Presenter: Emre Brookes

Topic:

Small angle X-ray and Neutron Scattering

Outline - SAS

- Experimental setup
- Theory
- Modeling
- Software
- Practical considerations, Sample preparation etc.
- Possibly other techniques introduced as time permits

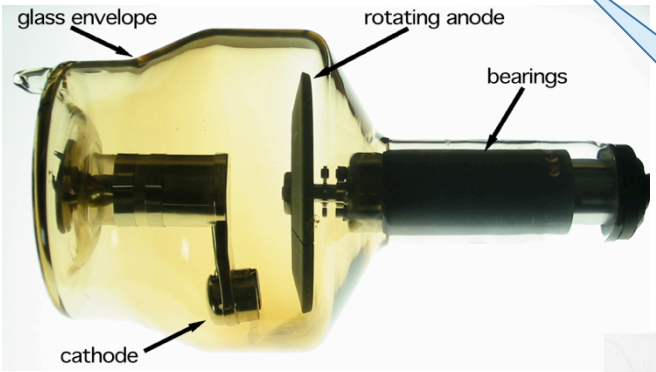
Experimental setup

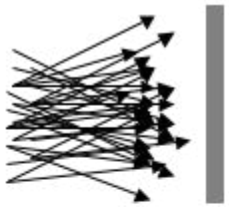
X-Ray Source

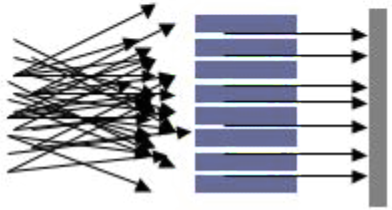
Collimation

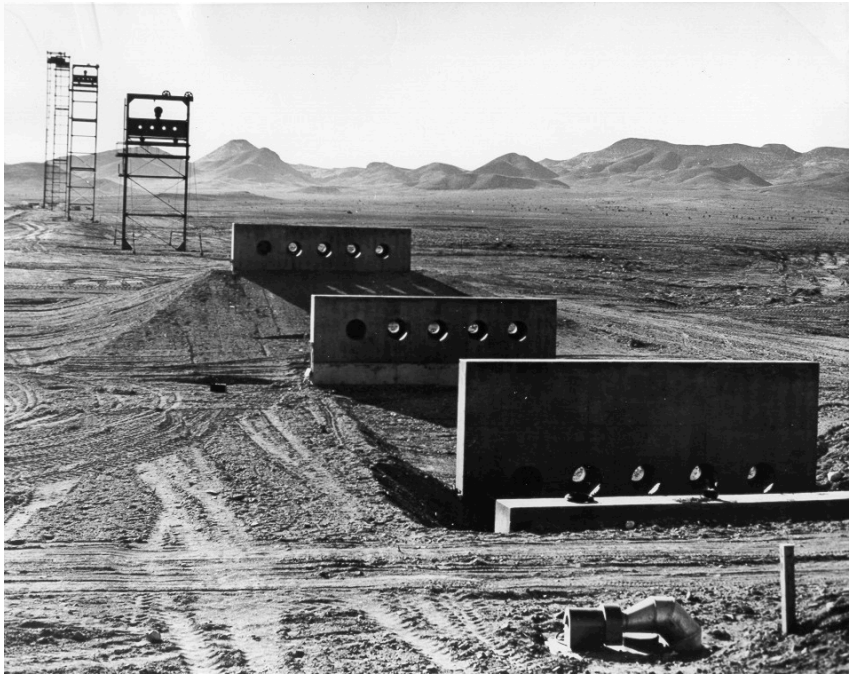
Sample

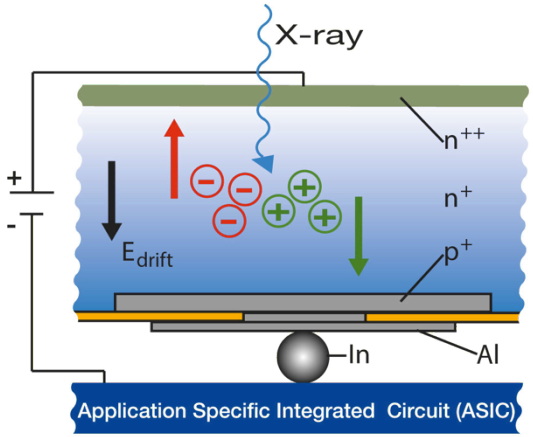
Detector





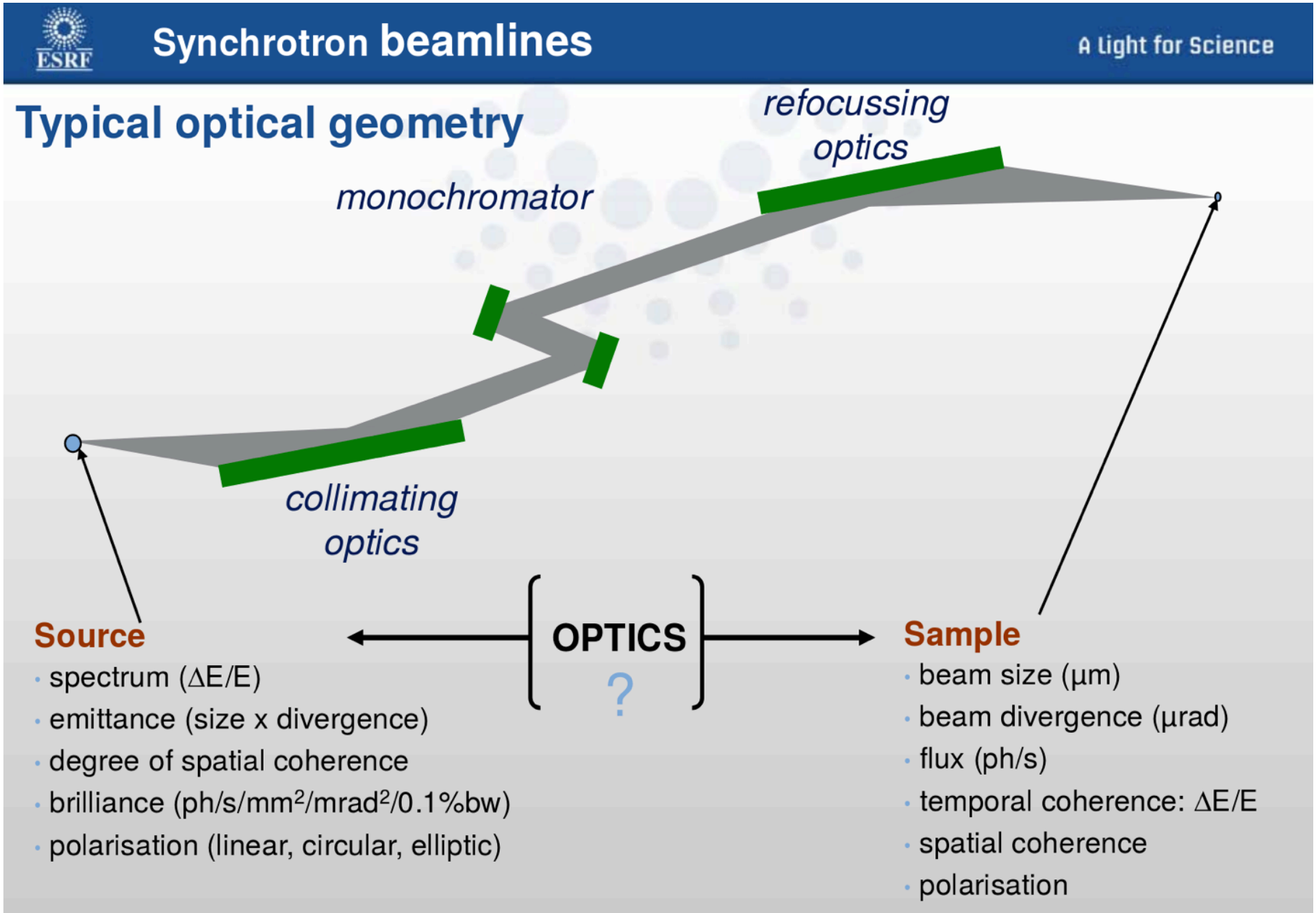






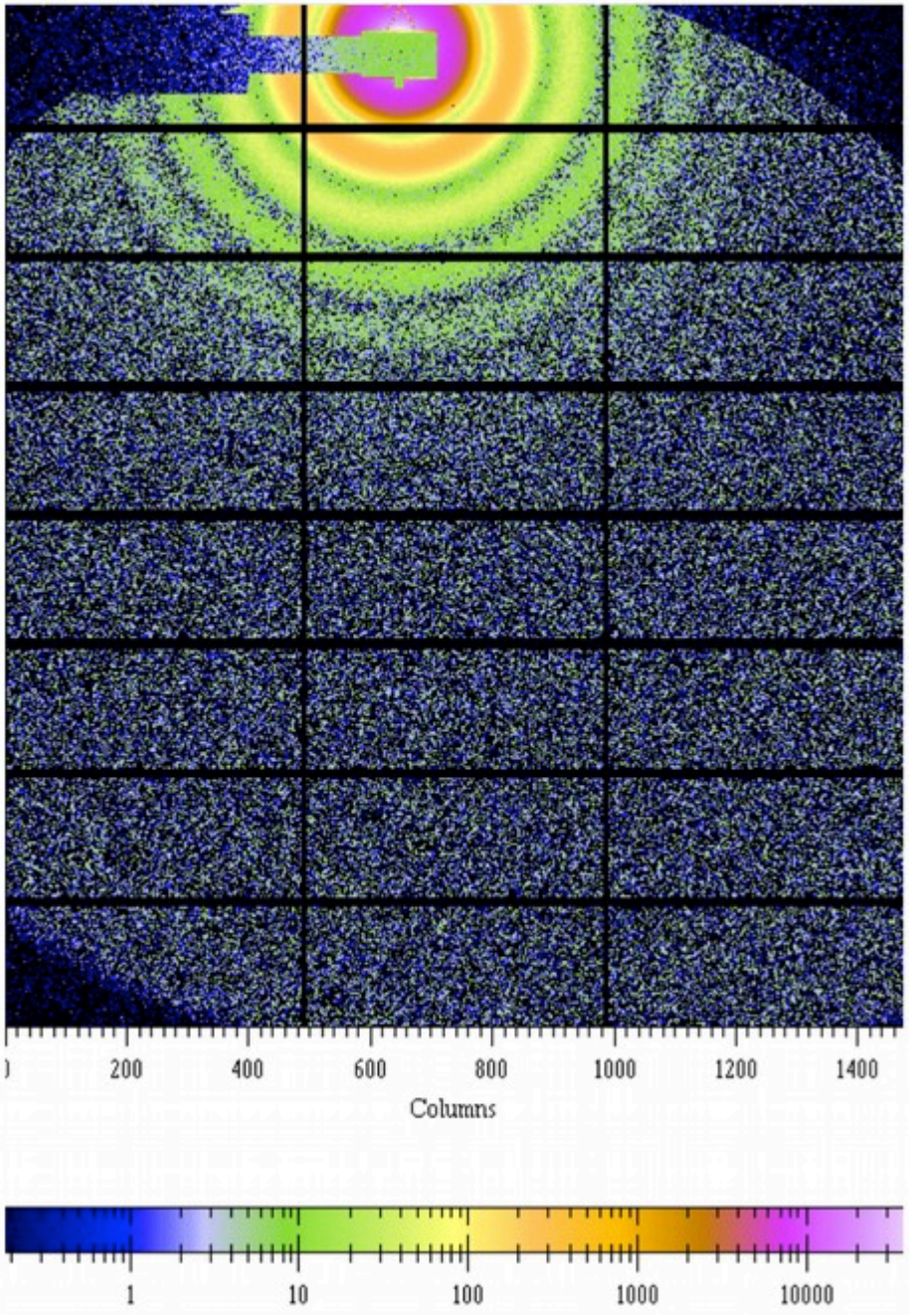
https://www.dectris.com/

Experimental setup



http://www.epn-campus.eu/fileadmin/_migrated/content_uploads/16.BARRETT_XRayOptics.pdf

