A brief introduction to SAXS

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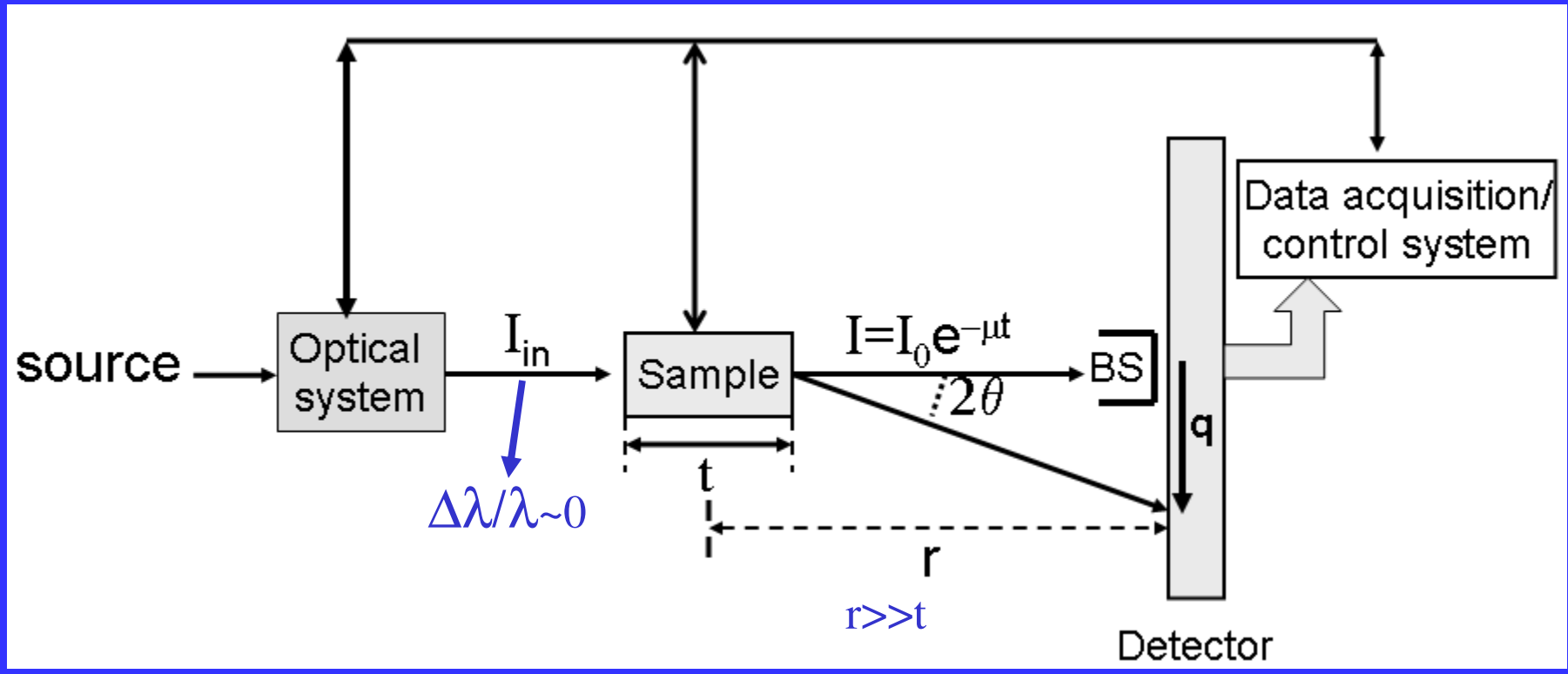
SAXS



For a single electron:

$$I_e(2\theta) = r_0^2\left(\frac{1+\cos^2(2\theta)}{2}\right) \qquad \frac{1}{r^2} I_0 = \frac{r_0^2}{r^2} I_0$$

very small!!

classical electron radius $= 2.82\ 10^{-15}$ m

For SAXS this factor =1

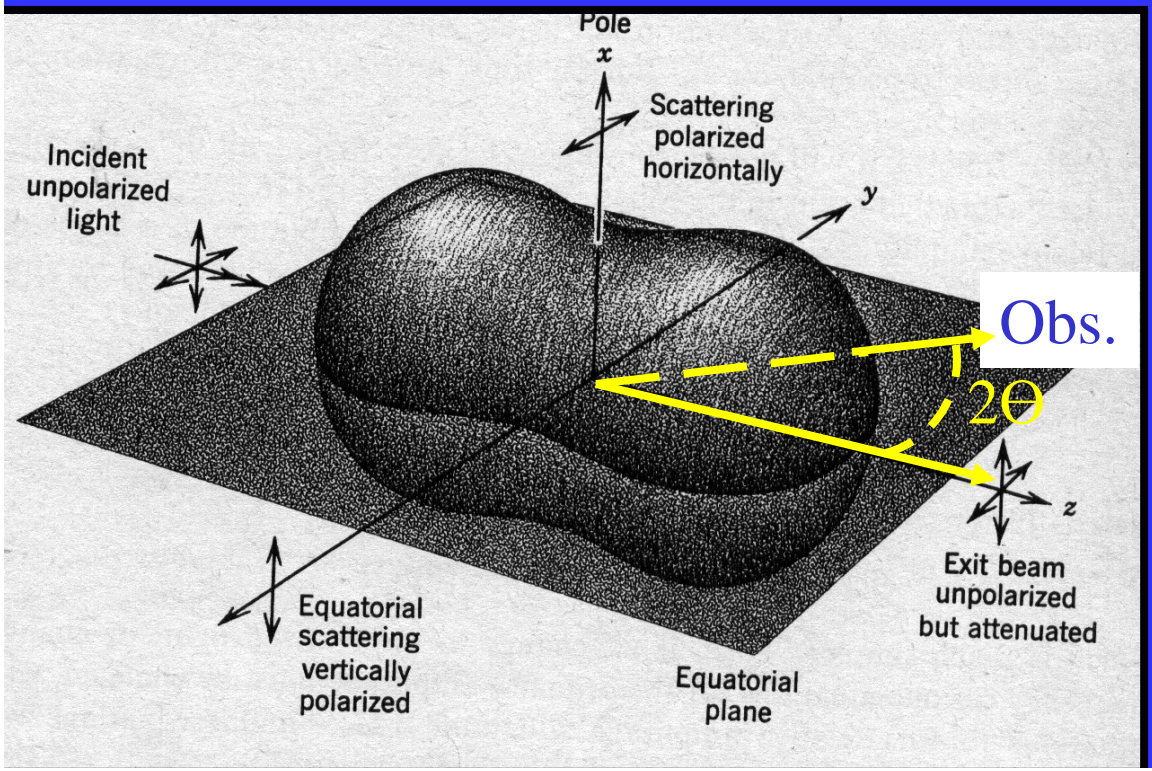
$\sin 2\theta = 2\theta$ $\cos 2\theta = 1$

$I_0 = I_{in}\exp(-\mu t)$

Electrons in an electromagnetic field

are accelerated and therefore emit radiation: they scatter. The spatial distribution of the scattered intensity depends on the geometry of the experiment. For unpolarized incident radiation the spatial distribution on the equator is:

$$I(2\theta) = r_0^2 \cdot \frac{1+\cos^2(2\theta)}{2} \cdot \frac{1}{r^2} \cdot \frac{\omega^4}{(\omega_0^2-\omega^2)^2} I_0 \qquad (1)$$



where 2θ is the angle between the incident and the scattered beam.

$\omega_0 = \sqrt{\frac{k}{m}}$ corresponds to the natural frequency ($\nu_0=\omega_0/2\pi$) of the oscillator and ω to the frequency of the incident radiation.

The most interesting factor

in the previous equation is the one describing the frequency dependence. For the AMPLITUDE (E with I= E·E*)

$$\frac{\omega^2}{(\omega_0^2 - \omega^2)}$$

The natural frequency of the oscillator (ω_0) corresponds to the binding strength of electrons in atoms and lies somewhere in the UV to X-ray region. If the incident radiation is visible light ($\lambda \approx$ 500 nm), $\omega << \omega_0$ and the factor reduces to:

$$\frac{\omega^2}{\omega_0^2}$$

The amplitude of the scattered radiation at **r** is proportional to ω^2 and in phase with the incident radiation. This is

Rayleigh scattering.

The scattered intensity is proportional to ω^4 hence the blue sky.

X-Rays

If the incident radiation is X-rays ($\lambda \approx$ 0.1 nm), $\omega_0 << \omega$ and the factor

$$\frac{\omega^2}{(\omega_0^2 - \omega^2)} = -1$$

The scattering amplitude is independent of the frequency and its phase is shifted by 180 degrees relative to the incident radiation. This is

Thomson scattering

As it is independent of the frequency of the incident radiation, the world of X-rays is colorless with shades of gray (i.e. contrast) only and Eq.1 above simplifies to:

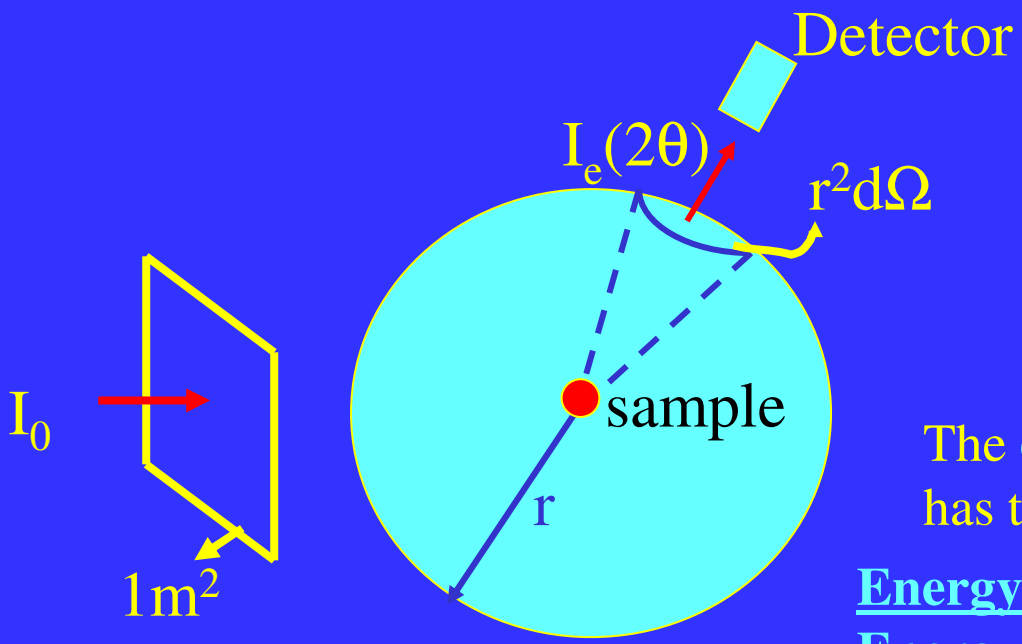
$$I(2\theta) = r_0^2 \cdot \frac{1+\cos^2(2\theta)}{2} \cdot \frac{1}{r^2} I_0$$

$$r_0 = \frac{e^2}{mc^2} = 2.817\,10^{-15}\,m \quad \text{classical electron radius}$$

