

Scattering of X-rays

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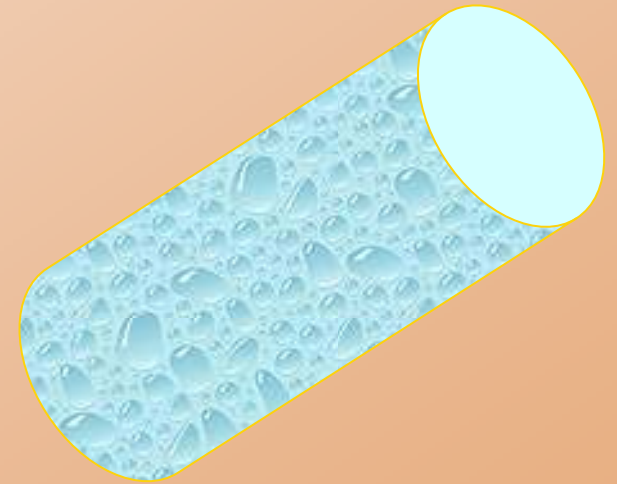
**EMBO Practical Course on Solution Scattering from Biological Macromolecules
Hamburg October, 25th – November 1st 2010**

SAXS measurement

Sample

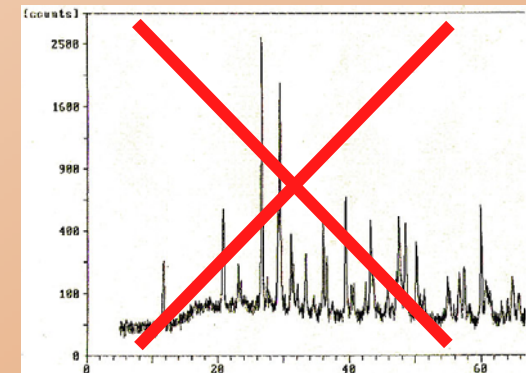
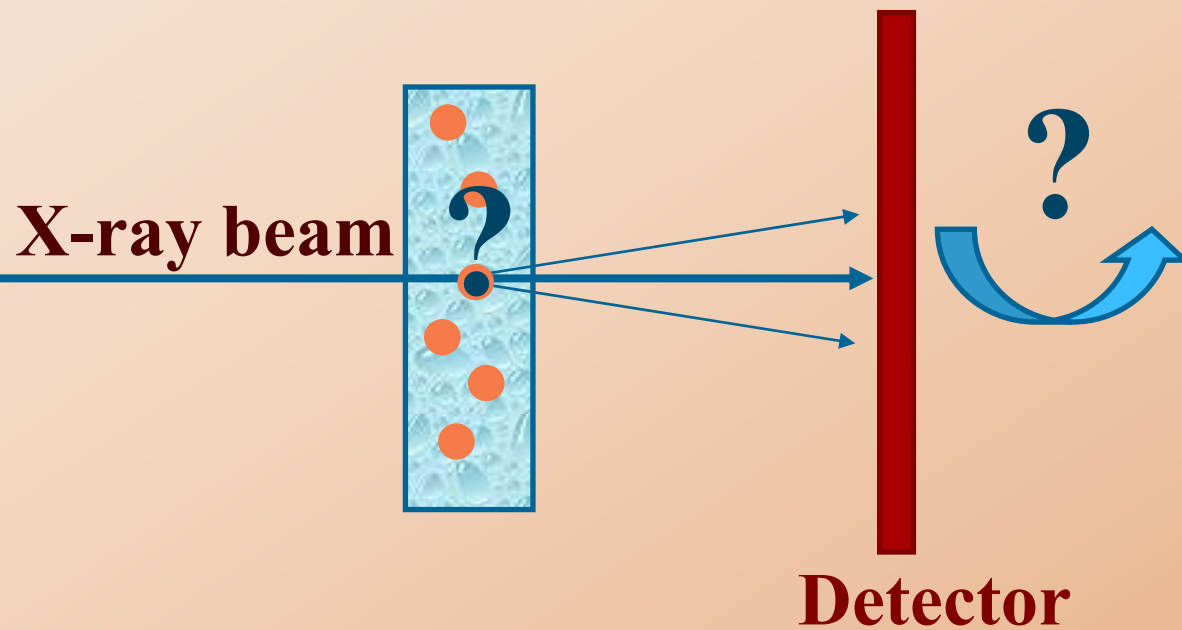


SAXS measuring cell



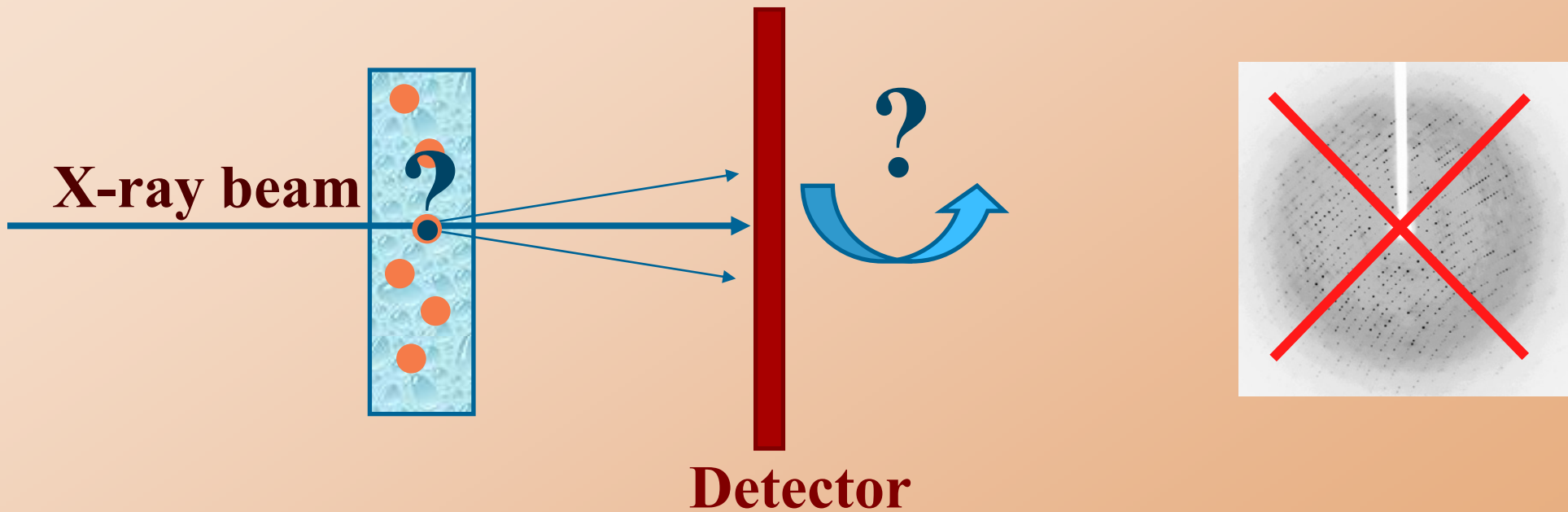
SAXS measurement

Scattering experiment



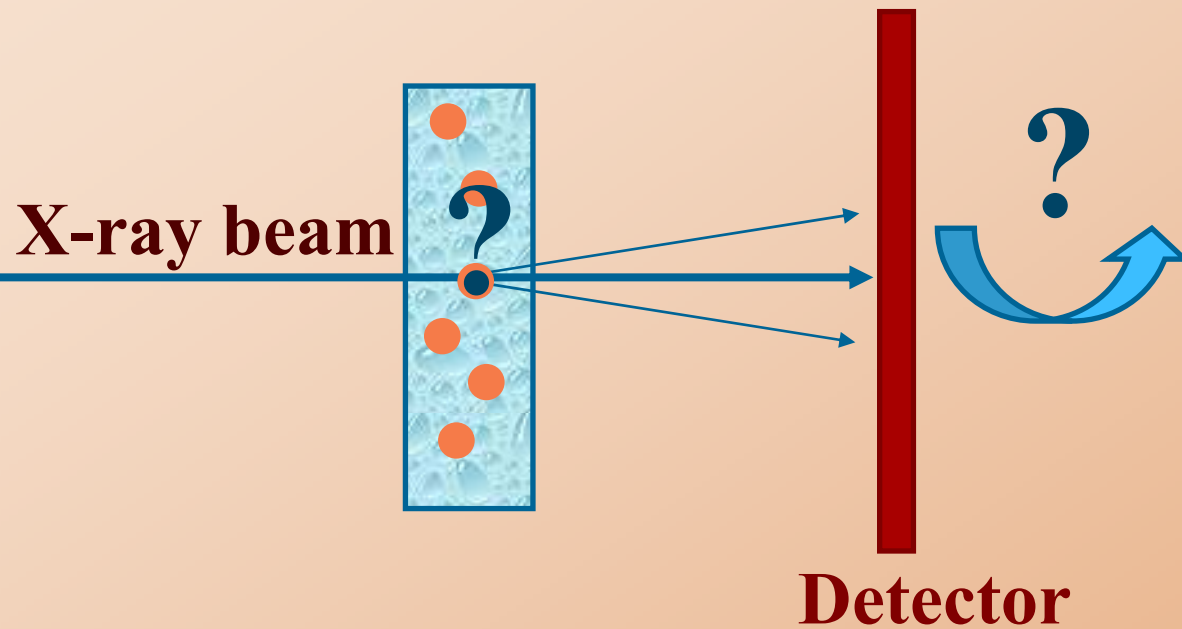
SAXS measurement

Scattering experiment

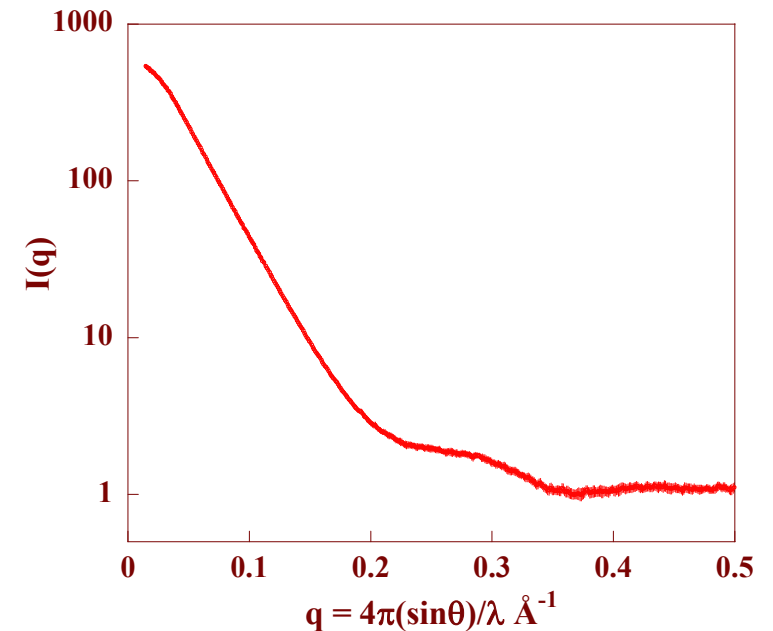


SAXS measurement

Scattering experiment

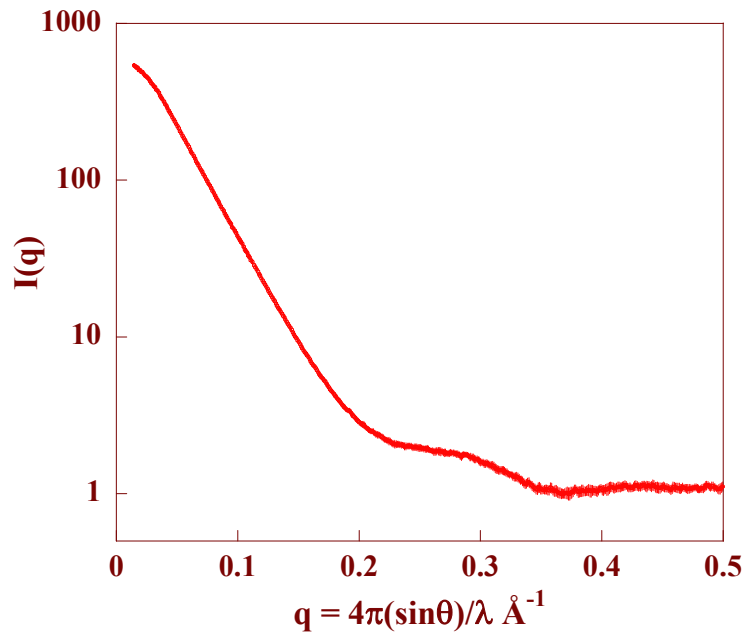


SAXS pattern



SAXS measurement

SAXS pattern



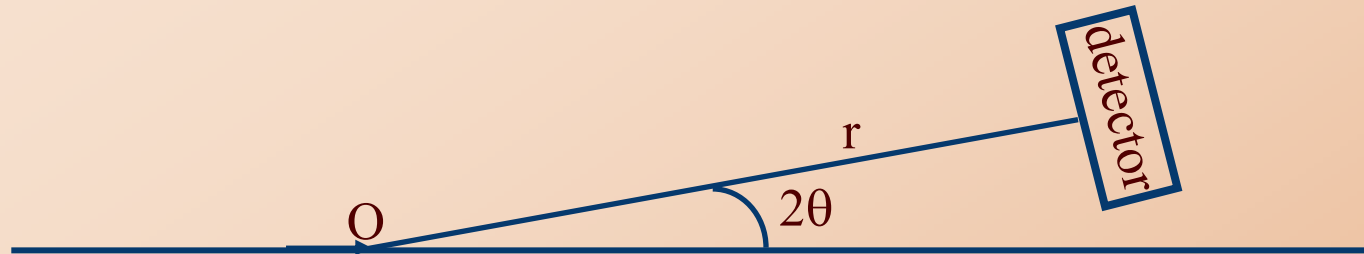
**Structural
parameters**

Summary

- **Reminder of elementary tools and notions**
 - *X-ray Scattering by an electron*
 - *X-ray Scattering by assemblies of electrons*
 - *Fourier transform*
 - *Convolution Product*

- **X-ray scattering by particles in solution.**

Elastic scattering by a single electron



-elastic : interaction without exchange of energy.

The scattered photon has the same energy (or wavelength) than the incident photon.

The *elastically* scattered intensity by an electron placed at the origin is given by the *Thomson formula below*:

$$I(2\theta) = r_0^2 \frac{1 + \cos^2(2\theta)}{2} \frac{1}{r^2} I_0$$

$$r_0 = \frac{e^2}{mc^2} = 0.282 \cdot 10^{-12} \text{ cm}$$

2θ : scattering angle,
 $\cos 2\theta$ close to 1 at small-angles

I_0 intensity (energy/unit area /s) of the incident beam.

r_0 classical radius of the electron.

✓ *differential scattering cross-section of the electron*

$$d\sigma/d\Omega = r_0^2 \frac{1 + \cos^2(2\theta)}{2} \approx r_0^2 = 7.95 \cdot 10^{-26} \text{ cm}^2$$

✓ *the scattering length of the electron b_e*

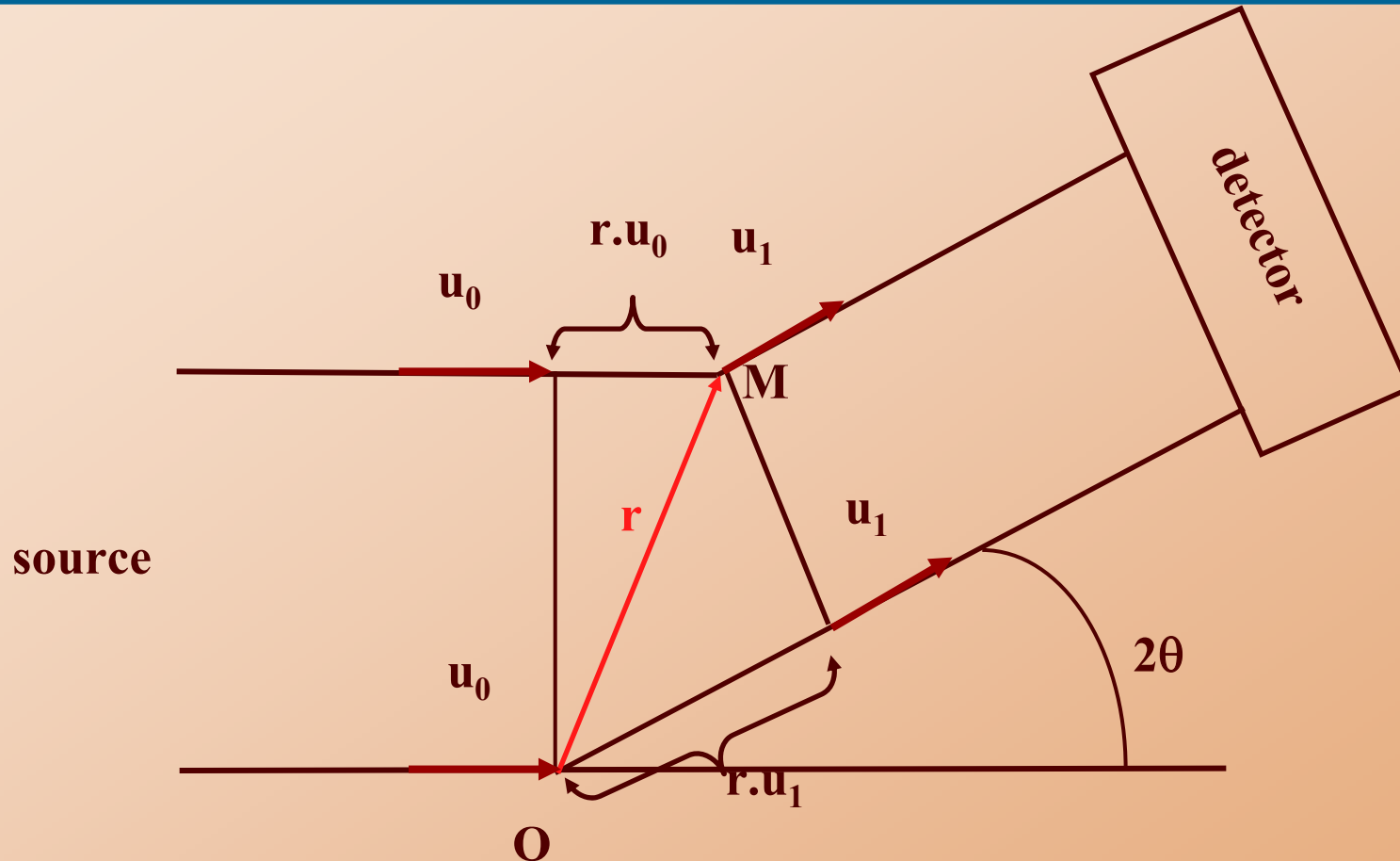
$$b_e^2 = d\sigma/d\Omega$$

Scattering factor

The *scattering factor* f of an object is defined as the ratio between the amplitude of the scattering of the object and that of one electron in identical conditions.

The scattering factor of a single electron $f_e \equiv 1$.
We therefore eliminate $d\sigma/d\Omega$ from all expressions.

Scattering by an electron at a position r



Path difference = $r \cdot u_1 - r \cdot u_0 = r \cdot (u_1 - u_0)$ corresponding to a phase difference $2\pi r \cdot (u_1 - u_0) / \lambda$ for X-rays of wavelength λ .