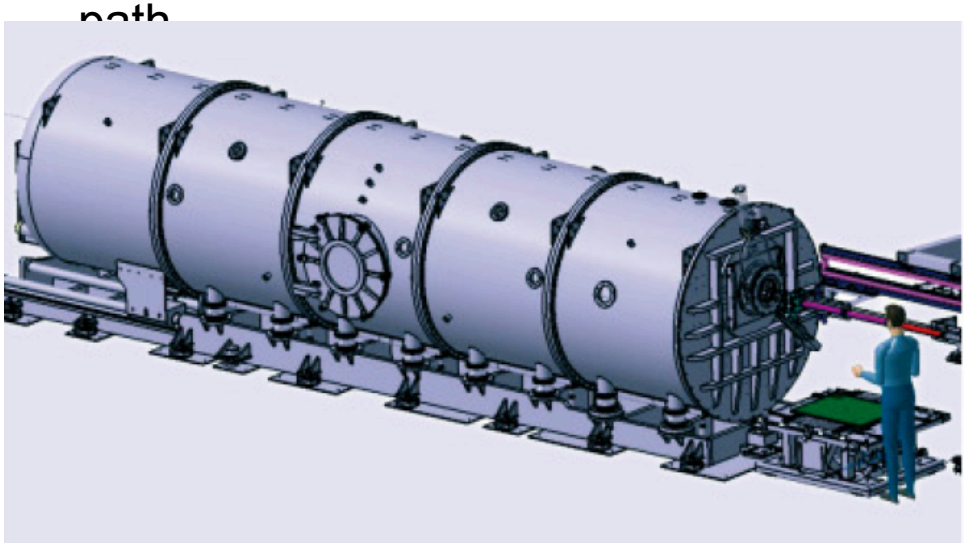
Experimental setup

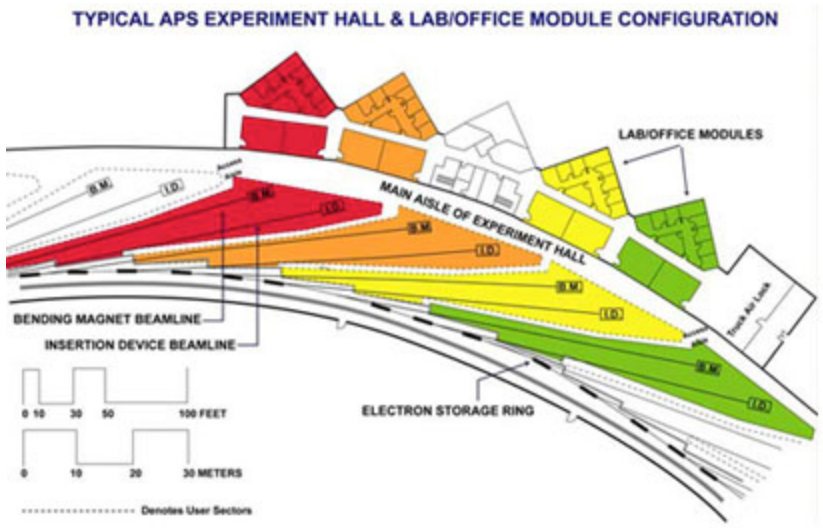


X-ray detector

Synchrotron SAXS

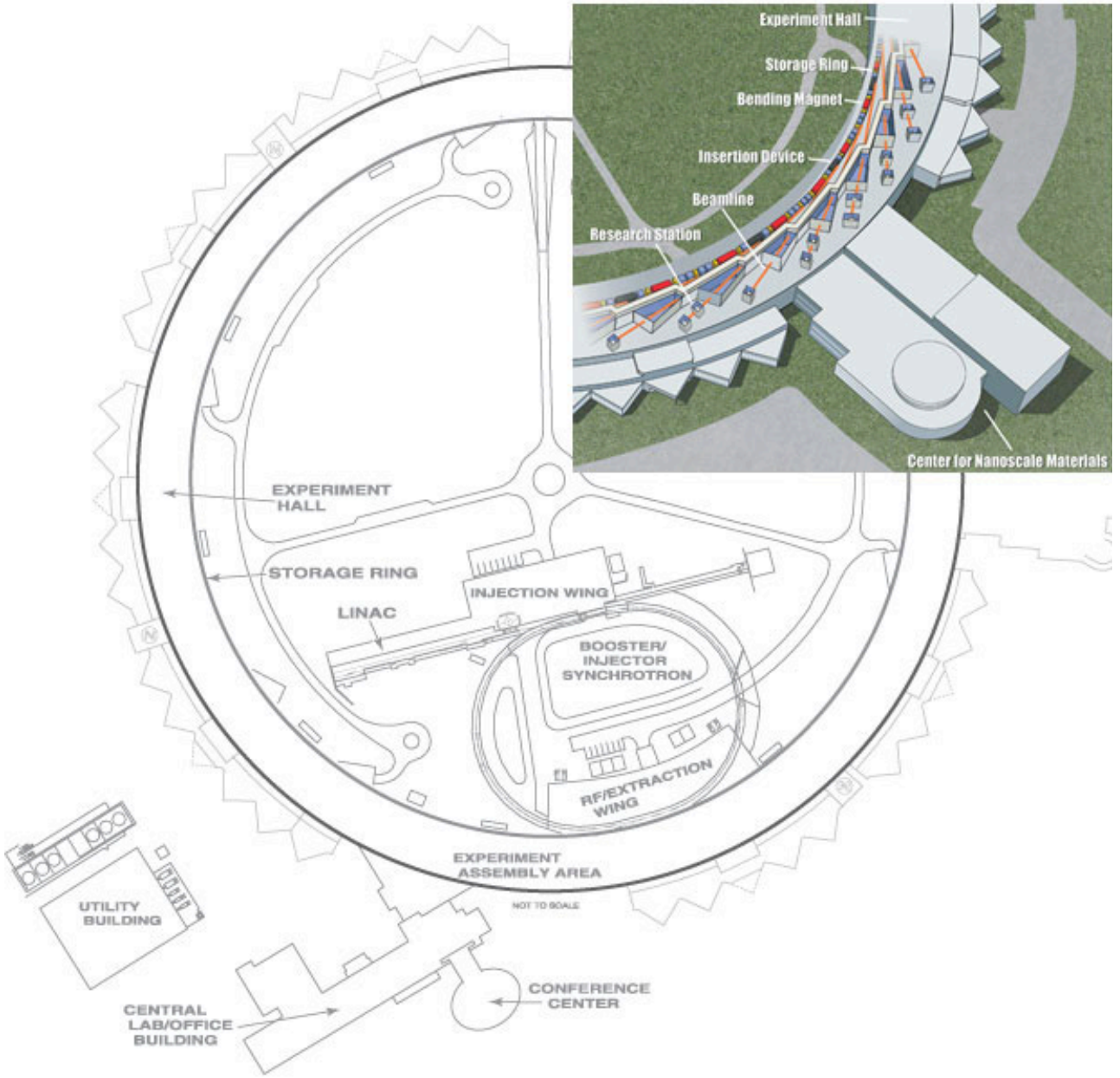
- APS
- The Electron Storage Ring
- The 7-GeV electrons are injected into the 1104-m-circumference storage ring, a circle of more than 1,000 electromagnets and associated equipment, located in a radiation-proof concrete enclosure inside the experiment hall. A powerful electromagnetic field focuses the electrons into a narrow beam that is bent on a circular path





http://www.aps.anl.gov/About/APS_Overview/

Synchrotron SAXS



http://www.aps.anl.gov/About/APS_Overview/

Synchrotron SAXS

APS SAXS beamlines

List of APS SAXS beamlines and their capabilities

Beamline	Experiments	Energy Range [keV]	Q range [1/A]	Operating group	Contact person
1ID	High Energy SAXS; Simultaneous SAXS/WAXS	50-100	0.005 - 0.4 1-10	XSD	Jon Almer, 630-252-1049
12-BM	SAXS, Solution scattering	5 - 23 keV	0.008 - 1	XSD-CMS	Sungsik Lee, 630-252-7491
12-ID-B	simultaneous SAXS/WAXS, bioSAXS, Grazing incidence SAXS, solution scattering	7 - 14 keV	0.003 - 2.8	XSD-CMS	Byeongdu Lee, 630-252-0395 Xiaobing Zuo, 630-252-1553
12-ID-C	SAXS/WAXS, ASAXS, Grazing incidence SAXS, Time-resolved SAXS	4.5 - 36 keV	0.006 - 2.0	XSD-CMS	Soenke Seifert, 630-252-0391
14-ID	Time-resolved SAXS	7 - 17 keV, pink beam 3% bandpasss	0.02 - 3.5	BioCARS	Irina Kosheleva 630-252-0467
15-ID	SAXS	5 - 70 keV	0.003 - 0.5	ChemMatCARS	Mrinal Bera collaborations only, no GUP
18-ID	SAXS/WAXS, static and time-resolved, Biological samples, solutions, 2D focused beam	3.5 - 40	0.002 - 3.0	BIOCAT	Tom Irving, 630-252-0524

$$E = \frac{hc}{\lambda}$$

3.5 to 0.31 Angstrom

https://small-angle.aps.anl.gov/aps-saxs-beamlines

Reactor SANS

SANS Instruments: For bioSANS we use ‚cold neutrons.'

Controlled fission: nuclear reactor: 'hot' neutrons – high energy, multiple λ.

20-30 m

10-40 m

Moderators: 'cool' the neutrons down (lower energy)

Collimation guides and apertures (neutron guides): plane wave geometry

Sample position

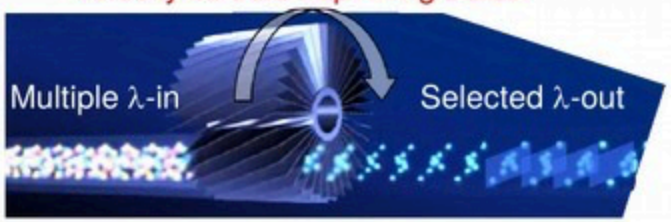
Detector tube (vacuum)

Movable detector

Velocity selector: fine tunes neutron energies (λ)

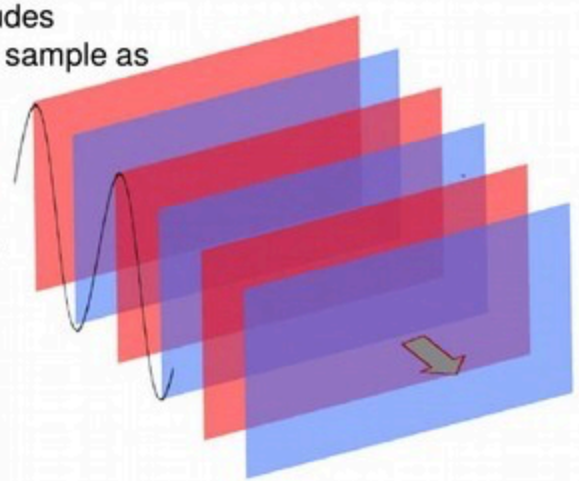
$n + {}^3He \rightarrow {}^3H + {}^1H(p) + 0.764$ MeV

Velocity selector: spinning blade.



Quokka and Bilby, ANSTO.

Neutron wave amplitudes propagate toward the sample as a plane wave.



'cold' neutrons: biology
λ = 4 to 7 Å

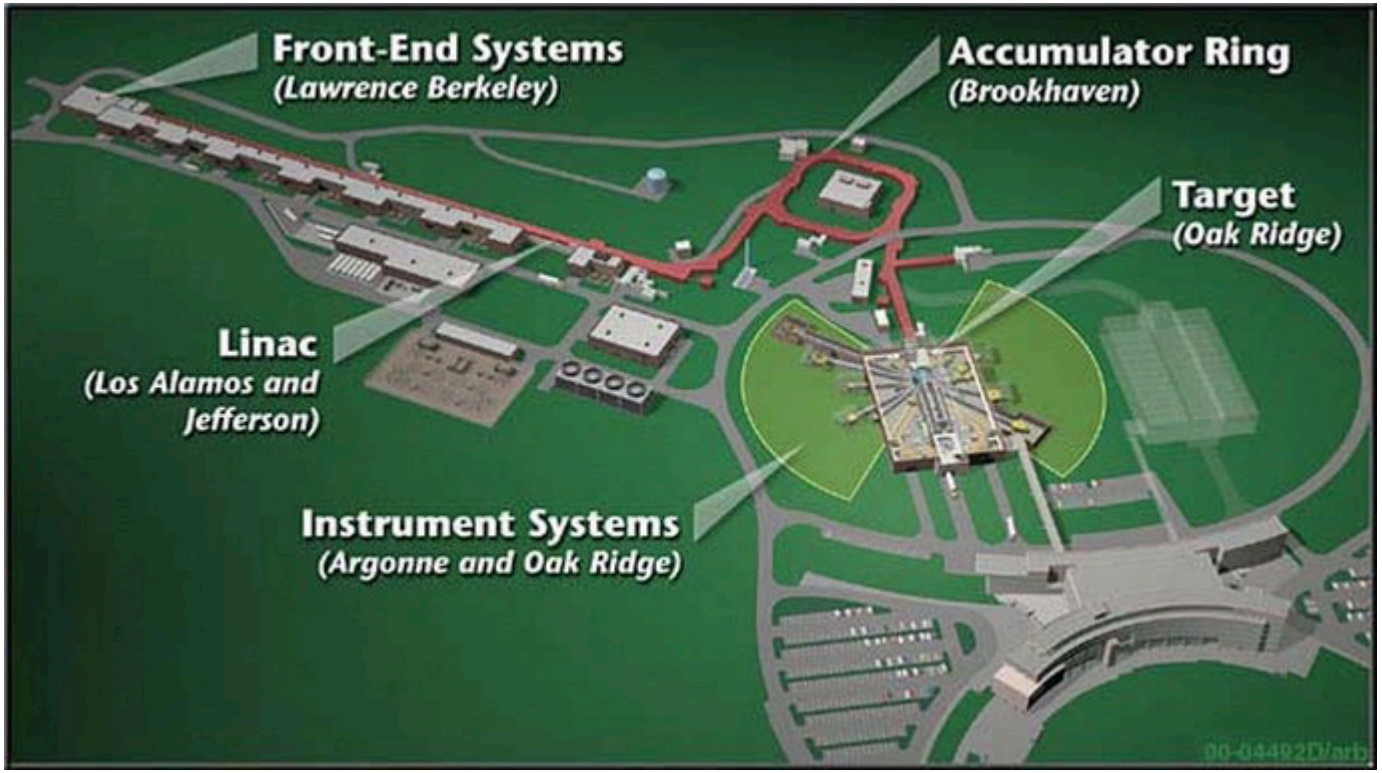
Wood et al., (2015) *Nucl. Instrum. Meth. A* 798:44–51

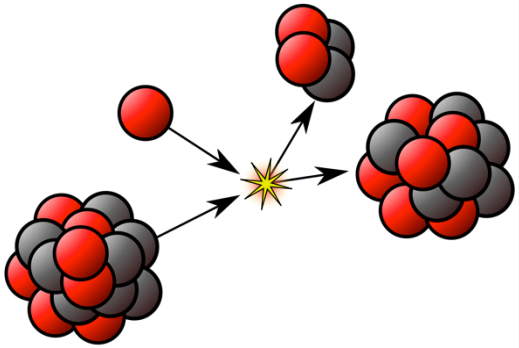
Courtesy Cy Jeffries, EMBL

Spallation SANS

SNS – Oak Ridge

Negatively charged hydrogen ions are produced by ion source.
Hydrogen ions injected into linear particle accelerator (~90% c)
H^- ions pass through a foil, leaving protons.
Protons enter accumulator ring, accumulate in “bunches”
“bunches” released as pulse
Pulses strike a target of liquid mercury, where spallation occurs.
Spalled neutrons slowed down and guided to beam lines.

