Presenter: Emre Brookes

Topic:

Small Angle Scattering - III

Copy of Lecture at:

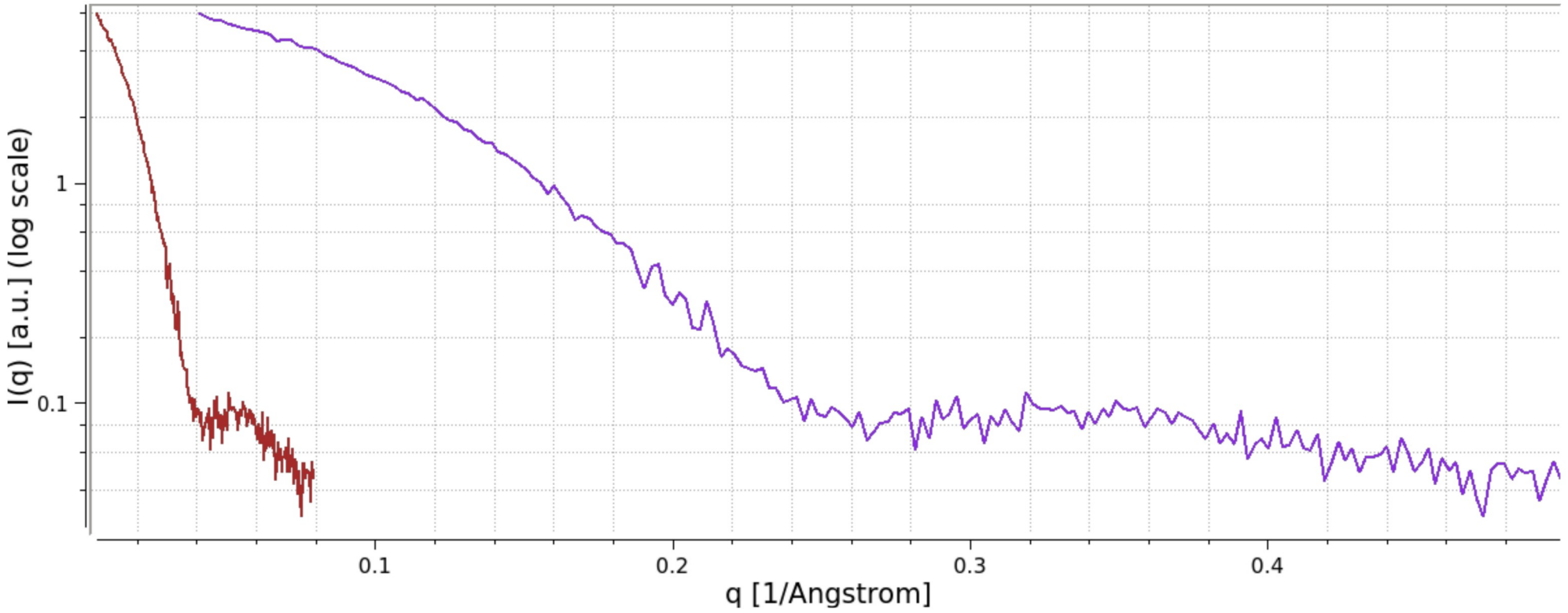
https://demeler.uleth.ca/biophysics/archive/Brookes

Outline

- Homework answers
- Brief review
- Practical considerations
- Sampling of other techniques

1. The dimensionless Kratky plot shows a peak of ~(1.75,1.1) for globular proteins. Would this peak change if the experimental data had a momentum transfer of $s = \frac{2\sin(\theta)}{\lambda}$? $q = \frac{4\pi\sin(\theta)}{\lambda}$

If so, what would be the peak?



1. The dimensionless Kratky plot shows a peak of ~(1.75,1.1) for globular proteins. Would this peak change if the experimental data had a momentum transfer of $s = \frac{2\sin(\theta)}{\lambda}$?

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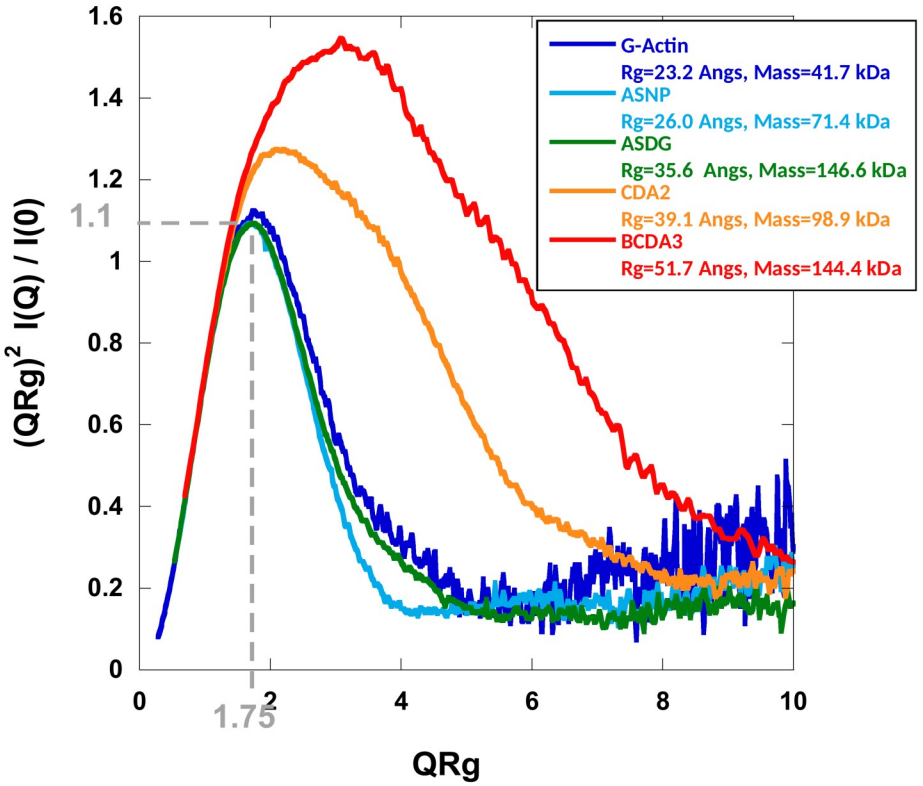


$$q = \frac{4\pi\sin(\theta)}{\lambda}$$

$$qR_g = 1.75$$

$$2\pi s R_g = 1.75$$

$$sR_g = \frac{1.75}{2\pi} \cong 0.279$$

$$(qR_g)^2 I(q)/I(0) = 1.1$$

$$(2\pi)^2 (sR_g)^2 I(s)/I(0) = 1.1$$

$$(sR_g)^2 I(s)/I(0) = \frac{1.1}{(2\pi)^2} \cong 0.0279$$

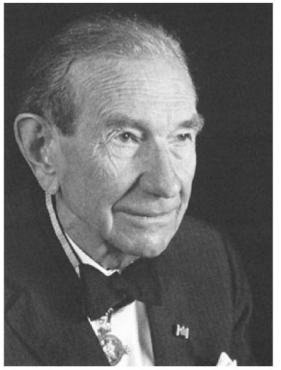
$I(q) \to I(s)$ o.w. taking values from another data point that might not exist

Peak: ~(0.279,0,0279)

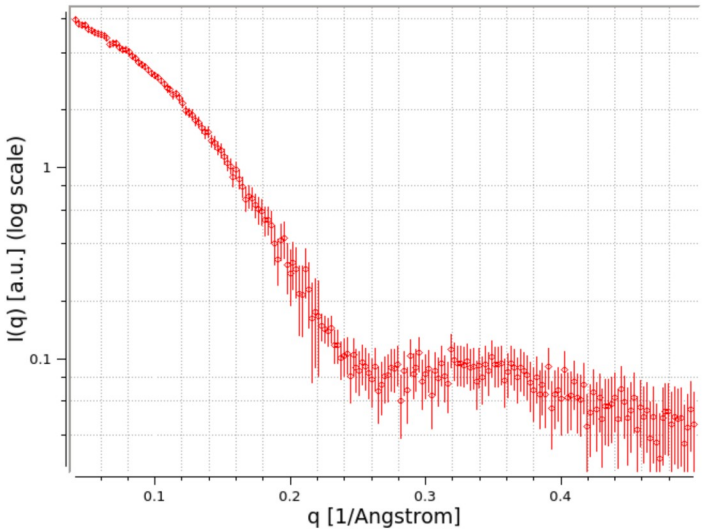
SAS provides a sensitive means to evaluate *the degree of compactness* of a protein:

- To determine whether a protein is globular, extended or unfolded
- To monitor the folding or unfolding transition of a protein

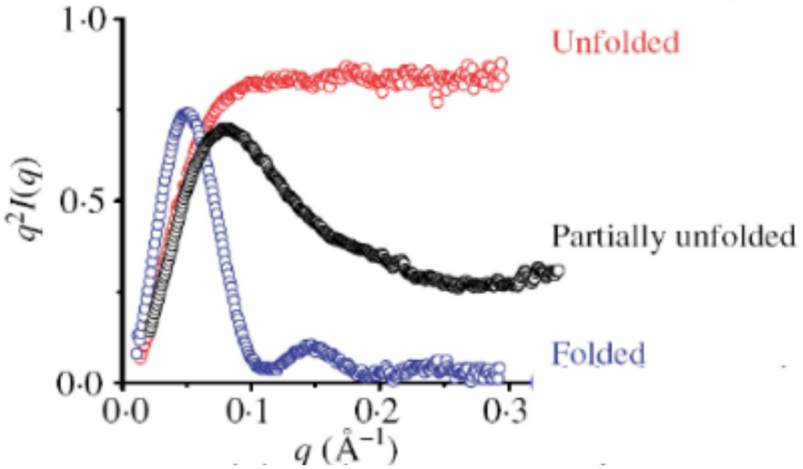
This is most conveniently represented using the Kratky plot:



Prof. Otto Kratky
1902-1995
Graz, Austria



$q^2 I(q)$ **versus** q



Putnam, D., et al. (2007) Quart. Rev. Biophys. 40, 191-285.

Folded particle : *bell-shaped curve* (asymptotic behavior $I(q) \sim q^{-4}$)

Random polymer chain : *plateau* at large q-values (asymptotic behavior $I(q) \sim q^{-2}$)

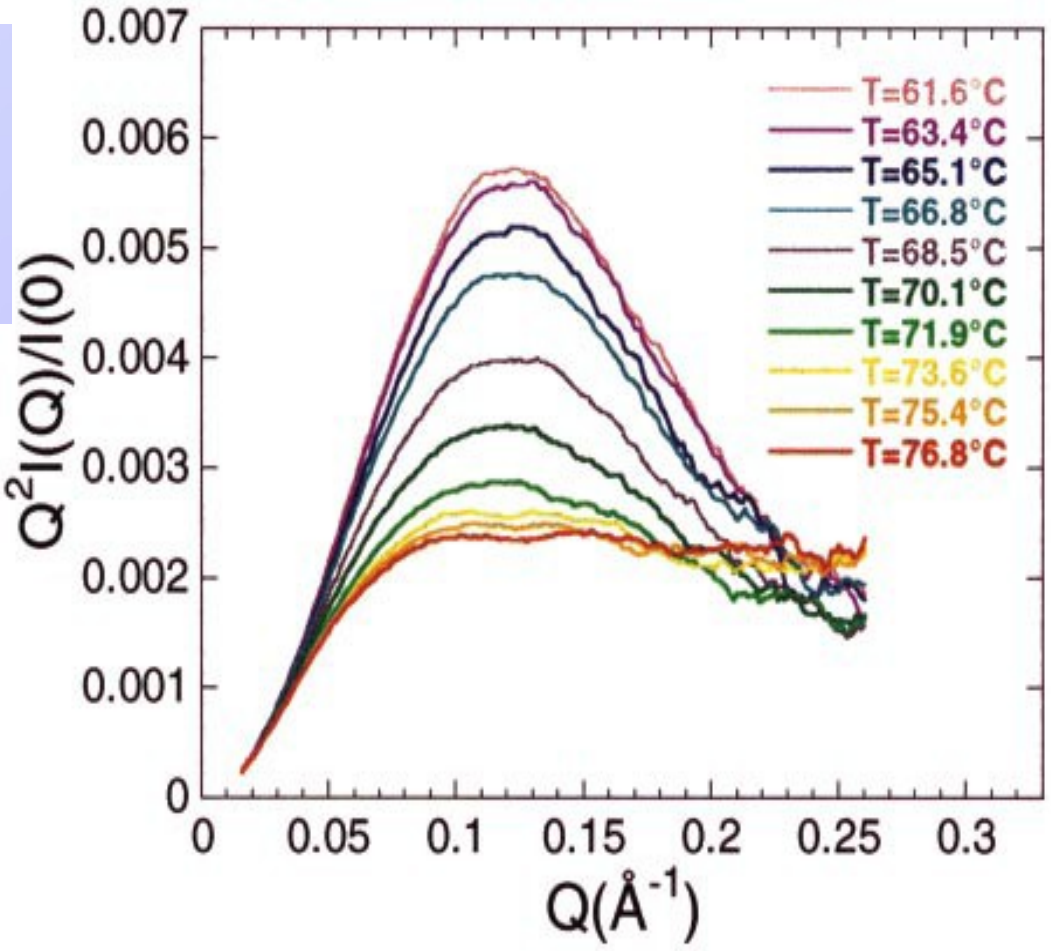
Extended polymer chain : *increase* at large q-values (asymptotic behavior $I(q) \sim q^{-1.x}$)

Review – Kratky plots of (partially) folded proteins

Pérez et al., J. Mol. Biol.(2001), 308, 721-743

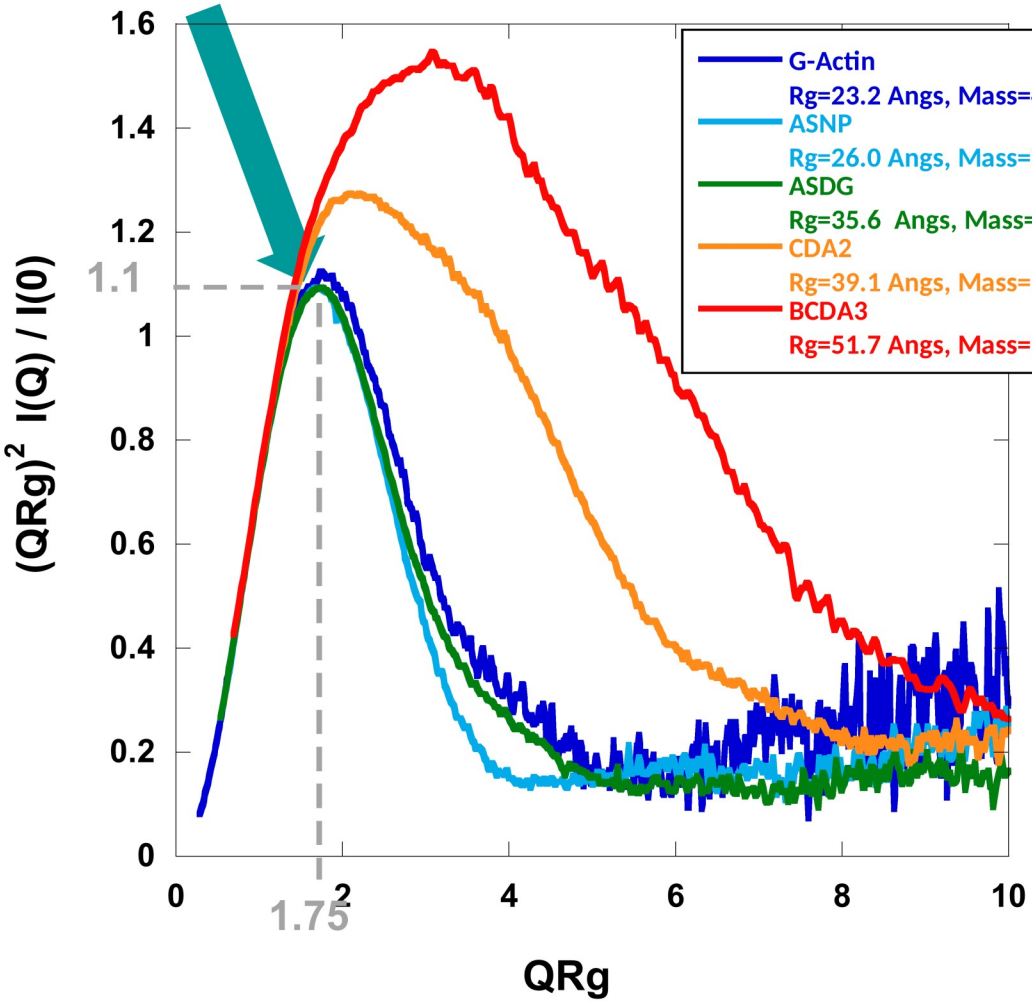
In practice, thin Gaussian chains do not exist.

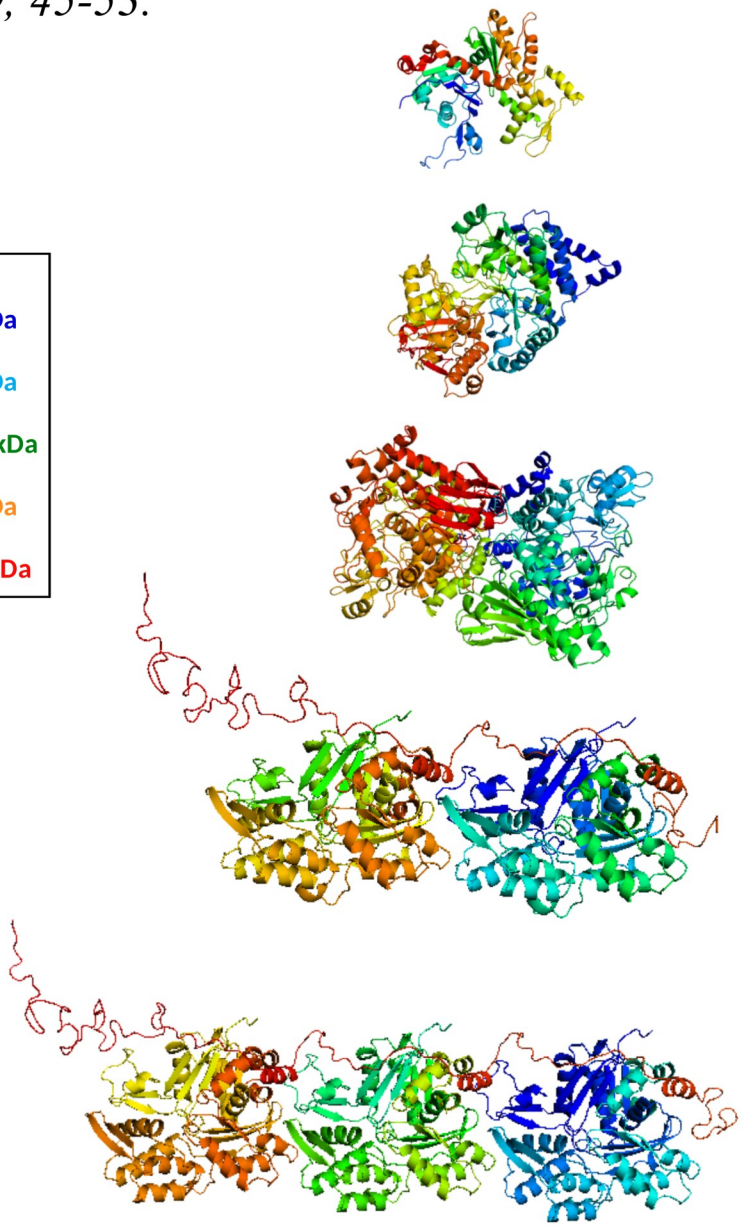
In spite of the plateau at T=76°C, NCS is not a Gaussian chain when unfolded, but a thick chain with persistence length.



Durand et al. (2010), J. Struct. Biol. 169, 45-53.

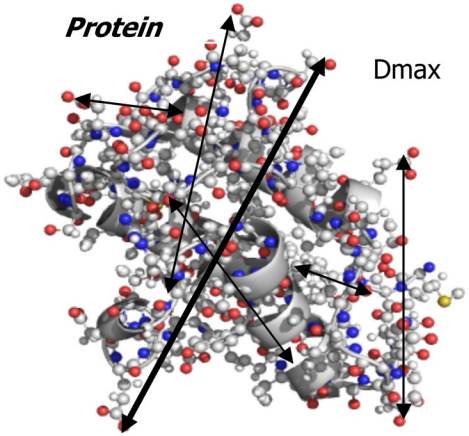
For globular structures, DLKPs fold into the same maximum



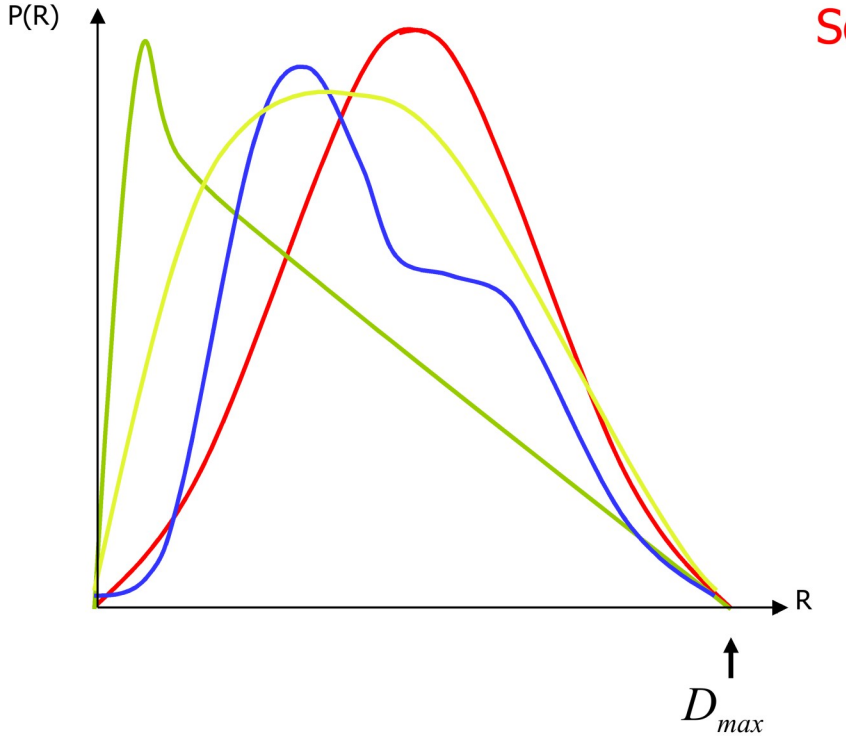


The maximum value on the dimensionless bell shape tells if the protein is globular.

