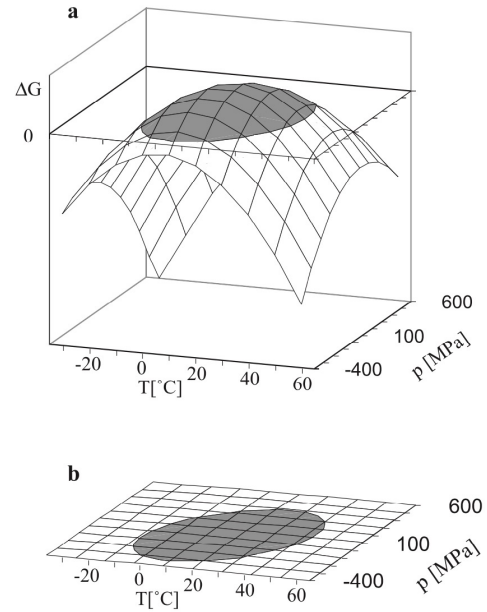


Fig. 3. (a) ΔG as a function of temperature and pressure for chymotrypsinogen, using the parameters determined by Hawley.

*Hawley's equation of state: (1) S. A. Hawley, *Biochemistry* **1971**, *10* (13), 2436–2442.
$\Delta G(P,T) = const$ curves are elliptical

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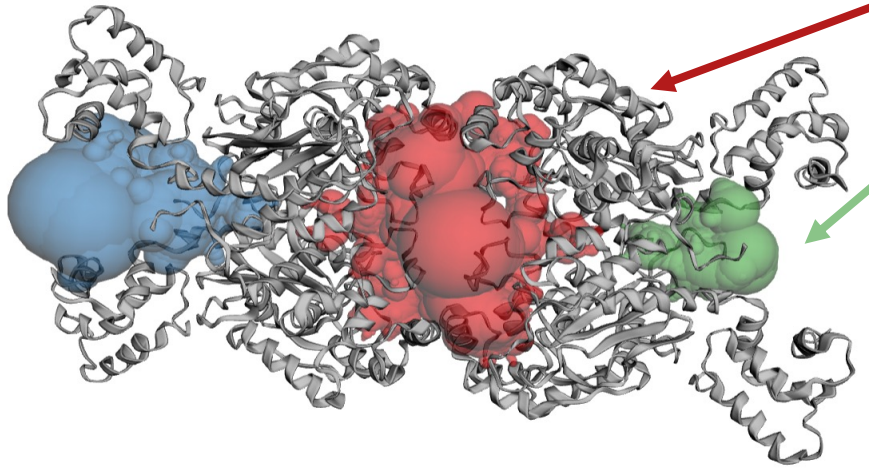
Anything a biomolecule does changes its volume!

- Hydrogen bonds strengthened
- Buried salt bridges weakened
- Hydration altered
- Hydrophobic interactions change
- Packing is altered (cavities, unfolding)



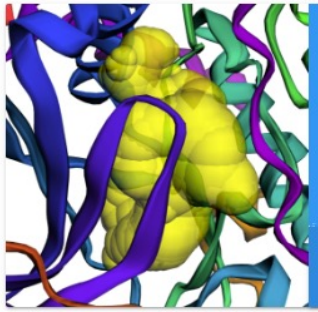
Pressure-induced changes can reveal voids and imperfect packing

*GAC (from Durgesh Rai calc.)

- Pressure unfolding of proteins
- Multimer association/disassociation
- Changes in ligand binding
- Altered membrane ion transduction
- Changes in transcription of nucleic acids
- Large shifts in chemical kinetic constants
- Changes in conformational states
- Greatly decreased viral infectivity

Winter, R. (2019). "Interrogating the Structural Dynamics and Energetics of Biomolecular Systems with Pressure Modulation." Annual Review of Biophysics **48**(1): 441-463.

sts.bioe.uic.edu/castp/calculation.html



CASTp

Computed Atlas of Surface

Visualization of molecules may be incorrect on Safari. Please use Chrome or Firefox for full funct

CASTp | Calculation | Background | Plugin

Please cite this paper if you publish or present results using CASTp analysis:

Tian et al., Nucleic Acids Res. 2018. PMID: 29860391 DOI: 10.1093/nar/gky473

For questions and bugs, please contact uic.lianglab{at}gmail.com .

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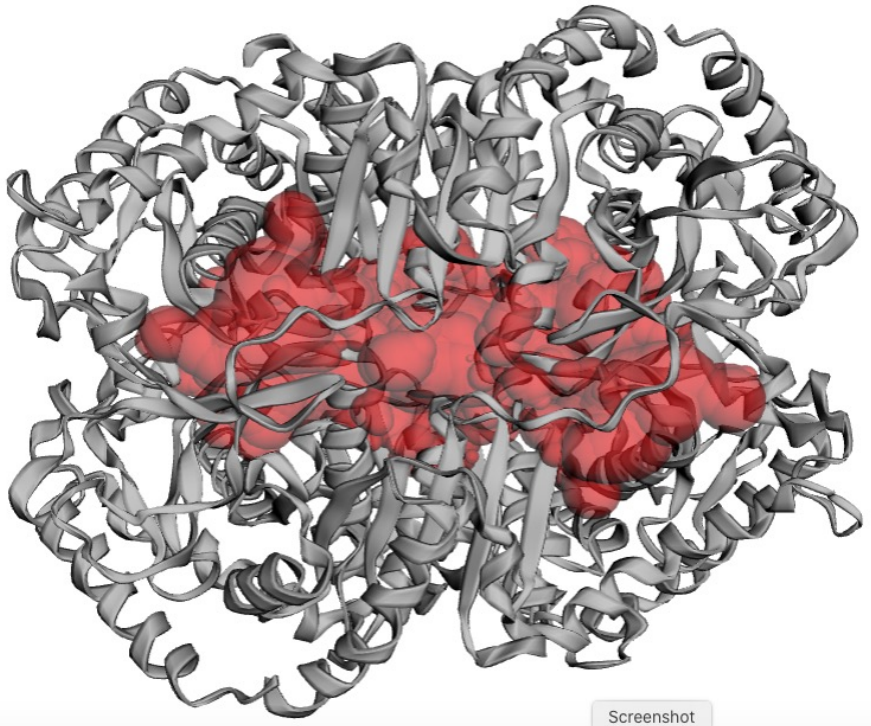
LDH



What can SAXS tell us about pressure-induced structural changes?

Intensity of a SAXS pattern

$$I(q) \propto M\, c\left(\rho_1 - \rho_2\right)^2 F(q) S(q)$$

M – molecular weight

c – concentration (mg/ml)

ρ – "scattering density": particle (ρ_1), solvent (ρ_2)

F(q) – Form factor* (scattering from single protein, rotationally averaged)

S(q) – Structure Factor (≈ 1 for dilute solutions)

* Not to be confused with crystallography naming where "structure factor" corresponds with form factor here and reciprocal lattice = our structure factor

* Stuhrmann, H. B. (1980). *Synchrotron Radiation Research, edited by H. Winick, Doniach, S., pp. 513-531. New York: Plenum Press.*

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Contrast variation is actually useful in neutron scattering, but is a potential problem in SAXS.

- keep salt and buffer < 1M
- keep glycerol < 10-15%
- be careful with protein+RNA/DNA
- avoid detergent/lipids

ρ

Component	e/nm^3	g/cm^3	Mol. Wt.	e/molec
H_2O	334	1	18	10
Protein	420			
100% glycerol	413	1.261	92	50
50% sucrose	400			
LD amorphous ice	321	0.96	18	10
HD amorphous ice	391	1.17	18	10
lipid	300			
65% sucrose	420			
nucleic acid	550			
ethylene glycol	368	1.1132	62	34
propylene glycol	344	1.036	76.1	42
ethanol	269	0.789	46	26
octane	245	0.703	114	66

Intensity of a SAXS Profile

$$I(q) = \eta T d \frac{P}{r^2} \left[\frac{v^2 \Delta\rho^2}{N_a} Mc \, FF(q) \, SF(q) \right]$$

η	quantum efficiency of detector (dimensionless)
T	x-ray transmission of sample (dimensionless)
d	thickness of sample (cm)
P	incident photon flux (ph/s on sample)
r	sample-to-detector distance (cm)
M	molecular mass (g/mol)
N_a	Avogadro's number = 6.023×10^{23} (mol^{-1})
v	specific volume of solute (cm^3/g)
Δρ	excess scattering length density (cm/cm^3)
c	concentration of solute (g/cm^3)

FF(q)	molecular form factor (dimensionless, FF(0)=1)
SF(q)	sample structure factor (dimensionless, SF(0)=1)

*Wang, C., Lin, Y., Bougie, D., & Gillilan, R. E. (2018).. *Acta Cryst. D, 74(8), 727-738*

