Small-Angle X-ray Scattering (SAXS)

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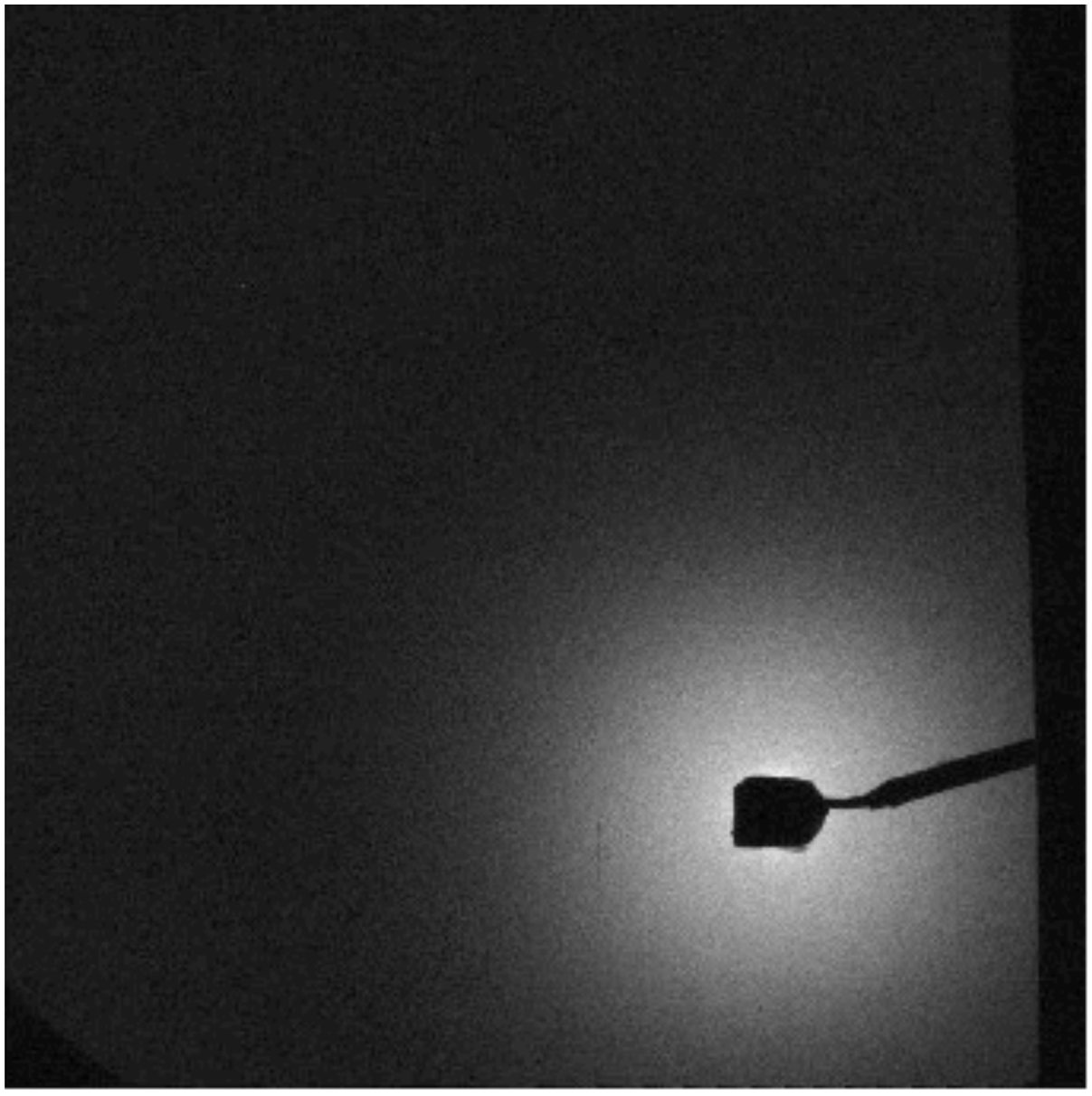
What do you see?



Ando, et al. (2012). JACS, 134(43), 17945–17954.

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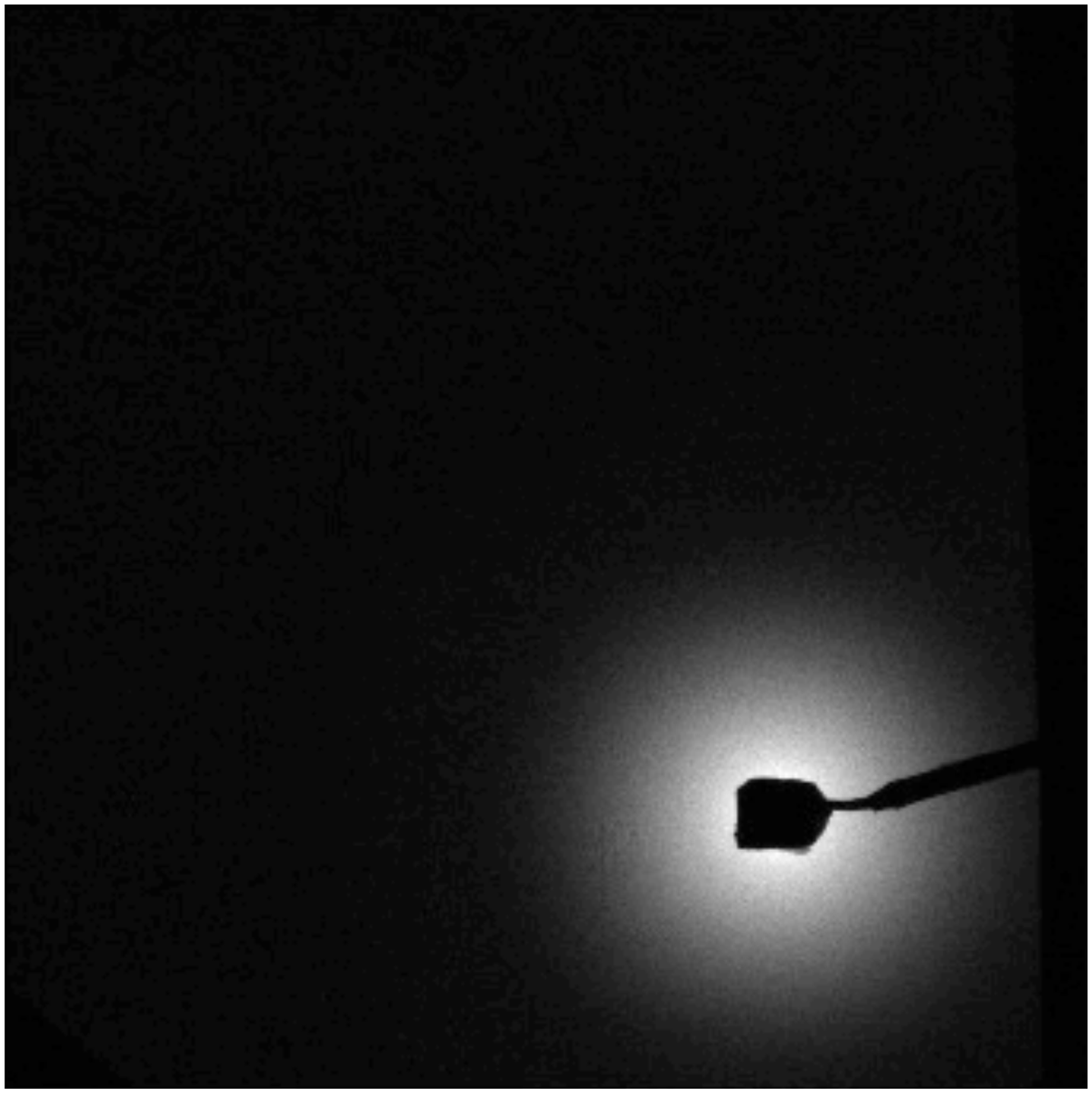


Ando, et al. (2012). JACS, 134(43), 17945–17954.

Outline

- Why use SAXS?
- Scattering theory
- Basic interpretation of SAXS data
- Experimental requirements
- An advanced example: ribonucleotide reductase
- Variations

Is this you?

- I have a protein that won't crystallize.

- I have a crystal structure, but I want to know what forms in solution.

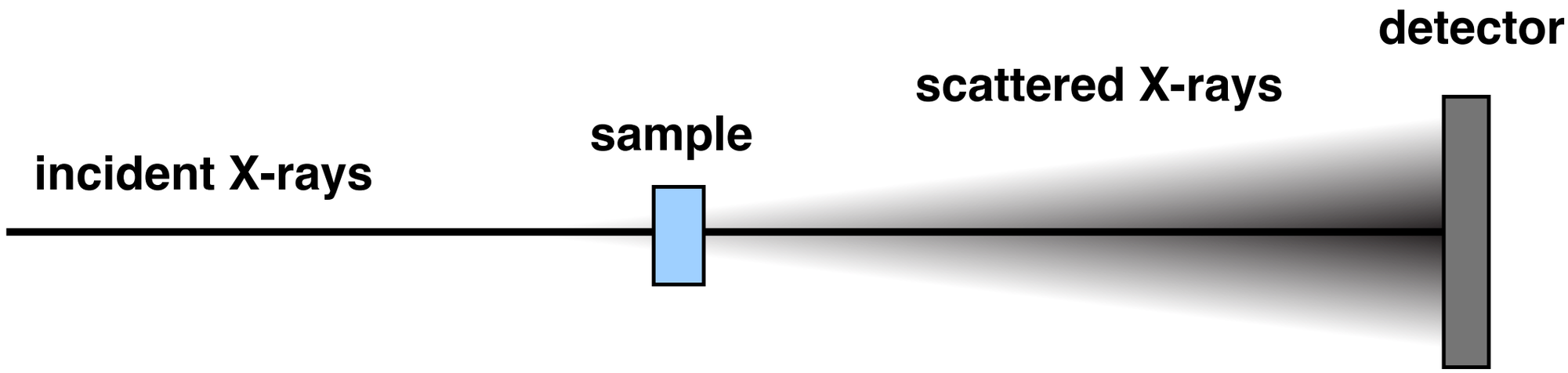
- I want to know the oligomerization state or the stoichiometry of a complex.

- I want to know what happens to my protein in the absence or presence of a cofactor or substrate.

- I want to know if my samples are properly folded.

- I want structural information on my protein under the same conditions used for activity assays.

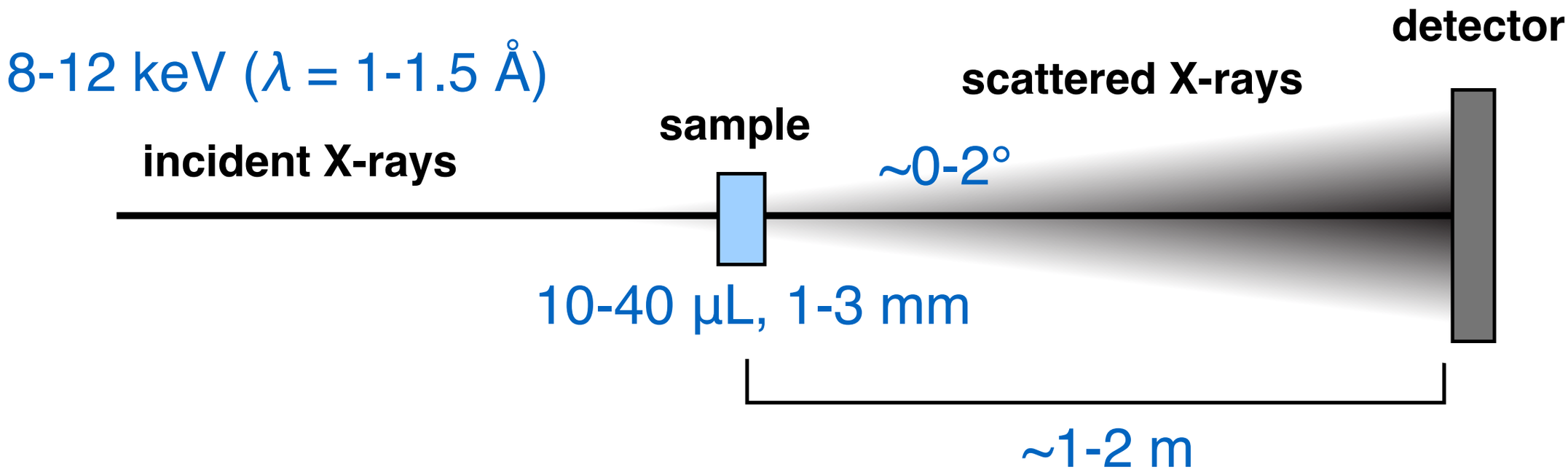
SAXS probes nanometer length scales



Samples:

- biomolecules in solution
- lipid membranes
- cellular organelles
- polymers
- emulsions
- liquid crystals
- alloys
- fibers

SAXS probes nanometer length scales

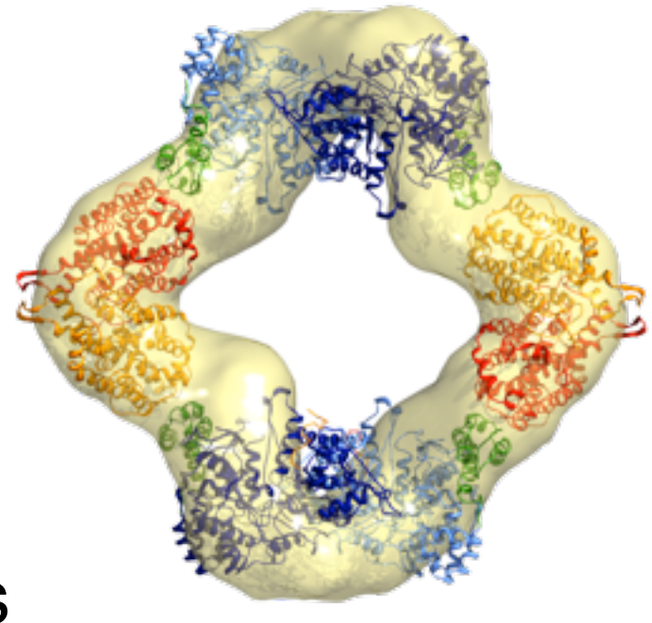


Samples:

- biomolecules in solution ⟶ **"solution SAXS", "bioSAXS"**

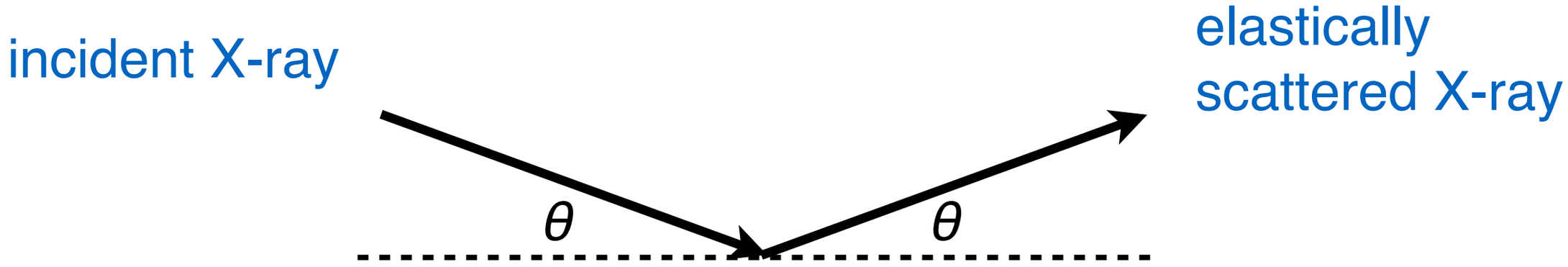
Information:

- mass, spatial size
- foldedness, packing, flexibility
- overall shape
- stoichiometry and shape of complexes
- conformational changes and #states as functions of time, ligands, solution conditions



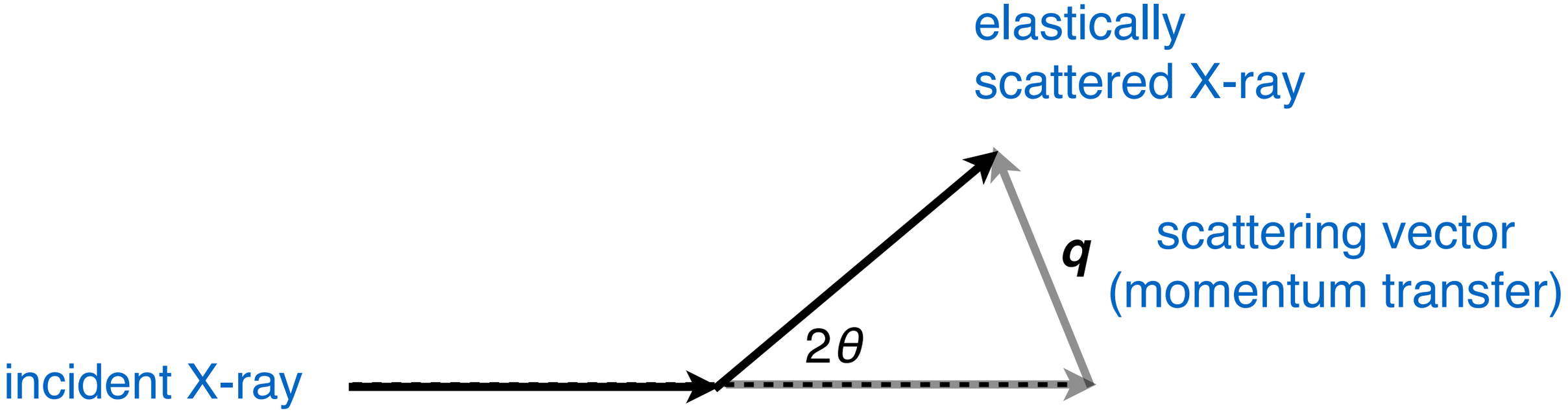
Part 1: Basic Theory

Scattering angles



By crystallography conventions, the scattering angle is defined as 2θ.

Scattering angles



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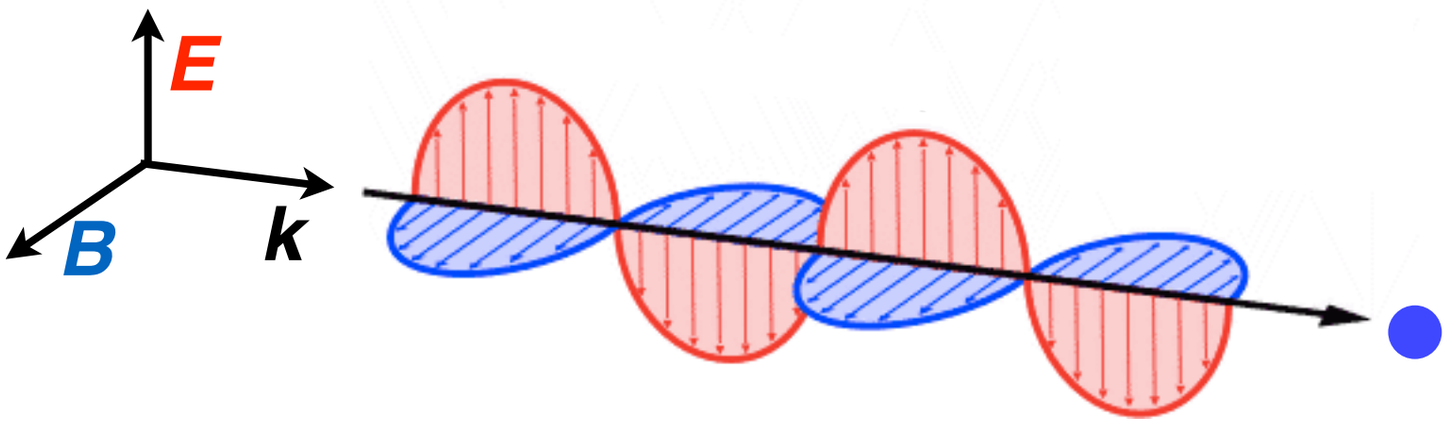
Scattering from a single electron



free electron

Driving force:

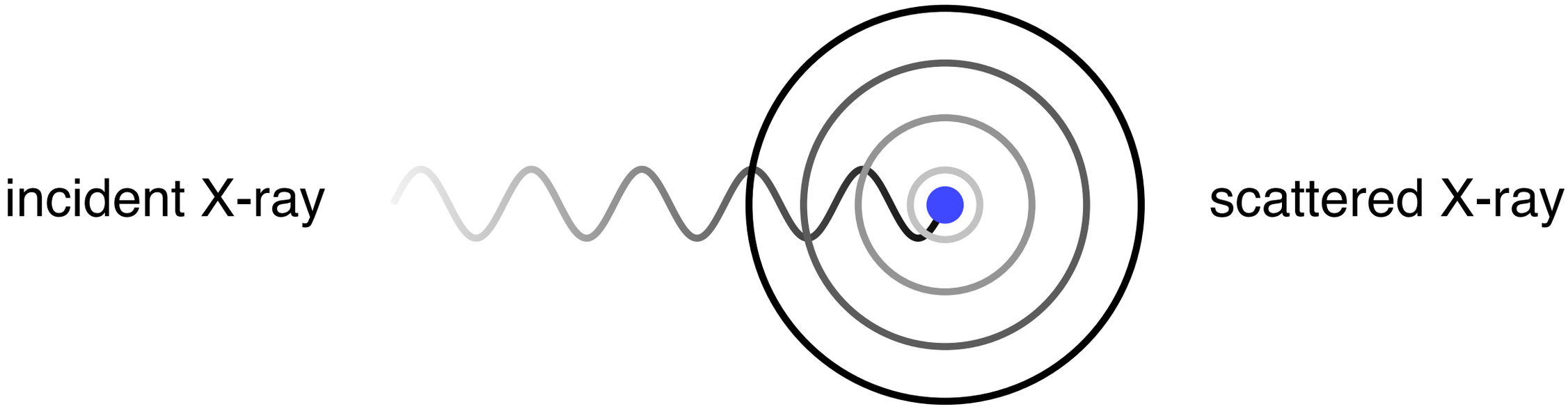
$$\vec{F} = q\vec{E}$$

Incident X-ray is a plane wave with wave vector, $\vec{k}_0 = \frac{2\pi}{\lambda}\hat{k}$

Electric field component of plane wave at electron position, ***r*** :

$$E(\vec{r},t) = E_0 e^{i(\vec{k}\cdot\vec{r}-\omega t)}$$

Scattering from a single electron



Scattered X-ray is a spherical wave with wave vector, $\left|\vec{k}\right| = \left|\vec{k}_0\right|$

Thomson scattering: Elastic scattering from free electron

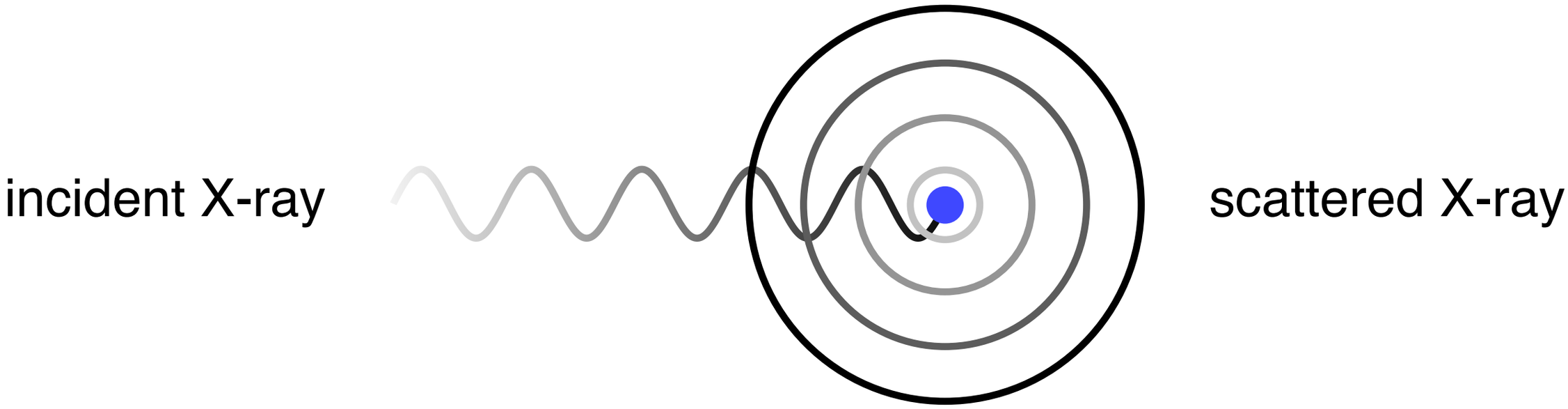
$$I_{elec} = \left|E_{elec}\right|^2 = E_0^2 \left(\frac{e^2}{4\pi\varepsilon_0 c^2 m}\right)^2 \left(\frac{1+\cos^2 2\theta}{2}\right)\frac{1}{R^2}$$

incident intensity

polarization factor

spherical wave

Scattering from a single electron



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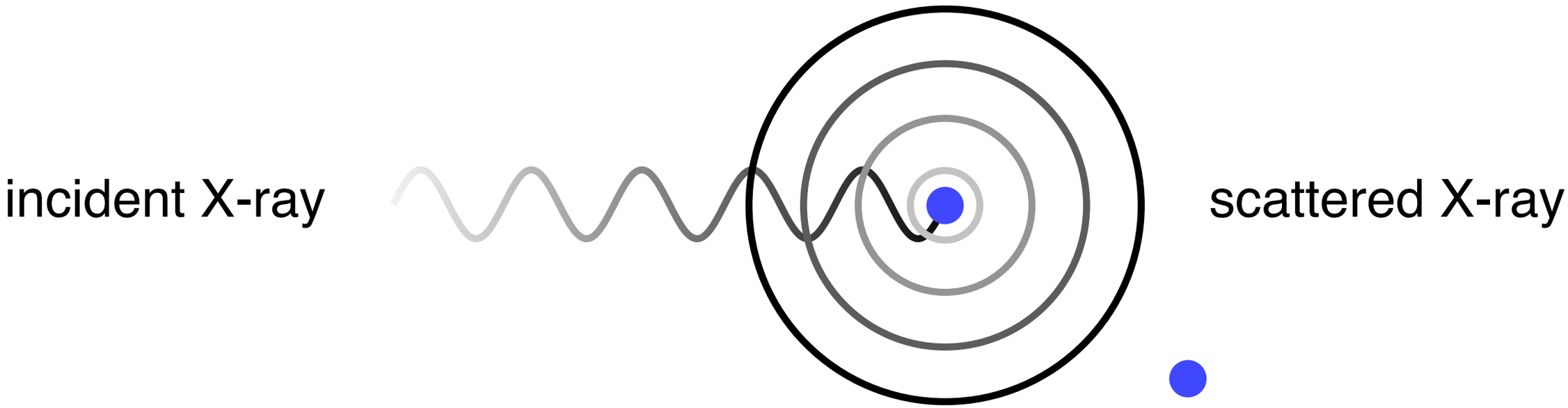
Scattering dominated by electrons: $(m_e/m_p)^2 \sim 3 \times 10^{-7}$

≈1 at small angles

Scattering from a pair of electrons



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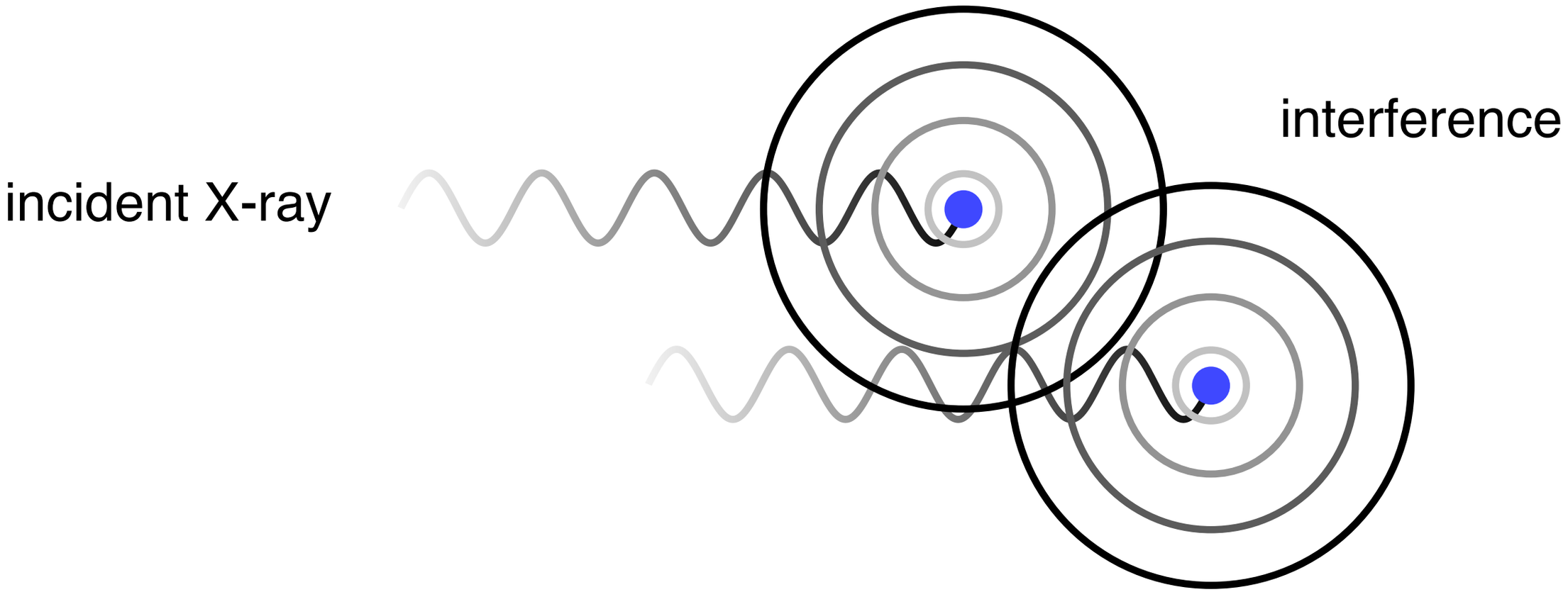

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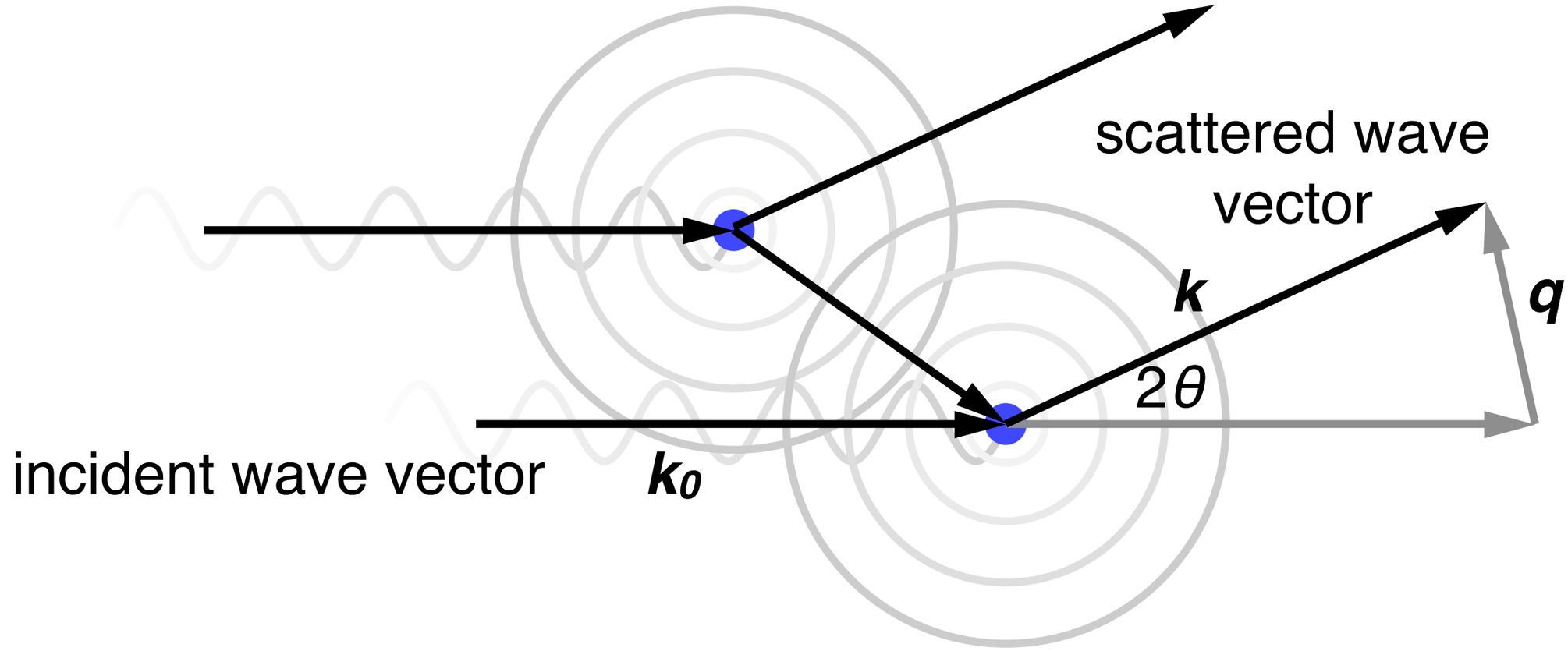
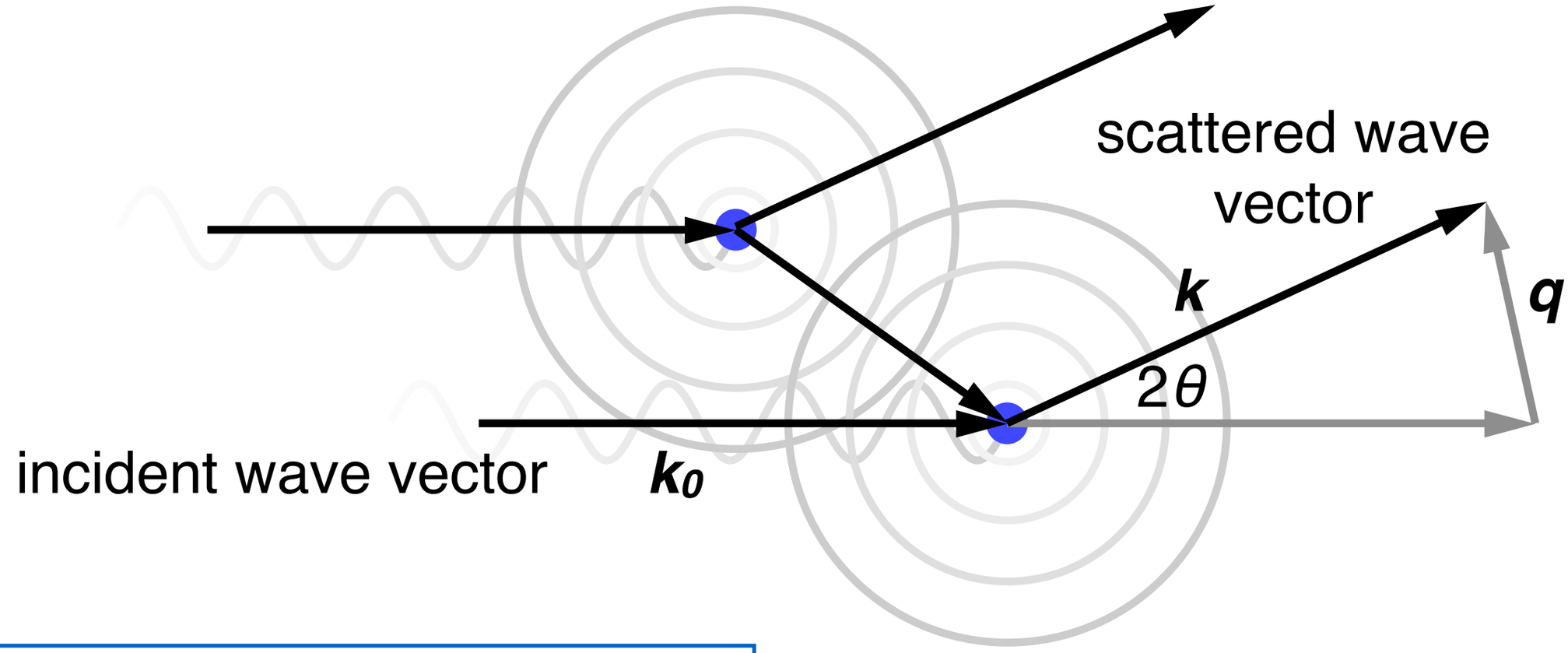