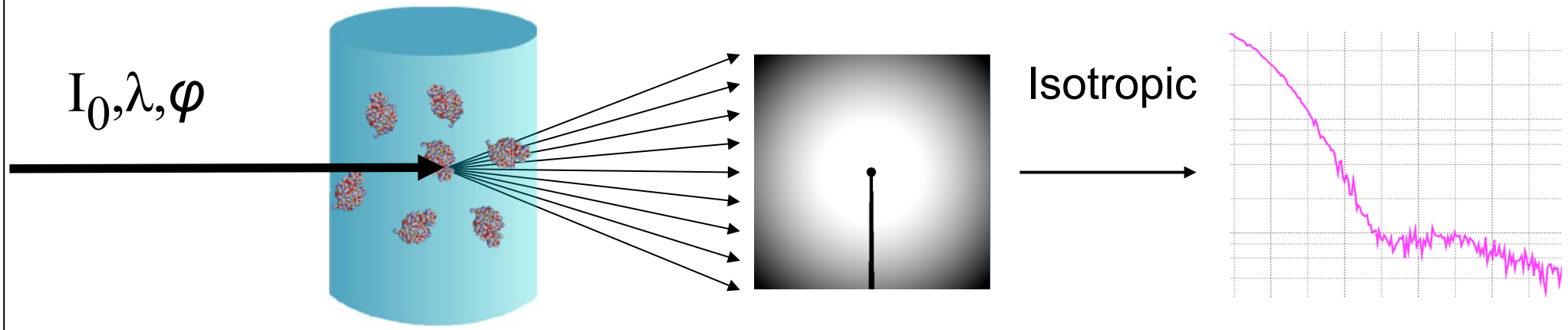




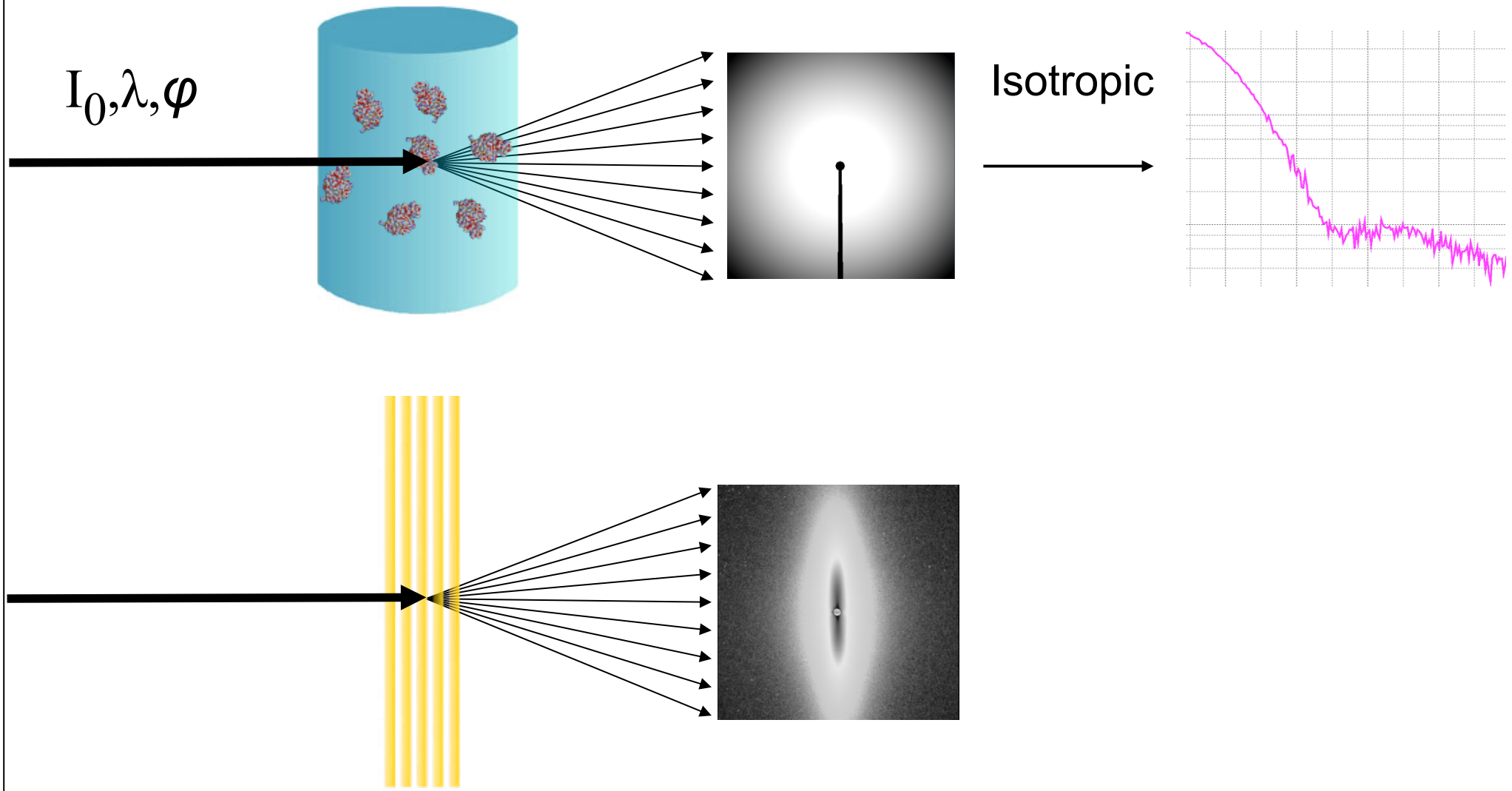
By Kjerish - Own work, CC BY-SA 4.0, https://commons.wikimedia.org/w/index.php?curid=54378478

ISIS – RAL
ESS – Lund 2025
Proton “bunches” spall off Tungsten

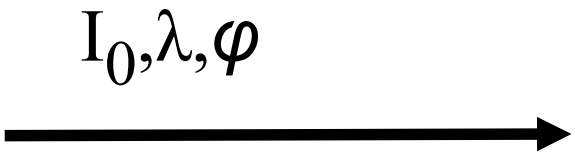
Solution BioSAXS

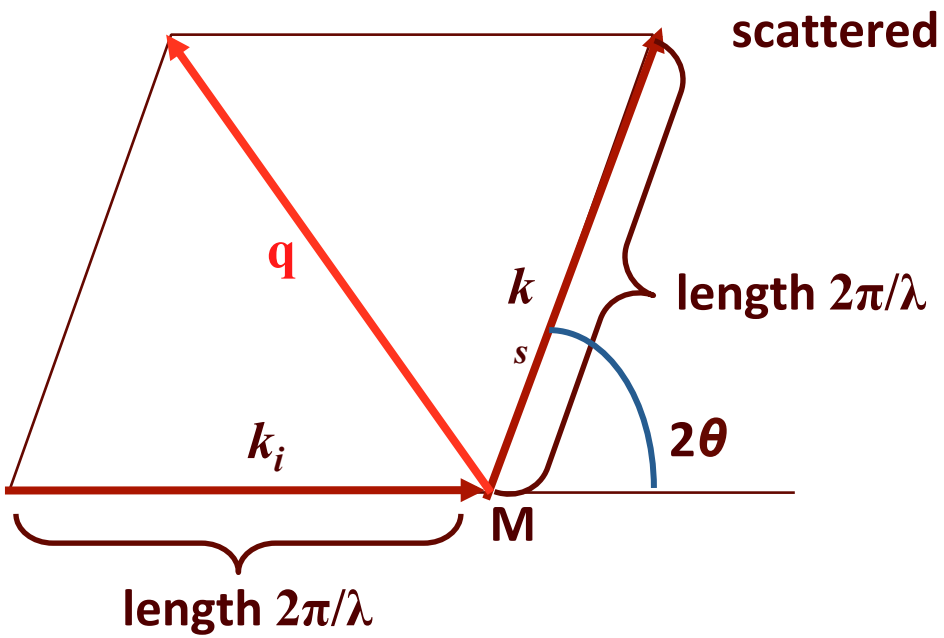


Solution BioSAXS vs Fibre SAXS



Momentum transfer





$$|\mathbf{k}_i| = |\mathbf{k}_s| = \frac{2\pi}{\lambda}$$

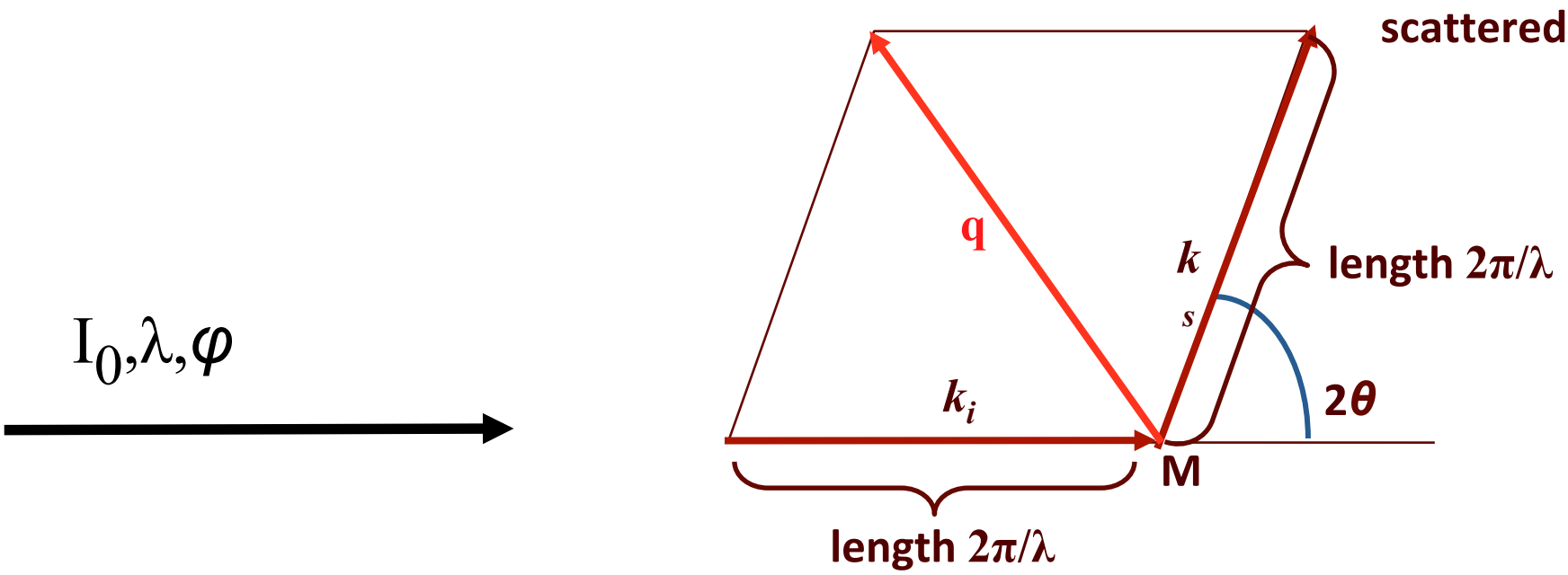
"wavevector" $\mathbf{k}$

$$\mathbf{q} = \mathbf{k}_s - \mathbf{k}_i$$

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

"momentum transfer" q

Momentum transfer N.B.



$$s = \frac{2\sin(\theta)}{\lambda}$$

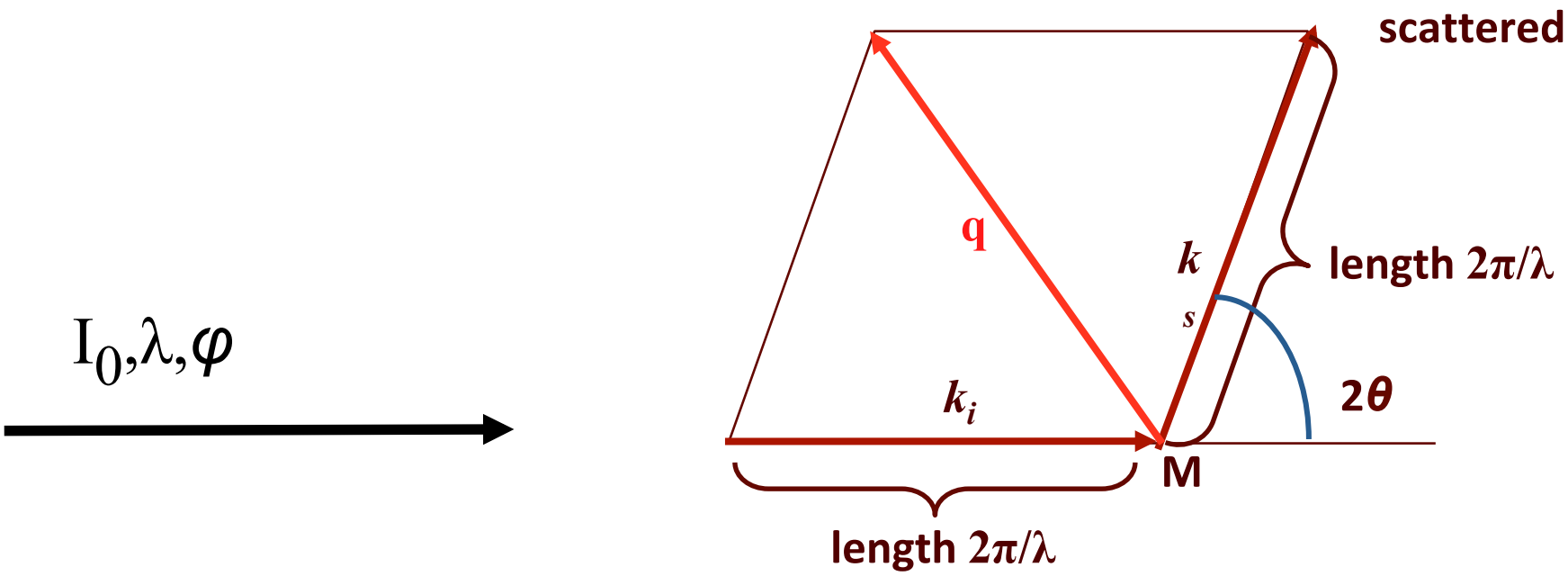
"momentum transfer" s

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

"momentum transfer" $s=q$ *D. Svergun and coll.*

Always report the momentum transfer used!

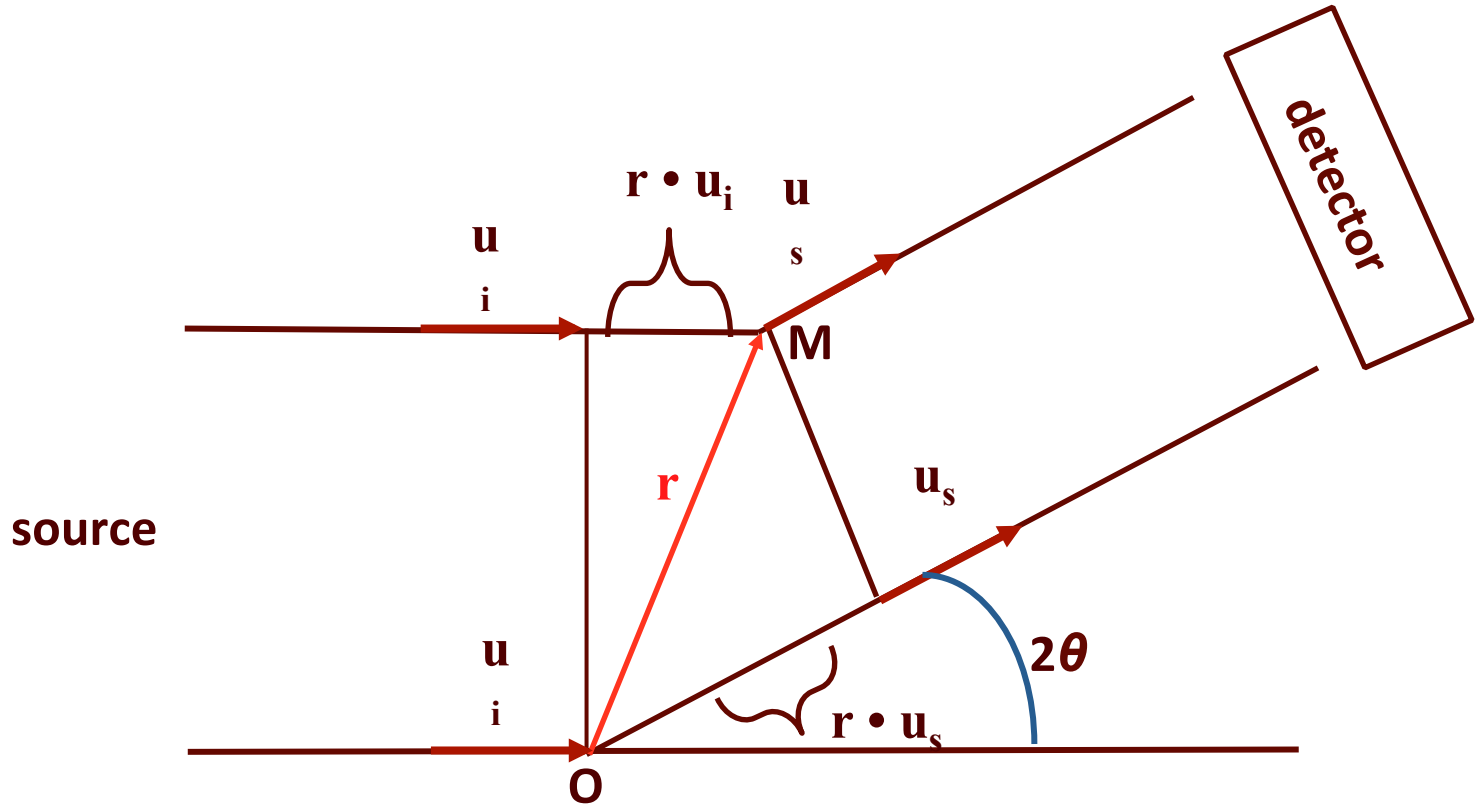
Momentum transfer N.B.



