Scattering of X-rays
Books on SAS

- "The origins" (no recent edition): *Small Angle Scattering of X-rays*
- *Small-Angle X-ray Scattering*
- *Structure Analysis by Small Angle X-ray and Neutron Scattering*
  P. Lindner and Th. Zemb (ed.), 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., **30**, (1997), next one soon to come out
Reminder - notations

Fourier Transform

\[ \rho(r) \xrightarrow{\text{F. T.}} F(s) = \int_{V_r} \rho(r) e^{2i\pi rs} dV_r \]

\[ F(s) \xrightarrow{\text{F. T.}^{-1}} \rho(r) = \int_{V_s} F(s) e^{-2i\pi rs} dV_s \]
Properties of the Fourier Transform

- 1 – linearity

\[ \text{FT} \left( \lambda_1 \rho_1 + \lambda_2 \rho_2 \right) = \lambda_1 \text{FT}(\rho_1) + \lambda_2 \text{FT}(\rho_2) \]

- 2 – value at the origin

\[ F(0) = \int_{V_r} \rho(r) dV_r \]

- 3 – translation

\[ \rho(r + R) \xrightarrow{\text{F. T.}} F(s) e^{2i\pi Rs} \]
Fourier Transforms of simple functions

- 1 – delta function at the origin
  \[ \rho(r) = \delta(r) \]

- 2 – Gaussian of width 1/K
  \[ f(r) = e^{-\pi K^2 r^2} \]

- 3 – rectangular function
  \[ f(x) = 1 \text{ for } -\frac{a}{2} \leq x \leq \frac{a}{2} \]

- 1 – constant unitary value
  \[ F(s) = 1 \]

- 2 – Gaussian of width K
  \[ F(s) = e^{-\frac{\pi}{K^2 s^2}} \]

- 3 – sinc
  \[ F(s) = a \frac{\sin \pi sa}{\pi sa} \]
Convolution product

\[ A(r) * B(r) = \int_{V_u} A(u) B(r - u) dV_u \]
Convolution product

\[ \text{Convolution product} \]

\[ B(r-u) \]

\[ A(u) \]

\[ r_B \]

\[ r_A \]

\[ r_A - r_B \]

\[ r_A + r_B \]
Fundamental Property of the convolution product

\[ \text{FT}(A \ast B) = \text{FT}(A) \cdot \text{FT}(B) \]

\[ \text{FT}(A \cdot B) = \text{FT}(A) \ast \text{FT}(B) \]
Autocorrelation function

\[ \gamma(r) = \rho(r) \ast \rho(-r) = \int_{V_u} \rho(r + u) \rho(u) dV_u \]

spherical average \( \gamma(r) = \langle \gamma(r) \rangle \)

\( \rho(r) = \rho \) (uniform density) \( \Rightarrow \gamma(0) = \rho^2 V \)

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

particle \( \cap \) ghost
Distance distribution function

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

\(\gamma_0(r)\): *probability* of finding a point at \(r\) from a given point

number of el. vol. \(i\) \(\propto V\) - number of el. vol. \(j\) \(\propto 4\pi r^2\)

*number* of pairs \((i,j)\) separated by the distance \(r \propto 4\pi r^2 V \gamma_0(r) = (4\pi/\rho^2)p(r)\)
Scattering by a free electron

The elastically scattered intensity is given by the Thomson formula

\[ I = r_0^2 I_0 \frac{1}{d^2} \frac{1 + \cos^2 2\theta}{2} \]

where \( 2\theta \) : scattering angle, \( \cos 2\theta \) close to 1 at small-angles

\[ r_0 = \frac{e^2}{mc^2} = 2.82 \times 10^{-13} \text{ cm} \]

\( I_e = r_0^2 = 7.95 \times 10^{-26} \text{ cm}^2 \) is the scattering cross-section of the electron

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

The scattered beam has an intensity \( I_e/d^2 \) at a distance \( d \) from the scattering electron.

