Basics of X-ray scattering by solutions

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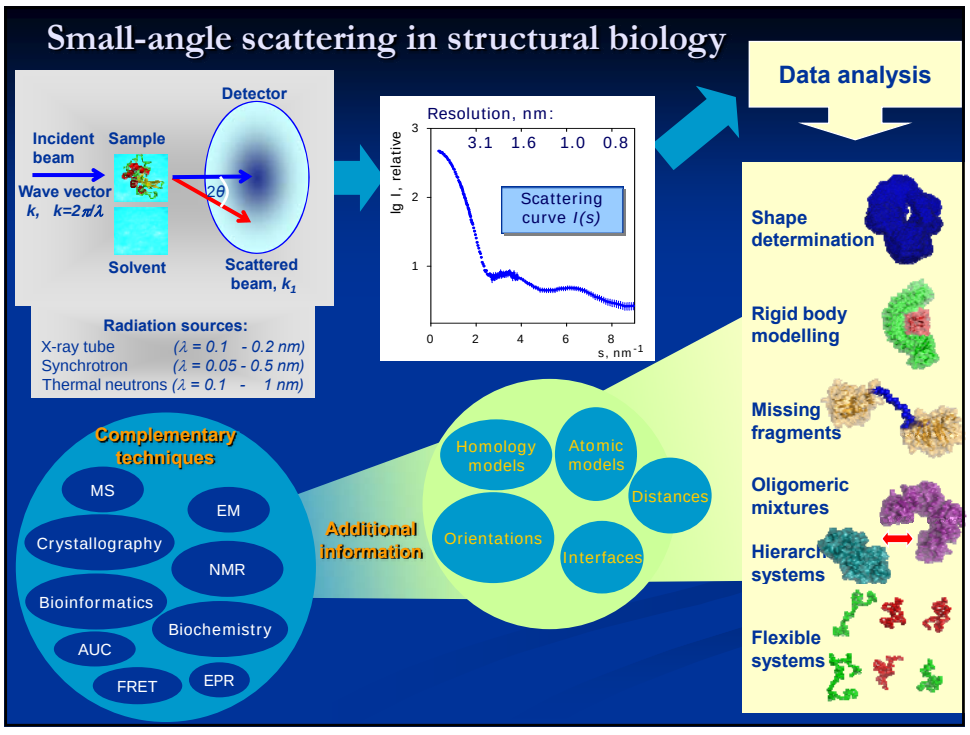
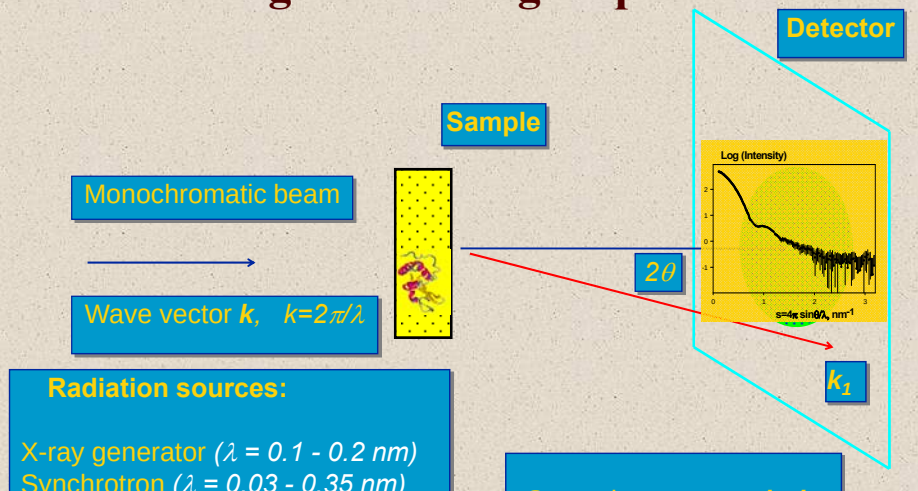
EMBO

EMBL



General princples of solution SAXS

Small-angle scattering: experiment



Scattering by matter

- **X-rays** are scattered mostly by electrons
- **Thermal neutrons** are scattered mostly by nuclei
- Scattering amplitude from an ensemble of atoms $A(\mathbf{s})$ is the Fourier transform of the scattering length density distribution in the sample $\rho(\mathbf{r})$
- Experimentally, scattering intensity $I(\mathbf{s}) = [A(\mathbf{s})]^2$ is measured.