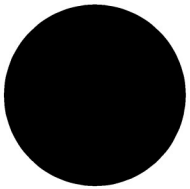
$p(r)$ is obtained by "histogramming" the distances between any pair of scattering elements within the particle. (weighted by scattering density)



$p(r)$ vanishes at $r = D_{max}$



Cylinder



Solid sphere

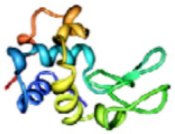
Disc

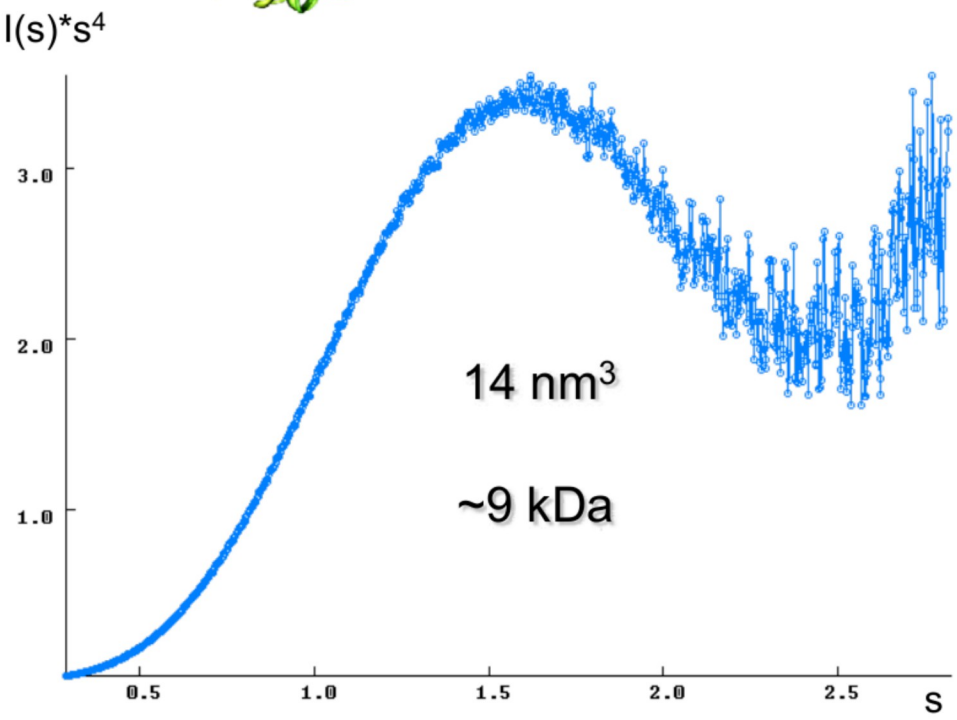
Domains

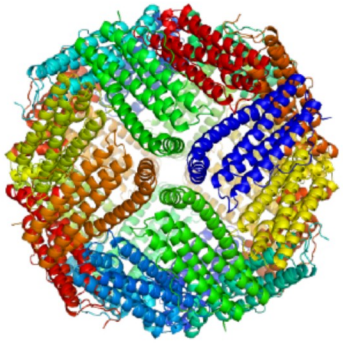
The distance distribution function characterizes the shape of the particle **in real space**

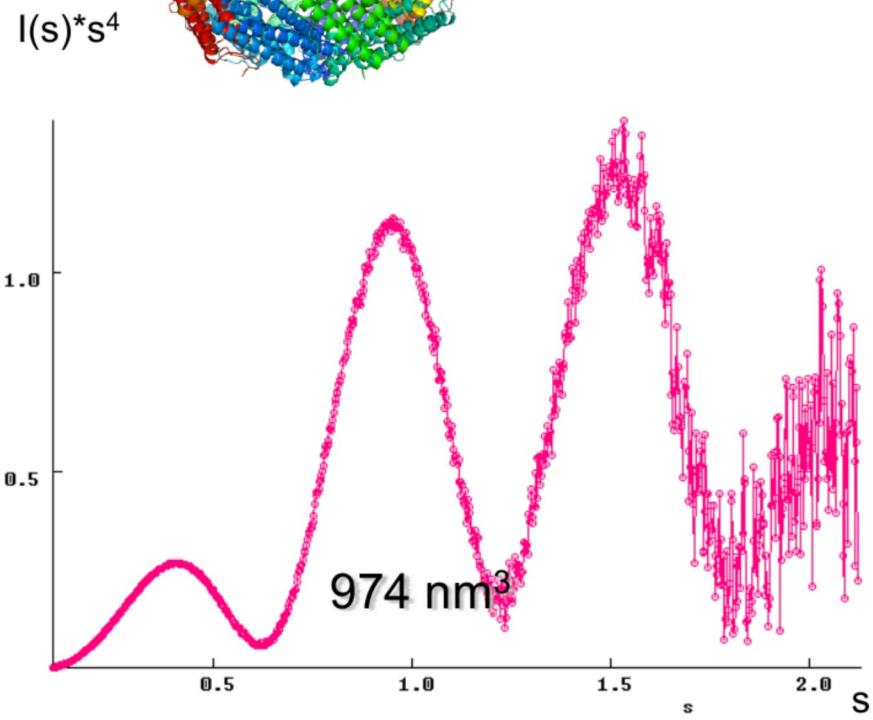
$$V_P = \frac{2\pi^2 I(0)}{\int_0^\infty [I(q) - K_4]\, q^2\, dq}$$

K_4 is a constant determined to ensure the asymptotic decay of $I(q)$ is proportional to q^{-4}







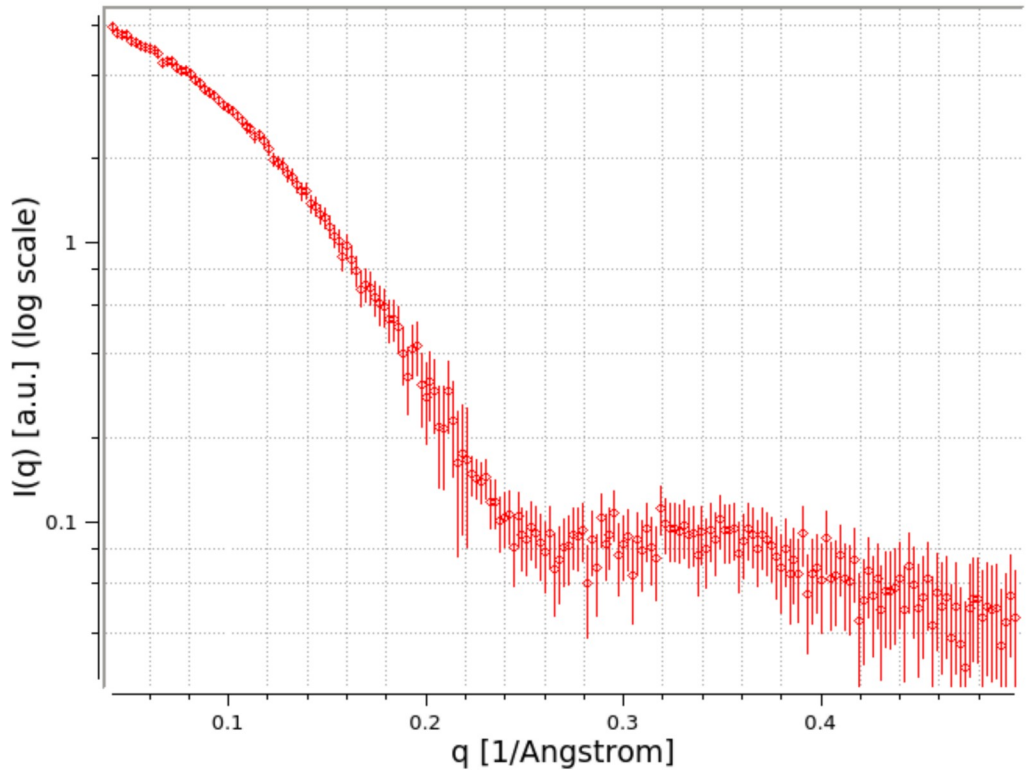


Images courtesy Al Kikhney, EMBL

~610 kDa

Svergun, D.I. & Koch, M.H.J. (2003) Small-angle scattering studies of biological macromolecules in solution. Rep. Prog. Phys. 66 1735-82

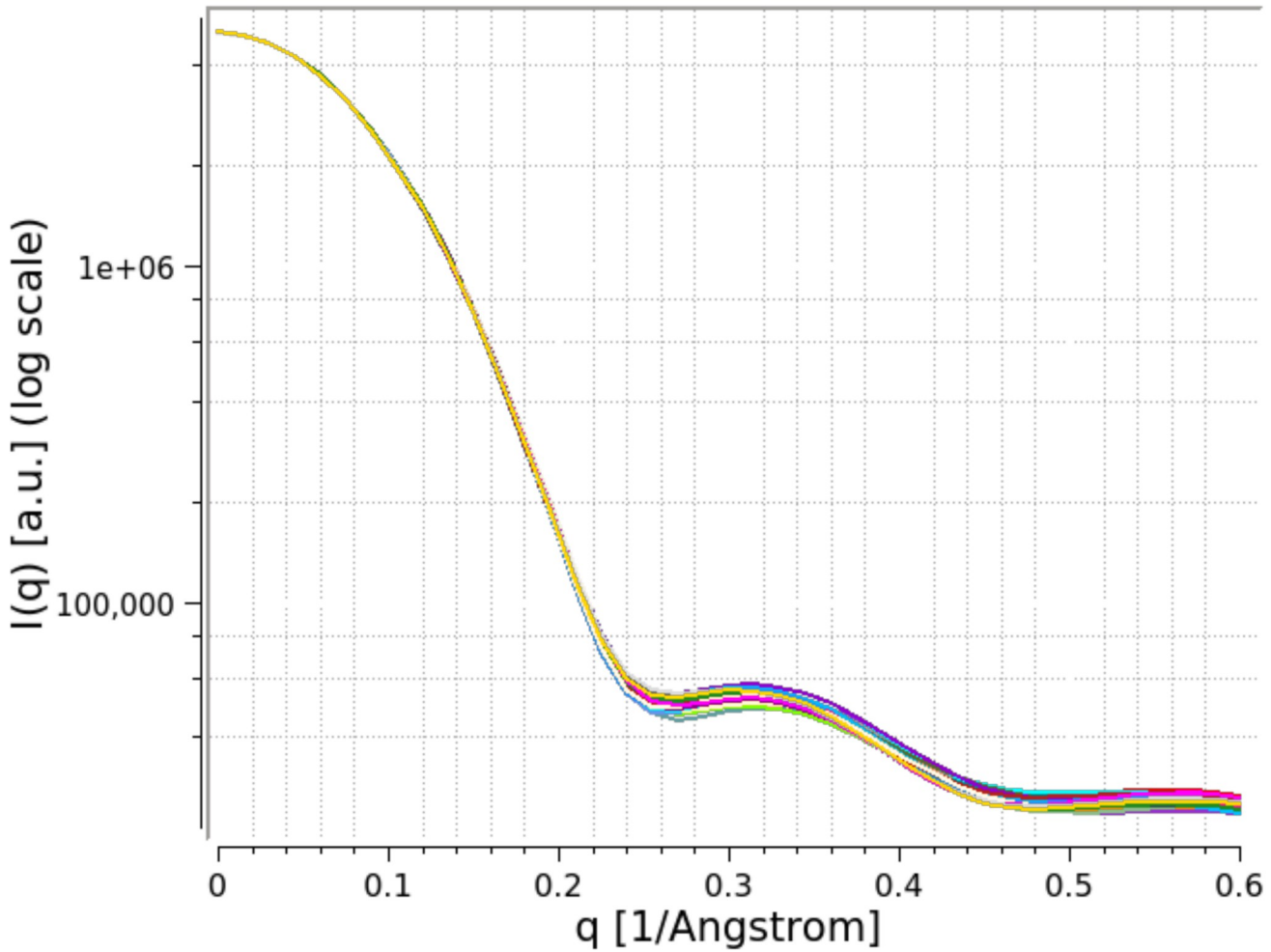
- Shannon channels = $D_{max} \cdot q\text{-}range / \pi$
- "the number of [obtainable parameters] typically does not exceed **10–15**"



Lyzosyme $D_{max} \sim 48$ Angstroms

Shannon channels = $48 * 0.5 / \pi \sim 8$

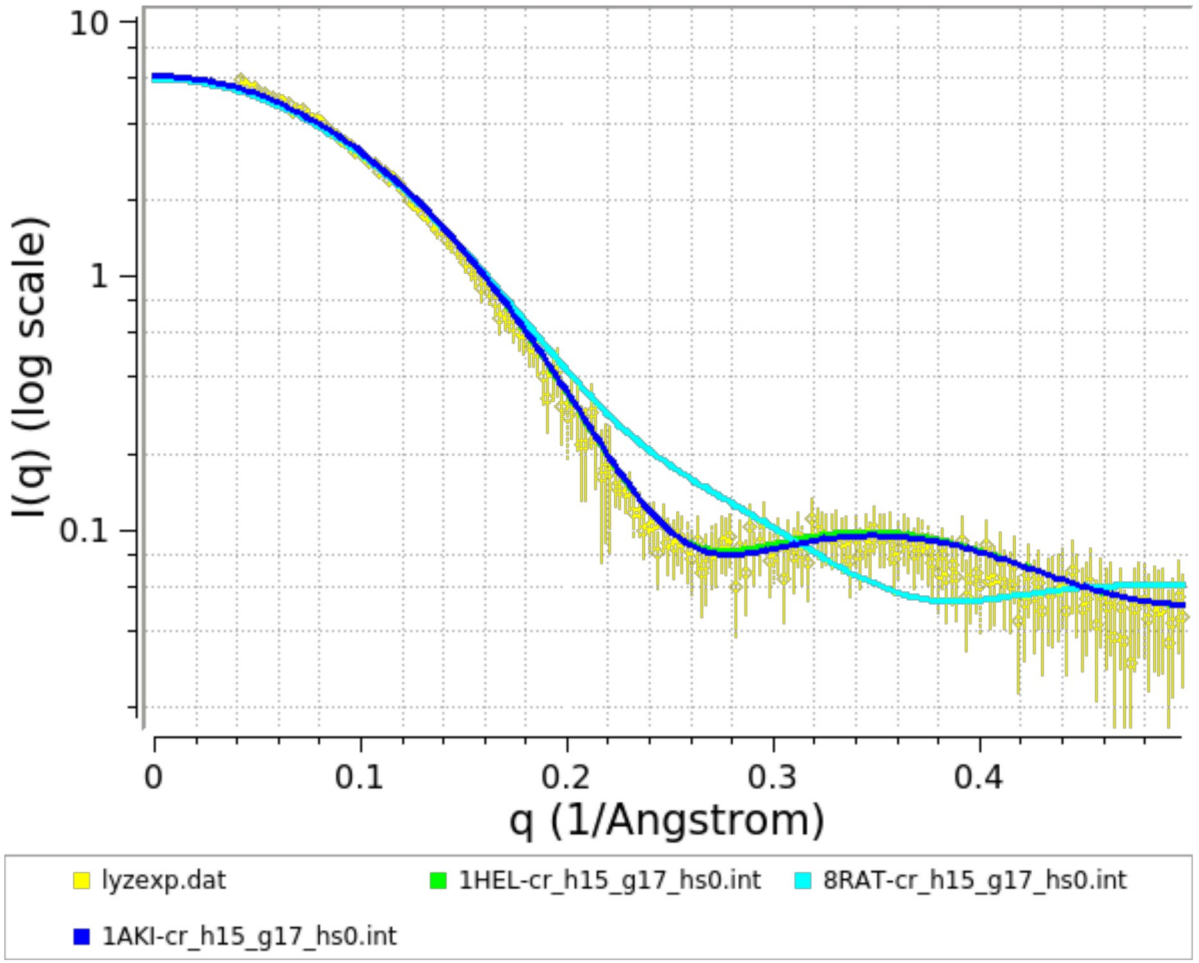
1AKI
48 frames NAMD
100mM NaCl
10 ps/frame



1HEL

8RAT

1AKI



Sorted best fit results
0.914138 : "1HEL-cr_h15_g17_hs0_03.int"
0.922224 : "1AKI-cr_h15_g17_hs0_03.int"
1.2017 : "8RAT-cr_h15_g17_hs0_03.int"
Best fit model: "1HEL-cr_h15_g17_hs0_03.int" nchi 0.914138

NNLS results:
1AKI-cr_h15_g17_hs0_03.int 0
1HEL-cr_h15_g17_hs0_03.int 0.726835
8RAT-cr_h15_g17_hs0_03.int 0.273165
Scaling factor: 1 chi^2=143.506 df=195
nchi=0.857863

UltraScan-SOMO https://somo.aucsolutions.com

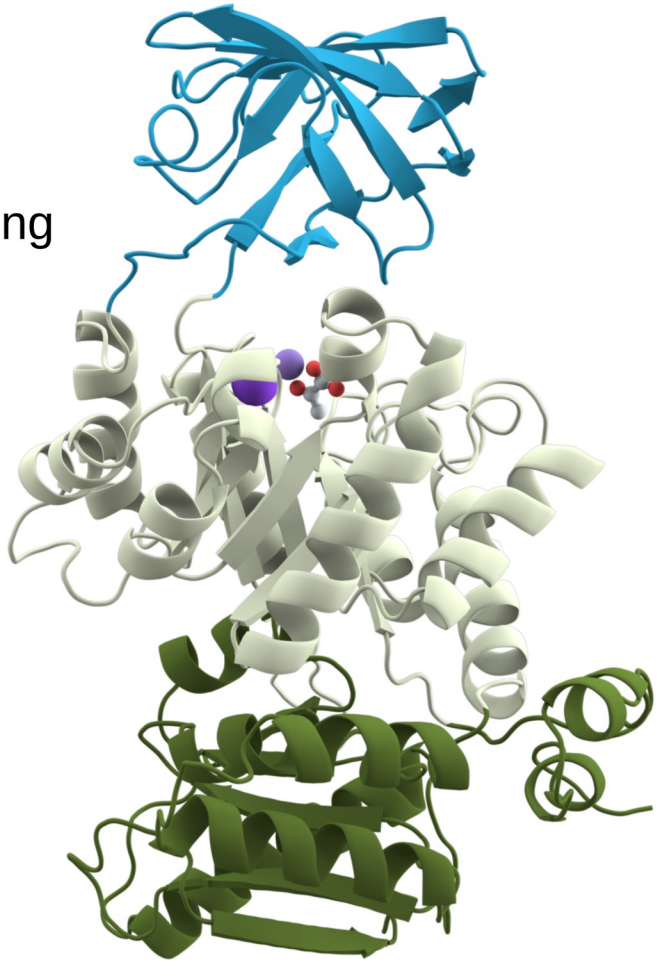
Structure of subunits are known

Arbitrary complex can be constructed by moving and rotating

Verify no steric clashes

→ scattering data subunits

+ contacts (chemical shifts by NMR or mutagenesis)
+ distances between residues (FRET or mutagenesis)
+ relative orientation (RDC by NMR)



Pyruvate kinase 1PKN

Petoukhov et al. 2006 Eur. Biophys J 35:567

Software for “data reduction”, “visualization”, “model fitting”, various “analysis” …
Grouped packages and stand alone components

ATSAS – Dmitri Svergun group
Scatter – Rob Rambo
BioXTAS Raw – Jesse Hopkins
SASView – multiple contributors
CCP-SAS – SCT/SCTPL / US-SOMO / SASSIE & others – multiple contributors
more at *http://smallangle.org/content/software*