Energy out /energy in

For unpolarized X-rays at $2\Theta = 0$:

$$I_e(0) = r_0^2 \frac{1}{r^2} I_0$$



$$\frac{d\sigma}{d\Omega} = \frac{I_e(2\theta)}{I_0} \cdot r^2 = |b|^2$$

b: **scattering length =**
$r_0 = 2.810^{-15}$ m for the electron

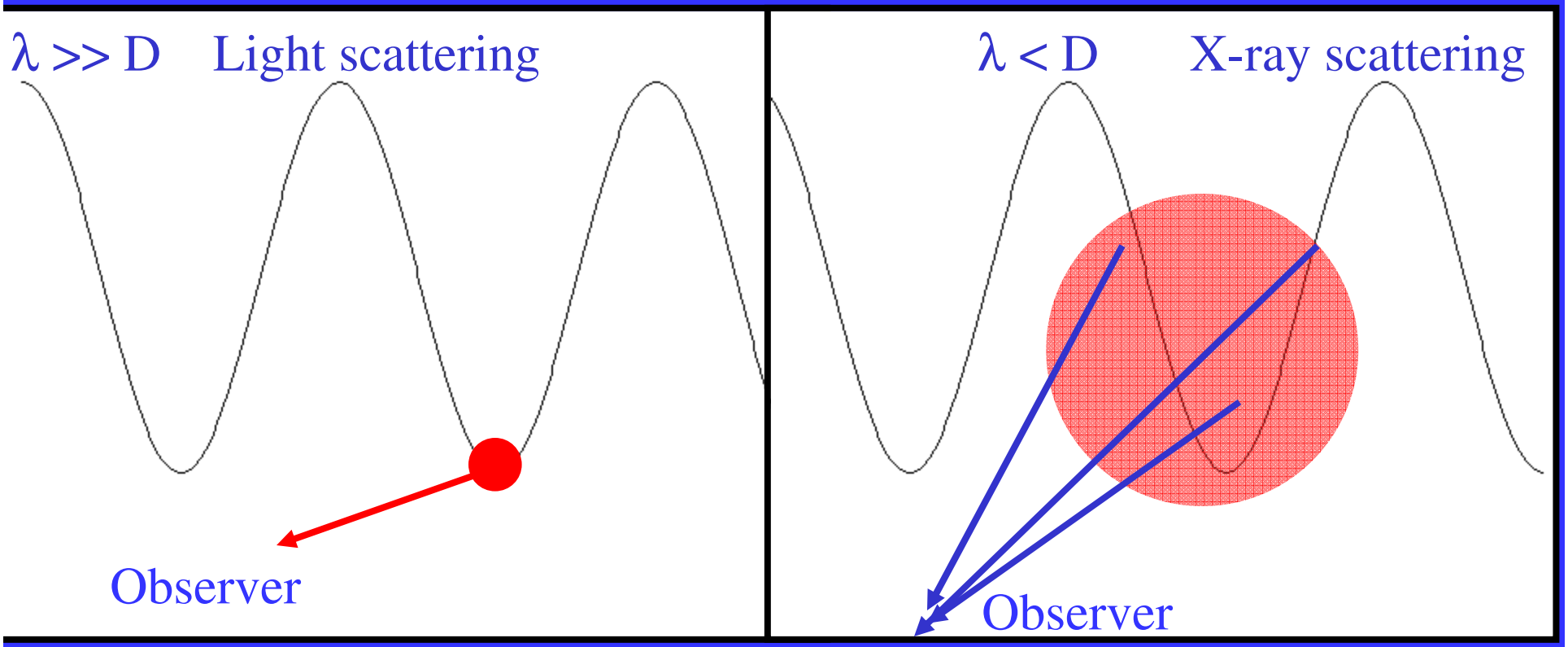
The **differential scattering cross-section** has the dimension of an **area** and represents

Energy scattered/unit solid angle/unit time
Energy incident/unit area/unit time

For one electron: the amplitude of scattering $|I_e(0)|^{1/2} = 2.810^{-15}|I_0|^{1/2}$ and as the scattering amplitude ≡ f =

$$\frac{\text{amplitude scattered by an object}}{\text{amplitude scattered by an electron in identical conditions}} \rightarrow f_e = 1$$

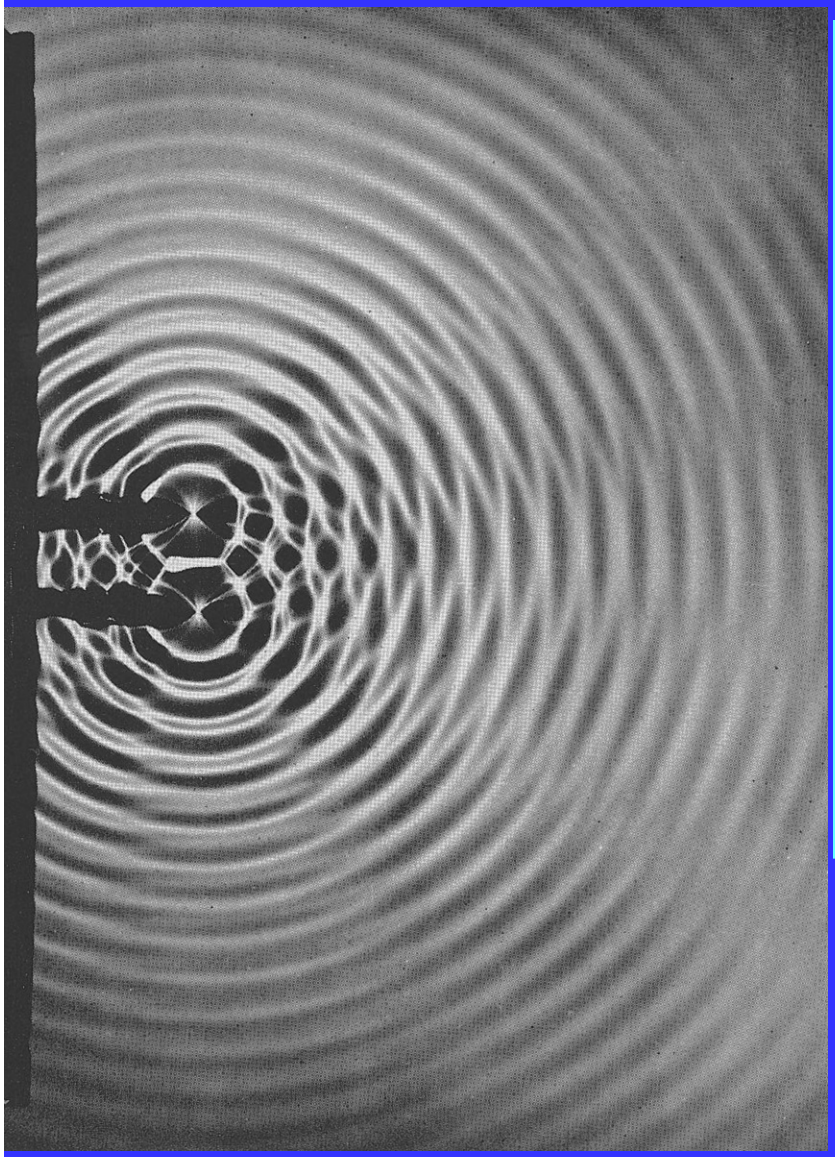
Particle size (D) and wavelength of the radiation



When λ >> D all N electrons in the particle are accelerated in phase the scattering amplitude is N times that of one electron.

When λ < D the electrons in the particle are no longer moving in phase and one has to take the phase shift of the waves into account.

Waves and Interference



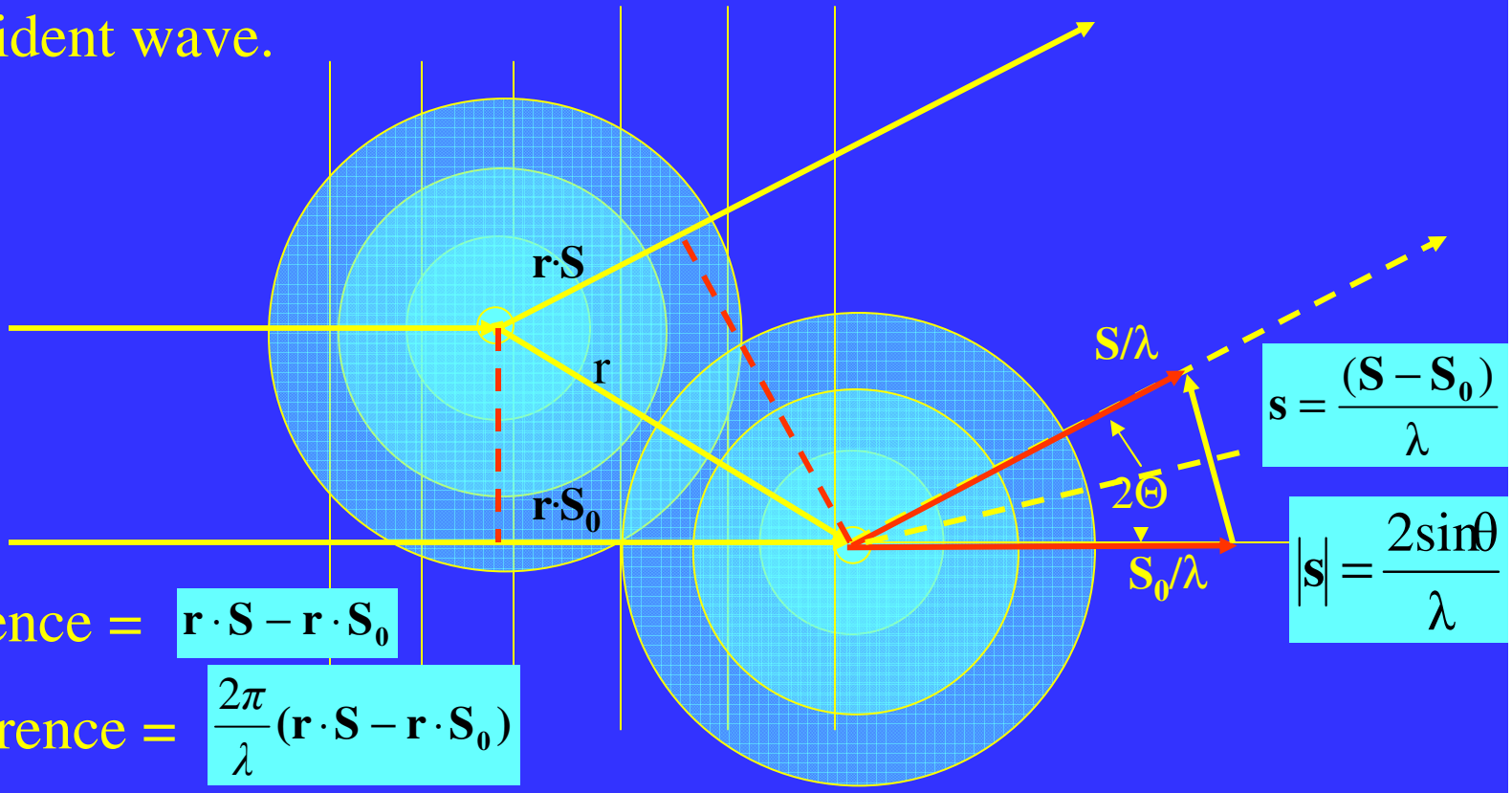
Interferences lead to fringe patterns. This is illustrated here with water waves.