Elastic scattering: $\left|\vec{k}\right| = \left|\vec{k}_0\right| = \frac{2\pi}{\lambda}$

Derive: $q = \frac{4\pi}{\lambda}\sin\theta$

(homework)

Scattering from a pair of electrons



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

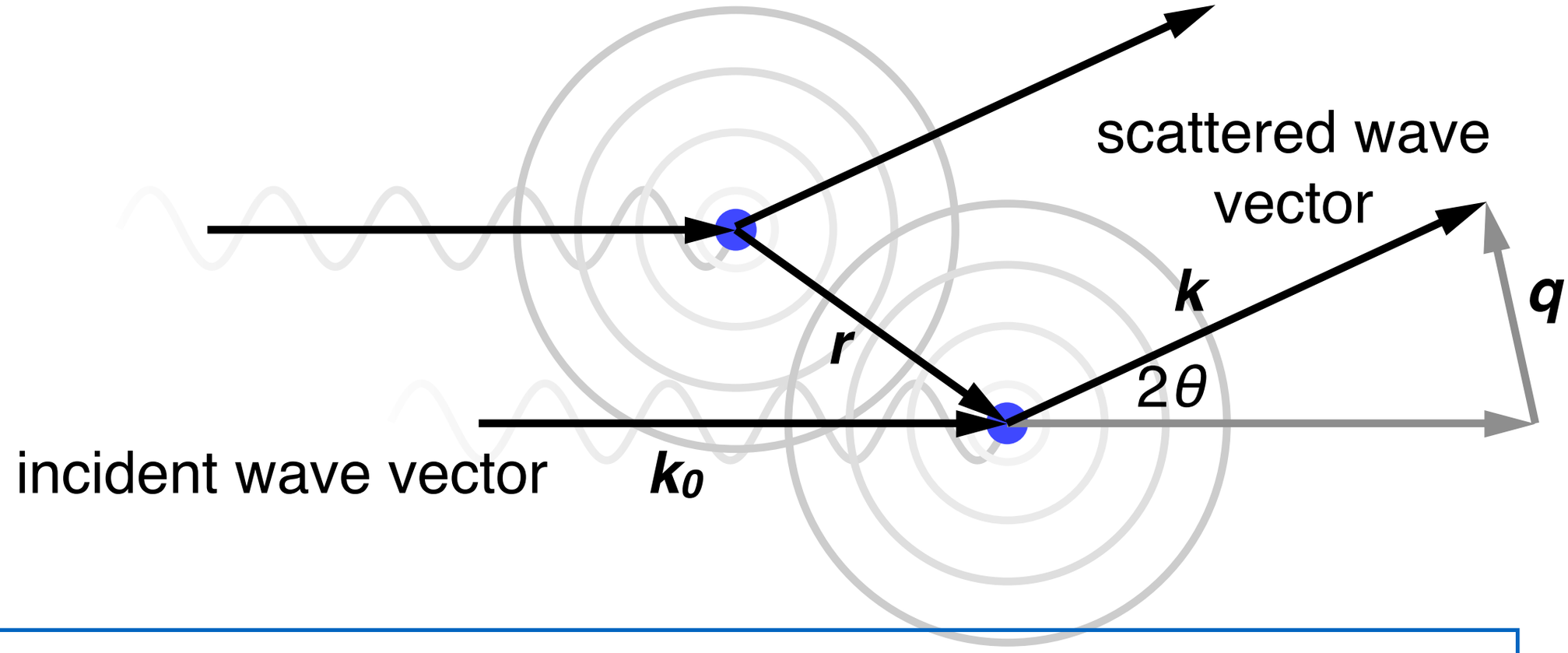
Another notation:

$$s = \frac{2}{\lambda}\sin\theta,$$

where $q = 2\pi s$

units are 1/Å or 1/nm for both *q* and *s*; **define** notation in publications

Scattering from a pair of electrons

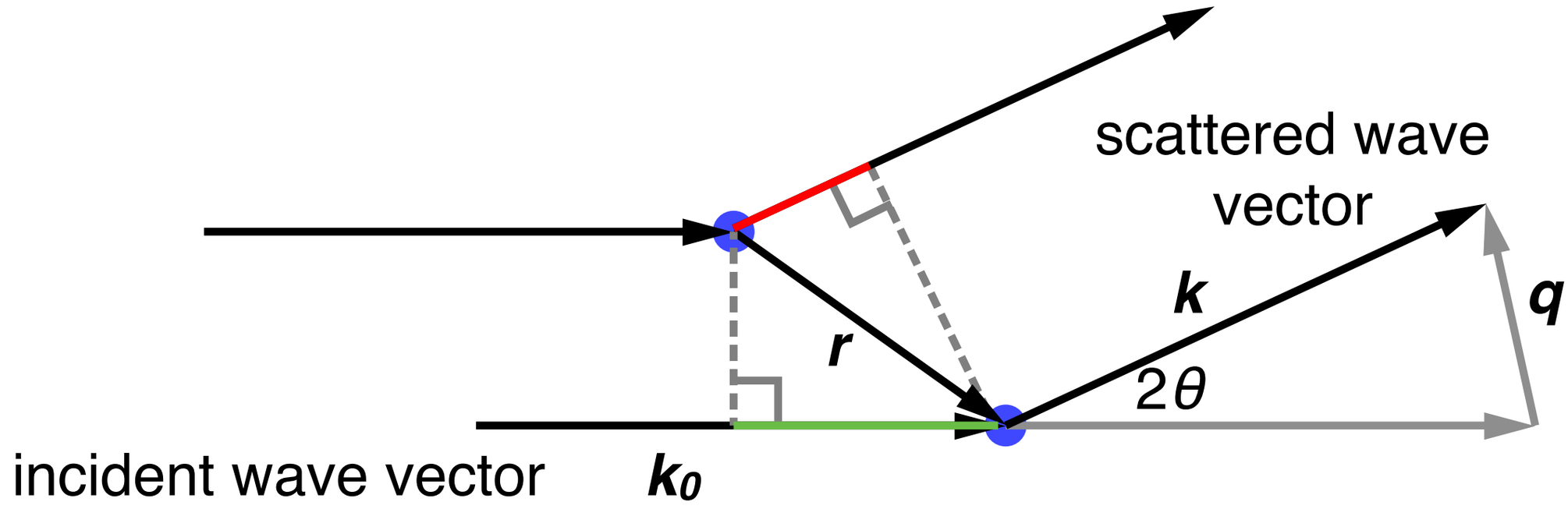


At small angles and far field ($R >> r$), individual scattered waves have ***identical amplitudes***.

$$I_{elec} = \left|E_{elec}\right|^2 = E_0^2 \left(\frac{e^2}{4\pi\varepsilon_0 c^2 m}\right)^2 \left(\frac{1+\cos^2 2\theta}{2}\right)\frac{1}{R^2} \Rightarrow \text{constant}$$

amplitude squared

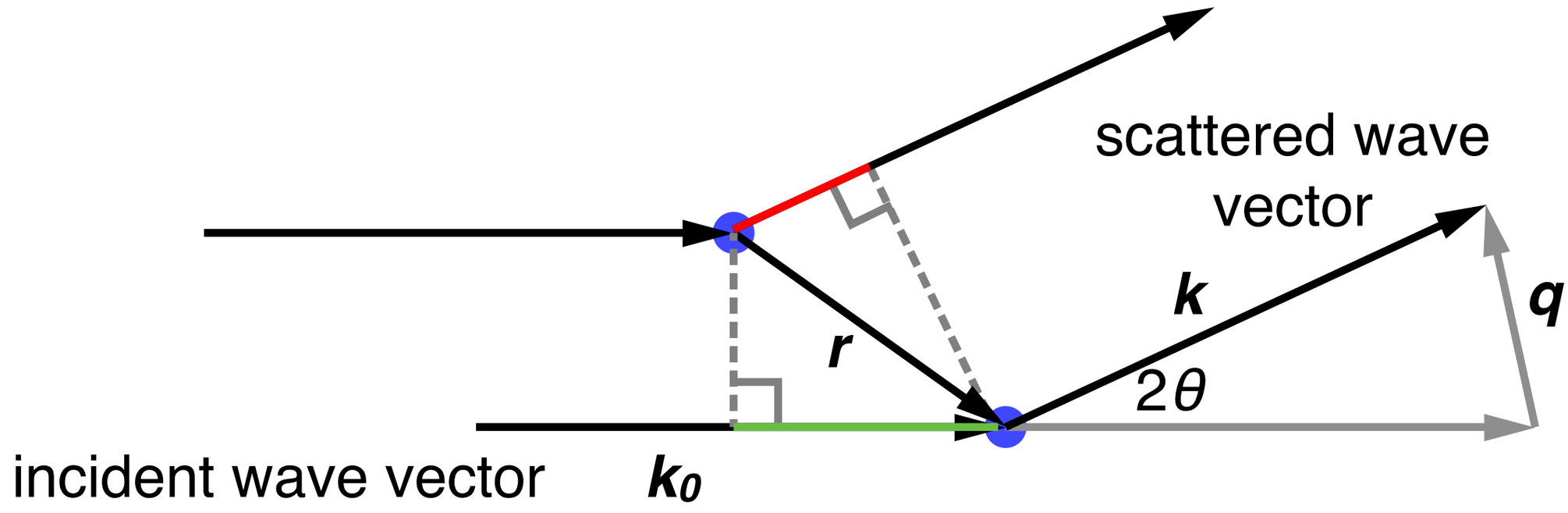
Scattering from a pair of electrons



At small angles and far field ($R >> r$), individual scattered waves have ***identical amplitudes***... but they differ by ***phase shift***, Φ.

$$\phi = \vec{k}_0 \cdot \vec{r} - \vec{k} \cdot \vec{r} = -\vec{q} \cdot \vec{r}$$

Scattering from a pair of electrons

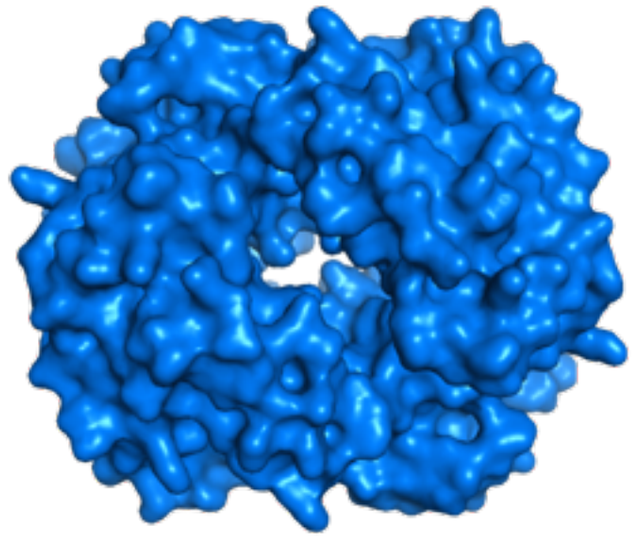


At small angles and far field ($R >> r$), individual scattered waves have ***identical amplitudes***... but they differ by ***phase shift***, Φ.

$$E_{pair}(q) = \text{wave 1} + \text{wave 2} = \sum_{j=1}^{2} \left|E_{elec}\right| e^{i\phi_j} = \left|E_{elec}\right| e^{0} + \left|E_{elec}\right| e^{-i\vec{q}\cdot\vec{r}}$$

Scattering from a protein (with *n* electrons)

incident X-ray

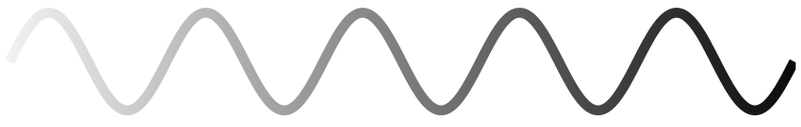


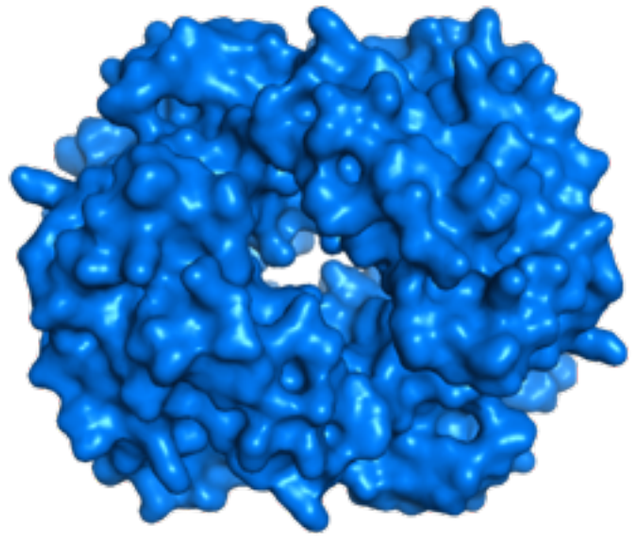
At X-ray energies far from binding energies (absorption edges), all electrons can be treated as if free.

$$E(q) = \left|E_{elec}\right| \sum_{j}^{n} e^{-i\vec{q}\cdot\vec{r}_j} \propto \int_V \rho(\vec{r}) e^{-i\vec{q}\cdot\vec{r}}\, d\vec{r} = F\left[\rho(\vec{r})\right]$$

Scattering from a protein (with *n* electrons)

incident X-ray





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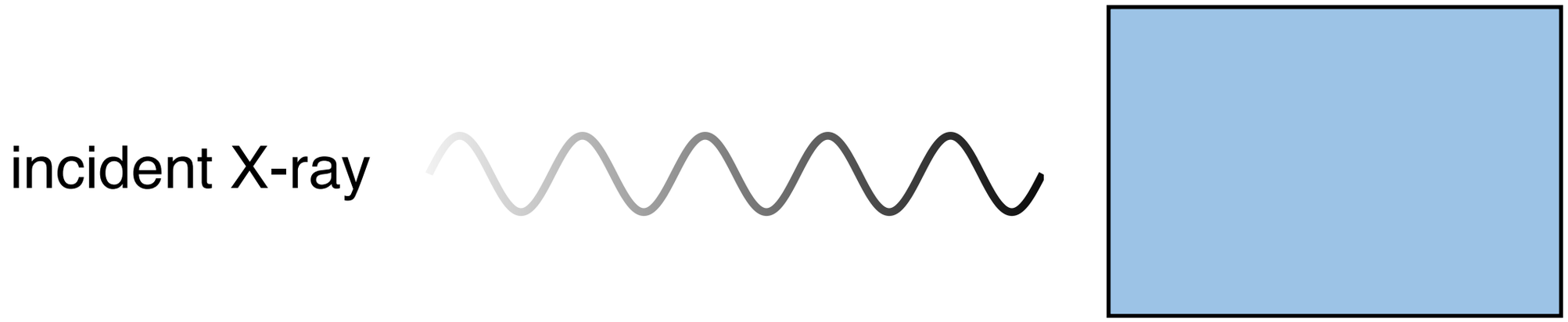
Scattering amplitude from a protein is the Fourier Transform of its electron density distribution, $\rho(r)$, ***in other words structure***!!!

Scattering from a uniform material (e.g. buffer)

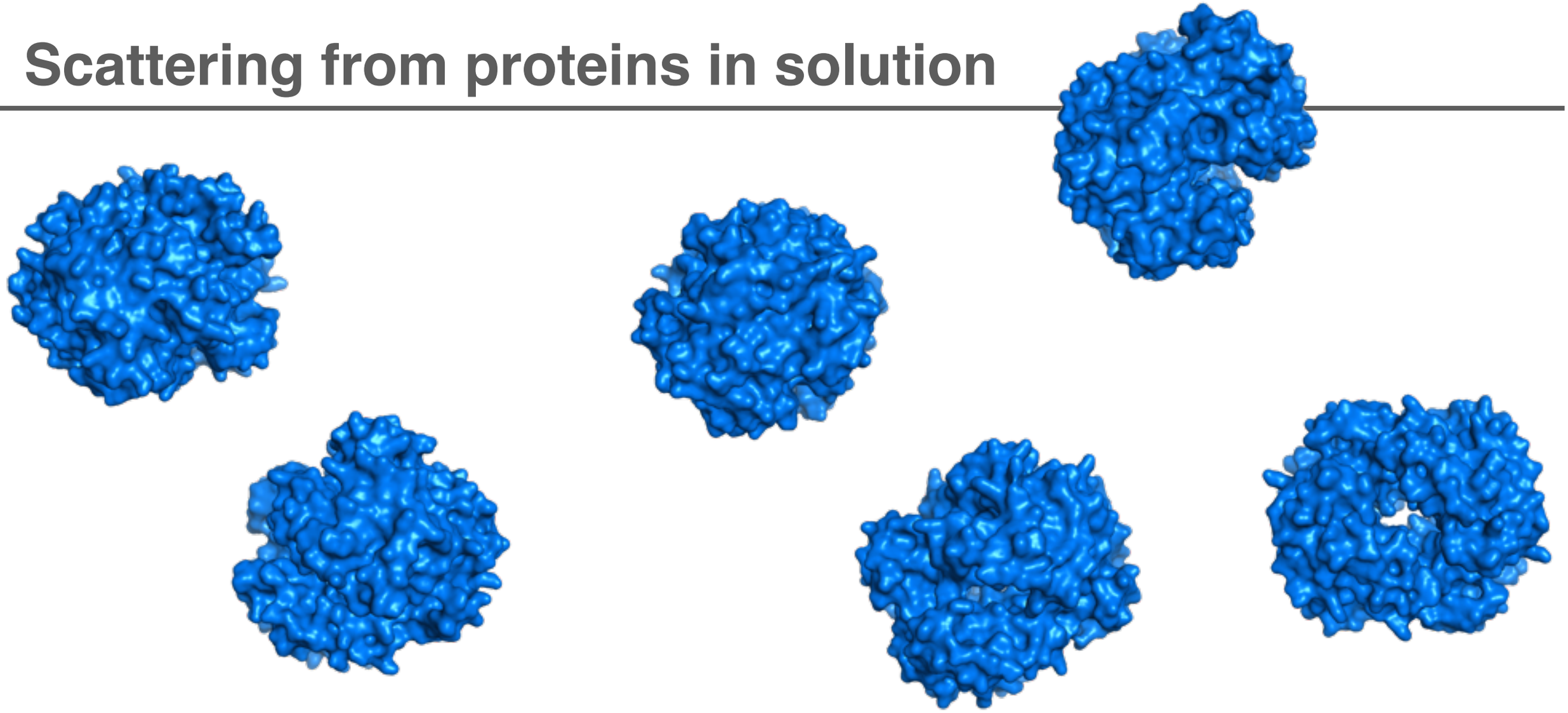


Scattering from a uniform material (constant ρ):

$$E(q) \propto \rho \int e^{-i\vec{q}\cdot\vec{r}} d\vec{r} = \rho \cdot \delta(\vec{q} - 0)$$

→zero everywhere except at zero-angle: $\boldsymbol{q} = 0$
In other words, a uniform sample does not deflect X-rays!

Scattering from proteins in solution

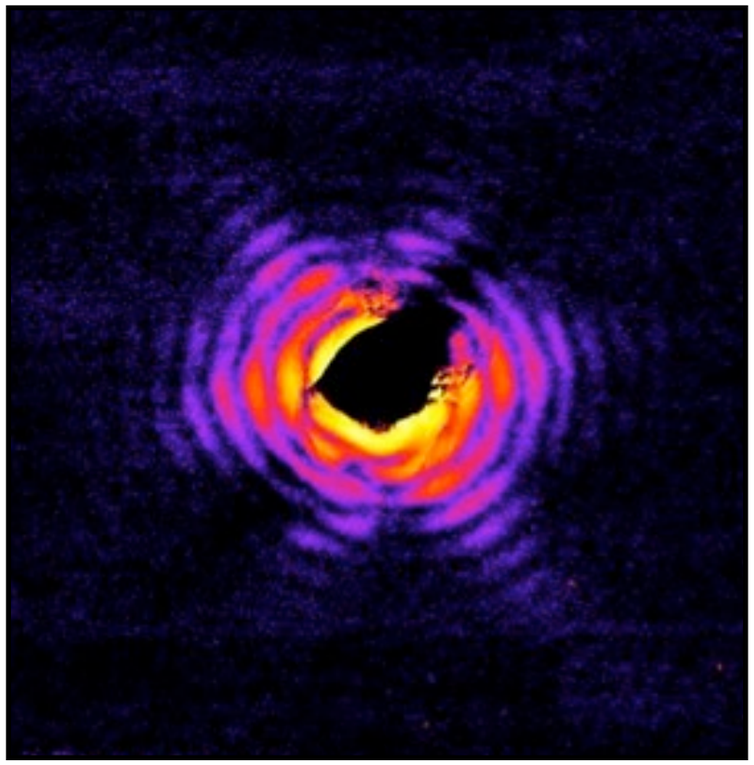


Scattering from *N* identical proteins under dilute conditions:

$$I(q) \propto N \left\langle \left| F\left[\rho(\vec{r})\right] \right|^2 \right\rangle_{\Omega}$$

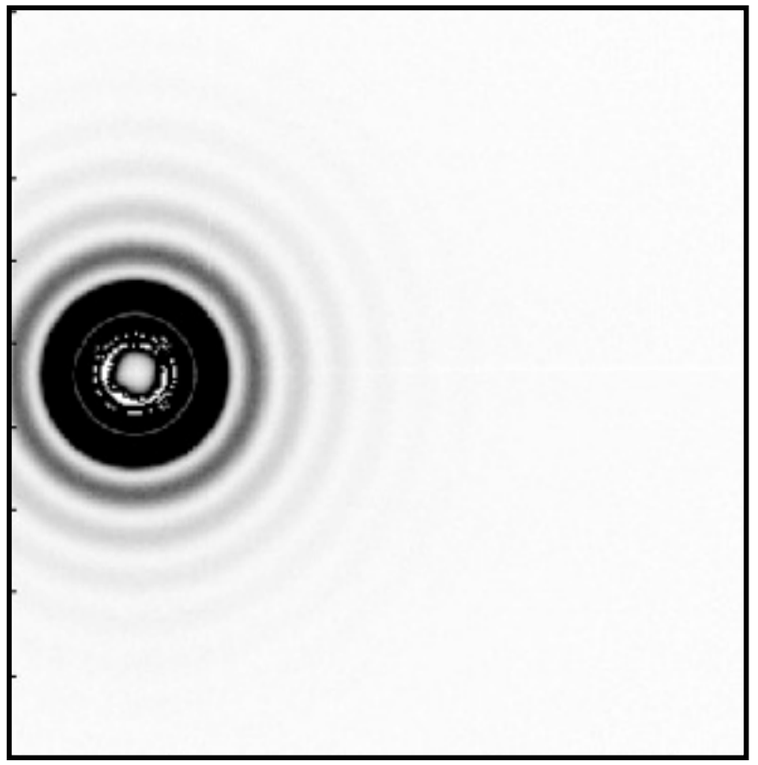
Appears like the rotational average of a single protein.

Scattering overview



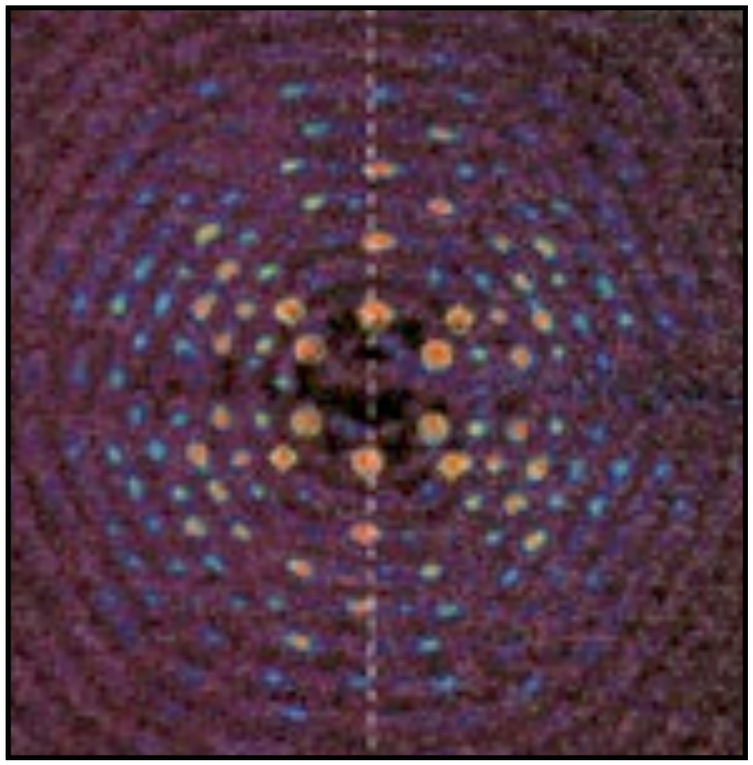
single-sphere scattering

[ESRF Sept. 2008 newsletter]



solution SAXS

[A. Finnefrock]

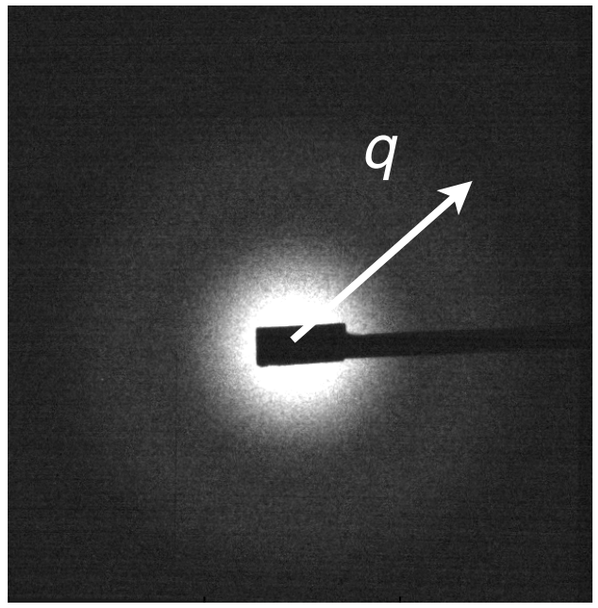


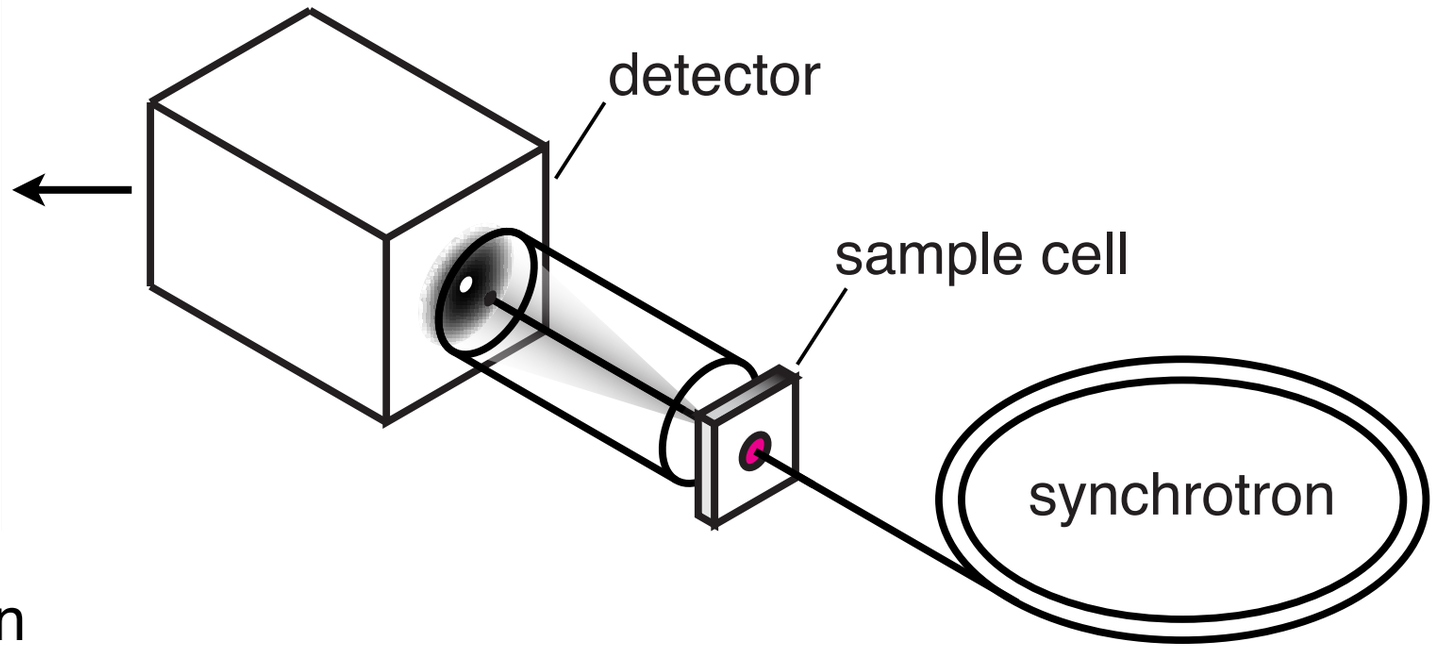
crystal diffraction

[W. L. Vos, et al. Langmuir 1997]

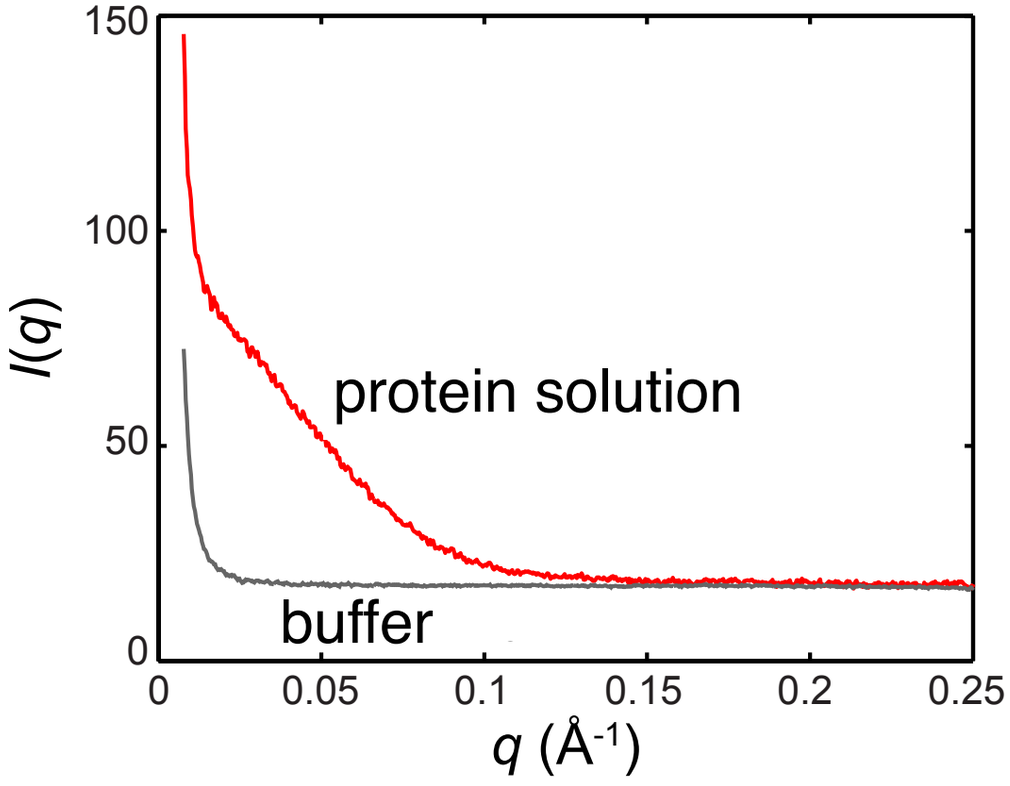
Part 2: Basic Interpretation of SAXS Data

SAXS begins with measurement of two solutions



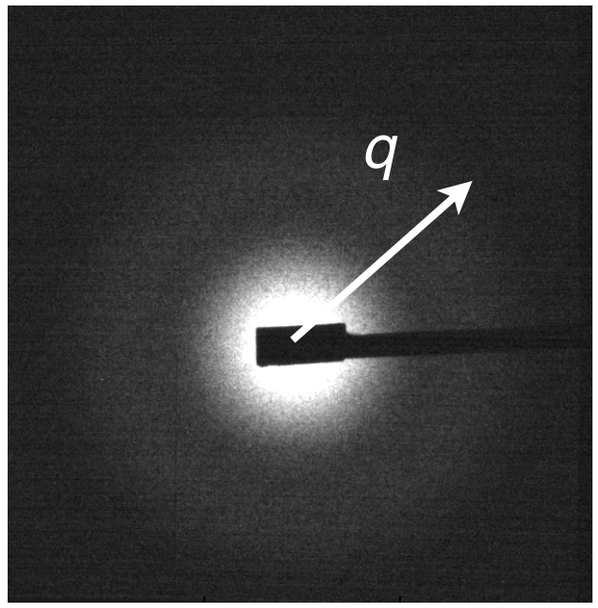


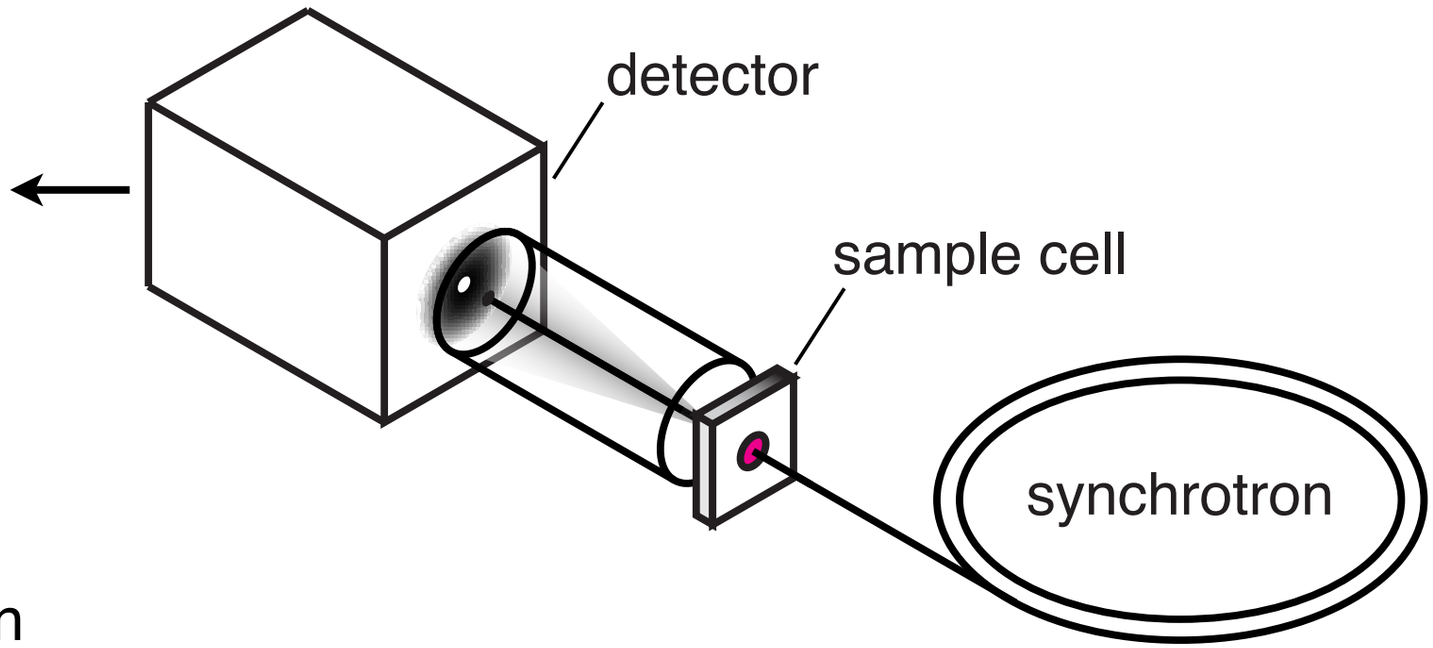
integration
$q = 4\pi/\lambda \sin\theta$



Note: Buffer must be matched **EXACTLY**, and the profiles must be accurately normalized for decay in synchrotron radiation.