Notations

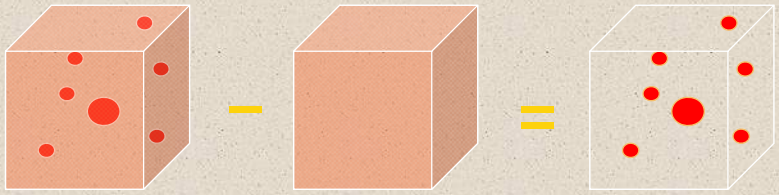
The momentum transfer (i.e. the modulus of the scattering vector is denoted here as $s=4\pi \sin(\theta)/\lambda$

There are also different letters used, like

$$Q = q = s = h = k = 4\pi \sin(\theta)/\lambda$$

Sometimes, the variable $S= 2\sin\theta/\lambda = 2\pi s$ is used, and to add to the confusion, also denoted as "s", or μ or yet another letter. Always check the definition for the momentum transfer in a paper

Small-angle scattering: contrast



$I_{sample}(s)$ $I_{matrix}(s)$ $I_{particle}(s)$

- To obtain scattering from the particles, matrix scattering must be subtracted, which also permits to significantly reduce contribution from parasitic background (slits, sample holder etc)
- **Contrast $\Delta\rho = \langle\rho(r) - \rho_s\rangle$**, where ρ_s is the scattering density of the matrix, may be very small for biological samples

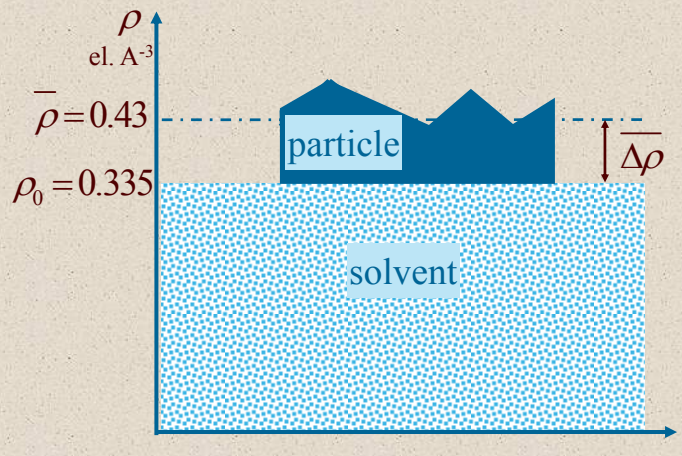
X-rays versus neutrons

- **X-rays:** scattering factor increases with atomic number, no difference between H and D
- **Neutrons:** scattering factor is irregular, may be negative, huge difference between H and D

Element	H	D	C	N	O	P	S	Au
At. Weight	1	2	12	14	16	30	32	197
N electrons	1	1	6	7	8	15	16	79
$b_X, 10^{-12}$ cm	0.282	0.282	1.69	1.97	2.16	3.23	4.51	22.3
$b_N, 10^{-12}$ cm	-0.374	0.667	0.665	0.940	0.580	0.510	0.280	0.760

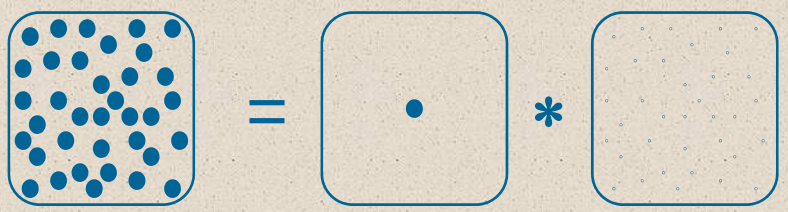
Neutron contrast variation

Contrast of electron density



In the equations below we shall always assume that the solvent scattering has already been subtracted

Solution of particles



Solution		Motif (protein)		Lattice
$\Delta\rho(\mathbf{r})$	=	$\Delta\rho_p(\mathbf{r})$	*	$d(\mathbf{r})$
$F(c,s)$		$F(0,s)$	•	$\delta(c,s)$

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

Ideal and monodisperse solution

$$A(\mathbf{s}) = \Im[\Delta\rho(\mathbf{r})] = \int_V \Delta\rho(\mathbf{r}) \exp(i\mathbf{sr}) d\mathbf{r}$$

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

Ideality and monodispersity $I(s) = Ni_1(s)$

Crystal versus solution

$$I(c,s) = I(0,s) \times S(c,s)$$

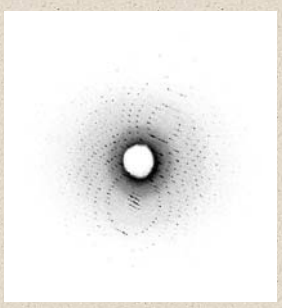
For an ideal crystal,
I (**s**) is the three-dimensional scattering intensity from the unit cell
S(**s**) is a sum of δ-functions along the directions of the reciprocal space lattice
s=(h**a***+k**b***+l**c***)

For an ideal dilute solution,
I(**s**)= I(s) is the orientationally averaged intensity of the single particle

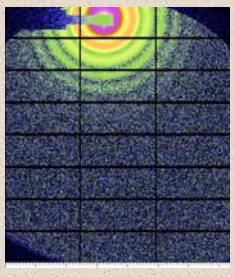
S(s) is equal to unity

Crystal versus solution

For an ideal crystal, measured signal is amplified into specific directions allowing measurements to high resolution ($d \approx \lambda$)



For an ideal dilute solution, I (**s**) is isotropic and concentrates around the primary beam (this is where the name "small-angle scattering" comes from): low resolution ($d >> \lambda$).