When “solving” a structure the problem is to go from the fringe pattern – in the case of X-ray diffraction from the intensities of the fringes – to the distribution of sources i.e. of scatterers.

Similar effects are observed with optical transforms obtained by shining coherent visible (laser) light through small apertures (see e.g. Cantor and Schimmel , Biophysical Chemistry, Part II, Ch. 13).

Interference and coherent scattering

In Thomson (coherent scattering) the scattered wave is 180° out of phase with the incident wave.



Path difference = $\mathbf{r}\cdot\mathbf{S} - \mathbf{r}\cdot\mathbf{S_0}$

Phase difference = $\frac{2\pi}{\lambda}(\mathbf{r}\cdot\mathbf{S} - \mathbf{r}\cdot\mathbf{S_0})$

The total amplitude from two centers (one at the origin and one at **r**) is thus:

$$F(s) = \sum_{i=1}^{2} f_e \exp(2\pi i\, s r_i) \quad = \quad f_e + f_e \exp(2\pi i\, \mathbf{s}\cdot\mathbf{r}_2)$$

The sum of amplitudes for N electrons:

$$F(\mathbf{s}) = \sum_{i=1}^{N} f_e \exp(2\pi i\, \mathbf{s} \cdot \mathbf{r_i})$$

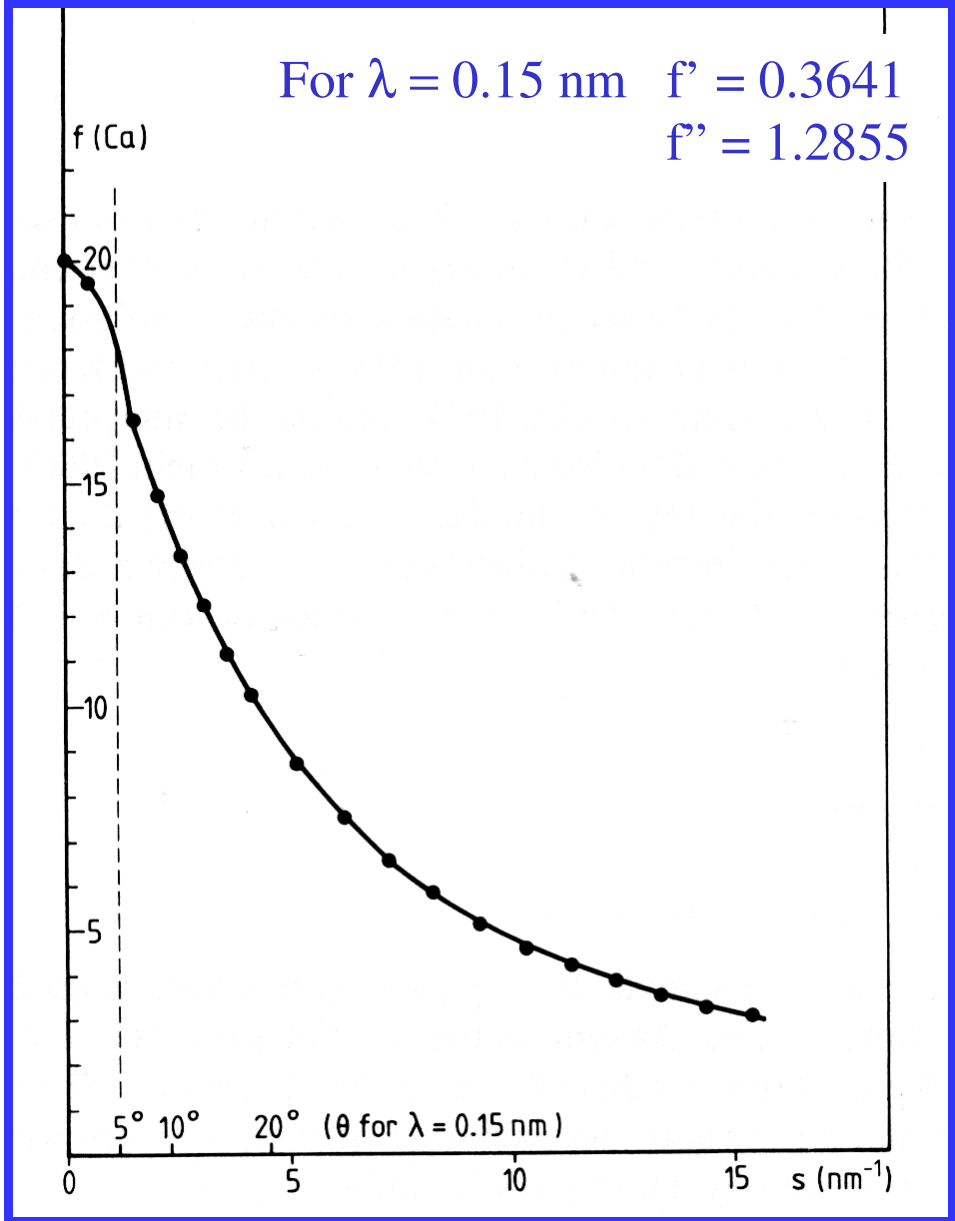
F(**s**) is the Fourier transform of the distribution of electrons

The average of the exponential factor over all orientations of **r** relative to **s** for randomly oriented particles (e.g. in solution) is:

$$\langle \exp(2\pi i \mathbf{s} \cdot \mathbf{r_i}) \rangle = \frac{\sin(2\pi sr)}{2\pi sr}$$

As this is a real number there is no phase problem but one has lost most of the structural information.

Scattering factor



For an atom with a continuous radial electron density ρ(r):

$$F(s) \equiv f(s) = 4\pi \int_0^\infty \rho(r)\ r^2\ \frac{\sin(2\pi sr)}{2\pi sr}\ dr$$

and since

$$\lim_{x\to 0}\left(\frac{\sin x}{x}\right) = 1$$

$$f(0) = Z$$

For modeling purposes one often uses larger spherical subunits (beads, dummy residues, etc) for which:

$$f(s)_{sphere} = \frac{3}{(2\pi sR)^3}\left(\sin(2\pi sR) - 2\pi sR\cos(2\pi sR)\right)$$

Anomalous scattering

Near an absorption edge, the dissipative effects due to the rearrangement of the electrons can no longer be neglected.

The scattering factor f_e must be modified to take anomalous scattering into account

$$f_e = 1 + f_e' + if_e''$$

(f'' is always $\pi/2$ ahead of the phase of the real part)

$$f''(\omega,0) = \frac{\omega A}{4\pi r_0 c N_A}\,\mu(\omega)$$

μ : linear (photoelectric) absorption coefficient,

N_A : Avogadro's number, A atomic weight

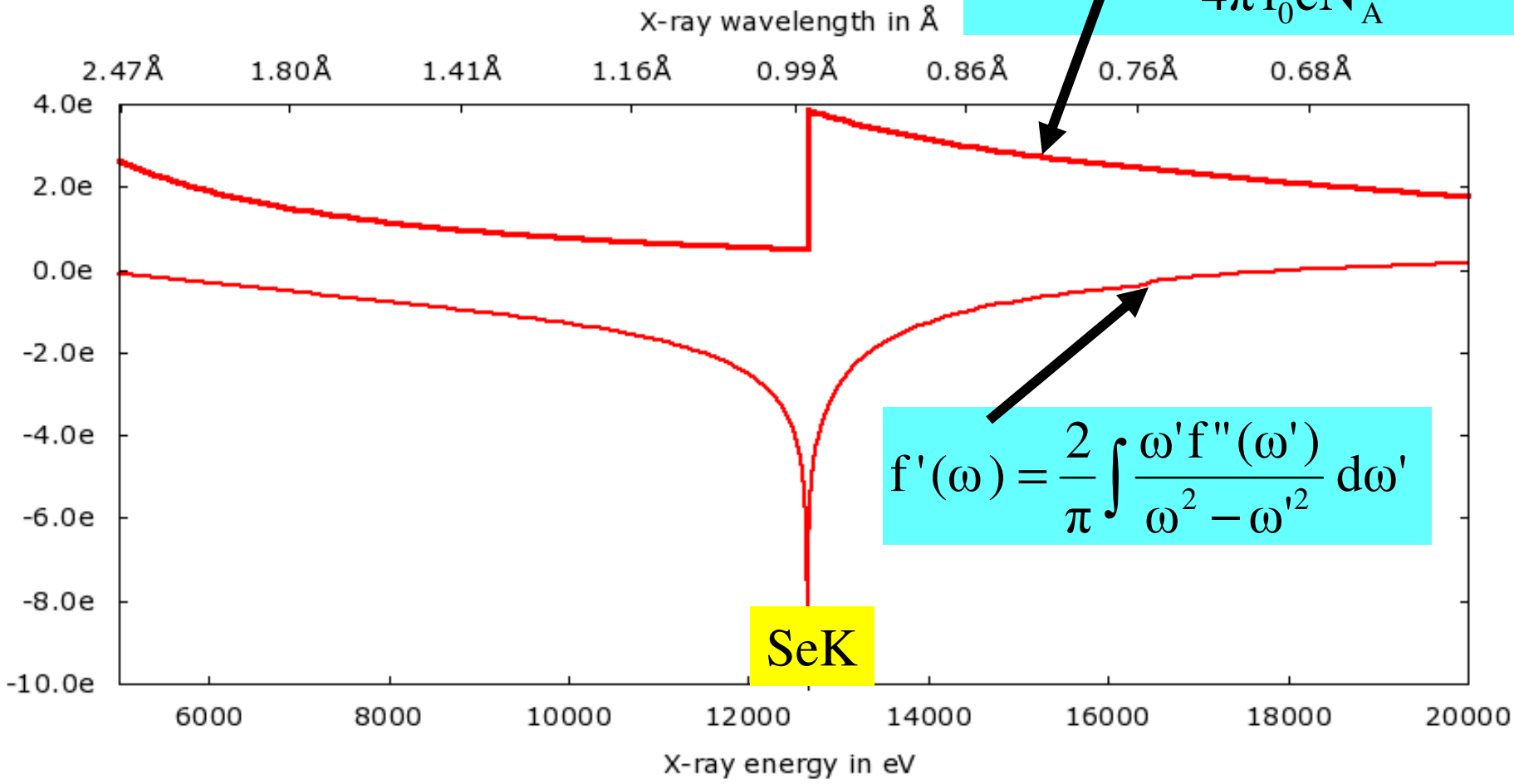
$$f'(\omega) = \frac{2}{\pi}\int \frac{\omega' f''(\omega')}{\omega^2 - \omega'^2}\,d\omega'$$