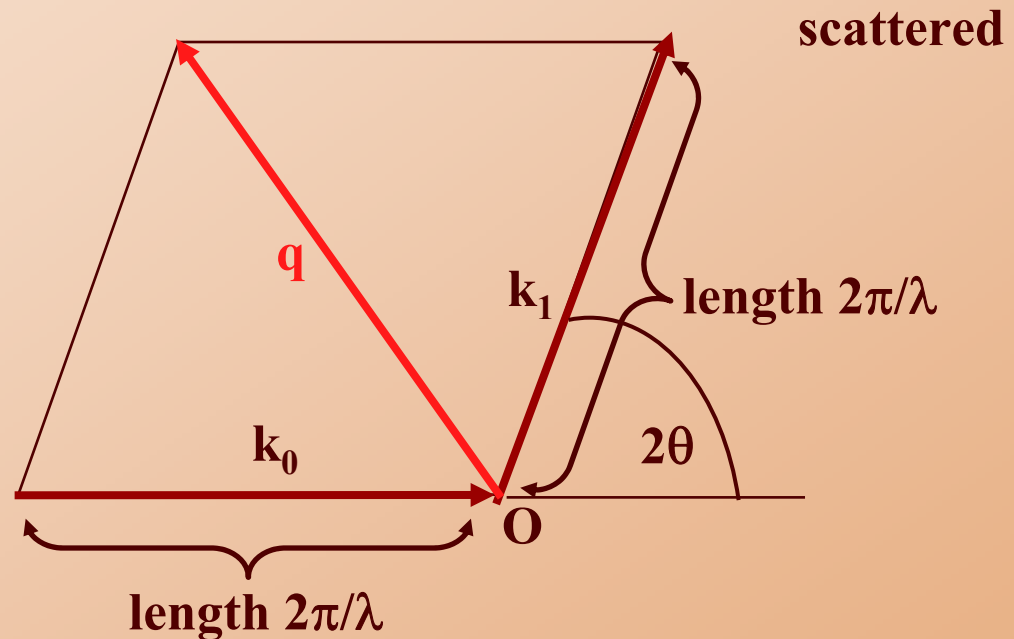
momentum transfer

wavevector \mathbf{k}

$$|\mathbf{k}_0| = |\mathbf{k}_1| = \frac{2\pi}{\lambda}$$

$$\mathbf{q} = \mathbf{k}_1 - \mathbf{k}_0$$

$$q = |\mathbf{q}| = \frac{4\pi \sin \vartheta}{\lambda}$$



q is the momentum transfer

The scattered amplitude by the electron at \mathbf{r} is $A(q)e^{i\mathbf{r}\cdot\mathbf{q}}$
where $A(q)$ is the scattered amplitude by an electron at the origin
Phase difference $\phi = \mathbf{q}\cdot\mathbf{r}$

scattering vector

$$s = \frac{2 \sin \vartheta}{\lambda}$$

Phase difference $\phi = 2\pi r \cdot s$



$$s = \frac{4\pi \sin \vartheta}{\lambda}$$

D. Svergun and coll.

scattering by assemblies of electrons

➤ the distance Δ between scatterers is fixed, e.g. atoms in a molecule :

➔ **coherent scattering** ➔ one adds up **amplitudes**

$$F(\mathbf{q}) = \sum_{i=1}^N f_i e^{i\mathbf{r}_i \cdot \mathbf{q}}$$

Use of a **continuous electron density** $\rho(\mathbf{r})$:

$$F(\mathbf{q}) = \int_{V_r} \rho(\mathbf{r}) e^{i\mathbf{r} \cdot \mathbf{q}} dV_r$$

and

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

➤ Δ is not fixed, e.g. two atoms in two distant molecules in solution :

➔ **incoherent scattering** ➔ one adds up **intensities**.

Fourier Transform

$F(\mathbf{q})$ is the *Fourier transform* of the electron density $\rho(\mathbf{r})$ describing the scattering object.

$$\rho(\mathbf{r}) \xrightarrow{\text{F. T.}} F(\mathbf{q}) = \int_{V_r} \rho(\mathbf{r}) e^{i\mathbf{r}\mathbf{q}} dV_r$$

Properties of the Fourier Transform

- 1 – linearity

$$\text{FT}(\lambda_1\rho_1 + \lambda_2\rho_2) = \lambda_1 \text{FT}(\rho_1) + \lambda_2 \text{FT}(\rho_2)$$

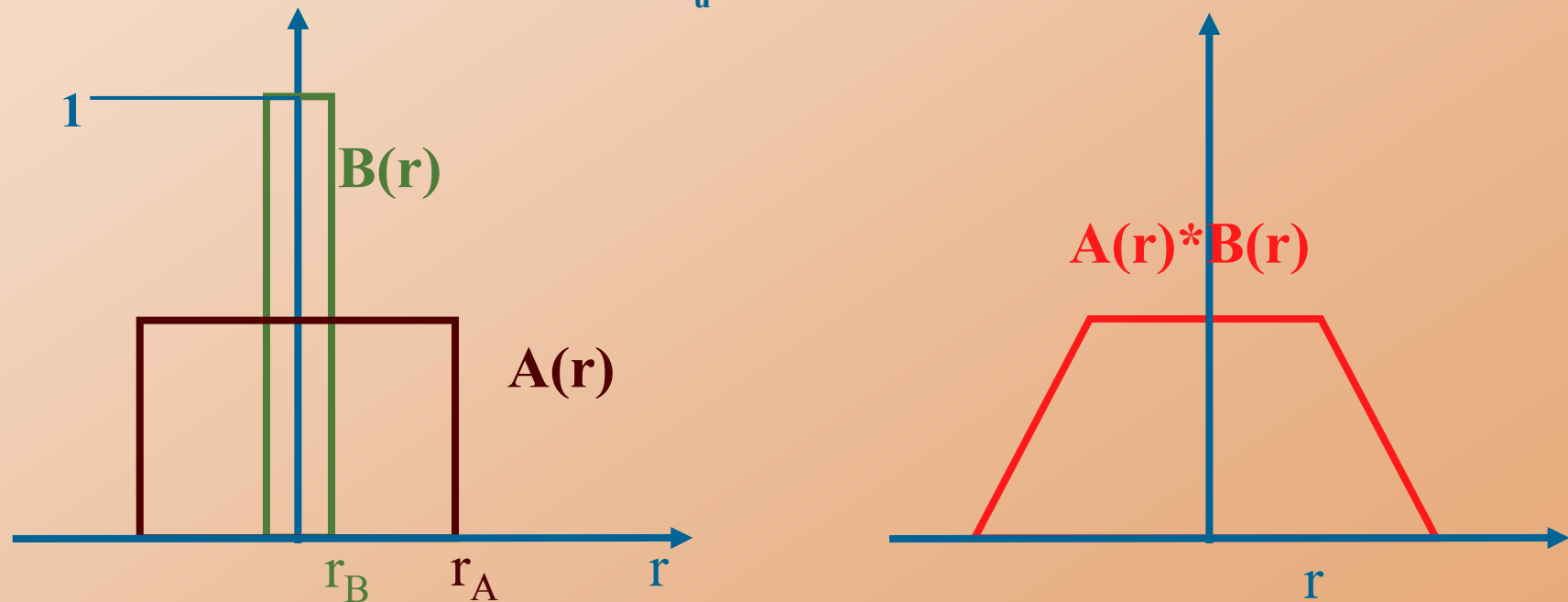
- 2 – value at the origin

$$F(0) = \int_{V_r} \rho(\mathbf{r}) dV_r$$

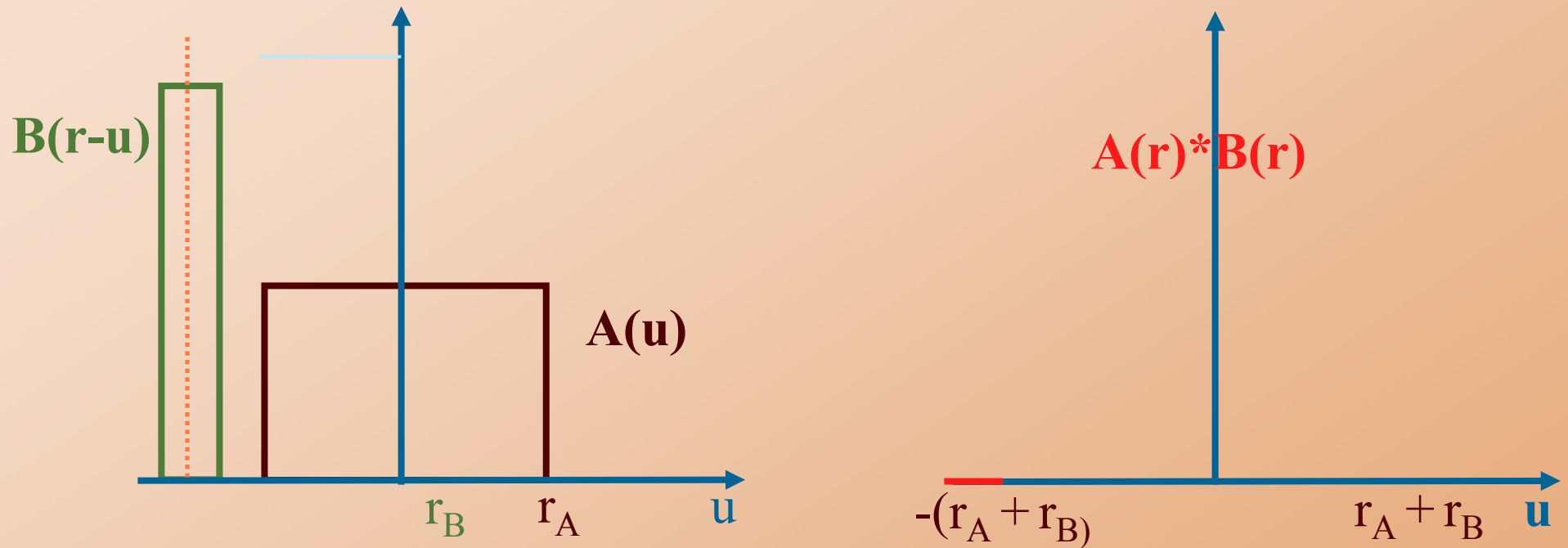
Convolution product

A convolution is an integral that expresses the amount of overlap of one function B as it is shifted over another function A.

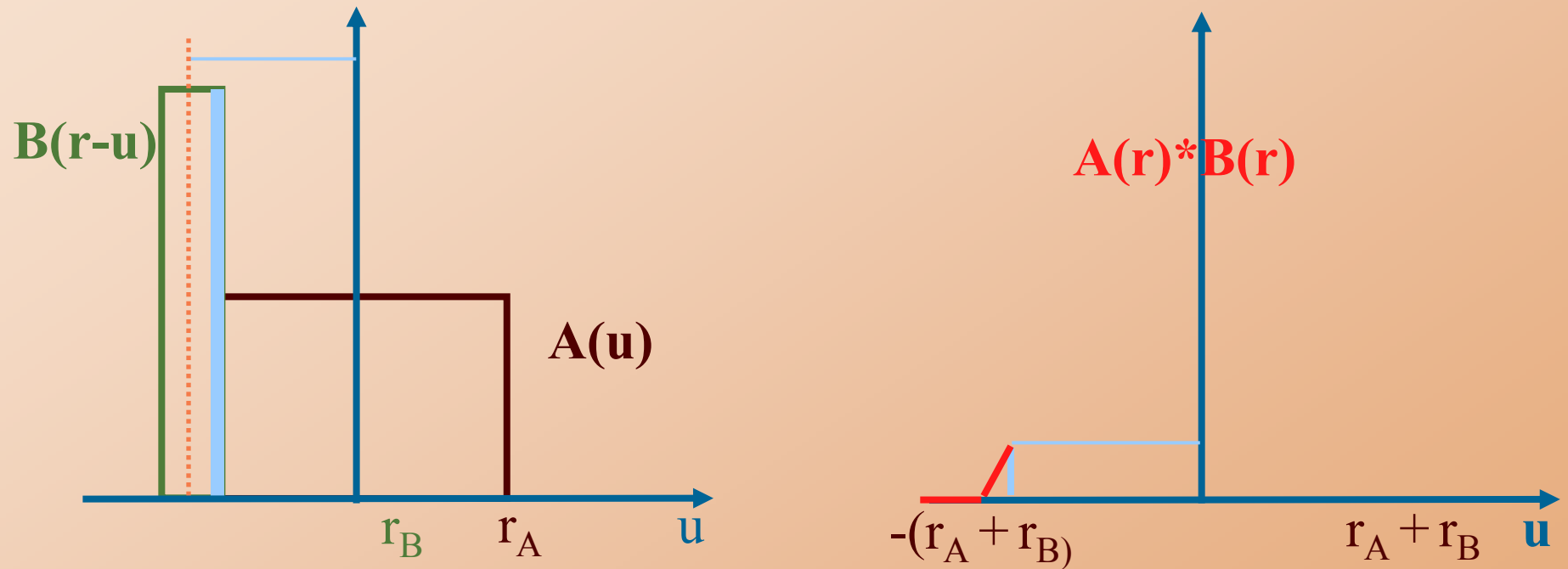
$$A(\mathbf{r}) * B(\mathbf{r}) = \int_{V_u} A(\mathbf{u}) B(\mathbf{r} - \mathbf{u}) dV_u$$