Main equations and overall parameters

Relation between real and reciprocal space

Using the overall expression for the Fourier transformation one obtains for the spherically averaged single particle intensity

$$I(s) = \left\langle A(\mathbf{s})A^*(\mathbf{s}) \right\rangle_\Omega = \left\langle \int_V \int_V \Delta\rho(\mathbf{r})\Delta\rho(\mathbf{r}')\exp\{i\mathbf{s}(\mathbf{r}-\mathbf{r}')\}d\mathbf{r}d\mathbf{r}' \right\rangle_\Omega$$

or, taking into account that $\langle exp(isr) \rangle_\Omega = sin(sr)/sr$ and integrating in spherical coordinates,

$$I(s) = 4\pi \int_0^{D_{max}} r^2 \gamma(r) \frac{\sin sr}{sr} dr$$

where

$$\gamma(r) = \left\langle \int \Delta\rho(\mathbf{u})\Delta\rho(\mathbf{u}+\mathbf{r})d\mathbf{u} \right\rangle_\omega$$

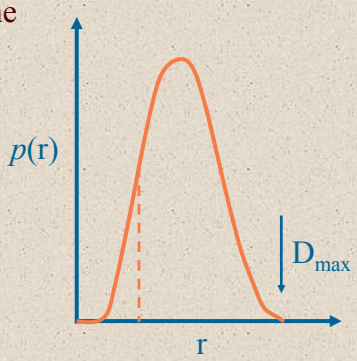
Distance distribution function

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

$\gamma_0(r)$: ***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 $\propto 4\pi r^2 V\gamma_0(r) = (4\pi/\rho^2)p(r)$



Debye formula

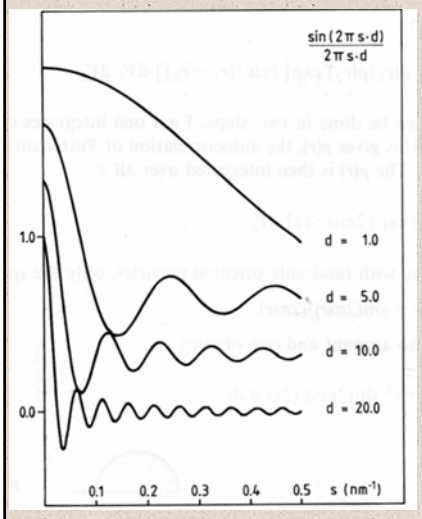
If the particle is described as a discrete sum of elementary scatterers,(e.g. atoms) with the atomic scattering factors $f_i(s)$ the spherically averaged intensity is

$$I(s) = \sum_{i=1}^{K} \sum_{j=1}^{K} f_i(s) f_j(s) \frac{\sin(sr_{ij})}{sr_{ij}}$$

where $r_{ij} = |\mathbf{r}_i - \mathbf{r}_j|$

The Debye (1915) formula is widely employed for model calculations

Contribution of distances to the scattering pattern

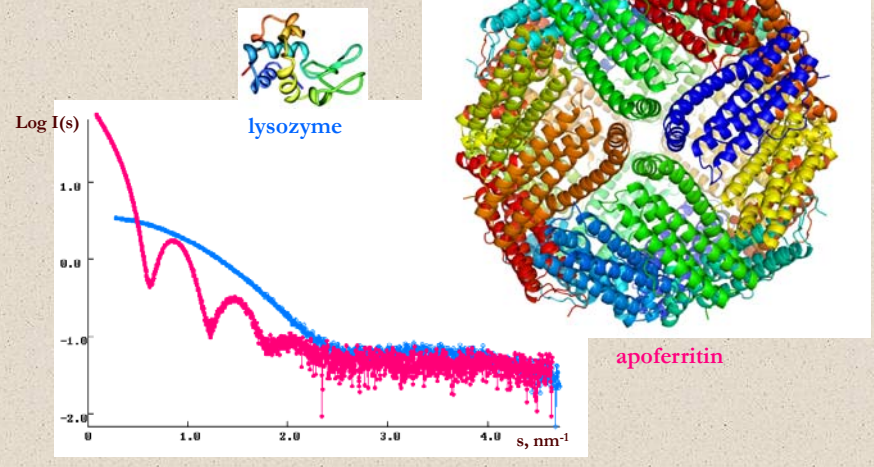


In isotropic systems, each distance $d = r_{ij}$ contributes a sinx/x –like term to the intensity.

Large distances correspond to high frequencies and only contribute at **low angles** (i.e. at low resolution, where particle shape is seen)

Short distances correspond to low frequencies and contribute over a large angular range.
Clearly at **high angles** their contribution dominates the scattering pattern.