"momentum transfer" q or s may be in units of 1/Angstrom or 1/nm

Always report the units used!

Scatterers not at the origin → Phase difference



Path difference = r • u_s – r • u_i = r • (u_s - u_i) corresponds to a phase difference:

$$\phi = \frac{2\pi \mathbf{r}\cdot(\mathbf{u}_s - \mathbf{u}_i)}{\lambda} = \mathbf{r}\cdot\mathbf{q} = 2\pi\,\mathbf{r}\cdot\mathbf{s} \quad \text{or} \quad \mathbf{r}\cdot\mathbf{s}$$

D. Svergun and coll.

$A(q)$ is the scattered amplitude by the scatterer at the origin

$A(q)e^{\mathbf{i}\phi}$ is the scattered amplitude by the scatterer at the position **r**

Scattering by assemblies of scatterers

Given a collection of N scatterers with scattering factors f_i, at positions $\mathbf{r}_i$ we define:

$$\mathbf{F}(\mathbf{q}) = \sum_{i=1}^{N} f_i \, \mathbf{e}^{\mathbf{i} r_i q}$$

Given a continuous electron density, $\rho(\mathbf{r})$, we define:

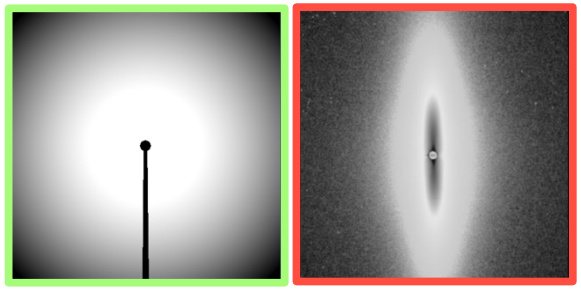
$$\mathbf{F}(\mathbf{q}) = \int_{V_\mathbf{r}} \rho(\mathbf{r}) \mathbf{e}^{\mathbf{i} r q} \, dV_\mathbf{r}$$

The real space intensity is in either case:

$$\mathbf{I}(\mathbf{q}) = \mathbf{F}(\mathbf{q}) \cdot \mathbf{F}^*(\mathbf{q})$$

Scattering by assemblies of scatterers under special conditions

Assuming the system is statistically isotropic:



spherical average $\left\langle e^{i\mathbf{r}\cdot\mathbf{q}}\right\rangle = \frac{\sin(rq)}{rq}$ *Debye 1915*

Peter J. W. Debye
1884-1966

The real space intensity *I(q)* can be simplified to real number computations:

$$F(q) = \sum_{i=1}^{N} f_i \frac{\sin(r_i q)}{r_i q}$$

$$I(q) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i f_j \frac{\sin(r_{ij} q)}{r_{ij} q}$$

Glatter, O. and Kratky, O. "Small Angle X-Ray Scattering" 1982

Scattering factors

What scatters?

X-Ray source → electrons
Neutron source → nuclei

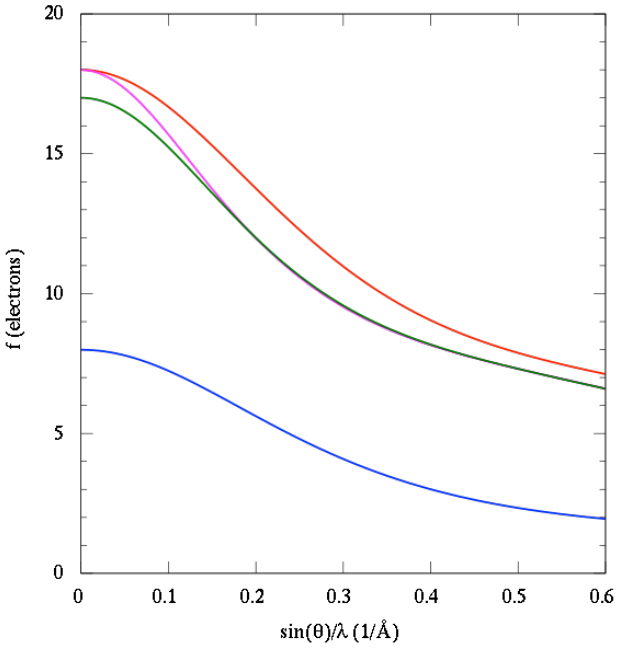
For X-Rays: one can compute atomic form factors from the electron density

$$f(q) = \int_{V_\mathbf{r}} \rho(\mathbf{r})\, dV_\mathbf{r}$$

These are typically taken from tables of Gaussian coefficients

$$f(q) = \sum_k a_k \mathbf{e}^{-b_k\left(\frac{q}{4\pi}\right)^2} + c$$

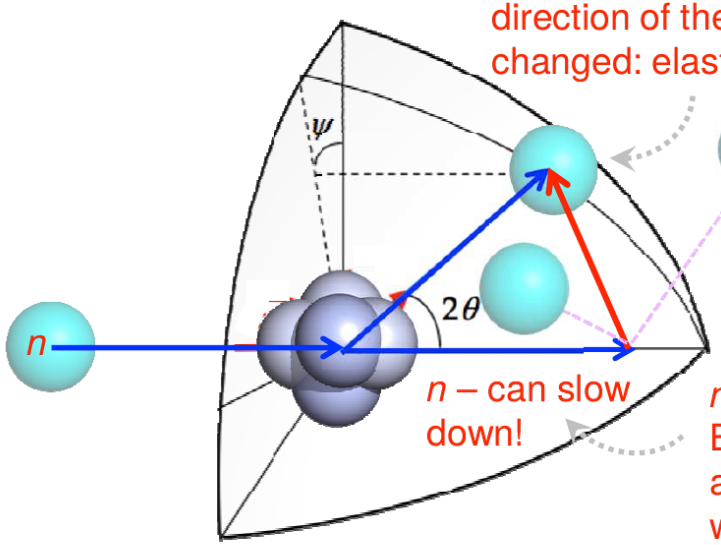
from the International Tables for Crystallography (4 terms) or *Waasmaier and Kirfel, Acta Cryst. A51:416-431, 1995* (5 terms)



X-ray atomic form factors of oxygen (blue), chlorine (green), Cl− (magenta), and K+ (red); smaller charge distributions have a wider form factor.

Scattering factors X-rays vs Neutrons

$$b_n = b_p + b_s$$



n – no energy change. Only the direction of the wave is changed: elastic scattering!

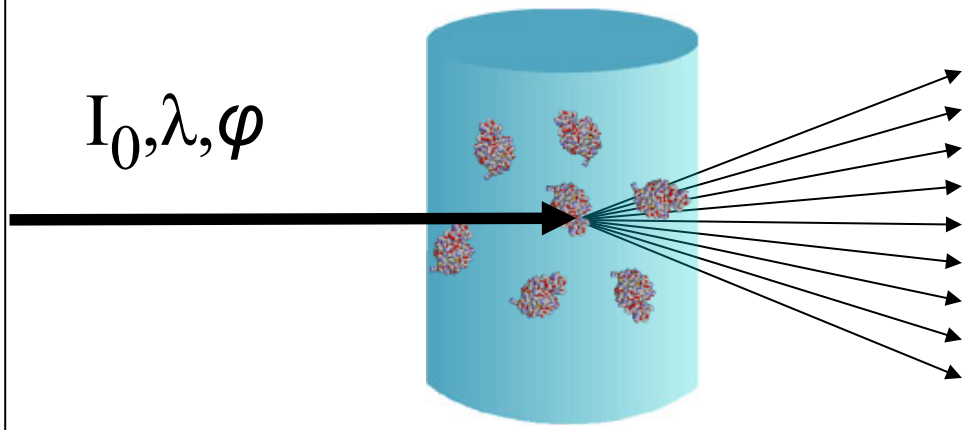
n – can speed up!

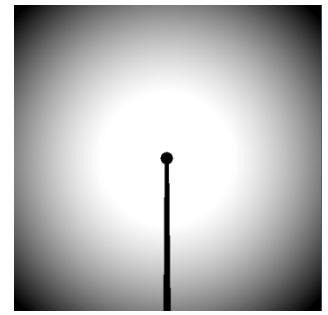
n – can slow down!

n – energy change. Both the direction and magnitude of the wave is changed. Inelastic scattering!

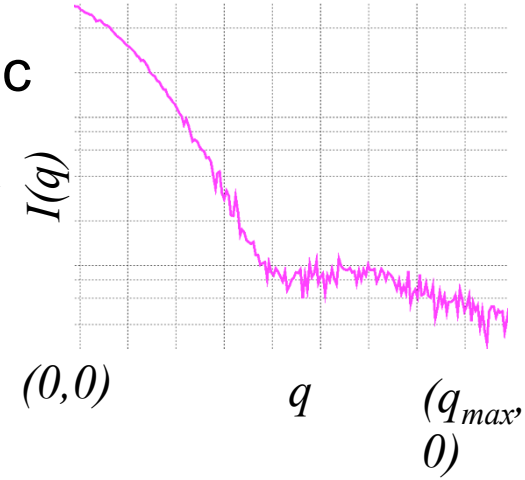
Courtesy Cy Jeffries, EMBL

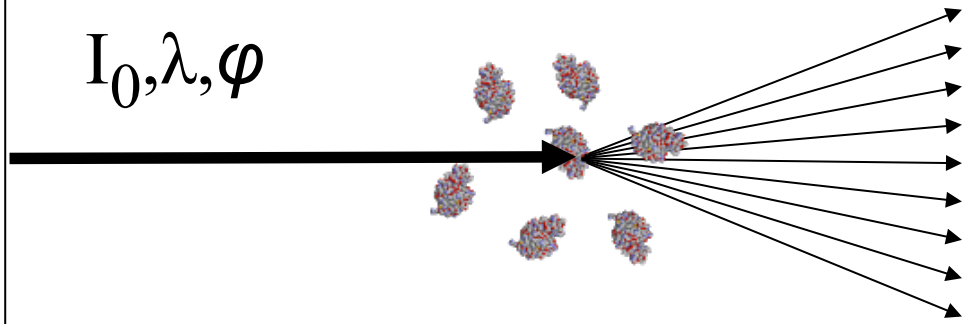
What we have so far





Isotropic



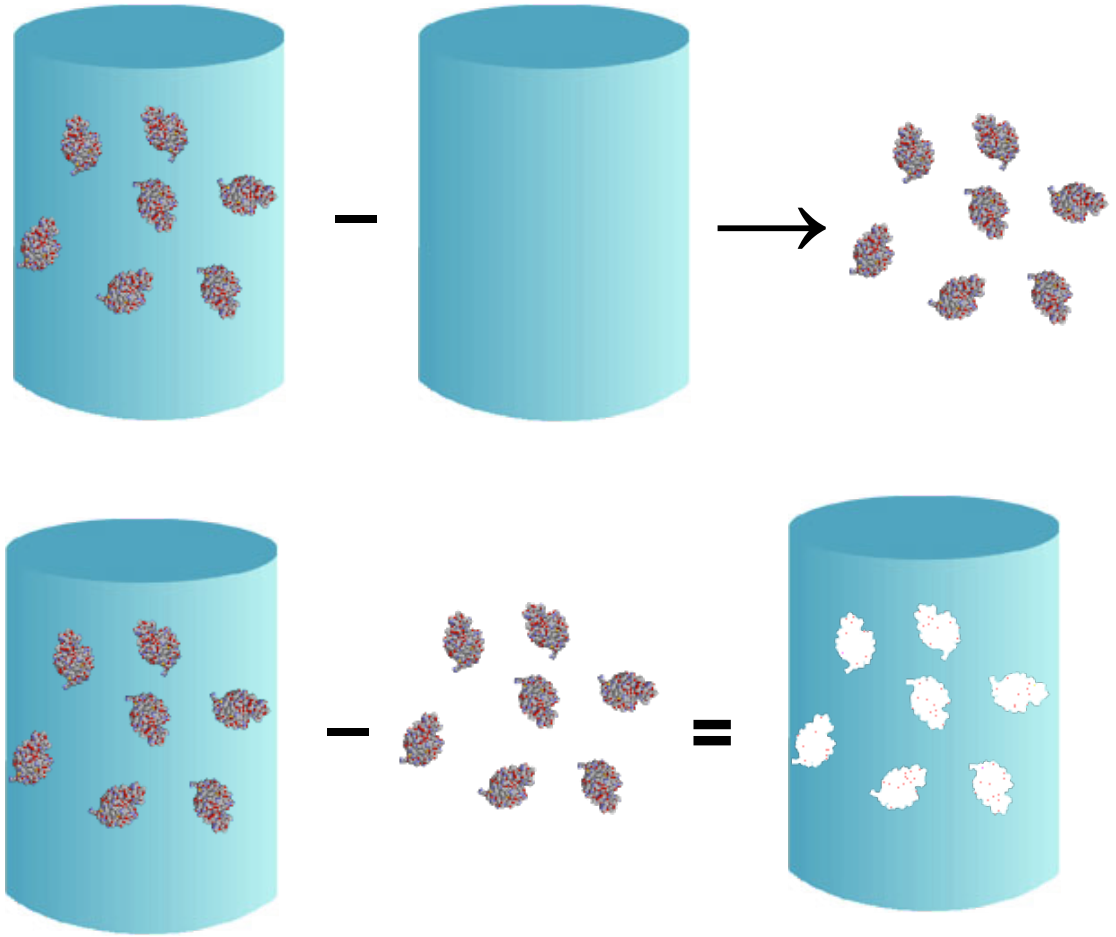


$I(q)$

Assuming statistically isotropic, monodisperse and sufficiently dilute (ideality - no correlation between molecules)

$$I(q) = N_{\text{molecules}} \sum_{i=1}^{N} \sum_{j=1}^{N} f_i f_j \frac{\sin(r_{ij} q)}{r_{ij} q}$$