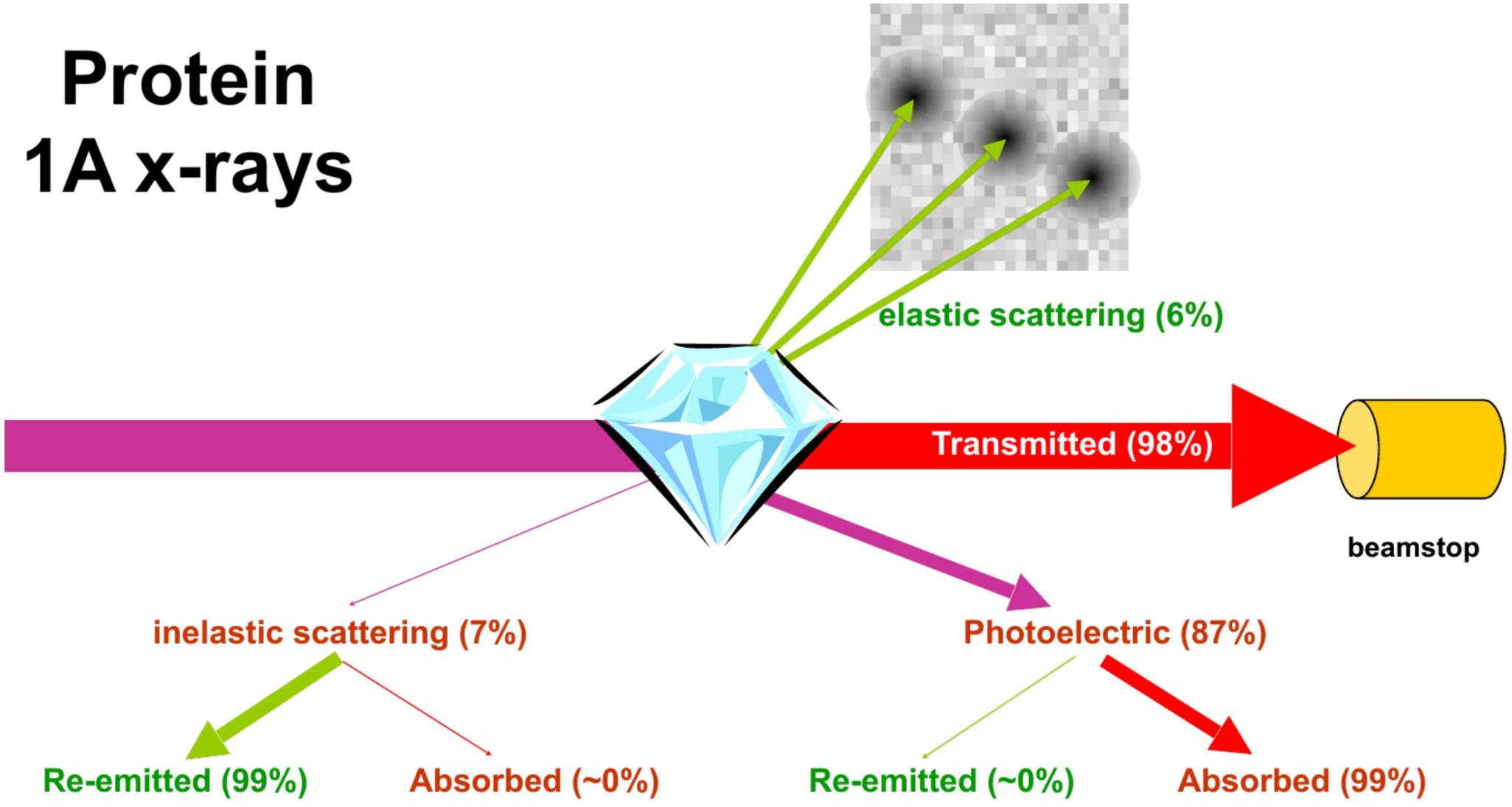
Table 1

Incomplete list of methods for predicting SWAXS curves from structural models: Fitting of hydration layer required ($\delta\rho_{fit}$, including method that ignore the hydration layer), using tabulated reduced form factors (f_{red}), resolution [atomistic or coarse grained (CG)], fluctuations included, free availability [Download (D), web server (W)]. Additional software is listed in Refs. [63,64]

ID	Name/authors	Year	$\delta\rho_{fit}/f_{red}$	Resol.	Fluct.	Avail.	Refs.
Implicit solvent methods							
1	CRYSOL	1995	Yes/yes	atom.	–	D/W	[25]
2	ORNL-SAS	2007	Yes/yes	atom.	–	D	[65]
3	SoftWAXS	2009	Yes/–	atom.	–	D	[66]
4	Fast-SAXS-pro	2009	Yes/yes	CG	Yes	D/W	[30,36]
5	FoXS	2010	Yes/yes	atom.	–	D/W	[67,29]
6	PHAISTOS	2010	Yes/yes	CG	–	D	[68]
7	AquaSAXS/AquaSol	2011	Yes/yes	atom.	–	W	[27]
8	SASbtx/Zernike	2012	Yes/–	atom.	–	W	[69]
9	RISM-SAXS	2014	–/yes	atom.	–	D	[70]
10	BCL::SAXS	2015	Yes/yes	atom.	–	D	[71]
11	Pepsi-SAXS	2017	yes/yes	atom.	–	D	[72*]
Explicit solvent methods							
12	SASSIM/Sassena	2002	–/yes	atom.	Yes	D	[73]
13	MD-SAXS	2009	–/–	atom.	Yes	–	[74,75]
14	AXES	2010	Yes/–	atom.	–	W	[26]
15	HyPred	2011	–/–	atom.	–	W	[76]
16	Park et al.	2009	–/–	atom.	–	–	[77]
17	Köfinger &Hummer	2013	–/–	atom.	Yes	D	[78]
18	WAXSiS	2014	–/–	atom.	Yes	D/W	[38,79]

Table from Jochen S Hub. Curr. Op. in Struct. Bio. 2018, 49:18-26

Protein
1A x-rays



James Holton

Practical considerations

Sample requirements for (SAXS) solution scattering

- size: >5kD
- purity: highly monodisperse !
- concentration: 0.25 – 10mg/ml (higher for small proteins and intermediate angle data
- sample volume 15-50 ul ;(so only a fraction of 1mg protein needed for a starting experiment!)
- enough material for at least 3 concentrations
- matching buffer solution is very important (lower salt better)
- most buffer components tolerated (e.g. glycerol (<30%) and salt (<0.5M) are OK)
- S-reducing agent can help protein to stay intact under irradiation

Additional requirements for time-resolved measurements

- lots of sample (at least 10mg, better more)
- sufficiently large change between initial and final state
- pre-characterization of kinetics by other techniques

Courtesy: Thomas Weiss, SLAC

Practical considerations

A good SAXS experiment starts in your home lab

- every protein has it's own "personality"
  - the more you know about your protein the better you can select the data acquisition parameters (buffer composition, pH, additives ….)

- Characterize your protein as much as possible with biochemical means
  - check for possible oligomerization with concentration
    - in case of complexes: for dissociation under dilution
  - determine highest concentration the protein is stable (and how long?)
  - simulate shipping conditions (e.g. freezing & thawing) and check sample quality afterwards

- know your numbers
  - sequence and MW
  - extinction coefficient and concentration of your stock solution

Courtesy: Thomas Weiss, SLAC

Practical considerations

Monodispersity

- check your samples:
  - Good solubility (clear solution), no obvious precipitates
  - Single species on native gels
  - SDS-PAGE should show no contamination
  - Single symmetric peak on an SEC column
- Other analytical techniques:
  - Dynamic light scattering (DLS)
  - Analytical ultracentrifugation
  - Mass spectrometry SEC-MALLS

Buffer conditions

- use a low salt concentration if possible
- for proteins PBS buffer is usually a good choice
- consider additives to prevent radiation damage (DDT, TCEP, Glycerol …)
- bring plenty of matched buffer

Courtesy: Thomas Weiss, SLAC

Practical considerations

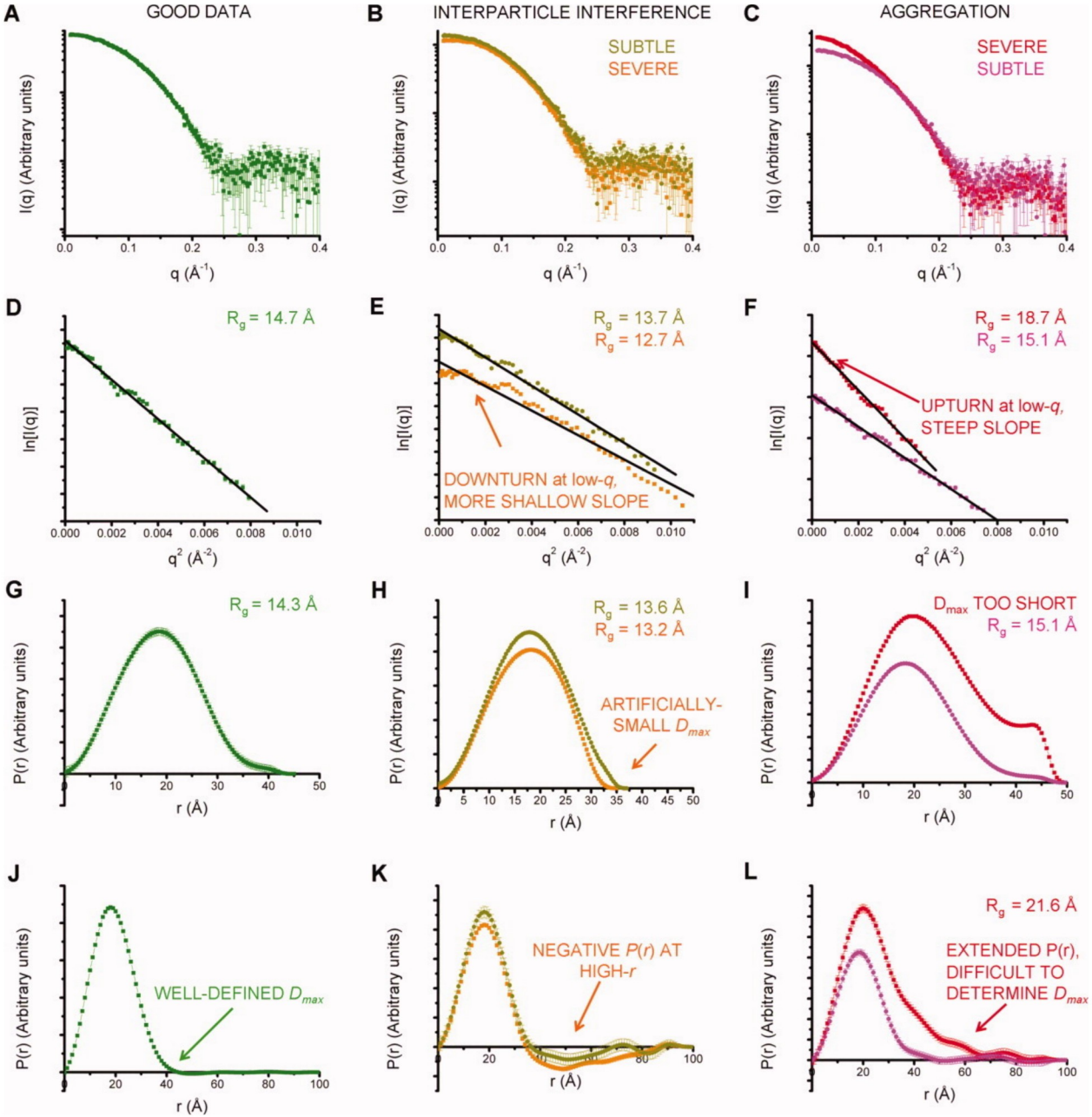
Before coming to SSRL

- provide accurate information in the beamtime request form
- ask beamline staff if you are unsure or have questions
- contact your beamline staff before experiment just in case something changed

At the beamline

- understand how the data collection works and how to load your samples
- take plenty of buffer images
- take advantage of the online data reduction: **monitor what's happening!**
- consider sample recovery for post exposure analysis
- bring additional radical scavengers in case of unexpected radiation damage

Courtesy: Thomas Weiss, SLAC



Immediate data quality checks

- aggregation:
  - upturn at low q
  - residuals in guinier plot will show upward curvature
- interparticle repulsion:
  - downturn at low q
  - residuals in guinier plot will show downward curvature
  - will increase with concentration

Checks with the p(r) function

- determine Dmax
  - no “nose-diving” !
  - no excessive oscillation around 0
  - rule of thumb: Dmax ≈ 3* Rg
  - Switch off P(dmax)=0 and use large Dmax to estimate
- determine Rg
  - should compare well with Rg from Guinier

Jacques D.A. and Trewhella. Prot. Sci. 2010 19(4):642-657

Courtesy: Thomas Weiss, SLAC

Practical considerations – Aggregated data

What if your Sample is Aggregated?

- centrifuge your sample (ideally keep it cold)
- dilute and centrifuge
- filter
- add more DTT if radiation damage is the problem
- run sample through SEC column if time permits
- change buffer condition (if you have enough material)

Courtesy: Thomas Weiss, SLAC

lightsources.org

Light sources of the world

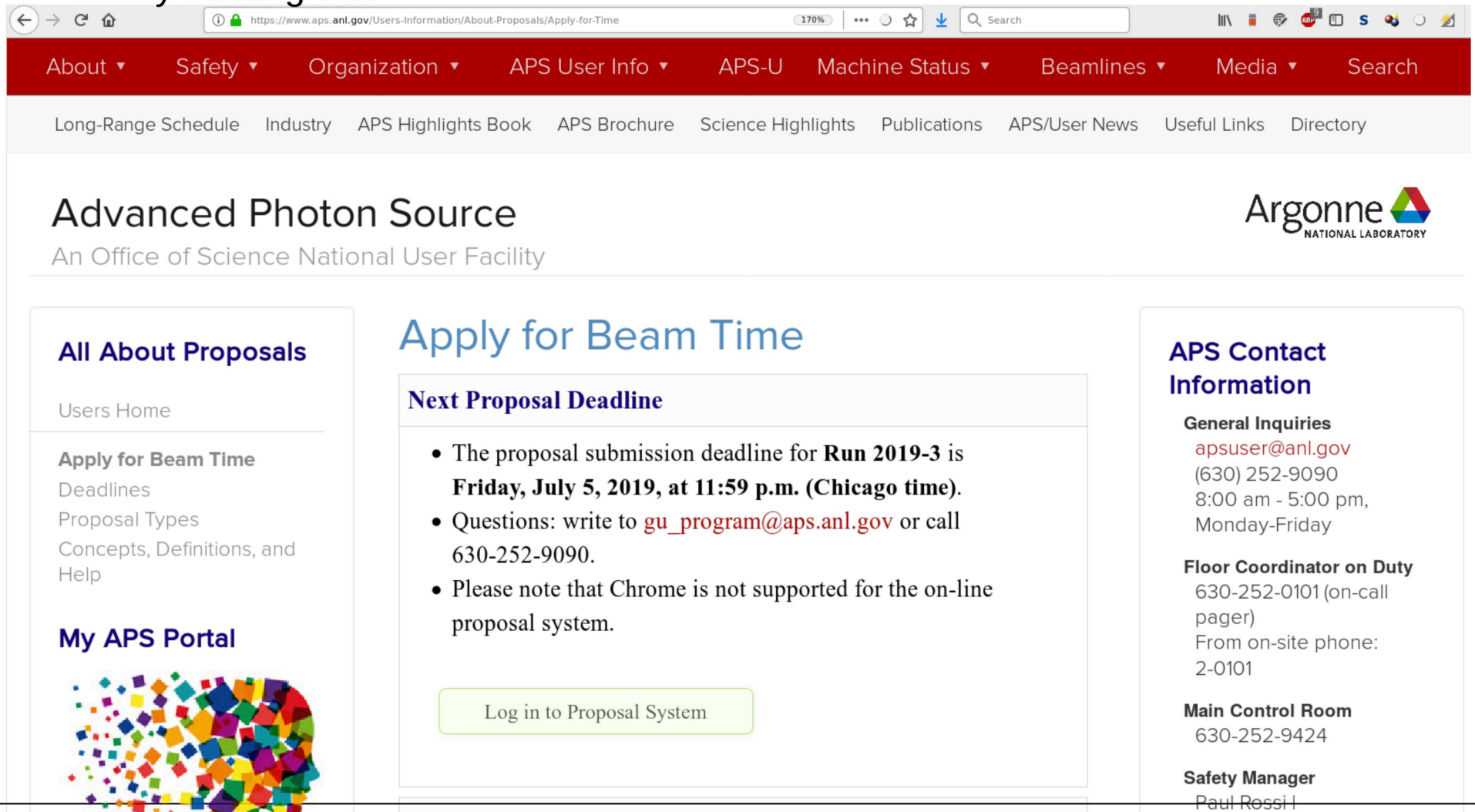
There are more than 50 light sources in the world (operational, or under construction). This page lists all the members of the lightsources.org collaboration.

Orange pins on the map represent members of the lightsources.org collaboration.


ALBA
ALS
ADVANCED LIGHT SOURCE
Argonne
NATIONAL LABORATORY
APS at Argonne National Laboratory
Australian
Synchrotron
HZB Helmholtz
Zentrum Berlin
BESSY II at HZB
Canadian
Light
Source
Centre canadien
de rayonnement
synchrotron
CHESS
CORNELL HIGH ENERGY
SYNCHROTRON SOURCE
diamond
Elettra Sincrotrone Trieste
ESRF
The European Synchrotron
LNLS
MAX IV
BROOKHAVEN
NATIONAL LABORATORY
NSLS-II at Brookhaven National Laboratory
NSRRC
PAL
POHANG ACCELERATOR LABORATORY
Pohang Light Source-II
DESY.
PETRA III at DESY
Photon Factory
Institute of Materials Structure Science
High Energy Accelerator Research Organization,KEK
SESAME
(Honorary Member)
SOLARIS
NATIONAL SYNCHROTRON
RADIATION CENTRE
SLAC
NATIONAL ACCELERATOR LABORATORY
SSRL at SLAC
SYNCHROTRON
THAILAND
CENTRAL LAB
PAUL SCHERRER INSTITUT
PSI
Swiss Light Source at PSI
SOLEIL
SYNCHROTRON

Steps are generally the same:

- Find a beamline
- Talk with a beamline scientist
- Register and submit a proposal
- If you are going to do the experiment yourself
  - Safety training etc.



If you are awarded time

- Bring a **TEAM!**
- Bring extra samples (ask colleagues).
- Expect to work every hour of your allocation!
  - e.g. if you have 2 days beamtime scheduled, expect to have someone working at the beamline 48 hours

Argonne NATIONAL LABORATORY

APS at Argonne National Laboratory

diamond

SANS beamlines

NCNR, NIST, Maryland
HFIR, ORNL, Tennessee
ISIS, RAL, UK
ILL, Grenoble, France
ANSTO, Sydney, Australia
ESS, Lund, Sweden (2025)
others...

NIST

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Arrange a visit to NCNR +

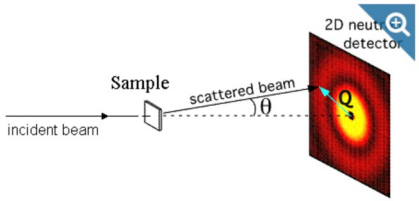
Planning Your Experiment +

Live Data

About NCNR +

Neutron Instruments −

Small Angle Neutron Scattering (SANS)



Small-Angle Neutron Scattering (SANS) probes material structure on the nanometer (10^{-9} m) to micrometer (10^{-6} m) scale. Structures on this length scale are critical to the performance of advanced engineering materials.

SANS Instruments

- CHRNS VSANS
- CHRNS 30m SANS
- CHRNS USANS
- nSoft 10m SANS
- NG7 30m SANS

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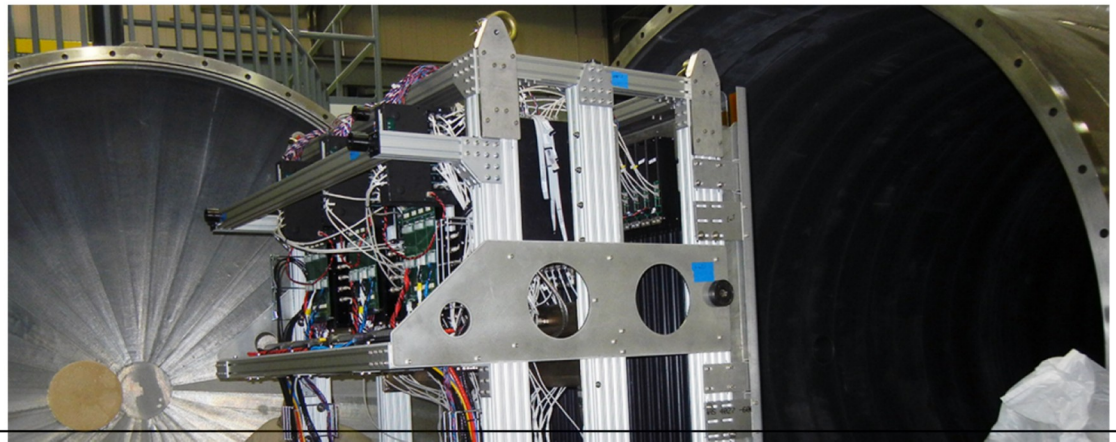


	SAXS	SANS
Features	msec resolution for time-resolved measurements	D labeling and H/D contrast variation
	Superior q-resolution	Magnetic scattering
	Anomalous scattering (ASAXS)	Conducive to extreme environments
	Small sample size	Nondestructive
Complications	Radiation damage to some samples	Incoherent scattering
	Parasitic scattering	H/D isotope effects
	Fluorescence	
	Beam stability	

Anomalous SAXS:
Allows limited contrast variation when the adsorption edge of one of the constituent elements is at an accessible energy range.

Theory pioneered by Heinrich B. Stuhrmann:

Q. Rev. Biophys. 14, 433 (1981)
Adv. Polym. Sci. 67, 123 (1985)

Stuhrmann analyzed metal containing proteins such as hemoglobin, ferritin, and the anomalous effect on the radius of gyration of DNA near the absorption edge of counterions.