Small and large proteins: comparison



Guinier law

Near *s=0* one can insert the McLaurin expansion *sin(sr)/sr≈1-(sr)²/3!+...* into the equation for I(s) yielding

$$I(s) = I(0)\left[1-\frac{1}{3}R_g^2 s^2 + O(s^4)\right] \cong I(0)\exp\left(-\frac{1}{3}R_g^2 s^2\right)$$

This is a classical formula derived by Andre Guinier (1938) in his first SAXS application (to defects in metals). The formula has two parameters, forward scattering and the radius of gyration

$$I(0) = \int_V \int_V \Delta\rho(\mathbf{r})\Delta\rho(\mathbf{r}')d\mathbf{r}d\mathbf{r}' = 4\pi \int_0^{D_{\max}} p(r)dr = (\Delta\rho)^2 V^2$$

$$R_g = \int_0^{D_{\max}} r^2 p(r)dr \left[2\int_0^{D_{\max}} p(r)dr\right]^{-1}$$

ideal monodisperse

Intensity at the origin

$$i_1(0) = \int_{V_\mathbf{r}} \int_{V_{\mathbf{r}'}} \Delta\rho(\mathbf{r})\Delta\rho(\mathbf{r}')dV_\mathbf{r}dV_{\mathbf{r}'}$$

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

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

$$I(0) = \frac{cMV}{N_A}\left[\bar{v}_P(\rho - \rho_0)\right]^2$$

ideal monodisperse

Intensity at the origin

If : the concentration c (w/v), $\bar{v}_P$
the partial specific volume $\bar{v}_P$,
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

In practice, MM can be determined from the data on relative scale by comparison with I(0) of a reference protein (e.g. BSA, lysozyme or cytochrom C)

ideal monodisperse

Radius of gyration

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

Ellipsoid of revolution (a, b) $$R_g = \sqrt{\frac{2a^2 + b^2}{5}}$$

Cylinder (D, H) $$R_g = \sqrt{\frac{D^2}{8} + \frac{H^2}{12}}$$

ideal monodisperse

Virial coefficient

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

$$I(0,c) = \frac{I(0)_{ideal}}{1 + 2A_2 Mc + \ldots}$$

Where A_2 is the second virial coefficient which represents pair interactions and $I(0)_{ideal}$ 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.

To obtain I(0, s), this extrapolation to infinite dilution is performed for different angles

Guinier plot example

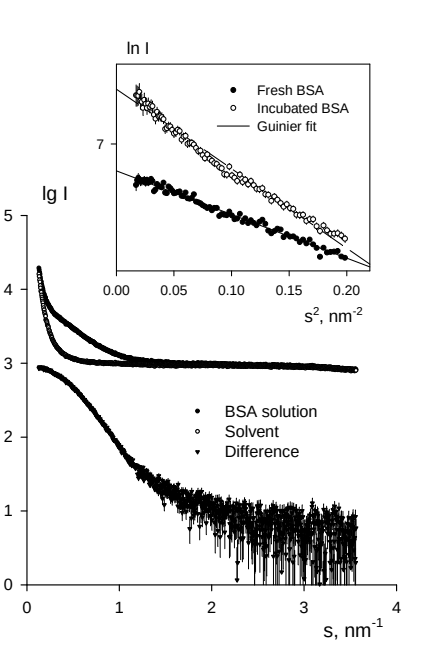
The law is generally used under its log form :

$$\ln[I(s)] = \ln[I(0)] - [sR_g]^2/3$$

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

Validity range :
$0 < sR_g < 1.3$

ideal monodisperse



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

$$sI(s) \cong I_C(0)\exp\left(-\frac{1}{2}R_c^2 s^2\right)$$

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

$$s^2 I(s) \cong I_T(0)\exp\left(-R_t^2 s^2\right)$$

with $R_t = T/\sqrt{12}$ T : thickness

ideal monodisperse

Distance distribution function

$$p(r) = \frac{r^2}{2\pi^2} \int_0^\infty s^2 I(s) \frac{\sin sr}{sr} dr$$

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

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

$\Rightarrow$ Fourier transform of ***incomplete and noisy data*** is an ***ill-posed problem***.

Solution : Indirect Fourier Transform (suggested by O. Glatter, 1977).
p(r) is parameterized on [0, D_{max}] by a linear combination of orthogonal functions, where D_{max} is the particle diameter.
Implemented in several programs, including GNOM (part of ATSAS)

ideal monodisperse

Distance distribution function

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

$$R_g^2 = \frac{\int_0^{D_{max}} r^2 p(r) dr}{2\int_0^{D_{max}} p(r) dr} \quad \text{and} \quad I(0) = 4\pi \int_0^{D_{max}} p(r) dr$$

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

ideal monodisperse

Porod invariant and volume

Following the Parseval theorem for Fourier transformations

$$Q = \int_0^\infty s^2 I(s) ds = 2\pi^2 \int_V (\Delta\rho(\mathbf{r}))^2 d\mathbf{r}$$

Q is called the Porod invariant, which is computed from the intensity but provides the mean square electron density contrast.

For homogeneous particles, $Q=2\pi^2(\Delta\rho)^2V$, and, taking into account that $I(0)=(\Delta\rho)^2V^2$, the excluded volume of hydrated particle in solution (Porod volume) is

$$\mathbf{V=2\pi^2 I(0)/Q}\ .$$

The asymptotic regime : Porod law

Integrating the Fourier transformation for I(s) by parts and using that for particles with a *sharp interface* $\gamma'(D_{max}) = 0$, one has

$$I(s) \cong 8\pi s^{-4}\gamma'(0) + O_1 s^{-3} + O_2 s^{-4} + o(s^{-5})$$

where O_1, O_2 are oscillating trigonometric terms of the form *sin* (sD_{max}). The main term responsible for the intensity decay at high angles is therefore proportional to s^{-4}, and this is known as Porod's law (1949). For homogeneous particles, $\gamma'(0)$ is equal to $-(\Delta\rho)^2S/4$, where S is the particle surface.