The buffer also scatters



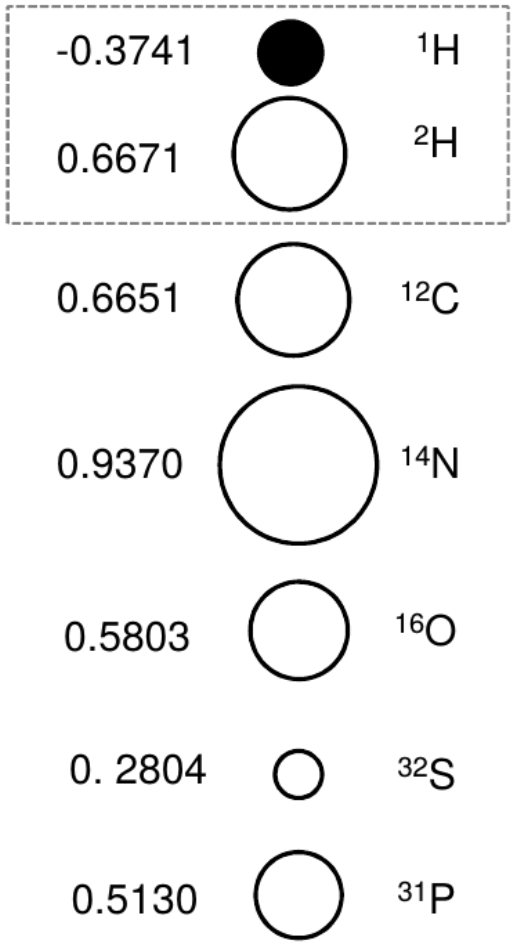
So we subtract an “excluded volume” term from the atomic form factors to compensate

$$f(q) = \sum_k a_k \mathbf{e}^{-b_k\left(\frac{q}{4\pi}\right)^2} + c - v_{\mathrm{ex}}\rho_0 \mathbf{e}^{-q^2\frac{v_{\mathrm{ex}}^{2/3}}{4\pi}}$$

Scattering factors Neutrons

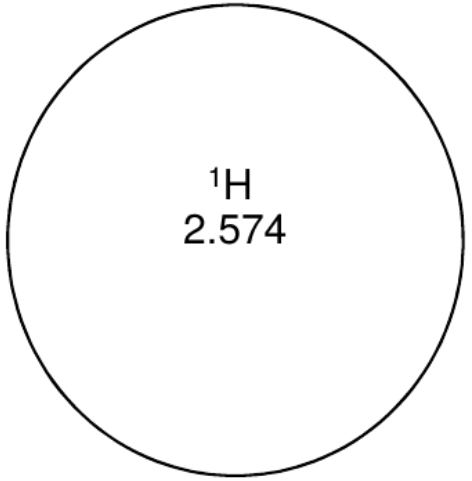
Scattering lengths, biological elements.

$b_{(coherent)}$ values (10^{-12} cm)

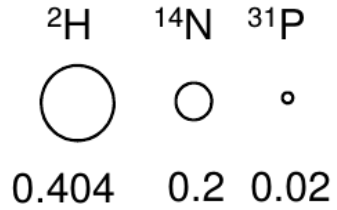


The coherent *b* for ^{1}H is negative:
Attractive interaction potential.

$b_{(incoherent)}$ (10^{-12} cm)



The spin state of ^{12}C, ^{16}O and ^{32}S disallow incoherent scattering events from the ½ spin state of neutrons.



The incoherent scattering length of ^{1}H is enormous – one of the longest incoherent scattering lengths!

Courtesy Cy Jeffries, EMBL

SANS contrast matching

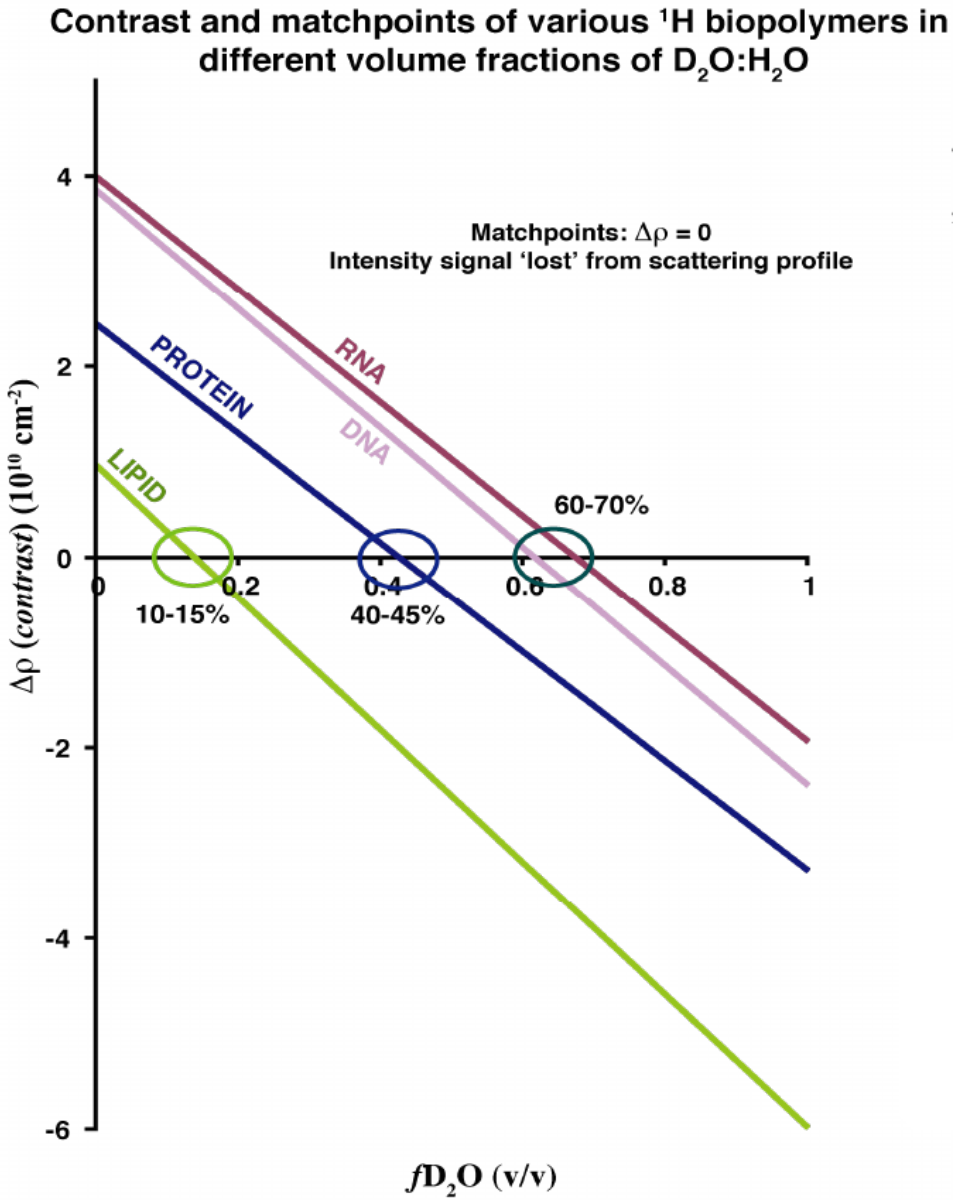
Different classes of macromolecules have different average scattering length densities.

The reason? Proteins, polynucleotides, and lipids naturally have different ^1H per unit volume.

If hetero-macromolecular complexes, e.g., protein bound to DNA, are placed into the appropriate % v/v $^1H_2O/^2H_2O$ solvents, it is possible to extract the scattering contributions for the whole complex (e.g., 100% v/v ^1H_2O buffers) *and* from the individual components at the respective match points (for example 43% and 65% v/v ^2H_2O).

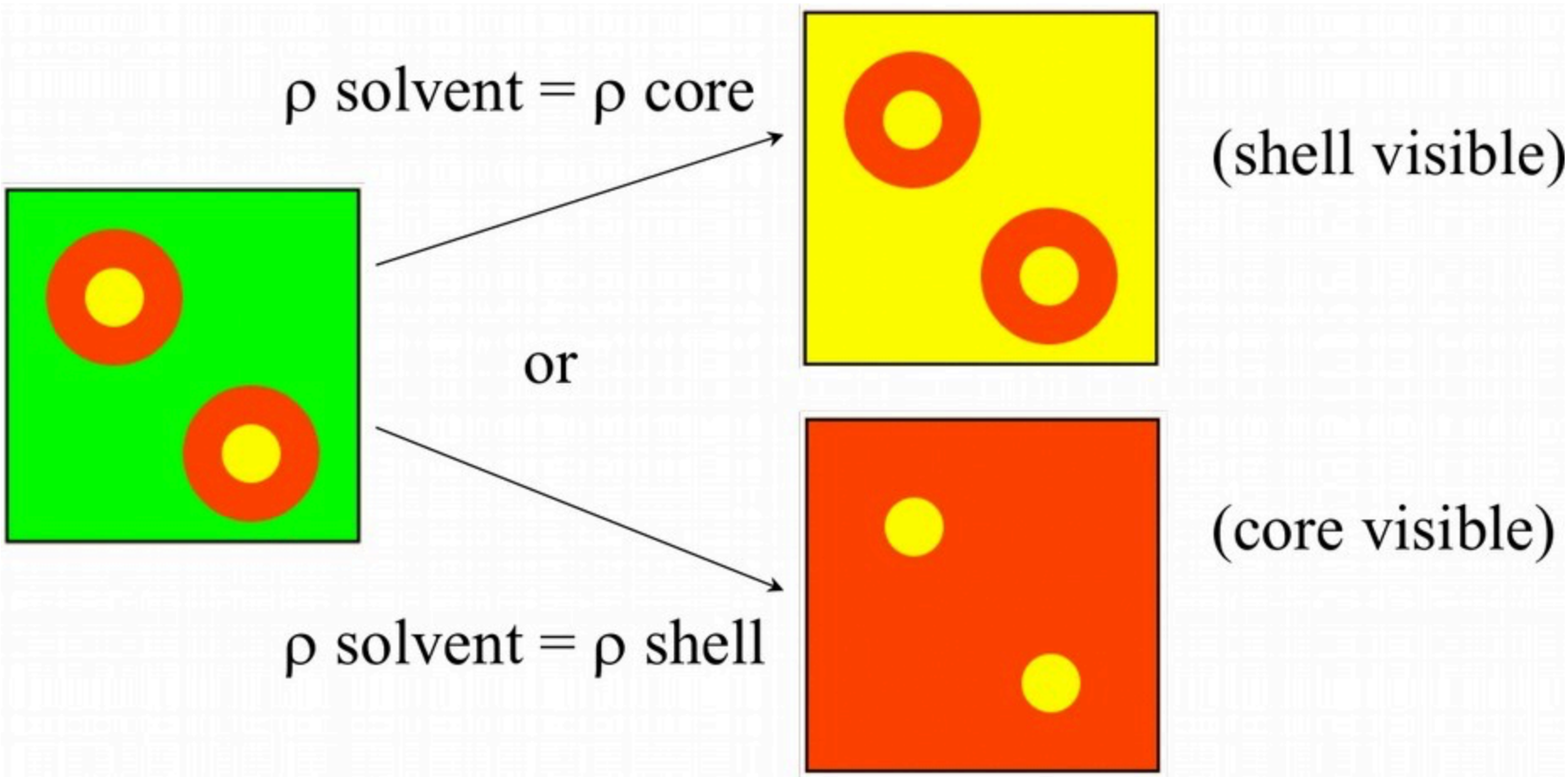
Match point: $\varnothing\rho = 0$

This type of experiment is called *contrast matching*.



Courtesy Cy Jeffries, EMBL

SANS contrast matching



Courtesy Charles Glinka NCNR

I(q) scaling

When comparing experimental results with a simulated curve, $I(q)$ is often scaled arbitrarily scaled by multiplying by a positive constant.

$$I(q) \cong kI(q)$$

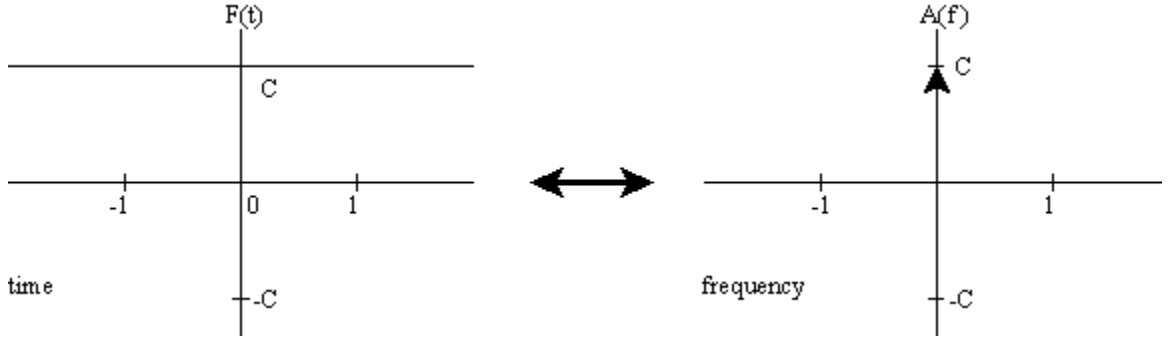
Linear offsets are not generally acceptable.

$$I(q) \ncong I(q) + c$$

Note that if you are comparing absolute intensities, you may want to avoid scaling. N.B. different sources and experimental setups generally have different beam intensities and detectors have different sensitivities.