Kramers – Kronig

Note that for most practical purposes, f' and f'' are independent of $s = 2\sin\theta/\lambda$

Selenium f‘ and f‘‘

$$f''(\omega,0) = \frac{\omega A}{4\pi r_0 c N_A}\ \mu(\omega)$$



$$f'(\omega) = \frac{2}{\pi}\int \frac{\omega' f''(\omega')}{\omega^2 - \omega'^2}\, d\omega'$$

Note the sign and absolute value of the corrections

Scattering from N spherical atoms:

$$F(\mathbf{s}) = \sum_{i=1}^{N} f_i(s)\exp(2\pi i\, \mathbf{s}\cdot\mathbf{r_i})$$

F(**s**) is the Fourier transform of the distribution of the spherical atoms. Crystallographers call this the structure factor. Note that in SAXS the structure factor refers to structure of the solution. The intensity is, of course:

$$I(\mathbf{s}) = \sum_{i=1}^{N}\sum_{j=1}^{N} f_i(s)f_j(s)\exp(2\pi\, i\, \mathbf{s}\cdot(\mathbf{r_i} - \mathbf{r_j}))$$

→ Crystal structure - atomic coordinates

→ This is a real number!

For random orientation

$$\langle \exp(2\pi i \mathbf{s}\cdot(\mathbf{r}_i - \mathbf{r}_j)) \rangle = \frac{\sin(2\pi s r_{ij})}{2\pi s r_{ij}}$$

and

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

→ Solution – Distance distribution only!

Debye (1915)

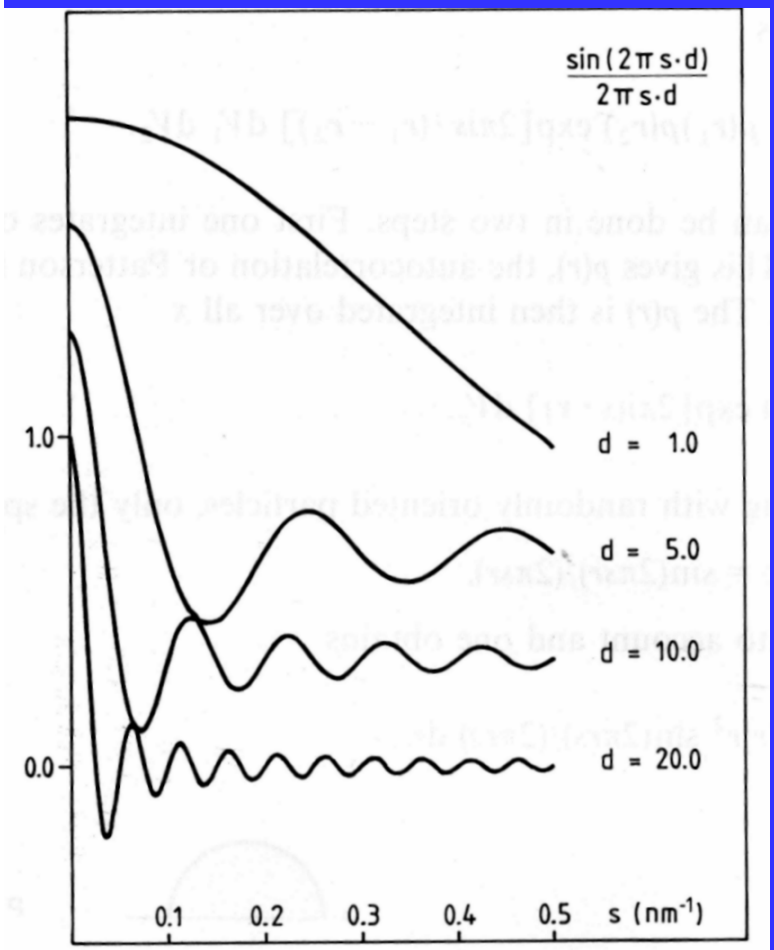
SAXS:

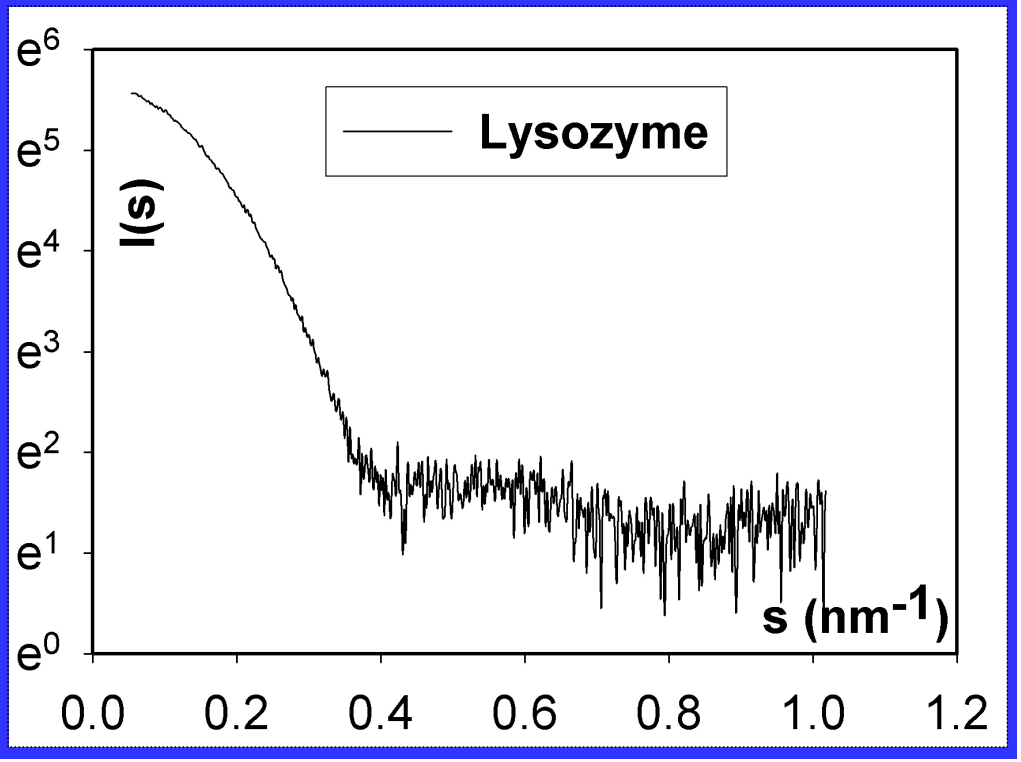
$$I(s) = \sum_{i=1}^{N}\sum_{j=1}^{N} f_i(s) f_j(s) \frac{\sin(2\pi s r_{ij})}{2\pi s r_{ij}}$$

In isotropic systems, each distance $d = r_{ij}$ contributes a sinx/x –like term to the intensity. A scattering pattern is a continuous function of s.

Short distances >> low frequencies dominate at high angles

Large distances >> high frequencies contribute only at low angles





The wider a function in real space the narrower its transform in reciprocal space

1) The Fourier transform of the Dirac delta function

$$\delta(0) = \infty \qquad \int_{-\infty}^{\infty} \delta(x)dx = 1$$

is the 1(x) function (i.e. the function which has a constant value of 1 over the interval $[-\infty,\infty]$.

2) Obviously, the Fourier transform of 1(x) is $\delta(x)$.

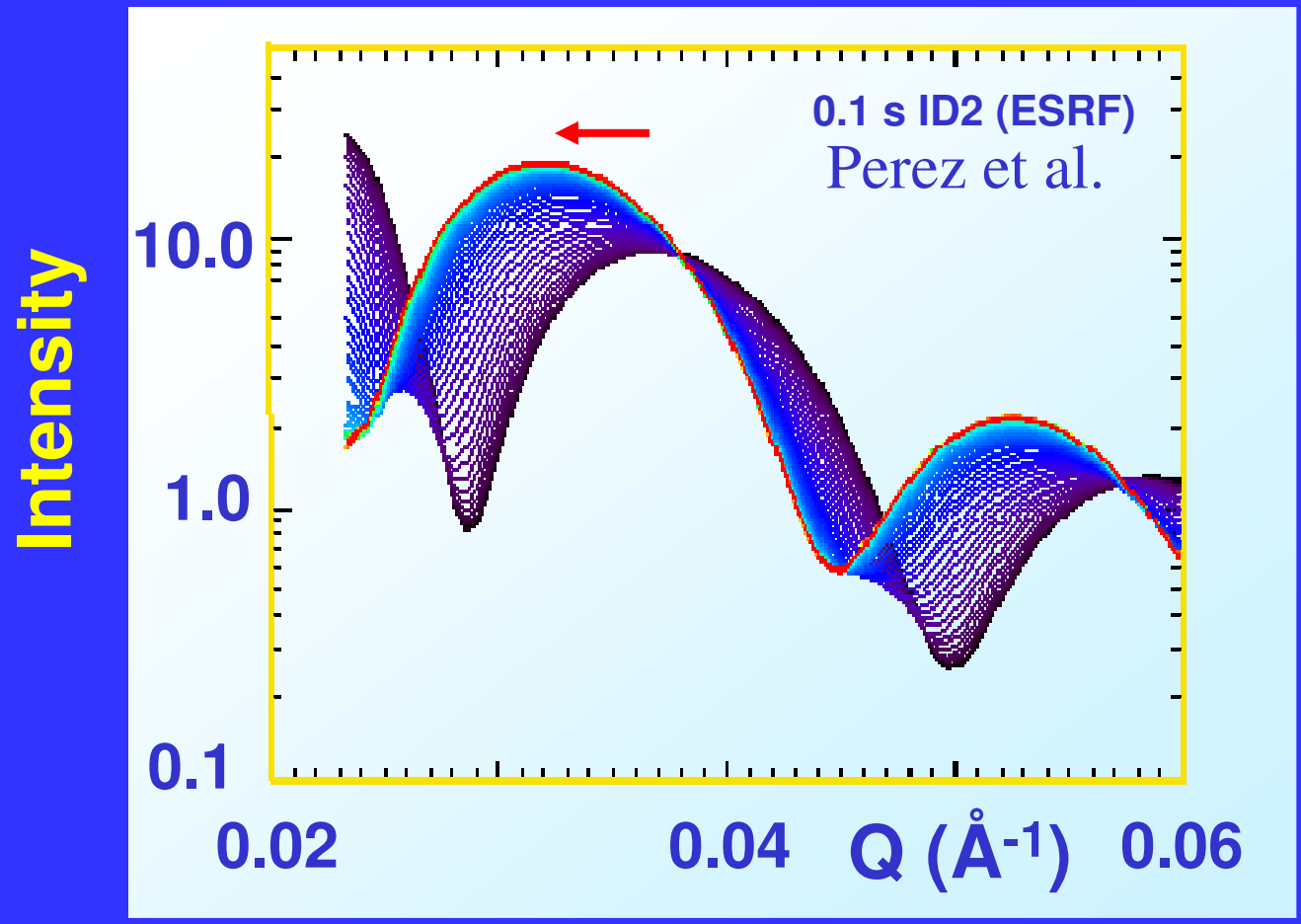
3) The Fourier transform of a Gaussian is also a Gaussian

$$FT(\exp(-ax^2)) = F(k) = \sqrt{\frac{\pi}{a}}\exp(-\pi^2 k^2 / a)$$

Note the relationship between the widths. If the Gaussian has a width $\sigma_R=(1/2a)^{1/2}$, its transform has a width $\sigma_F=(a/2\pi^2)^{1/2}$ and $\sigma_R\sigma_F=1/2\pi$.