In what follows, \( r_0^2 \) is omitted and only the number of electrons is considered.
Scattering by an electron at a position $r$

Path difference $= r.s_1 - r.s_0 = r.(s_1 - s_0)$ or $r.(s_1 - s_0)/\lambda$ in cycles, for X-rays of wavelength $\lambda$
the scattering vector \( s \)

\[
|s_0| = |s_1| = \frac{1}{\lambda}
\]

\( s = s_1 - s_0 \)

\( s \) is called the scattering vector

\[
s = |s| = \frac{2\sin \vartheta}{\lambda}
\]

The scattered amplitude by the electron at \( r \) is \( E(s)e^{2i\pi r.s} \)

where \( E(s) \) is the scattered amplitude by an electron at the origin.
scattering vector

\[ q = h = \frac{4\pi \sin \vartheta}{\lambda} \]

SAXS measurements are restricted to small-angles:

\[ 2\theta \leq 5 \text{ deg} = 85 \text{ mrad} \text{ at } \lambda = 1.5 \text{ Å} \]

In this case, the further approximation is valid:

\[ s = |s| = \frac{2\sin \vartheta}{\lambda} \frac{2\theta}{\lambda} \]
Scattered amplitude

\[ F(s) = \int_{V_r} \rho(r) e^{2i\pi sr} \, dV_r \]

\[ |s| = \frac{2\sin \vartheta}{\lambda} \]

\( F(s) \) is the \textit{Fourier transform} from the electron density \( \rho(r) \) describing the scattering object

Scattered intensity

\[ I(s) = F(s).F^*(s) \]

Remark

\( s = 0 \)

\[ F(0) = \int_{V_r} \rho(r) \, dV_r \]

For a particle \( I(0) = m^2 \) \( m \) : number of electrons
Solution X-ray scattering

Diagram of the experimental set-up

\[ s = \frac{2 \sin \theta}{\lambda} \]

X-ray beam

Sample

Detector

X-ray scattering curve
Particles in solution

The relevant quantity is now the contrast of electron density between the particle and the solvent

\[ \Delta \rho(r) = \rho(r) - \rho_0 \]

with \( \rho_0 = 0.3346 \text{ el. } \text{A}^{-3} \) the electron density of water
Contrast of electron density

\[ \rho = 0.43 \]

\[ \rho_0 = 0.335 \]

\[ \Delta \rho \]
X-ray scattering power of a protein solution

Let $W$ be the probability of an incident photon being scattered by a solution of spherical proteins:

$$W = (\Delta \rho)^2 c \bar{v} \lambda d r = 0.3(\Delta \rho)^2 c \bar{v} r / \lambda$$

using the expression for the optimal thickness $d(\text{cm}) = 0.3/\lambda^2 (\text{A}^3)$

Protein: $\rho = 0.43 \text{ el.} \text{A}^{-3}$ solvent $\rho_0 = 0.335 \text{ el.} \text{A}^{-3}$

$\Delta \rho = 0.1 \text{ el.} \text{A}^{-3} = 2.8 \times 10^{10} \text{ cm}^{-2}$

$W = 2.3 \times 10^{-4} c \bar{v} r / \lambda$

Example: 10 mg/ml solution of myoglobin $v= 0.74 \text{ cm}^3 \cdot \text{g}^{-1}$ $r=20\text{A}$, $\lambda = 1.5\text{A}$

$W=2 \times 10^{-5}$ (x efficiency x geometrical factor)

from H.B. Stuhrmann
Synchrotron Radiation Research
Solution of particles

\[
\text{Solution} = \Delta \rho(r) \cdot F(c,s) = \Delta \rho_p(r) \cdot F(0,s) \ast \text{Lattice} = \delta(c,s)
\]
Solution of particles

For spherically symmetrical particles

\[ I(c,s) = I(0,s) \times S(c,s) \]

- form factor of the particle
- structure factor of the solution

Still valid for globular particles though over a restricted s-range
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 and 4 dealt with at a later stage in the course
Ideal and monodisperse solution

\[ F_1(s) = \int_{V_r} \Delta \rho(r) e^{2i\pi rs} dV_r \]

Particles in solution => thermal motion => particles have a random orientation / X-ray beam. The sample is isotropic. Therefore, only the spherical average of the scattered intensity is experimentally accessible.

\[ i_1(s) = \langle i_1(s) \rangle = \langle F_1(s) \cdot F_1^*(s) \rangle \]

\[ I(s) = \langle I(s) \rangle = \langle F(s) \cdot F^*(s) \rangle \]