Basic law of reciprocity in scattering

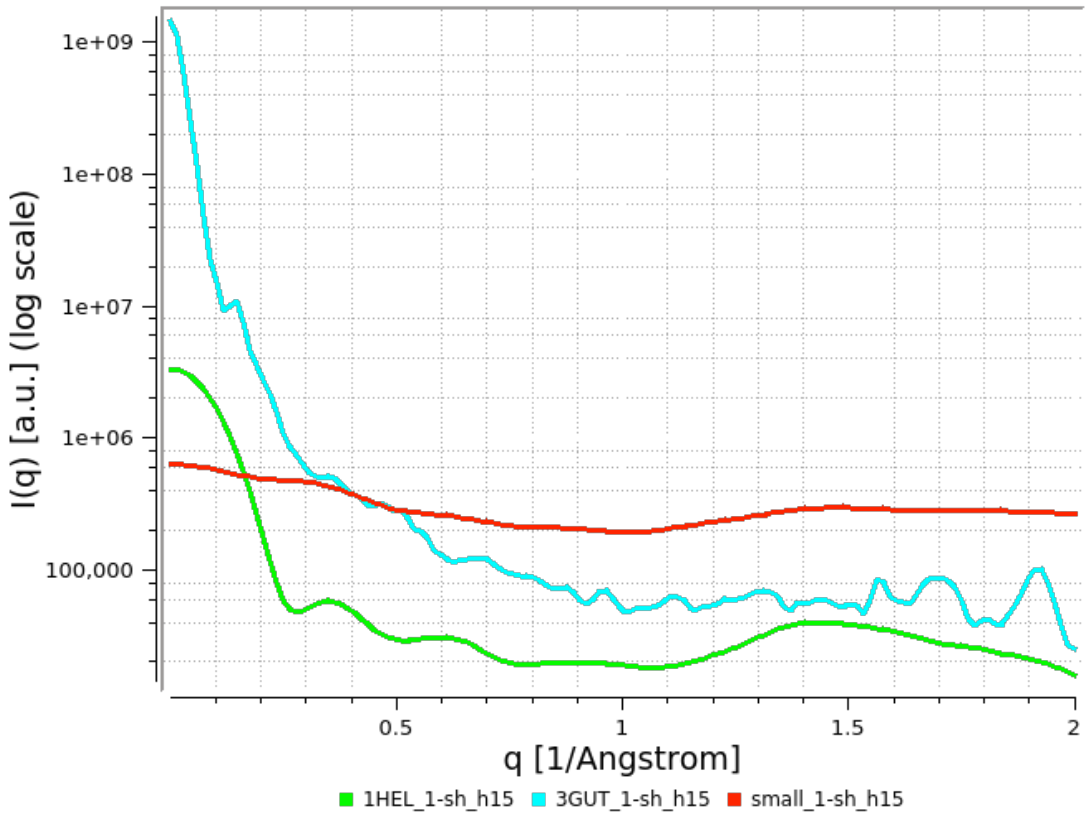
large dimensions → small scattering angles
small dimensions → large scattering angles



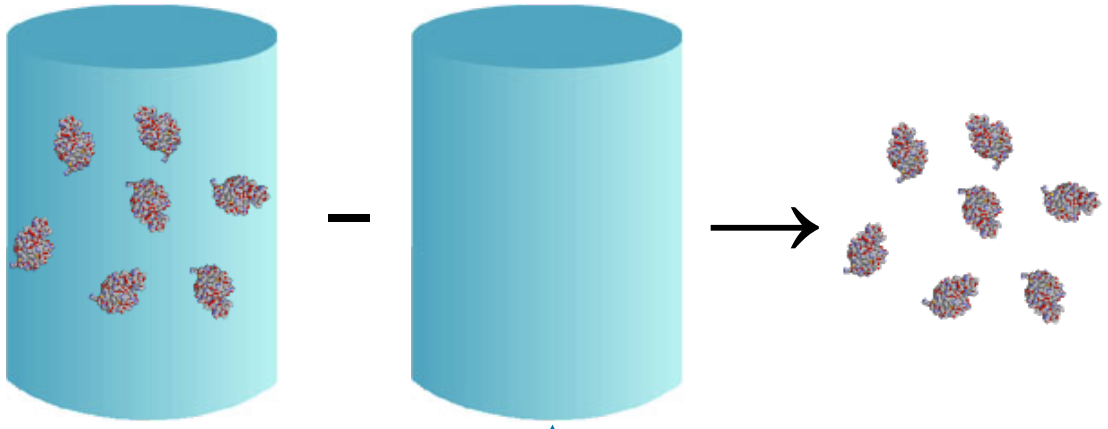
$$\mathfrak{F}(-t) = \mathfrak{F}(\mathfrak{F}(t))$$

$$c = \mathfrak{F}(\delta(t))$$

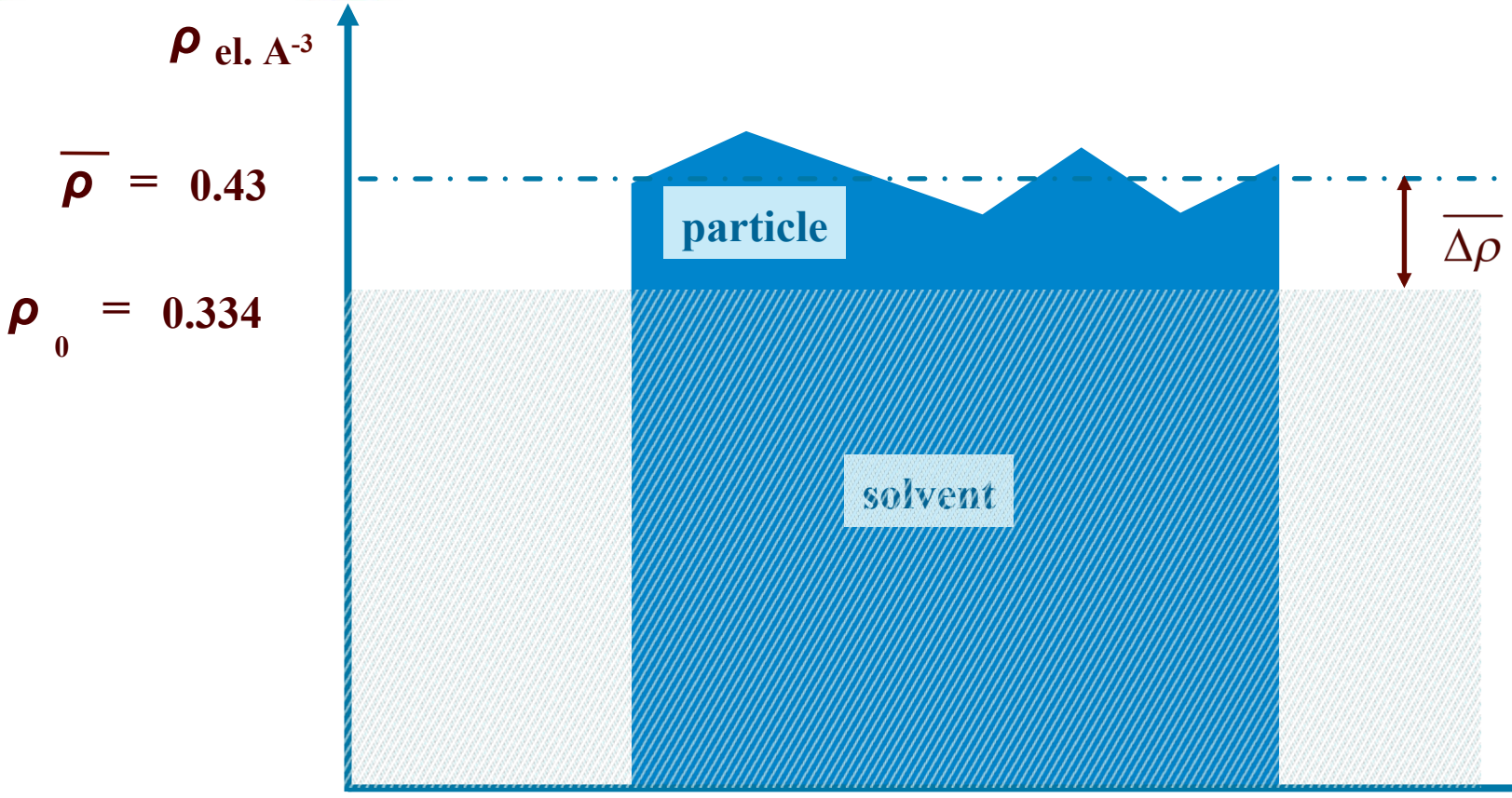
615 Da
14k Da
295k Da



Given the results of a SAS experiment

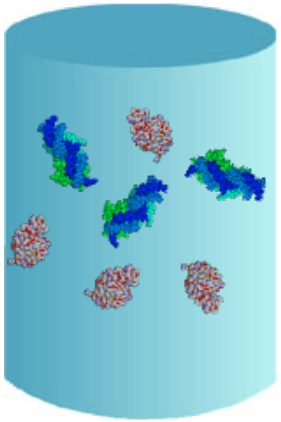


Note that the scattering difference can be quite small. Buffer should exactly match! Can be used to advantage, e.g.: SANS contrast matching.

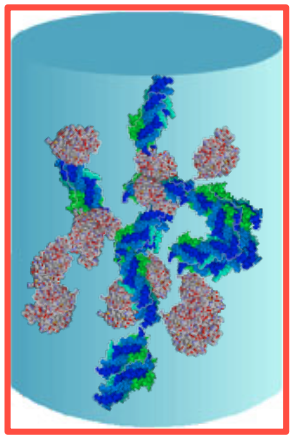


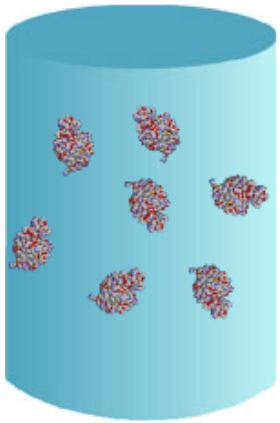
Ideal and monodisperse solution

Given N molecules of n types in solution $N = \sum_{j=1}^{n} n_j$



Ideality $I(q) = \sum_{j=1}^{n} n_j I_j(q)$

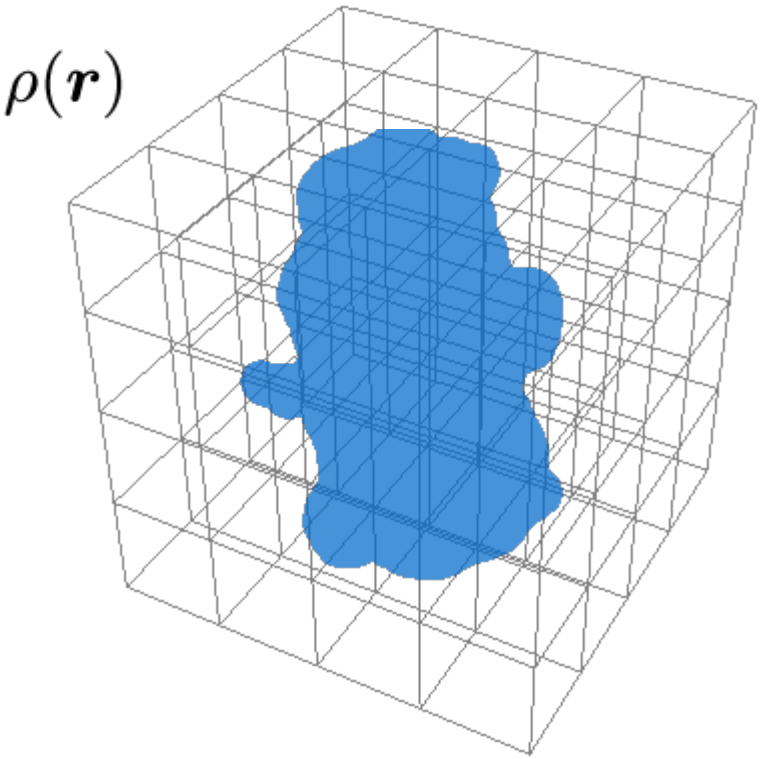


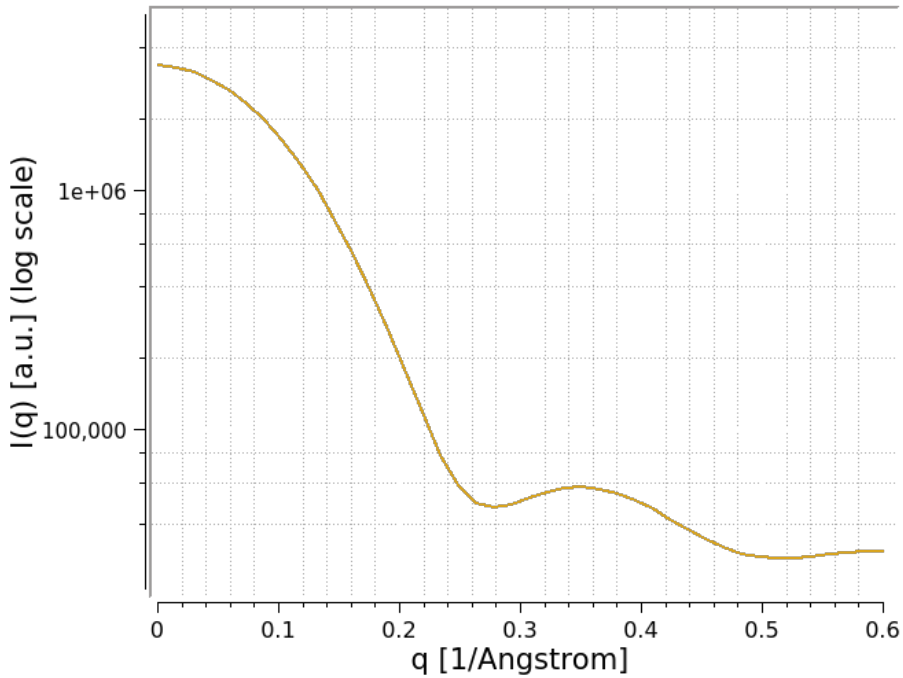


Monodispersity $I_j(q) = I_1(q) \qquad \forall j$

Ideality & Monodispersity $I(q) = N I_1(q)$

Simulating a SAS curve



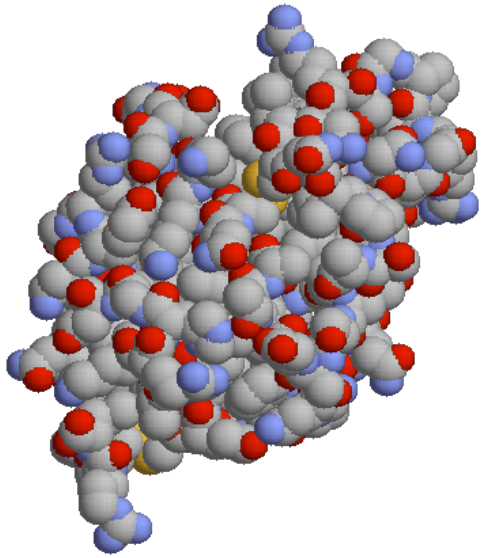


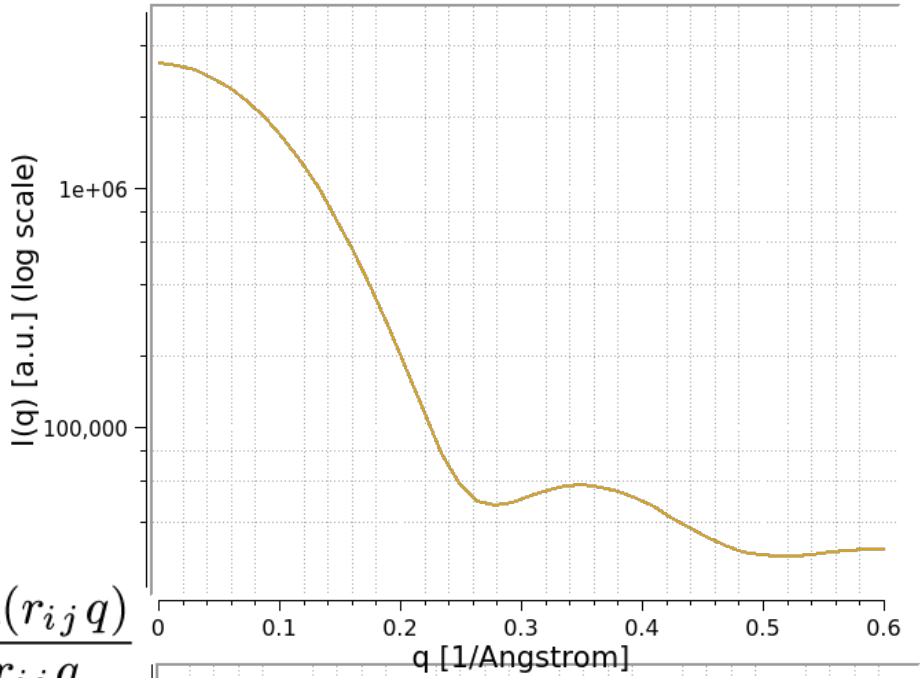
$$\mathbf{F}(\mathbf{q}) = \int_{V_\mathbf{r}} \rho(\mathbf{r}) \mathbf{e}^{\mathbf{i} r q} \, dV_\mathbf{r}$$