In the case of the large subunit of ribosome (1500 kD), measurements near phosphorous K-edge allowed separation of all three partial intensities. *Stuhrmann. J. Appl. Cryst. 2007. 40:s23*

Heinrich B Stuhrmann
Guinier prize 2006

M. Sztucki et al., 2012 Eur. Phys J. Special Topics. 208:319-331

$$f(\lambda) = f_0 + f'(\lambda) + \mathbf{i} f''(\lambda)$$

$$|f| = [(f_0 + f')^2 + f''^2]^{\frac{1}{2}}$$

KK:
Re/Im of Fourier
1-1 Even odd

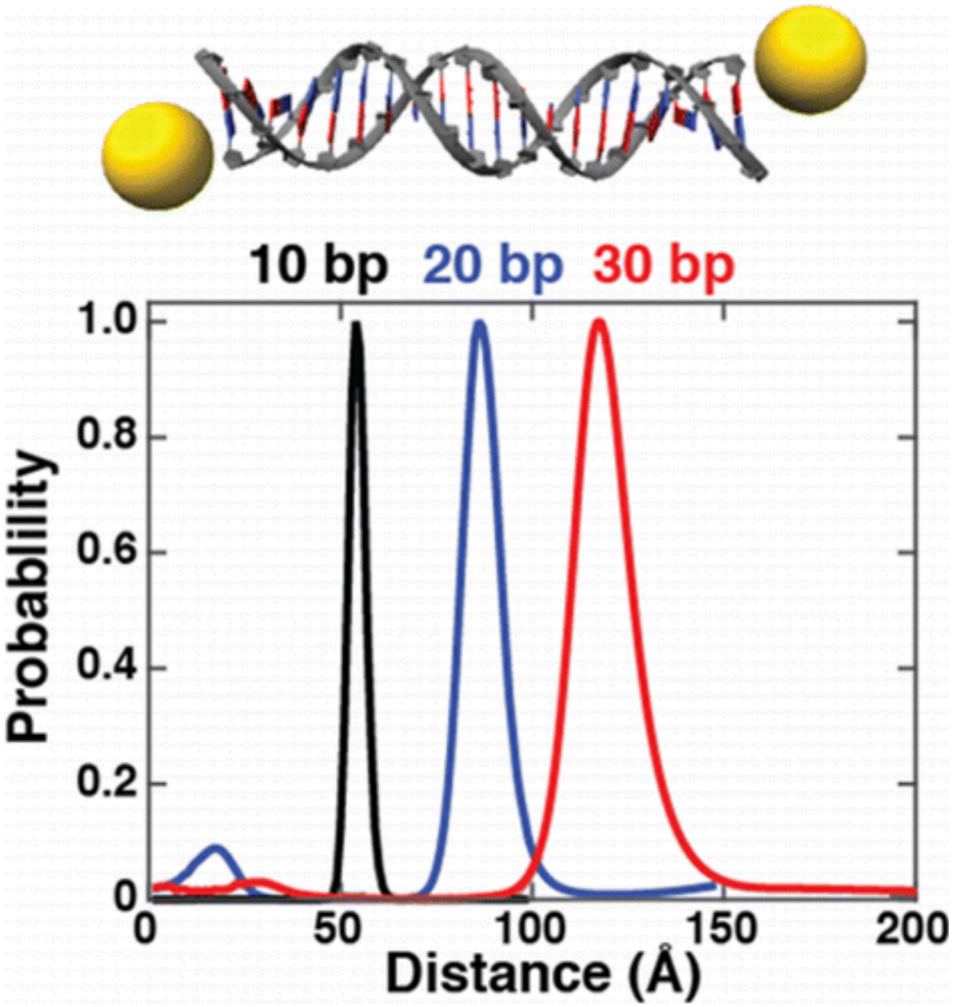
Stuhrmann 1981: *f''* via absorption vs wavelength for bound iron.
f' via *f''* using the Kramers-Kroning relation…
Tabulated values are available for most elements.
Corrected I(q) curves were produced, compared.
Multipole expansion for scattering density
→ distance distributions for iron were estimated.

Generalized in *V. J. Pinfield and D.J. Scott. PLoS ONE. 2014 9(4): e95664*

Table 2. Distances between label atoms or nanocrystals.

Molecule	Actual distance between labels/Å	Calculated distance between labels/Å
10 bp DNA, atom labels	37.3	----
10 bp DNA, nanocrystal	50.5	51
20 bp DNA, nanocrystal	60.7	61
50 bp DNA, nanocrystal	142.0	143
100 bp DNA, nanocrystal	269.6	270
200 bp DNA, nanocrystal	672.0	673

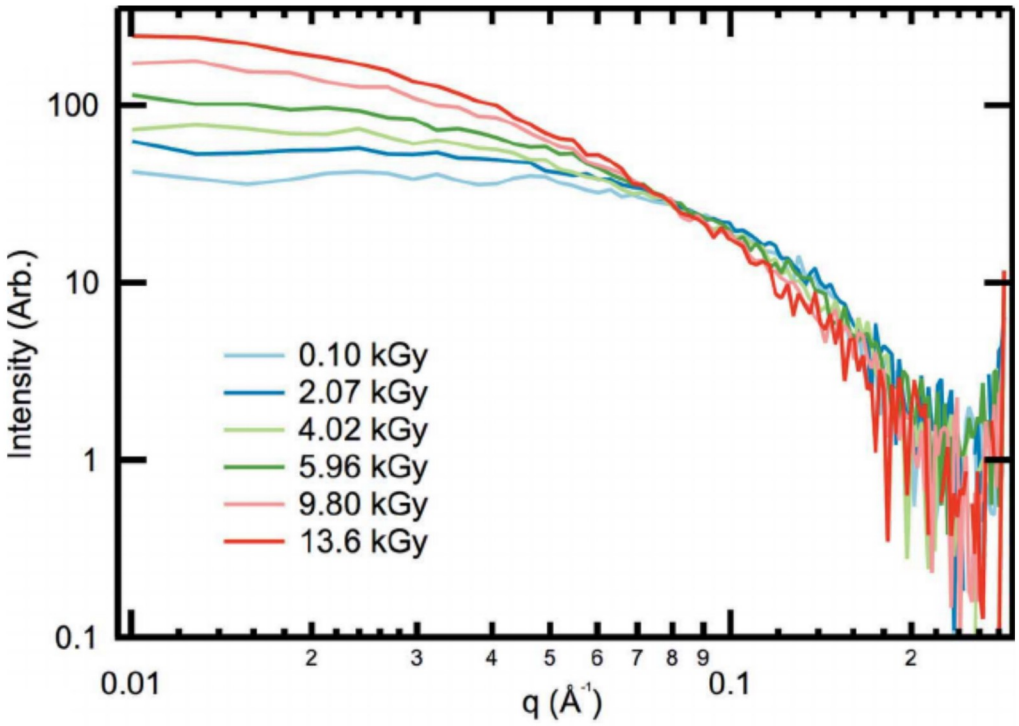
The distance between the label atoms or nanocrystals, as defined in the coordinate files, and determined by the anomalous SAXS simulation.
doi:10.1371/journal.pone.0095664.t002



APS
ID-12

Thomas Zettl et al. Nano Letters 2016 16 (9), 5353-5357

X-ray-induced radiation damage can cause macro- molecule aggregation, fragmentation, conformation changes and unfolding, all of which can be detected by SAXS. Radiation damage is therefore a major obstacle for SAXS, and descriptions of dedicated biological SAXS beamlines acknowledge the need to check for and avoid radiation damage.



Radiation damage in most contexts is a function of Dose (Gy = J kg^{-1}).

$$\text{Dose} = \frac{ftAE_\gamma}{\rho l}$$

f – flux density
t – exposure time
A – fraction of incident energy absorbed
E_γ – energy of photon
ρ – sample density
l – path length

Jesse Hopkins and Robert Thorne, J. Appl. Cryst. (2016) 49:880-890

Minimize:

Reduce exposure time

$$\text{Dose} = \frac{ftAE_\gamma}{\rho l}$$

Decrease volume irradiated

Oscillating or continuous flow

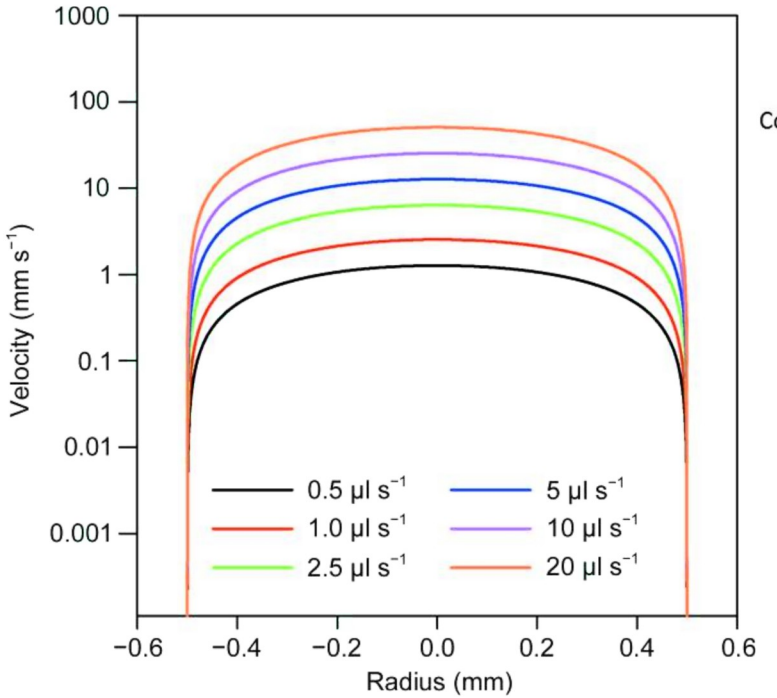
Defocusing the beam

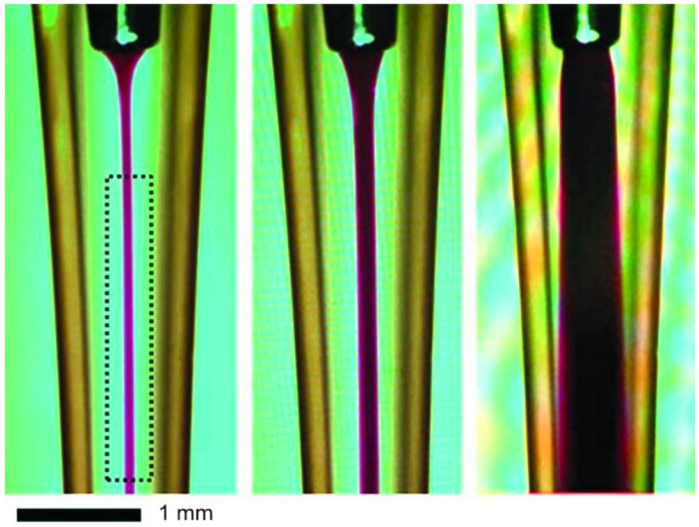
Buffer additives to competitively bind with free radicals or by inhibit aggregration

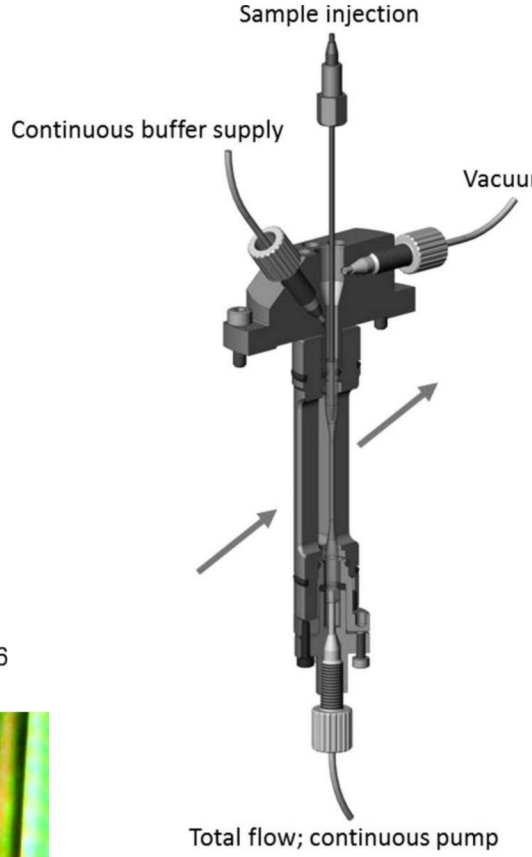
Glycerol

Cryo-SAXS

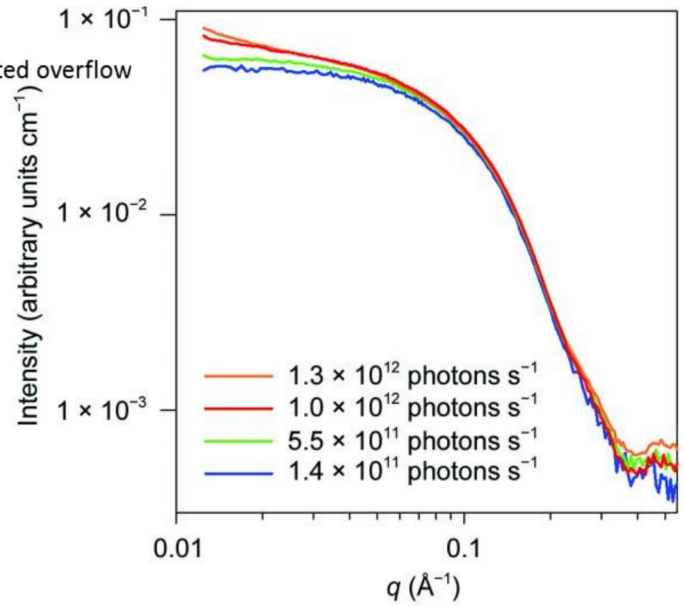
Jesse Hopkins and Robert Thorne, J. Appl. Cryst. (2016) 49:880-890



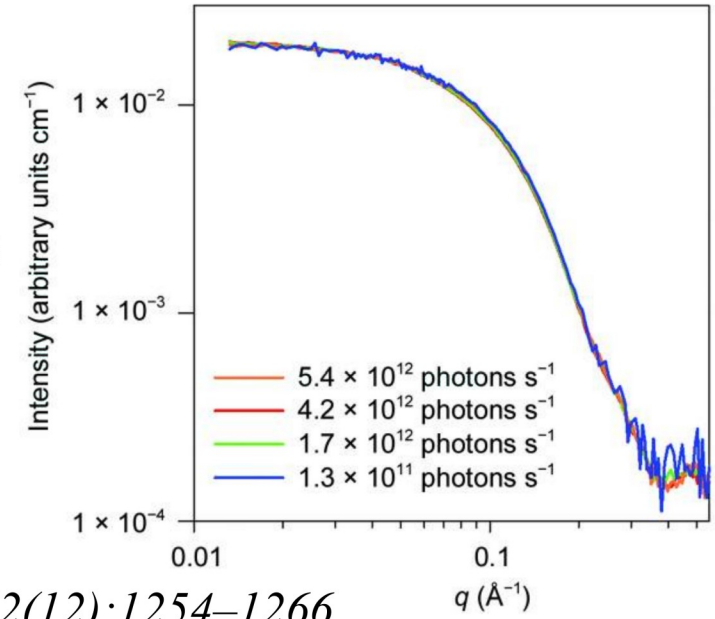




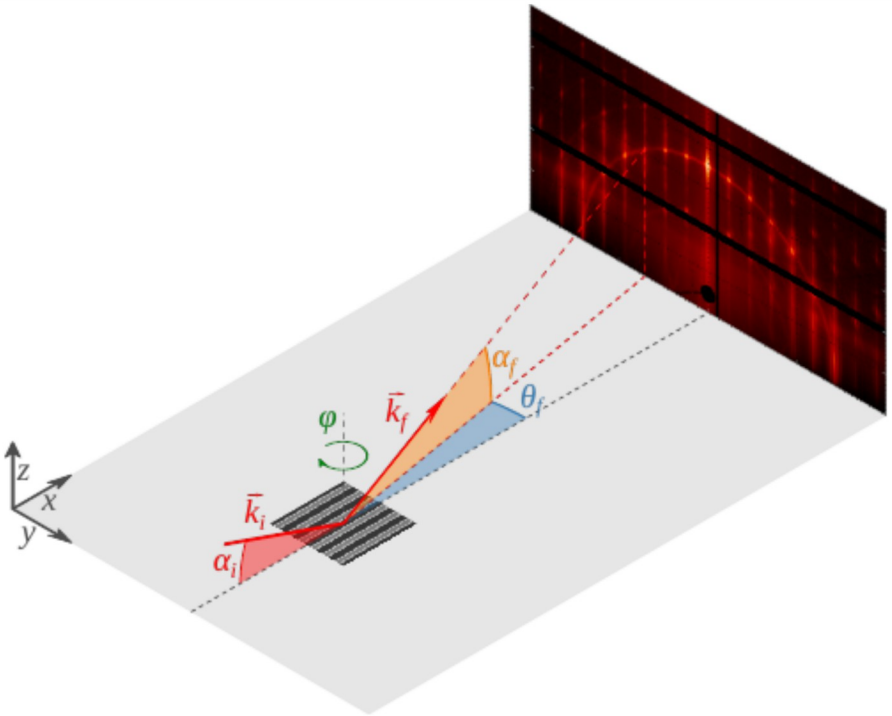
RNase A



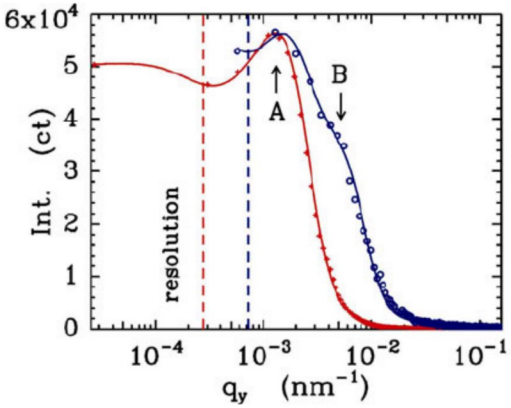
ANSTO / SAXS/WAXS
APS / BioCAT
SSRL / BL4.2
SOLEIL / SWING
MAX IV / CoSAXS

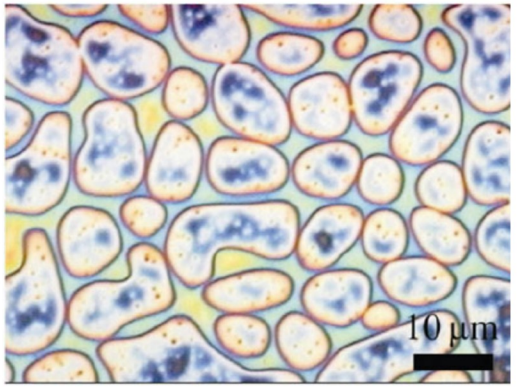


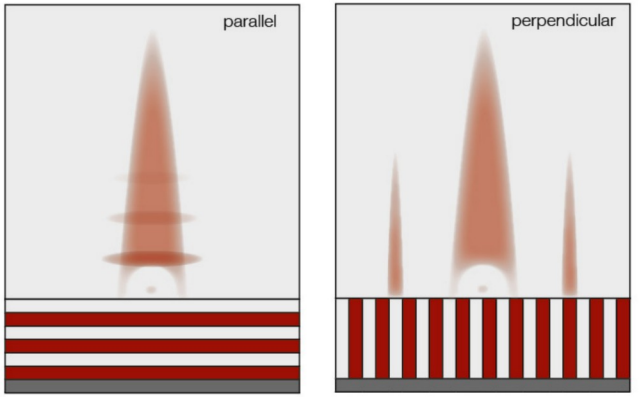
Nigel Kirby et al., Acta Cryst. D Struct. Biol. 2016 72(12):1254–1266.