Ideality and monodispersity

\[ I(s) = NI_1(s) \]
Let us use the properties of the Fourier transform and of the convolution product

\[ i_1(s) = \langle FT[\Delta \rho(r)].FT[\Delta \rho(-r)] \rangle = \langle FT[\Delta \rho(r) \ast \Delta \rho(-r)] \rangle \]

\[ i_1(s) = \langle FT[\gamma(r)] \rangle = \left\langle \int_{V_r} \gamma(r)e^{2i\pi rs} dV_r \right\rangle \]

\[ i_1(s) = 4\pi \int_0^\infty p(r) \frac{\sin(2\pi rs)}{2\pi rs} dr \]

with \[ p(r) = r^2 \gamma(r) \]
Or one of the symmetrical expressions:

\[ s_i_1(s) = 2 \int_0^\infty r \gamma(r) \sin(2\pi rs) dr \]

\[ r \gamma(r) = \frac{1}{\pi} \int_0^\infty s I(s) \sin(2\pi rs) ds \]
Relationships real space – reciprocal space

$$\rho(r) \rightarrow p(r) \rightarrow F(s) \rightarrow I(s)$$

$$FT \quad FT \quad FT$$

$$< > \quad < > \quad < >$$

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If the particle is described as a discrete sum of elementary scatterers, (e.g. atoms) the scattered intensity is:

\[
i_1(s) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i(s) f_j(s) e^{2i\pi(r_i-r_j)s}
\]

where the \(f_i(s)\) are the atomic scattering factors, and the spherically averaged intensity is (Debye):

\[
i_1(s) = \sum_{i=1}^{N} \sum_{j=1}^{N} f_i(s) f_j(s) \frac{\sin 2\pi r_{ij} s}{2\pi r_{ij} s}
\]

where \(r_{ij} = |r_i - r_j|\)

The Debye formula is widely used for model calculations.
If the particle is described using a density distribution, the Debye formula is written:

\[ i_1(s) = \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) \frac{\sin(2\pi r_{12}s)}{2\pi r_{12}s} \, dr_1 \, dr_2 \]
Porod invariant

\[ \gamma(r) = 2 \int_0^\infty s^2 I(s) \frac{\sin(2\pi rs)}{2\pi rs} ds \]

For \( r=0 \):

\[ \gamma(0) = 2 \int_0^\infty s^2 I(s) ds = 2Q \]

By definition:

\[ \gamma(0) = \int_0^\infty \Delta \rho(r)^2 dV_r = \Delta \rho^2 V \]

\( Q \) is called the Porod invariant

\[ Q = \frac{\Delta \rho^2 V}{2} = \int_0^\infty s^2 I(s) ds \]

\( Q \) depends on the mean square electron density contrast
Intensity at the origin

\[ i_1(0) = \int_{V_r} \int_{V_{r'}} \Delta \rho(r) \Delta \rho(r') dV_r dV_{r'} \]

\[ i_1(0) = \Delta m^2 = (m - m_0)^2 = \left[ \frac{M}{N_A} \bar{v}_P(\rho - \rho_0) \right]^2 \]

\[ c = \frac{N M}{N_A V} \] is the concentration (w/v), e.g. in mg.ml\(^{-1}\)

\[ I(0) = \frac{c M V}{N_A} \left[ \bar{v}_P(\rho - \rho_0) \right]^2 \]
Intensity at the origin

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

Then, the **molecular weight** of the particle can be determined from the value of the intensity at the origin
### Intensity on an absolute scale

The experimental intensity $I_{\text{exp}}(0)$ is expressed as:

$$I_{\text{exp}}(0) = \frac{I_0 I_e c M d}{N_A a^2} \left[ \nu_P (\rho - \rho_0) \right]^2$$

- d: thickness of the sample
- a: sample-detector distance
- $I_e = r_0^2 = 7.95 \times 10^{-26} \text{ cm}^2$ scattering cross-section of the electron
- $I_0$ total energy of the incident beam
The determination of $I_0$ is performed using:

- attenuation of the direct beam by calibrated filters
  (valid in the case of monochromatic radiation, beware of harmonics)
- by using a well-known reference sample like the Lupolen (Graz)
- using the scattering by water as proposed by O. Glatter in

Thorough presentation of calculations on an absolute scale


Clear presentation of the geometrical calculations and all the procedures used on ID2 (ESRF) to put intensities on an absolute scale.
**Guinier law**

\[ I(s) = 4\pi \int_0^\infty p(r) \frac{\sin(2\pi rs)}{2\pi rs} dr \]

For small values of \( x \), \( \sin x/x \) can be expressed as:
\[
\frac{\sin(2\pi rs)}{2\pi rs} = 1 - \frac{(2\pi rs)^2}{3!} + \frac{(2\pi rs)^4}{5!} - ... 
\]