Analysis is done on background-subtracted profiles





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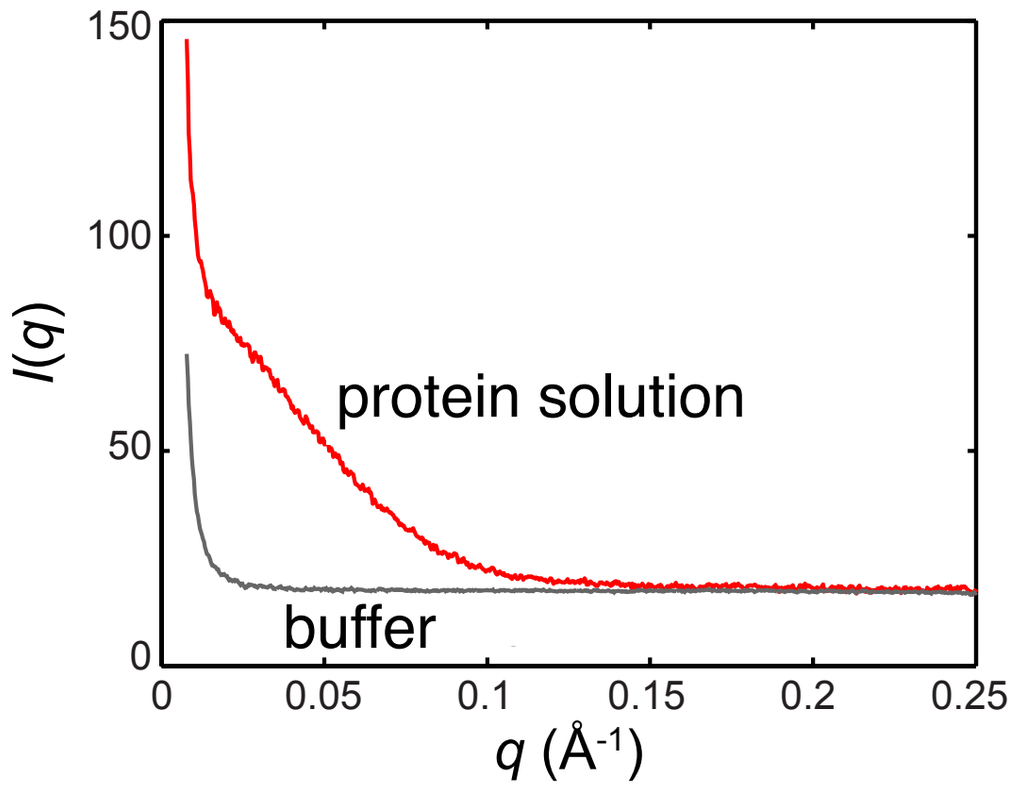
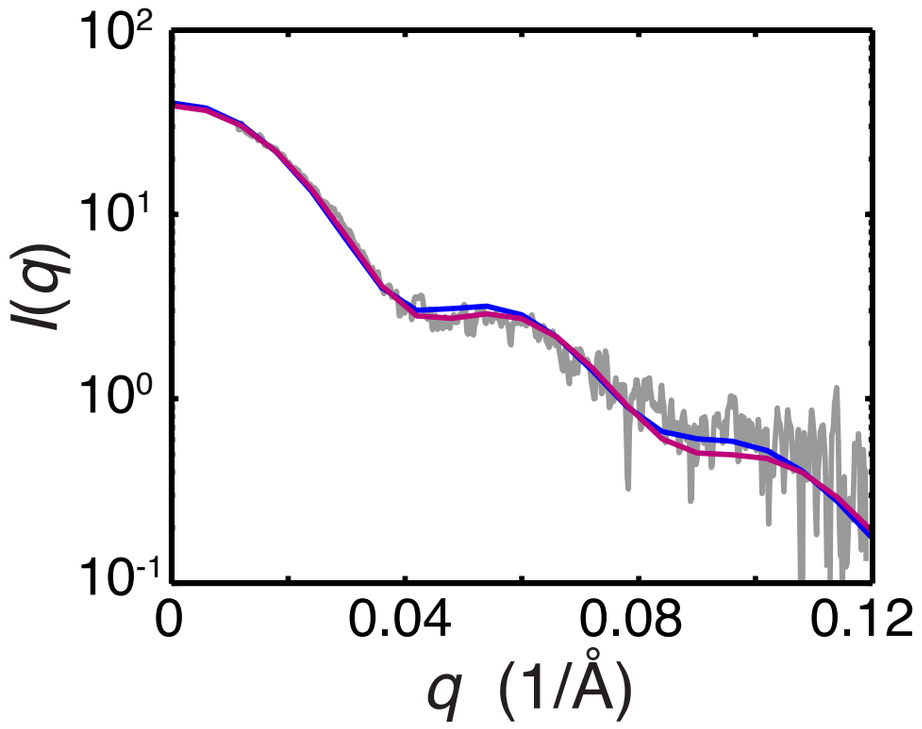


background-subtracted profile



Radius of gyration and the zero-angle intensity

From rotational averaging, we can re-write:

$$I(q) = 4\pi \int_0^{D\max} P(r) \frac{\sin qr}{qr} dr$$

P(*r*) is the pair-distance distribution, a histogram of all distances in a protein.

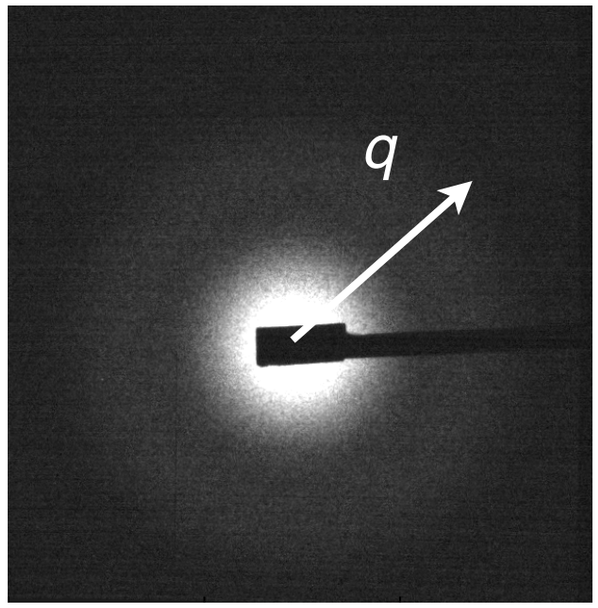
Radius of gyration = root mean square distance of all electrons:

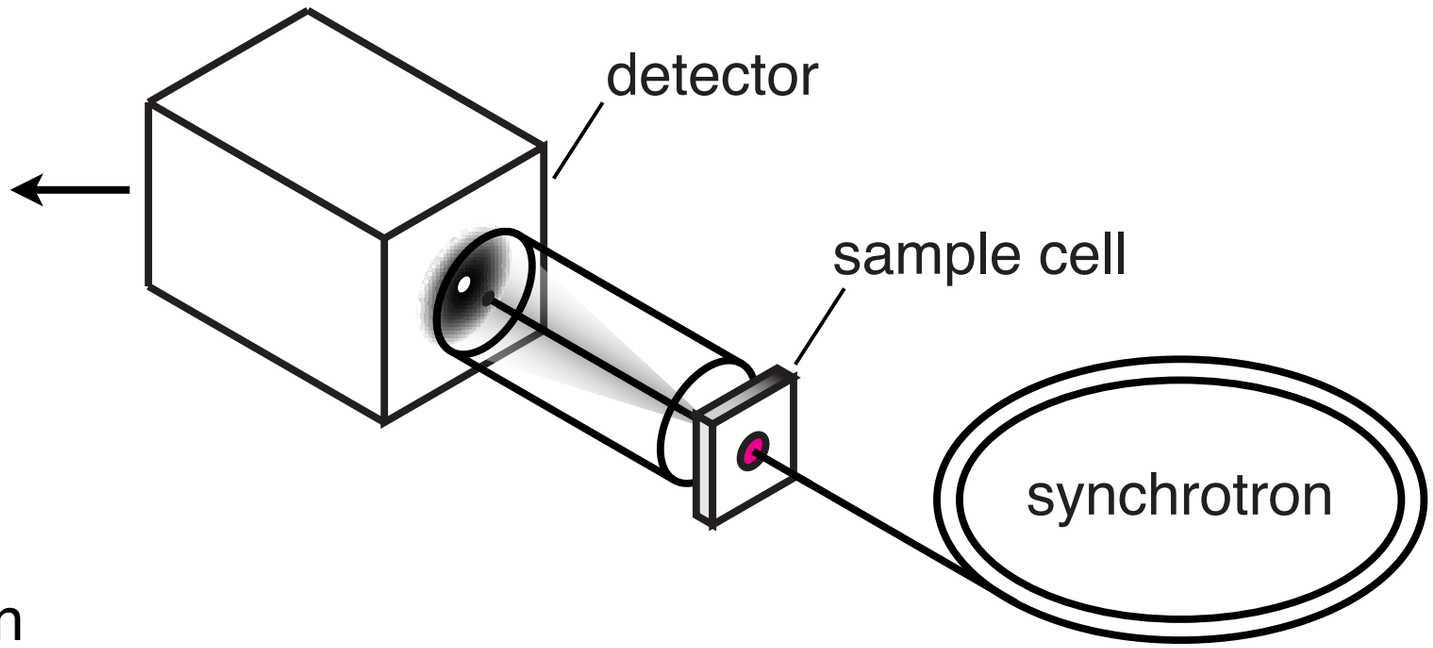
$$R_g^2 = \frac{\int_0^{D\max} r^2 P(r) dr}{2\int_0^{D\max} P(r) dr}$$

The zero-angle scattering intensity (or forward scattering intensity) is a function of the number of electrons (*n*) and the constrast, $\Delta\rho$:

$$I(0) = n / V(\Delta\rho \cdot V_p)^2 \approx f(\text{mass})$$

Interpretation of background-subtracted profiles





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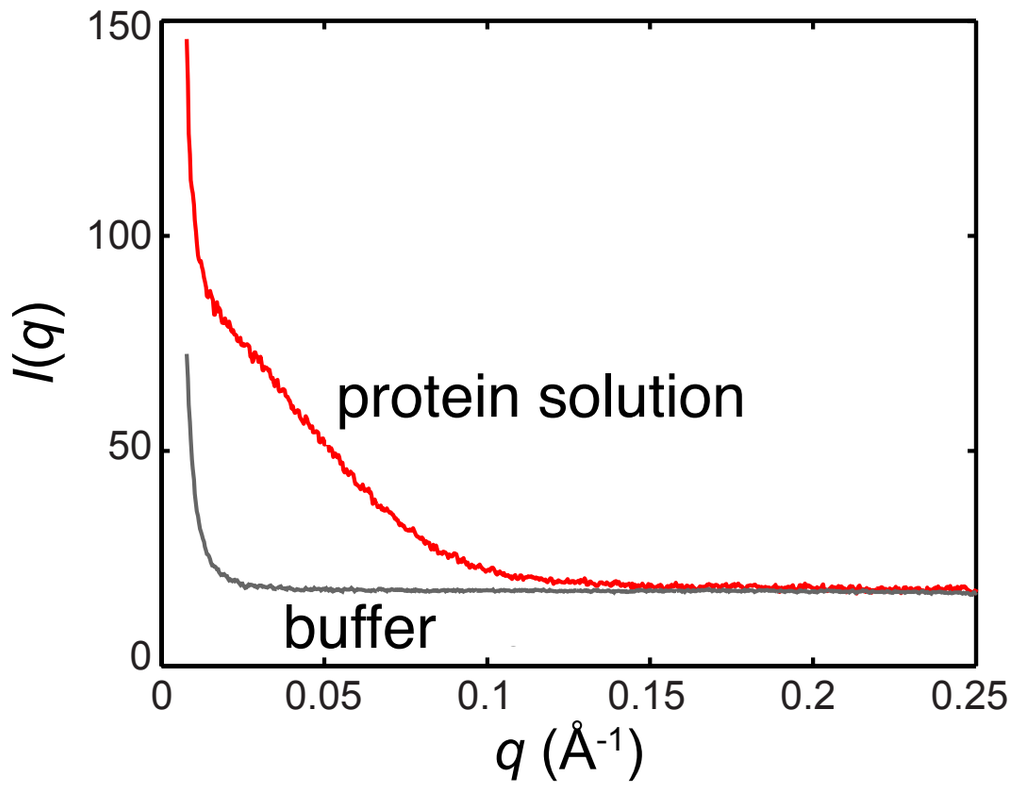

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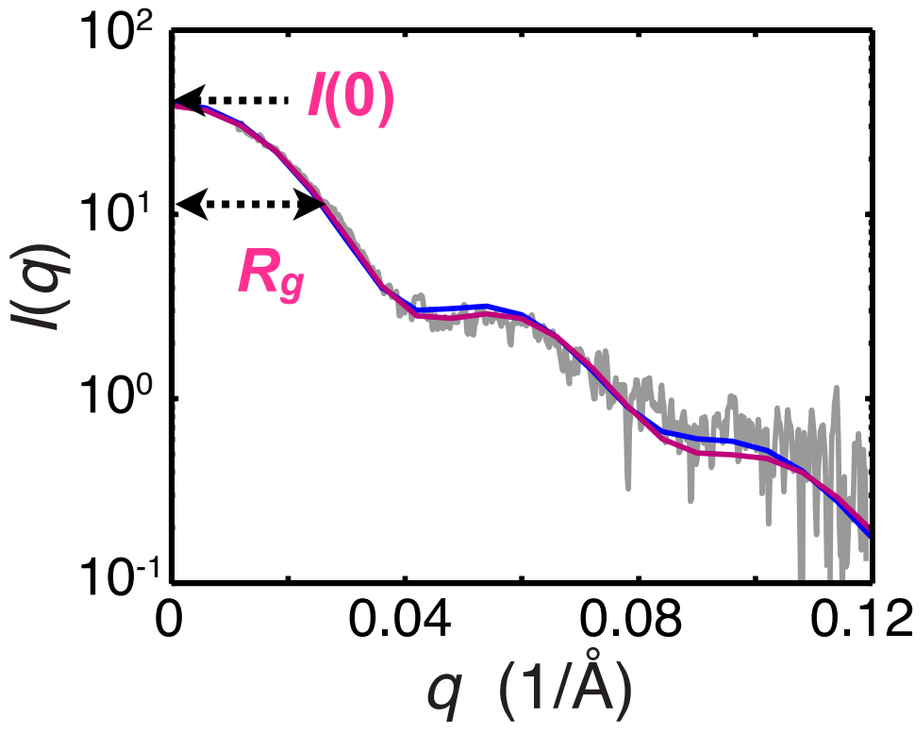
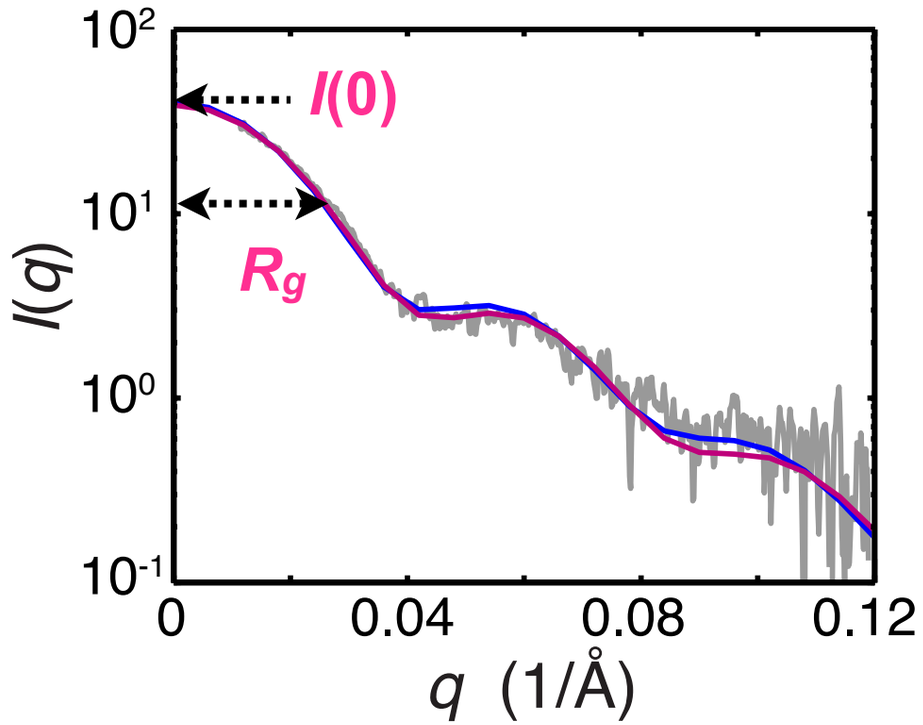


Obtaining R_g and $I(0)$ from Guinier analysis



Guinier approximation at small angles (qR_g <1.3):

$$I(q) = I(0)e^{-R_g^2 q^2/3}$$

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

Skou, *et al.* (2014) Nature Procols,in press.

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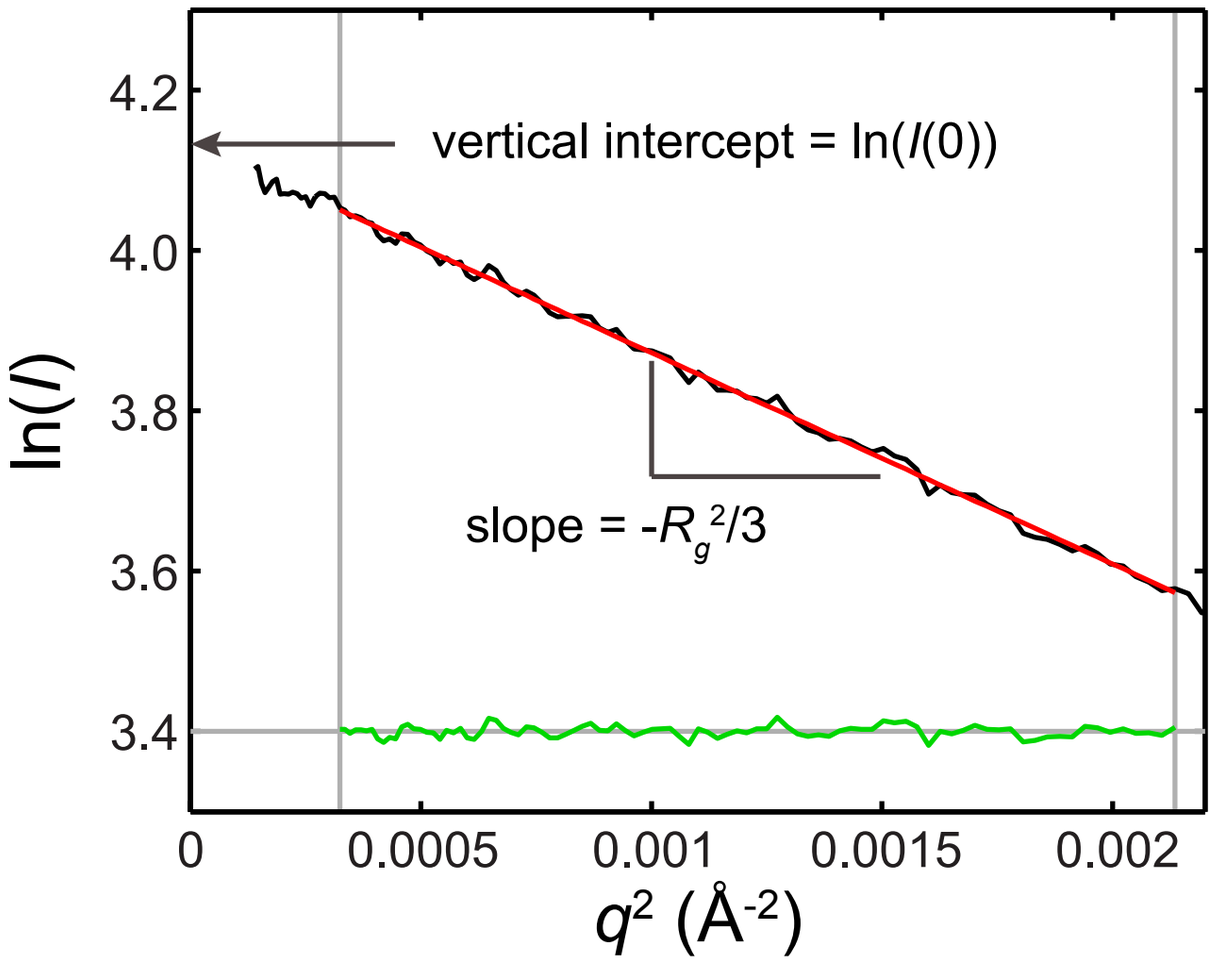


"Guinier plot"

Guinier approximation at small angles (qR_g <1.3):

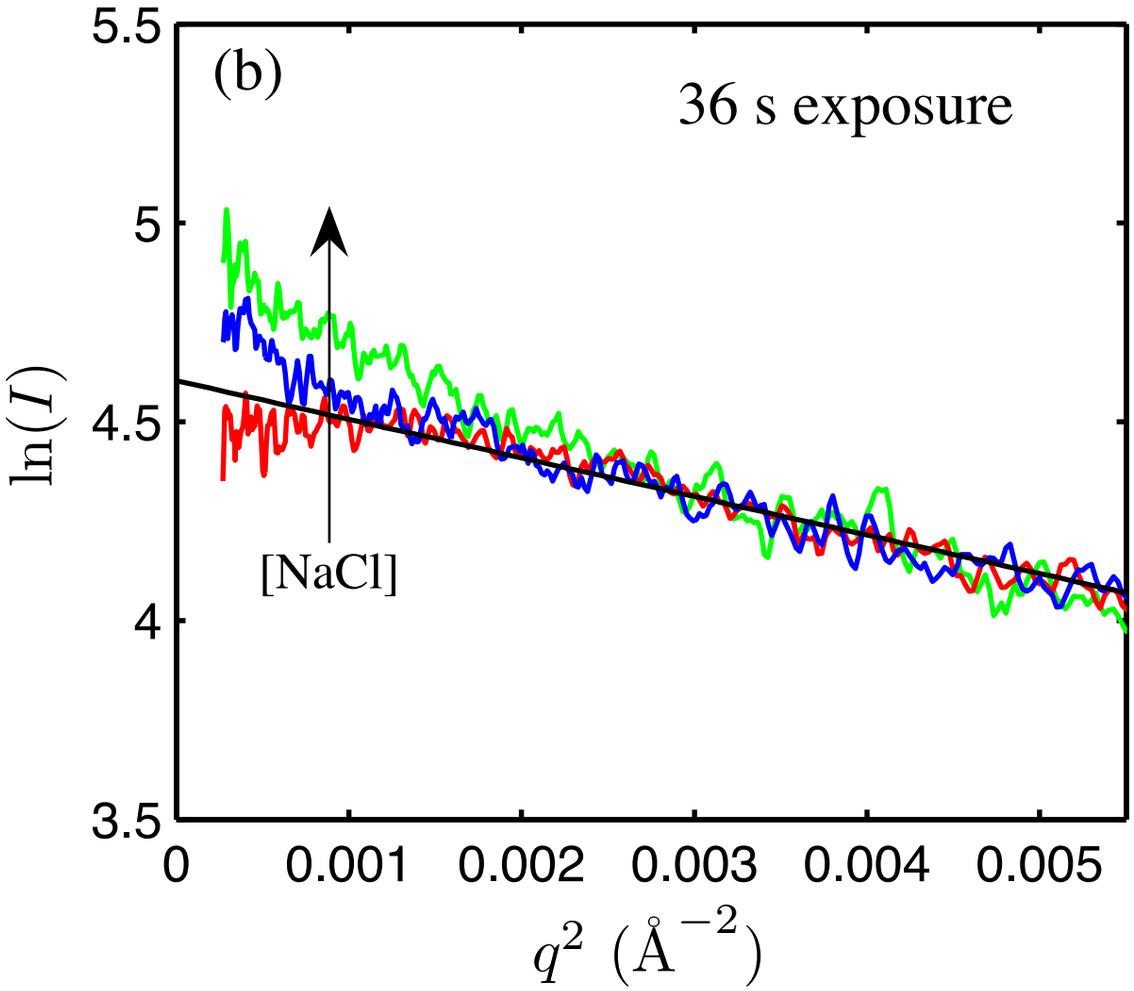
$$I(q) = I(0)e^{-R_g^2 q^2/3}$$

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

Reviewers want to see a linear Guinier plot, which is an indication of high sample/data quality.

Skou, *et al.* (2014) Nature Procols,in press.

Guinier plots are sensitive to aggregation



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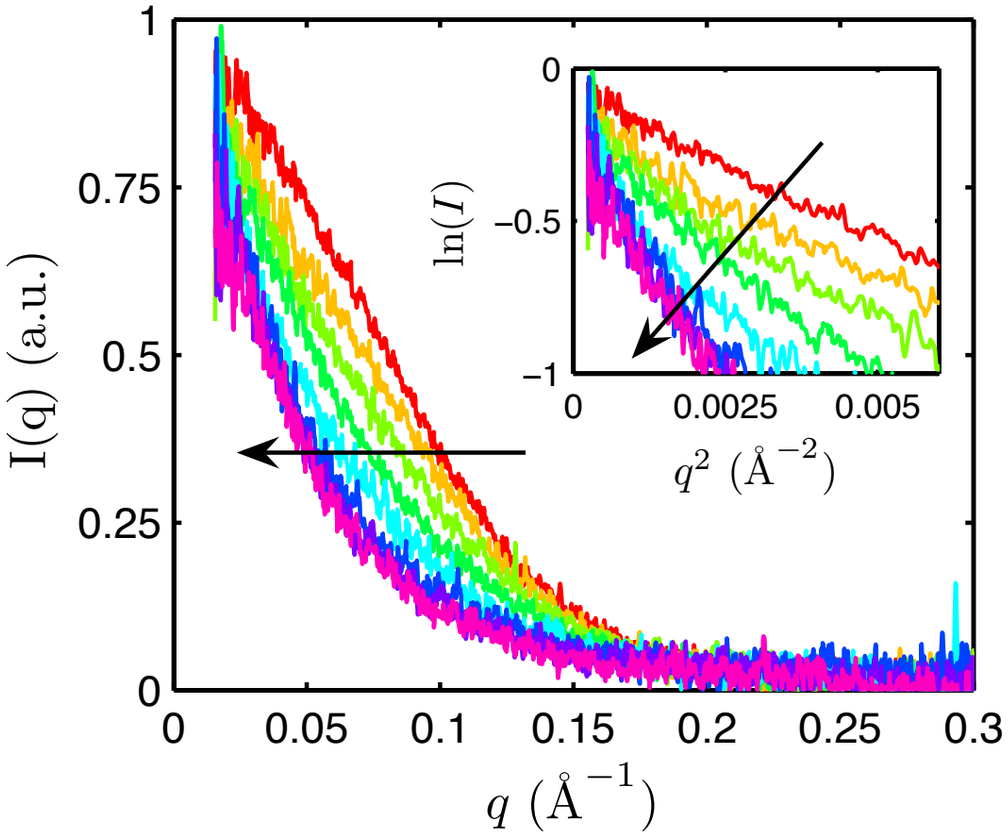
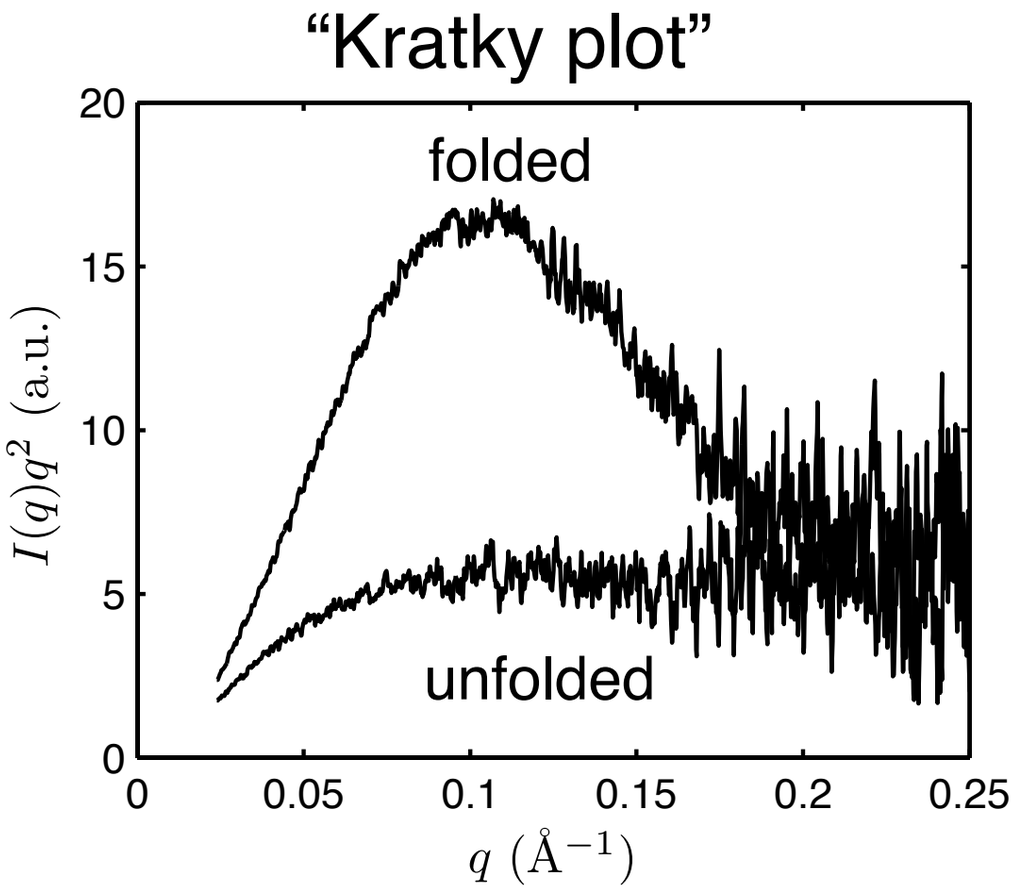
Radiation damage manifests as aggregation, which leads to an upturn at low *q*.