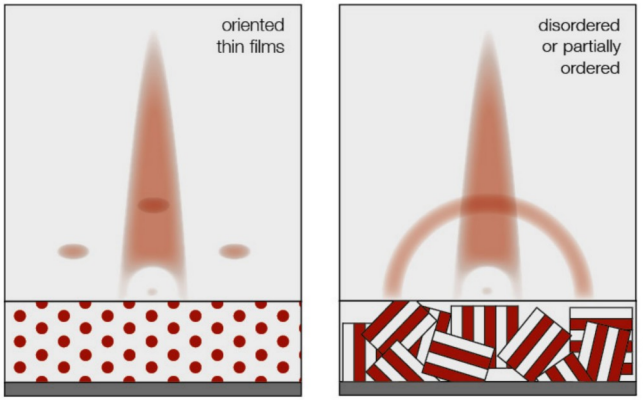


Müller-Buschbaum P. (2009) A Basic Introduction to Grazing Incidence Small-Angle X-Ray Scattering. Lecture Notes in Physics, vol 776, Springer







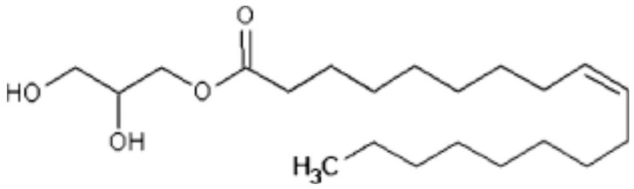


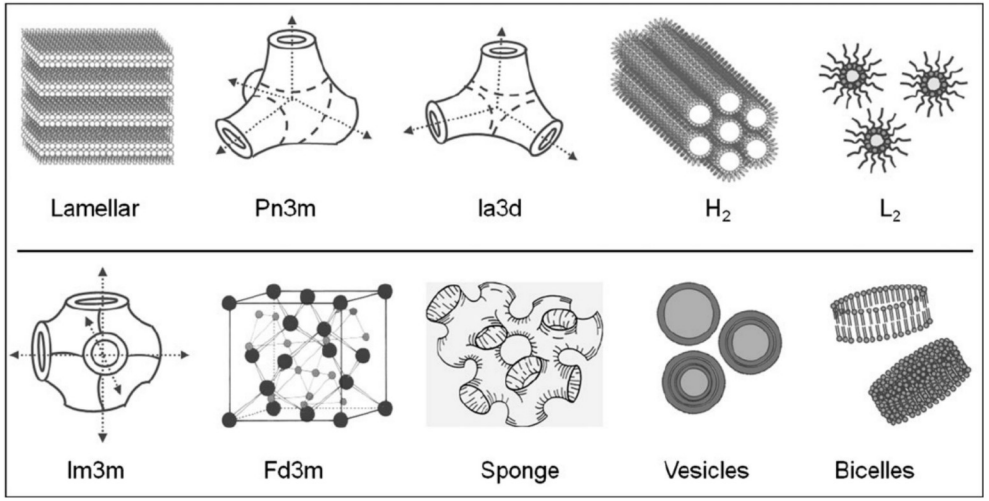
https://wiki.anton-paar.com/en/grazing-incidence-small-angle-x-ray-scattering-gisaxs

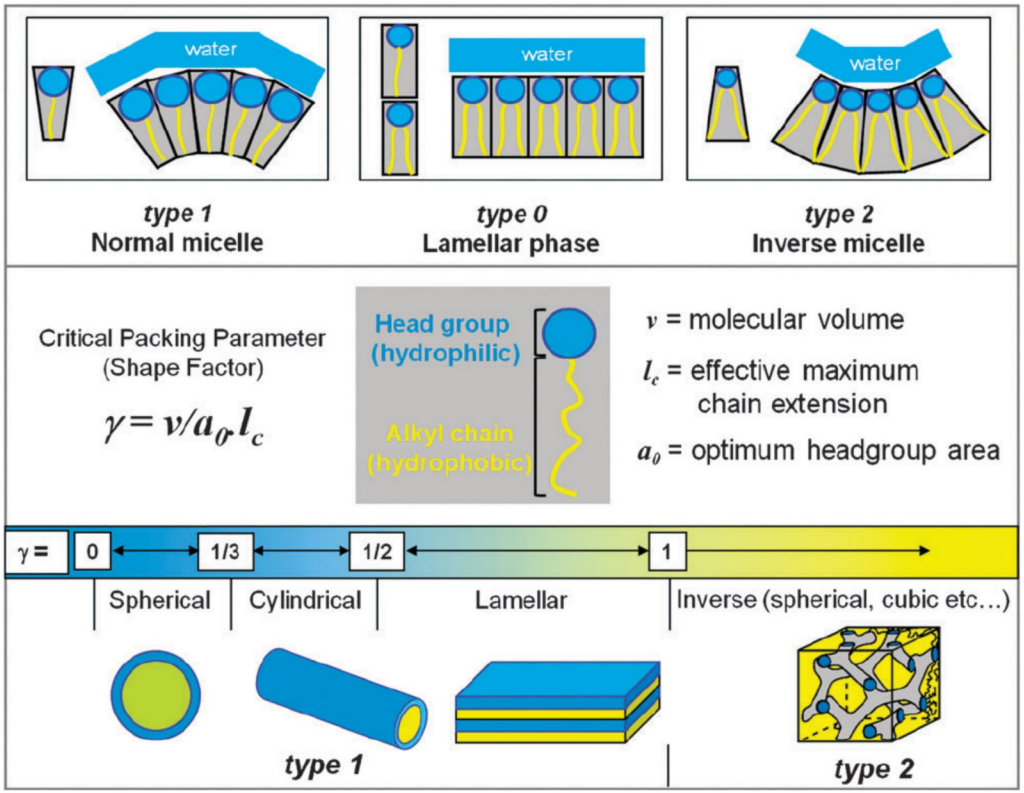
Blend films of PS and PnBA
"A" most prominent in-plane length

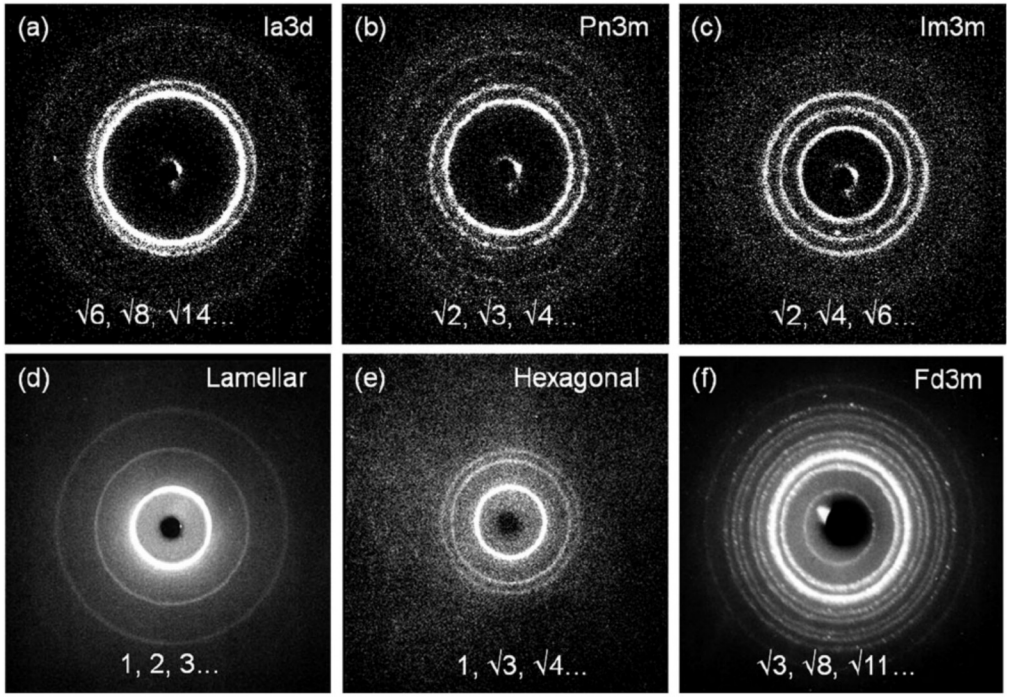
Müller-Buschbaum P., Prog. in Colloid & Polymer Sci. 2006 doi:10.1007/2882_031

Liquid crystal structures
e.g. Monoolein thermotropic and lyotropic









C. V. Kulkarni et al. Phys. Chem. Chem. Phys. 2011, 13:3004-3021

Monodispersity revisited

Svergun – 2003

Shannon channels = $D_{max} \cdot q\text{-}range / \pi$

"the number of [obtainable parameters] typically does not exceed **10–15**"

Hub – 2018

"… generally accepted that experimental SWAXS curves do not contain more than 10–30 independent data points."

Monodispersity → maximize information content / species

Even if you purify immediately before SAXS measurements and inject each fraction or a pool of fractions, you still have a chance that the sample will either aggregate or degrade during operations

SEC-SAXS

- High pressure liquid chromatography or FPLC (Fast protein liquid chromatography) on line with the SAXS cell
- Individual peaks are more likely to be monodisperse
- First use paper, available to users who could self-manage FPLC
  - *Mathew, E., Mirza, A., & Menhart, N. (2004)*. Liquid-chromatography-coupled SAXS for accurate sizing of aggregating proteins. *J. Synchrotron Rad. 11, 314-318.*
- First setup with user HPLC support
  - *David, G. & Pérez, J. (2009).* Combined sampler robot and high-performance liquid chromatography: a fully automated system for biological small-angle X-ray scattering experiments at the Synchrotron SOLEIL SWING beamline. *J. Appl. Cryst. 42, 892-900*
- Implementations (not guaranteed exhaustive)
  - ID-18 BioSAXS/APS
  - BL4.2/SSRL
  - CHESS/MacCHESS
  - SWING/SOLEIL
  - BM-29/ESRF
  - I22/Diamond
  - P12/Petra
  - SR13 ID01/Australian Synchrotron

Inline HPLC/MALS system

SWING/SOLEIL

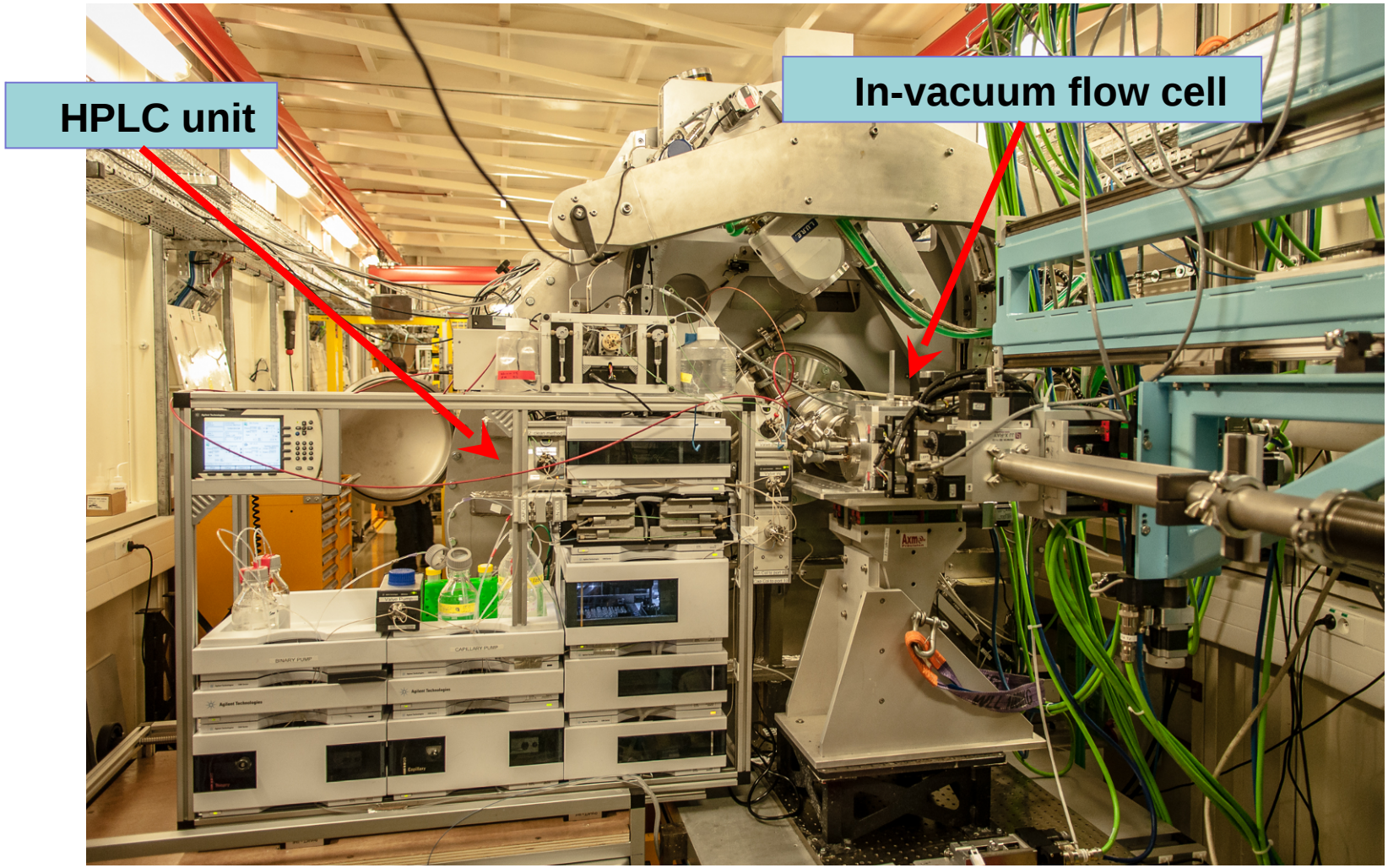
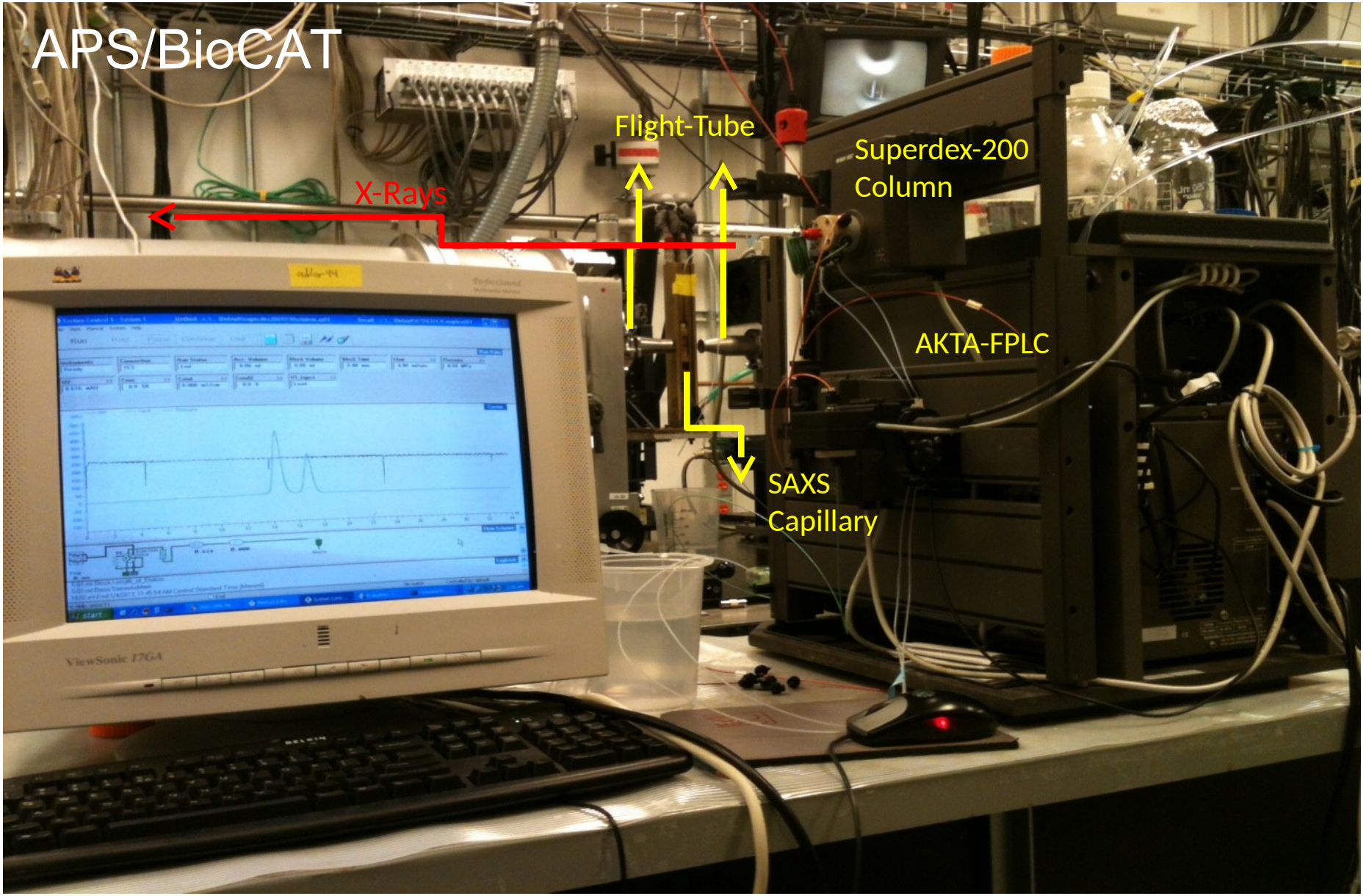


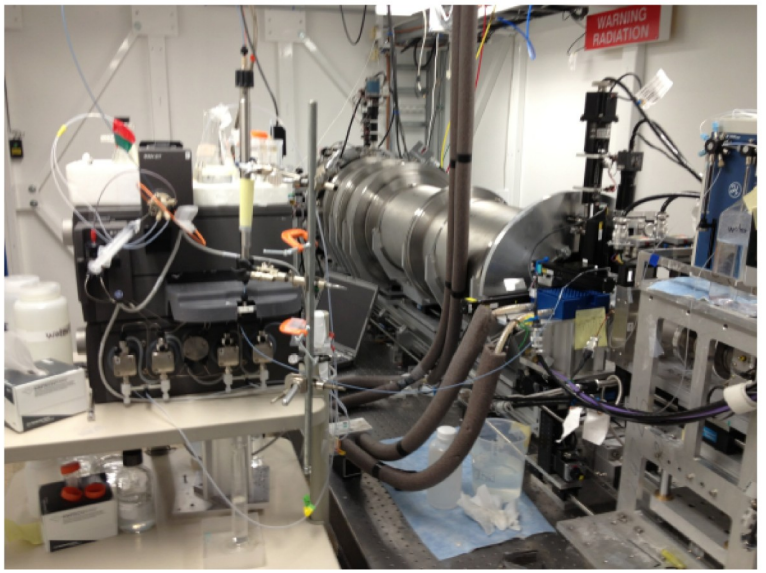
Photo credit: Javier Perez

SEC-SAXS



Slide Credit: Srinivas Chakravarthy

SSRL/BL4.2



- The online FPLC-SAXS system at the BioSAXS beamline BL4-2
- consists of an Akta Ettan with low volume (2.5ml) SEC columns:
  - Superdex 200
  - Superose 6
  - Or bring your own

- The system uses the same flow path as the
- regular “autosampler” setup at the beamline:
  - rapid switch-in of the FPLC system during normal data collection
  - FPLC-SAXS and “autosampler” results can be compared quickly

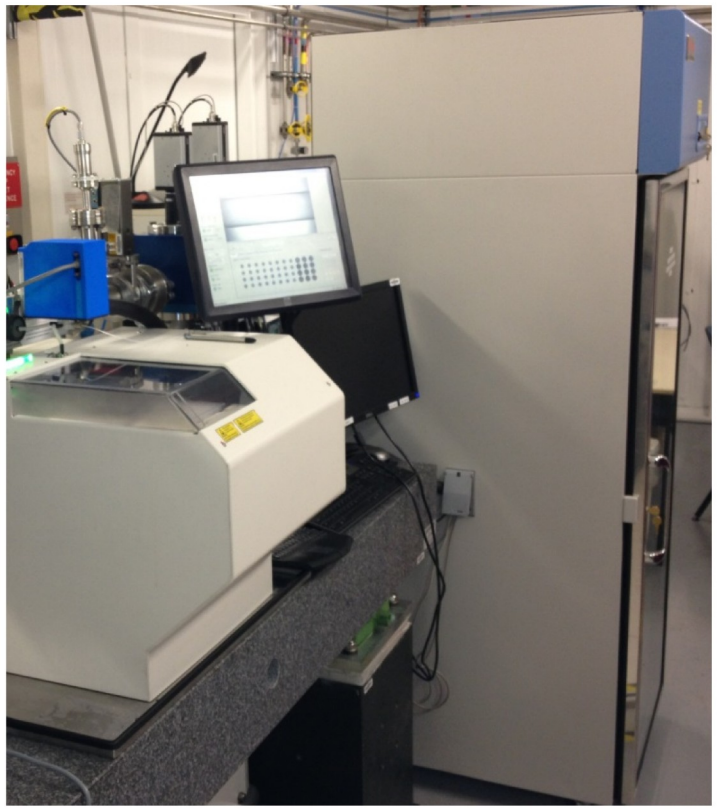
- sample requirement:
  - typically 50ul of 5mg/ml sample
  - each run requires 3 ml of buffer and takes roughly an hour

- Automated data analysis scripts allow easy tracking of experimental results during experiment
- More information on our website:
  - **http://www-ssrl.slac.stanford.edu/~saxs/**