Intensity of a SAXS Profile

$$I(q) = \eta T d \frac{P}{r^2}\left[\frac{\nu^2 \Delta\rho^2}{N_a} Mc\, FF(q)\, SF(q)\right]$$

$$T = e^{-\mu_m \rho_P d}$$

ρ_0 = density at 0 MPa

ρ_P = density at pressure P

$$c = c_0 \frac{\rho_0}{\rho_P}$$

$$\Delta\rho = \left[\rho_{M,sample} / \nu - \rho_{buffer}\right] \times r_0$$

$$\rho_{M,protein} = 3.22 \times 10^{23}\ e\, g^{-1}$$

r_0 = classical electron radius

Intensity of a SAXS Profile

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SAXS data are normalized by transmitted intensity

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Intensity of a SAXS Profile

constant

$$I(q) = \eta T d \frac{P}{r^2}\left[\frac{v^2 \Delta\rho^2}{N_a} Mc\, FF(q)\, SF(q)\right]$$

$$T = e^{-\mu_m \rho_P d}$$

SAXS data are normalized by transmitted intensity

$$c = c_0 \frac{\rho_0}{\rho_P}$$

constant

$$\Delta\rho = \left[\rho_{M,sample} / v - \rho_{buffer}\right] \times r_0$$

$$\rho_{M,protein} = 3.22 \times 10^{23}\ e\, g^{-1}$$

r_0 = classical electron radius

$$\beta_T = -\frac{1}{v}\left(\frac{\partial v}{\partial P}\right)_T \ll 1$$

Isothermal compressibility
very meaningful, but small

Intensity of a SAXS Profile

$$\frac{I(0)_{pressure}}{I(0)_{ambient}} = r_V \frac{\left(\rho_{protein} - r_V \rho_{buffer}\right)^2}{\left(\rho_{protein} - \rho_{buffer}\right)^2} \qquad r_V = V_{ambient}/V_{pressure}$$

$$\rho_{water} = 3.3 \times 10^{21}\ \mathrm{cm}^{-3} \qquad \rho_{protein} = 4.4 \times 10^{21}\ \mathrm{cm}^{-3}$$

Notice: r_0's cancel!

Pressure (MPa)	r_V (25 °C)	$I_{pressure}/I_{ambient}$
100	1.04	0.805
200	1.08	0.624
300	1.10	0.539
400	1.13	0.420

Intensity of a SAXS Profile

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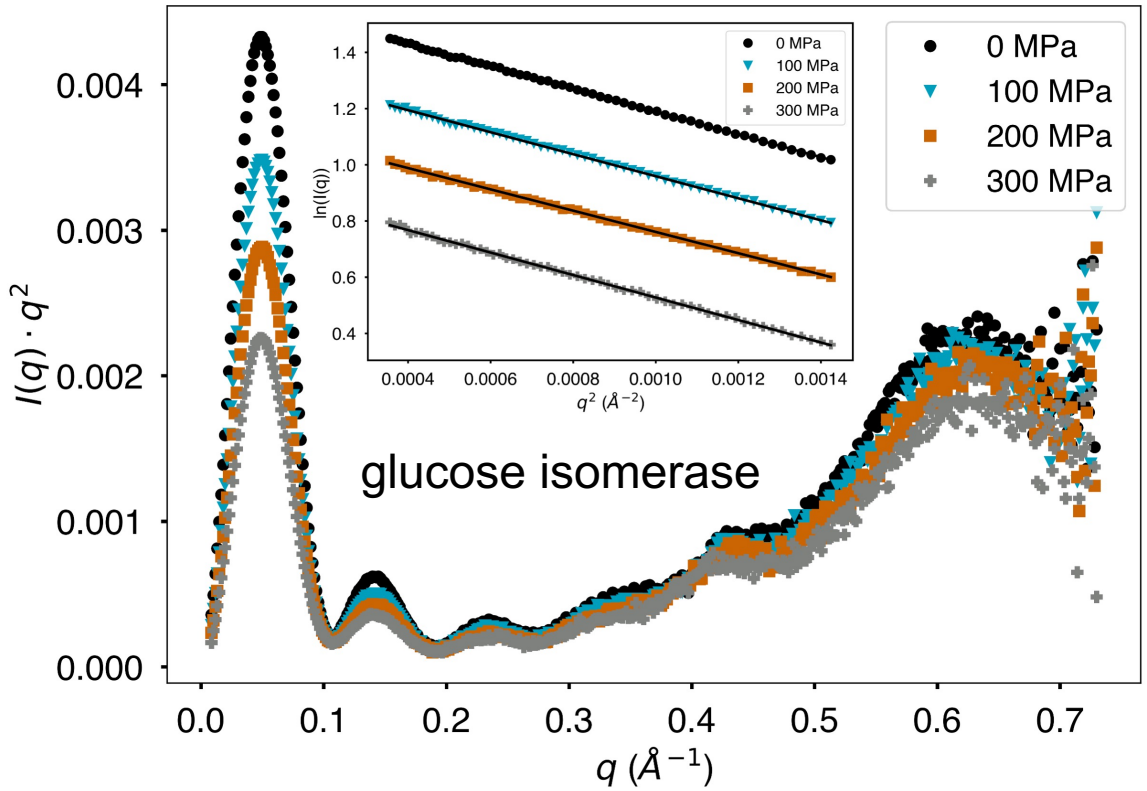

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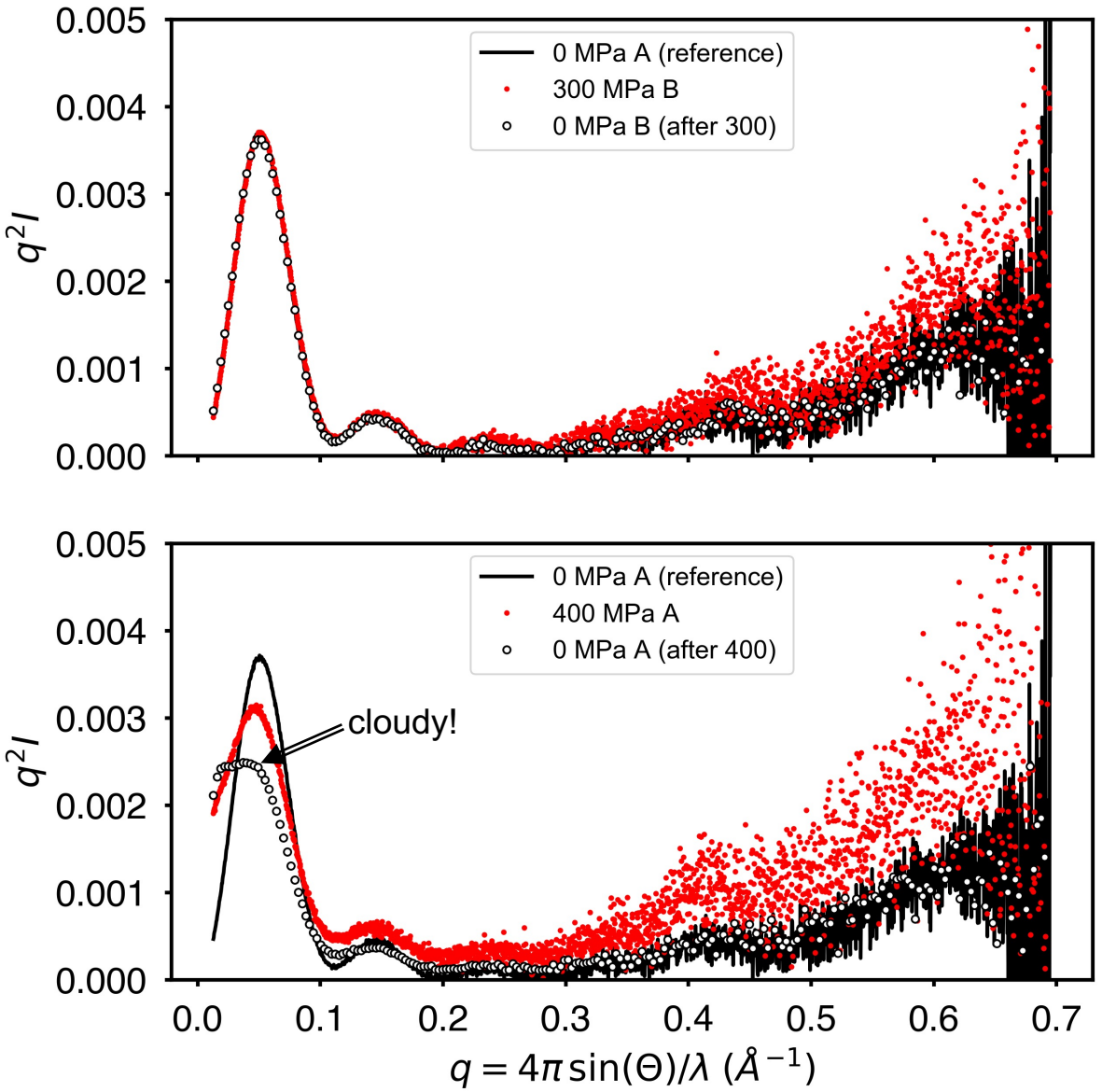
Concentrated glucose isomerase (17.9 mg/ml)