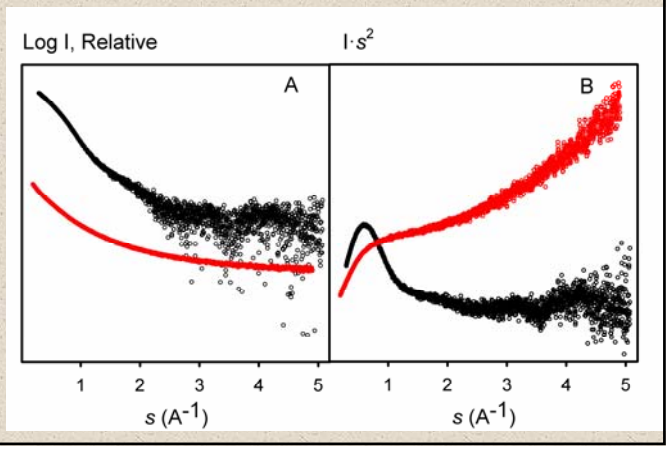
ideal monodisperse

Kratky plot

A plot of $s^2I(s)$ vs s provides a sensitive means of ***monitoring the degree of compactness*** of a protein.

Globular particle : bell-shaped curve

Unfolded particle: plateau or increase at large s-values



Summary of model-independent information

I(0)/c, i.e. molecular mass *(from Guinier plot or p(r) function)*

Radius of gyration R_g *(from Guinier plot or p(r) function)*

Radii of gyration of thickness or cross-section *(anisometrc particles)*

Second virial coefficient A_2 *(extrapolation to infinite dilution)*

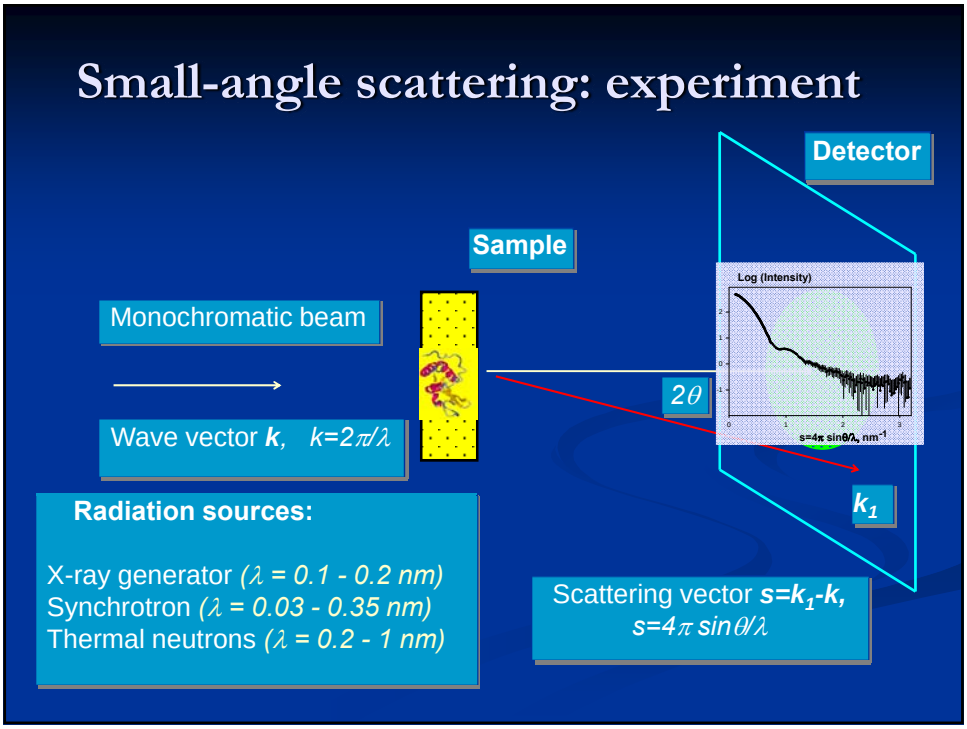
Maximum particle size D_{max} *(from p(r) function)*