Slide Credit: Thomas Weiss

SSRL

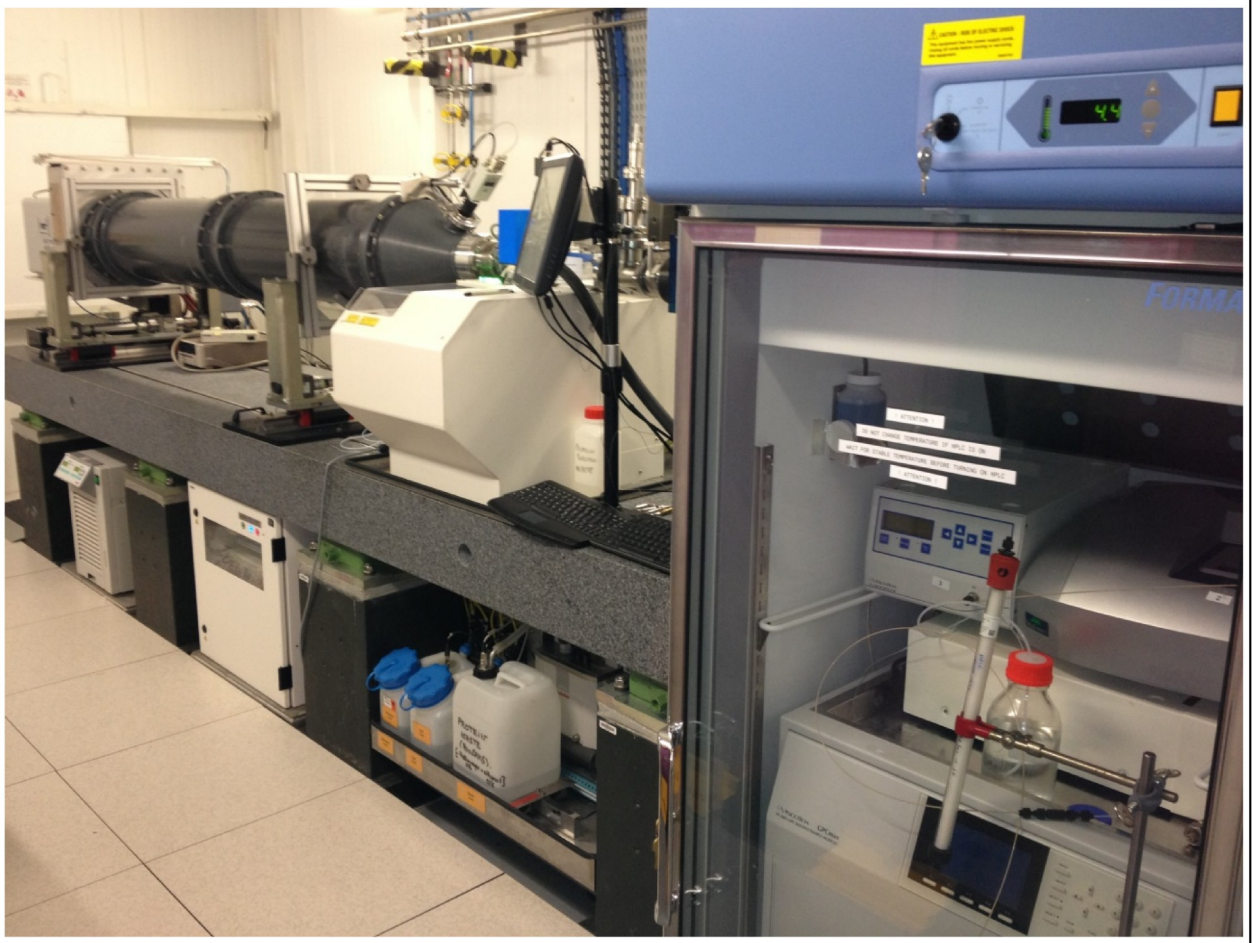
BM29/ESRF



Automated switching between SEC and SC for efficient use

Integrated Sample changer and Online-SEC

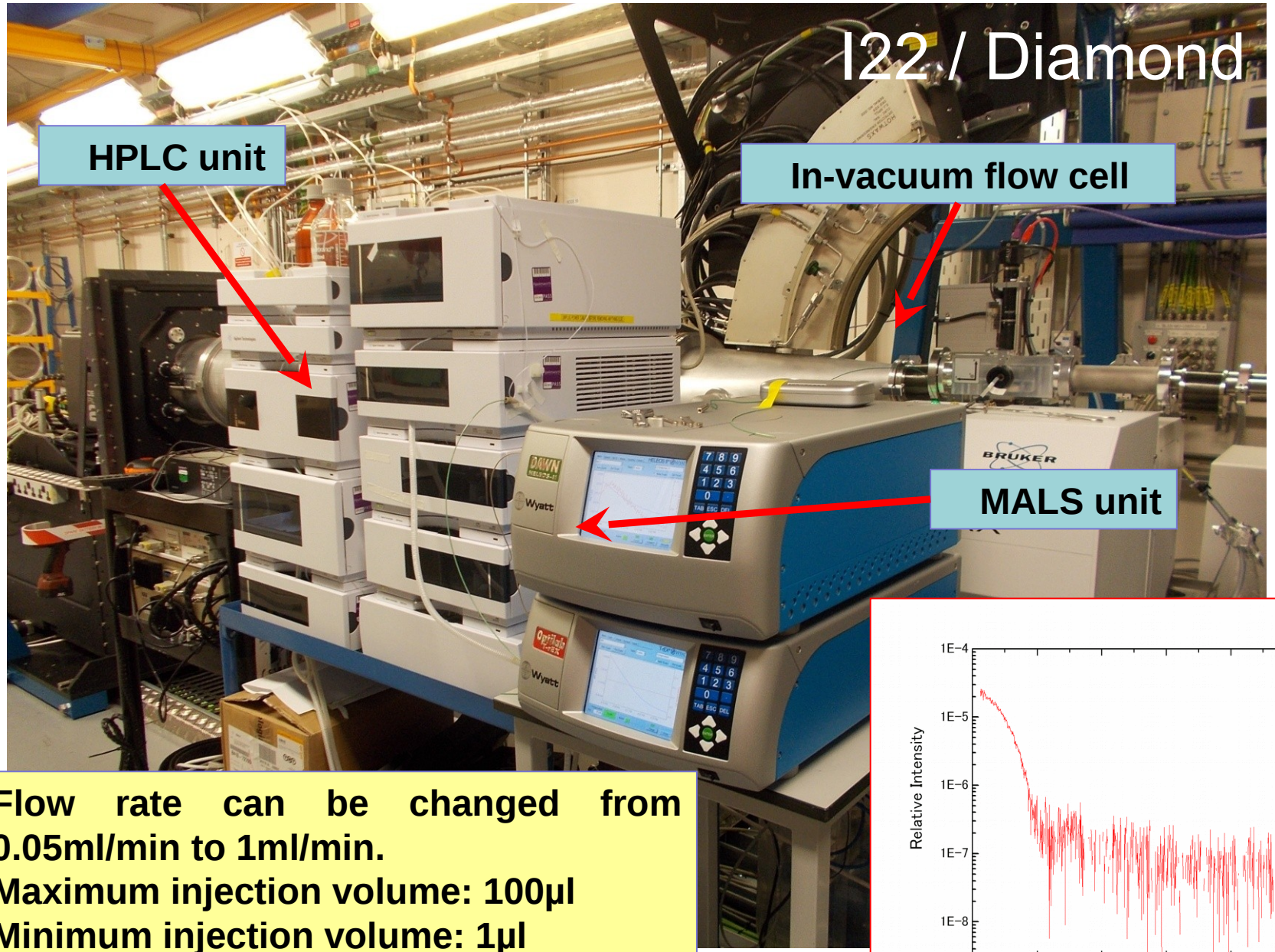
SEC units housed in temperature controlled cabinet (4 -25 °C)



Slide Credit: Adam Round

ESRF EMBL

SEC-SAXS



- **Flow rate can be changed from 0.05ml/min to 1ml/min.**
- **Maximum injection volume: 100µl**
- **Minimum injection volume: 1µl**

Slide Credit: Katsuaki Inoue

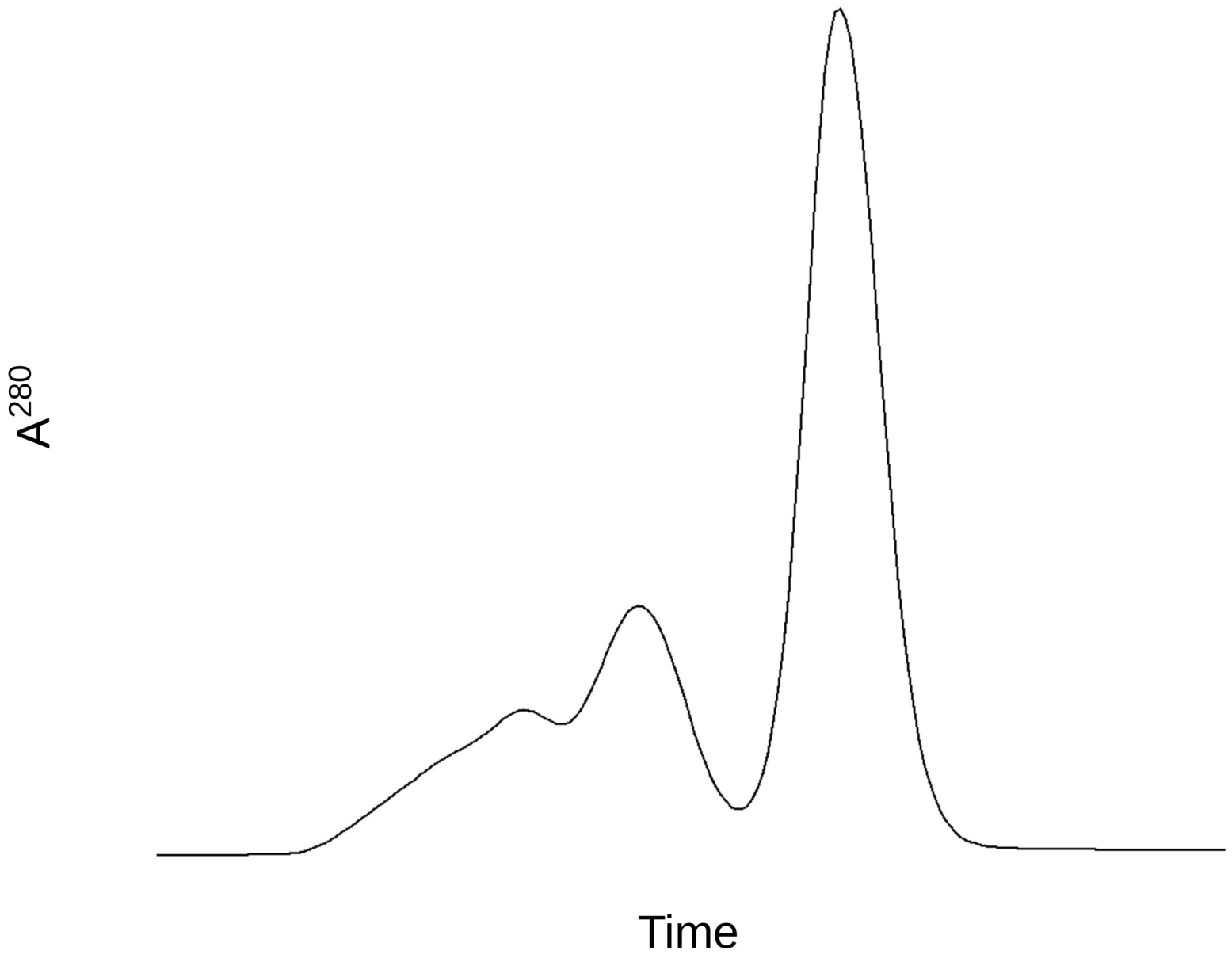
- Separate immediately before measuring
- Individual peaks are more likely to be monodisperse
- Now available as primary method of analysis at multiple beamlines

- Conclusion of ACA 2014 session 4.2.4 [ACA Reflexions Fall 2014]:

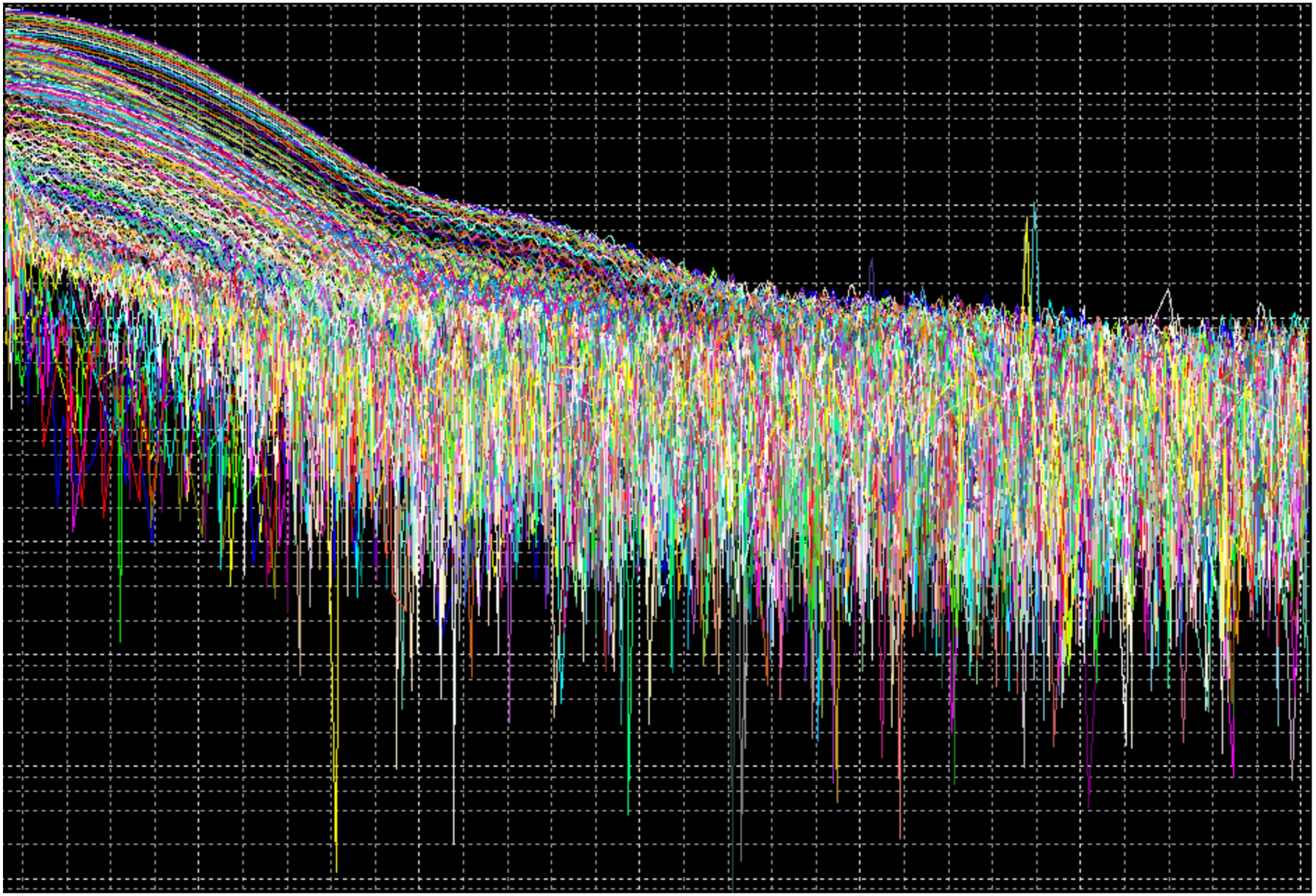
"The consensus that emerged was that SEC-SAXS may become the standard data collection strategy for biological samples, as a large number of samples that were heretofore believed to be monodisperse have been shown to be polydisperse when analyzed with online SEC-SAXS setups."

ACA Structure Matters — Albuquerque ACA Meeting — Fall 2014

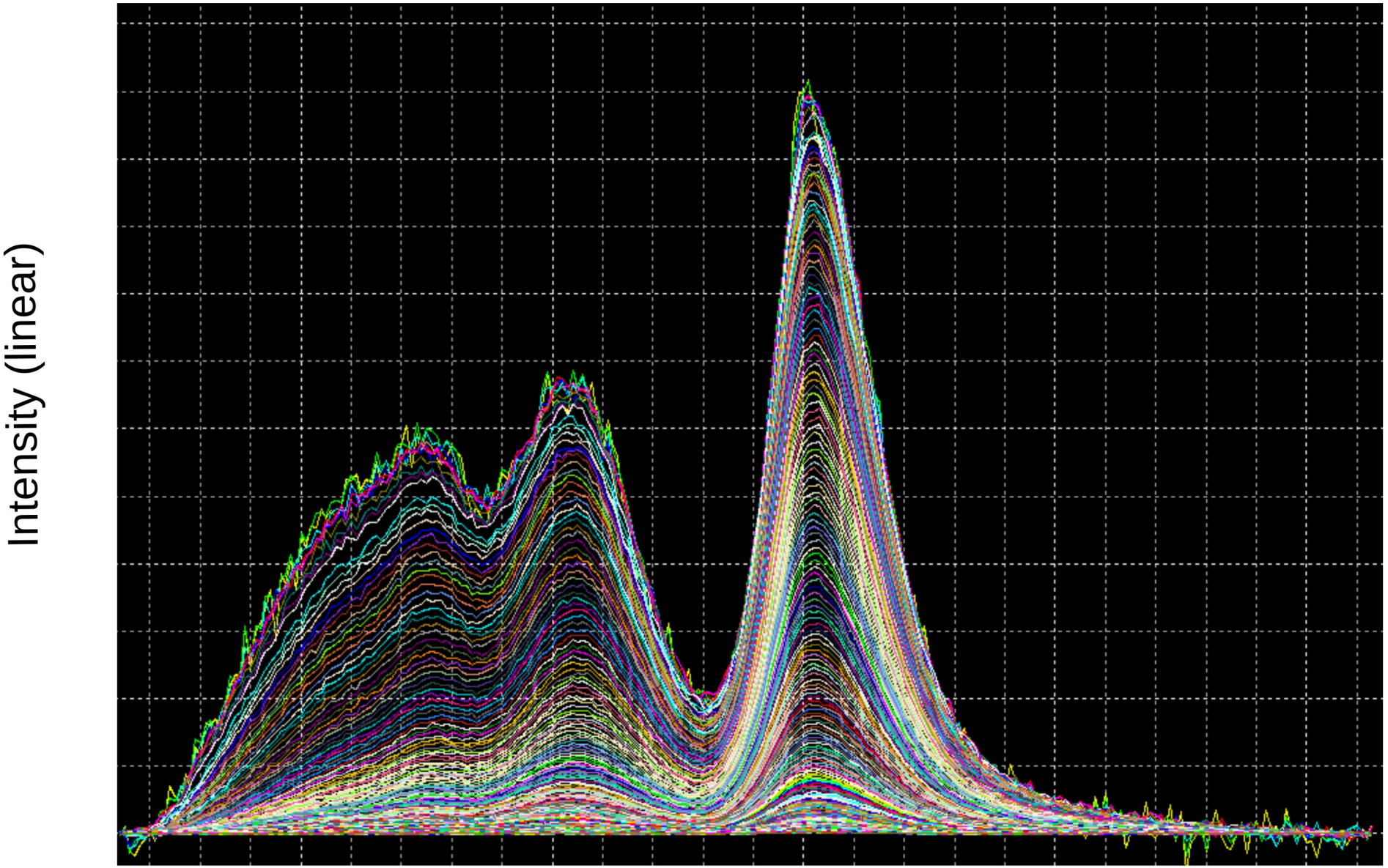
Nicolas Wolff, Sophie Zinn-Justin, Nigel Kirby, Alvin Acerbo, Srinivas Chakravarthy, Javier Pérez, Alexey Kikhney, Emre Brookes, Adam Round, David Lambright.



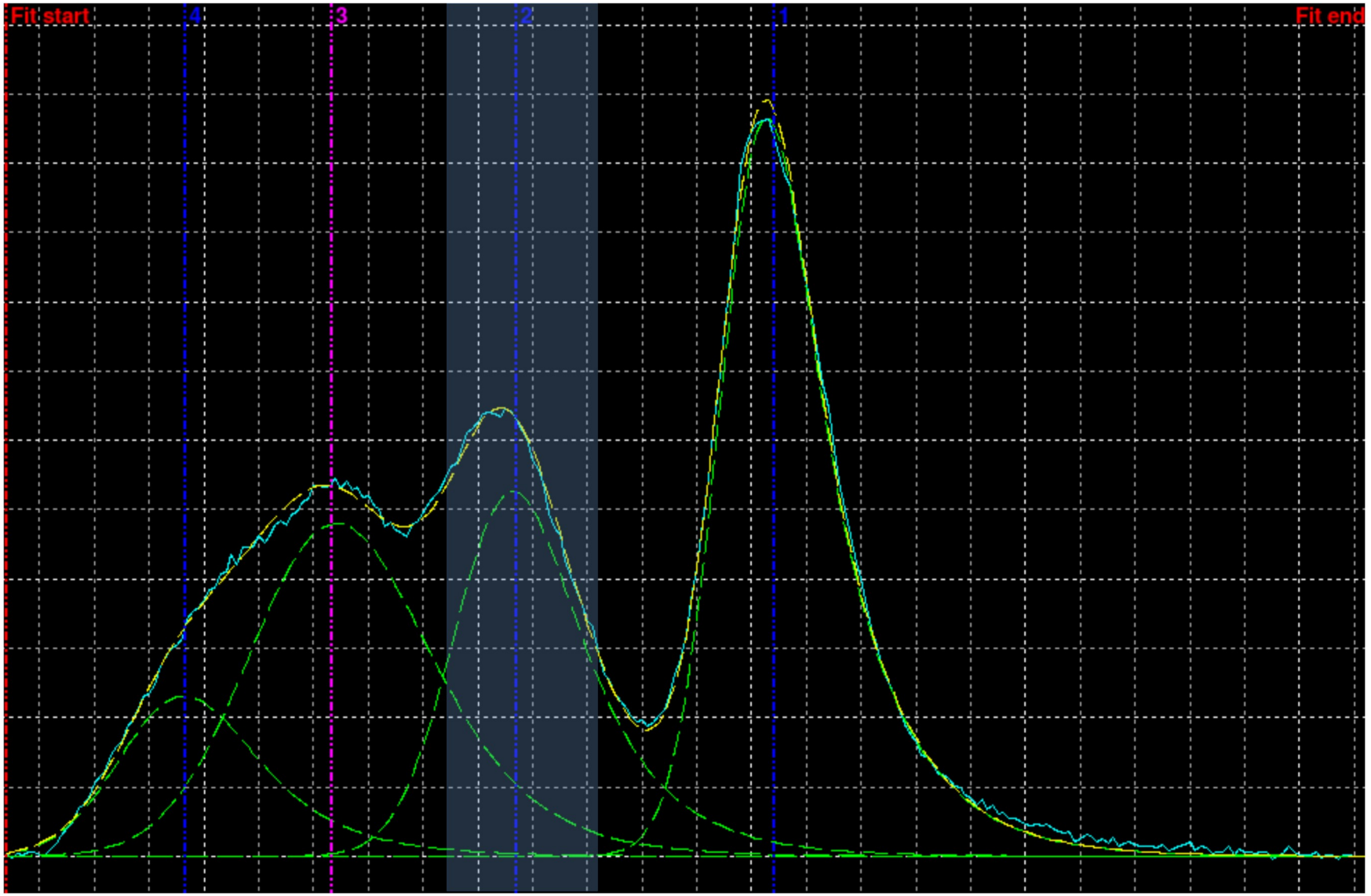
Brookes et al, J. Appl. Cryst. 46 (2013) 1823-33



Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

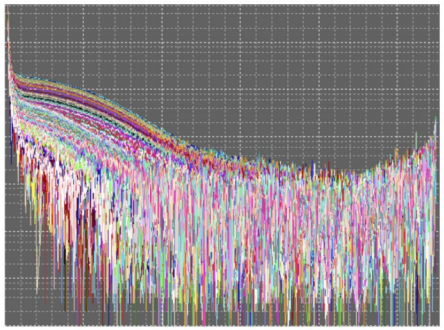


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

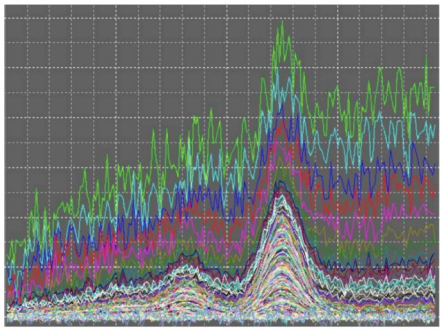


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

Collect SEC-SAXS data

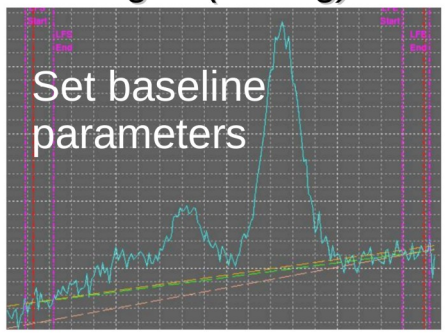


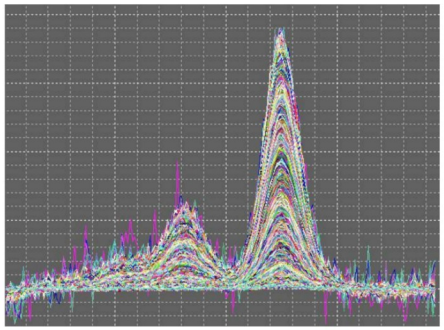
Make I(t)