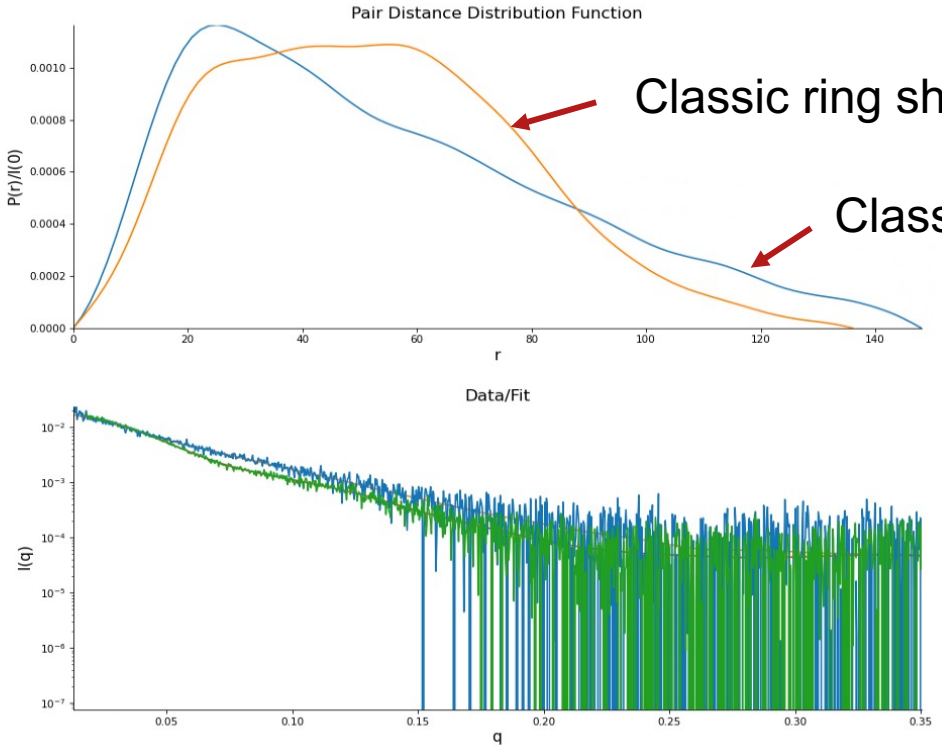
- Don’t expect arbitrary proteins, especially stable standards, to do anything interesting under pressure.
- Unless you apply **extreme** pressure
- Concentrated solutions may aggregate
- High-resolution changes appear reversible



* GI is known to dissociate into distorted dimers under denaturing conditions. Tetramers have also been re-constituted.

Ghatge, M. S.; Phadatare, S. U.; Bodhe, A. M.; Deshpande, V. V. Unfolding and Refolding of Glucose/Xylose Isomerase from Streptomyces Sp. NCIM 2730. *Enzyme and Microbial Technology* **1994**, *16* (4), 323–327.

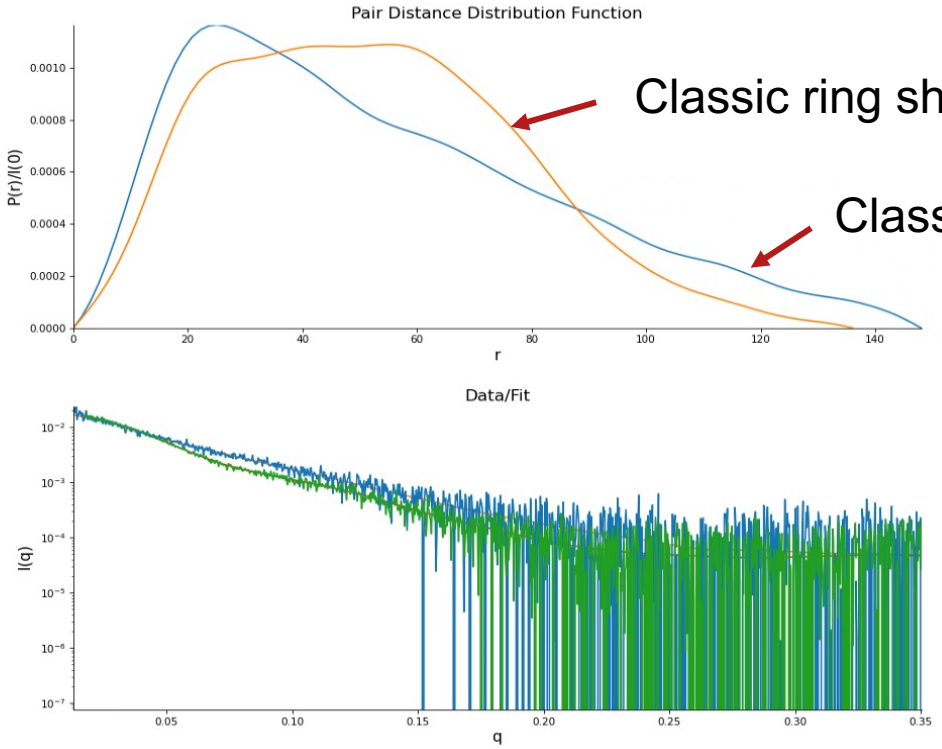
Pressure dissociation



Classic ring shape (0 MPa): this is the known trimer

Classic rod shape (300 MPa): a linear oligomer?!

* Ying Li sample (Abbaspourrad)



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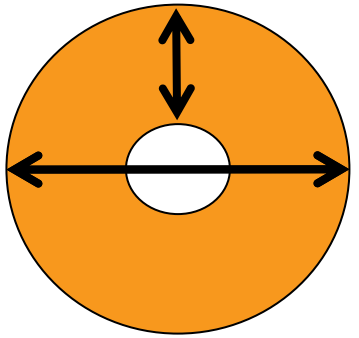
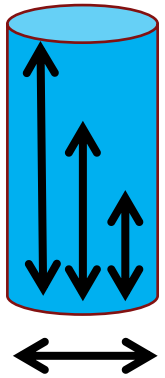
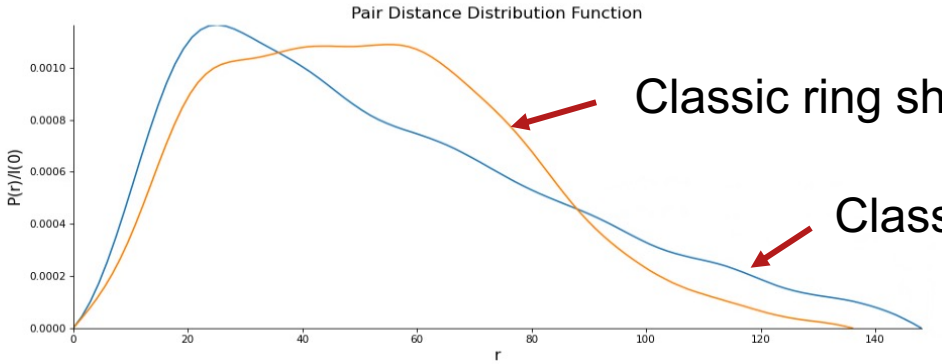


Ring: has two length scales the show up as two peaks in the P(r).



Cylinder: one of the two size scales is a linear distribution of lengths. P(r) has one peak which falls linearly to zero.

* Ying Li sample (Abbaspourrad)



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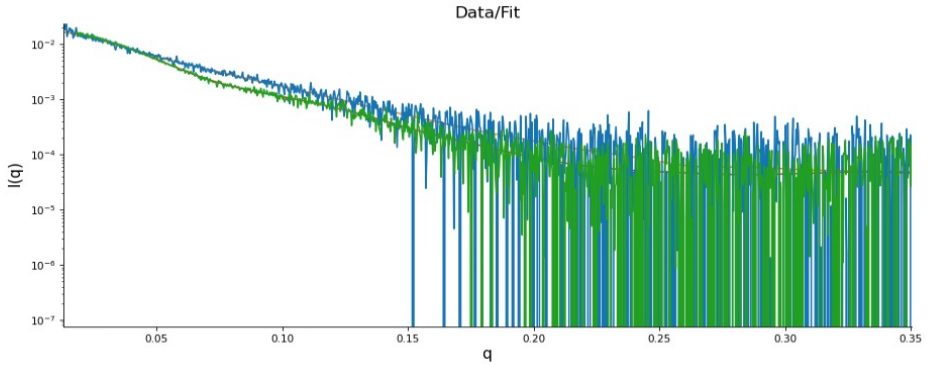
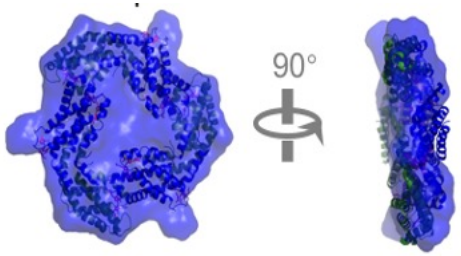