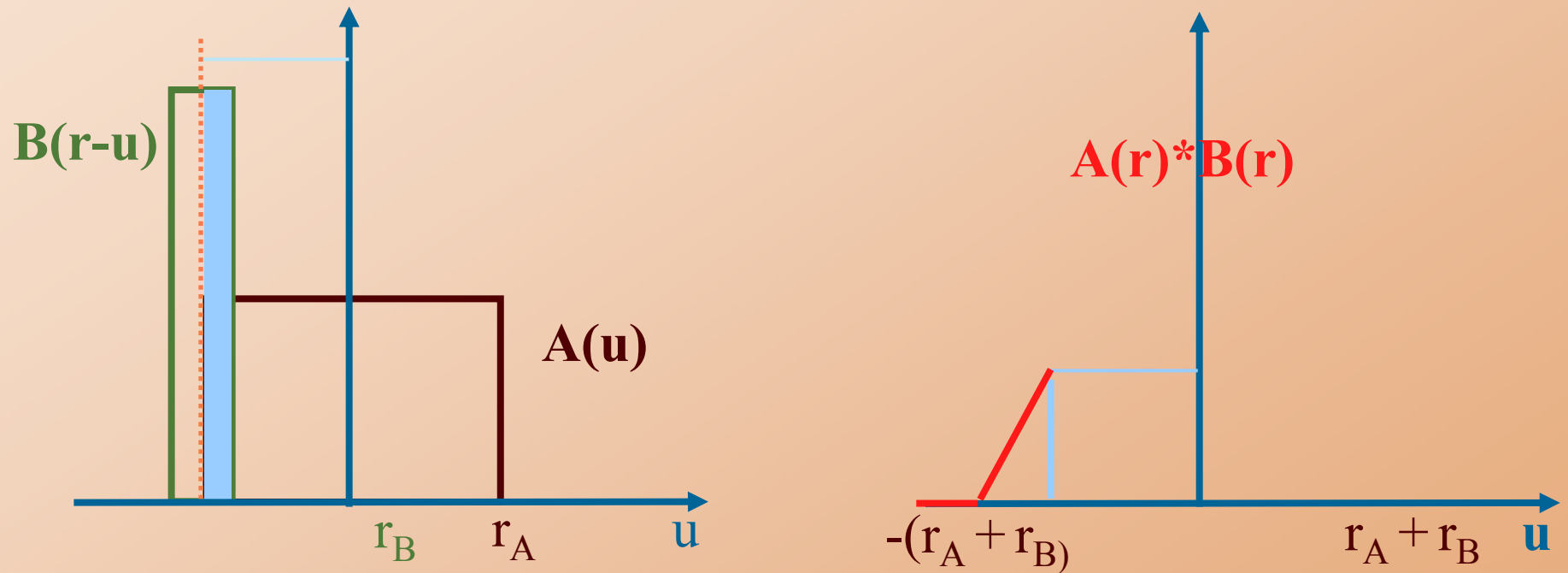
Convolution product



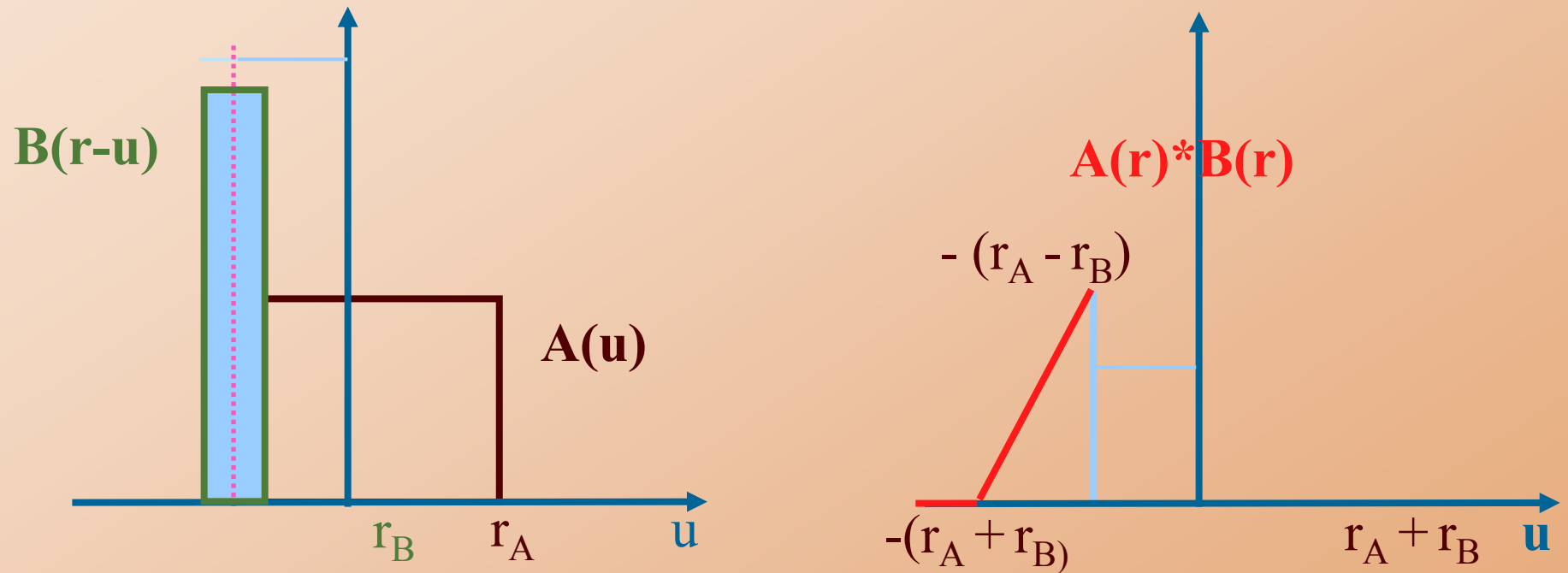
Convolution product



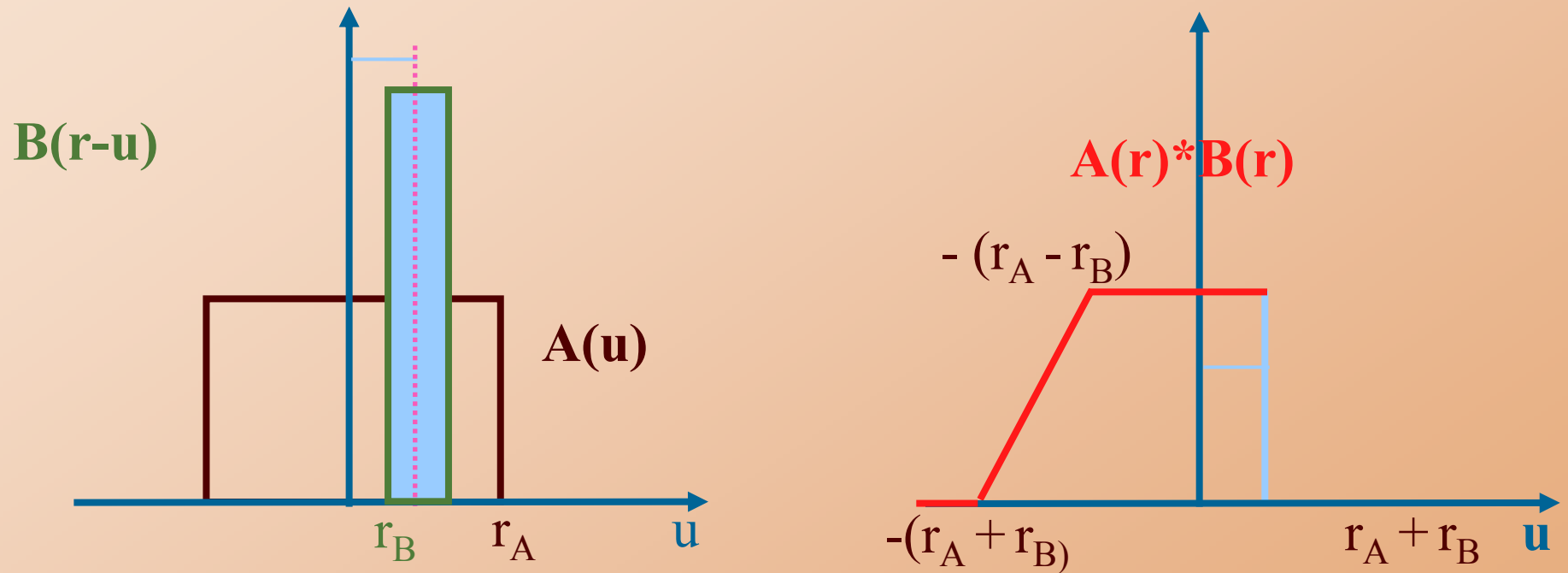
Convolution product



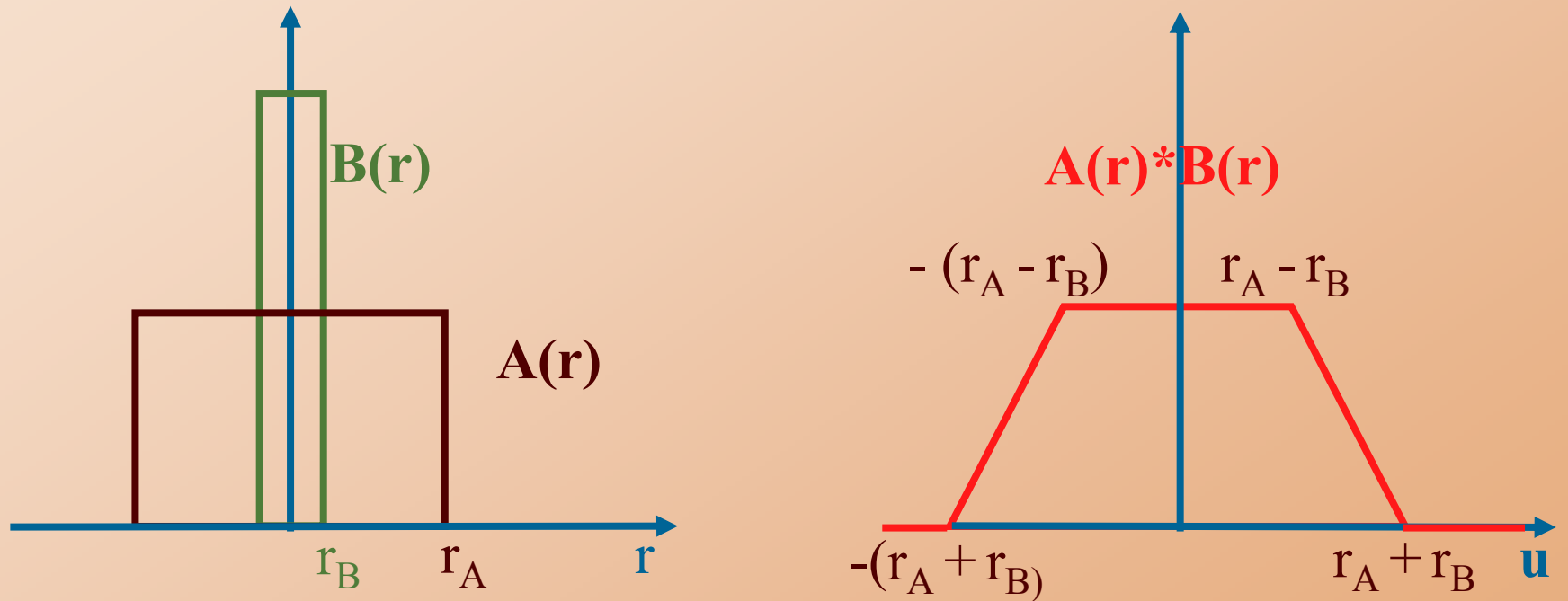
Convolution product



Convolution product



Convolution product



Fourier transform of a convolution product

$$\text{FT}(A * B) = \text{FT}(A) \cdot \text{FT}(B)$$

$$\text{FT}(A \cdot B) = \text{FT}(A) * \text{FT}(B)$$

Autocorrelation function

$$\gamma(\mathbf{r}) = \rho(\mathbf{r}) * \rho(-\mathbf{r}) = \int_{V_u} \rho(\mathbf{r} + \mathbf{u}) \rho(\mathbf{u}) dV_u$$

$\rho(\mathbf{r}) = \rho$ (uniform density)

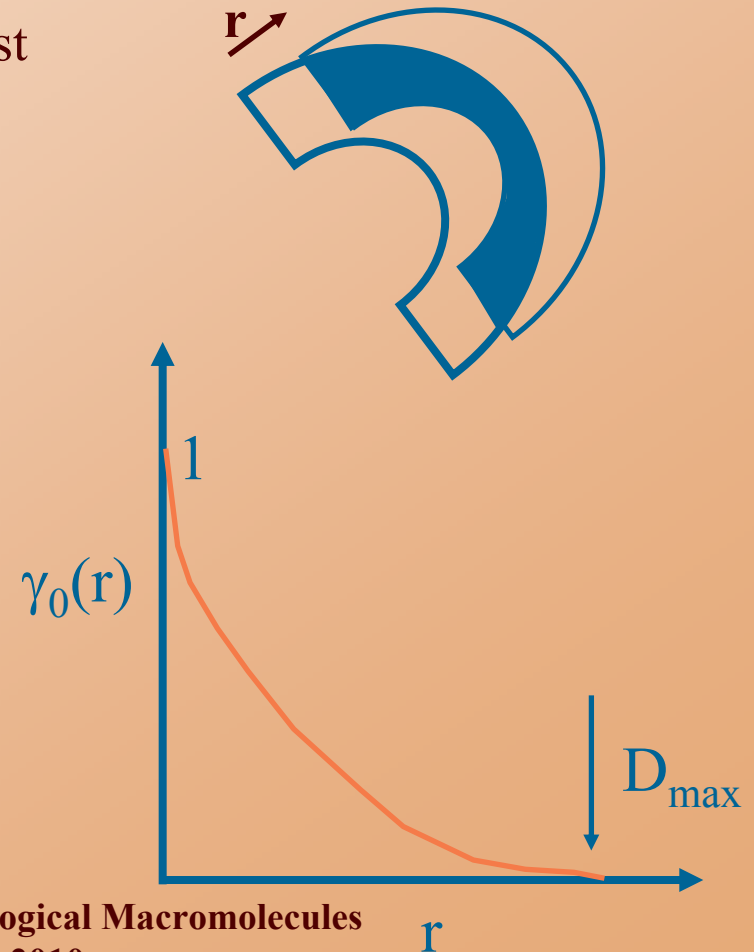
particle \cap ghost

$$\Rightarrow \gamma(\mathbf{r}) = \rho^2 V_{ov}(\mathbf{r}) \quad \text{and} \quad \gamma(0) = \rho^2 V$$

spherical average $\gamma(r) = \langle \gamma(\mathbf{r}) \rangle$

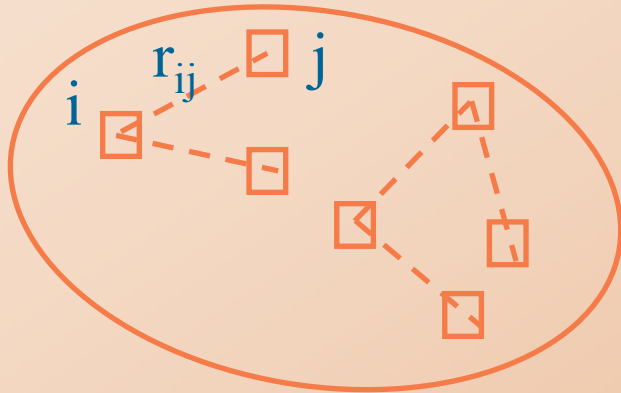
characteristic function $\gamma_0(r) = \gamma(r) / \gamma(0)$

$\gamma_0(r)$: *probability* of finding a point within the particle at a distance r from a given point



Distance (pair) distribution function

- ✓ $p(r)$ is the distribution of distances between all pairs of points within the particle weighted by the respective electron densities



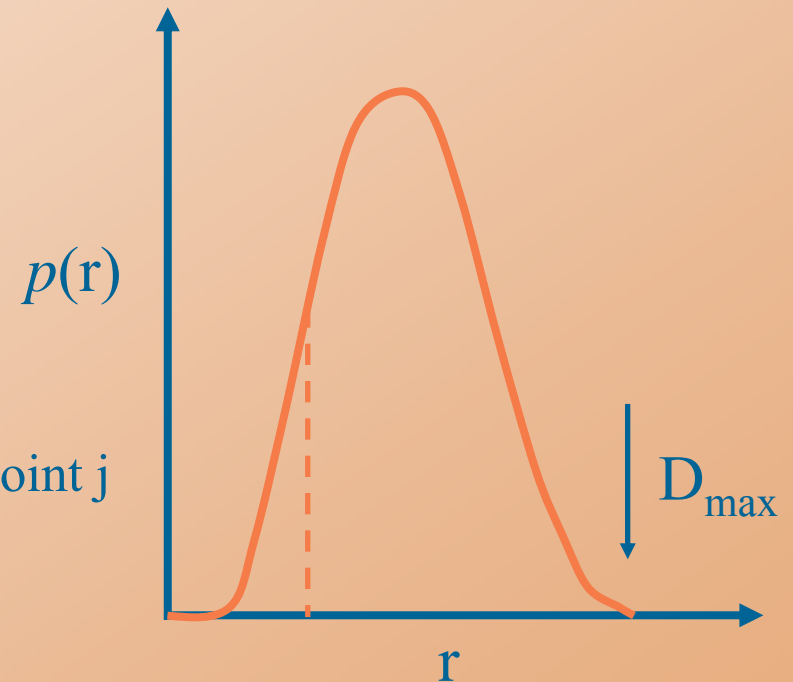
– $\gamma_0(r)$: **probability** of finding within the particle a point j at a distance r from a given point i

- number of el. vol. $i \propto V$

- number of el. vol. $j \propto r^2$

number of pairs (i,j) separated by the distance $r \propto r^2 V \gamma_0(r)$

$$p(r) = \rho^2 \gamma_0(r) V r^2 = r^2 \gamma(r)$$



Solution X-ray scattering

Diagram of the experimental set-up

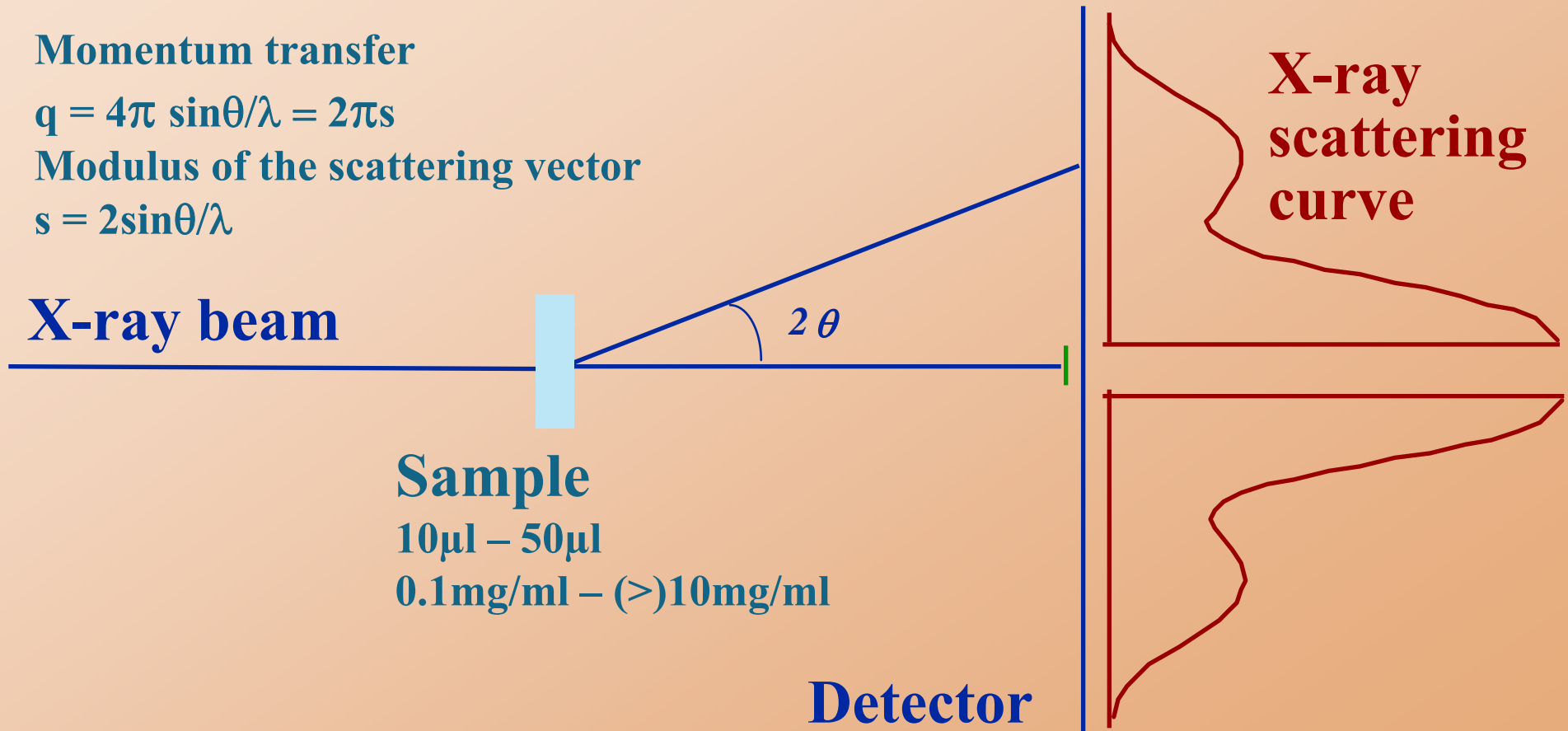
Momentum transfer

$$q = 4\pi \sin\theta/\lambda = 2\pi s$$

Modulus of the scattering vector

$$s = 2\sin\theta/\lambda$$

X-ray beam



Sample

10 μ l – 50 μ l

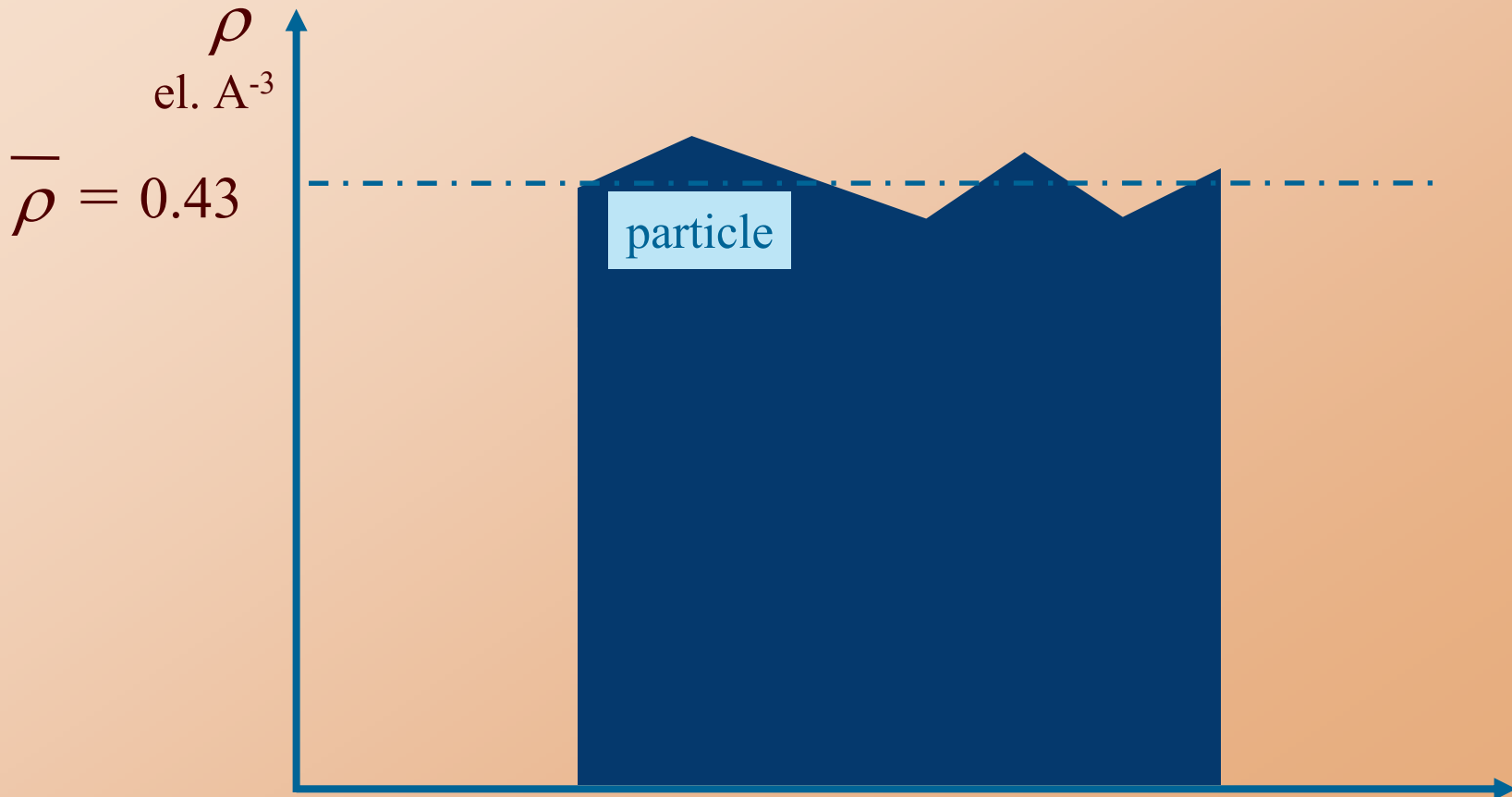
0.1mg/ml – (>)10mg/ml

Detector

X-ray
scattering
curve

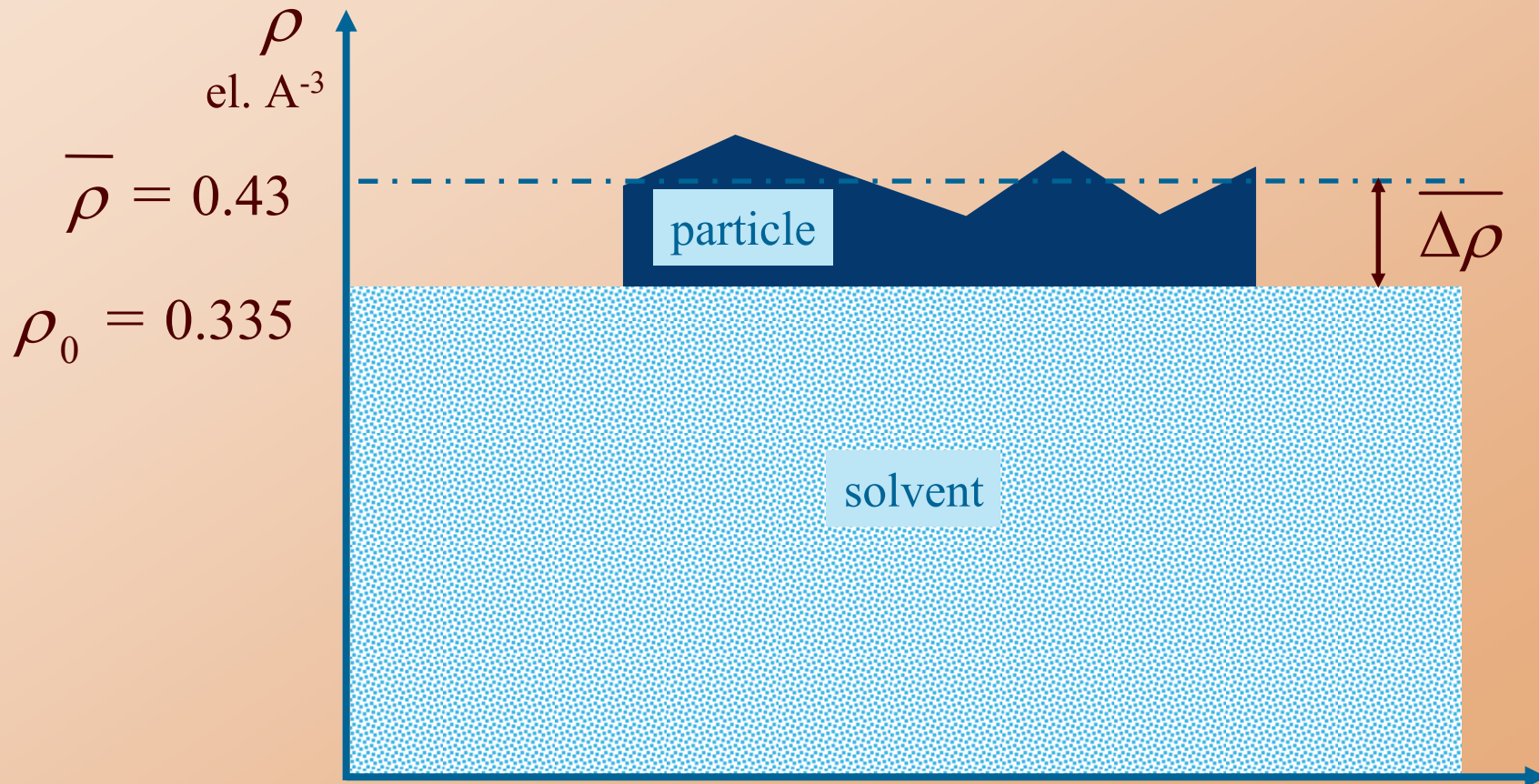
Particles in solution

✓ A particle is described by the associated electron density distribution $\rho_p(\underline{r})$.