Select typical curve

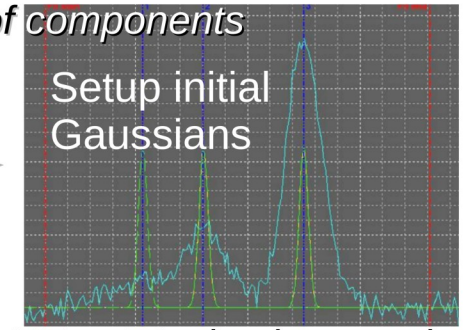
Linear (drift)
Integral (fouling)





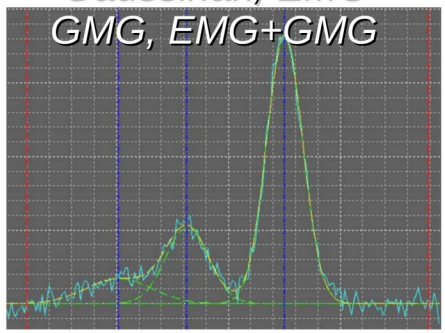
optional SVD to inform number of components

Select typical curve



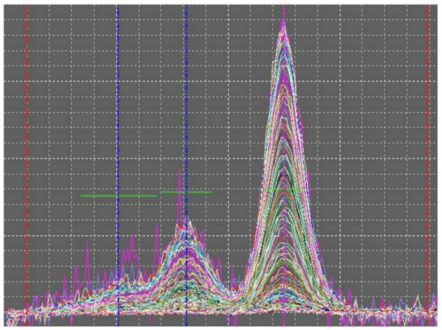
Gauss Fit

Gaussinan, EMG
GMG, EMG+GMG

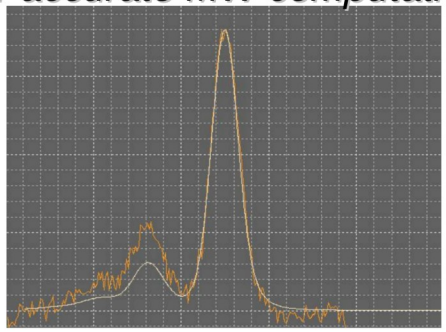


UV or RI, simultaneously fit for accurate MW computations

Global Gauss Fit



Opt. apply to conc. curve

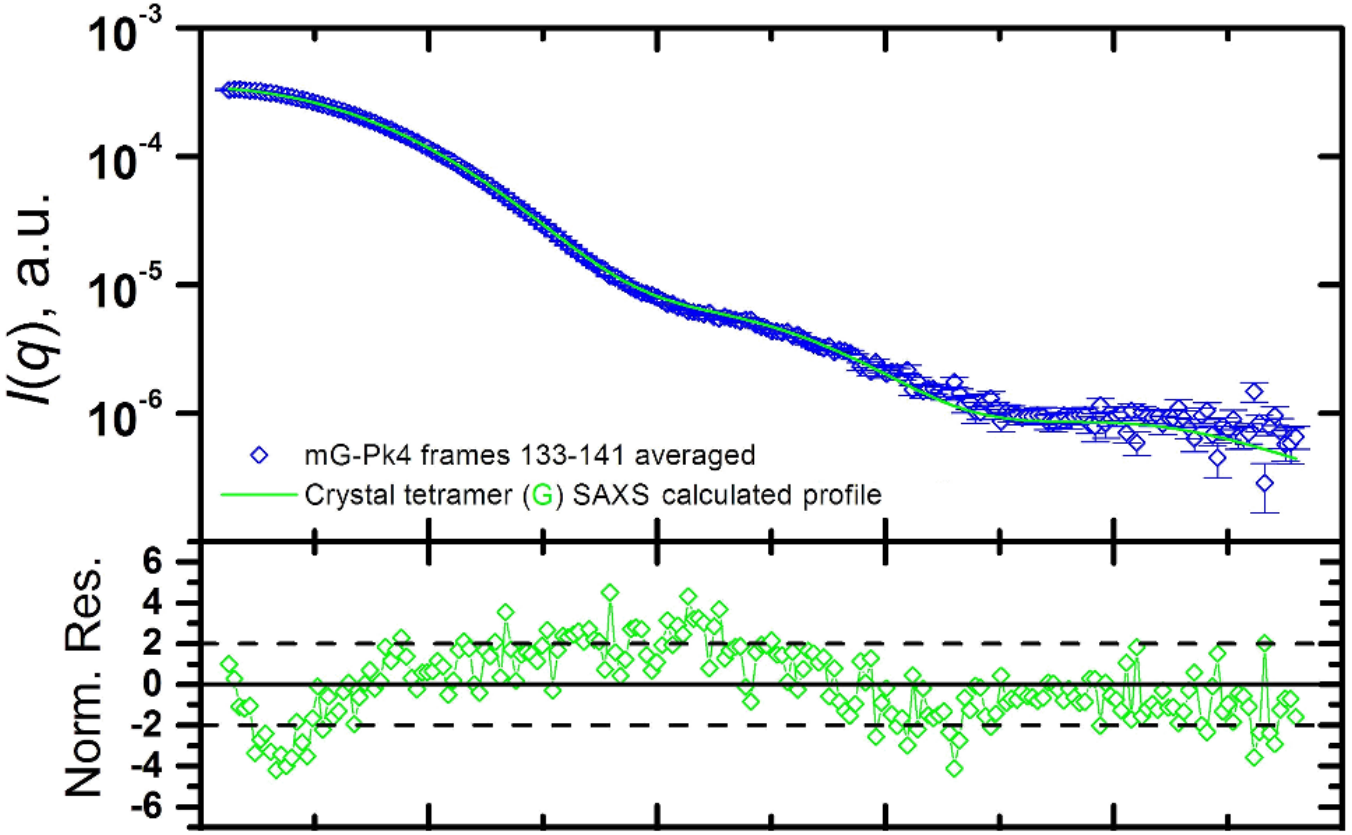


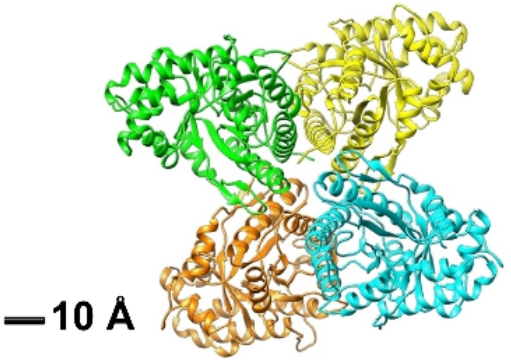
Make I(q)

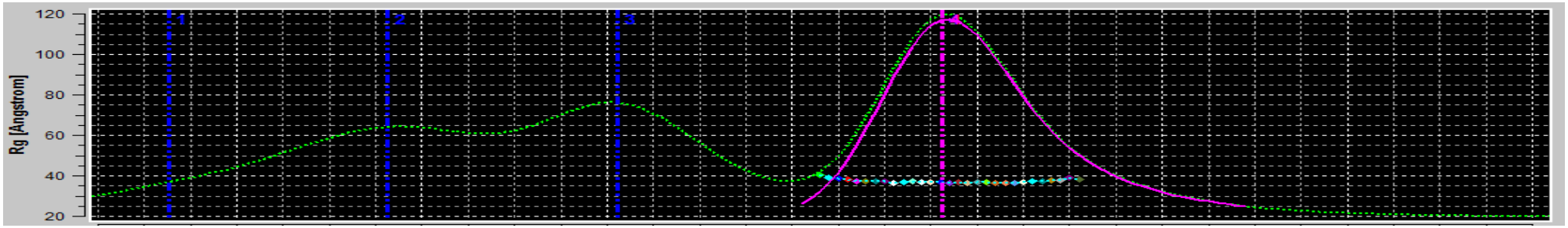
Set of I(q) curves for each Gaussian peak

Brookes et al, J. Appl. Cryst. 46 (2013) 1823-33
Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