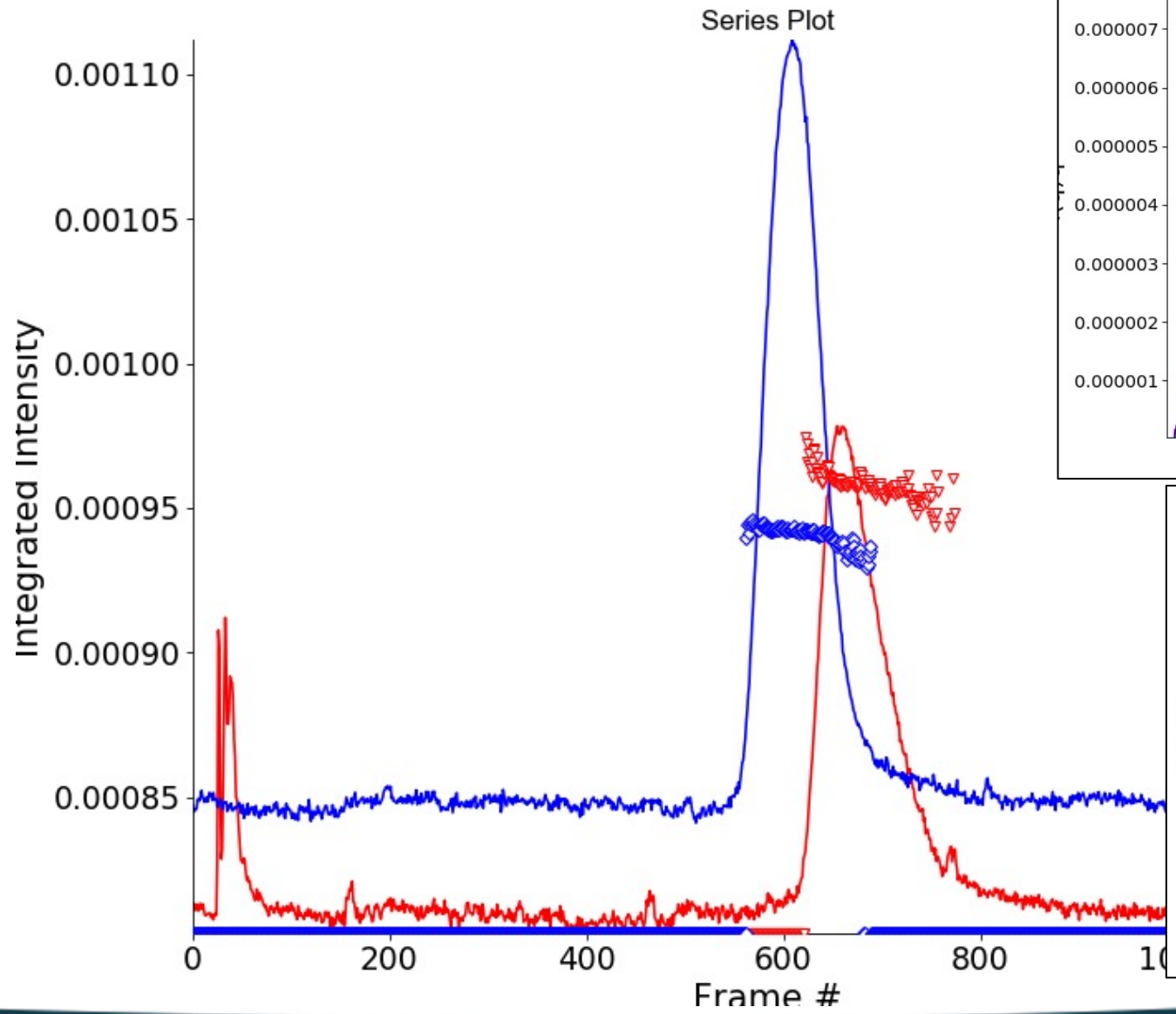


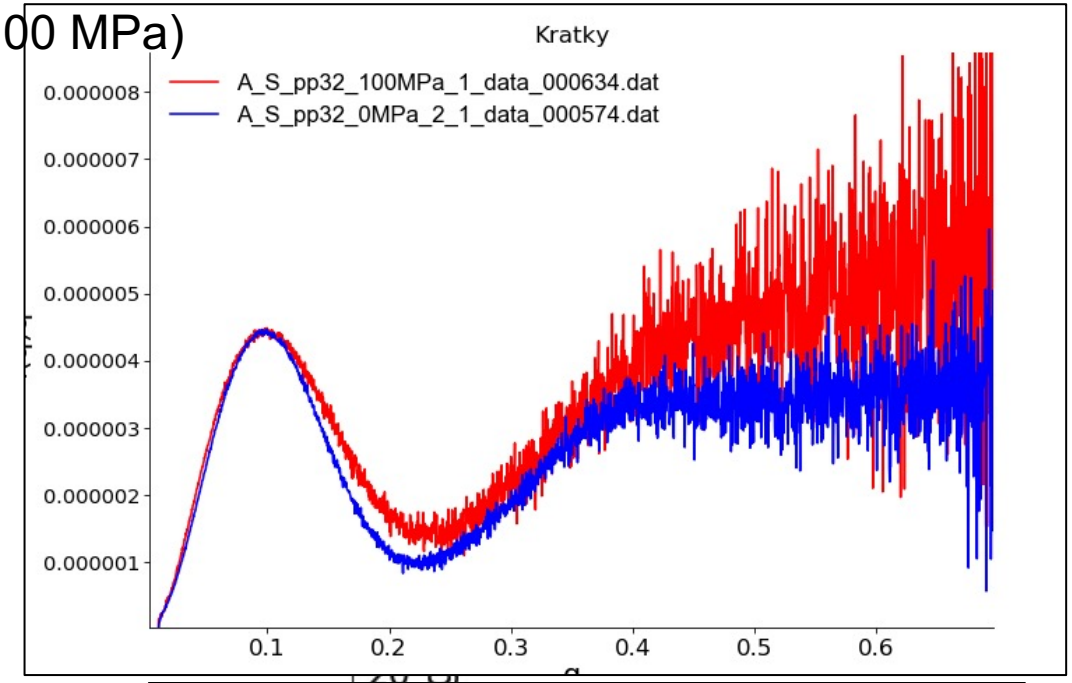
Known ambient (0 Mpa) structure: trimer

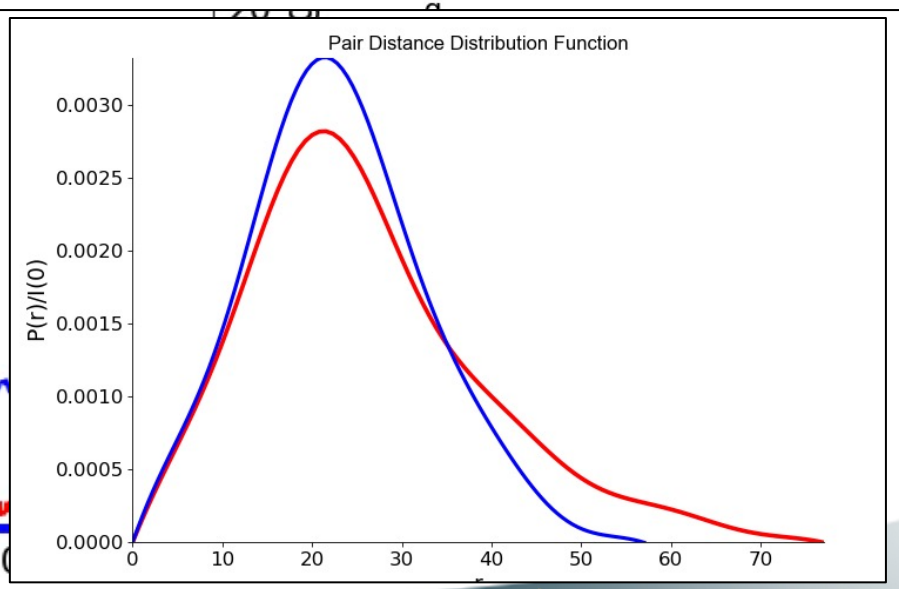
* Ying Li sample (Abbaspourrad)

You will find this dataset included with the Rest of the tutorial data!