$$f(q) = \int_{V_\mathbf{r}} \rho(\mathbf{r}) \, dV_\mathbf{r}$$

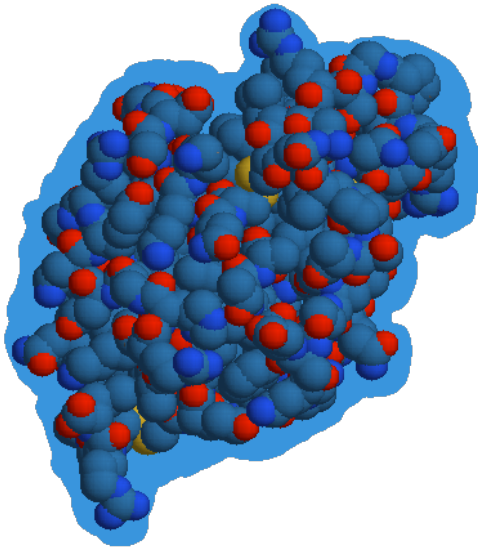
$$I(q) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i f_j \frac{\sin(r_{ij} q)}{r_{ij} q}$$

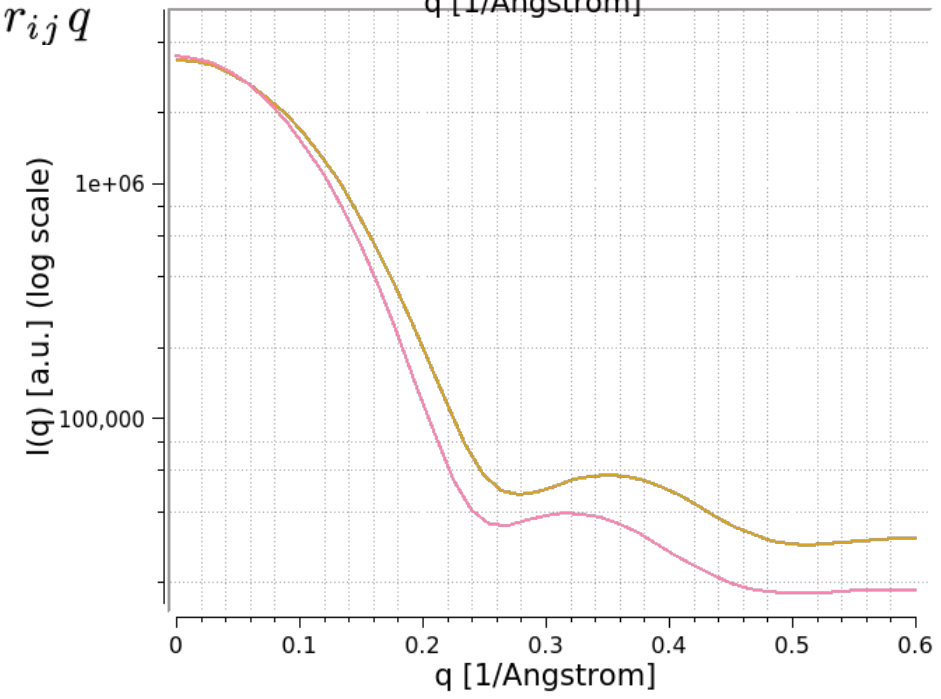
Simulating a SAS curve from a “dry” and “wet” model





$$I(q) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i f_j \frac{\sin(r_{ij}\, q)}{r_{ij}\, q}$$

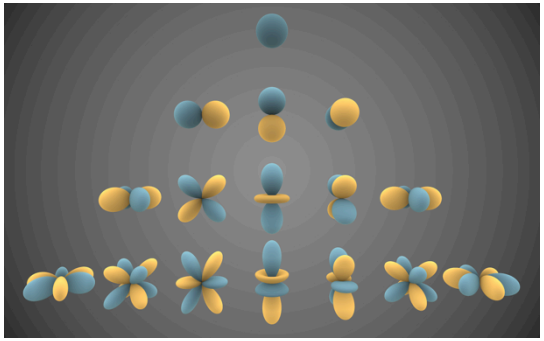




Calculators for a SAS curve & how they hydrate

$$< f_i, g_j > = \int f_i(\mathbf{x})\, f_j(\mathbf{x})\, d\mathbf{x} = \delta_{ij}$$

CRYSOL – Spherical Harmonics – Radial expansion
Svergun D.I. et al (1995) J. Appl. Cryst. , 28, 768-773.

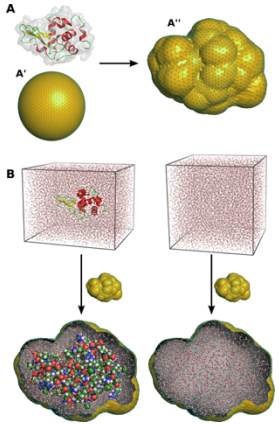


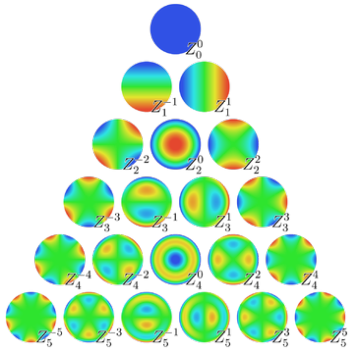
$$Y_l^m(\theta, \phi)$$

FoXS – *P(r)* → *I(q)* – Radial expansion
Schneidman-Duhovny et al. Biophys. J. 2013

Pepsi-SAXS – Spherical Harmonics – Grid
Sergei Grudinin et al., Acta Cryst., 2017, D73, pp.449 – 464

SASTBX – Spherical Harmonics & Zernike polynomials – Radial expansion
Haiguang Liu et al., J. Appl. Cryst., 2012 45:587-593





$$Z_n^m(\rho, \varphi)$$

SasCalc – Golden ratio – None (Hypred)
M.C. Watson et al., J. Appl. Cryst., 2013, 46, 1171-1177

WAXSiS – Debye – MD
Po-chia Chen et al., Biophys. J., 2014, 107, 435-447

AXES – Complex space – MD based
A. Grishaev et al., J. Am. Chem. Soc. 2010, 132, 15484-15486

US-SOMO – Full Debye, Spherical harmonics, complex space, & wraps others – None natively
Brookes et al. (2012). ACM DOI:10.1145/2335755.2335839

Calculators for a SAS curve and fitting parameters

CRYSOL[1]
FoXS[1]
Pepsi-SAXS[1]
SASTBX[1]
SasCalc[1],[2]
WAXSiS[1]
SoftWAXS[1]
AXES[1]

Explicit water model [Not supported yet]
Use explicit hydrogens [only CRYSOL, FoXS, Pepsi-SAXS]
Automatic background subtractions [only CRYSOL, Pepsi-SAXS]

CRYSOL specific

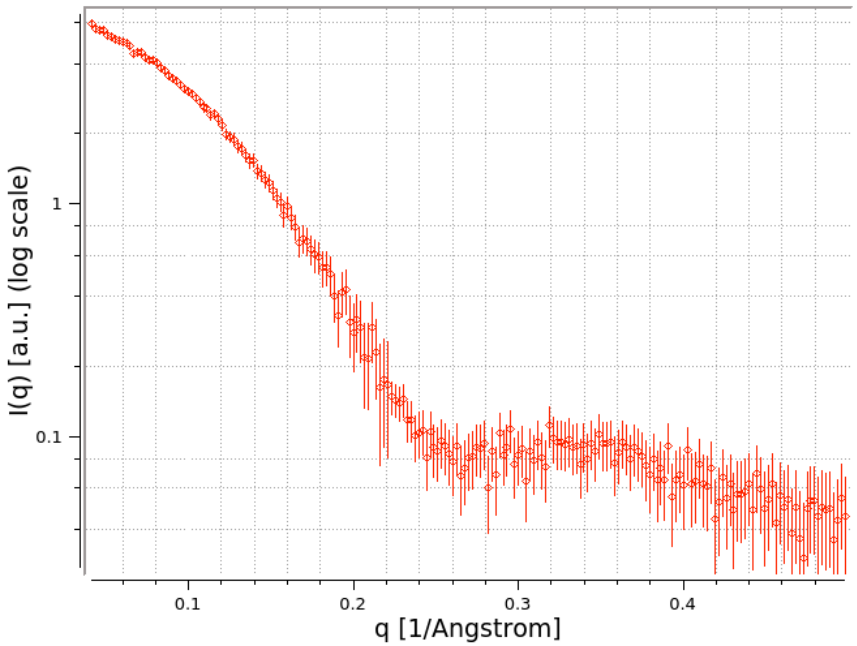
chain identifier
maximum number of harmonics 15
fibonacci grid 17
solvent density 0.334
contrast of hydration shell (Dro) Crysol Default
atomic radius (Ra) Crysol Default
excluded volume (Vol) Crysol Default

FoXS specific

score log
coarse grained (CA only)
use offset
partial profile
background subtractions
min c1 0.99
max c1 1.05
min c2 -2
max c2 4

Pepsi_SAXS specific

Guinier analysis



$\rightarrow R_g$

Guinier law

The scattering intensity of a particle can be described by a Gaussian curve in the vicinity of the origin.
The validity domain actually depends on the shape of the particle and is around $q < 1.2 / R_g$ for a globular shape.

Prof. André Guinier
1911-2000
Orsay, France

$$I(q) = I(0)\, \mathbf{e}^{\frac{-q^2 R_g^2}{3}}$$

Extrapolated intensity at origin

Radius of gyration

Guinier law, in log form **:**

$$\ln[I(q)] = \ln[I(0)] - \frac{q^2 R_g^2}{3}$$

ln[I(q)] vs q^2 : Guinier plot.
Linear regression on the experimental Guinier plot directly provides R_g and *I(0)*.

Radius of Gyration

$$R_g^2 = \frac{\int_{V_\mathbf{r}} \Delta\rho(\mathbf{r}) r^2 \, dV_\mathbf{r}}{\int_{V_\mathbf{r}} \Delta\rho(\mathbf{r}) dV_\mathbf{r}}$$

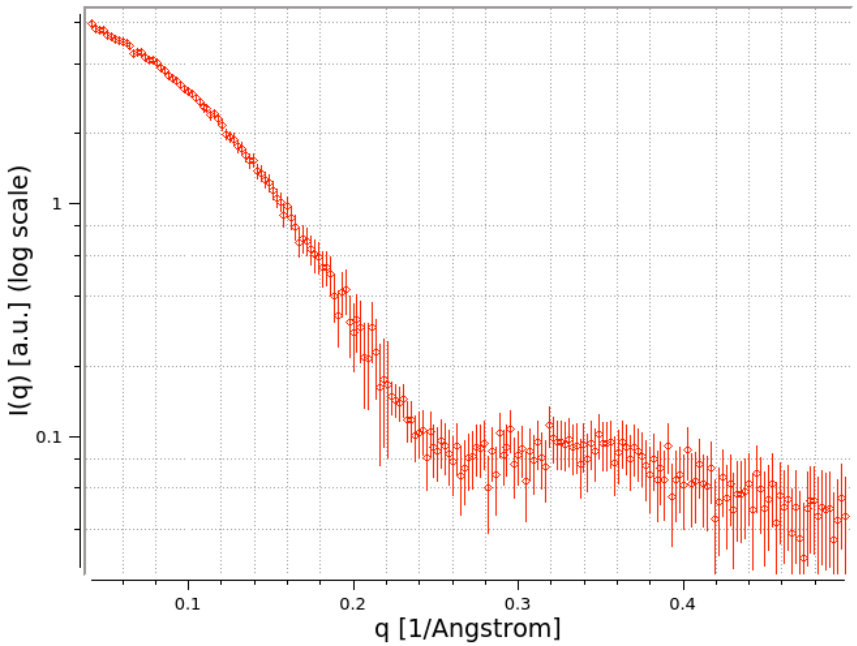
R_g^2 is the mean square distance to the center of mass **weighted by the contrast of electron density**.

If $\Delta\rho(r)$ is approximately constant then R_g is a geometrical quantity.