Hence, close to the origin:
\[
I(s) \approx I(0) \left[ 1 - ks^2 \right] \quad I(0) e^{-ks^2} 
\]

with \( I(0) = 4\pi \int p(r) dr \) and \( k = \frac{4\pi^2}{6} \int r^2 p(r) dr / \int p(r) dr \)

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

Let us introduce the expansion of \( \sin x/x \) into the Debye formula:

\[
I(s) = \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) \frac{\sin(2\pi r_1 s)}{2\pi r_1 s} dr_1 dr_2
\]

We obtain the following expression:

\[
I(s) = \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) dr_1 dr_2 - \frac{4\pi^2 s^2}{6} \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) |r_1 - r_2|^2 dr_1 dr_2
\]

hence

\[
k = \frac{4\pi^2}{6} \int_{V_1} \int_{V_2} \Delta \rho(r_1) \Delta \rho(r_2) |r_1 - r_2|^2 dr_1 dr_2
\]
Guinier law

writing \[ |\mathbf{r}_1 - \mathbf{r}_2| = |\mathbf{r}_1 - \mathbf{r}_0 + \mathbf{r}_0 - \mathbf{r}_2| \]

where \( \mathbf{r}_0 \) is the center of mass

it comes

\[ k = \frac{4\pi^2}{6} \frac{2 \int_{V_r} \Delta \rho(\mathbf{r}) r^2 dV_r}{\int_{V_r} \Delta \rho(\mathbf{r}) dV_r} = \frac{4\pi^2}{3} R_g^2 \]

Radius of gyration

Guinier law

\[ I(s) \equiv I(0) \exp \left( - \frac{4\pi^2}{3} R_g^2 s^2 \right) \]
Radius of gyration:

\[ R_g = \sqrt{\frac{\int_{V_r} \Delta \rho(r) r^2 dV_r}{\int_{V_r} \Delta \rho(r) dV_r}} \]

\( R_g \) is the quadratic mean of distances to the center of mass weighted by the contrast of electron density. \( R_g \) is an index of non sphericity.

For a given volume the smallest \( R_g \) is that of a sphere: \( R_g = \sqrt{\frac{3}{5}}R \)

Ellipsoidal of revolution \((a, b)\)

\[ R_g = \sqrt{\frac{2a^2 + b^2}{5}} \]

Cylinder \((D, H)\)

\[ R_g = \sqrt{\frac{D^2}{8} + \frac{H^2}{12}} \]
The law is generally used under its log form:

\[ \ln[I(s)] \equiv \ln[I(0)] - \frac{4\pi^2}{3} R_g^2 s^2 \]

A linear regression yields two parameters: \( I(0) \) (y-intercept) and \( R_g \) from the slope.

**Validity range:**

\[ 0 < 2\pi R_g s < 1.2 \]

*For a sphere*
Attractive Interactions

\( \gamma \)-crystallins \( c = 160 \text{ mg/ml} \) in 50mM Phosphate pH 7.0

A. Tardieu et al., LMCP (Paris)
Repulsive Interactions

ATCase in 10mM borate buffer pH 8.3

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Virial coefficient

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

\[ I(0, c) = \frac{I(0)_{\text{ideal}}}{1 + 2A_2Mc + ...} \]

Where \( A_2 \) is the second virial coefficient which represents pair interactions and \( I(0)_{\text{ideal}} \) is \( \propto \) to \( c \).

\( A_2 \) is evaluated by performing experiments at various concentrations \( c \).

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

In the case of very elongated particles, the radius of gyration of the cross-section can be derived using a similar representation, plotting this time $sI(s)$ vs $s^2$

$$sI(s) \propto \exp(-2\pi^2 R_c^2 s^2)$$

Finally, in the case of a platelet, a thickness parameter is derived from a plot of $s^2I(s)$ vs $s^2$:

$$s^2I(s) \propto \exp(-4\pi^2 R_t^2 s^2)$$

with $R_t = T/\sqrt{12}$, $T$: thickness
Volume of the particle

**Hypothesis**: the particle has a *uniform density*

\[ i_1(0) = (V_1\Delta\rho)^2 \quad \text{and} \quad Q = \frac{\Delta\rho^2 V_1}{2} \quad \text{(Porod invariant)} \]

*uniform density* \[ \Rightarrow \Delta\rho^2 = \Delta\rho^2 \]