Particles in solution

✓ In solution, what matters is the *contrast* of electron density between the particle and the solvent $\Delta\rho(\underline{\mathbf{r}}) = \rho_p(\underline{\mathbf{r}}) - \rho_0$ that may be **small** for biological samples.



X-ray scattering power of a protein solution

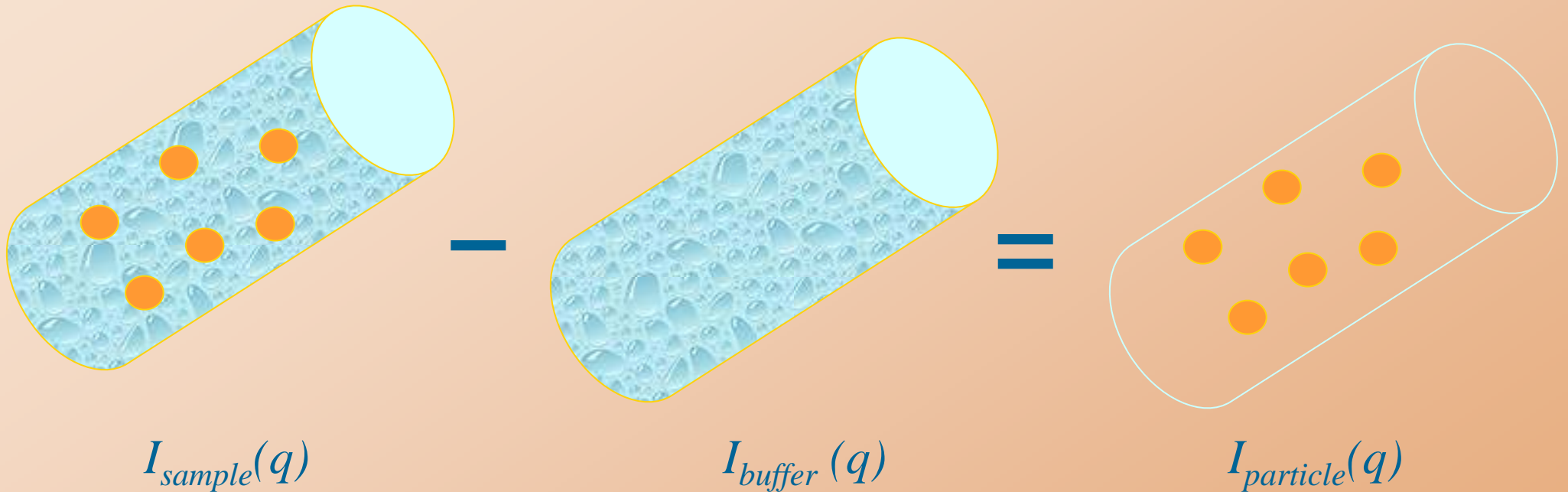
A 1 mg/ml solution of a globular protein 15kDa molecular mass such as lysozyme or myoglobin will scatter in the order of

1 photon in 10^6 incident photons

from H.B. Stuhrmann
Synchrotron Radiation Research
H. Winick, S. Doniach Eds. (1980)

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Solution X-ray scattering: a pair of measurements



- To obtain scattering from the particles, buffer scattering must be subtracted, which also permits to eliminate contribution from parasitic background (slits, sample holder, etc) which should be reduced to a minimum.

particle in solution

scattering amplitude and intensity

$$F_1(\mathbf{q}) = \int_{V_r} \Delta\rho(\mathbf{r}) e^{i\mathbf{r}\cdot\mathbf{q}} dV_r$$

and

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

✓ Particle in **solution** \Rightarrow thermal motion \Rightarrow during the measurement, the particle adopts all orientations / X-ray beam. Therefore, only the *spherical average* of the scattered intensity is experimentally accessible.

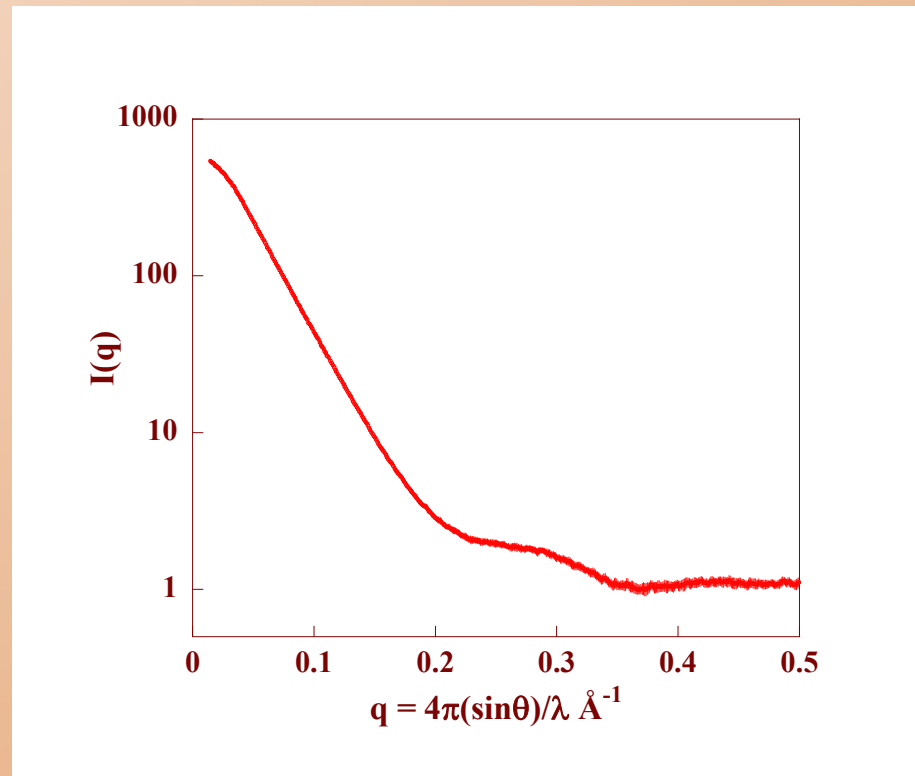
$$\overline{\text{time}} \quad i_1(q) = \langle i_1(\mathbf{q}) \rangle = \langle F_1(\mathbf{q}) \cdot F_1^*(\mathbf{q}) \rangle$$

$$\overline{\text{particles}} \quad I(q) = \langle I(\mathbf{q}) \rangle = \langle F(\mathbf{q}) \cdot F^*(\mathbf{q}) \rangle$$

particle in solution

- ✓ The sample is isotropic and the vectorial (3D) scattering intensity distribution $i(\mathbf{q})$ reduces to a scalar (1D) intensity distribution $i(q)$.

continuous, 1-dimensional
SAXS profile



particle in solution

- ✓ The sample is isotropic and the vectorial (3D) scattering intensity distribution $i(\mathbf{q})$ reduces to a scalar (1D) intensity distribution $i(q)$.
- ✓ This entails a loss of information which constitutes the most severe limitation of the method.

**Let us use the properties of the Fourier transform
and of the convolution product**

$$i_1(q) = \langle I(\mathbf{q}) \rangle = \langle F(\mathbf{q}) \cdot F^*(\mathbf{q}) \rangle$$

$$i_1(q) = \langle FT[\Delta\rho(\mathbf{r})] \cdot FT[\Delta\rho(-\mathbf{r})] \rangle = \langle FT[\Delta\rho(\mathbf{r}) * \Delta\rho(-\mathbf{r})] \rangle$$

$$i_1(q) = \langle FT[\gamma(\mathbf{r})] \rangle = \left\langle \int_{V_r} \gamma(\mathbf{r}) e^{i\mathbf{r}\cdot\mathbf{q}} dV_r \right\rangle$$

$$i_1(q) = \langle FT[\gamma(\mathbf{r})] \rangle = \left\langle \int_{V_r} \gamma(\mathbf{r}) e^{i\mathbf{r}\mathbf{q}} dV_r \right\rangle$$

spherical average: $\langle \exp(i\mathbf{q}\mathbf{r}) \rangle = \frac{\sin(qr)}{qr}$ $dV_r = r^2 \sin \theta dr d\theta d\varphi$

$$i_1(q) = 4\pi \int_0^\infty p(r) \frac{\sin(qr)}{qr} dr$$

with $p(r) = r^2 \gamma(r)$

Solution of particles

- 1 – *monodispersity*: identical particles
- 2 – size and shape polydispersity
- 3 – *ideality* : no intermolecular interactions
- 4 – non ideality : existence of interactions
between particles

In the following, we make the double assumption 1 and 3

2 (mixtures) and 4 (interactions) are dealt with at a later stage in the course.

Ideal and monodisperse solution

Ideality

$$I(q) = \sum_j n_j i_j(q)$$

Monodispersity

$$i_j(q) = i_1(q) \quad \forall j$$

Ideality and monodispersity

$$I(q) = N i_1(q)$$

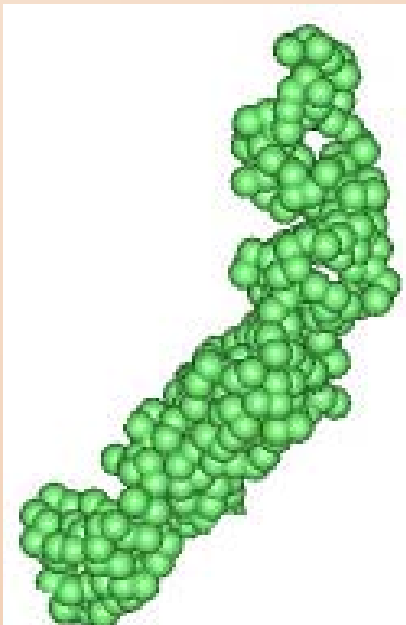
ideal
monodisperse

$$i_1(q)$$

Ideality

$$I(q)$$

Monodispersity



One must check that both assumptions are valid for the sample under study.



molecule

experimental

Checking the validity of both assumptions for the sample under study.

➤ **Monodispersity: purification protocol**
- Mass Spec., DLS, AUC, MALS + RI, etc.

➤ **Ideality : reached by working at infinite dilution**
In practice : one performs measurements at decreasing concentrations and checks whether the scattering pattern is independent of concentration.
- DLS, AUC

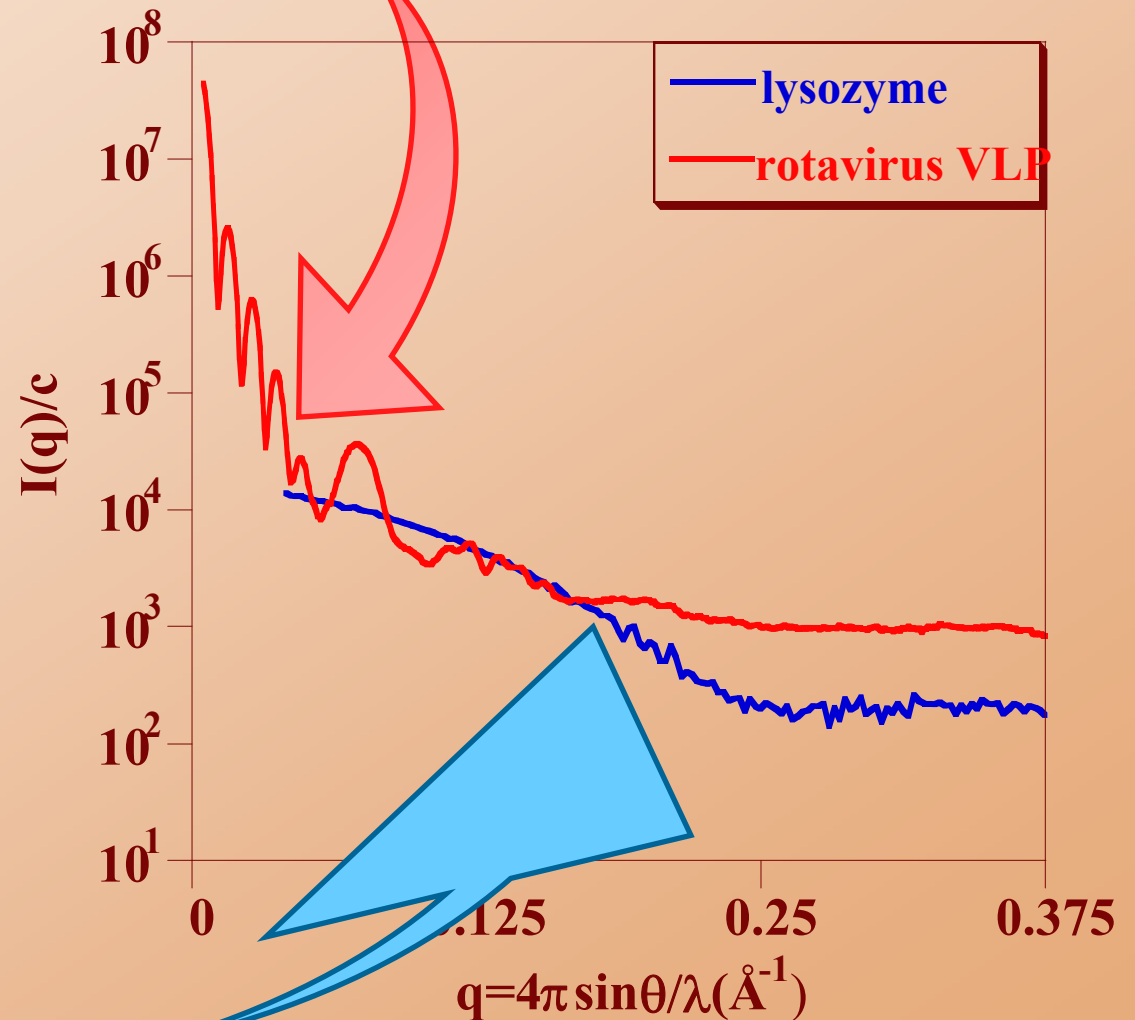
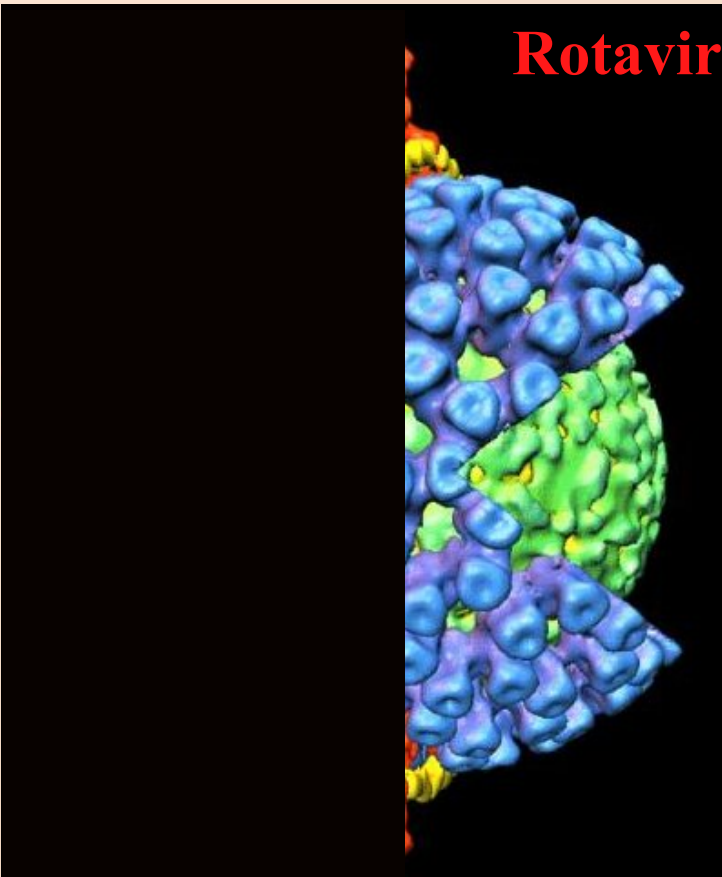
Basic law of reciprocity in scattering

- large dimensions **r**  small scattering angles **q**

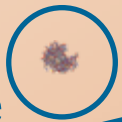
-small dimensions **r**  large scattering angles **q**

argument **qr**

Rotavirus VLP : diameter = 700 Å, 44 MDa MW



Lysozyme
 $D_{\text{max}} = 45 \text{ \AA}$
14.4 kDa MW



Guinier law

The scattering curve of a particle can be approximated by a Gaussian curve in the vicinity of the origin

$$I(q) \cong I(0)\exp(-Kq^2)$$

Guinier law

$$\ln[I(q)] \cong \ln[I(0)] - Kq^2$$

$\ln[I(s)]$ vs q^2 : linear variation.

Linear regression on experimental data yields slope and y-intercept.

ideal
monodisperse

Radius of gyration

Guinier law: slope value

$$K = \frac{R_g^2}{3}$$

Radius of gyration :

$$R_g^2 = \frac{\int_{V_r} \Delta\rho(\mathbf{r}) r^2 dV_r}{\int_{V_r} \Delta\rho(\mathbf{r}) dV_r}$$

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

If $\Delta\rho(\mathbf{r}) \approx$ 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.