Skou, *et al.* (2014) Nature Procols,in press.

Shape information from Kratky analysis



Re-plot as:
Iq^2 vs q



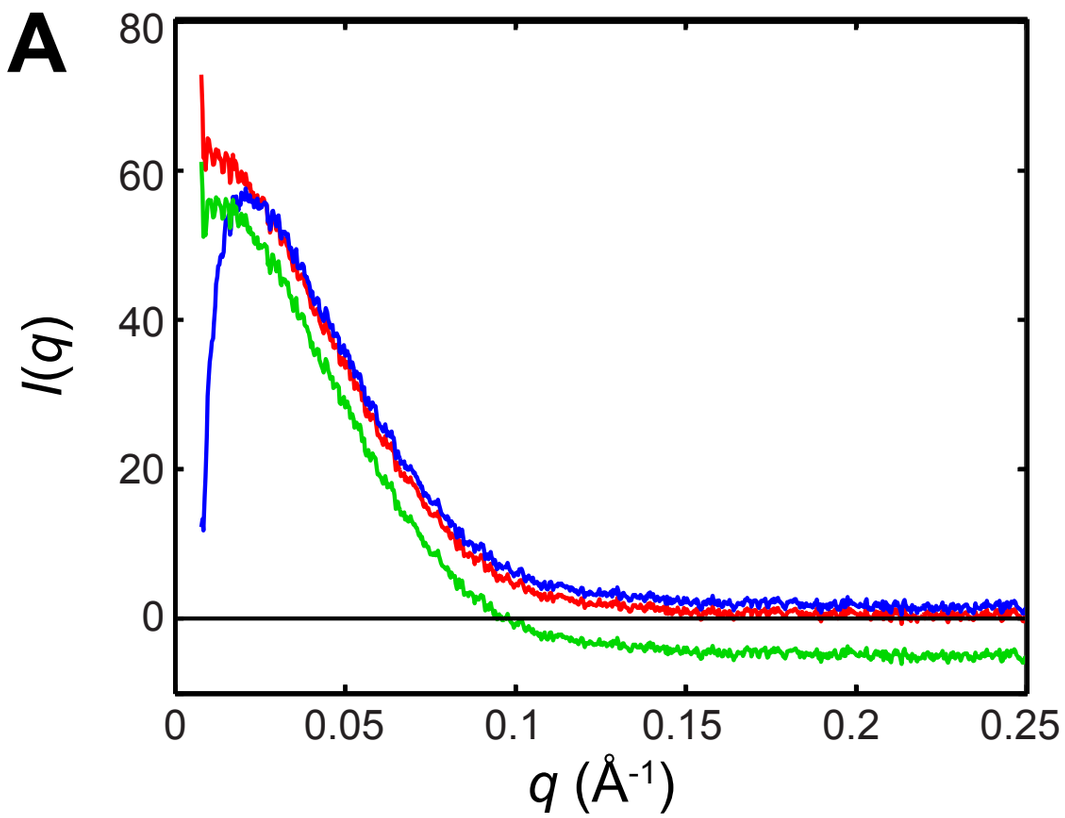
Emphasizes the power-law dependence in mid-q region ($\sim q^{-n}$) that contains information about foldedness, compactness, flexibility.

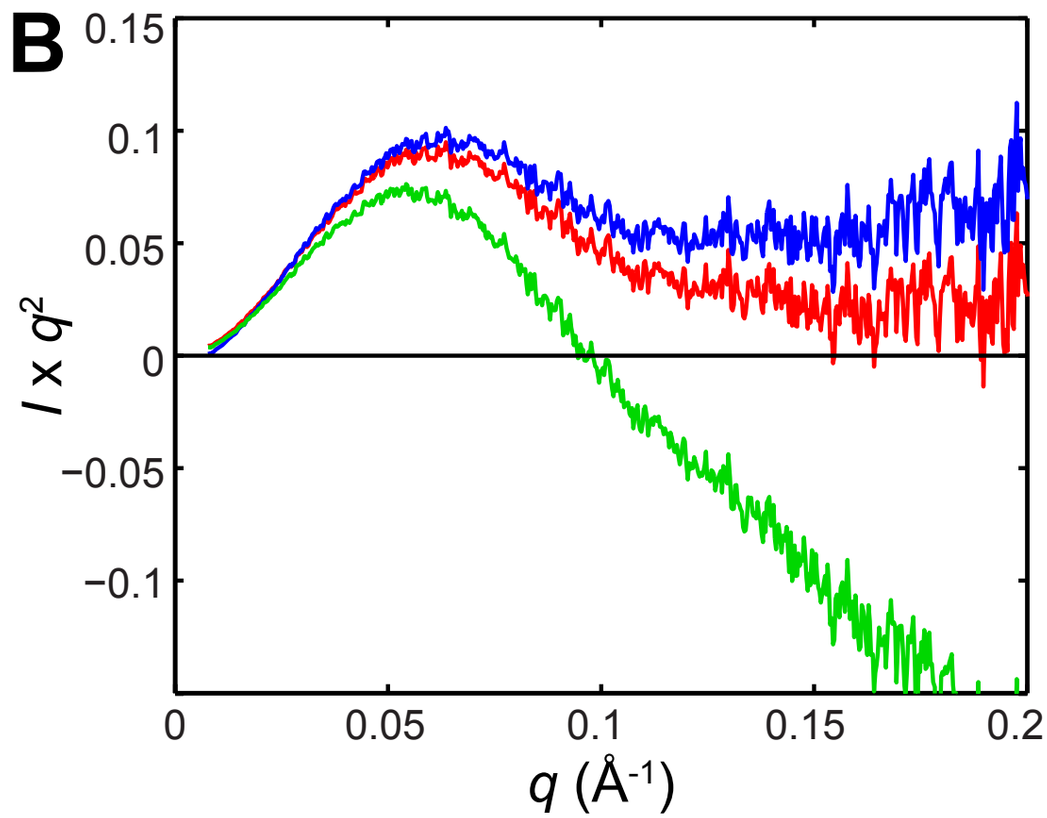
folded, compact: $n \sim -4$ $\Longrightarrow$ peak in $P(r)$
random polymer chain: $n \sim -2$ $\Longrightarrow$ plateau in $P(r)$
extended polymer chain: $n \sim -1$ $\Longrightarrow$ rise in $P(r)$

Ando, *et al.* (2008). Biochemistry, 47(42), 11097–11109.

Kratky plots are sensitive to background subtractions

Extreme examples of buffer mismatch:



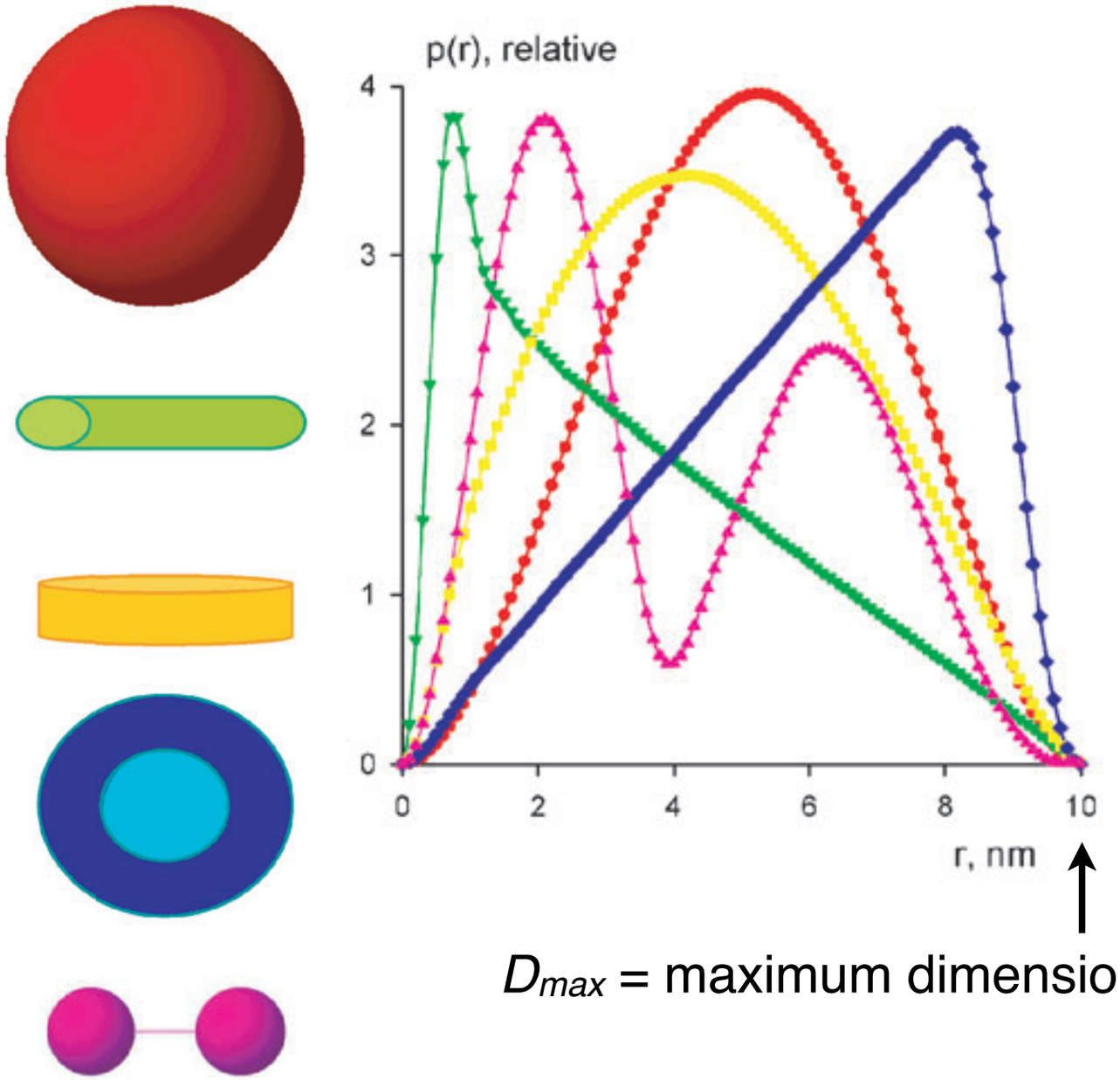


properly matched buffer
similar buffer with glycerol added
different buffer

Skou, *et al.* (2014) Nature Procols,in press.

Pair-distance distribution function

P(*r*) is the inverse Fourier Transform of *I*(*q*) and represents a histogram of electron-pair distances in a protein.



D_{max} = maximum dimension

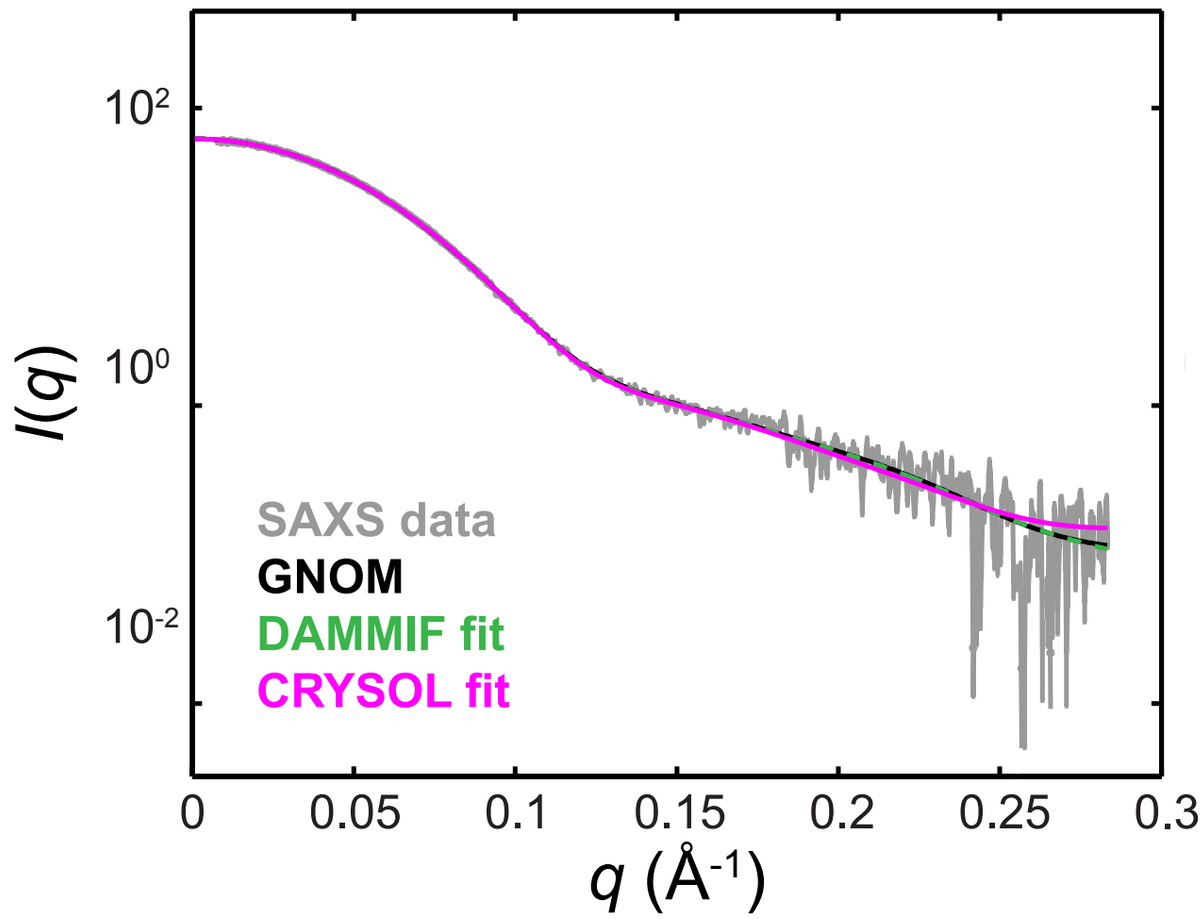
When D_{max} is well-defined, gives: R_g, $I(0)$, shape info

$$R_g^2 = \frac{\int_0^{D\max} r^2 P(r)\,dr}{2\int_0^{D\max} P(r)\,dr}$$

Svergun & Koch (2003). Reports on Progress in Physics, 66, 1735–1782.

Fitting data to theoretical scattering

- CRYSOL from ATSAS package (Svergun group, EMBL)
- FoXS server (Sali group, UCSF)



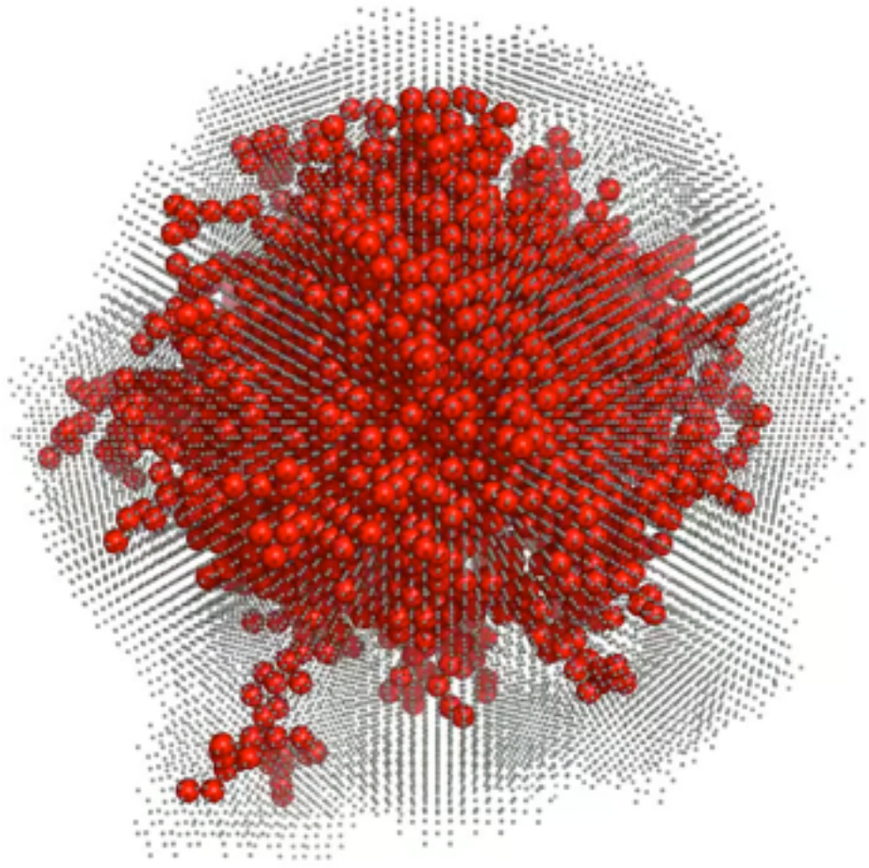
Skou, *et al.* (2014) Nature Procols,in press.

Ab initio shape reconstructions

Following *P*(*r*) analysis, a low-resolution 3D model can be reconstructed from SAXS data.

From ATSAS package:

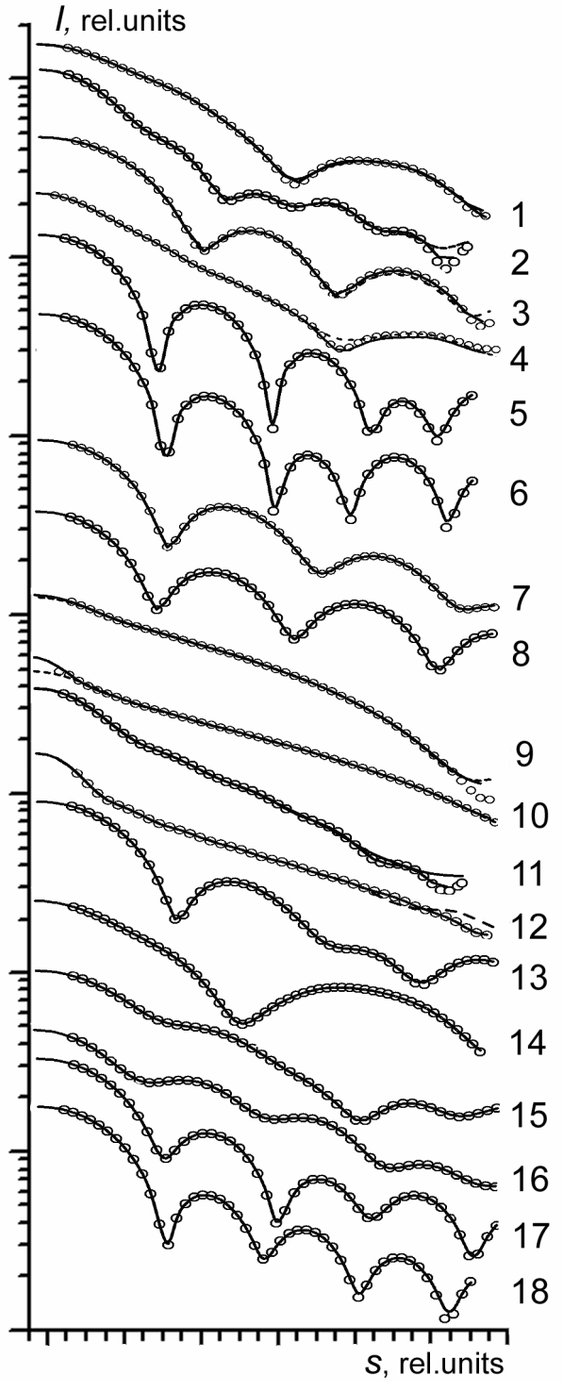
- dammif
- dammin
- gasbor
- damaver
- supcomb

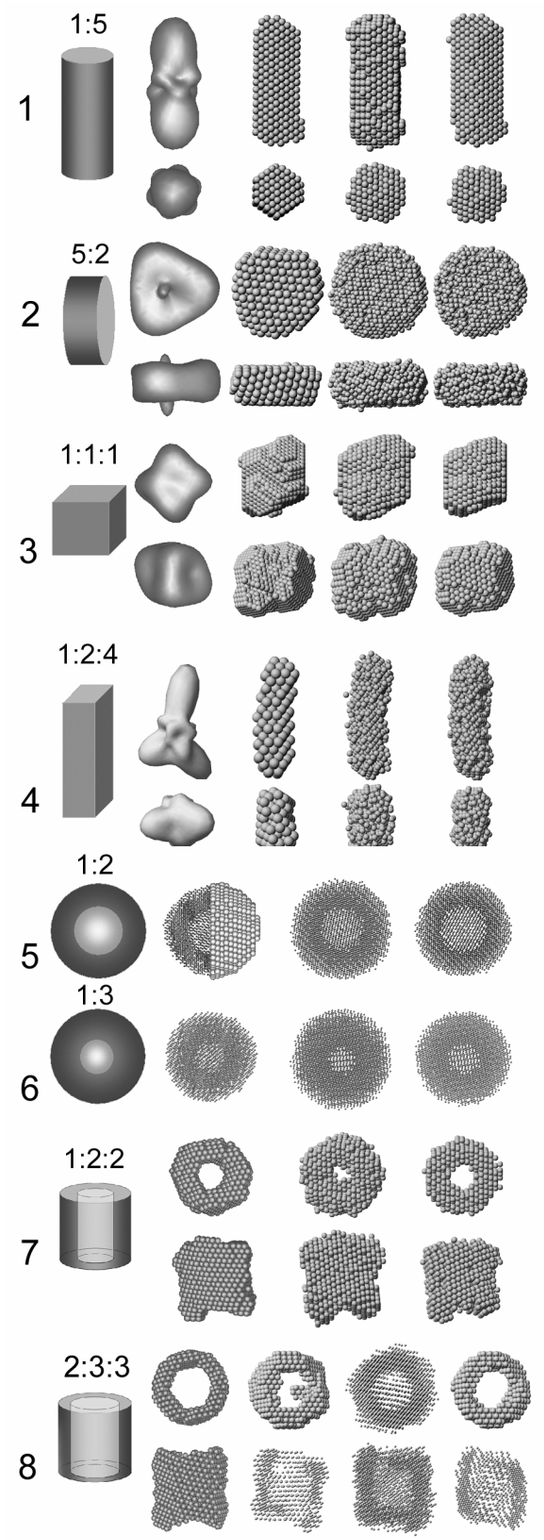


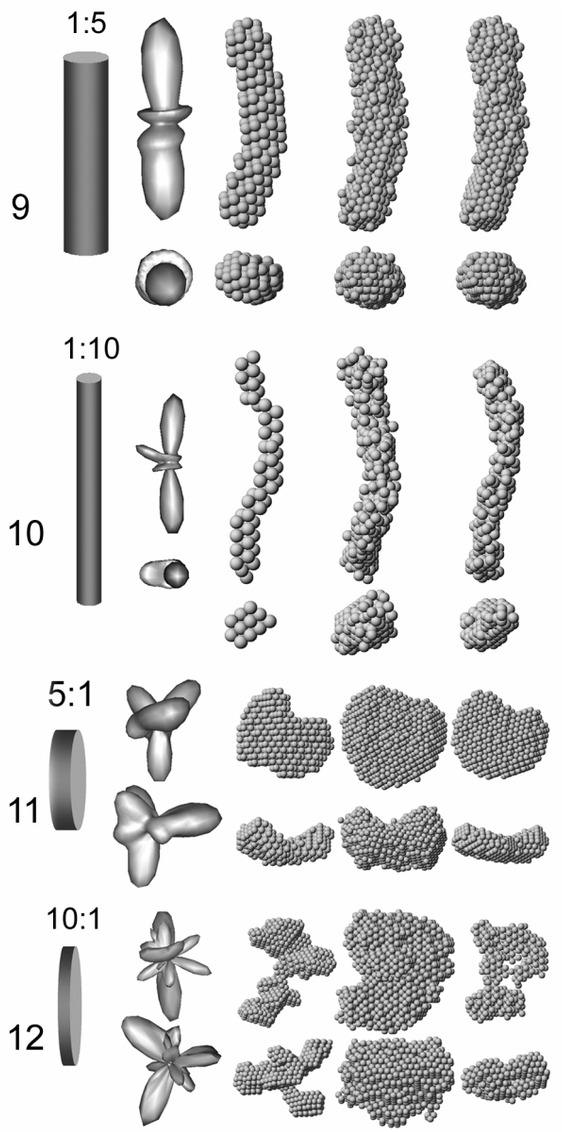
Repeat at least 10 times, align and average in *damaver*.

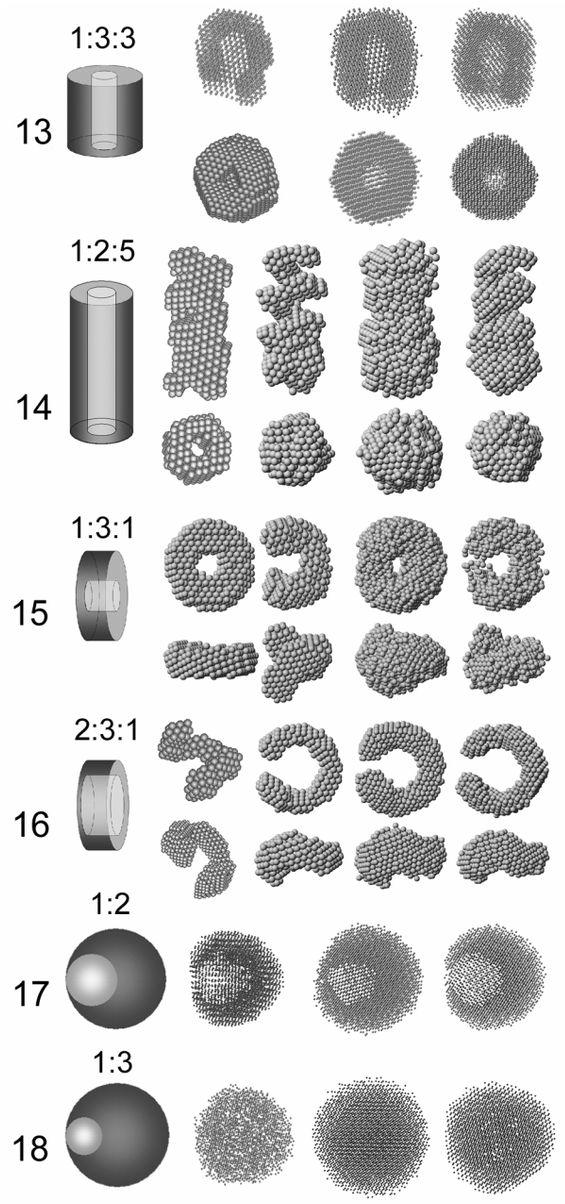
Skou, *et al.* (2014) Nature Procols,in press.

Ab initio shape reconstructions are models



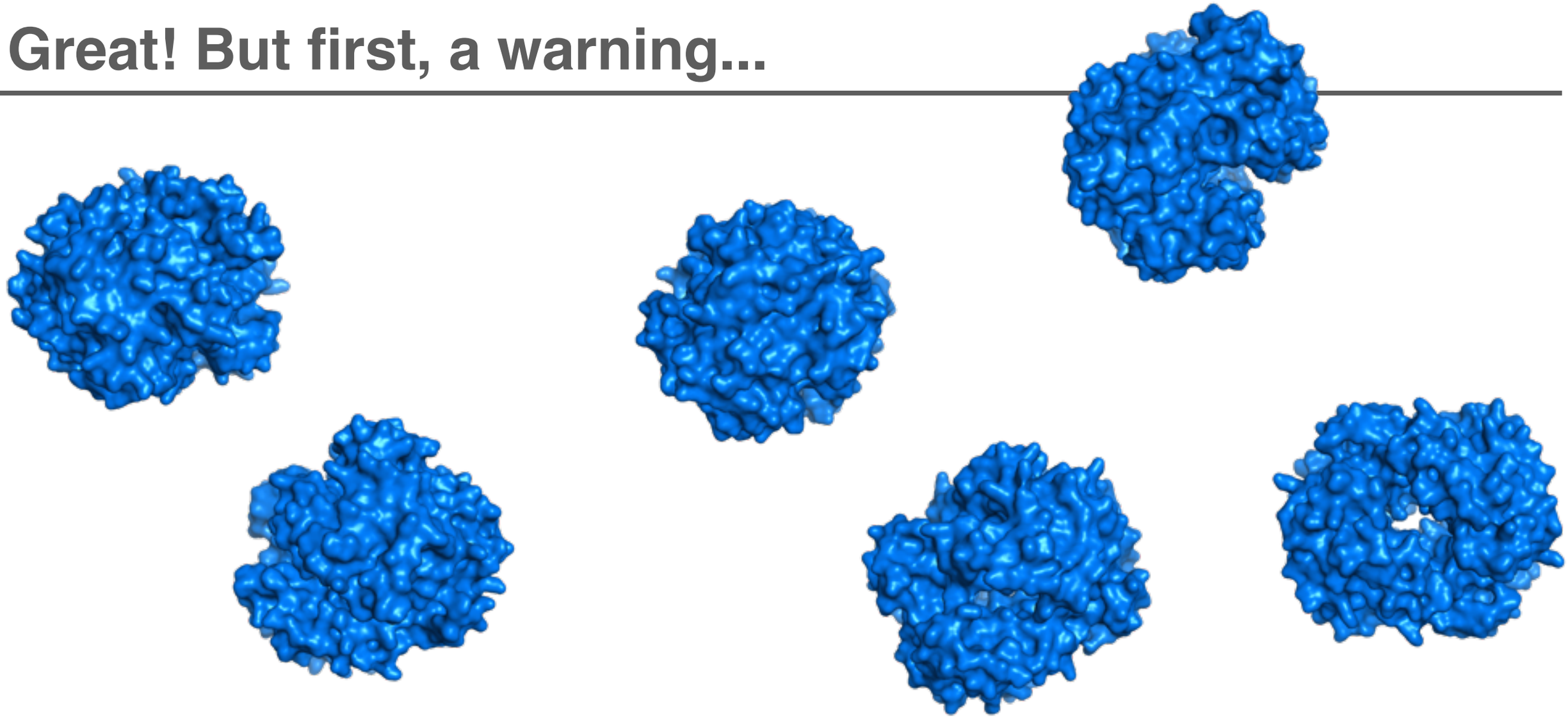






Volkov & Svergun (2003). J. Appl. Cryst. 36(3), 860–864.

Great! But first, a warning...