Hence the expression of the volume for a particle of *uniform density*:

\[ V_1 = \frac{i_1(0)}{2Q} \]
The asymptotic regime: Porod law

**Hypothesis**: the particle has a *sharp interface* with the solvent with a *uniform electron density*

Porod showed that the asymptotic behaviour of the scattering intensity is given by:

\[
8\pi^3 \lim_{s \to \infty} \left[ s^4 i_1(s) \right] = S\Delta\rho^2 (+Bs^4)
\]

*S is the area of the solute / solvent interface*

*B* is a *correction term* accounting for:
- short distance density fluctuations
- uncertainties of *i*(s) at large *s* (weak signal)
Distance distribution function

\[ p(r) = 2r^2 \int_0^{\infty} s^2 I(s) \frac{\sin(2\pi rs)}{2\pi rs} ds \]

In theory, the calculation of \( p(r) \) from \( I(s) \) is simple.  

**Problem**: \( I(s) \) - is only known over \([s_{\text{min}}, s_{\text{max}}]\) : truncation  
- is affected by experimental errors  
- might be affected by distortions due to the beam-size and the bandwidth \( \Delta \lambda / \lambda \) (neutrons)

\( \Rightarrow \) Calculation of the Fourier transform of *incomplete and noisy data*, which is an *ill-posed problem*.

**Solution**: Indirect Fourier Transform. See lectures by O. Glatter and D. Svergun
Calculation of $p(r)$

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

**Basic hypothesis:**
The particle has a *finite size*

\[
i_1(s) = 4\pi \int_0^{D_{Max}} p(r) \frac{\sin(2\pi rs)}{2\pi rs} dr
\]

$p(r)$ is parameterized on $[0, D_{Max}]$ by a linear combination of orthogonal functions
Distance distribution function

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

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

and

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

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.
Aspartate transcarbamylase from *E.coli* (ATCase)
Heterododecamer \((c_3)_{2}(r_2)_3\) quasi \(D_3\) symmetry
Molecular weight : 306 kDa
Allosteric enzyme

2 conformations:
\(T\) : inactive, compact
\(R\) : active, expanded
ATCase: scattering patterns

\[ I(s) = \text{ideal monodisperse} \]

\[ s = \frac{2 \sin(\theta)}{\lambda} \]
ATCase: $p(r)$ curves

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Information content of a SAXS curve

**Basic hypothesis**: The particle has a *finite size* $D_{\text{max}}$

Sampling theorem: the curve is entirely determined from the values of the intensity at the nodes of the lattice $s_k = k/2D_{\text{max}}$ where $k$ is an integer.

Number of independent structural parameters:

$$J = 2D_{\text{Max}}(s_{\text{Max}} - s_{\text{min}})$$

Example: spherical protein with 0.3 hydration (w/w) $\nu=0.73 \text{ cm}^3\text{ g}^{-1}$

$D_{\text{max}} = 1.5 M^{1/3}$

$\Rightarrow \quad J = 3.0 M^{1/3}(s_{\text{Max}} - s_{\text{min}})$

for $s_{\text{min}} = 0.002 \text{ Å}^{-1}$, $s_{\text{Max}} = 0.05 \text{ Å}^{-1}$ and $M = 10^5$

$\Rightarrow \quad J = 7$
The case of the sphere

Unique case in which an analytical expression of the scattered intensity can be derived:

\[
I(s) = \left( 3 \frac{\sin x - x \cos x}{x^3} \right)^2 x = 2\pi R_s
\]

\( R \) : radius of the sphere

\( I(s) = 0 \) for values of \( s \) solutions of \( 2\pi R_s = \tan(2\pi R_s) \)

first minima at \( R_s = 0.7158, 1.23, 1.73, \text{ etc.} \)
Scattering intensity of the sphere

I(s) \text{ sphere} 

Rs

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Overview of Rotavirus Capsid Architecture

F. Rey, V. Prasad et al., EMBO J. (2001) 20, 1485-1497

Enzyme Complex
Rotavirus derived particles

DLP: double layer particles containing RNA
VLP2/6: Virus-like particles double-layer VP6 and VP2
About 700 Å diameter

\[ I(s) = \frac{2\sin \theta}{\lambda} \]
Rotavirus derived particles

Icosahedral symmetry on the capsid surface: strong modulation of the electron density => modulation of I(s)

around 25-30 Å: signal associated with the RNA layers inside the VLP.
Rotavirus DLP

F. Rey et al., EMBO J. (2001), 20, 1485-1497
Scattering by an extended chain

In the case of an *unfolded protein*: models developed for *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**: \( \frac{I(s)}{I(0)} = \frac{2}{x^2} (x - 1 + e^{-x}) \) where \( x = (2\pi R_g s)^2 \)

I(s) depends on a single parameter, \( R_g \).