Pp32 HP-SEC-SAXS (0 vs 100 MPa)







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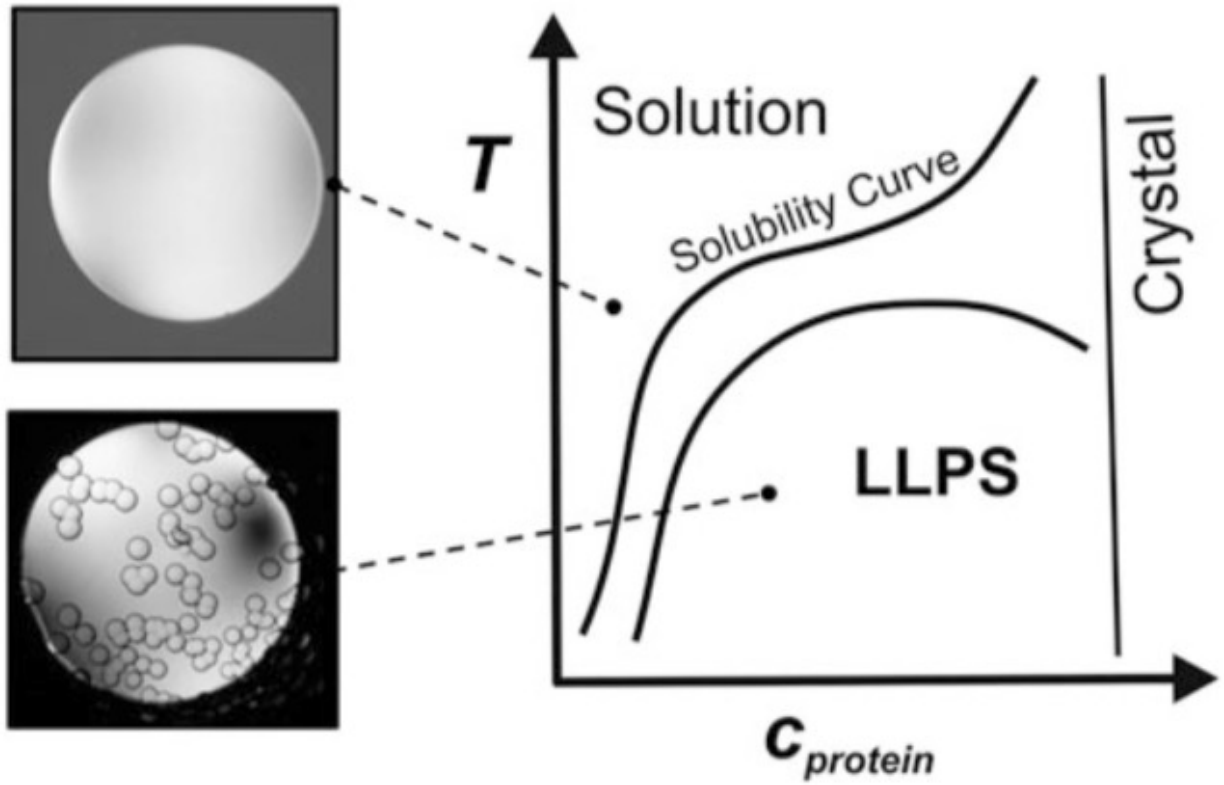
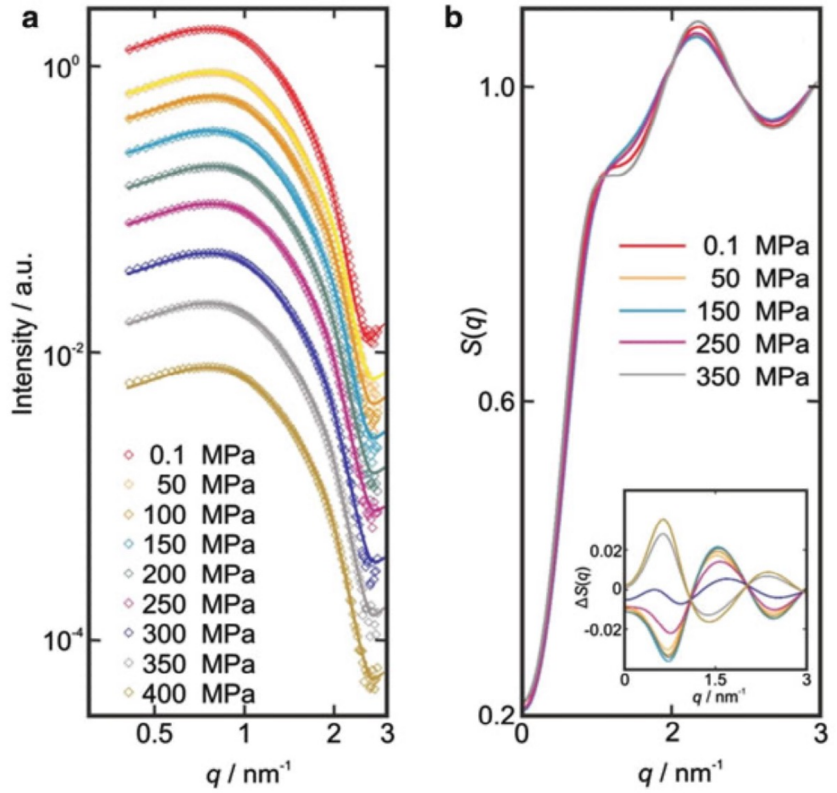
Use of structure factors to study inter-protein interactions

LLPS – Liquid Liquid Phase Separation

$$I(q) \propto M\, c\, (\rho_1 - \rho_2)^2\, F(q)\, S(q)$$





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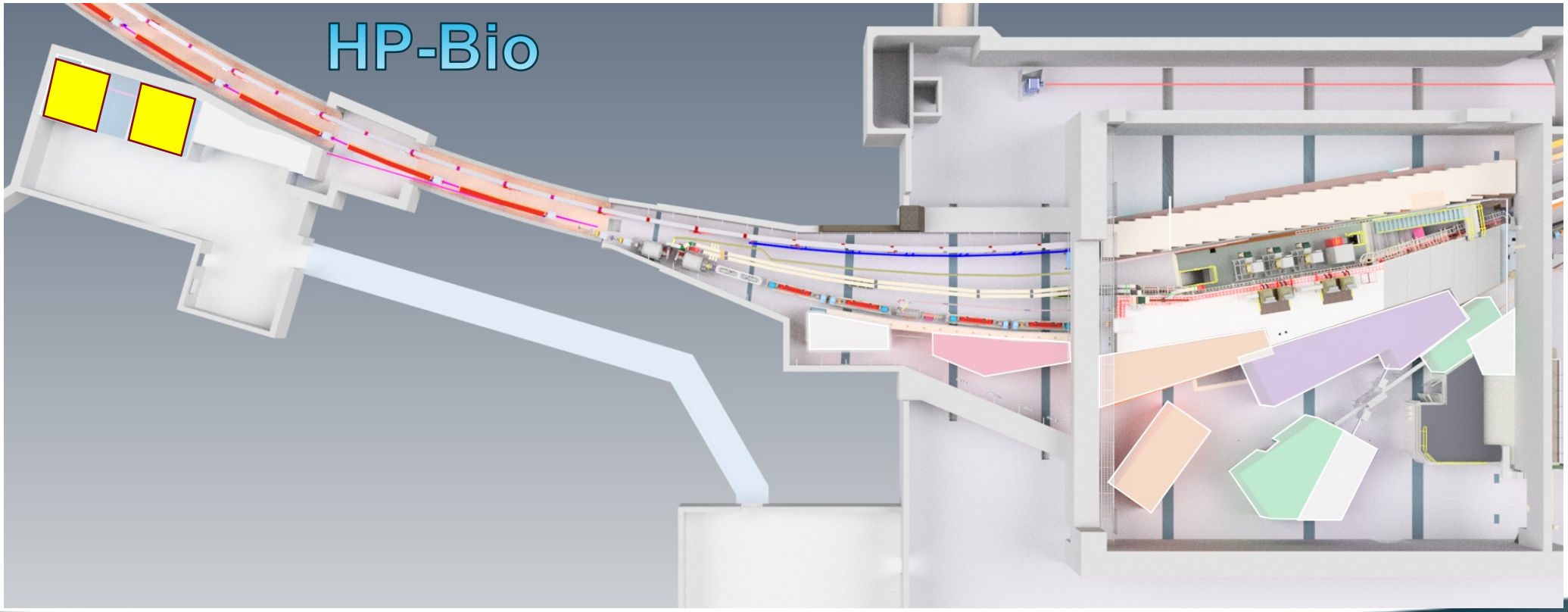
ID7A1 | ID7B2

BioSAXS | MX | High Pressure Structural Biology

Science Mission | *Fundamental biology and biomedicine* - characterizing biomolecular structure and interactions; conformational changes and flexibility under physiological and **extreme conditions**

Core Capabilities | High-pressure scattering technology – high flux MX, BioSAXS and specialized experiments