The δ-function is an infinitely narrow Gaussian.

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



Larger objects scatter at lower angles!

In an ideal solution

The solute particles are randomly oriented and their positions are uncorrelated in space and time. Consequently their scattering in isotropic and incoherent. The total scattering intensity is the sum of the coherent scattering intensity of all molecules. It is a function of the scattering angle or modulus of the scattering vector only: I(s).

Usually one plots log(I(s)) vs s, because the intensity falls off rapidly due to the interferences.

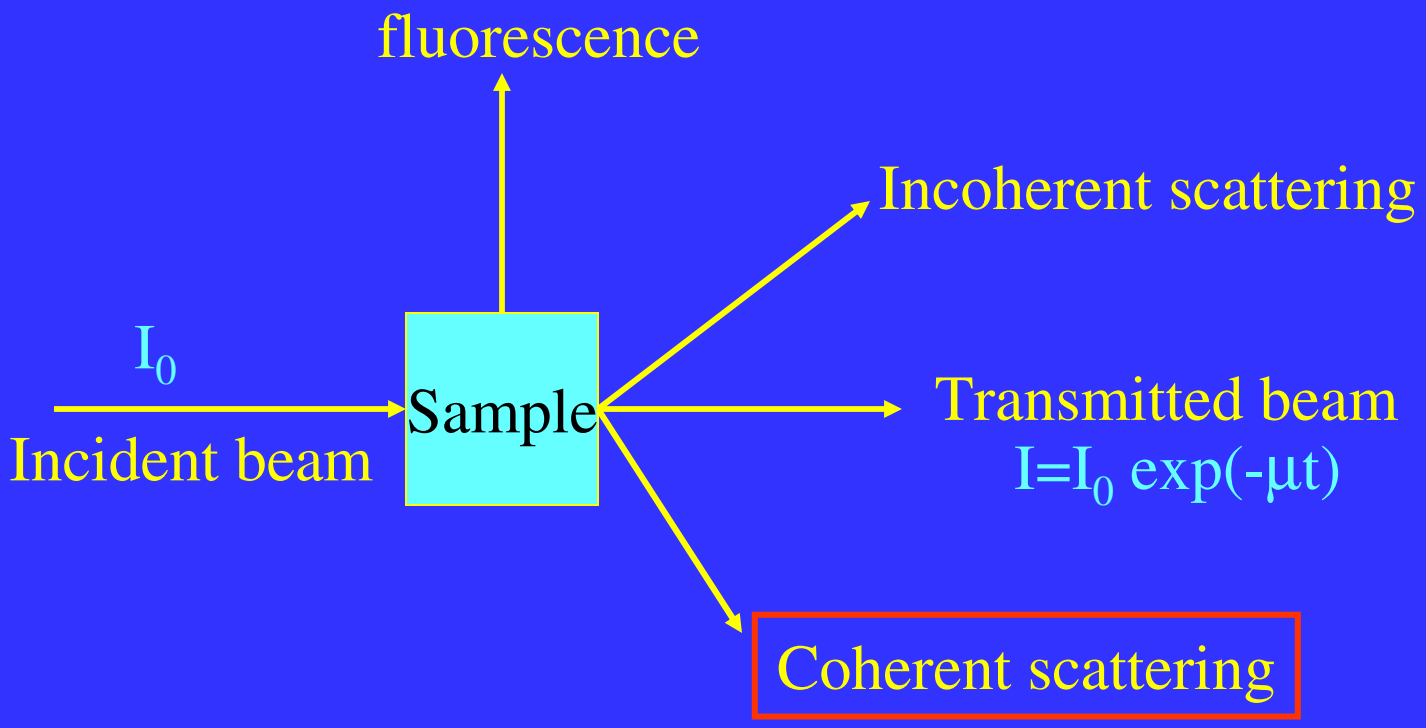
$$i_1(s) = \sum_{i=1}^{N}\sum_{j=1}^{N} f_i f_j \frac{\sin(2\pi s r_{ij})}{2\pi s r_{ij}}$$

i.e. for atoms one can neglect the s-dependence of f_i

If one uses a continuous density distribution ρ(r) this becomes

$$i_1(s) = \int\int_V \rho_1(\mathbf{r}_1)\rho_2(\mathbf{r}_2)\frac{\sin(2\pi s r_{12})}{2\pi s r_{12}} d\mathbf{r}_1 d\mathbf{r}_2$$

Interactions of X-rays with matter



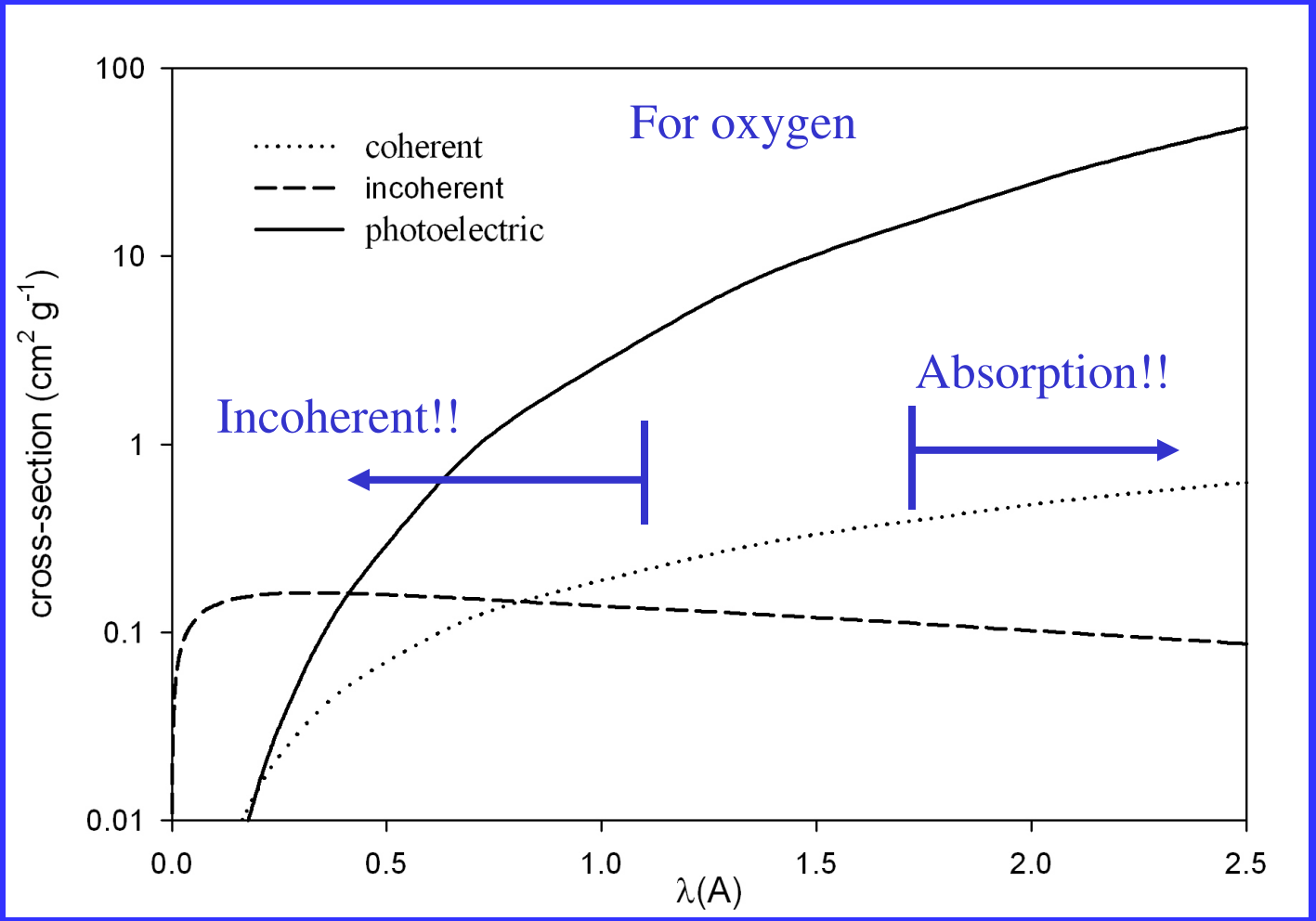
Structural information at the atomic/molecular level is in: coherent scattering

and to a limited extent in absorption/fluorescence near edges (EXAFS, XANES)