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

ideal
monodisperse

Guinier plot example

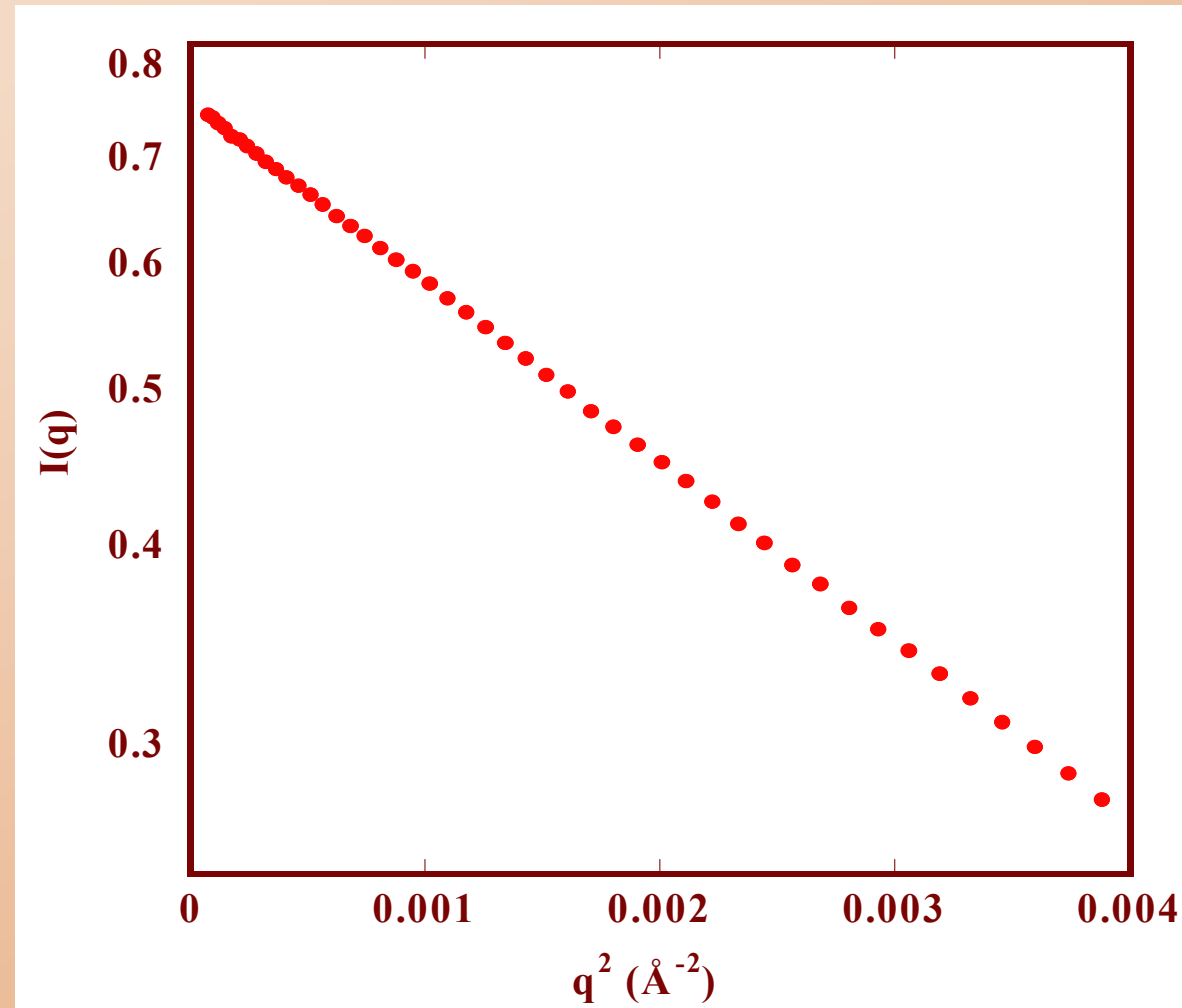
$$\ln[I(q)] \cong \ln[I(0)] - \frac{R_g^2}{3} q^2$$

Validity range :

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

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

ideal
monodisperse



*Swing – SAXS Instrument, resp. J. Pérez
SOLEIL (Saclay, France)*

Guinier plot example

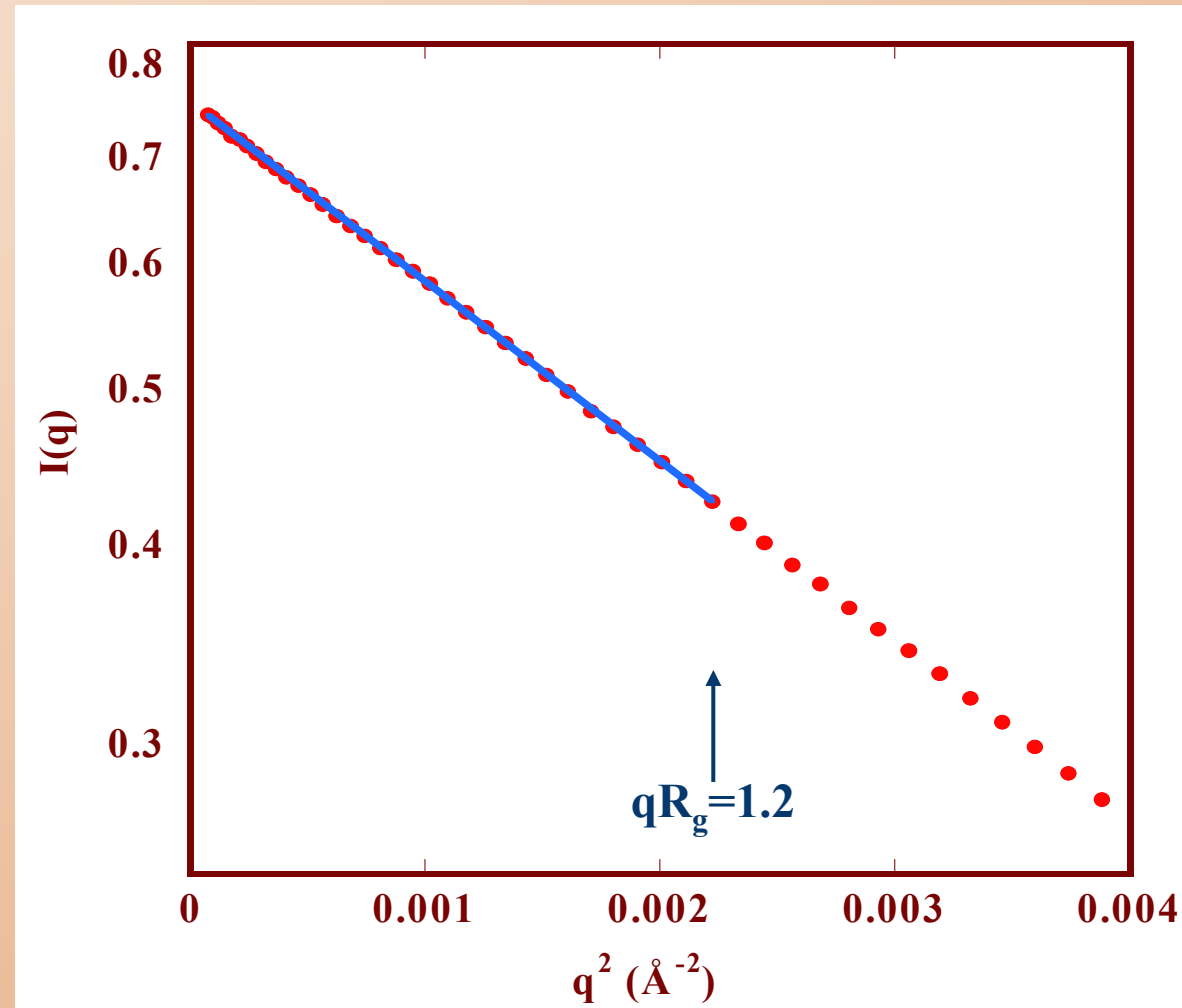
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ideal
monodisperse



*Swing – SAXS Instrument, resp. J. Pérez
SOLEIL (Saclay, France)*

Intensity at the origin

$$\frac{I(0)}{c} \propto M$$

If : the concentration c (w/v), —
the partial specific volume V_P , —
the intensity on an absolute scale, —
i.e. the number of incident photons *are known*,

Then the **molecular mass** of the particle can be determined from the value of the intensity at the origin.

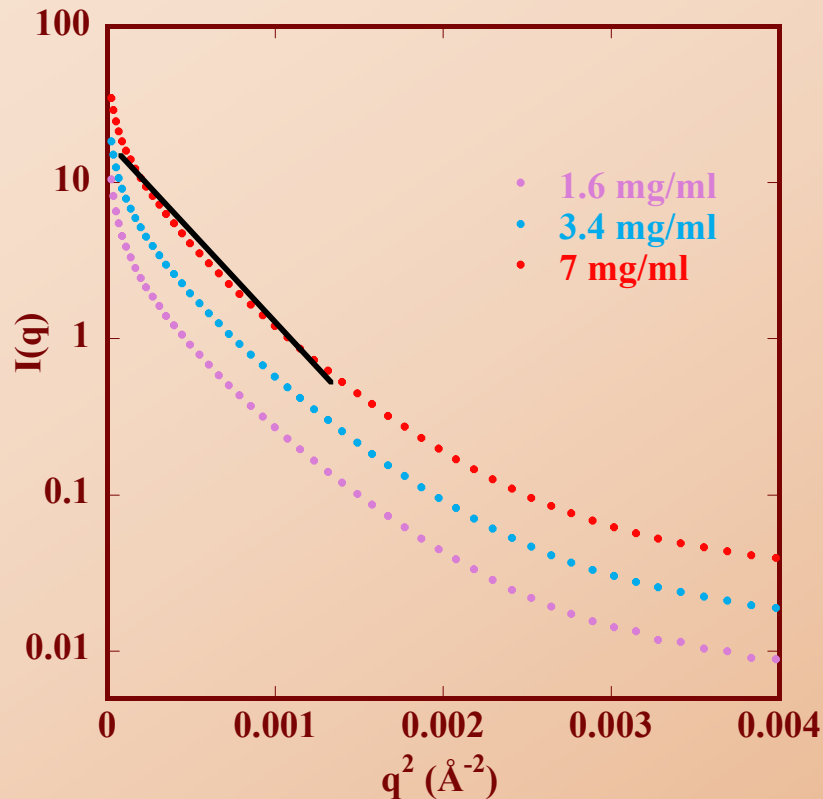
In actual fact one only gets an estimate of the MM.

Its determination is a useful check of ideality and monodispersity.

ideal
monodisperse

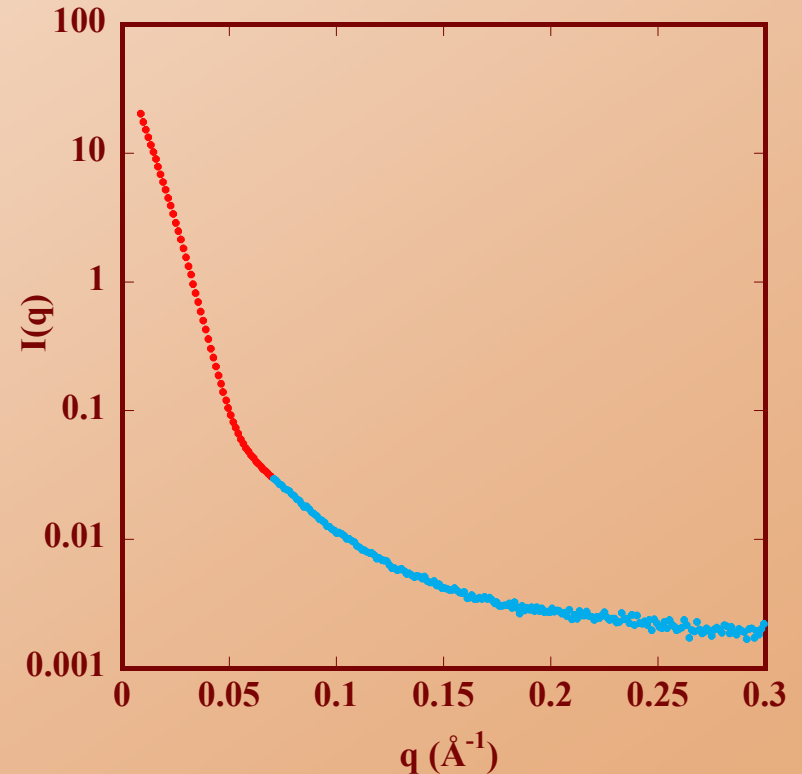
Evaluation of the solution properties

Irreversible aggregation



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

Useless data: the whole curve is affected

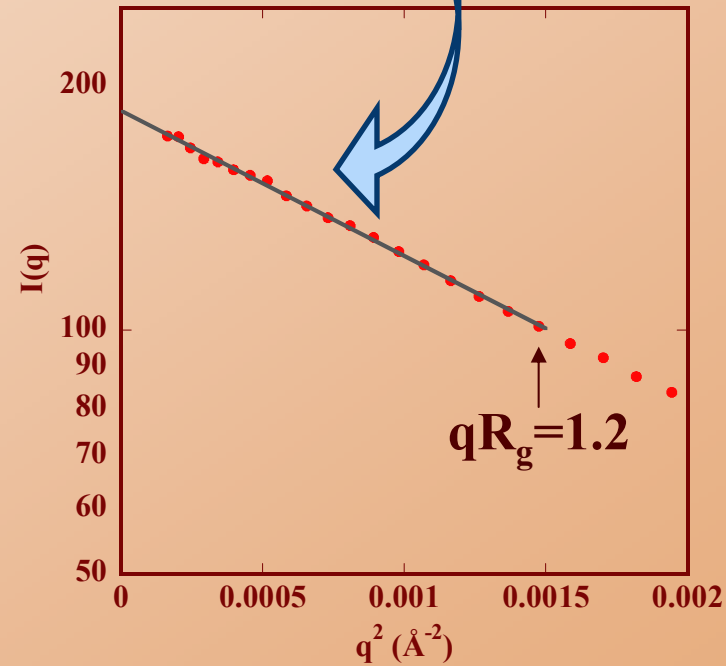
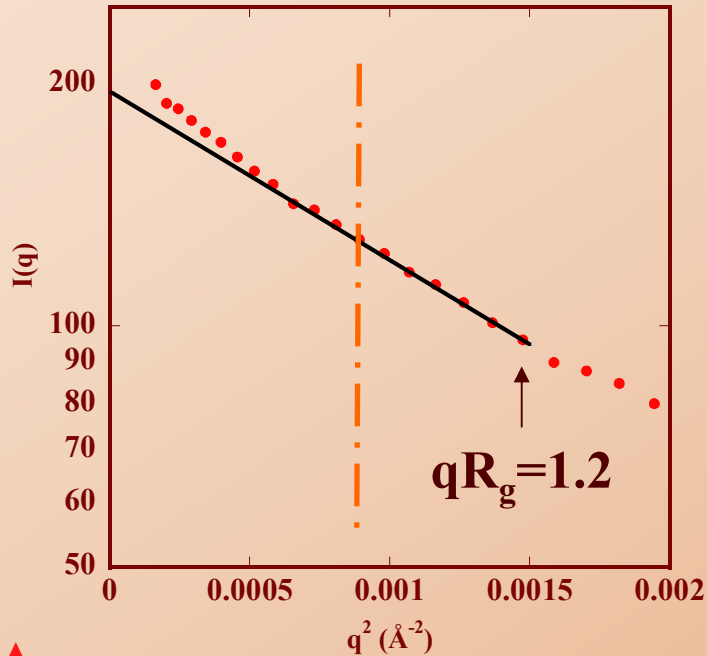


Swing – Domaine 1-242 de RRP44 – 07/08

Evaluation of the solution properties

weak aggregation → possible improvement
centrifugation, buffer change

Nanostar –PR65 protein

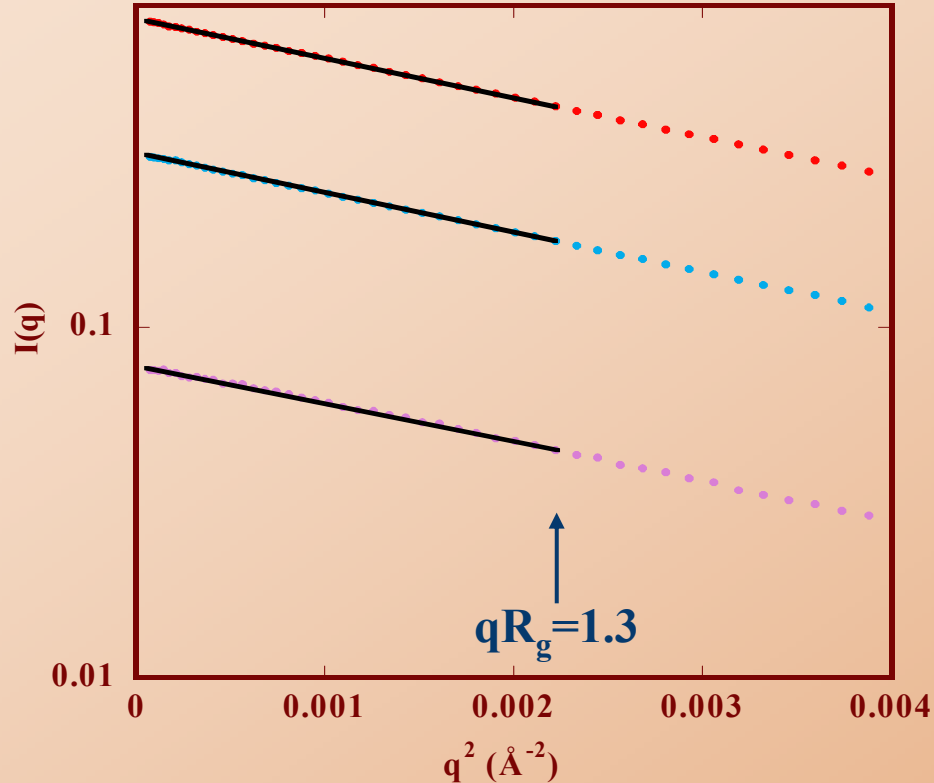


$R_g \sim 38 \text{ \AA} - \text{too high!!}$

$R_g \sim 36 \text{ \AA}$

Evaluation of the solution properties

Guinier plot



same R_g at all three
concentrations



**No aggregation,
no interactions.**

Swing – Polymérase – 07/08

N. Leulliot *et al.*, JBC (2009), 284, 11992-99.

ideal
monodisperse

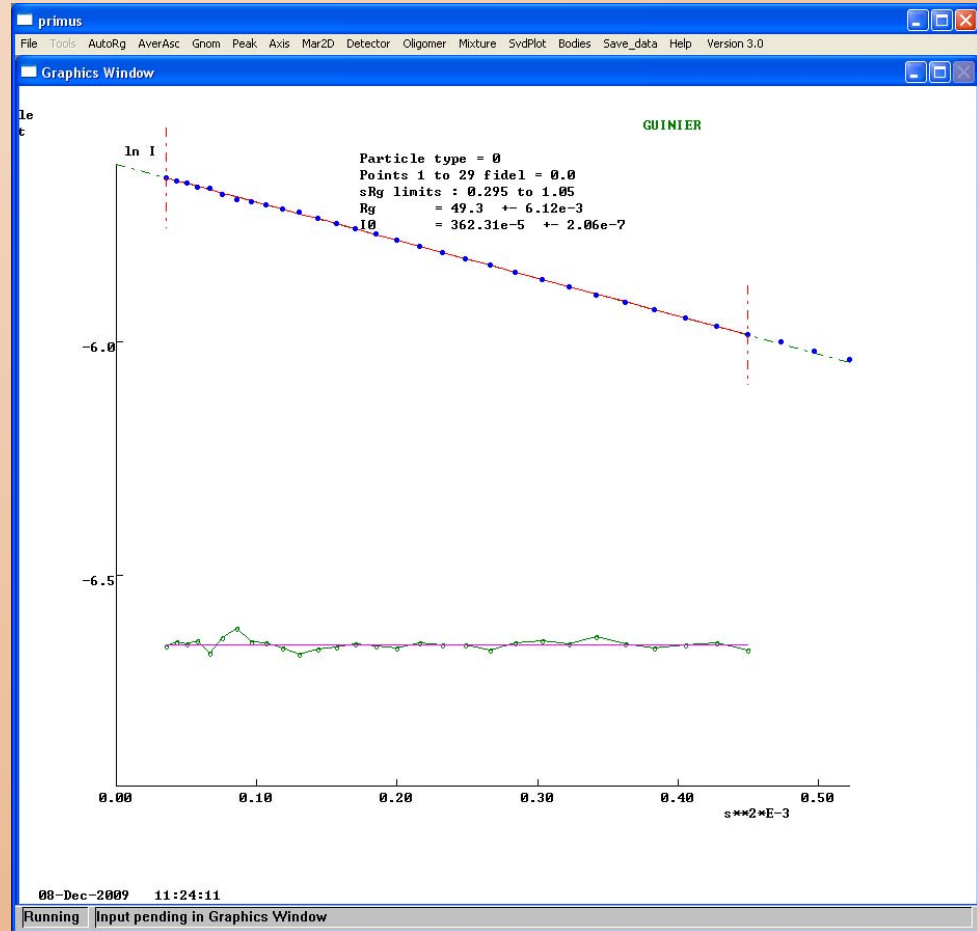
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Evaluation of the solution properties

Guinier plot

$$c_4$$
$$R_g = 49.3 \text{ \AA}$$

RNA molecule
L. Ponchon, C. Mériçoux *et al.*



ideal
monodisperse

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Evaluation of the solution properties