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CHEXS
NIH NIGMS
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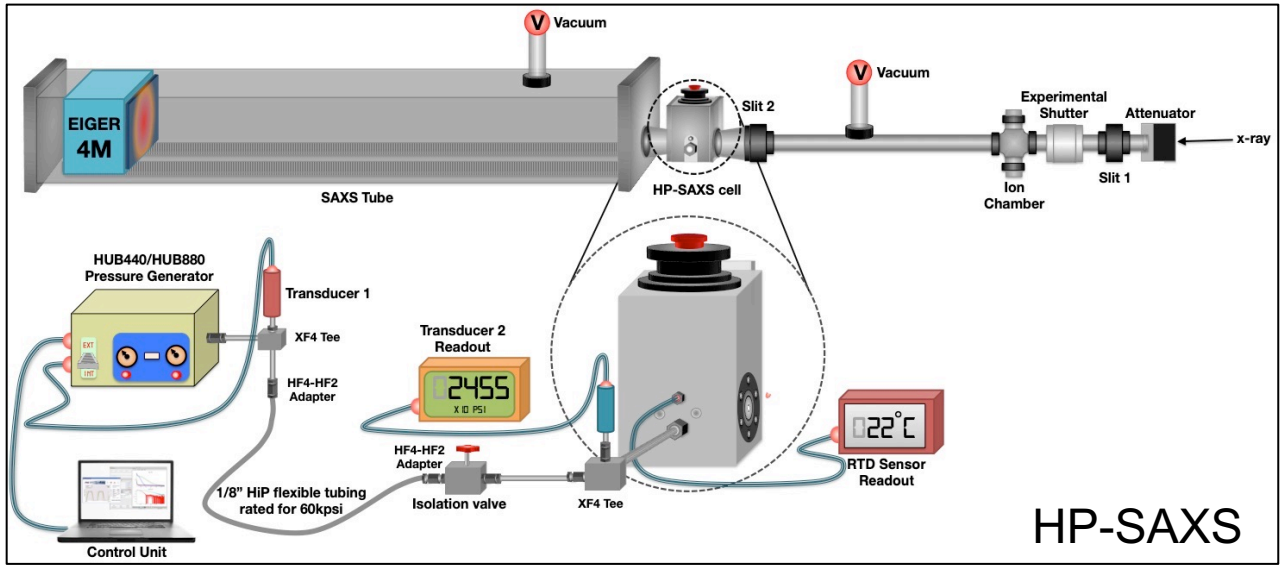
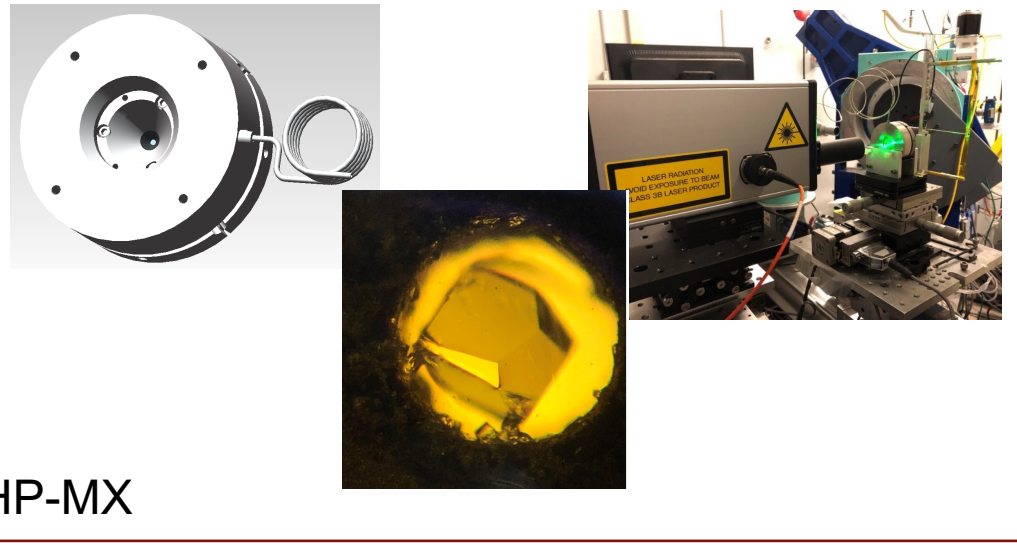
NYSTAR

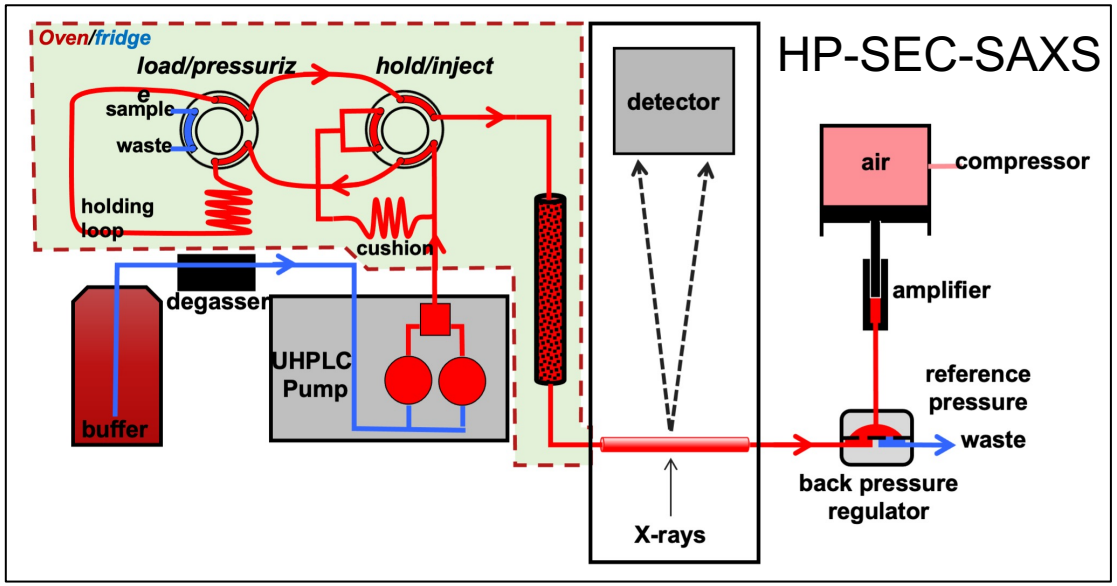
NSF
DMR-1829070
CHEXS
CENTER FOR HIGH ENERGY X-RAY SCIENCES

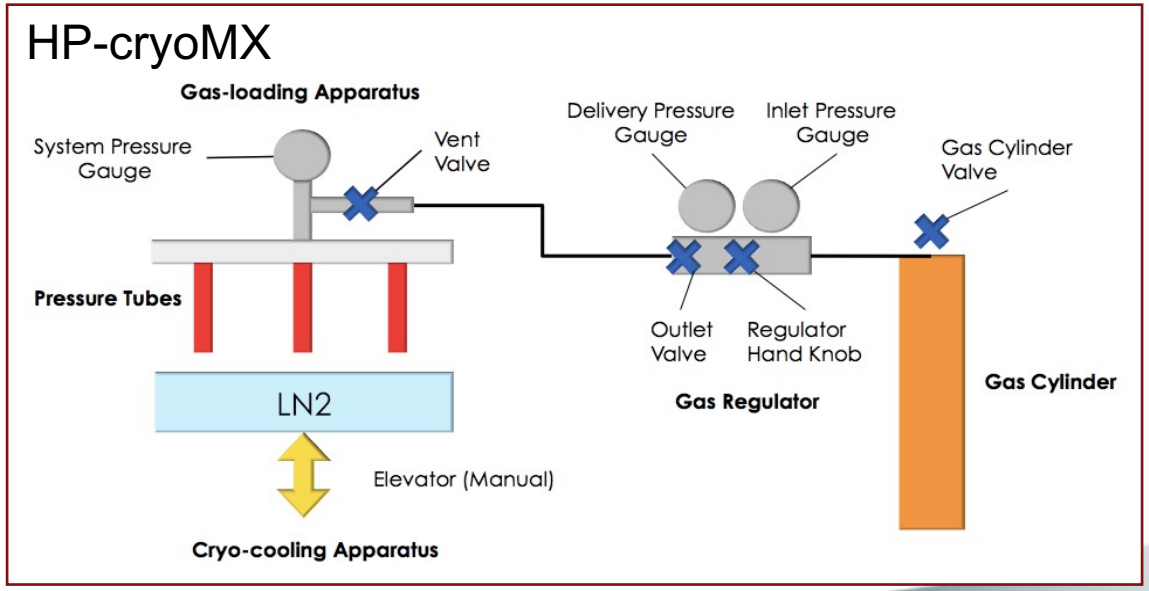

NO FLAMMABLE SOLVENTS
Chemicals Only
No Food
solo
0%
Zoom
Preset positions
1:1

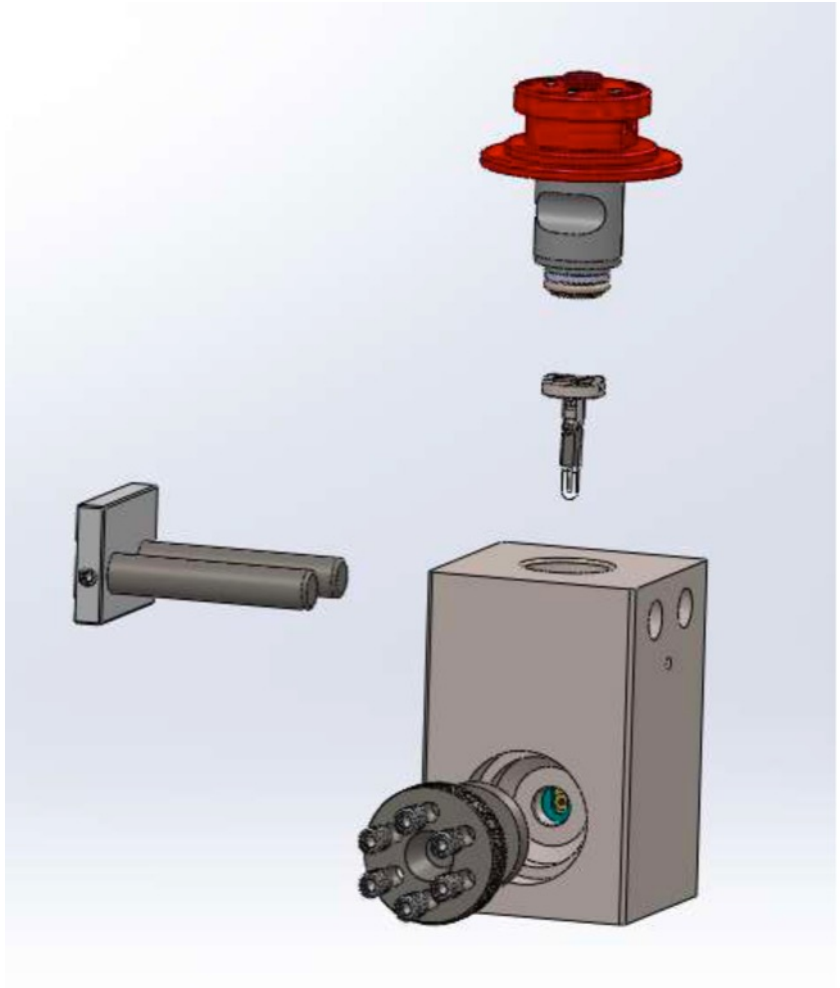
High Pressure Structural Biology Instrumentation: HP-MX, HP-SAXS