Deconvolution of Aldolase & Model

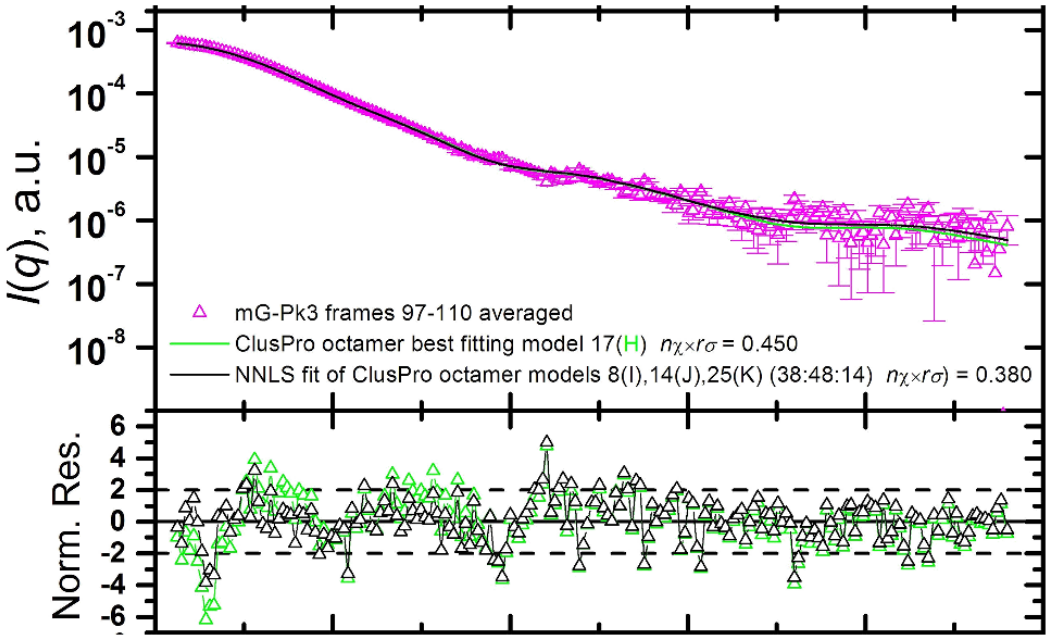


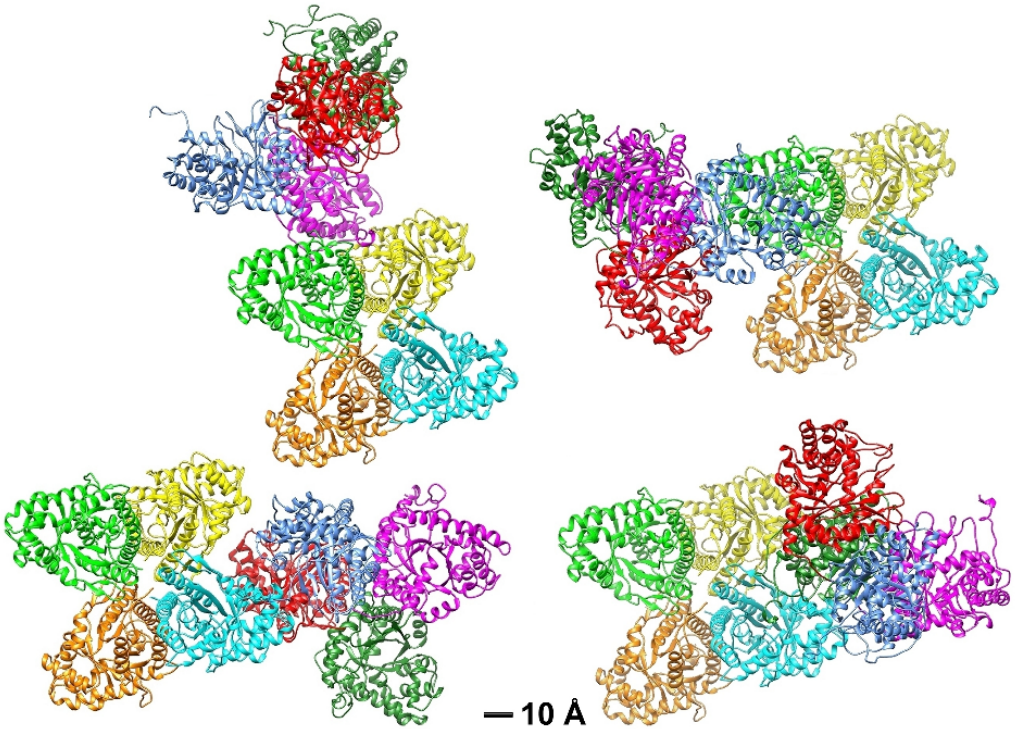


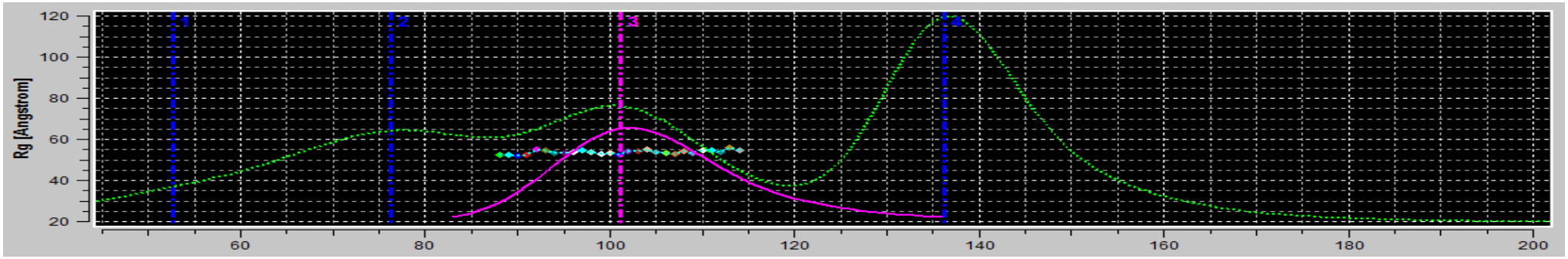


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

Deconvolution of Aldolase & Model

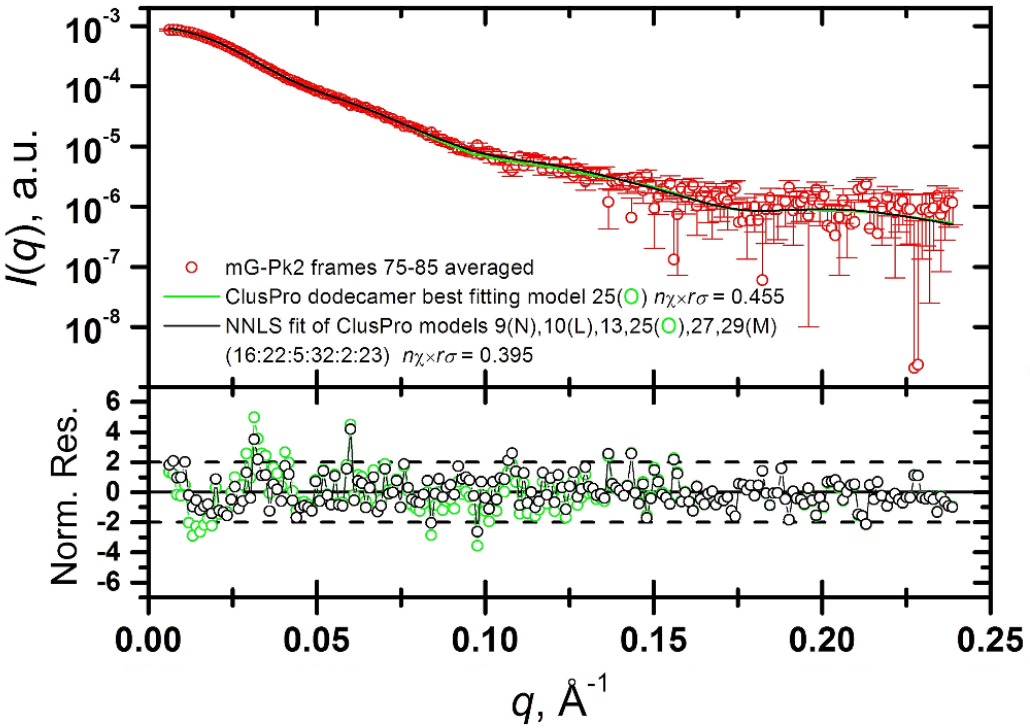


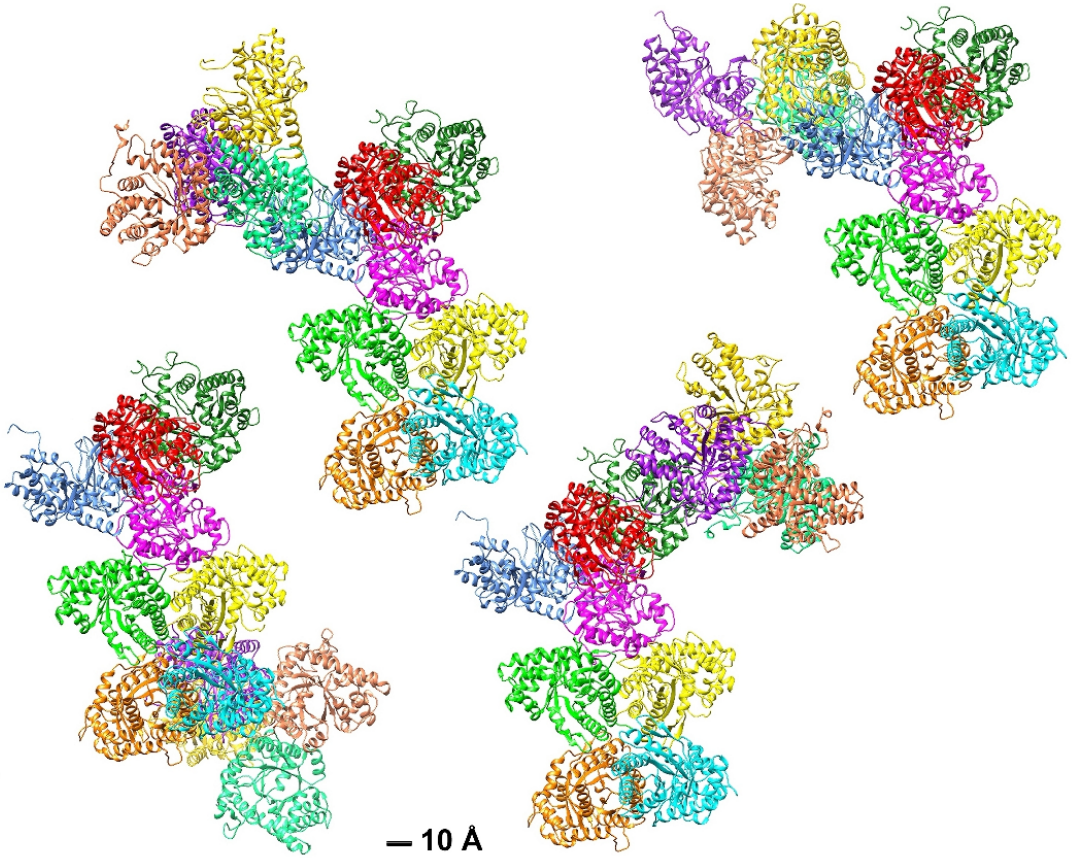


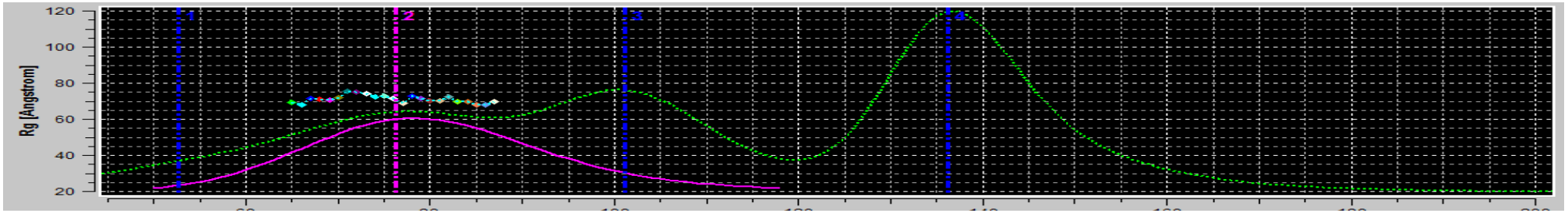


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

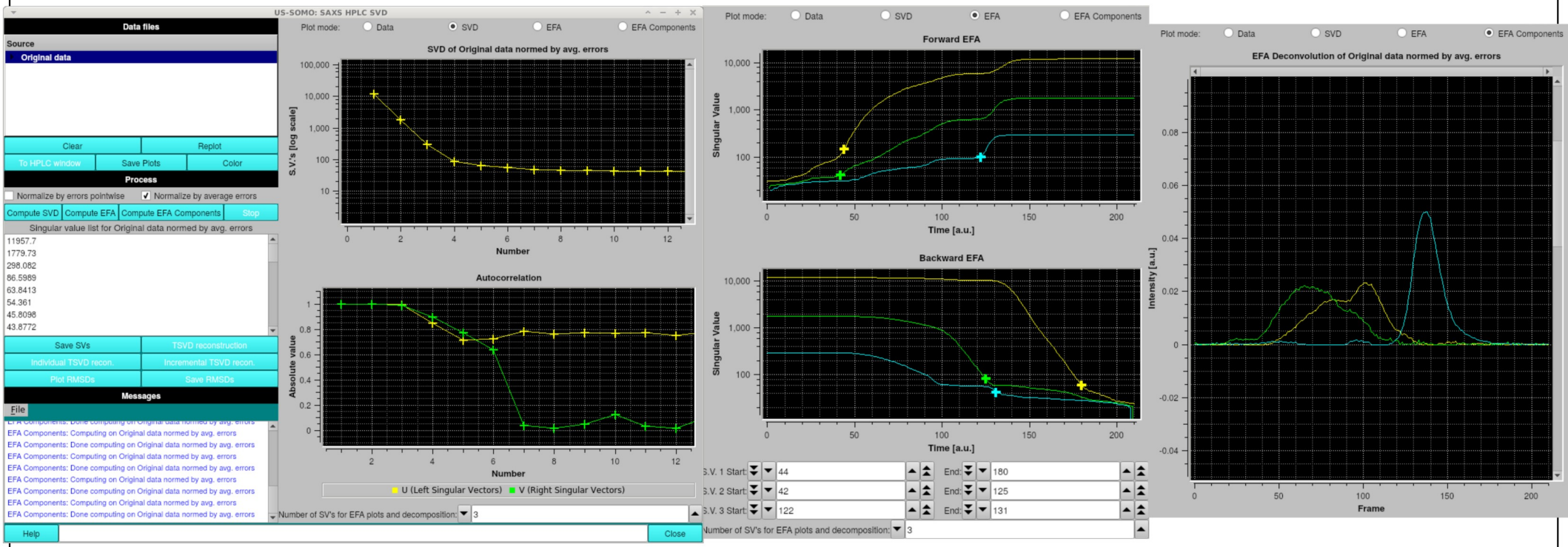
Deconvolution of Aldolase & Model





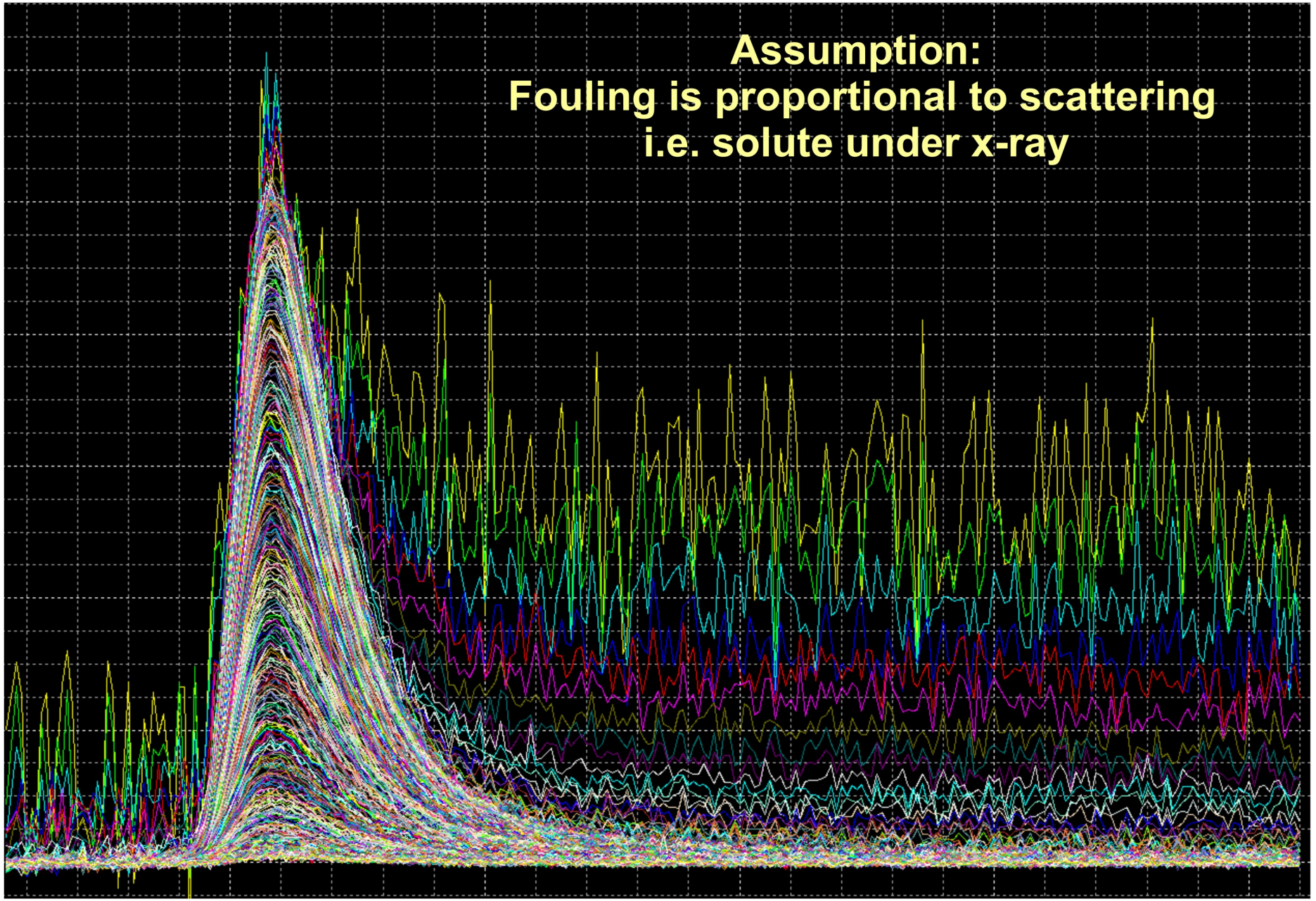


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

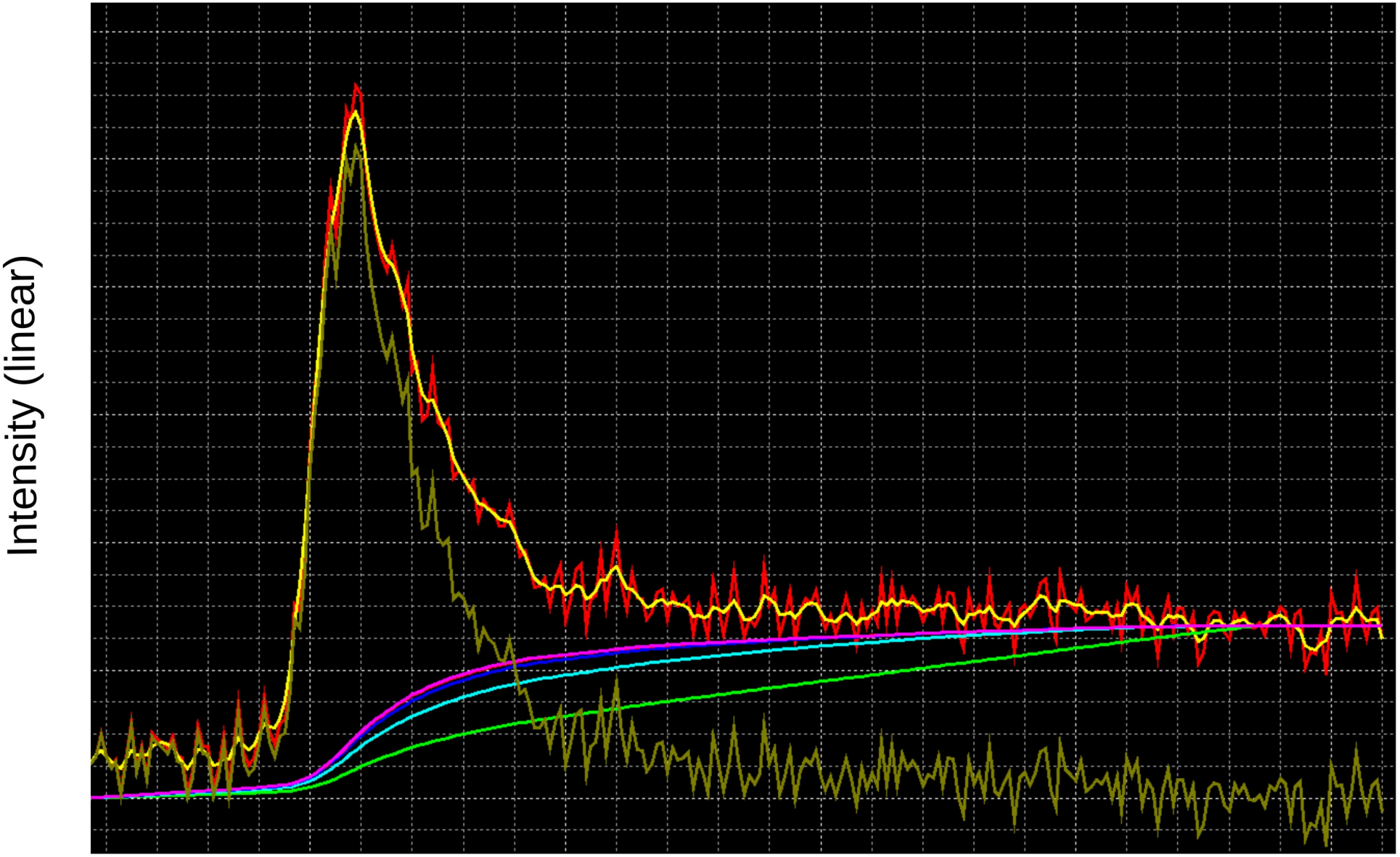


Hopkins et al. (2017) J. Appl. Cryst. 50(5) 1545-53
Meisburger et al. (2016) J. Am. Chem. Soc. 138 6506-16

Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

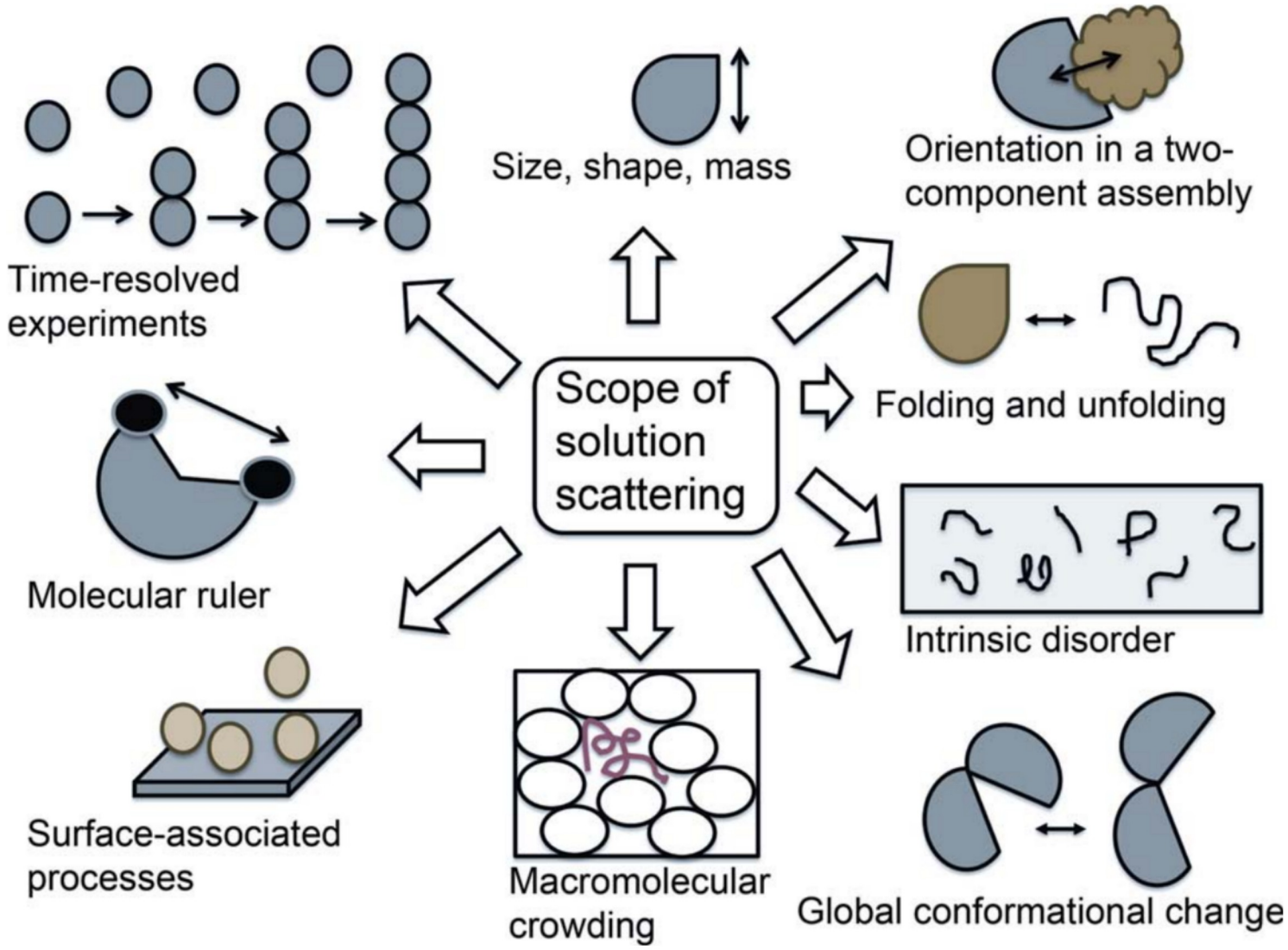


Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41



Brookes et al, J. Appl. Cryst. 49 (2016) 1827-41

- If you have sufficient sample – use SEC-SAXS for biological macromolecules
- If you have true baseline separation, excellent, you should probably be ok simply taking the peak data, but global Gaussian decomposition will use all of the data
- If you do not have true baseline separation, be very careful and you should use these techniques



B. N. Chaudhuri. Prot. Sci. 2015 24:267-276


Injector
Linac
e- Beam Transport
Undulator
Office Building
Near Experimental Hall
X-ray Transport
Far Experimental Hall

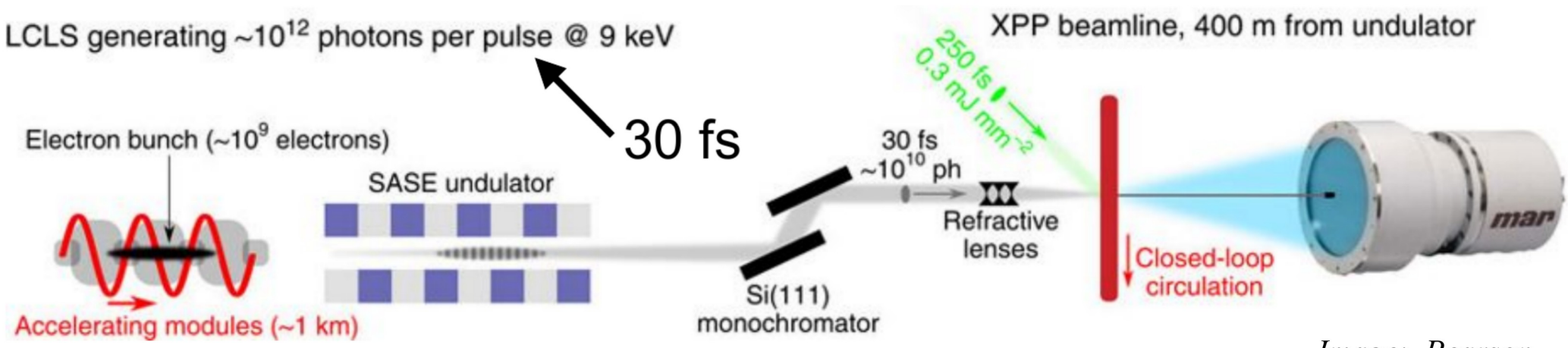
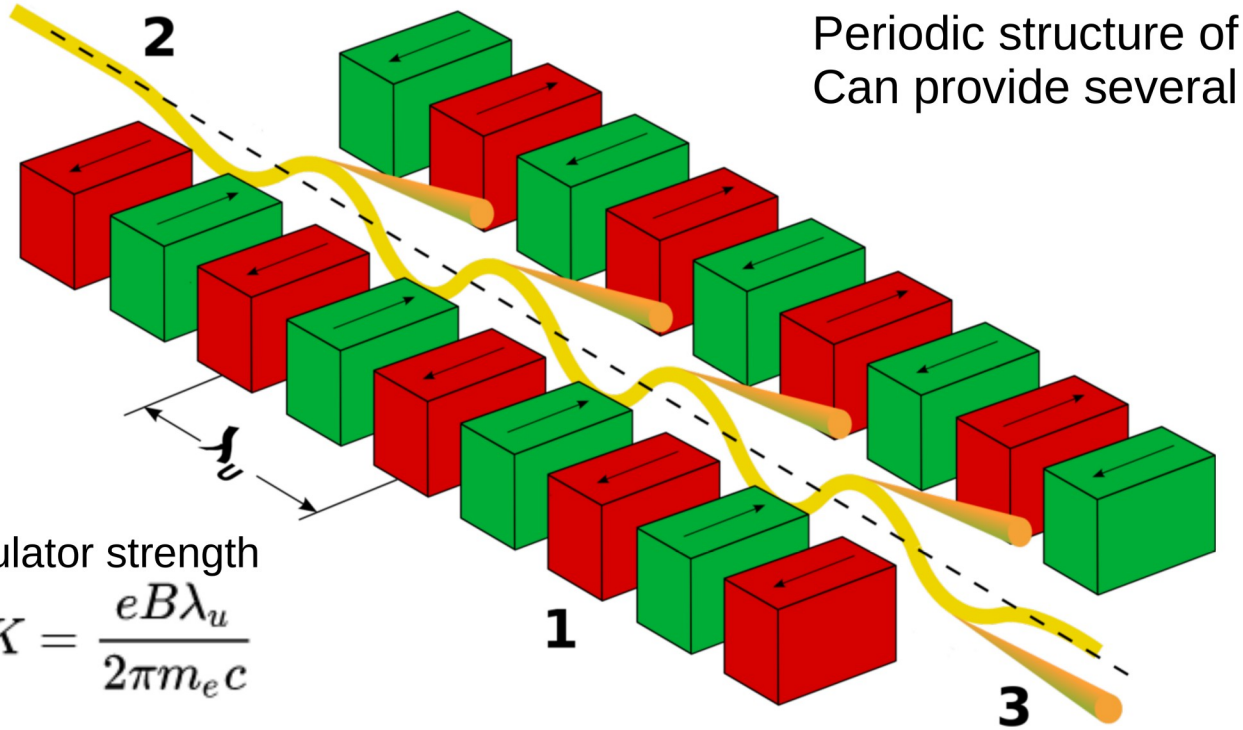


Image: Pearson



Periodic structure of dipole magnets
Can provide several orders of magnitude higher flux

Undulator strength

$$K = \frac{eB\lambda_u}{2\pi m_e c}$$

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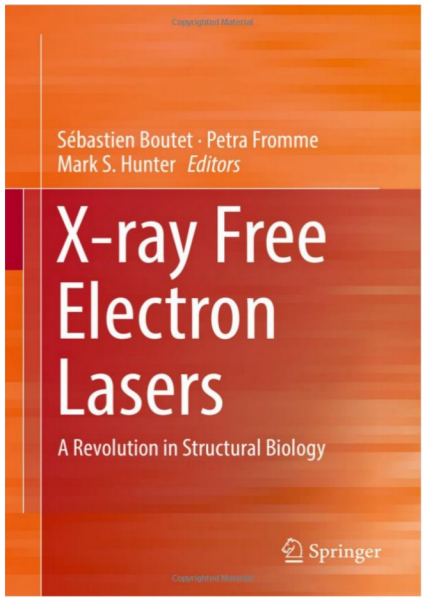

2 point correlations : $p(r)$ → 4 point correlations promises possibility of better 3d models

Still early days.

Few XFELs instruments

Serial access (vs synchrotron)

lightsources.org

European XFEL

Elettra Sincrotrone Trieste

FERMI at ELETTRA

DESY.

Flash at DESY

SLAC NATIONAL ACCELERATOR LABORATORY

LCLS at SLAC

PAL

POHANG ACCELERATOR LABORATORY

PAL-XFEL

PAUL SCHERRER INSTITUT PSI

Swiss FEL at PSI

Argonne NATIONAL LABORATORY

Beyond Rg Bio

https://small-angle.aps.anl.gov/future-courses#BeyondRgBio

OAK RIDGE National Laboratory

National School on Neutron and X-Ray Scattering

https://neutrons.ornl.gov/nxs

HERCULES European School

Organized by Université Grenoble Alpes & Grenoble Institute of Technology

http://hercules-school.eu/

EMBO

Practical courses

http://embo.org/funding-awards/courses-workshops/practical-courses

Books

"La diffraction des rayons X aux très petits angles: Application a l'etude de phénomènes ultramicroscopiques":

A. Guinier (1939), Ann. de Phys., 11:12

pdf in course papers

"Small Angle Scattering":

A. Guinier and A. Fournet, (1955), in English, ed. Wiley, NY

"Small Angle X-Ray Scattering":

O. Glatter and O. Kratky (1982), Academic Press.

pdf available

http://physchem.kfunigraz.ac.at/sm/Software.htm

"Structure Analysis by Small Angle X-ray and Neutron Scattering":

L.A. Feigin and D.I. Svergun (1987), Plenum Press.

pdf available

http://www.embl-hamburg.de/ExternalInfo/Research/Sax/reprints/feigin_svergun_1987.pdf

"Neutrons, X-Rays and Light, Scattering methods applied to soft condensed matter":

P. Lindner and T. Zemb Eds, (2002) Elsevier, North-Holland.

The Proceedings of the SAS Conferences held every three years are usually published in the Journal of Applied Crystallography