Particle volume V *(from I(0) and Porod invariant)*

Specific surface S/V *(from I(0), Porod invariant and asymptotics)*

Globular or unfoded *(From Kratky plot)*

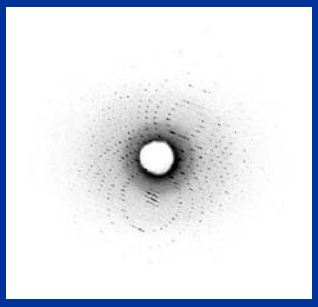
Small-angle scattering: experiment



Crystal versus solution

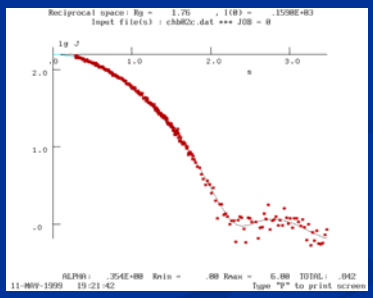
Crystal versus solution

- Thousands of reflections
- 3D, high resolution



- Data undersampled, $\Delta s = 2\pi / D$

- A few Shannon channels
- 1D, low resolution



- Data oversampled, $\Delta s << \pi / D$

Crystal versus solution

- In solution, no crystallographic packing forces are present

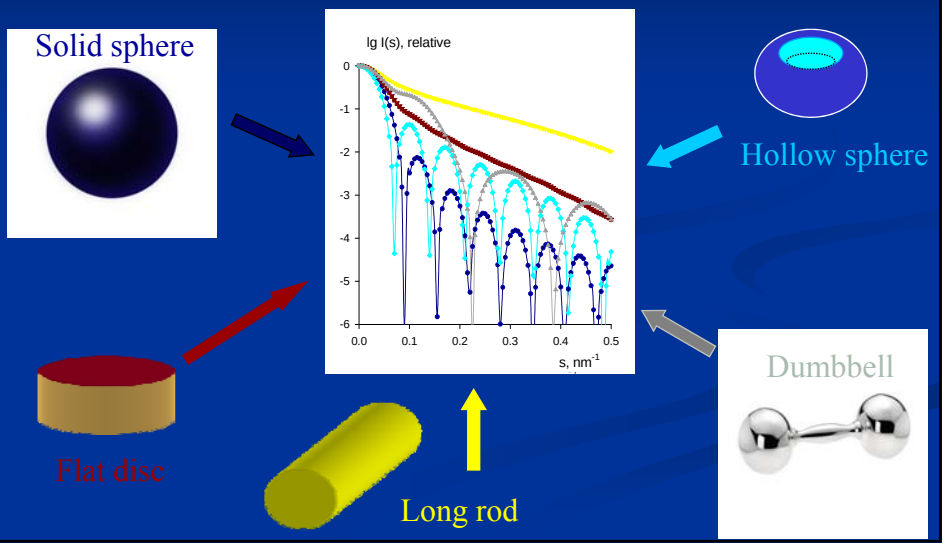
- For SAXS solution studies, one does not need to grow crystals
- SAXS is not limited by molecular mass and is applicable under nearly physiological conditions
- Using solution SAXS, one can more easily observe responses to changes in conditions
- SAXS permits for quantitative analysis of complex systems and processes

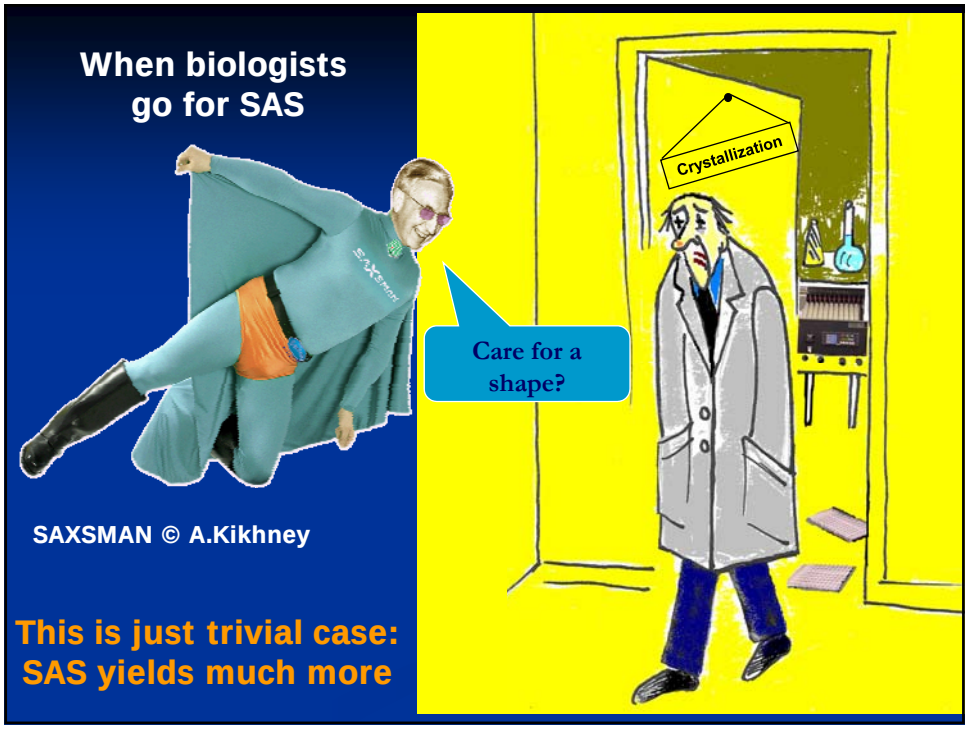
Scattering from dilute macromolecular solutions (monodisperse systems)