R_g is an *index of non sphericity*.
For a given volume the smallest R_g is that of a sphere.

$$R_g = \sqrt{\frac{3}{5}} R$$

Guinier analysis



$\rightarrow M$

Mass retrieval from Guinier analysis

$$I(0) = \frac{cMr_0^2}{N_A}[v_{\mathrm{mol}}(\rho_{\mathrm{mol}} - \rho_{\mathrm{buffer}})]^2$$

$$I(q) = I(0)\,\mathbf{e}^{\frac{-q^2R_g^2}{3}}$$

$I(0)$ from Guinier analysis, in absolute units cm^{-1}

c mass concentration

r_0 is the classical electron radius

v_{mol} volume of molecule

ρ_{mol} electron density of molecule

ρ_{buffer} electron density of solvent

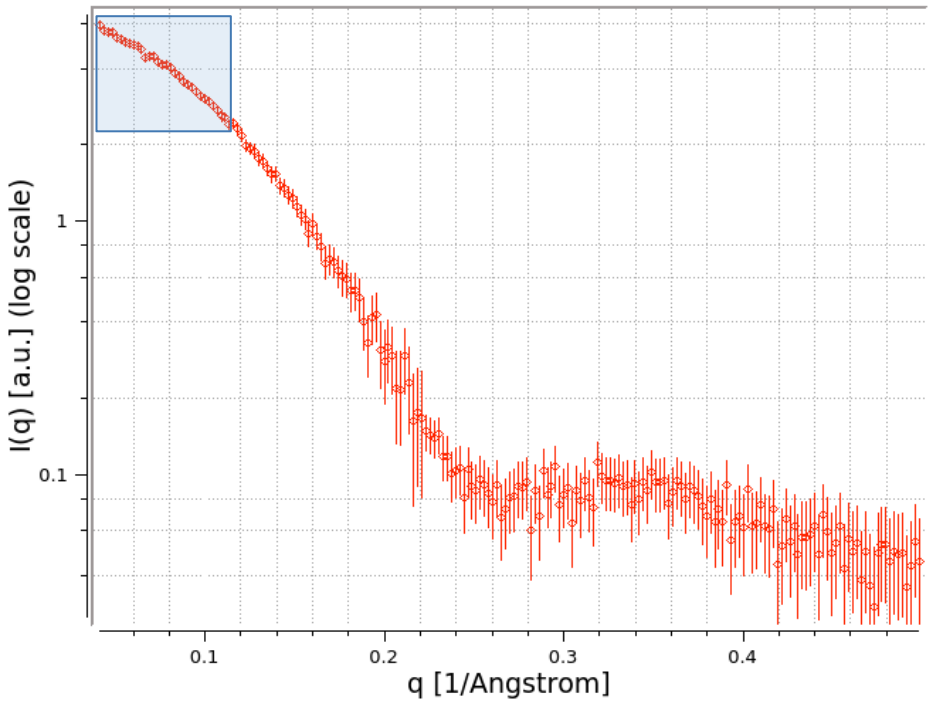
$\rightarrow$ molar mass M

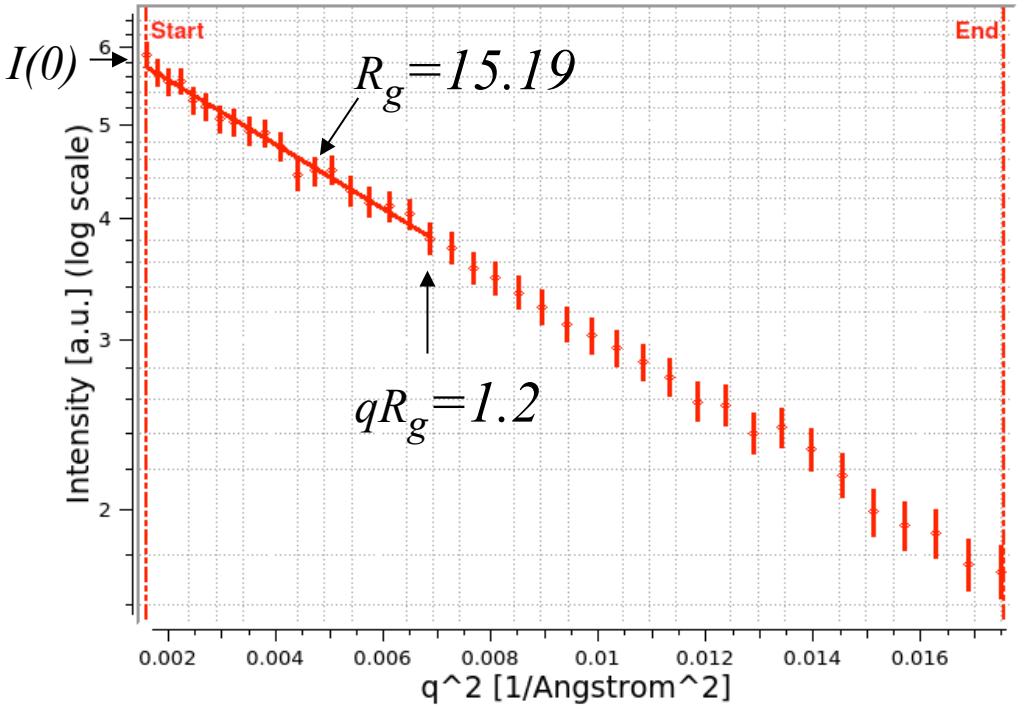
Mass retrieval from Guinier analysis

$$I(0) = \frac{cMr_0^2}{N_A}[v_{\mathrm{mol}}(\rho_{\mathrm{mol}} - \rho_{\mathrm{buffer}})]^2$$

$$\rightarrow \qquad I(0) \propto M$$

Guinier Plot





$$I(q) = I(0)\,\mathbf{e}^{\frac{-q^2R_g^2}{3}}$$

$$\ln[I(q)] = \ln[I(0)] - \frac{q^2R_g^2}{3}$$

Validity range “Guinier region”:

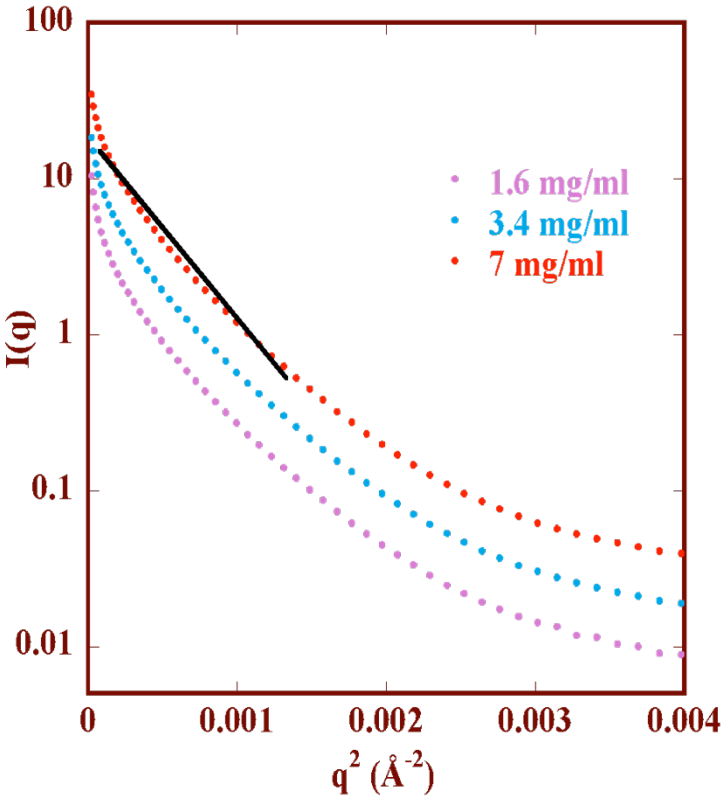
$0 < R_g q < 1$ for a solid sphere

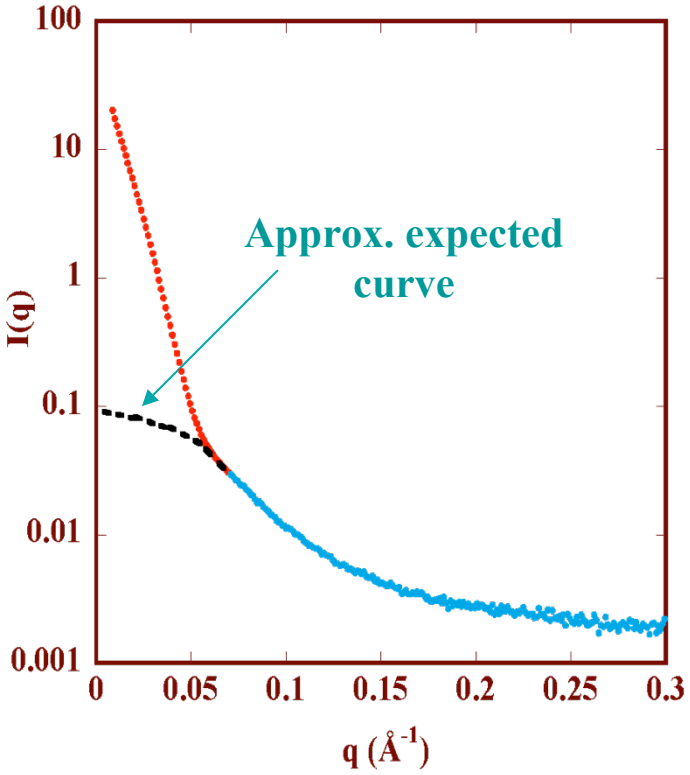
$0 < R_g q < 1.2$ rule of thumb for a globular protein

Guinier Plot – evaluation of solution properties

Irreversible aggregation

→ Useless data: the whole curve is affected





I(0): > 150 fold the expected value for the given MM

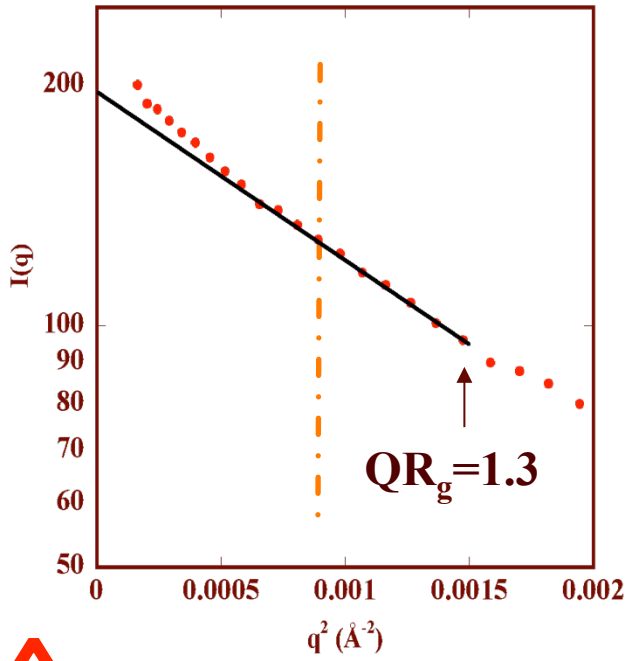
(Courtesy D. Durand, IBBMC, Orsay)

Guinier Plot – evaluation of solution properties

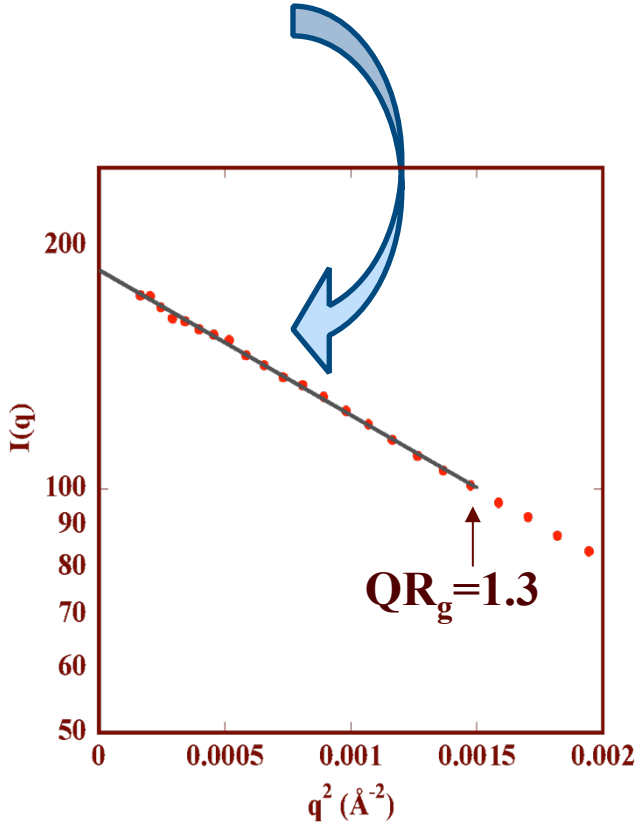
Weak aggregation → **possible improvement**

centrifugation, buffer change

Nanostar –PR65 protein



R_g ~ 38 Å – too high!!

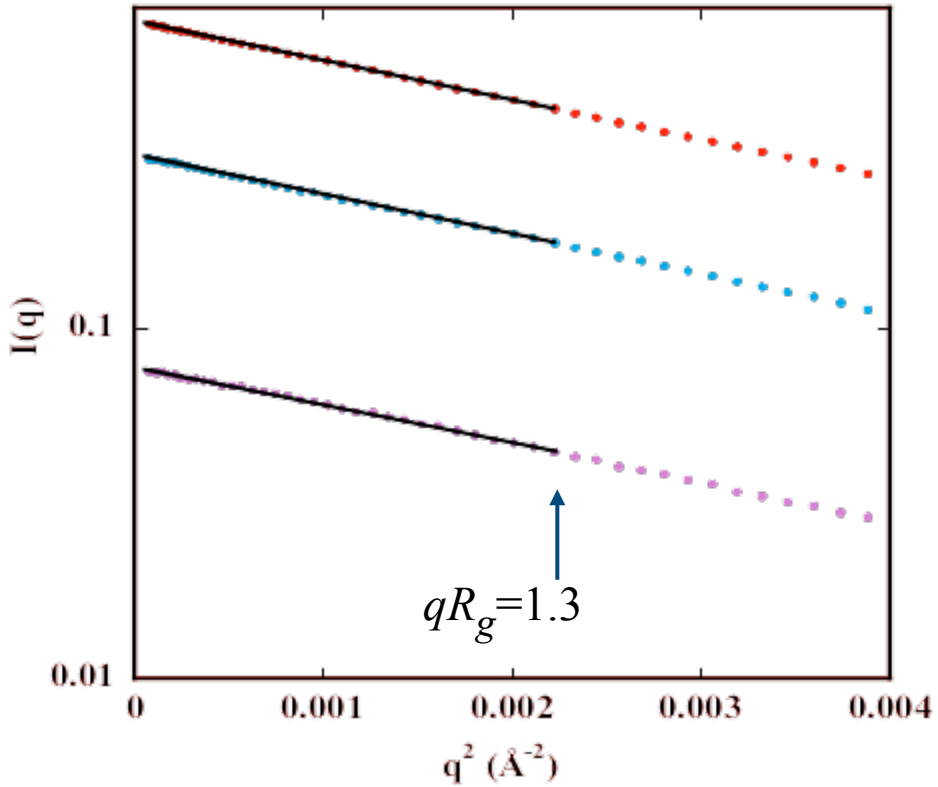


R_g ~ 36 Å

(Courtesy D. Durand, IBBMC, Orsay)

Guinier Plot – concentration series

$$I(0) \propto M$$



same R_g at all three concentrations

→ No aggregation, no interactions.

Guinier Plot

A linear Guinier plot is a requirement, but it is **not a sufficient condition** ensuring monodispersity and ideality of the sample.