Guinier plot

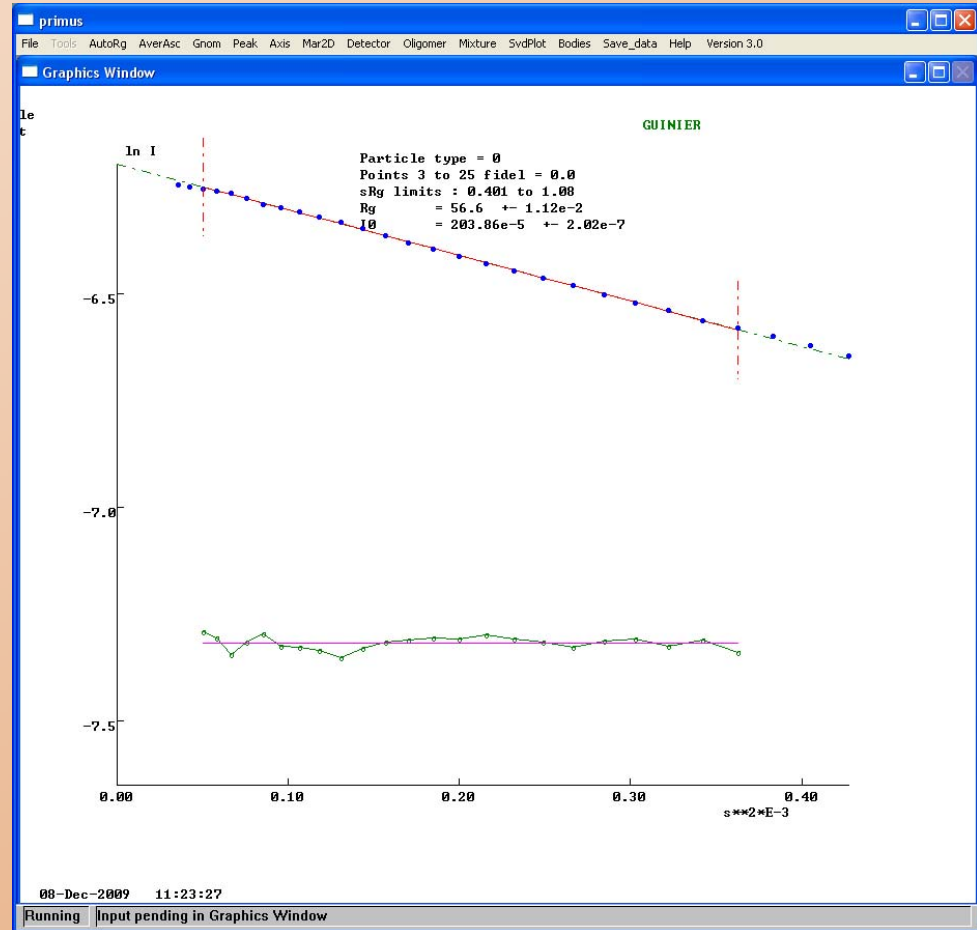
$$c_3 \\ R_g = 56.6 \text{ \AA}$$

$$c_4 \\ R_g = 49.3 \text{ \AA}$$

RNA molecule

L. Ponchon, C. MÉRIGOUX *et al.*

ideal
monodisperse



Evaluation of the solution properties

Guinier plot

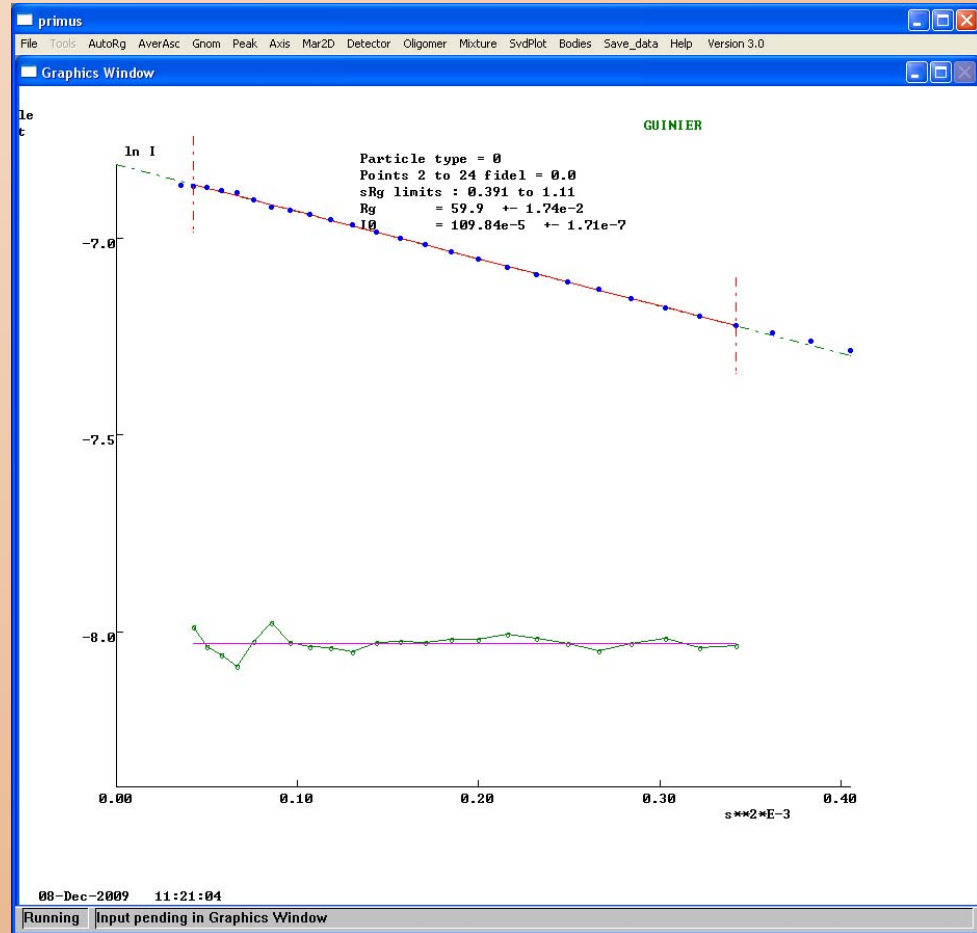
$$c_2 \\ R_g = 59.9 \text{ \AA}$$

$$c_3 \\ R_g = 56.6 \text{ \AA}$$

$$c_4 \\ R_g = 49.3 \text{ \AA}$$

RNA molecule

L. Ponchon, C. MÉRIGOUX *et al.*



ideal
monodisperse

Evaluation of the solution properties

Guinier plot

$$c_1 \\ R_g = 60.8 \text{ \AA}$$

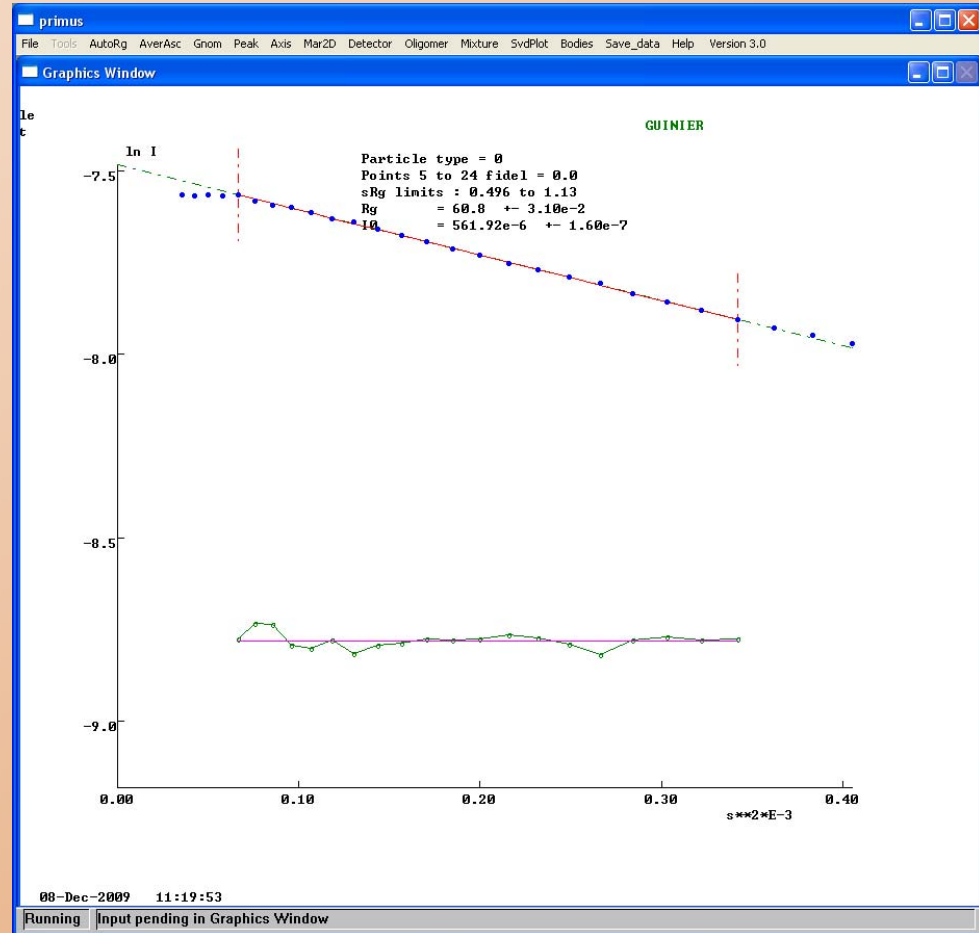
$$c_2 \\ R_g = 59.9 \text{ \AA}$$

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RNA molecule

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ideal
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Evaluation of the solution properties

Guinier plot

➤ A linear Guinier plot is a requirement, but it is **NOT** a sufficient condition ensuring ideality (nor monodispersity) of the sample.

ideal
monodisperse

Virial coefficient

In the case of moderate interactions, the intensity at the origin varies with concentration according to :

$$I(0, c) = \frac{I(0)_{ideal}}{1 + 2A_2Mc + \dots}$$

Where A_2 is the second virial coefficient which represents pair interactions and $I(0)_{ideal} = K \cdot c$ ($K = c^{te}$).

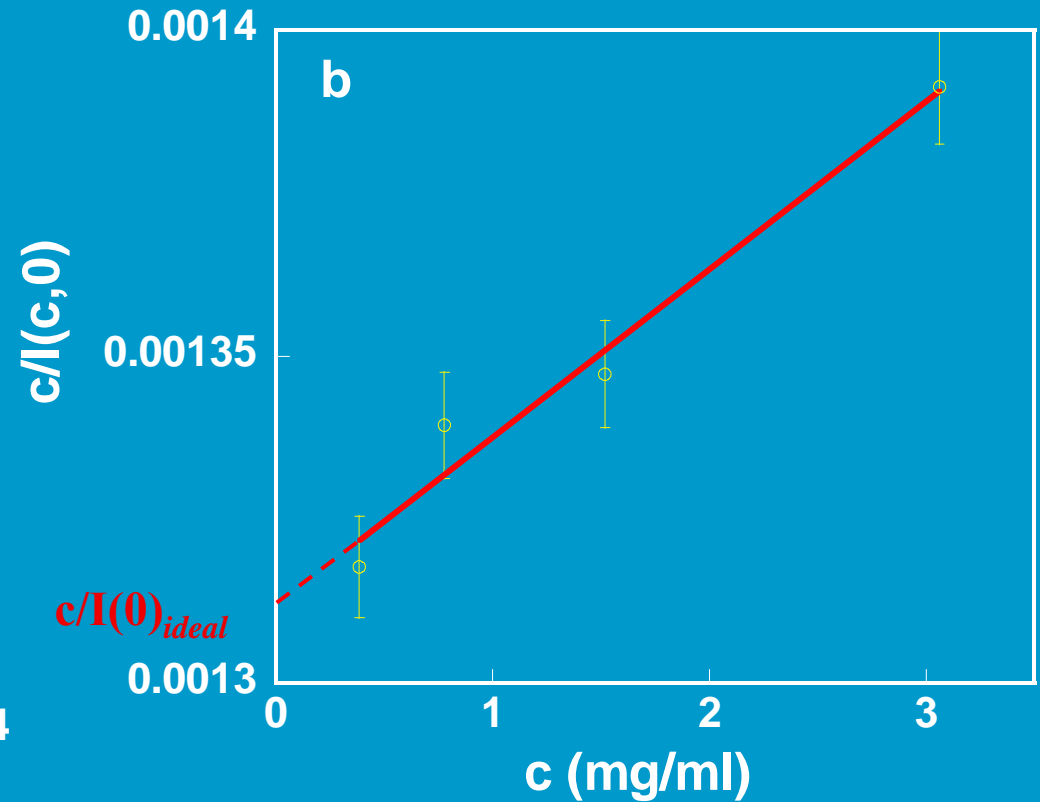
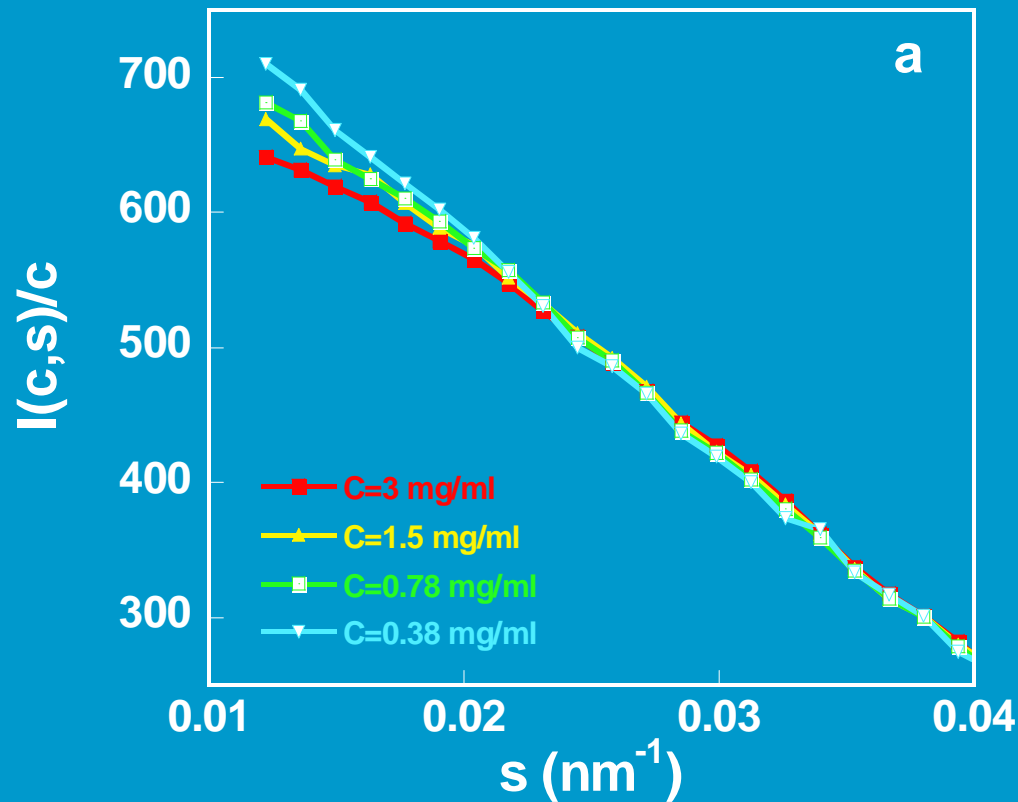
$I(0)_{ideal}$ and A_2 are evaluated by performing experiments at various concentrations c .

A_2 is \propto to the slope of $c/I(0,c)$ vs c .

$$\frac{c}{I(0, c)} = K(1 + 2A_2Mc)$$

Virial coefficient

- I - Example of repulsive interactions

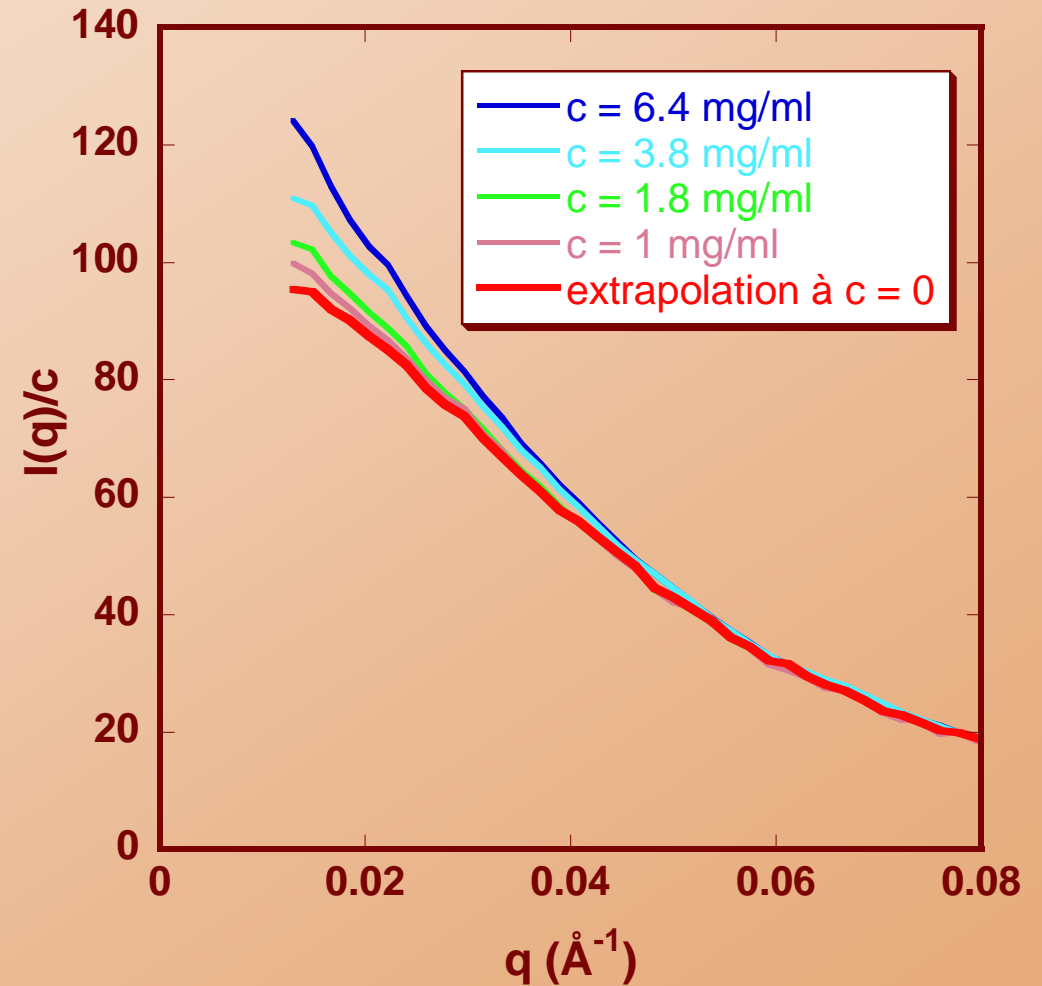


nucleosome core particles in a 10 mM Tris buffer, pH 7.6 with 15 mM NaCl
(Courtesy D. Durand, IBBMC, Orsay)

Virial coefficient

- II - Example of attractive interactions

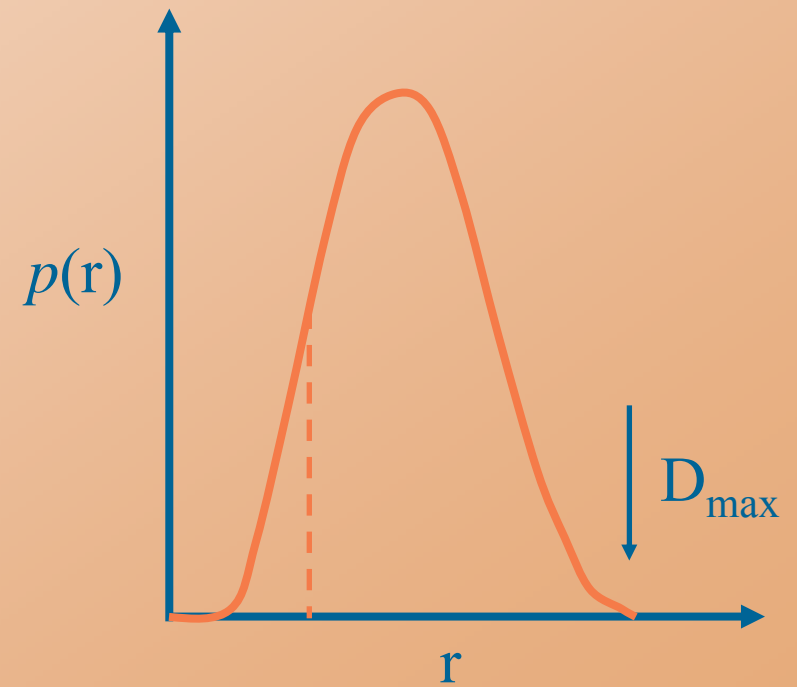
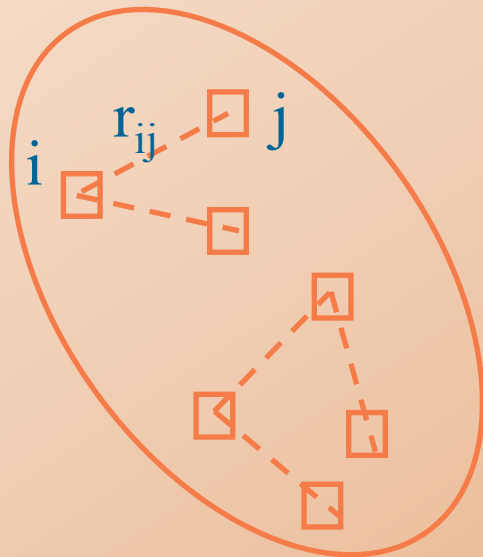
p47 : component of the NADPH oxidase from neutrophile.



D. Durand *et al.*, *Biochemistry* (2006), 45, 7185-93.

Distance distribution function

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



ideal
monodisperse

Distance distribution function

$$p(r) = \frac{r^2}{2\pi^2} \int_0^\infty q^2 I(q) \frac{\sin(qr)}{qr} dq$$

In theory, the calculation of $p(r)$ from $I(q)$ is simple.