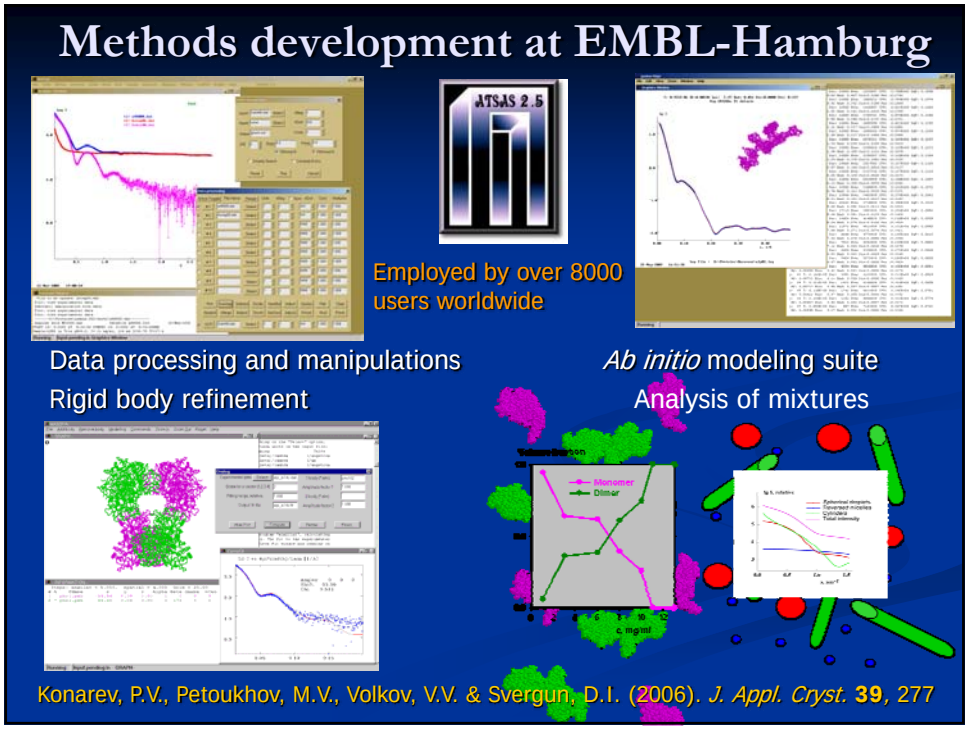
$$I(s) = 4\pi \int_0^D p(r) \frac{\sin sr}{sr} dr$$

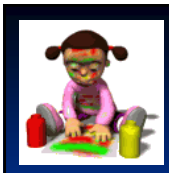
The scattering is proportional to that of a single particle averaged over all orientations, which allows one to determine size, shape and internal structure of the particle at low (*1-10 nm*) resolution.

The scattering is related to the shape (or low resolution structure)

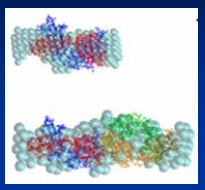



When biologists go for SAS
SAXSMAN © A.Kikhney
This is just trivial case: SAS yields much more
Crystallization
Care for a shape?


Methods development at EMBL-Hamburg
ATSAS 2.5
Employed by over 8000 users worldwide
Data processing and manipulations
Rigid body refinement
Ab initio modeling suite
Analysis of mixtures
Monomer
Dimer
Konarev, P.V., Petoukhov, M.V., Volkov, V.V. & Svergun, D.I. (2006). J. Appl. Cryst. 39, 277