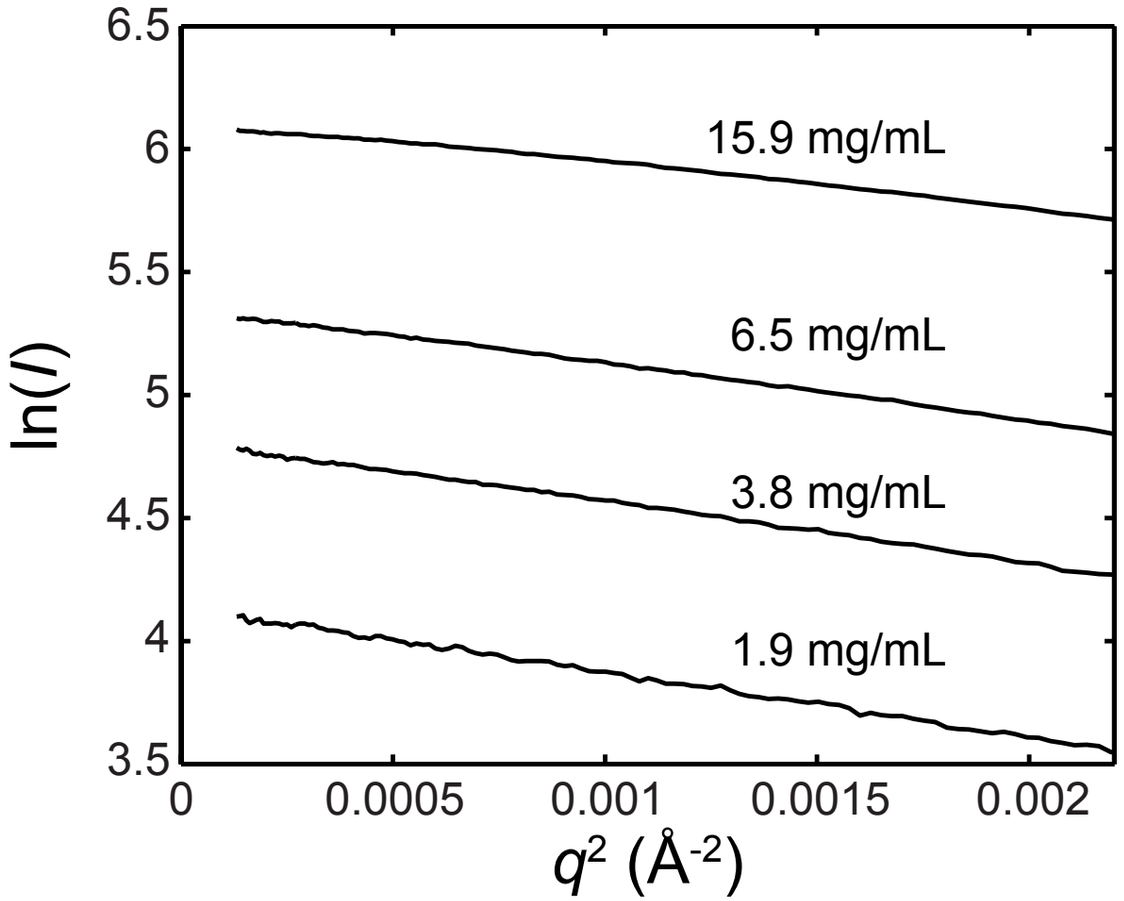


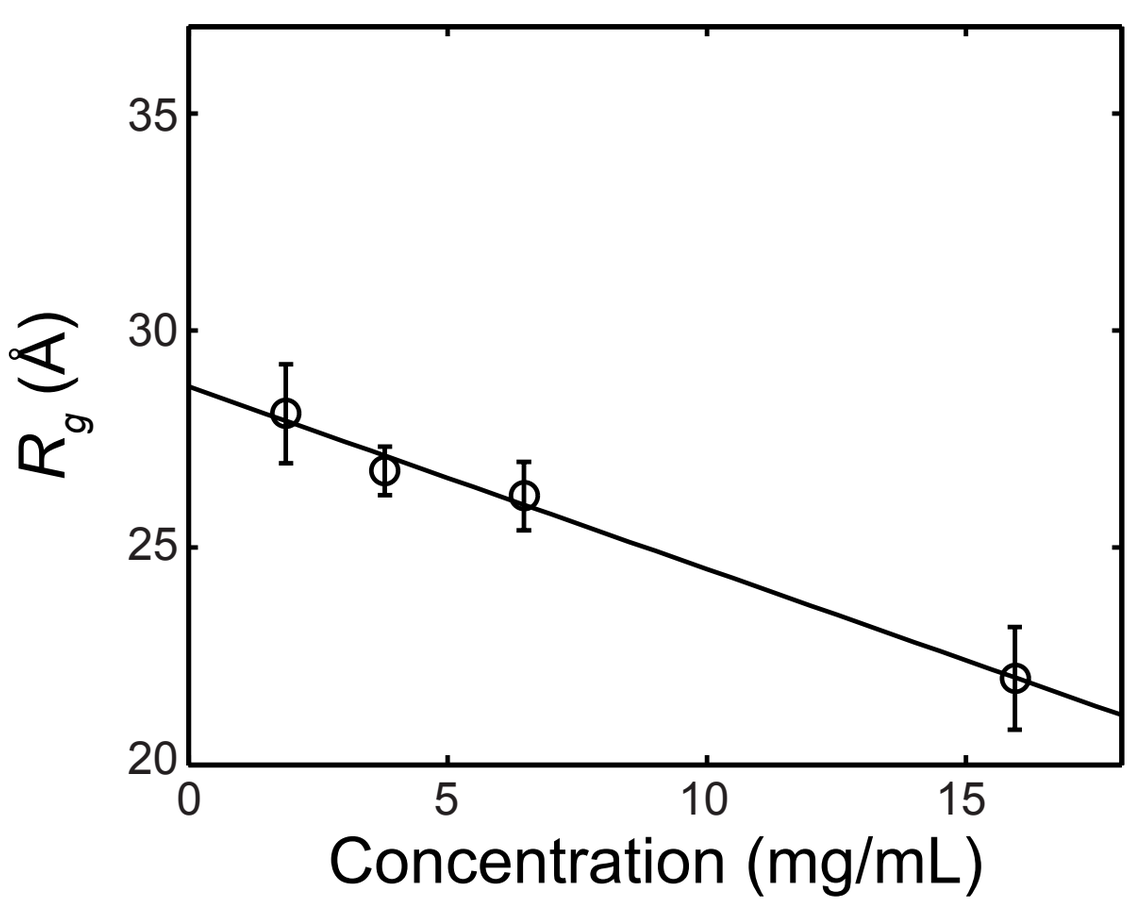
Recall that scattering from *N* proteins under **dilute** conditions:

$$I(q) \propto N \left\langle \left| F\left[\rho(\vec{r})\right] \right|^2 \right\rangle_{\Omega}$$

As long as N>1, we must be aware of **inter-particle interference**.

Concentration effects must be examined





Always, examine sample at multiple concentrations.

Report the R_g value extrapolated to infinite dilution (vertical intercept), where we can approximate “ideal-gas” like conditions.

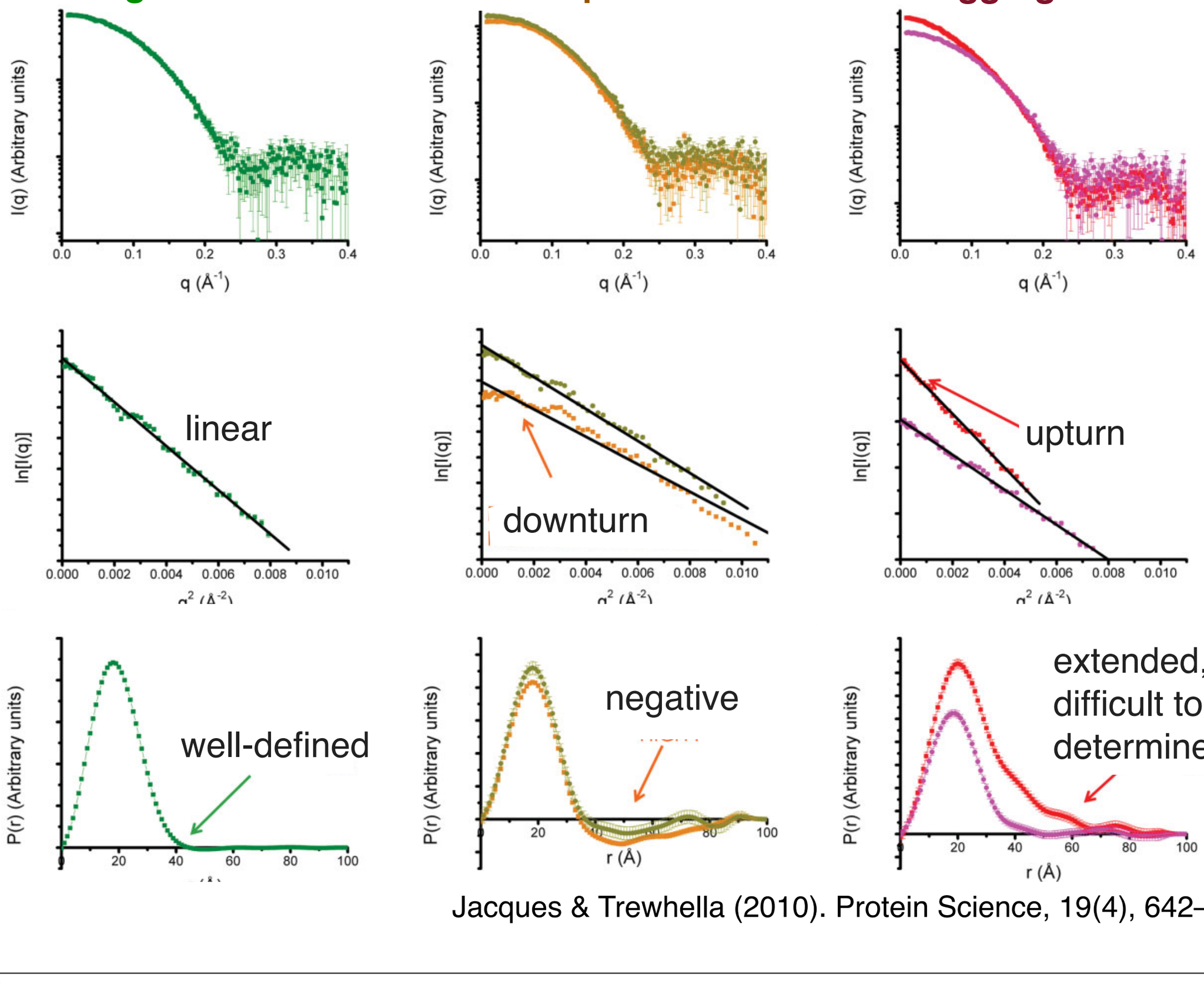
Ando, Dissertation (2008)

P(*r*) is extremely sensitive to inter-particle effects

I(q):

Guinier:

P(r):



Jacques & Trewhella (2010). Protein Science, 19(4), 642–657.

Part 3: Experimental Requirements

What do you need for SAXS?

A protein stock solution:

- Must be well characterized by other methods for high purity, conformational homogeneity, no aggregates.

- Must have accurately measured concentration (typically 1-10 mg/ml required for good signal, but depends on MW).

- Typical sample volumes are only 10-40 µL, but you will do many measurements, so bring as much as you can.

What do you need for SAXS?

Buffer solution:

• Almost all buffers useful for biochemistry are compatible.

• Should contain all ingredients needed for protein stability.

• Unless necessary, avoid adding excess of ingredients that can reduce contrast (e.g. >15% glycerol, excess metallocofactors).

• Most importantly, must be exactly matched to protein solution (use dialysate or elution buffer).

What do you need for SAXS?

Equipment requirements:

• X-ray source.

• Good PIN-diode beamstop to accurately measure transmission intensity.

• Good detector.

• Beamline and sample cell with low background scatter.

• Computers and software to process raw images and analyze processed data.

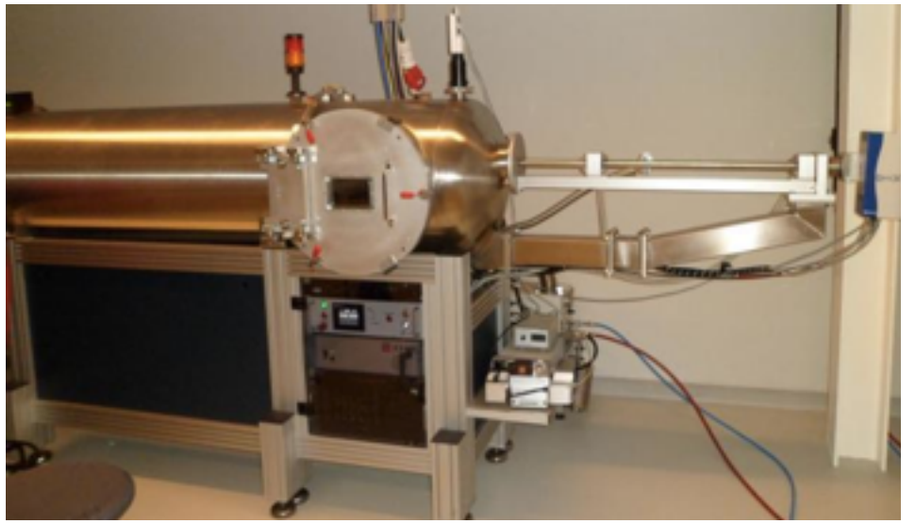
What do you need for SAXS?

Good experimental technique:

- Document everything.
- Accurate pipetting.
- Be observant (watch detector images as they are generated, monitor flux, etc).
- Back up data, and don’t discard raw images.

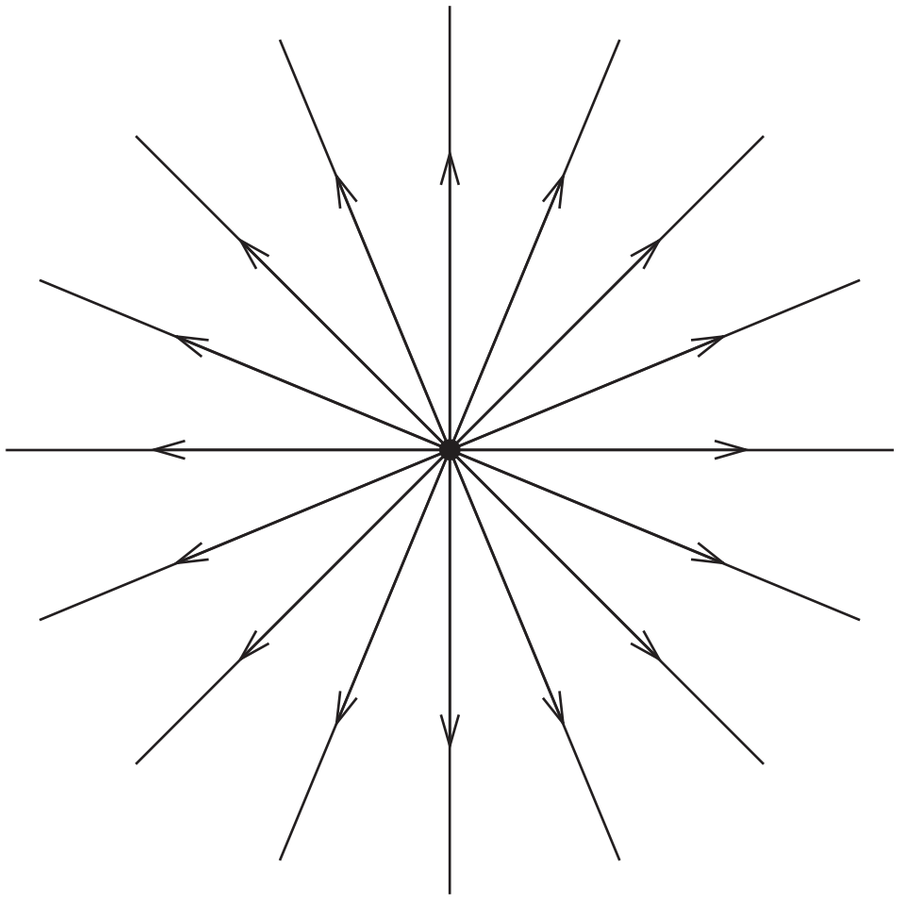
Laboratory anode-based X-ray generators

- Bruker NanoStar
- Anton Paar SAXSess
- Rigaku BioSAXS
- SAXSLab Ganesha



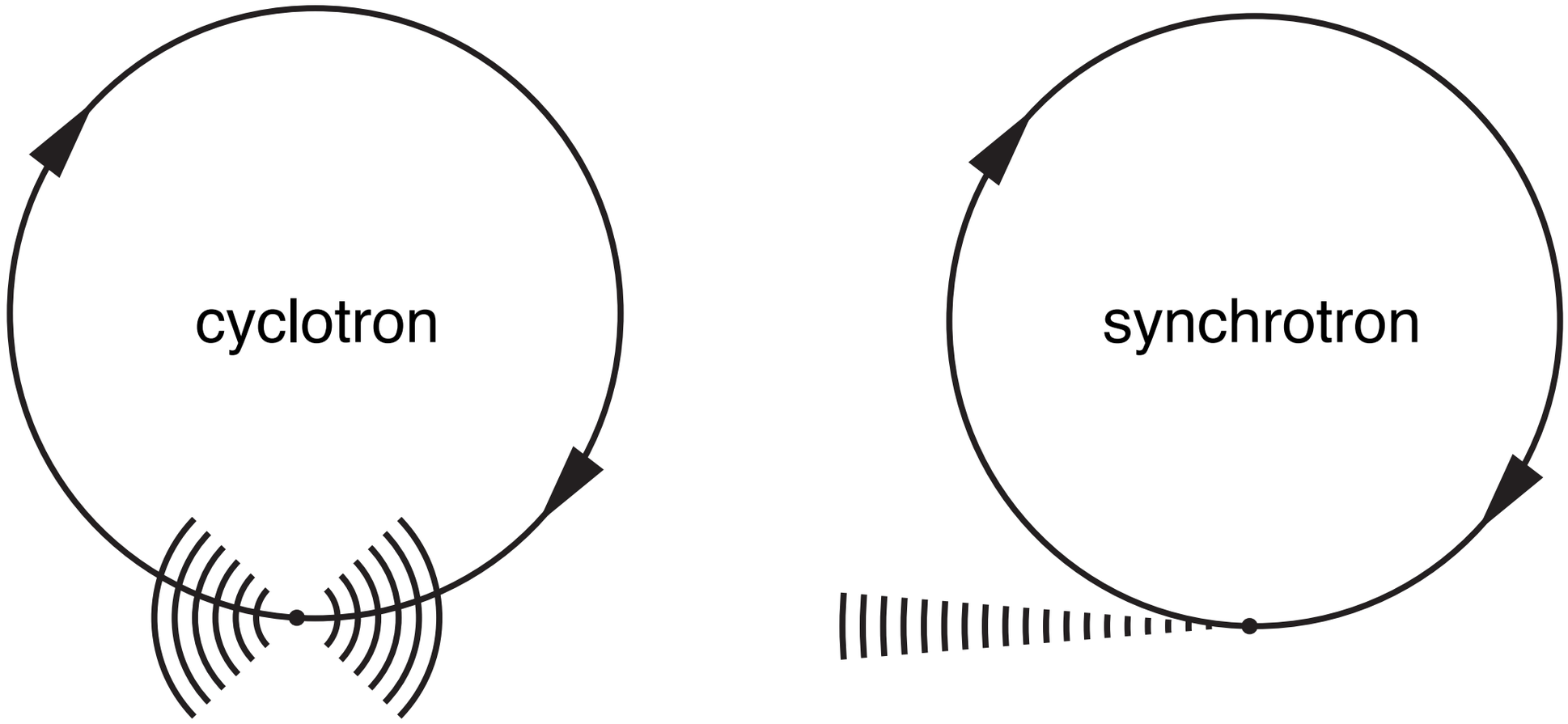
Synchrotron radiation

Recipe for EM waves: charged particle supplies electric field



Accelerate the charged particle in order to create a wave front in the electric field and induce a magnetic field.

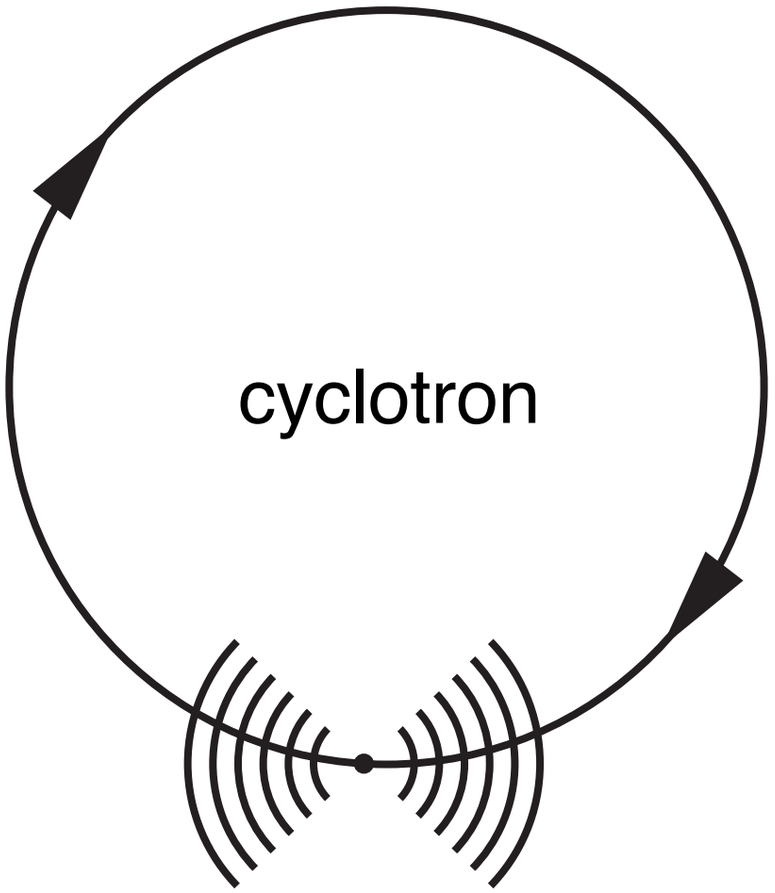
Synchrotron radiation comes from relativistic charge



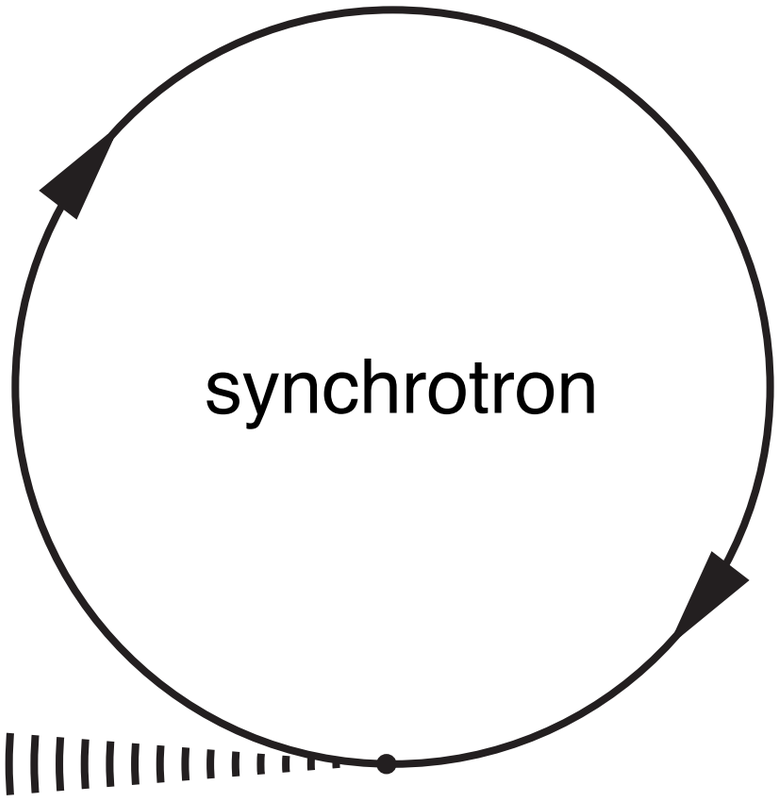
Synchrotron: 5 GeV electrons move at .9999999 c.

- Relativistic beaming = highly intense, collimated beams.
- Spectrum of radiation = tunable wavelength source.

Synchrotron radiation comes from relativistic charge



electron frame

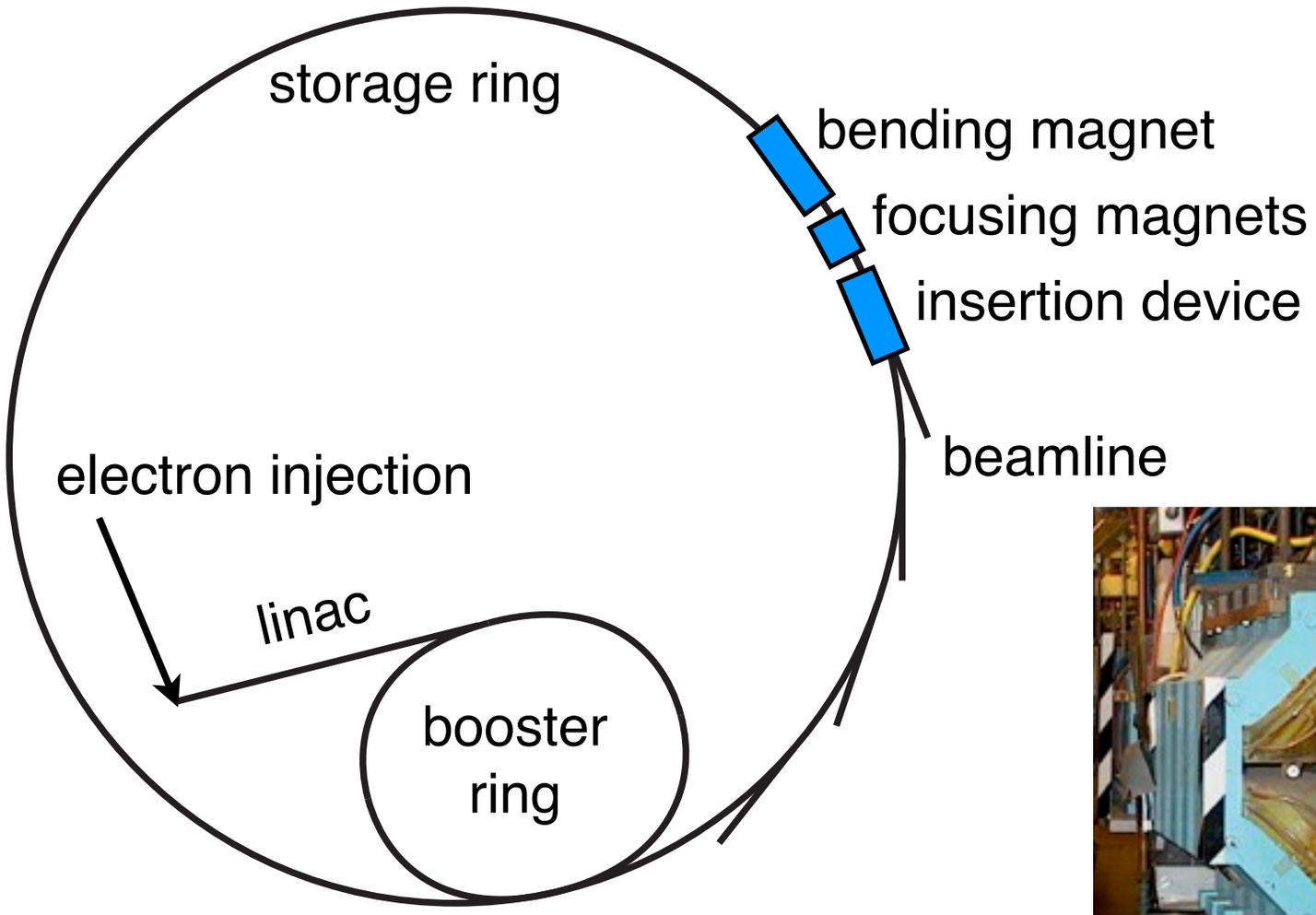


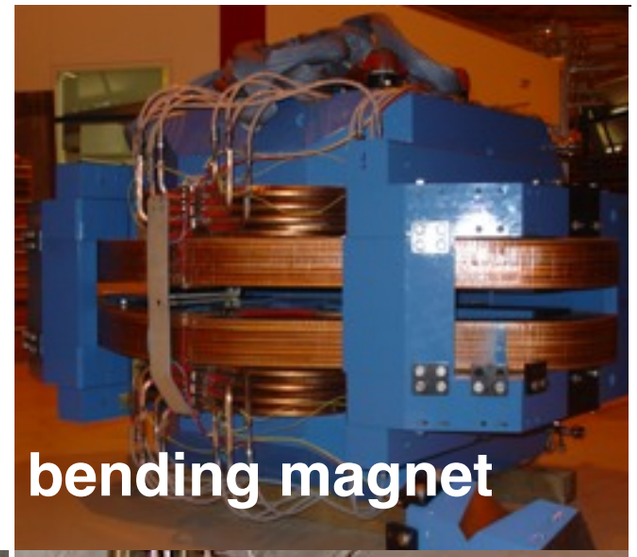
lab frame

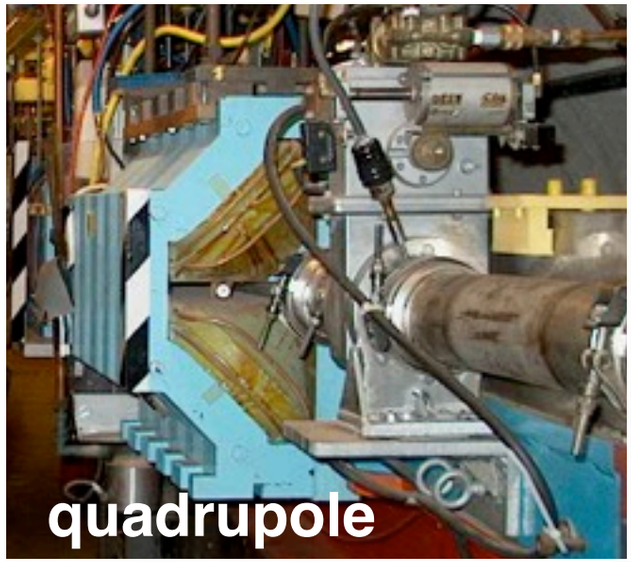
Synchrotron: 5 GeV electrons move at .9999999 c.

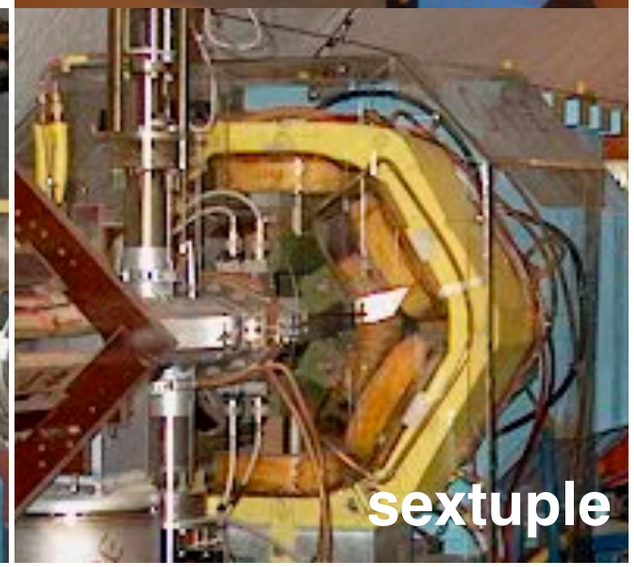
- Relativistic beaming = highly intense, collimated beams.
- Spectrum of radiation = tunable wavelength source.

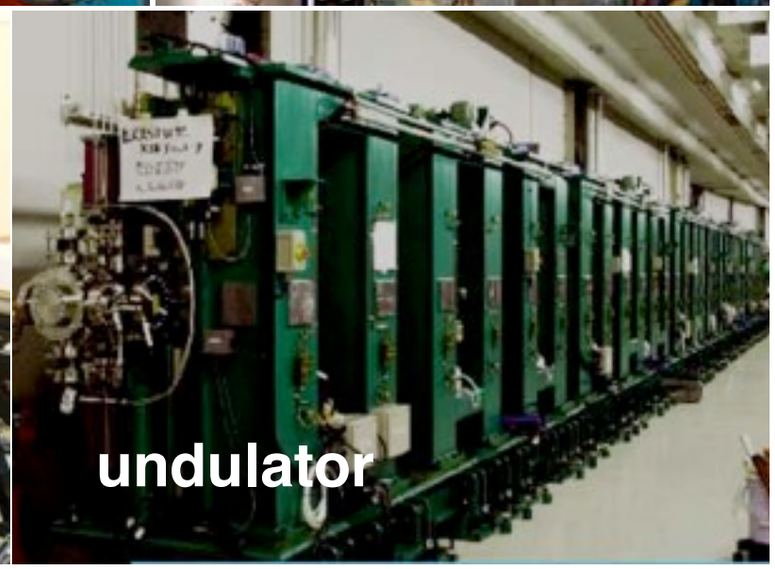
Elements of storage ring synchrotrons











Access to synchrotron facilities

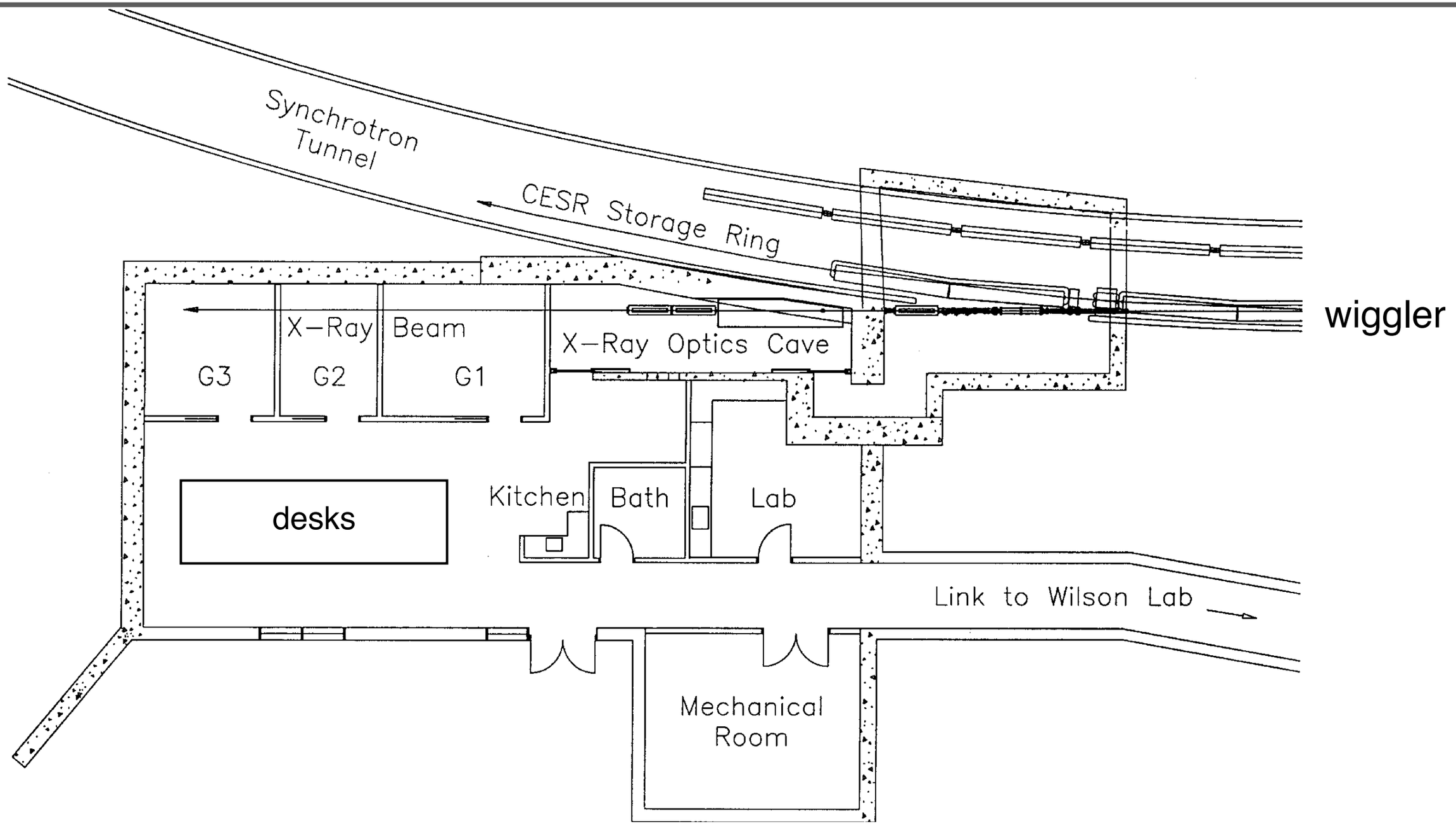
Most operate on a rapid-access proposal mechanism.