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

Limit at large s: \( \lim_{s \to \infty} [s^2 I(s)] = \frac{1 - 1/(2\pi R_g s)^2}{2\pi^2 R_g^2} \)

I(s) varies like \( s^{-2} \) instead of \( s^{-4} \) for a globular particle (Porod law).
Guinier plot: NCS heat unfolding

Neocarzinostatine: small (113 residue long) all-β protein.

arrows: angular range used for $R_g$ determination

Pérez et al., *J. Mol. Biol.* (2001) 308, 721-743
Debye law : NCS heat unfolding

arrows : angular range used for $R_g$ determination

Pérez et al., *J. Mol. Biol.* (2001) 308, 721-743
This provides a sensitive means of monitoring the degree of compactness of a protein as a function of a given parameter.

This is most conveniently represented using the so-called

\textit{Kratky plot of } s^2I(s) \textit{ vs } s.\textit{

Globular particle : bell-shaped curve
Gaussian chain : plateau at large s-values
but beware: a plateau does not imply a Gaussian chain
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
Extended conformation of a modular protein: Hemocyanin from *O. vulgaris*

*O. Vulgaris* hemocyanin (Hc), a dioxygen binding protein, is a decameric structure having the shape of a hollow cylinder with a five-fold symmetry axis.

At alkaline pH, or at neutral pH in the presence of a divalent cation chelator (EDTA), the protein dissociates into its constitutive subunits.

Each subunit comprises seven functional units of *ca* 50kDa joined by flexible linkers.

Study of the dissociation product (subunit) at pH 7.5 and 9.5.
Extended conformation of a modular protein: Hemocyanin from *O. vulgaris*

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Hemocyanin from *O. vulgaris*

**pH 7.5**

**Guinier plot**

- $R_g = 61.7$ Å
- $MW = 400$ kDa

**Distance distribution function**

- $R_g = 62.5$ Å
- $MW = 400$ kDa
Hemocyanin from *O. vulgaris*

**pH 9.5**

**Guinier plot**

- $R_g = 90.6 \text{ Å}$
- Slight upward curvature

**Distance distribution function**

- $R_g = 102.3 \text{ Å}$

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Hemocyanin from *O. vulgaris*

**Distance distribution function**

\[ p(r) \]

\[ r \ (\text{A}) \]

\[ R_g = 102.3 \ \text{A} \]

**Debye plot**

\[ I/c \ (\text{a.u.}) \]

\[ q \ (\text{A}^{-1}) \]

\[ R_g = 103.15 \ \text{A} \]

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Some experimental considerations
Practical aspects

Small-angle data: Guinier analysis, $R_g$ and $I(0)$

- **requirements**: monodisperse and ideal solution
to estimate the MW form the $I(0)$ value, the partialspecific volume must be known, and the intensity calibration performed witha reference sample (Lupolen, other protein).

  monodispersity: previous check by SEC-HPLC or LS

  ideality: extrapolation to infinite dilution by recordingexperiments at several, precisely determined, concentrations in the range1mg/ml (or less) – 5 mg/ml according to the MW of the particle.

  volume: less than 100µl for each sample (down to 30µl)

- **total amount**: less than 1mg/ml
Practical aspects

Wider-angle data: p(r), modeling

- requirements: 5mg/ml <c<10mg/ml with an area detector can provide a reasonable curve with adequate statistics. If available, a higher concentration will give better data (no stringent monodispersity and ideality requirements)

Note: case of a special sensitivity to X-ray irradiation:
- addition of radical scavengers and/or
- sample circulation through the beam cross-section (a few μls in the beam), with a correlative increase in volume.
Lysozyme

Lysozyme : 14 289 Da MW
5 mg/ml solution in Acetate buffer pH 4.5

$R_g=15.2$ Å in the air

$R_g=15.0$ Å under vacuum
Yeast 3-phosphoglycerate kinase (PGK) : 44 570 Da MW
5 mg/ml solution in Tris buffer pH 7.5
R\textsubscript{g} = 24.7 Å in the air
R\textsubscript{g} = 24.4 Å under vacuum
Cell under vacuum

Ratio of buffer scattering
air and windows / under vacuum
5 cm air
25 µm Kapton window upstream
25 µm mica window downstream

\[ s = 2 \sin \frac{\theta}{\lambda} \text{ Å}^{-1} \]
S/B ratio

S/B ratio lysozyme 5mg/ml

S/B ratio PGK 5mg/ml

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SAXS provides spherically averaged intensity, which entails a loss of structural information.