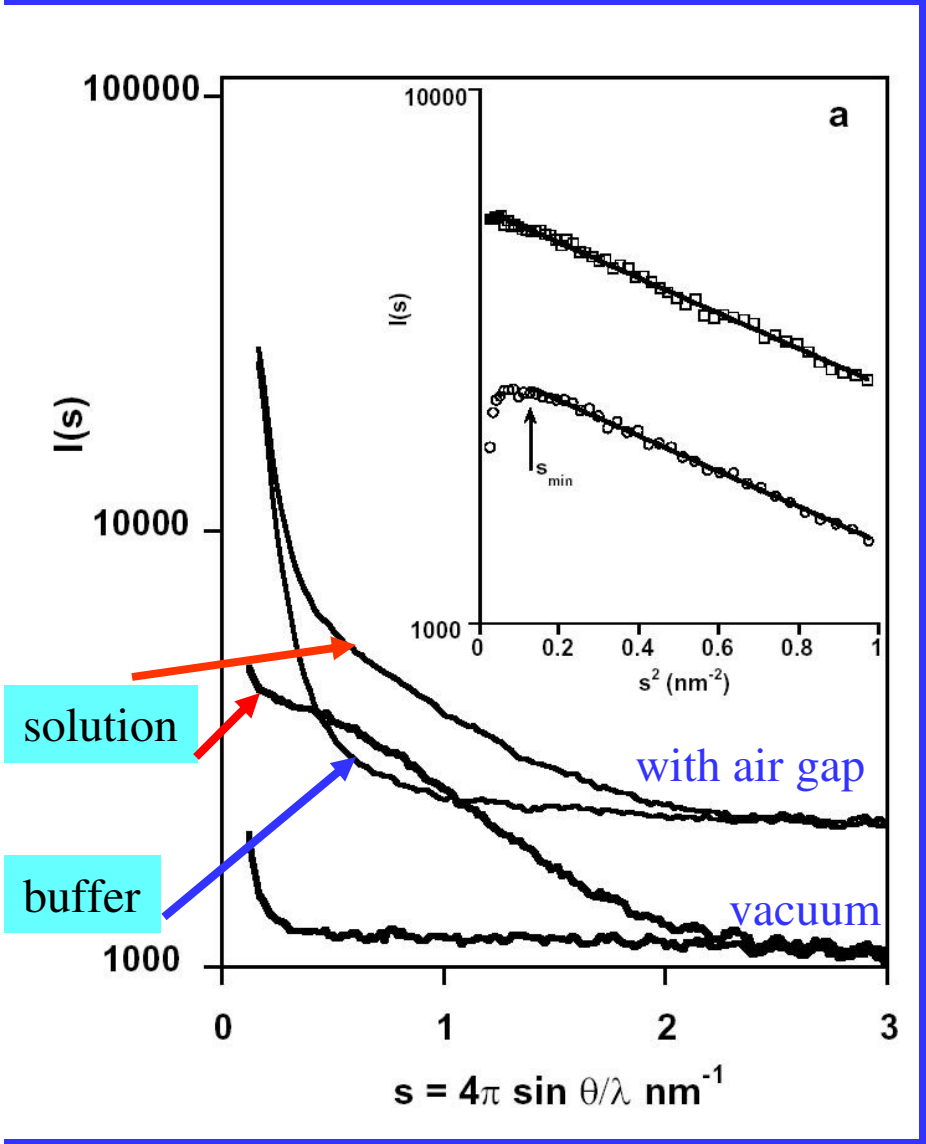
At lower resolution transmission/phase contrast imaging is also useful

Choice of wavelength

Optimal thickness of the sample: $t_{opt} = 1/\mu \approx 1$ mm for H_2O @ 1.5Å



Background



Background arises from the incoherent (Compton) scattering from the sample and from coherent and incoherent scattering due to air gaps, windows, solvent ….

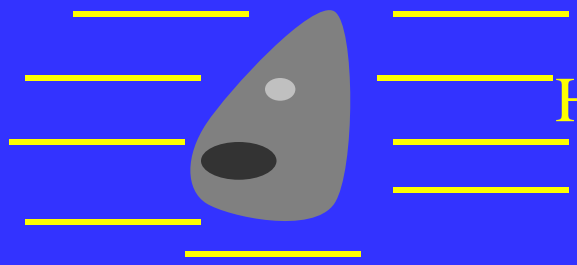
To obtain the coherent scattering of the solute normalize the intensities of solution and buffer to transmitted beam and subtract :

$$I(s) = [I(s)/I_{0T}]_{solution} - [I(s)/I_{0T}]_{buffer}$$

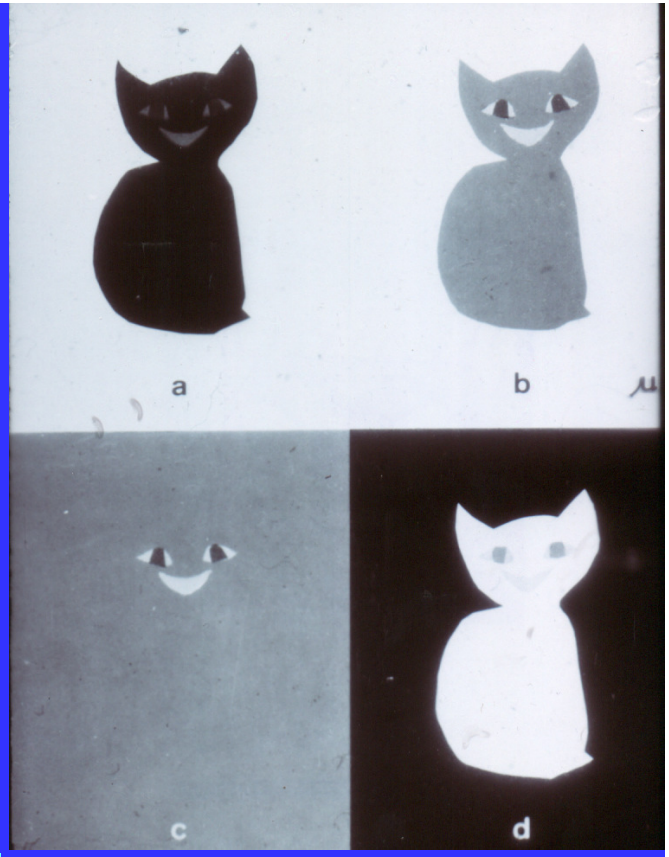
Divide by c to normalize for concentration

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

CONTRAST: $< \rho_{particle}(r) > - \rho_{buffer}$



Homogeneous solvent



Particle: $$F_p(\mathbf{s}) = \int_V \rho_p(\mathbf{r}) \exp(2\pi i \mathbf{s} \cdot \mathbf{r}) d\mathbf{r}$$

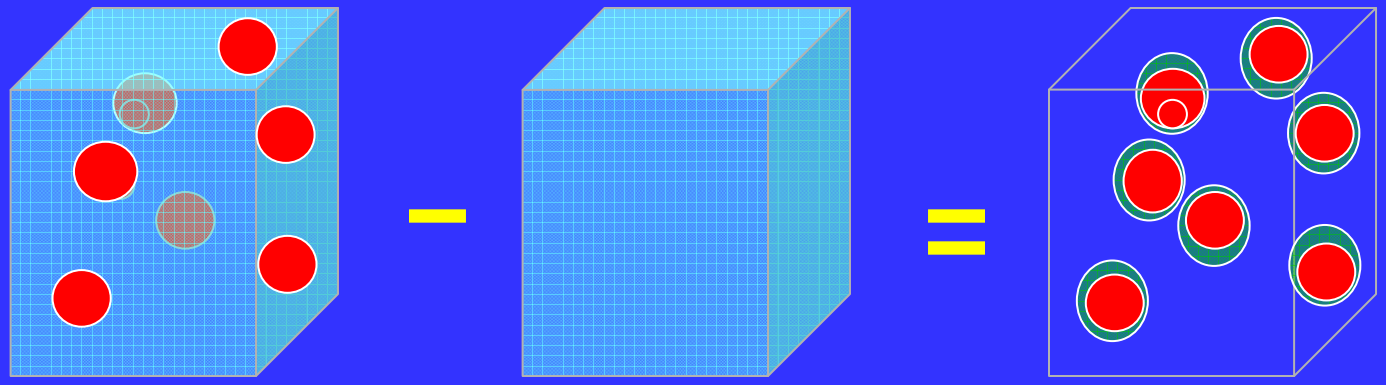
Solvent: $$F_b(\mathbf{s}) = \rho_b \int_V \exp(2\pi i \mathbf{s} \cdot \mathbf{r}) d\mathbf{r}$$

Ideally, a Dirac $\delta(0)$ and constant in practice not

Only fluctuations in electron density contribute to the scattering:

$$I_{obs}(\mathbf{s}) = I_p(\mathbf{s}) - I_b(\mathbf{s})$$

Solvent scattering and contrast



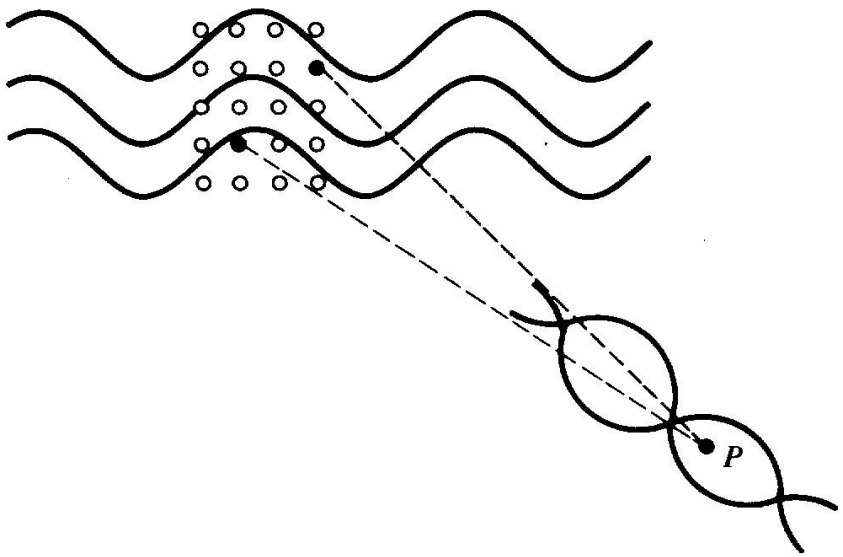
$I_{solution}(s)$ $I_{solvent}(s)$ $I_{particle}(s)$

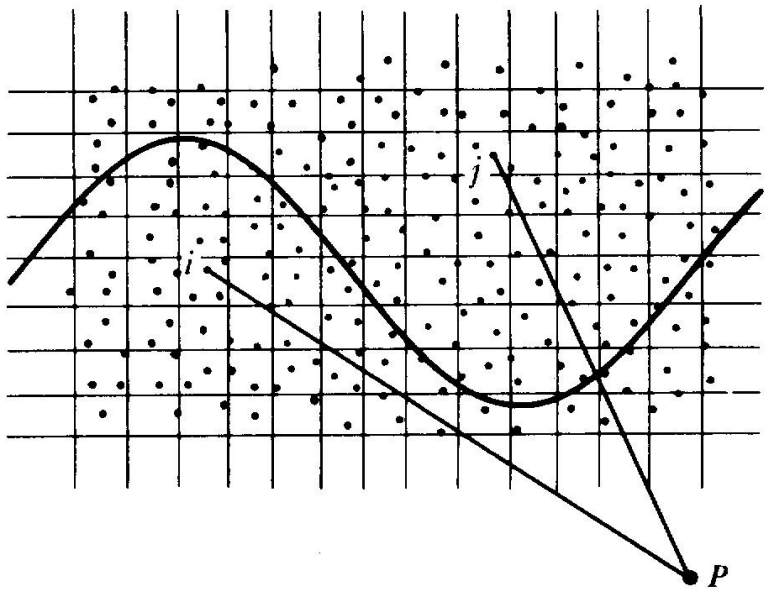
- To obtain scattering from the particles, solvent scattering must be subtracted to yield the excess scattering

$$\rho_p(r) = \rho_{solution}(r) - \rho_b$$

where ρ_b is the scattering density of the solvent

Scattering arises from fluctuations





In a perfectly homogeneous body the contribution of each small scattering volume element to the scattering amplitude would always be cancelled out by that of another one which is out of phase. This is why perfect crystals do not scatter visible light.