Problem : $I(q)$ - is only known over $[q_{\min}, q_{\max}]$: truncation
- is affected by experimental errors

⇒ Calculation of the Fourier transform of *incomplete and noisy data*, requires (hazardous) extrapolation to lower and higher angles.

Solution : Indirect Fourier Transform. First proposed by O. Glatter in 1977.

Calculation of $p(r)$

$p(r)$ is calculated from $i(q)$ using the indirect Fourier Transform method

Basic hypothesis :

The particle has a *finite size*

$$I(q) = 4\pi \int_0^{D_{Max}} p(r) \frac{\sin(qr)}{qr} dr$$

- ✓ $p(r)$ is parameterized on $[0, D_{Max}]$ by a linear combination of orthogonal basis functions.

$$p(r) = \sum_{n=1}^M c_n \varphi_n(r)$$

- ✓ The coefficients c_n are found by least-squares methods.
Ill-posed problem solved using stabilisation methods.

ideal
monodisperse

Distance distribution function

The radius of gyration and the intensity at the origin can be derived from $p(r)$ using the following expressions :

$$R_g^2 = \frac{\int_0^{D_{\max}} r^2 p(r) dr}{2 \int_0^{D_{\max}} p(r) dr}$$

and

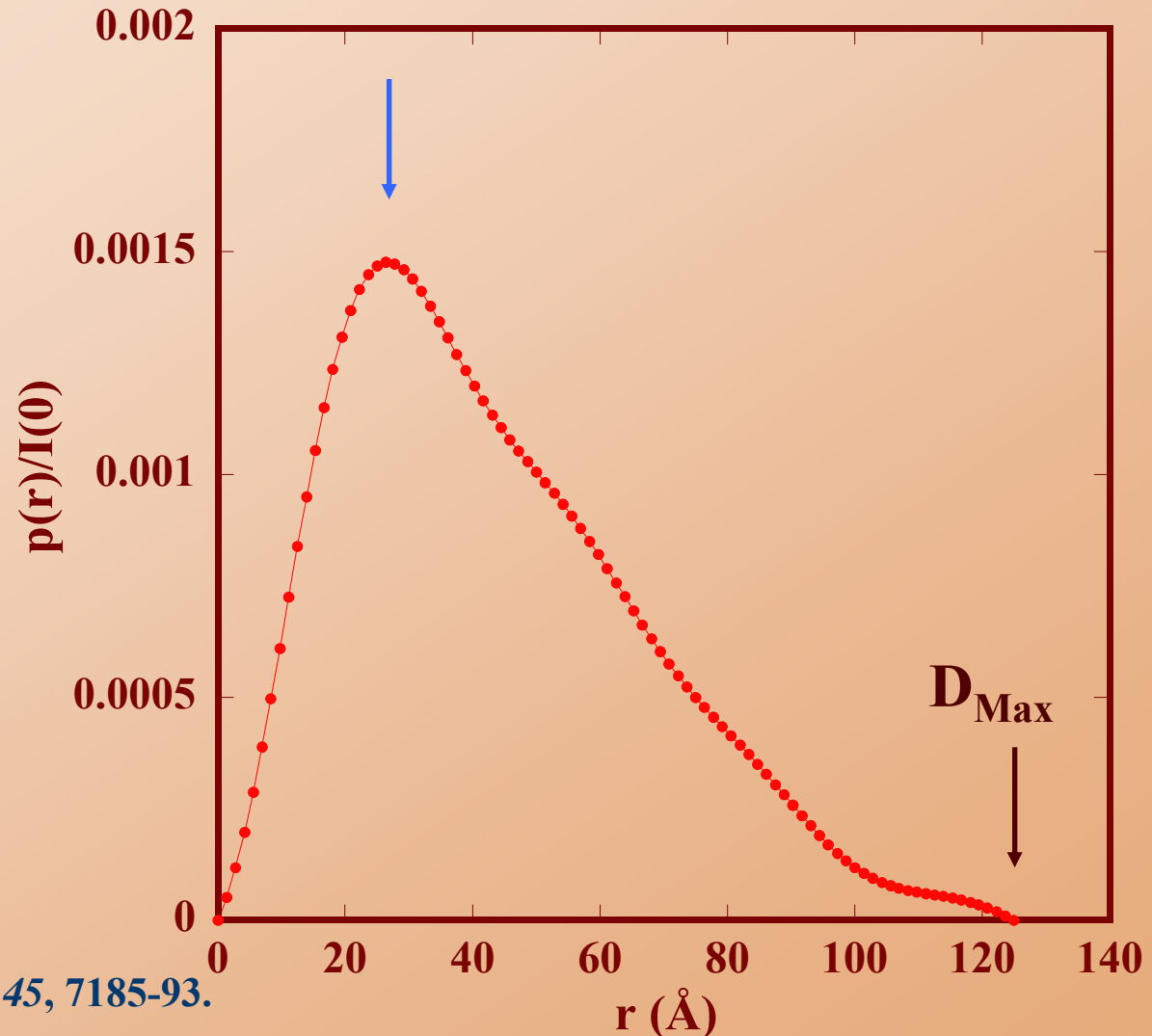
$$I(0) = 4\pi \int_0^{D_{\max}} p(r) dr$$

This alternative estimate of R_g makes use of the whole scattering curve, and is much less sensitive to interactions or to the presence of a small fraction of oligomers.

Comparison of both estimates : useful cross-check

Distance distribution function

Elongated particle
p47 : component of
NADPH oxidase from
neutrophile, a 46kDa
protein



D. Durand *et al.*, *Biochemistry* (2006), 45, 7185-93.

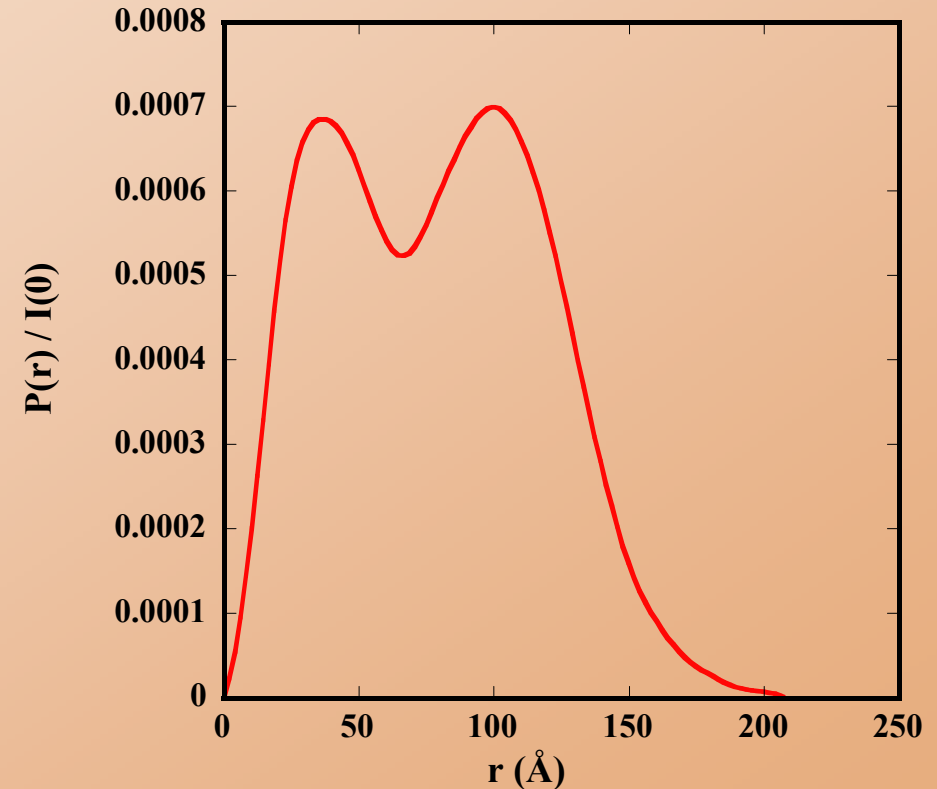
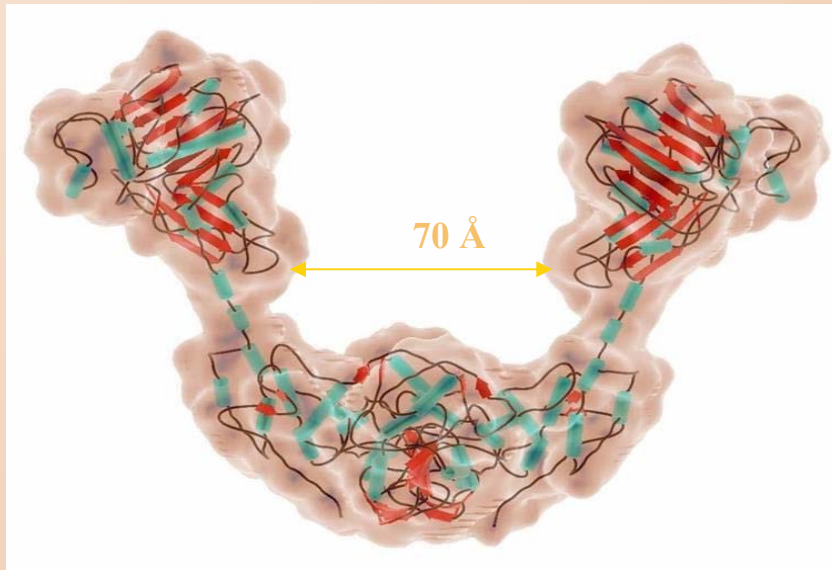
ideal
monodisperse

EMBO Practical Course on Solution Scattering from Biological Macromolecules
Hamburg October, 25th – November 1st 2010

Distance distribution function

Bimodal distribution

Topoisomerase VI

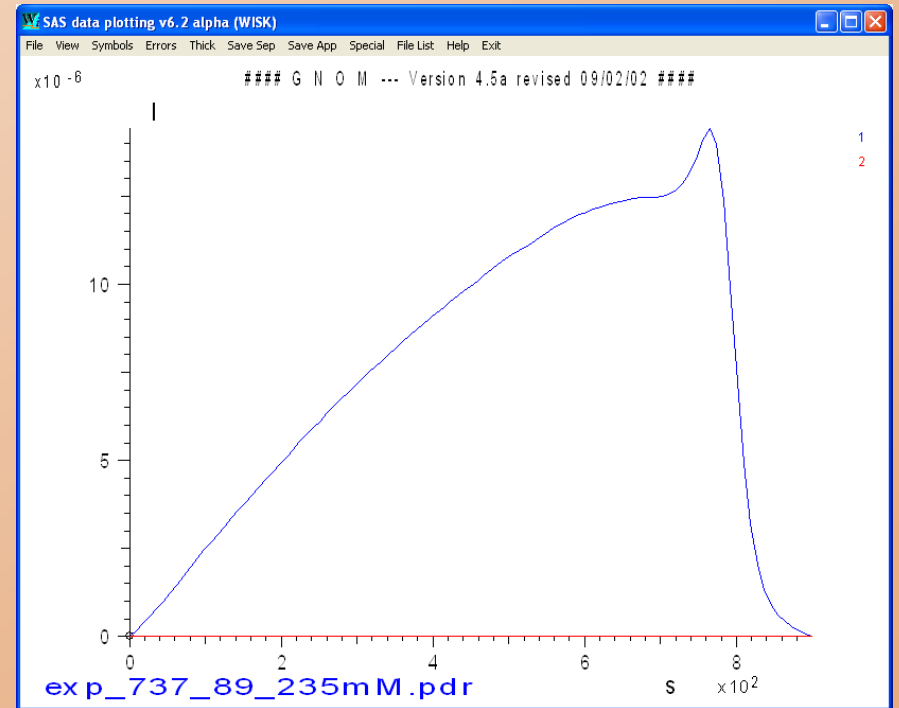
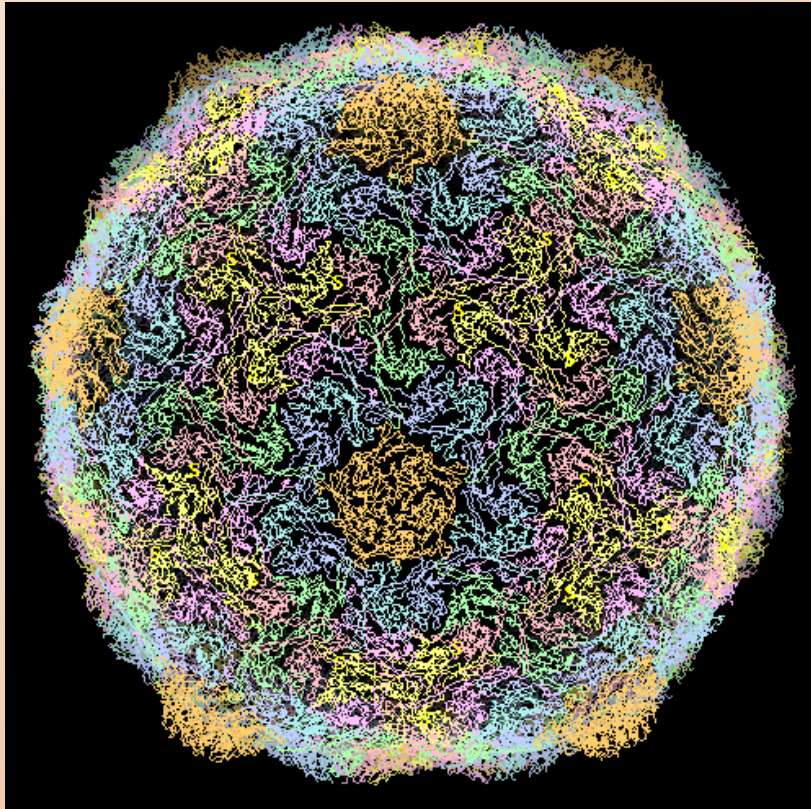


M. Graille et al., *Structure* (2008), *16*, 360-370.

Distance distribution function

Empty sphere

Phage T5 capsid



courtesy A. Huet, O. Preux & P. Boulanger,
IBBMC (Orsay, France)

Scattering by an extended chain

In the case of an *unfolded protein* :

- when studying the folding or unfolding transition of a protein
- when studying natively unfolded proteins.

one uses models derived for statistical polymers.

Gaussian chain : linear association of N monomers of length l with no persistence length (no rigidity due to short range interactions between monomers) and no excluded volume (i.e. no long-range interactions).

Debye formula :
$$\frac{I(q)}{I(0)} = \frac{2}{x^2} (x - 1 + e^{-x}) \quad \text{where} \quad x = (qR_g)^2$$

I(q) depends on a single parameter, R_g .

Valid over a restricted q-range in the case of interacting monomers

Guinier plot : NCS heat unfolding

Neocarzinostatin.

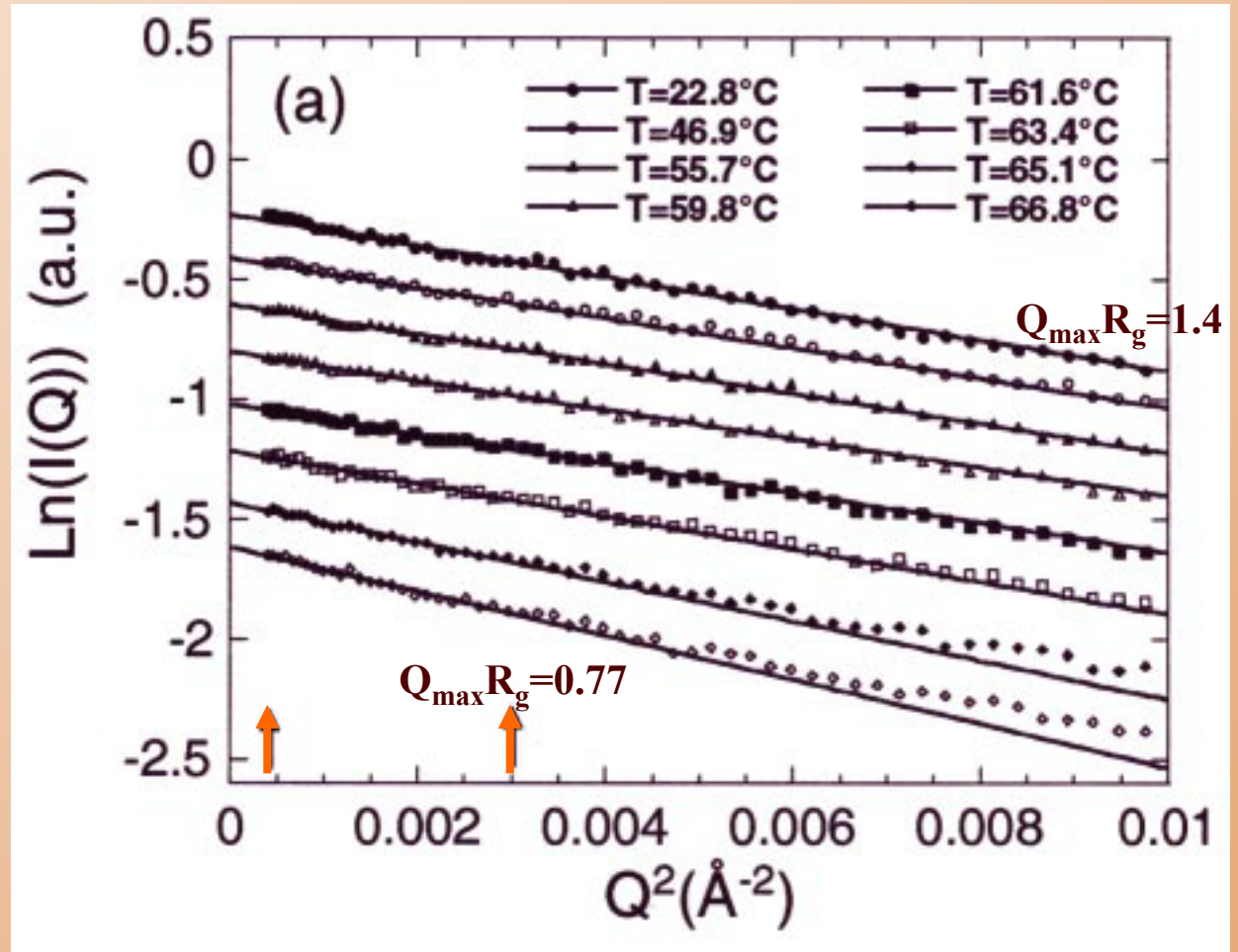
small (113 residue long) all- β protein.

arrows : angular range used for R_g determination

Native



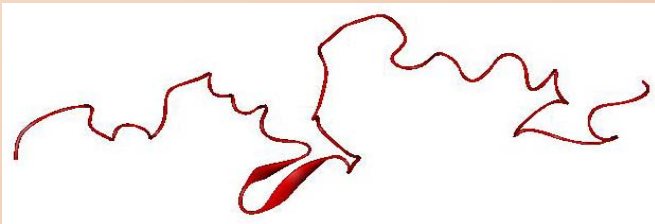
Pérez et al., *J. Mol. Biol.*(2001)
308, 721-743



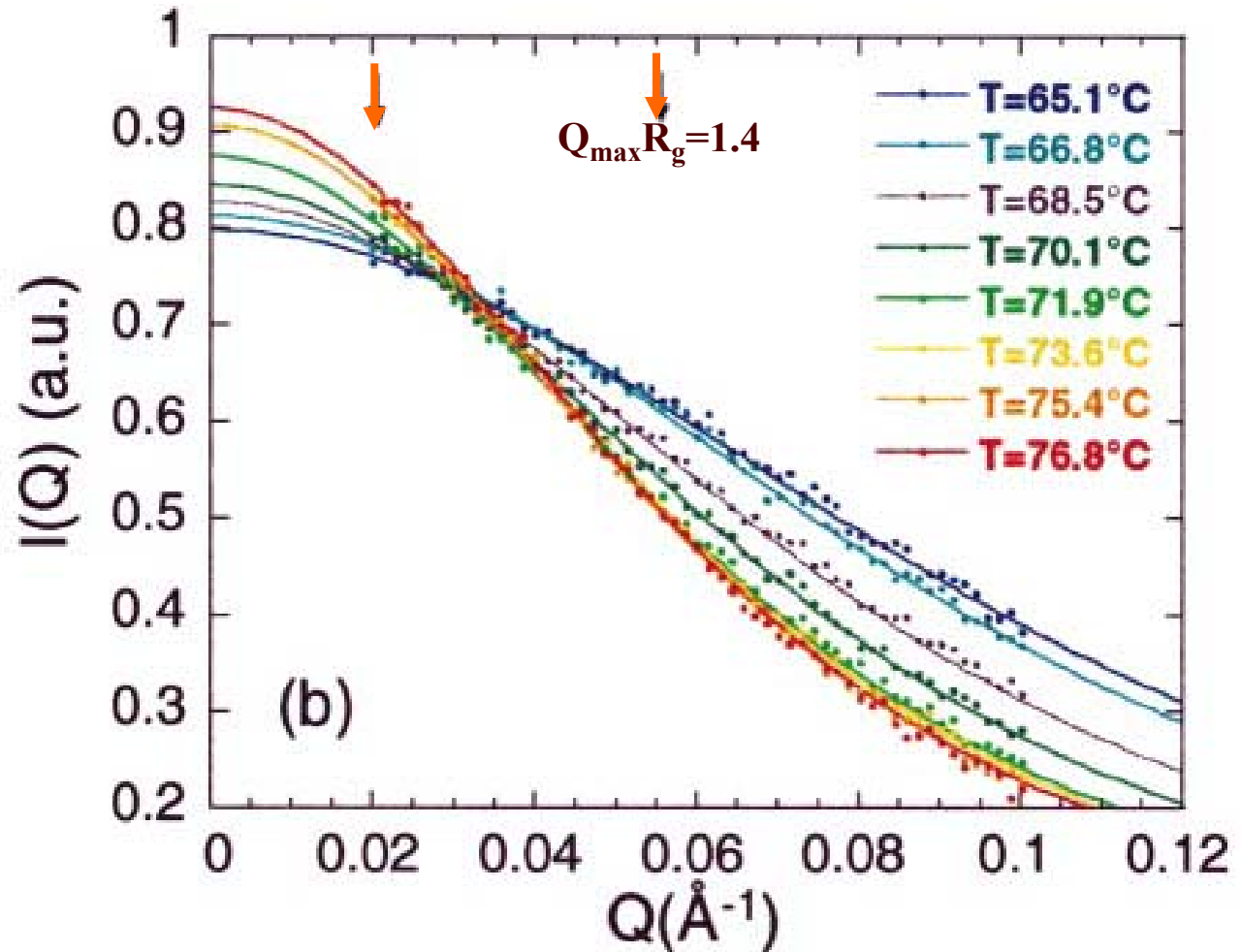
Debye law : NCS heat unfolding

arrows : angular range used
for R_g determination

Heat-unfolded



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



Kratky plot

SAXS provides a sensitive means of *monitoring the degree of compactness* of a protein:

- when studying the folding or unfolding transition of a protein
- when studying a natively unfolded protein.