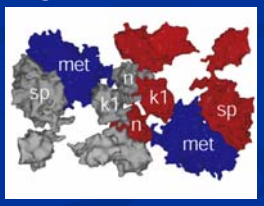


"Simple" monodisperse systems

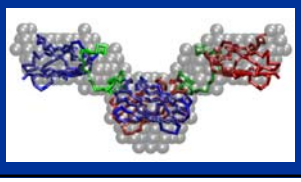


Shape and conformational changes of macromolecules and complexes

Validation of high resolution models and oligomeric organization



Rigid body models of complexes using high resolution structures



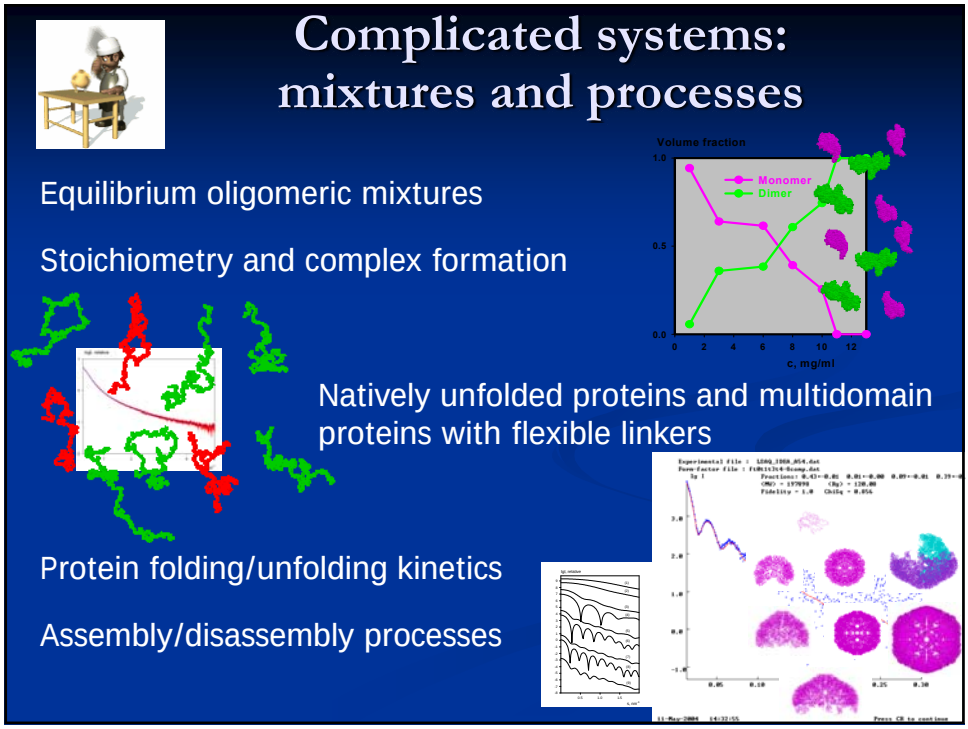
Addition of missing fragments to high resolution models

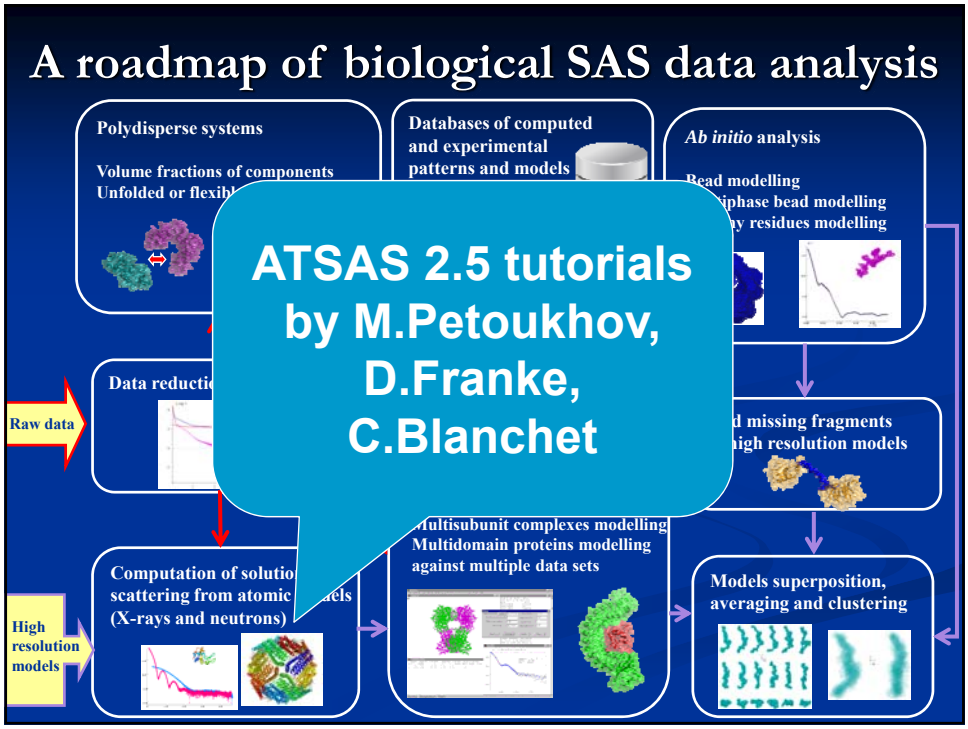
Scattering from mixtures

$$I(s)=\sum_k v_k I_k(s)$$

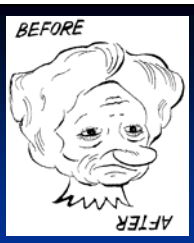
The scattering is proportional to that of a single particle averaged over all orientations, which allows one to determine size, shape and internal structure of the particle at low (*1-10 nm*) resolution.

For equilibrium and non-equilibrium mixtures, solution scattering permits to determine the number of components and, given their scattering intensities $I_k(s)$, also the volume fractions


Complicated systems:
mixtures and processes
Equilibrium oligomeric mixtures
Volume fraction
Monomer
Dimer
c, mg/ml
Stoichiometry and complex formation
Natively unfolded proteins and multidomain proteins with flexible linkers
Protein folding/unfolding kinetics
Assembly/disassembly processes


A roadmap of biological SAS data analysis
Polydisperse systems
Volume fractions of components
Databases of computed and experimental patterns and models
Ab initio analysis
Bead modelling
Raw data
Data reducti
Computation of solutio scattering from atomic (X-rays and neutrons)
High resolution models
Multisubunit complexes modelling
Multidomain proteins modelling against multiple data sets
Models superposition, averaging and clustering
ATSAS 2.5 tutorials by M.Petoukhov, D.Franke, C.Blanchet

A word of caution



- Sample preparation
- Experiment
- Data processing
- Unambiguous interpretation
- Changing conditions
- Relation to function

Books on SAXS

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