Solutions of macromolecules or colloidal suspensions strongly scatter visible light because of fluctuations in concentration.

The fluctuations are also what links scattering to diffusion (e.g. DLS) and thermodynamics (compressibility).

EXCESS SCATTERING DENSITY

$$\rho(\mathbf{r}) = \rho_x \rho_c(\mathbf{r}) + \rho_s(\mathbf{r}) - \rho_b \rho_c(\mathbf{r})$$

shape

equivalent volume of solvent

(Stuhrmann and Kirste, 1965)

$\rho_c(r)$ has a value of 1 inside the particle and 0 outside and thus represents the shape. $\rho_s(r)$, the internal structure, represents the fluctuations around the average electron density of the particle ρ_x.

$$\rho(\mathbf{r}) = (\rho_x - \rho_b)\,\rho_c(\mathbf{r}) + \rho_s(\mathbf{r}) \quad = \bar{\rho}\,\rho_c(\mathbf{r}) + \rho_s(\mathbf{r})$$

contrast

The corresponding intensity is:

$$I(s) = \overline{\rho^2} I_c(s) + \bar{\rho} I_{cs}(s) + I_s(s)$$

For a homogeneous particle: $I_s(s) = I_{cs}(s) = 0$

A communication problem

$$I(s) = \overline{\rho^2} I_c(s) + \bar{\rho} I_{cs}(s) + I_s(s)$$

These two terms are the most important in SAXS

This is essentially what crystallographers talk about

For a homogeneous particle (i.e. a shape): $I_s(s) = I_{cs}(s) = 0$

$I_s(0) = I_{cs}(0) = 0$ always!

Scattered intensity

$$FT[\rho(\mathbf{r})] = F(\mathbf{s})$$
$$FT[\rho(-\mathbf{r})] = F(-\mathbf{s}) = F^*(\mathbf{s})$$

ρ(**r**) is the excess electron density

$$i_1(\mathbf{s}) = F(\mathbf{s}) \cdot F^*(\mathbf{s}) = F(\mathbf{s}) \cdot F(-\mathbf{s})$$

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

$\gamma(\mathbf{r})$

$\gamma(\mathbf{r})$: Autocorrelation function of the excess electron density and:

$$i_1(s) = \langle FT(\gamma(\mathbf{r})) \rangle = \left\langle \int_{V_r} \gamma(\mathbf{r}) \exp(2\pi i \mathbf{s} \cdot \mathbf{r}) dV_r \right\rangle$$

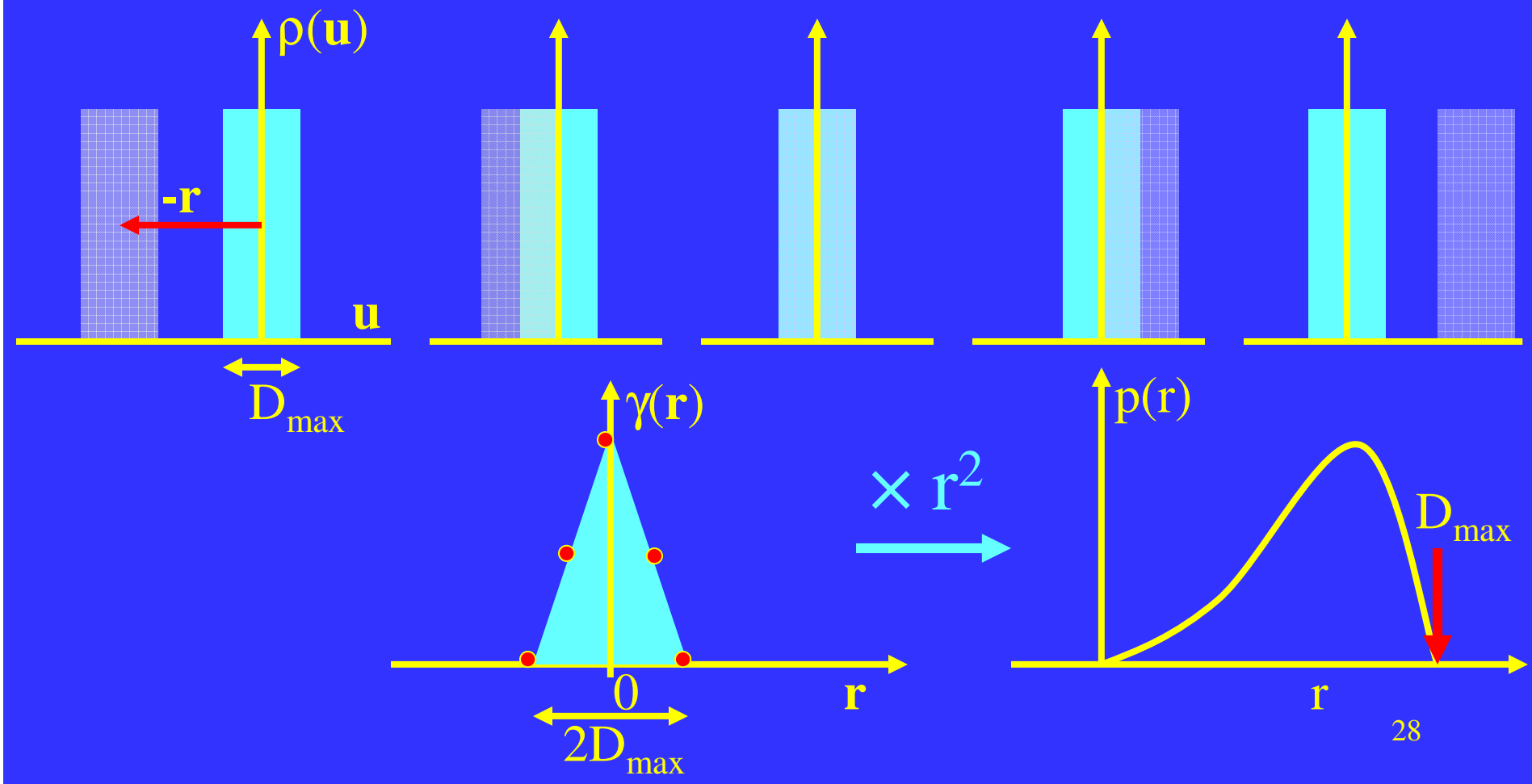
After spherical averaging:

$$\gamma(r) = \langle \gamma(\mathbf{r}) \rangle \longrightarrow i_1(s) = 4\pi \int_0^\infty p(r) \frac{\sin(2\pi rs)}{2\pi rs} dr$$

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

Autocorrelation: Shift-Multiply-Integrate

$$\gamma(\mathbf{r}) = \rho(\mathbf{r}) * \rho(-\mathbf{r}) = \rho(\mathbf{r}) \circ \rho(\mathbf{r}) = \int_{V_\mathbf{u}} \rho(\mathbf{r}+\mathbf{u})\rho(\mathbf{u})\,dV_\mathbf{u}$$

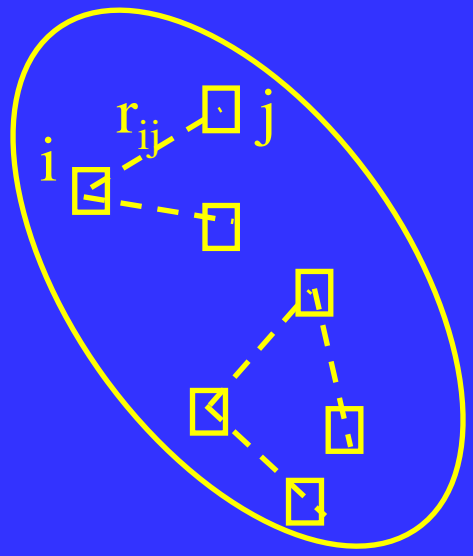


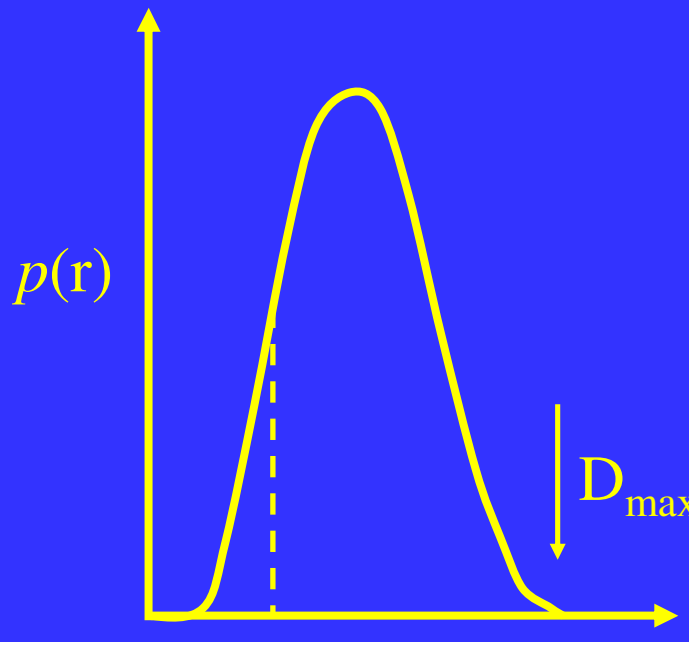
For a homogeneous body $\rho(r) = \rho$, $\gamma(0) = \rho^2 V$