This is most conveniently represented using the so-called

Kratky plot: $q^2I(q)$ vs q .

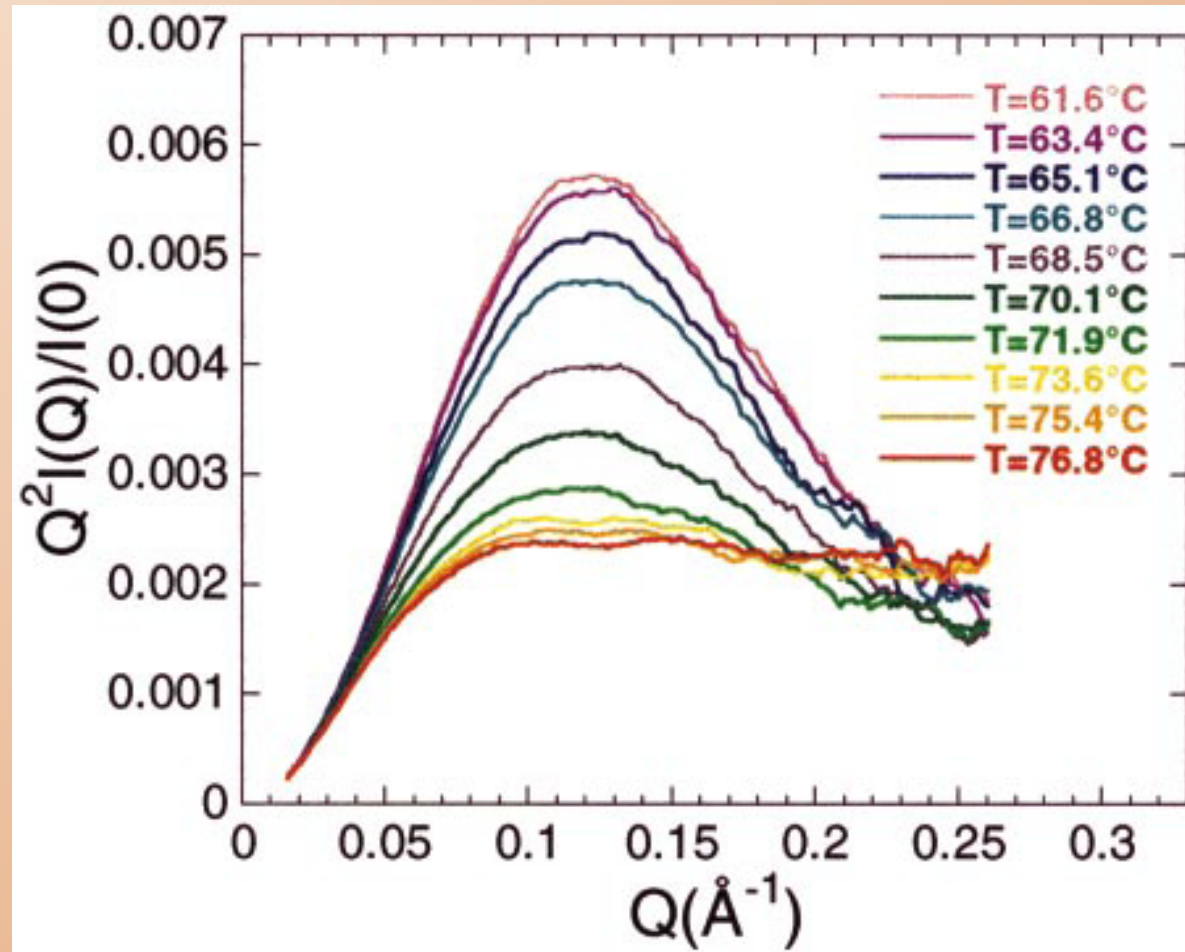
Globular particle : *bell-shaped curve* (asymptotic behaviour in q^{-4})

Gaussian chain : *plateau* at large q -values (asymptotic behaviour in q^{-2})

$$\lim_{q \rightarrow \infty} (q^2 I(q)) = \frac{2(1 - (qR_g)^{-2})}{R_g^2}$$

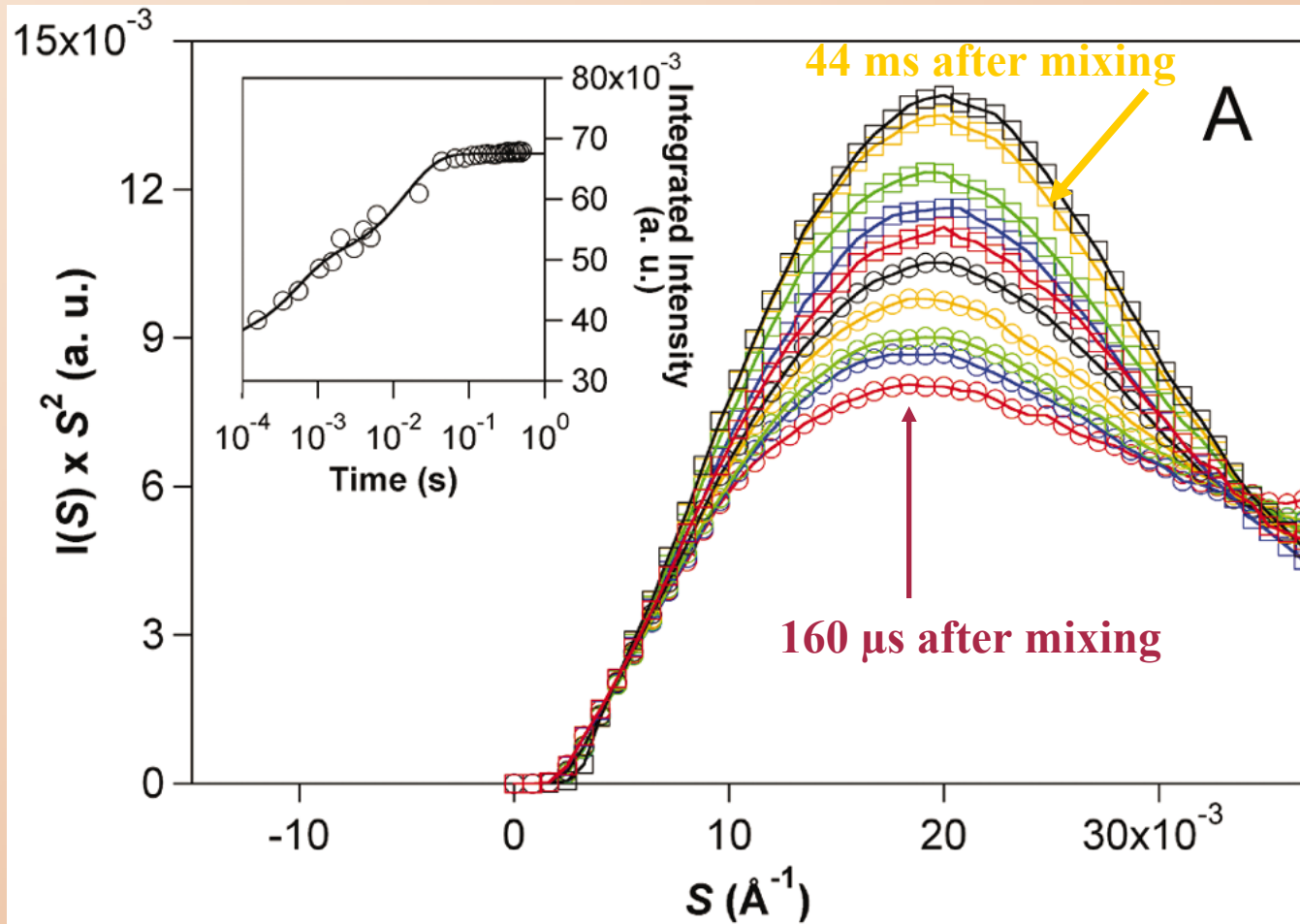
Kratky plot : NCS heat unfolding

In spite of the plateau,
not a Gaussian chain when unfolded.
Can be fit by a thick persistent chain



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

cytochrome c folding kinetics



S. Akiyama et al. (2002), PNAS, 99, 1329-1334.

ApoMb : T. Uzawa et al. (2004), PNAS, 101, 1171-1176

Books on SAS

- " The origins" (no recent edition) : *Small Angle Scattering of X-rays*
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 on the Internet at*
<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 on the Internet at*
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.
- The latest proceedings are in the J. Appl. Cryst., **40**, (2007).

Recent reviews

Small angle scattering: a view on the properties, structures and structural changes of biological macromolecules in solution.

*Michel H. J. Koch, Patrice Vachette and Dmitri I. Svergun
Quarterly Review of Biophysics (2003), 36, 147-227.*

X-ray solution scattering (SAXS) combined with crystallography and computation: defining accurate macromolecular structures, conformations and assemblies in solution

*Christopher Putnam, Michal Hammel, Greg Hura and John Tainer
Quarterly Review of Biophysics (2007), 40, 191-285.*

Structural characterization of proteins and complexes using small-angle X-ray solution scattering

*Haydin D.T. Mertens and Dmitri I. Svergun
Journal of Structural Biology (2010), 172, 128-141.*

Robust, high-throughput solution structural analyses by small angle X-ray scattering (SAXS). *Nat Methods* 6, 606-612.

*Hura, G.L., Menon, A.L., Hammel, M., Rambo, R.P., Poole, F.L., 2nd, Tsutakawa, S.E., Jenney, F.E., Jr., Classen, S., Frankel, K.A., Hopkins, R.C., Yang, S.J., Scott, J.W., Dillard, B.D., Adams, M.W., and Tainer, J.A. *Nat Methods* (2009), 6, 606-612.*

Small-angle scattering and neutron contrast variation for studying biomolecular complexes.

*Whitten, A.E., and Trewella, J. *Methods Mol Biol* (2009), 544, 307-323.*

Bridging the solution divide: comprehensive structural analyses of dynamic RNA, DNA, and protein assemblies by small-angle X-ray scattering.

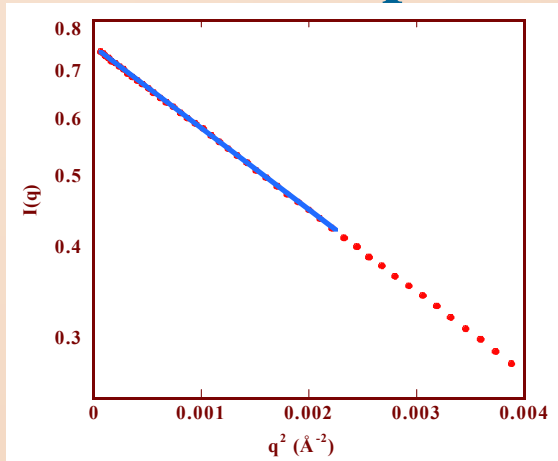
*Rambo, R.P., and Tainer, J.A. *Curr Opin Struct Biol* (2010), 20, 128-137.*

Small-angle scattering for structural biology--expanding the frontier while avoiding the pitfalls.

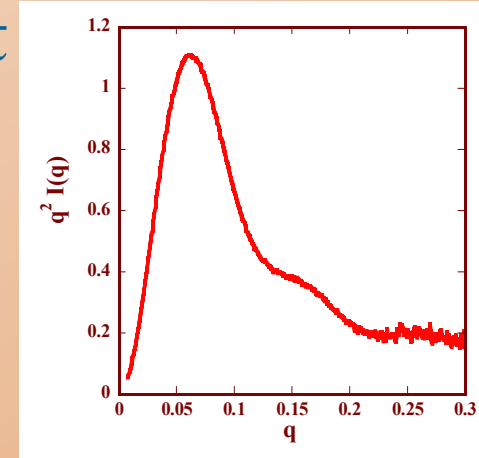
*Jacques, D.A., and Trewella, J. *Protein Sci* (2010), 19, 642-657.*

A survival kit for the travel you are embarking on

Guinier plot

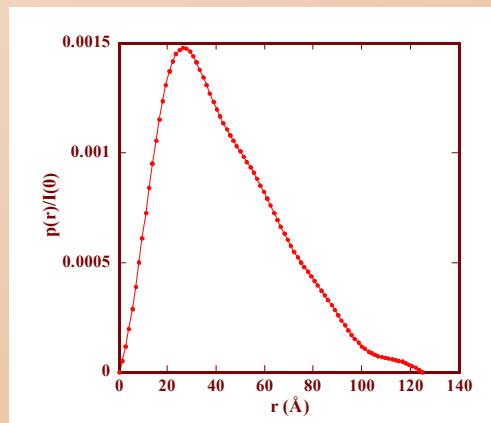
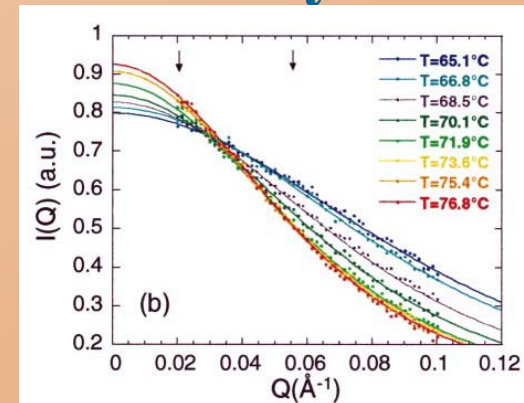


Kratky plot



monodispersity
ideality

Debye law



$p(r)$

Remember

- ✓ The method is simple but deceptively so:
 - ✓ analysis and modelling require a monodispersed and ideal solution.
 - ✓ it is critical to check the validity of these assumptions. Otherwise ...



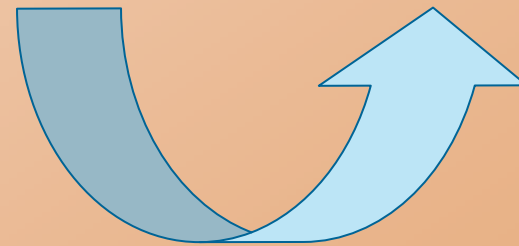
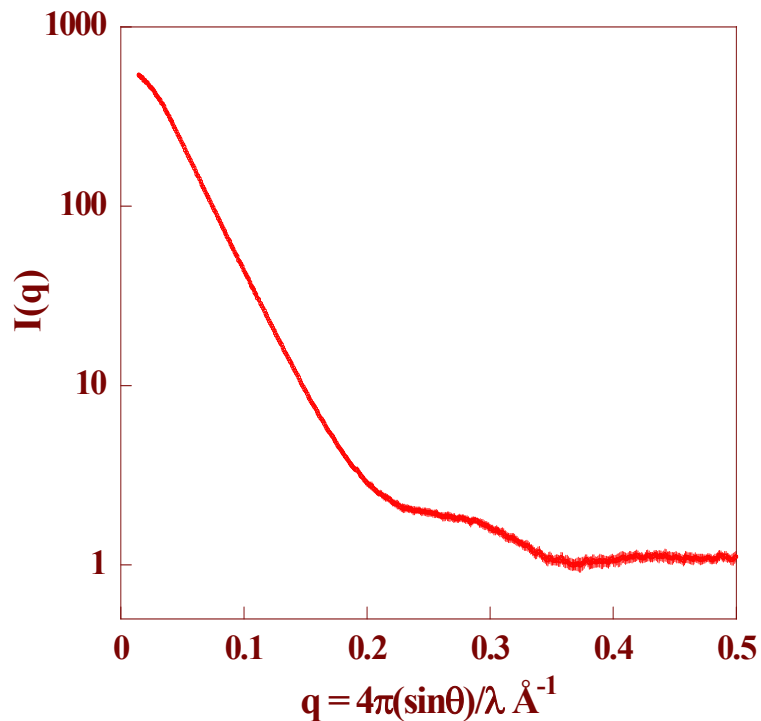
IN



OUT

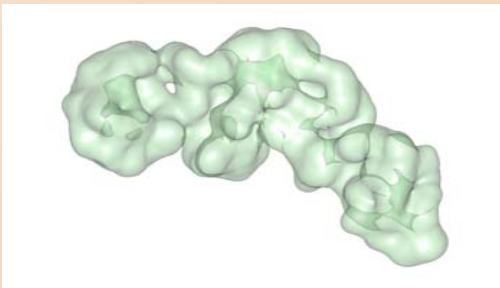
**with good quality,
validated data**

**you can apply to your
system any of the
modelling approaches
that you will discover
during the course:**

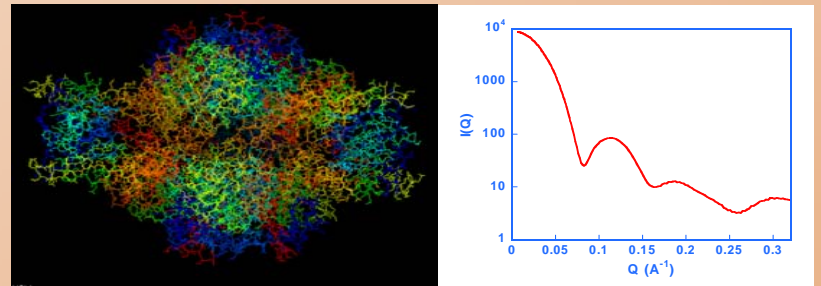


Various modelling approaches

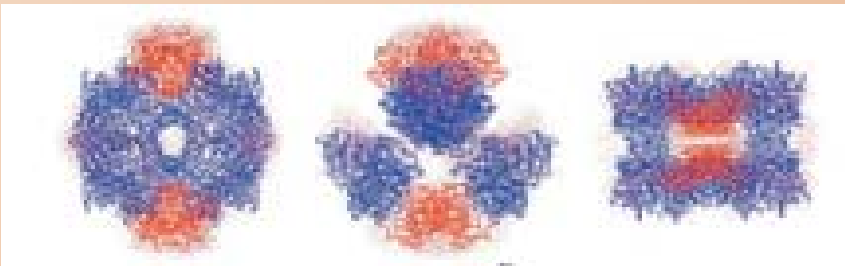
☀ *ab initio* modelling : *DAMMIN*, *GASBOR*



☀ Scattering pattern calculation from atomic coordinates : *CRYSOL*



☀ Rigid body analysis : quaternary structure of complexes : *SASREF*



☀ Rigid body analysis coupled with addition of missing fragments : *BUNCH*