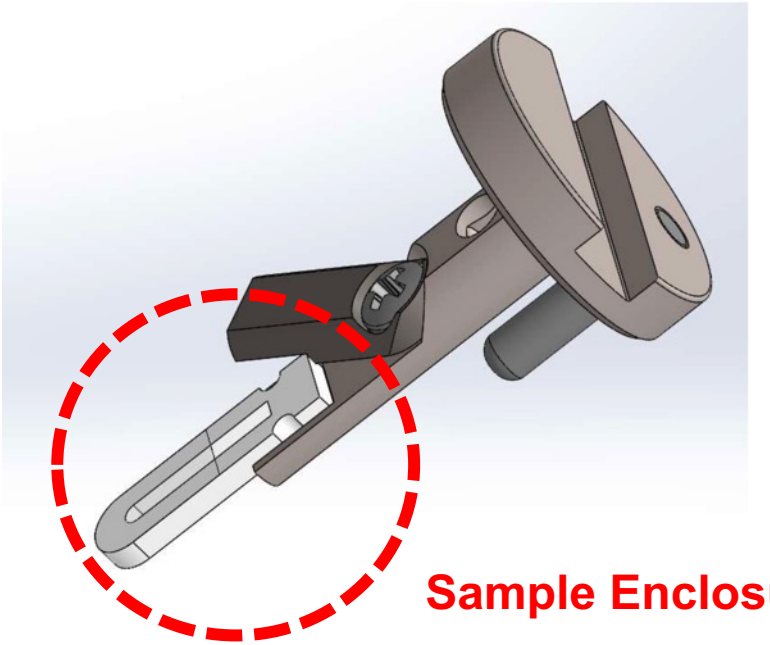


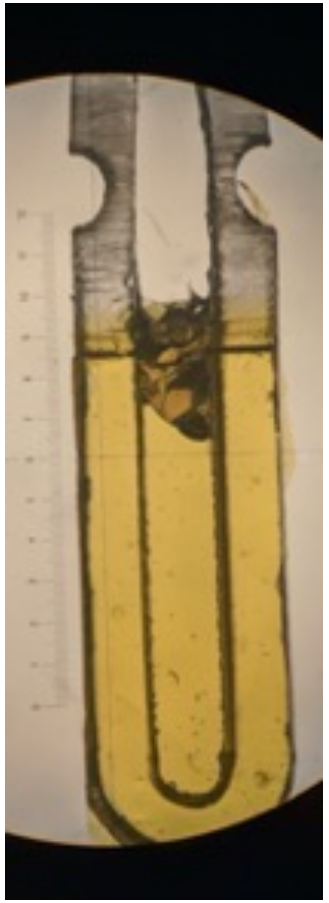






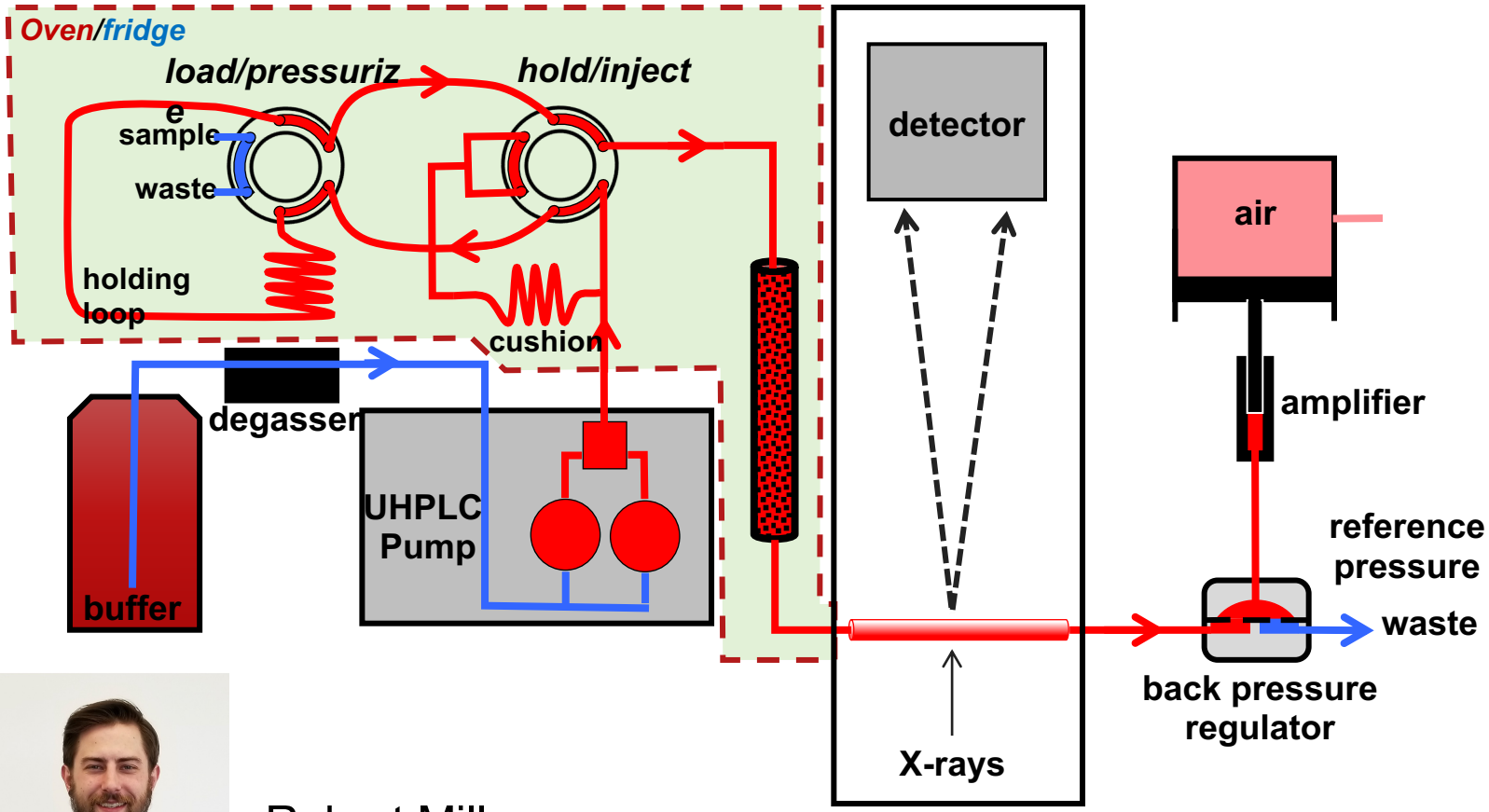


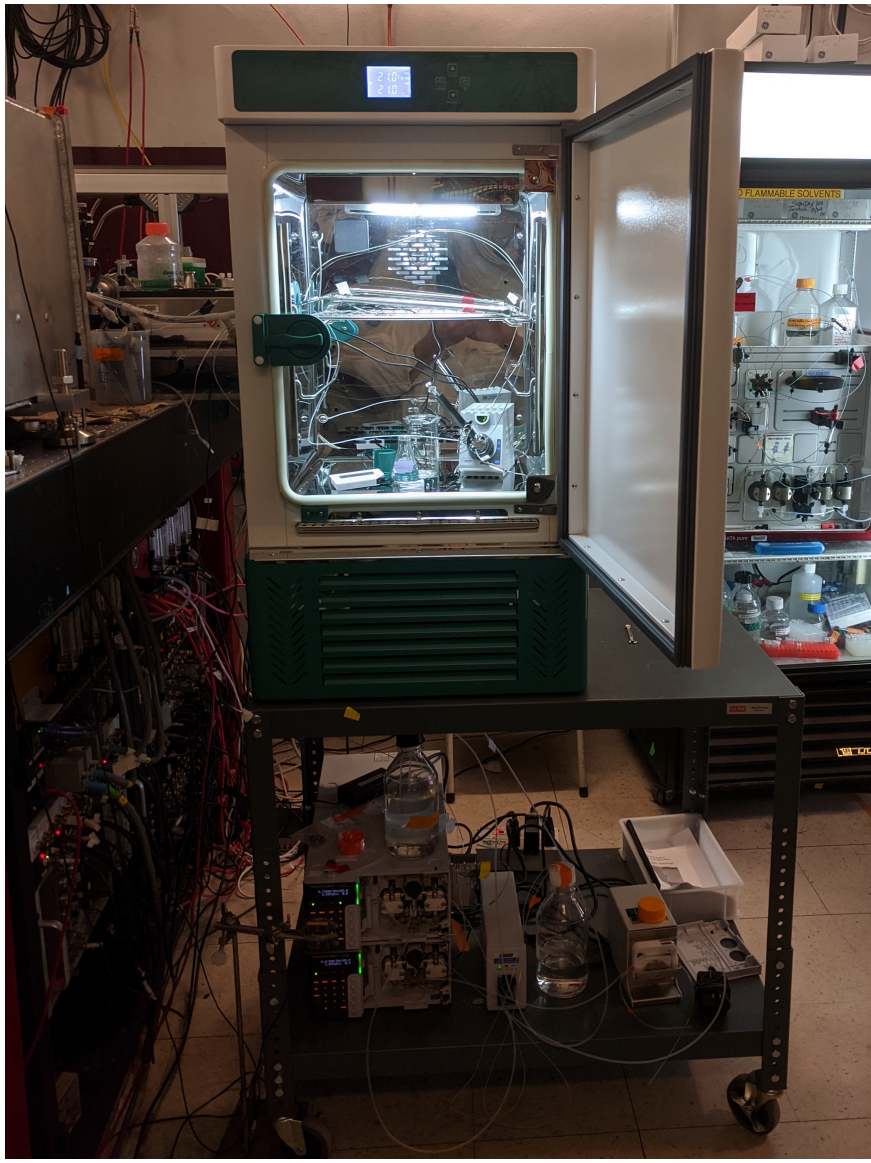




*In collaboration with Pressure BioSciences Inc.

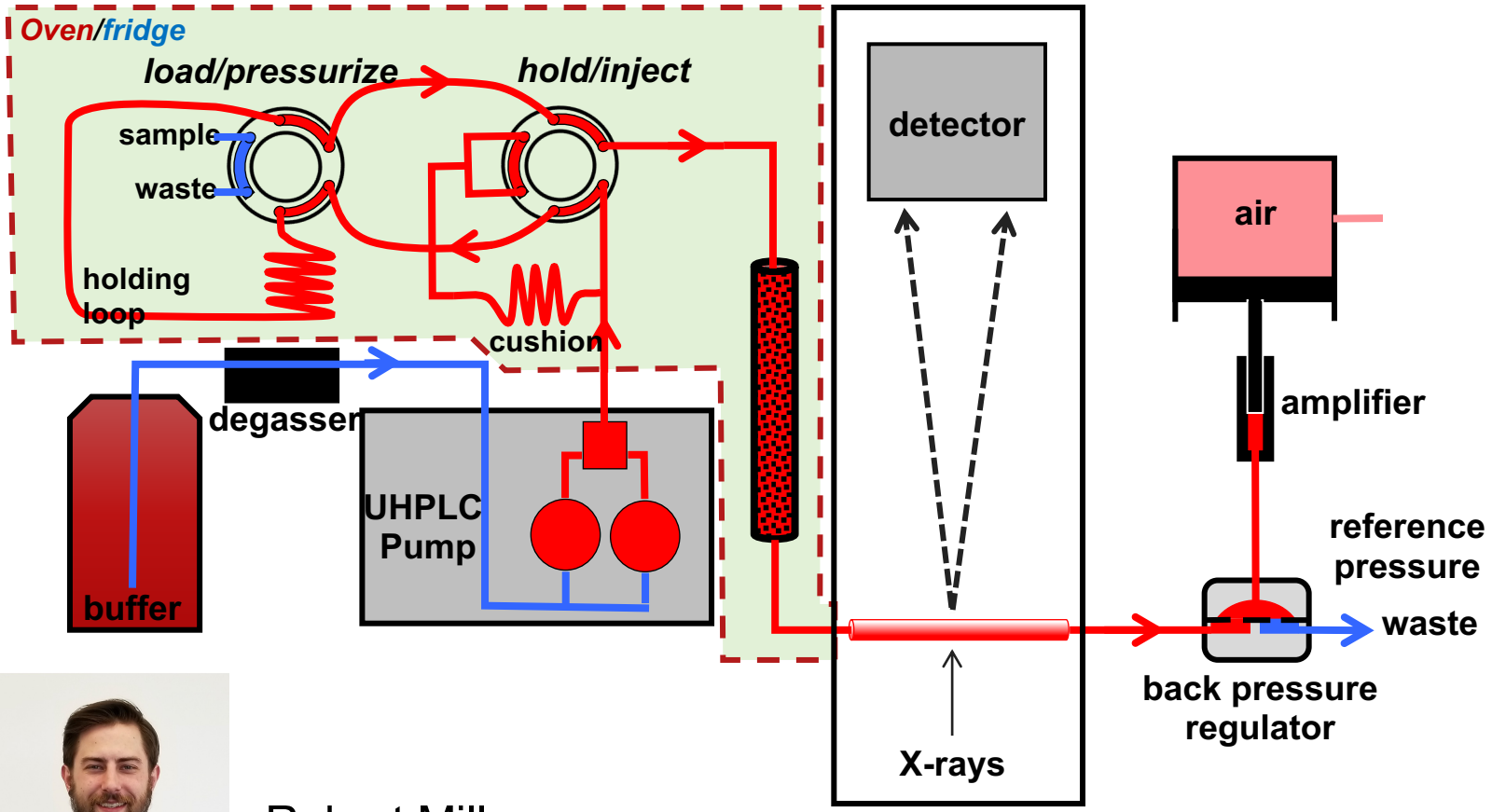
Size Exclusion Chromatography at 100 MPa: adding temperature control (0º-50º C)
And ability to hold samples under pressure prior to injection

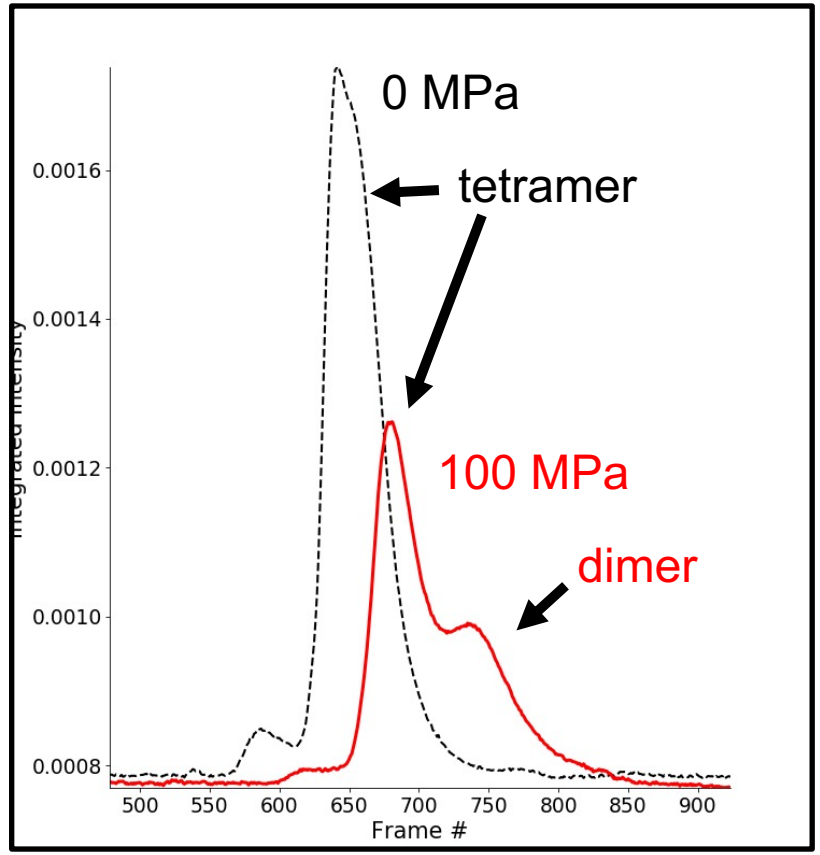




Robert Miller
grad student

Size Exclusion Chromatography at 100 MPa: adding temperature control (0º-50º C)
And ability to hold samples under pressure prior to injection





Robert Miller
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HP-SAXS Guidelines: things you should know

- more difficult than normal BioSAXS
- temperature and pressure changes take time
- background subtractions are less precise (for batch)
- less sensitive due to reduced contrast (use higher concentrations)
- more potential radiation damage (for batch)

Interpretation of data more difficult:

- effects can be small
- can you tell the difference between pressure effect and radiation damage?

Good practice: check samples with normal BioSAXS first

- assess radiation damage
- Assess sample monodispersity
- can you see the features you need to see?
- enough concentration?
- concentration effects?

Use pressure-resistant buffer!

Useful References

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