Many host SAXS workshops.

Get in touch with a beamline scientist.

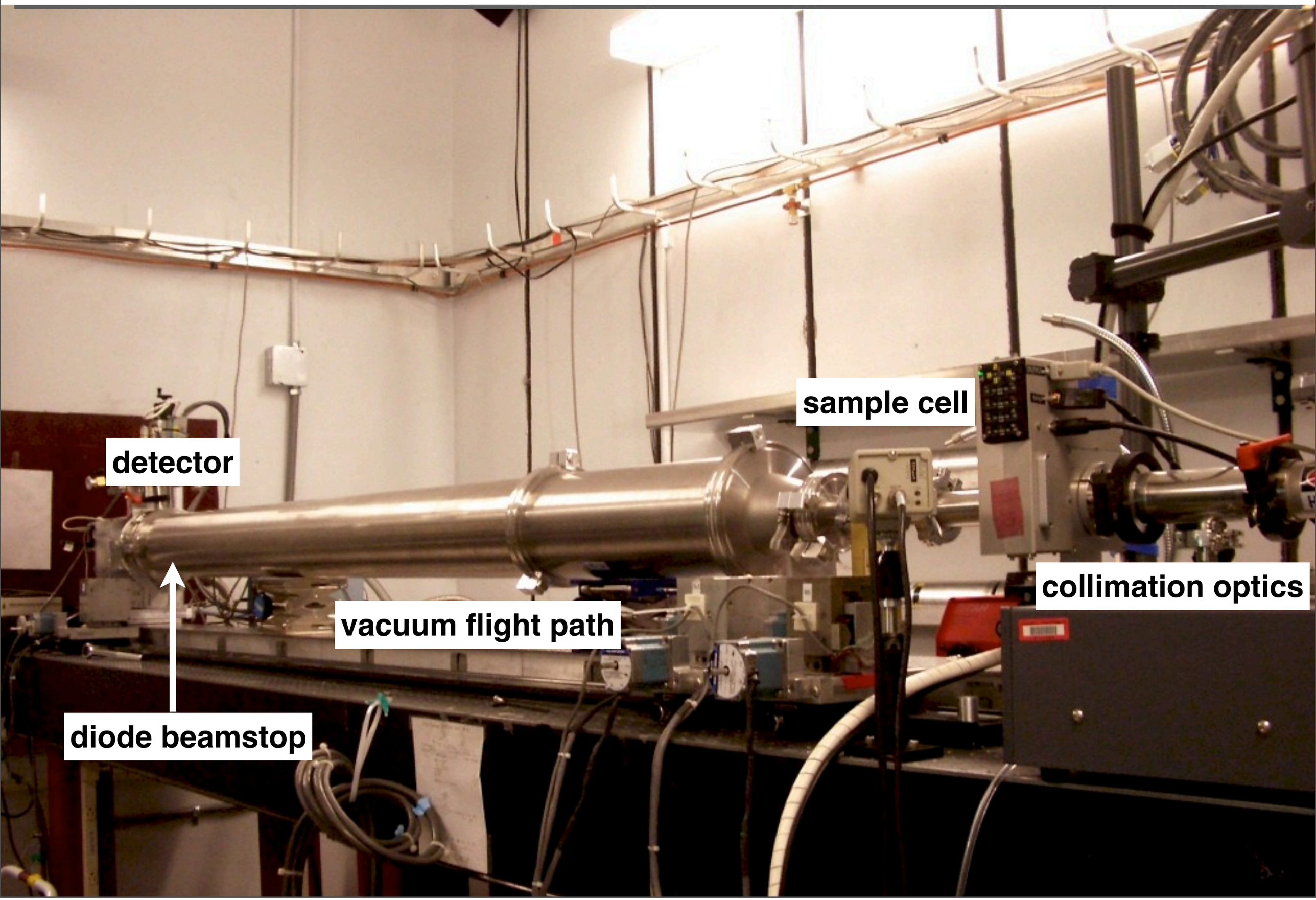
Beam time:
- 1-2 day (for routine SAXS, beamline is set up by staff)
- ~1 week or more (for non-conventional SAXS)

SAXS beamline at synchrotron

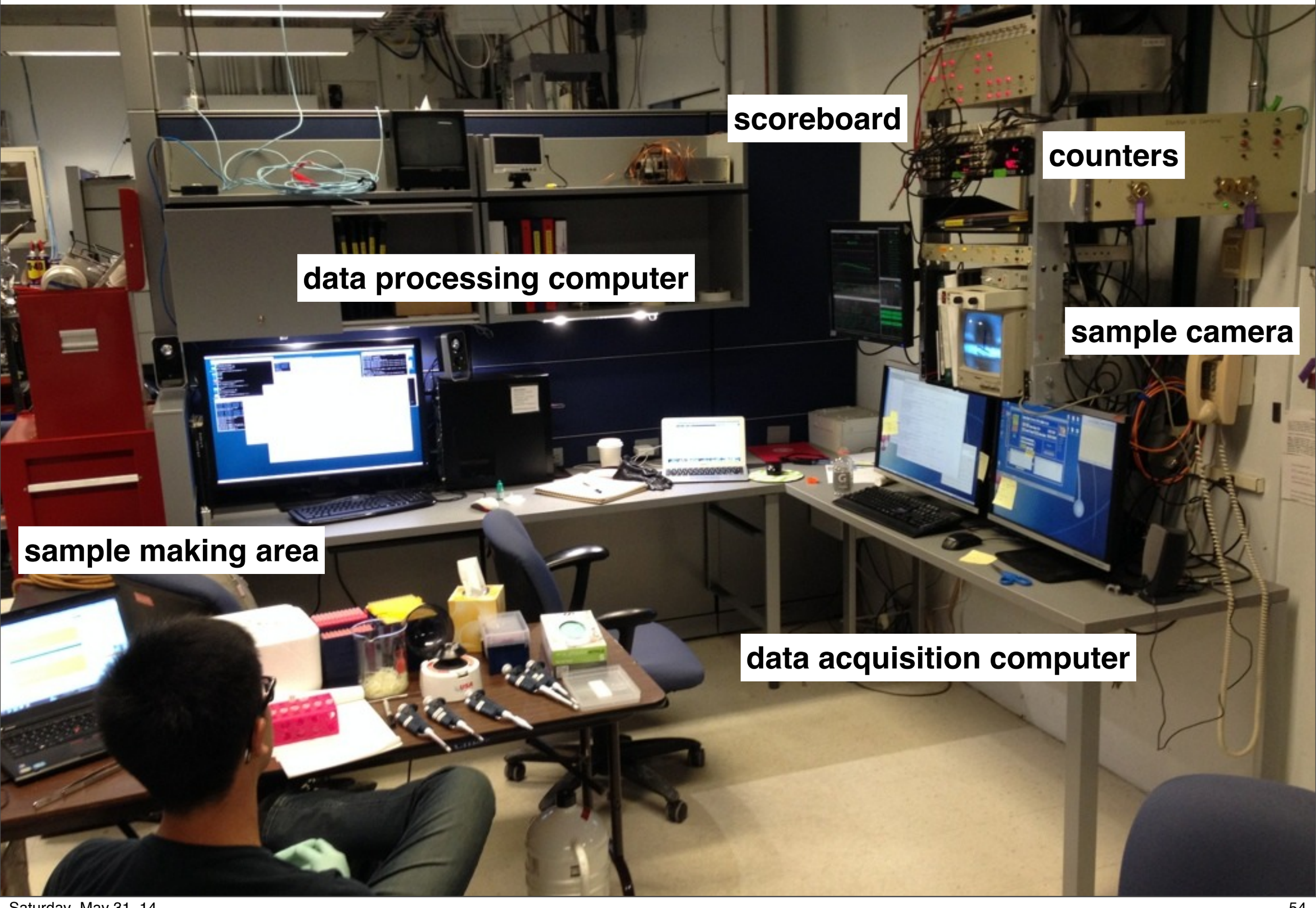


Cornell High Energy Synchrotron Source (CHESS)

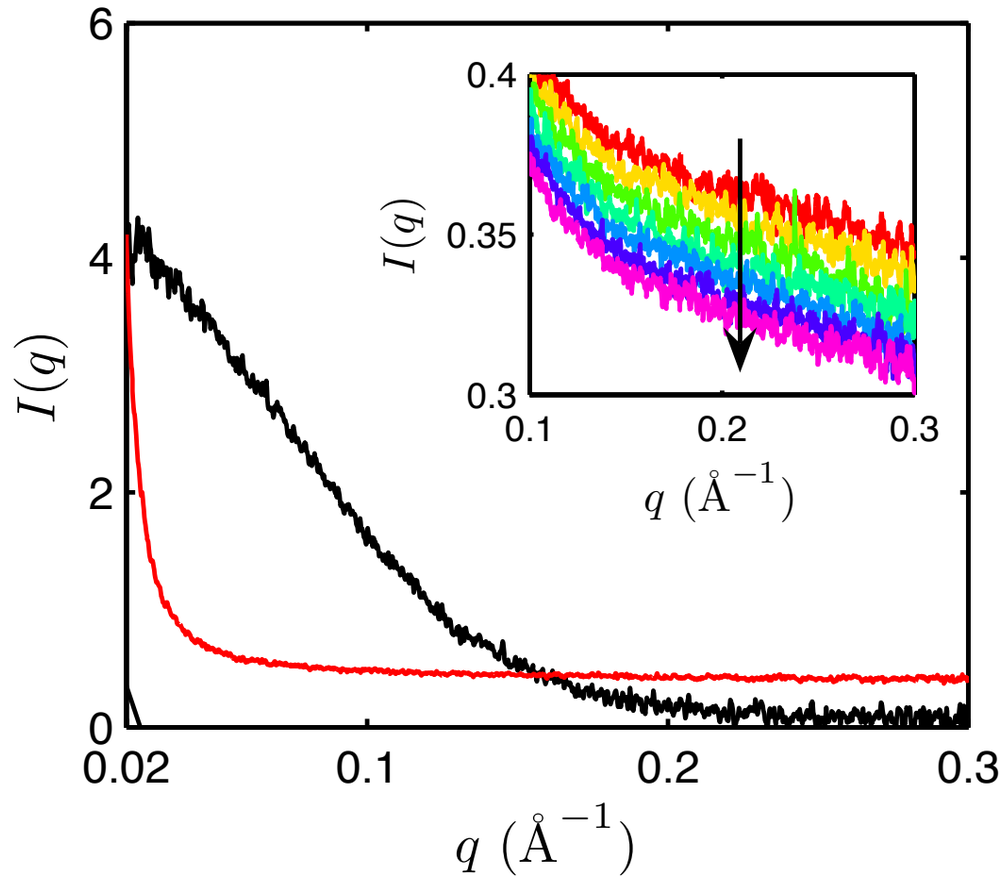
Experimental hutch



Outside of experimental hutch



Potential pitfall: Background subtraction



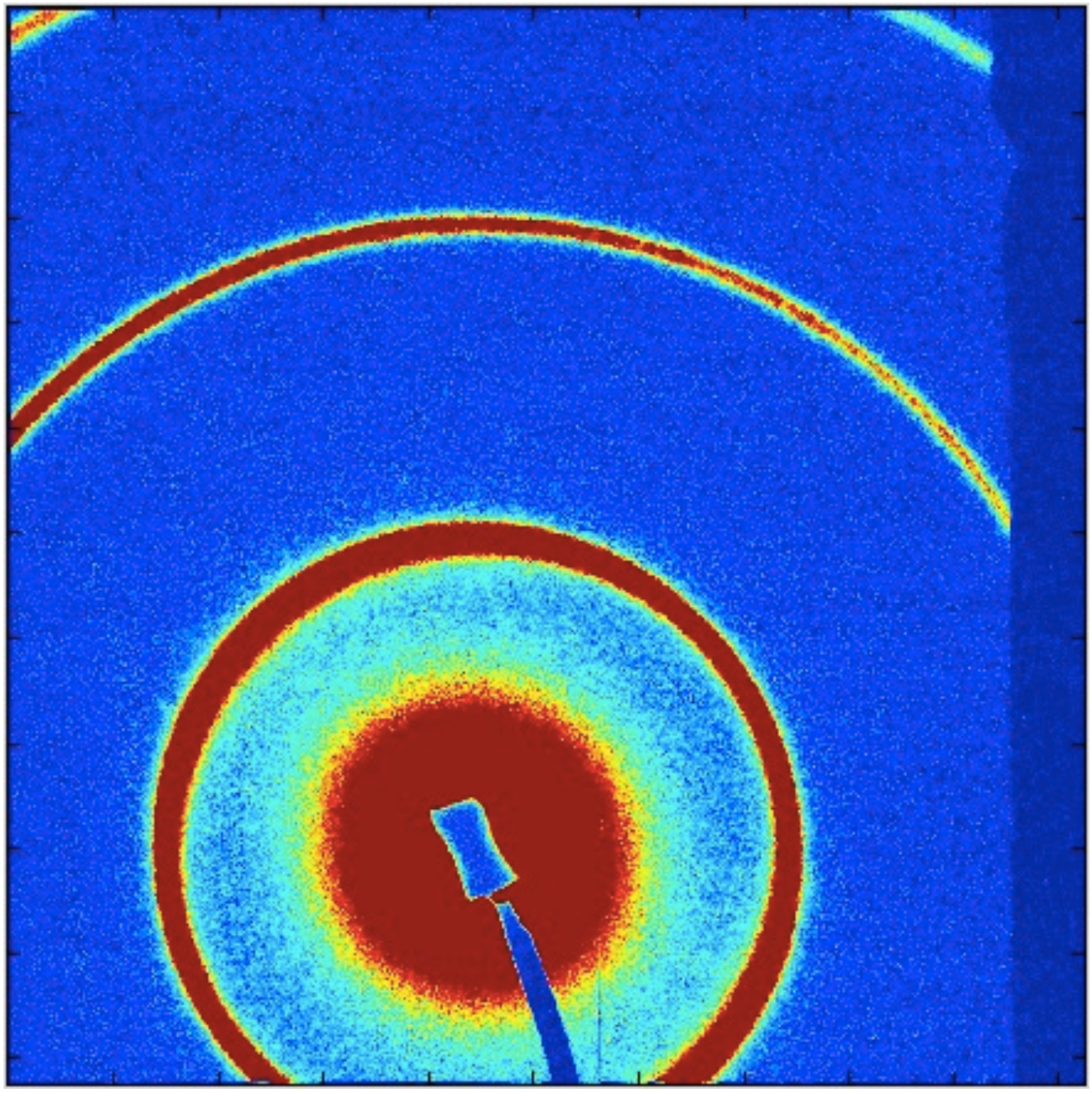
Protein signals at high *q* will be <0.5% of background level.

Need:

- exactly matched background solution
- very good detector and understanding of its limitations
- very good PIN-diode beamstop for accurate intensity normalization

Ando, *et al.* (2008) J. Appl. Cryst., 41, 167–175.

Detectors



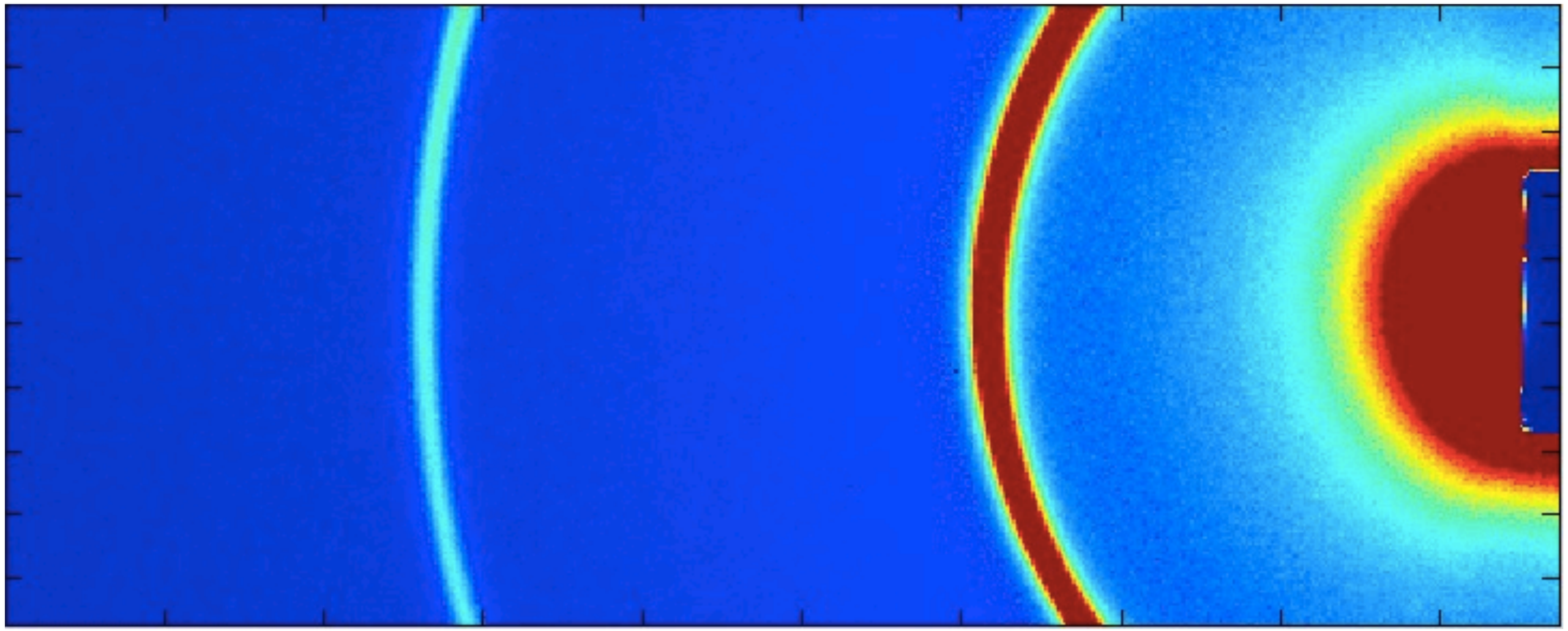
FLIcam detector @ CHESS

CCD Detectors:

- Images should be corrected for intensity and distortion.
- Detector offset should be measured.
- Dark current images should be measured frequently.

Note: If you can see a pattern in the detector image that is not from SAXS (e.g. faint shadows, stripes, sharp scattering, bright spots), then it will affect data.

Detectors



PIlatus 100K-S @ CHESS

Photon-counting Detectors:

- No image corrections, low read-out noise.
- Fast read-out.

Part 4: An Advanced Example

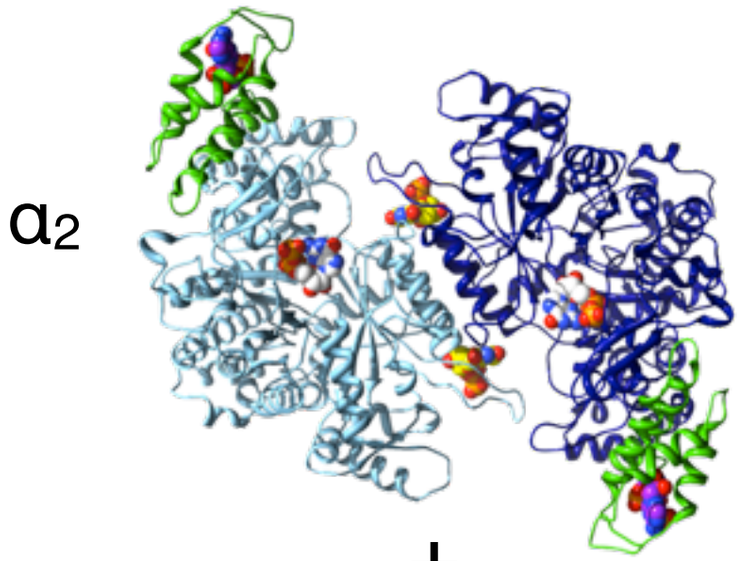
Example: Ribonucleotide reductase (RNR)

ribonucleotides
(RNA precursors)

deoxyribonucleotides
(DNA precursors)

Ando, et al. (2011). PNAS, 108(52), 21046–21051.

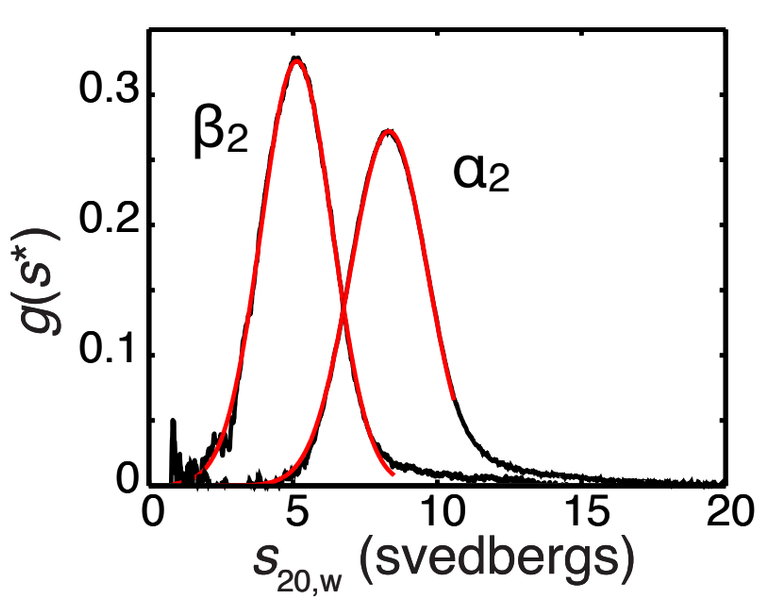
Two subunits form at least two unknown complexes.

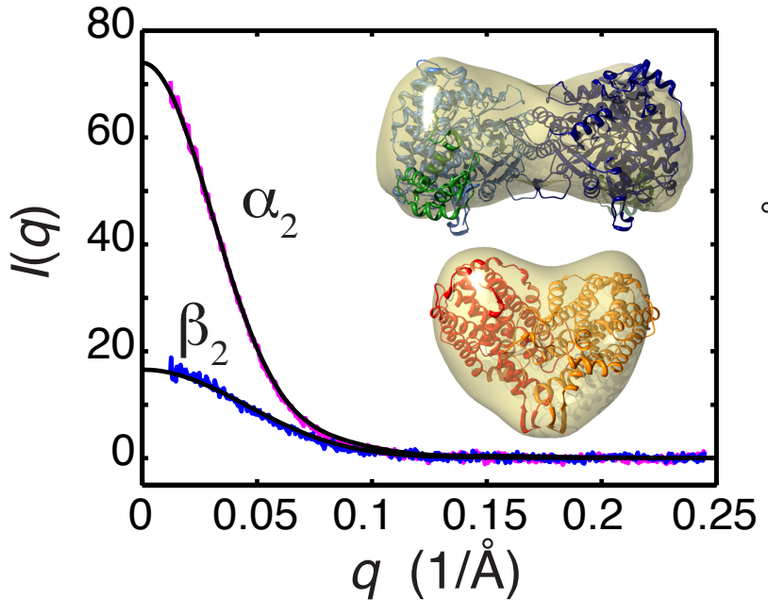


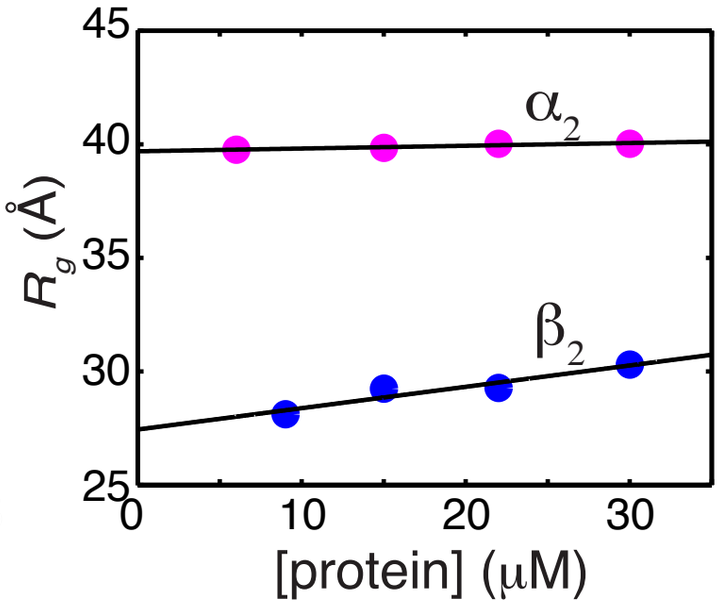
Catalytic mechanism requires long-range radical transfer between β_2 and α_2. Allosterically inhibited by dATP.

1. Check solution behavior of free subunits

- Confirm purity, oligomerization state (MW), and concentration dependence.
- Compare with any known structures.







A. Purity, molecular weight, hydrodynamic quantities

B. Protein shape in real and reciprocal space

C. Concentration effects (aggregation, dissociation?)

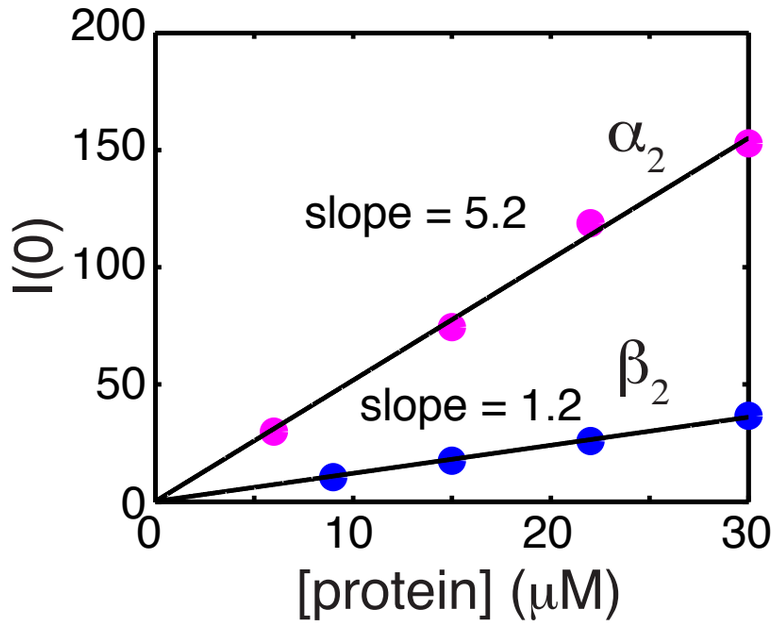
Compare with theoretical values calculated from crystal structures.

- AUC: hydropro
- SAXS: crysol

	$s_{20,w}$ (expt)	$s_{20,w}$ (theor)	R_g (expt)	R_g (theor)
β_2	5.2 S	5.8 S	27.4 ± 1.9 Å	27.0 Å
α_2	8.4 S	8.4 S	39.7 ± 0.3 Å	39.3 Å

2. Check molar ratio of protein stock solutions

- Confirm protein concentrations with SAXS.



$I(0) \propto c \times MW^2 \propto c' \times MW$

c = molar concentration
c' = concentration in mg/ml

1. Make sure the proteins are in the same buffer.
2. Measure SAXS data at several different protein concentrations.
3. Determine I(0) by Guinier analysis or by P(r) analysis.
4. Make sure I(0) vs. concentration is linear. Then determine slope through origin.

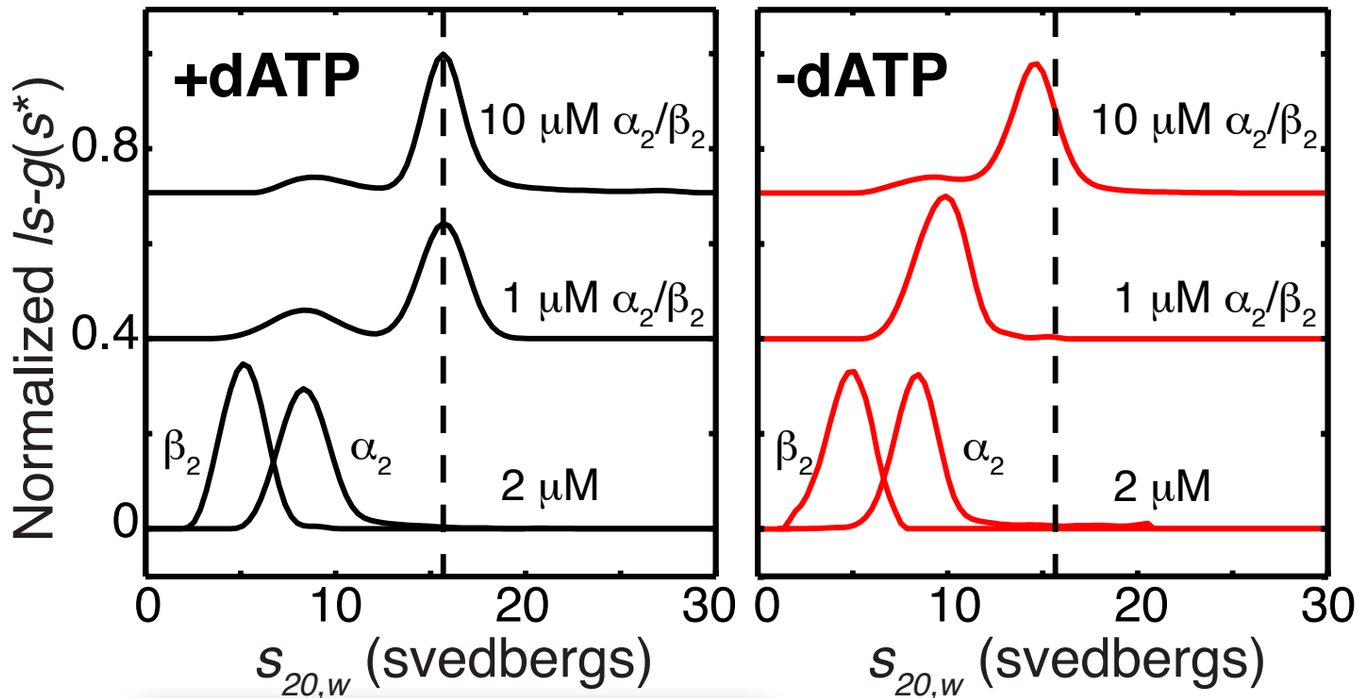
Example using molar concentrations:

MW of α_2 = 172 kDa; MW of β_2 = 87 kDa
→ Actual MW ratio ~ 2.0

Slope of I(0) vs [α_2] = 5.2; Slope of I(0) vs [β_2] = 1.2
→ Calculated MW ratio = $(5.2/1.2)^{1/2}$ = 2.1

3. Check stability of complex

• One way: examine concentration dependence with sedimentation velocity analytical ultracentrifugation (AUC)



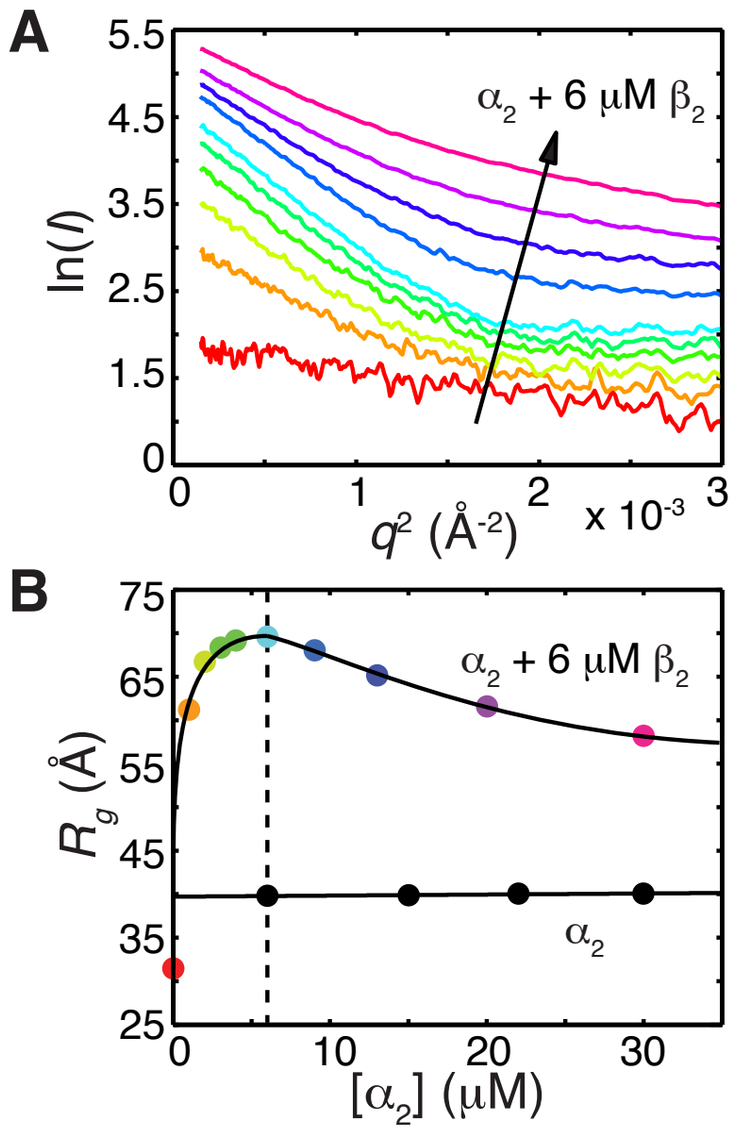
Inactive complex homogeneous

Active complex in mixture

• Concentration-independent sedimentation is a sign that the species is non-interacting (slowly dissociating). MW and sedimentation coefficient are tractable by peak fitting.
• Concentration-dependent sedimentation is a sign that there are multiple species rapidly exchanging (mass action).
• This is also true of size exclusion chromatography, but controlling/varying concentration is much more difficult.