The intensity strongly decreases with the scattering angle, thereby restricting the resolution of the available information. The method is therefore not sensitive to details, to potentially crucial changes in the structure involving e.g. side-chain movements.

The weak signal restrict the characteristic times of kinetics to typically the ms range as compared to the ns or less with Laue TR-PX.
Advantages of the method

SAXS provides time-resolved structural information as well as information on the interactions between particles.

A solution method, SAXS allows one to vary at will most physico-chemical parameters (T, P, pH, ionic strength, substrate, etc.) and assess their effect on the conformation of the particle under investigation.

It can be used as a stand-alone experimental approach (structural parameters, ab initio modeling) or, often most advantageously, coupled to other techniques: structural (EM, PX), spectroscopic (XR or UV-vis absorption, fluorescence, NMR), hydrodynamic, biochemical, molecular biology, etc.
Background on the PC: 240,200,130
But pbs with the HH projector:
Changed to 230,170,120
Contrast of electron density

\[ \rho_0 = \bar{\rho} \]
Contrast of electron density

\[ \rho_0 - \rho \]

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Contrast of electron density

Formulation of Stuhrmann & Kirste:

\[ \Delta \rho(r) = (\rho - \rho_0) \varphi_p(r) + \rho_i(r) = \Delta \rho \varphi_p(r) + \rho_i(r) \]

\( \varphi_p(r) \) : shape function = 1 inside the particle, = 0 outside

\[ \rho_i(r) = \rho(r) - \rho \]

\[ F(s) = \Delta \rho \Phi_p(s) + F_i(s) \]

The intensity is decomposed in three basic scattering functions:

\[ I(s) = \Delta \rho^2 I_s(s) + \Delta \rho I_{si}(s) + I_i(s) \]

The shape is observed at infinite contrast.

Only fluctuations are observable at vanishing contrast.

\( I_s \) and \( I_i \) are positive functions which can be directly observed.

The cross-term \( I_{si} \) can be positive or negative and cannot be measured directly.
Contrast of electron density

\[ \rho_{\text{el. A}^{-3}} \]

\[ \bar{\rho} \]

\[ \Delta \rho(r) \]

\[ \rho_i \]

\[ \rho_0 \]

\[ \bar{\Delta \rho} \]

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Kinetics of the Ca\textsuperscript{2+}-dependent swelling transition of Tomato Bushy Stunt Virus

D24 LURE quadrant gas detector, 1200 s

Intensity

\( s (\text{Å}^{-1}) \)

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Kinetics of the Ca$^{2+}$-dependent swelling transition of Tomato Bushy Stunt Virus

ID2 ESRF, XRII-CCD detector, 0.1s
Kinetics of the Ca^{2+}-dependent swelling transition of Tomato Bushy Stunt Virus

Intensity

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Optimal thickness of the sample

\[ I \propto d e^{(-\mu d)} \]

\[ \frac{dI}{dd} = e^{(-\mu d)} \left[ 1 - \mu d \right] \]

\[ d_{\text{opt}} = \frac{1}{\mu} \]

This corresponds to a transmission of \( \tau = e^{-1} = 0.37 \)
Optimal thickness of the sample

\[ \frac{I}{I_{\text{opt}}} = \frac{d}{d_{\text{opt}}} \]

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Optimal thickness of the sample

\[ d^* = \frac{d}{d_{\text{opt}}} \]

\[ \frac{I}{I_{\text{opt}}} \]

\[ \frac{1}{\mu} \]
Parseval’s theorem

\[ \int f(\mathbf{r}) g^*(\mathbf{r}) d\mathbf{r} = \int F(s) G^*(s) ds \]

Special case \( f(\mathbf{r}) = g(\mathbf{r}) \)

\[ \int |f(\mathbf{r})|^2 d\mathbf{r} = \int |F(s)|^2 ds \]