4. Determine subunit stoichiometry of complex

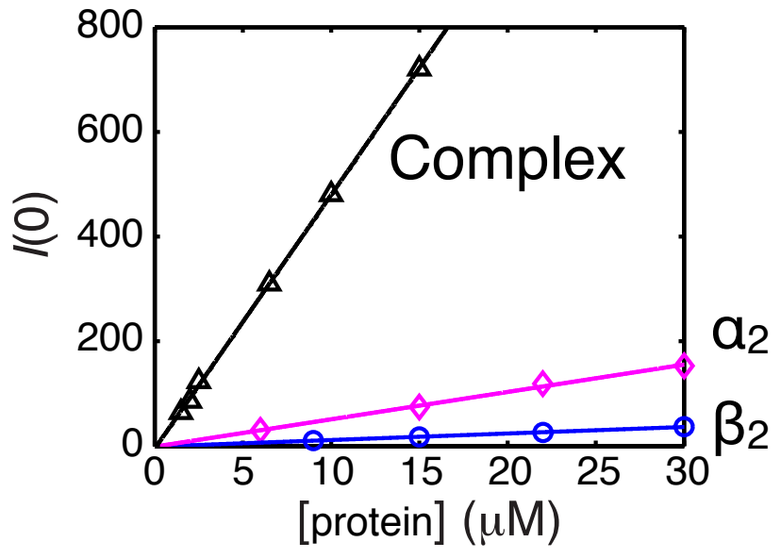
- Subunit titration with SAXS.



→ 1:1 complex

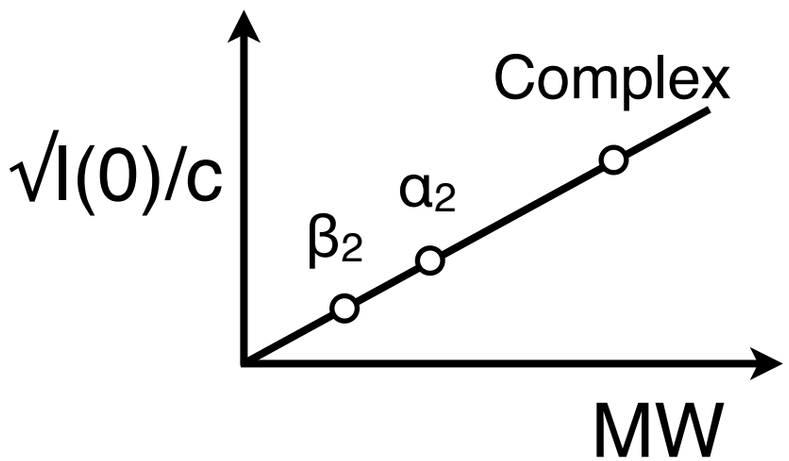
5. Oligomerization state of inactive complex

- Use free subunits as standards and check molecular weight of complex.
- Measure I(0) of complex at multiple concentrations.



$$I(0) \propto c \times MW^2 \propto c' \times MW$$

c = molar concentration
c' = concentration in mg/ml

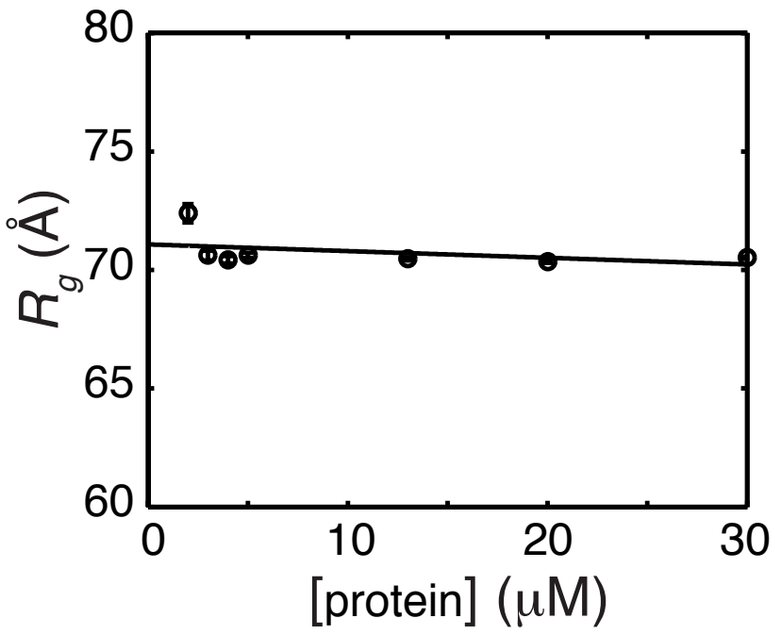


Gives predicted MW of 512 kDa.
We know complex has 1:1 subunit stoichiometry.

→ Consistent with $\alpha_4\beta_4$ (actual MW = 517 kDa).

6. Concentration dependence of inactive complex

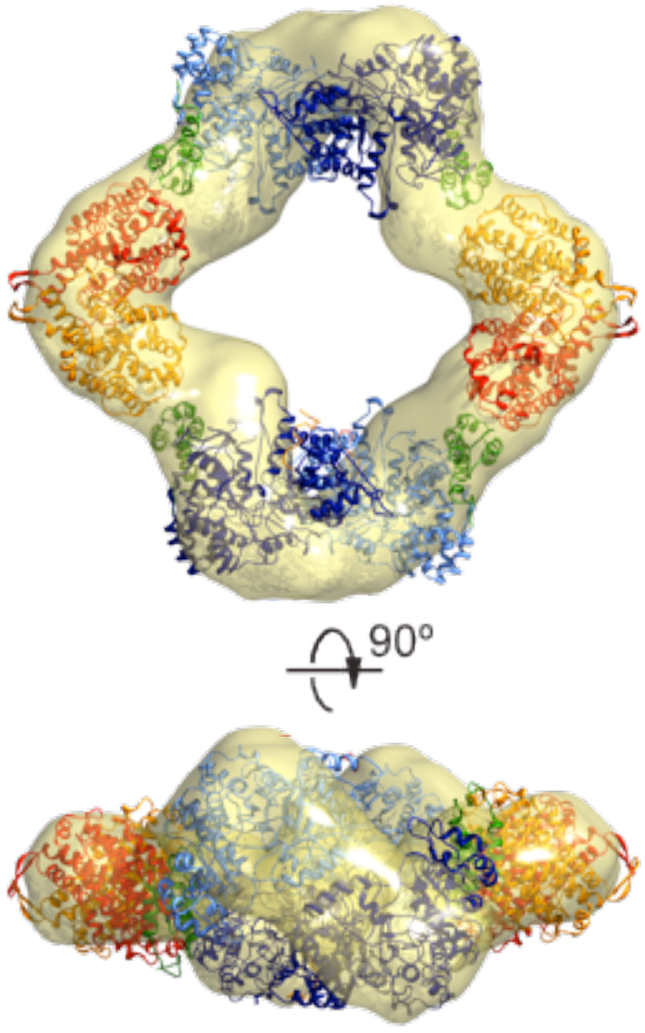
- Measure Rg of complex at multiple concentrations.



→ Complex is stable over a wide range of protein concentrations.

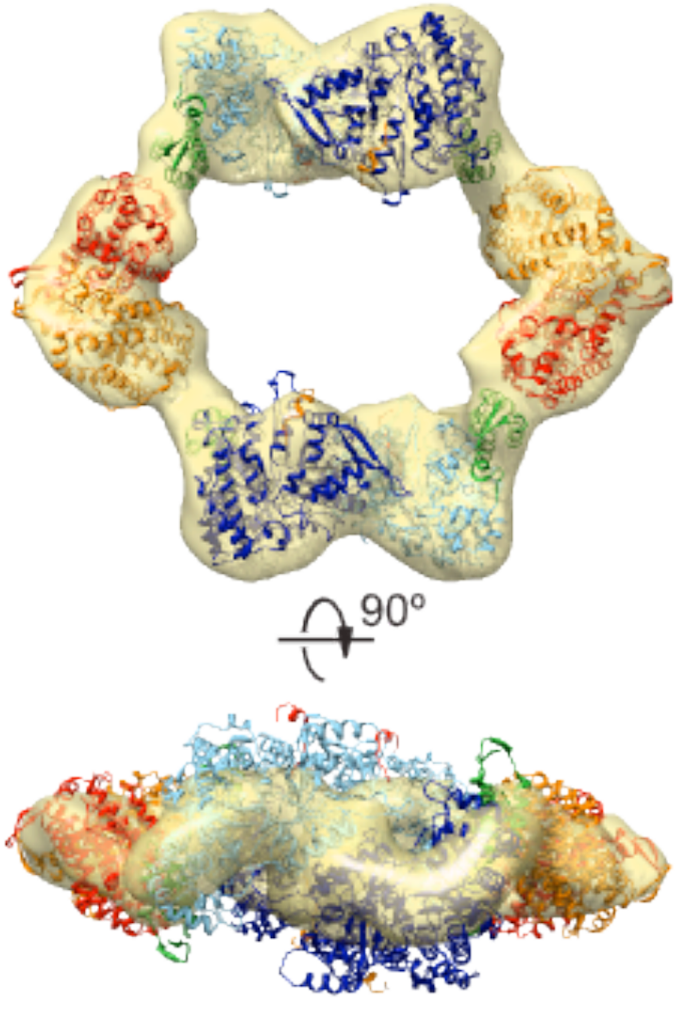
7. Structure determination of inactive complex

SAXS (2 μM RNR)



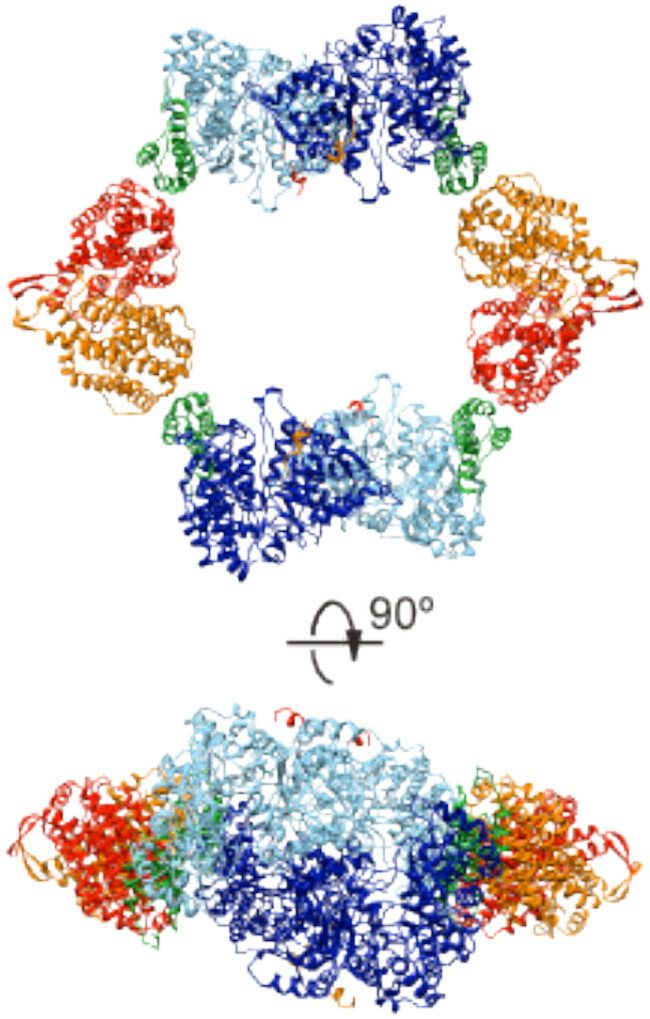
57 Å

EM (0.15 μM RNR)



23 Å

XTAL (25 μM RNR)

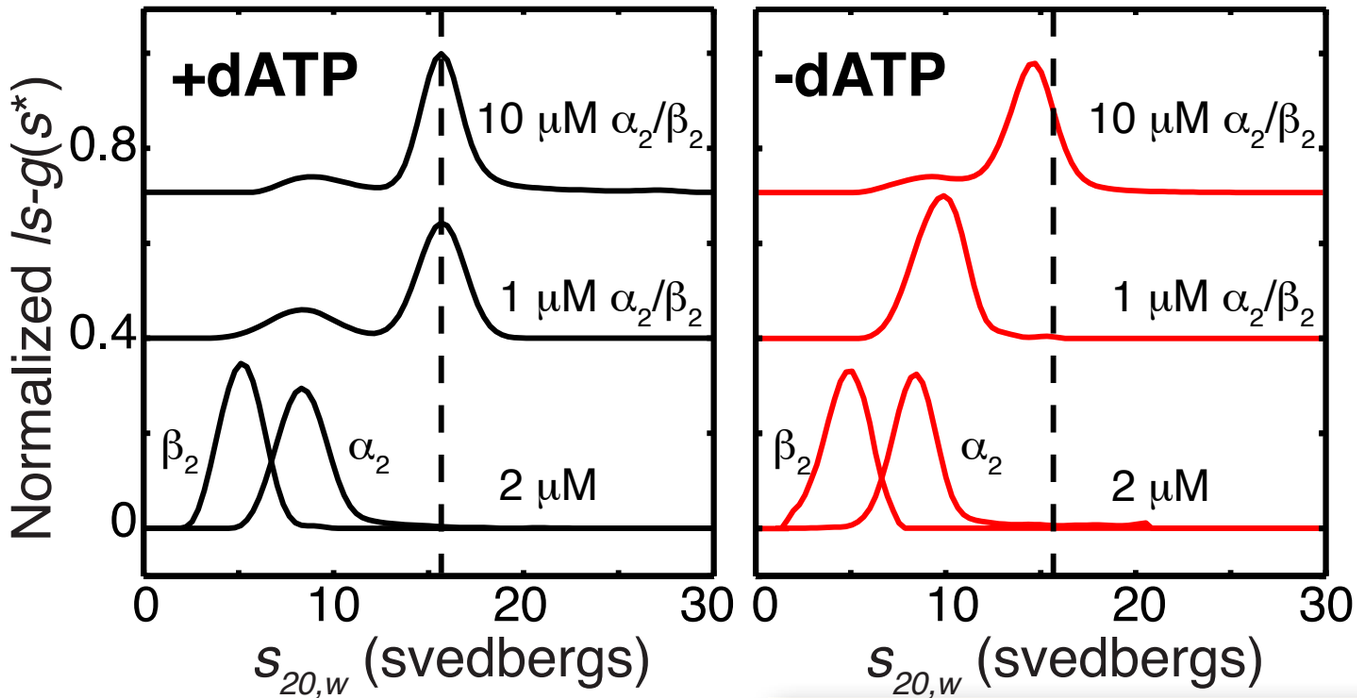


5.65 Å

Inactive complex is an $\alpha_4\beta_4$ ring.

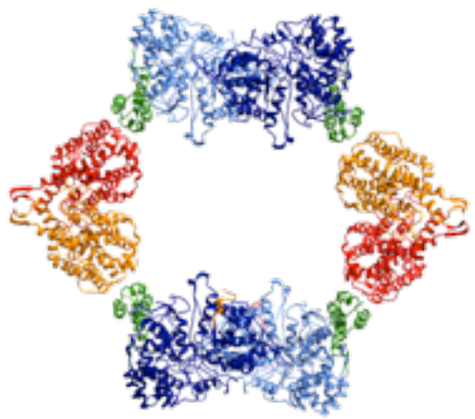
8. Extracting the active complex from mixture

- Active complex is embedded in equilibrium mixture, likely exchanging with inactive complex.



Inactive complex homogeneous

Active complex in mixture



8. Extracting the active complex from mixture

C. Equilibrium Data

In special cases it is possible also to obtain equilibrium data from SAXS. The accuracy of such results obtained from more or less polydisperse solutions is, of course, rather limited; but it can be high enough, as shown by Österberg *et al.* (1975), to decide which type of complex is formed between two macromolecules A and B, when the equilibrium of the system is described by the general reaction

$$pA + qB \leftrightarrows A_pB_q$$

which has the associated equilibrium constant β_{pq}. Österberg *et al.* (1975) showed by analysis of SAXS data that the predominant complex (AB) formed in solution between lysine: *t*RNA ligase (A) from yeast and *t*RNA (B) consists of two ligase molecules and one molecule of *t*RNA (A_2B-type).

For a sufficiently diluted system the scattered intensity I at any angle can be written as the sum of intensities scattered from all types of particles in the solution, i.e.

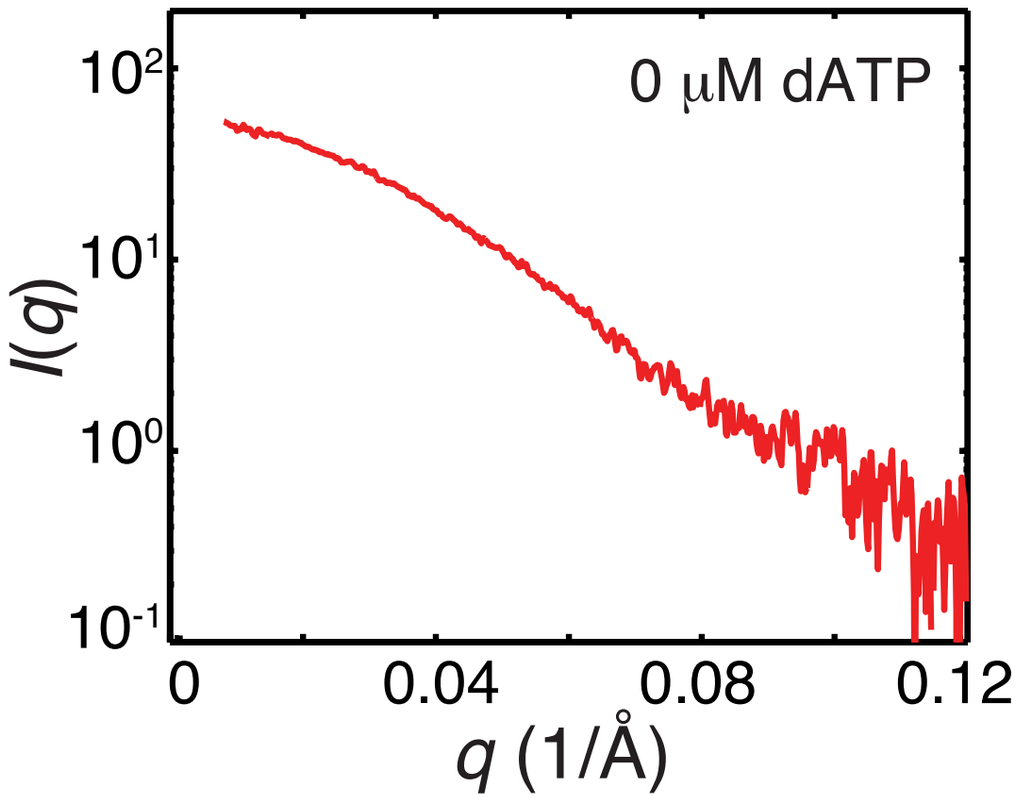
$$I = I_A + I_B + \sum\sum I_{pq}$$

where I_A, I_B and I_{pq} are the scattered intensities from the molecules A, B and the complexes A_pB_q, respectively. The intensity contributed by each type of particle at a particular small angle depends on its concentration, its molecular weight and the excess electrons Δz (see Chapter 4). The scattered intensity increases with an increase in any of these parameters.

Glatter & Kratky Ch. 8, p. 286 (1982)

9. Shifting the equilibrium from active to inactive

- Titration of dATP (negative activity effector) with SAXS.



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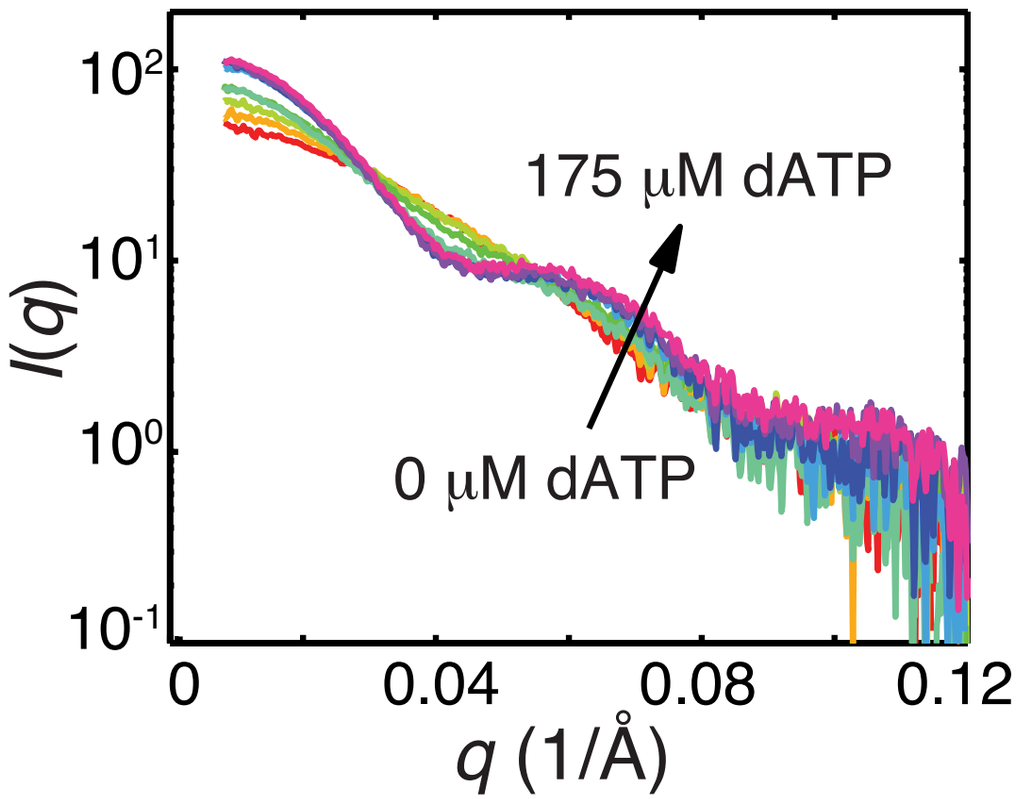

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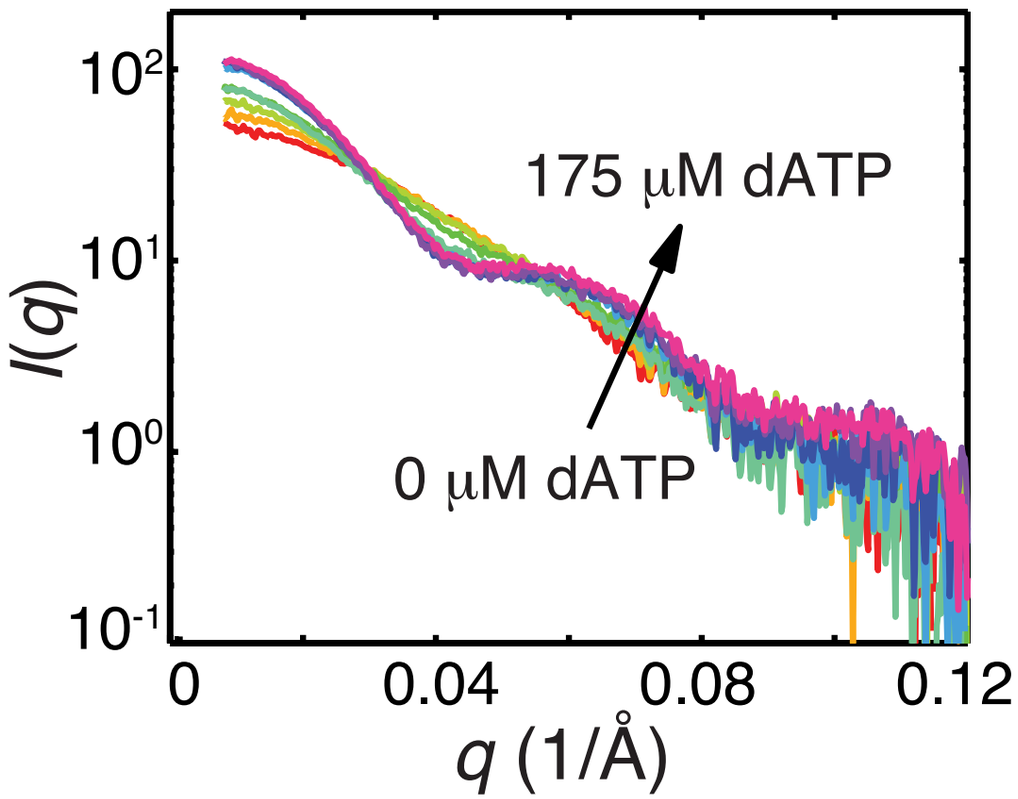
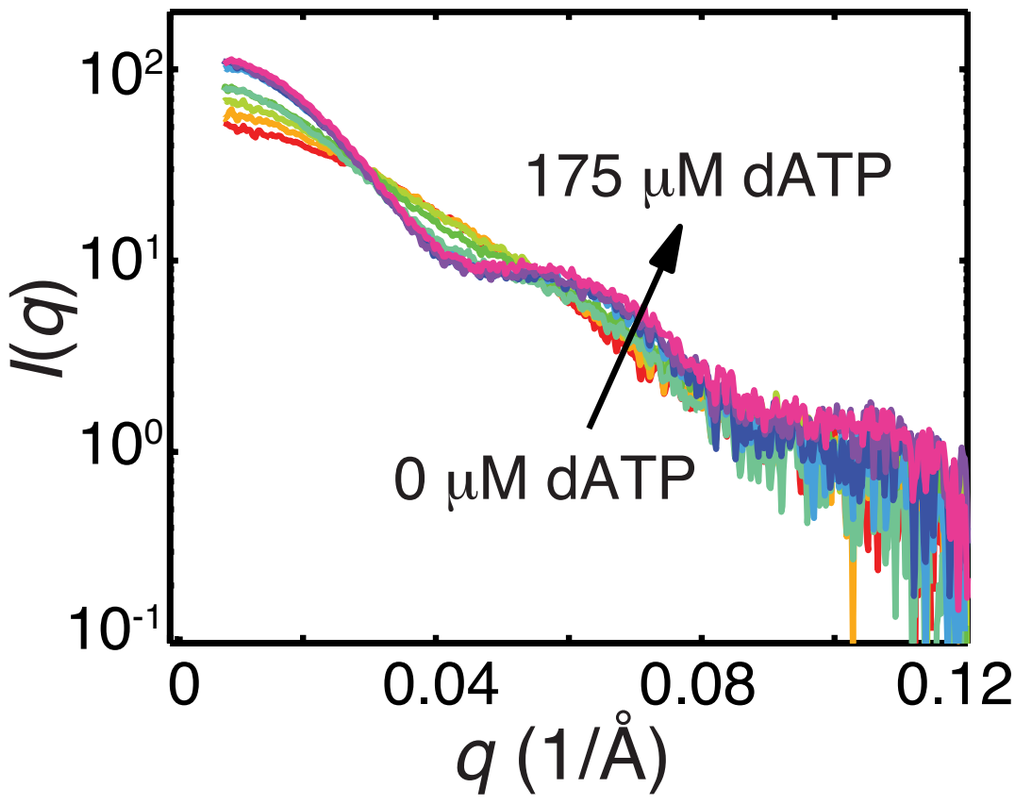


How many states in this transition?
A) observation of iso-scattering points
B) singular value decomposition → 2 “eigenstates”

dATP drives a two-state transition.

9. Shifting the equilibrium from active to inactive

- Titration of dATP (negative activity effector) with SAXS.



A) Increase in scattering signal: I(0) ~ c x MW^2 223 → 512 kDa

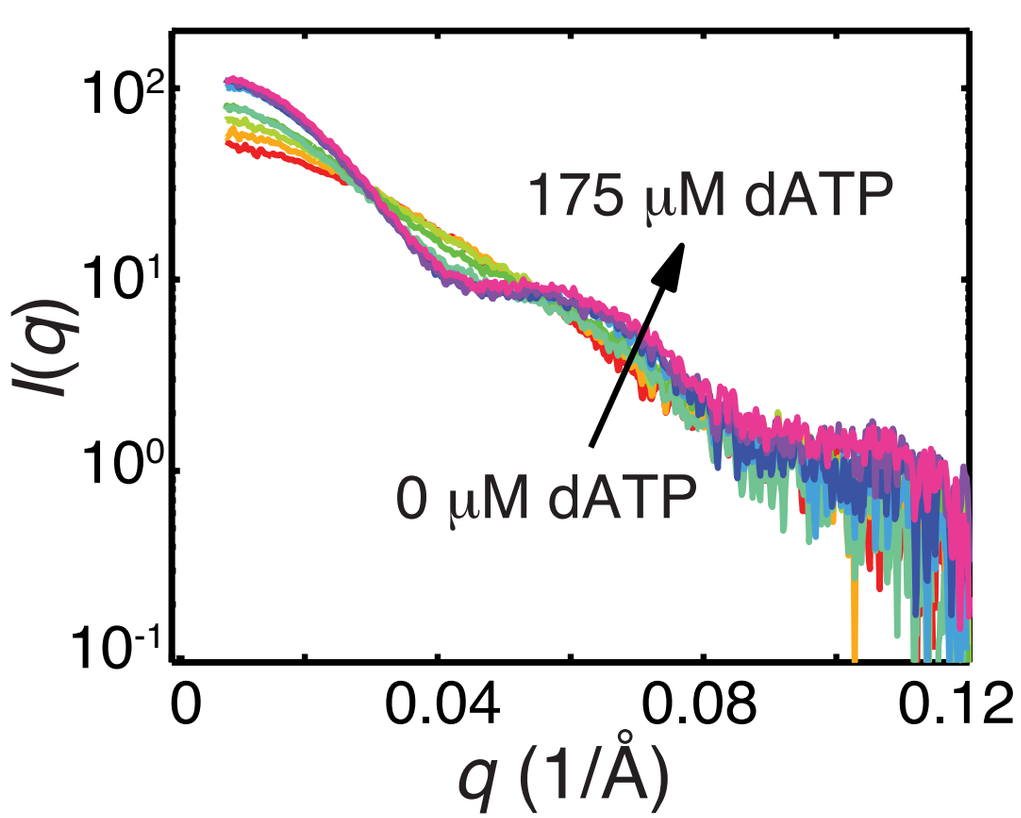
B) Porod invariant method* (area under the curve) 269 → 549 kDa

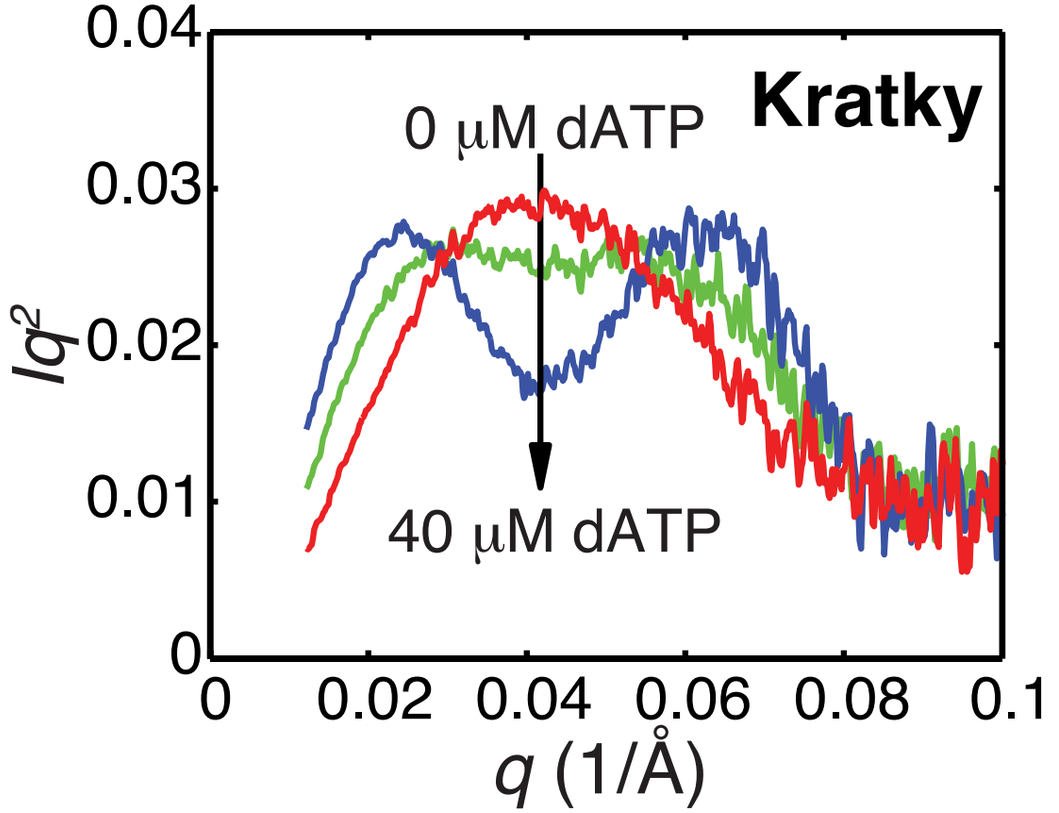
$\alpha_2\beta_2$ (259 kDa) → $\alpha_4\beta_4$ (517 kDa)

*http://www.ifsc.usp.br/~saxs/saxsmow.html

9. Shifting the equilibrium from active to inactive

- Titration of dATP (negative activity effector) with SAXS.



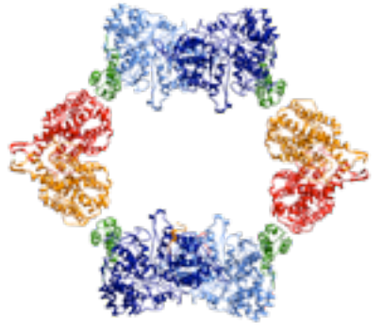


Kratky analysis (Iq^2 vs. q)

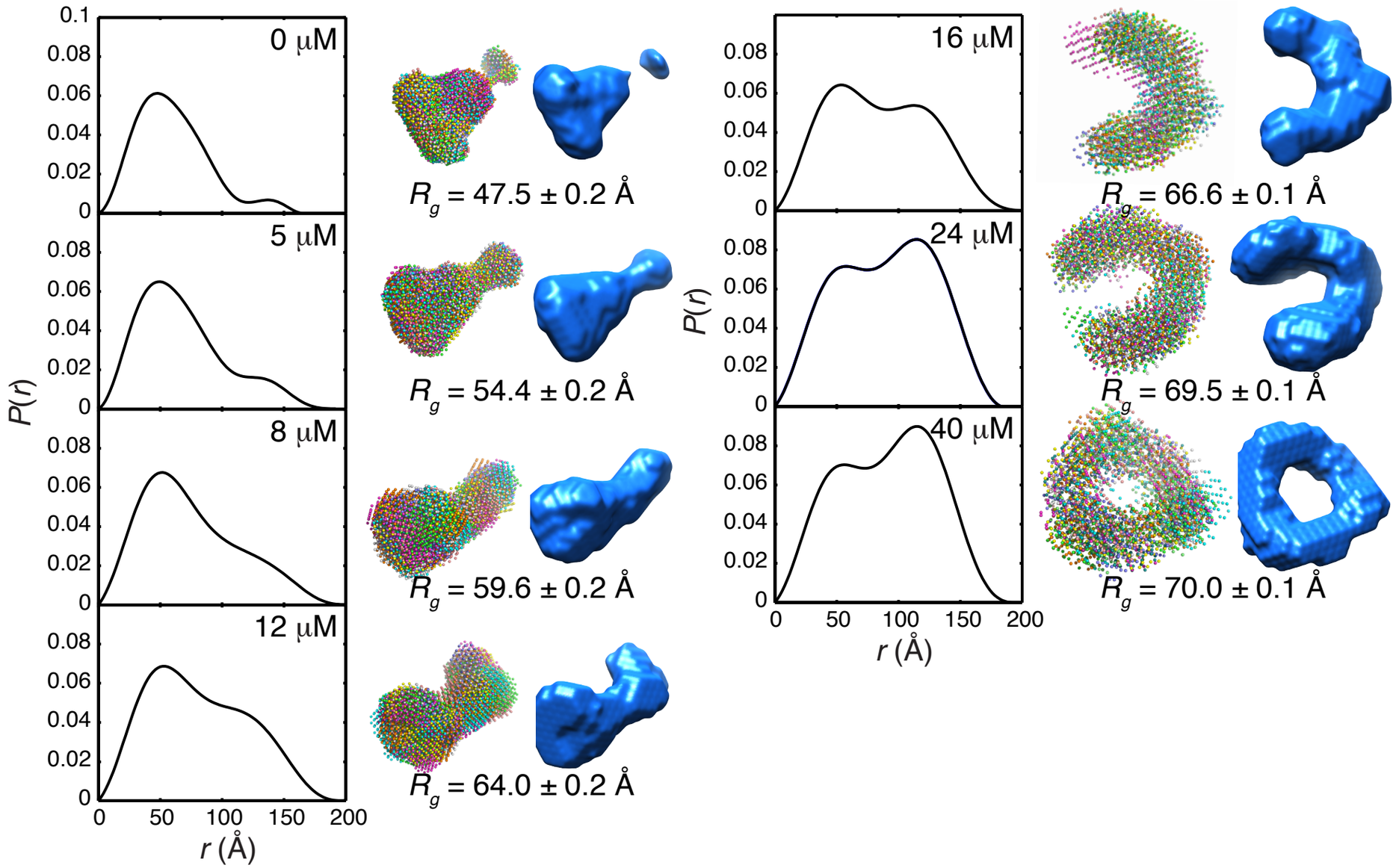
single peak = globular, compact

multiple peaks = non-globular arrangement of multi-domains

globular $\alpha_2\beta_2$ → non-globular $\alpha_4\beta_4$

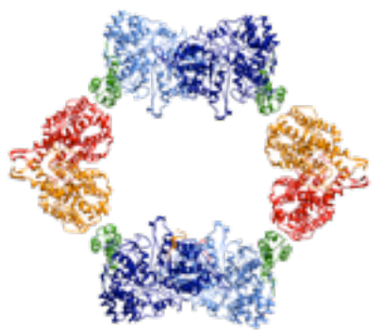


10. Visualizing the two-state transition



- Shapes are “average” of two species (active and inactive complexes)

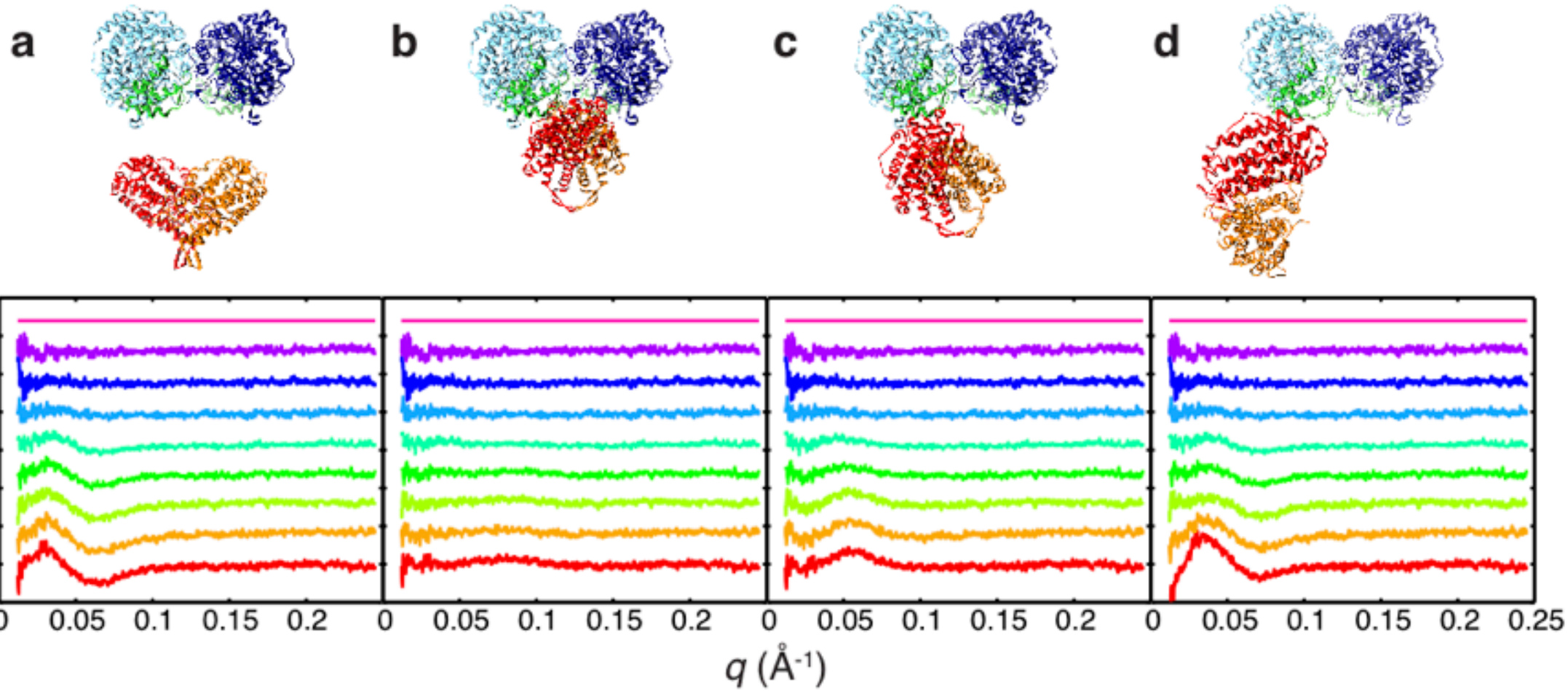
globular $\alpha_2\beta_2$ → non-globular $\alpha_4\beta_4$



11. Rigid body modeling of active complex

• Two-component fitting of dATP titration data with several candidate models for the active complex in equilibrium with inactive complex. Compare residuals.

$$I(q) = f_1\, I_1(q) + f_2\, I_2(q)$$



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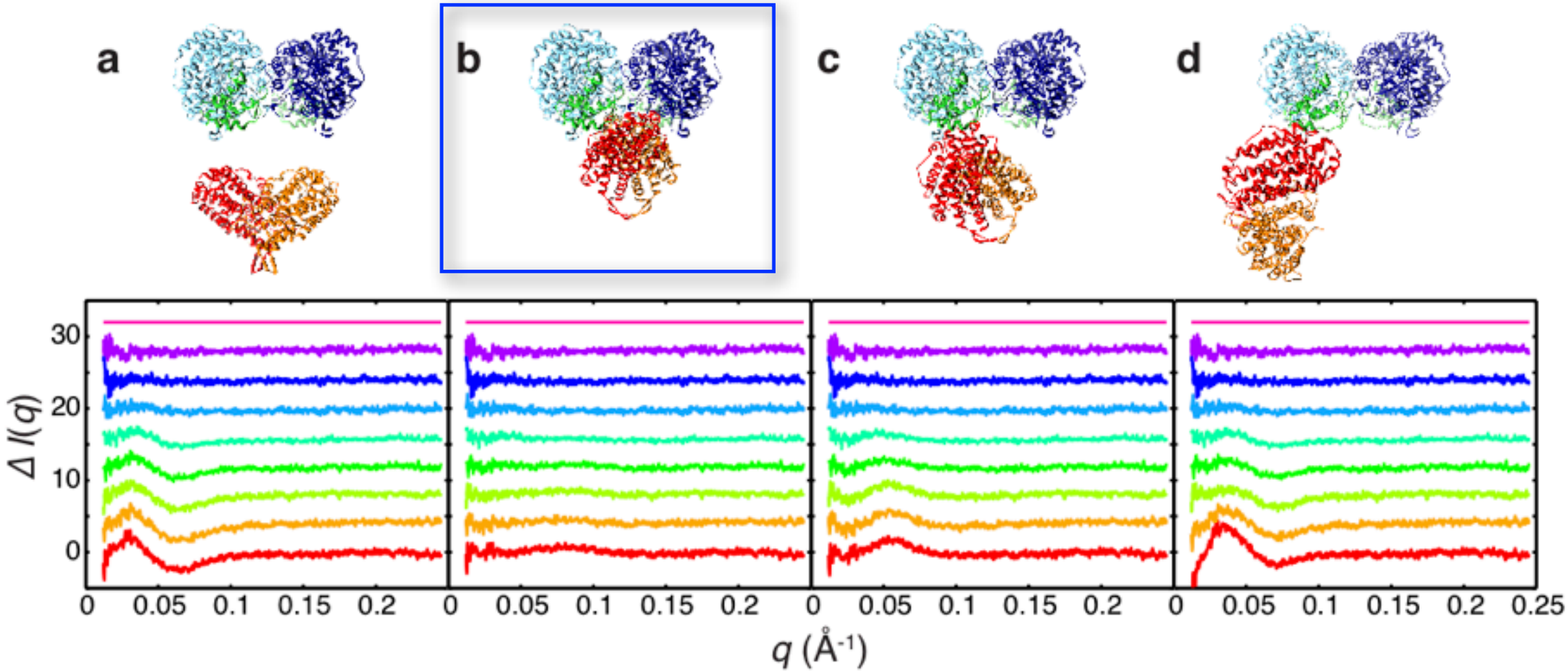

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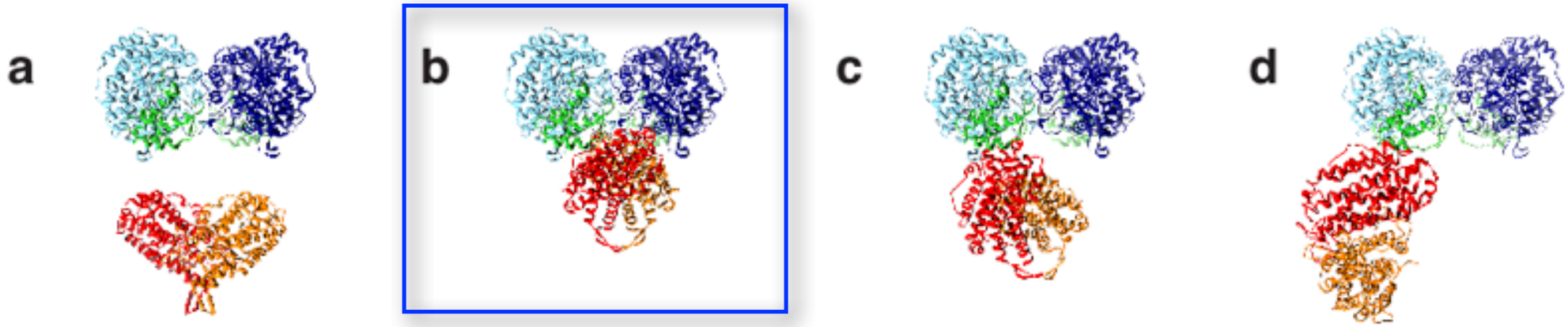

$\alpha_2\beta_2$ “docking” model

[Uhlin and Eklund. Nature (1994)]

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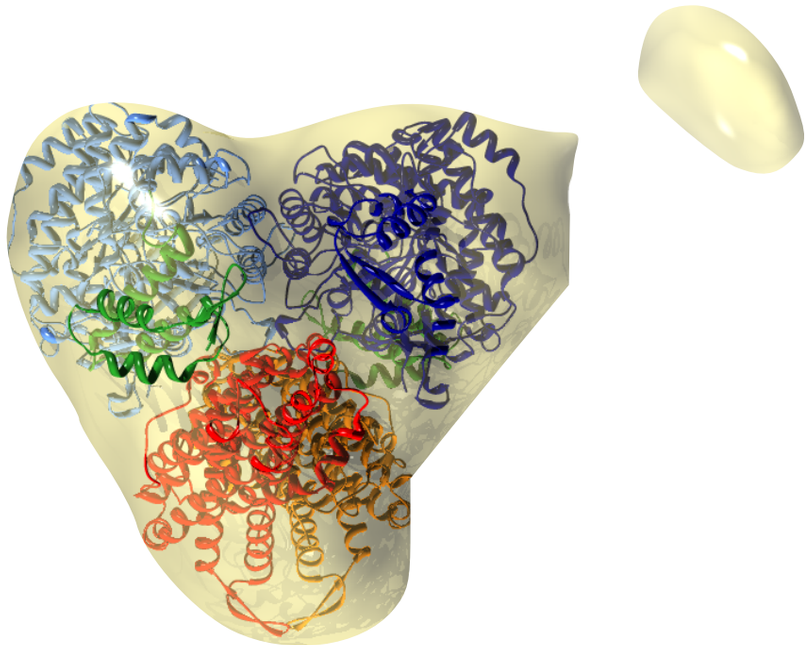
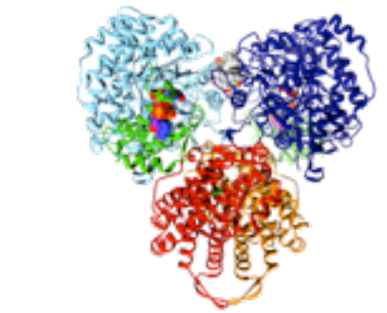


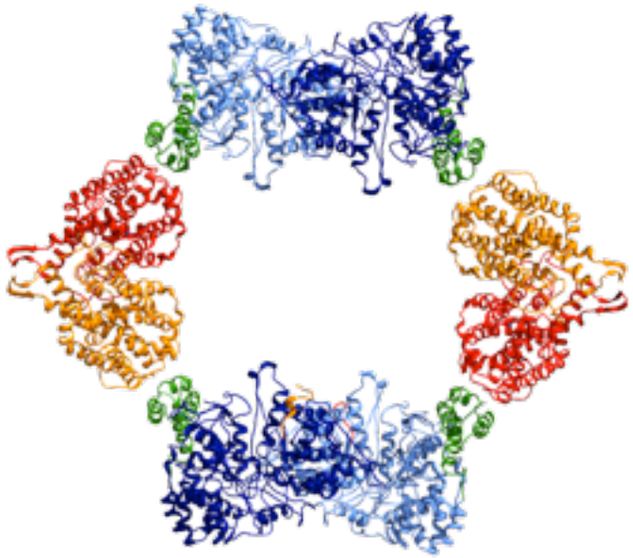
$\alpha_2\beta_2$ “docking” model fits well in shape reconstruction at 0 uM dATP

12. Fractions of species in transition

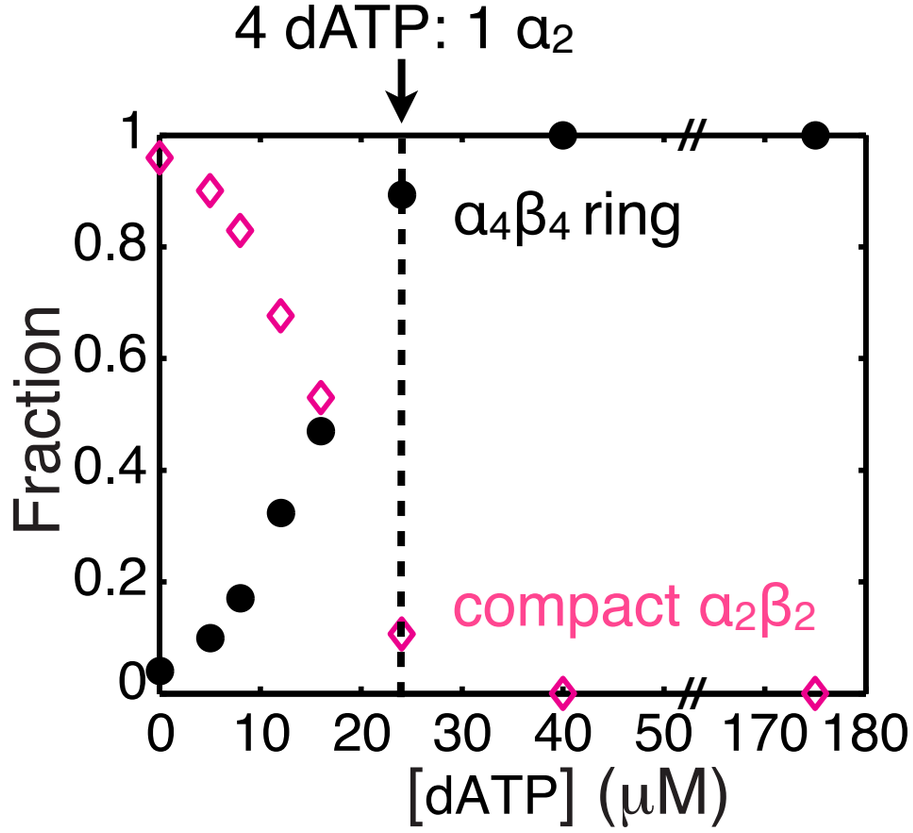
- Best-fit model for active complex is one in which the lobes of β_2 are docked against the active sites of α_2 along symmetry axis.
- This so-called “docking model” was originally proposed by Uhlin and Eklund [Nature (1994)] based on shape complementarity of subunits.



$\alpha_2\beta_2$
docking model



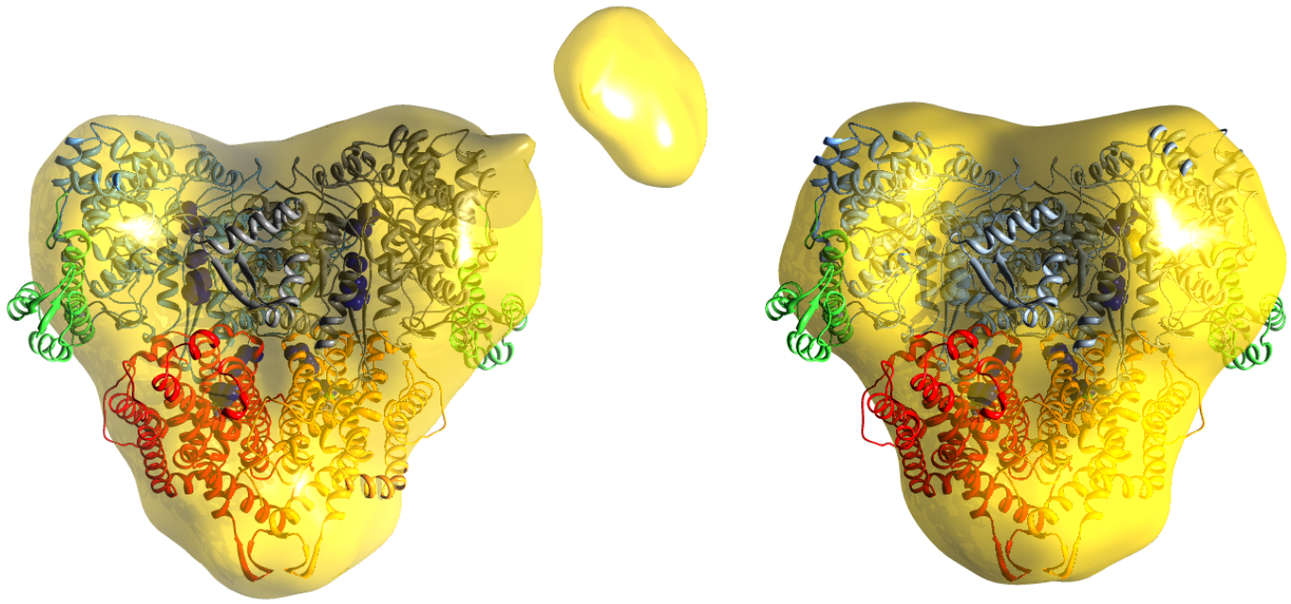
$\alpha_4\beta_4$ ring



Even at 0 uM dATP, there is 97% $\alpha_2\beta_2$ and 3% $\alpha_4\beta_4$.

12. Fractions of species in transition

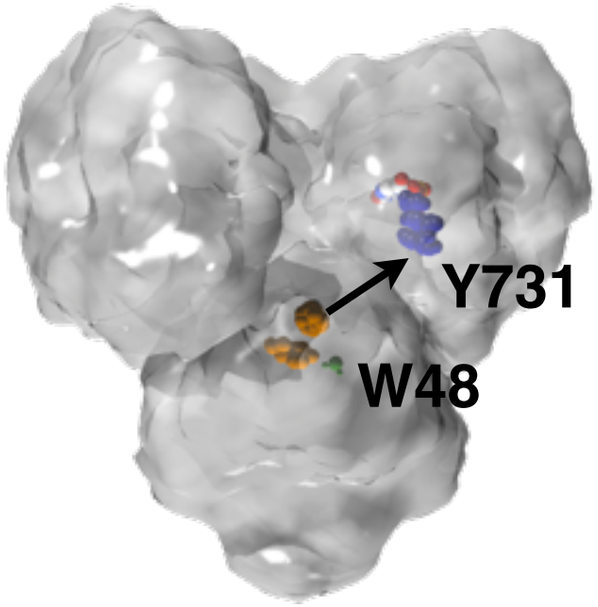
Even at 0 uM dATP, there is 97% $\alpha_2\beta_2$ and 3% $\alpha_4\beta_4$.



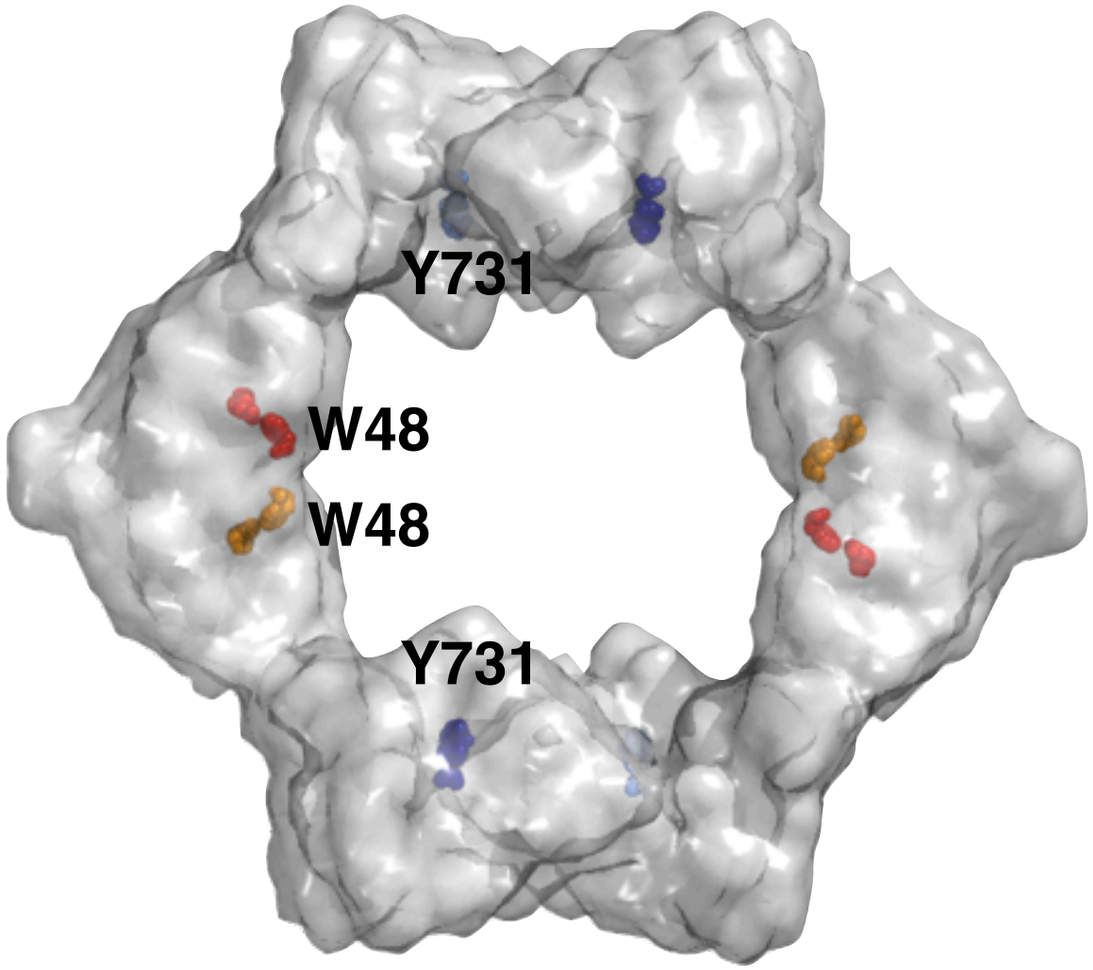
Subtracting 3% $\alpha_4\beta_4$ from 0 uM dATP data gives shape reconstruction that is more representative of $\alpha_2\beta_2$ complex.

13. Conformation affects radical transfer path

β_2 Y122 → W48 → Y356 → Y731 → Y730 → C439 α_2



$\alpha_2\beta_2$
docking model

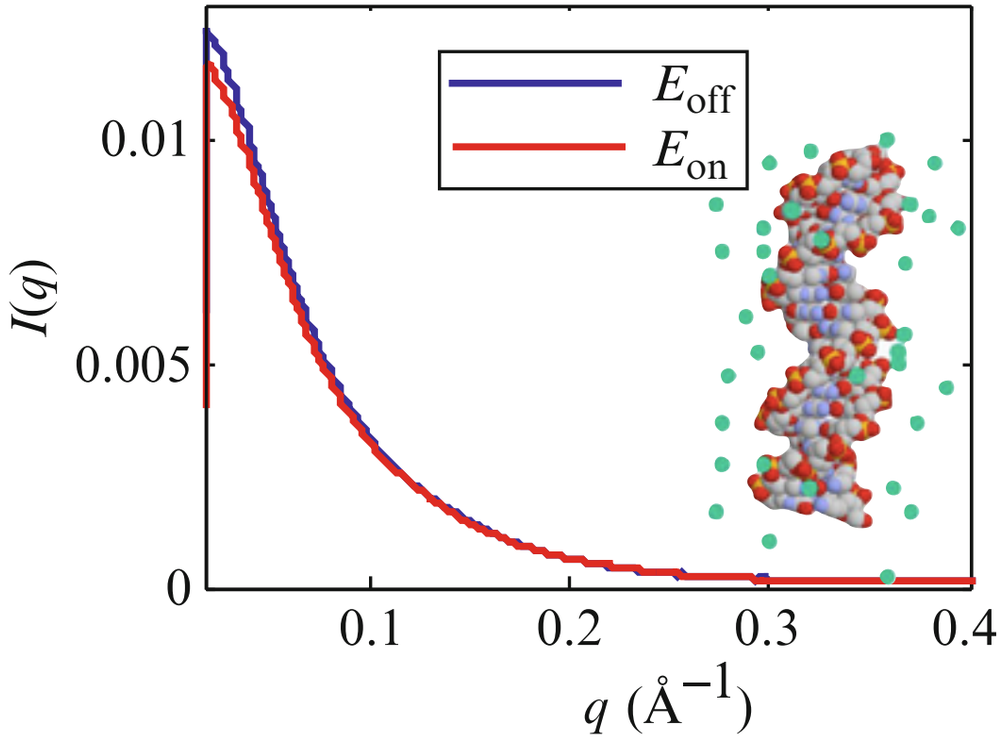


$\alpha_4\beta_4$ ring

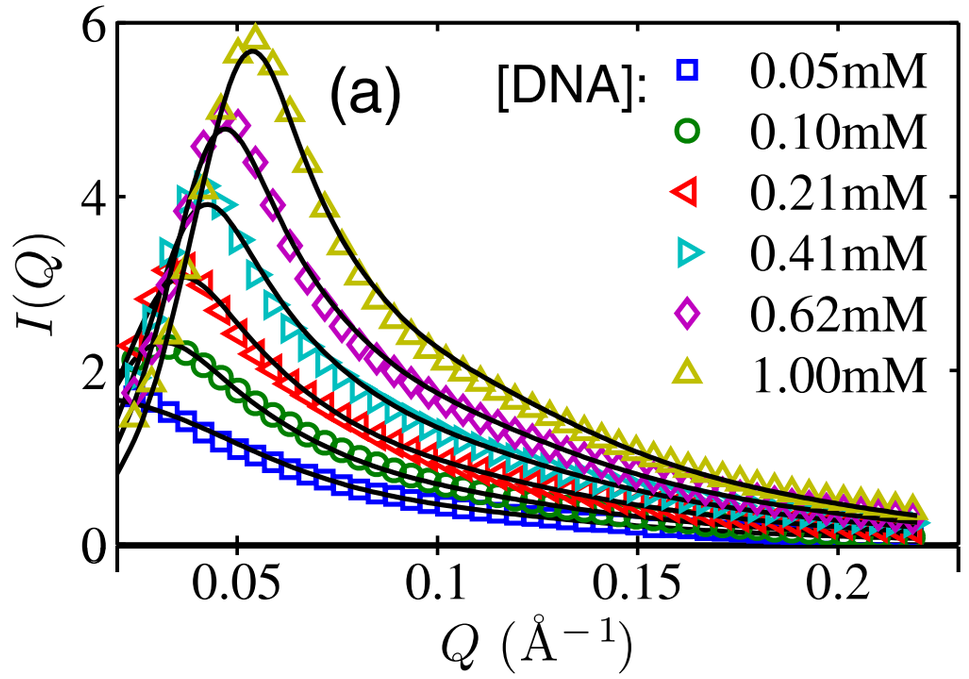
Ando, et al. (2011). PNAS, 108(52), 21046–21051.

Part 5: Variations

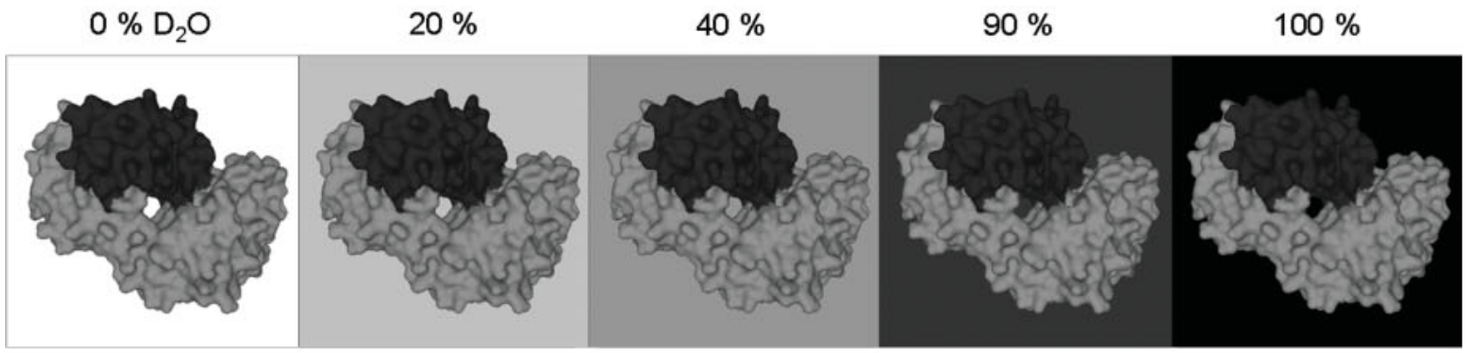
Anomalous SAXS: Tune to absorption edge of atom (Z >24)



Structure factor: Study strongly non-ideal solutions to probe inter-particle potentials



Contrast Variation: Tune relative scattering strength (neutron scattering)



Pabit, et al. (2009). Meth. Enz. 469, 391–410.
Qiu, et al. (2006). PRL, 96(13), 138101.
Jacques & Trewhella (2010). Protein Science, 19(4), 642–657.

Literature

A great introduction to SAXS with practical advice:
Jacques & Trewhella (2010). Protein Science, 19(4), 642–657.

An extensive review with comparison to crystallography:
Putnam, Hammel, Hura, & Tainer (2007). Q Rev Biophys, 40(3), 191–285.

Review with discussion of scattering of basic geometric shapes:
Svergun & Koch (2003). Reports on Progress in Physics, 66, 1735–1782.

Data collection protocols and trouble-shooting guide:
Skou, Gillilan, & Ando (2014) Nature Protocols, *in press*.

Discussion of SAXS publication guidelines:
Jacques, Guss, Svergun, & Trewhella (2012). Acta Cryst D, 68(Pt 6), 620–626.

Essential reading on *ab initio* shape reconstructions:
Volkov & Svergun (2003). Journal of Applied Crystallography, 36(3), 860–864.

On anomalous SAXS of metal ion distributions:
Pabit, Finkelstein, & Pollack (2009). Methods in Enzymology, 469, 391–410.

Classic text on SAXS for the serious SAXS user:
Glatter & Kratky, editors. Small Angle X-ray Scattering. Academic Press, New York, 1982.