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

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

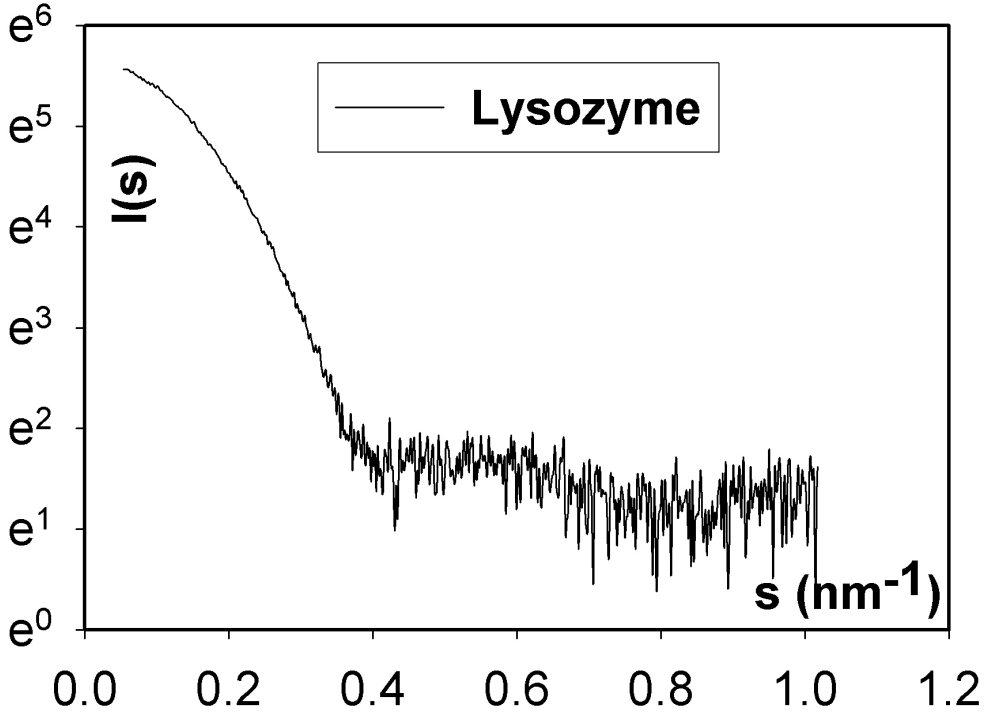
$\gamma(r)$: probability of finding a point at r from a given point (i).
Number of volume elements i $\propto$ V;
Number of volume elements j $\propto 4\pi r^2$.
Number of pairs (i,j) separated by the distance r $\propto 4\pi r^2 V\gamma_0(r) = (4\pi/\rho^2)\, p(r)$.

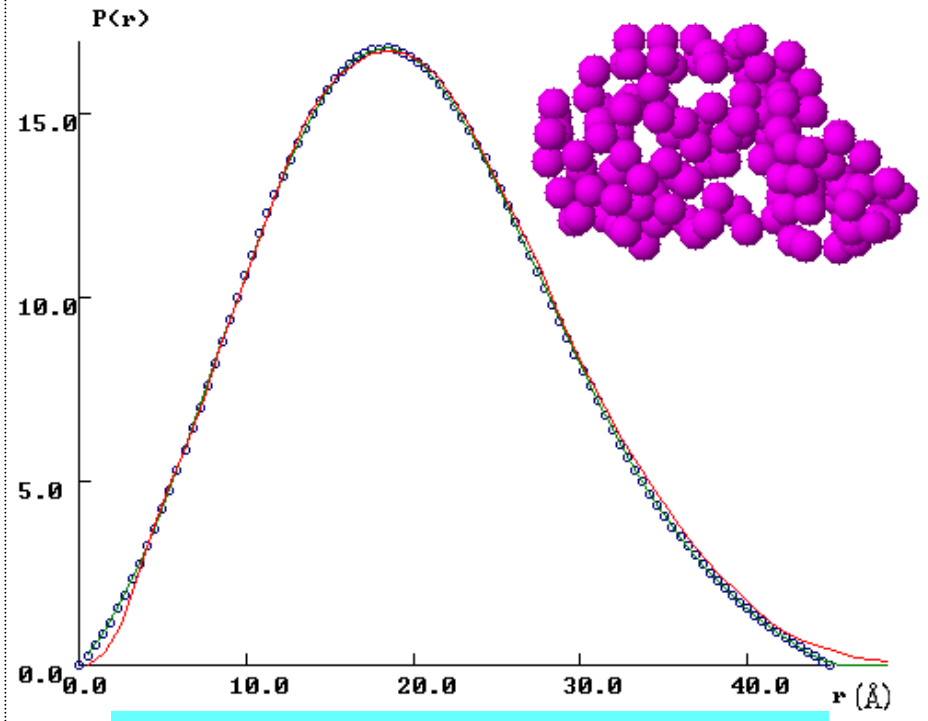




I(s) and p(r)

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





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

For a homogeneous particle p(r) represents the histogram of distances between pairs of points within the particle.

At low angles

Since $\frac{\sin(2\pi sr)}{2\pi sr} = 1 - \frac{(2\pi sr)^2}{6} + \frac{(2\pi sr)^4}{120} - \ldots$

$$i_1(s) = \sum_{i=1}^{N}\sum_{j=1}^{N} f_i f_j \frac{\sin(2\pi s r_{ij})}{2\pi s r_{ij}} \longrightarrow i_1(s) = \overline{\rho^2} V^2 \left(1 - \frac{4}{3}\pi^2 R_g^2 s^2 + \ldots\right)$$

$$\left(\sum_i f_i\right)^2$$

At very low angles one can use the approximation:
$\exp(x) = 1 + x + x^2/2 + \ldots$ which yields the Guinier formula

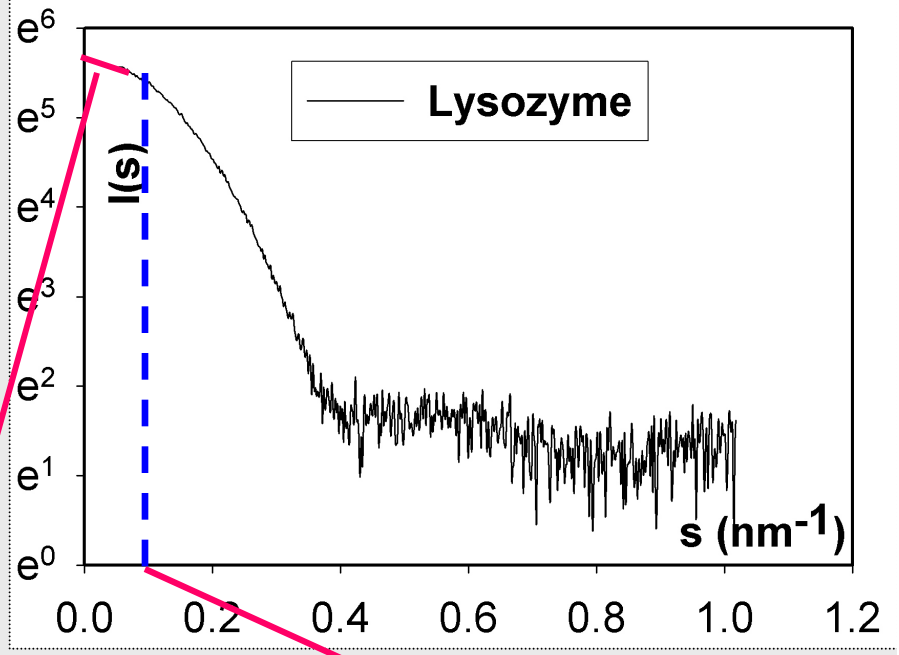
$$I(s) = I(0)\exp\left(-\frac{4}{3}\pi^2 R_g^2 s^2\right) \qquad \textit{with } I(0) = \overline{\rho^2} V^2 \sim \text{Molecular mass}$$

R_g is the mean squared distance to the centre of scattering mass weighted by the excess electron density.

Guinier plot for ideal solutions

The plot of ln[I(s)] vs s^2

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

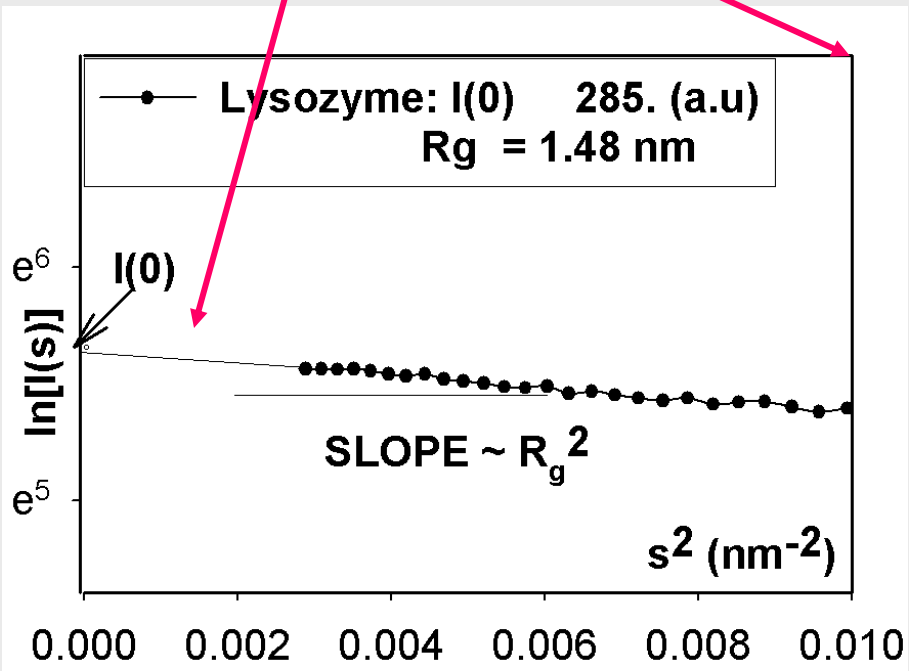


yields two parameters :

y-intercept: I(0)

R_g: from the slope

Valid for a sphere for $0 < 2\pi R_g s < 1.2$



Forward scattering I(0) and molar mass (M)

$$I(0) = \int_{V_r} \int_{V_{r'}} \rho(r)\,\rho(r')\,dV_r\,dV_{r'} = \left(\sum_i f_i \right)^2_{excess}$$

or

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

$$I(0) = (m - m_0)^2 = \left[\frac{M}{N_A} \overline{\nu}_\rho (\rho - \rho_0) \right]^2$$

Number of electrons

$$I(0) = \frac{cMV}{N_A} \left[\overline{\nu}_\rho (\rho - \rho_0) \right]^2$$

Partial specific volume

contrast

Molar mass

number of molecules

$$c = \frac{NM}{N_A V}$$

Avogadro's number

concentration (w/v)

Tip: Use a sample of similar contrast e.g. a known protein (BSA) as standard to calibrate the measurements:

$MM_{sample} = MM_{stand}.I(0)/c)_{sample}/(I(0)/_{c)stand.}$

Radius of gyration

$$R_g^2 = \frac{\int\int \rho(\mathbf{r}_1)\rho(\mathbf{r}_2)\left|\mathbf{r}_1 - \mathbf{r}_2\right|^2 d\mathbf{r}_1 d\mathbf{r}_2}{2\int\int \rho(\mathbf{r}_1)\rho(\mathbf{r}_2) d\mathbf{r}_1 d\mathbf{r}_2}$$

$$R_g^2 = \frac{\int_V \rho(\mathbf{r}) r^2 d\mathbf{r}}{\int_V \rho(\mathbf{r}) d\mathbf{r}}$$

If one places the origin of the coordinates at r_0, the centre of scattering mass of the particles, this yields the expression for the radius of gyration which is obtained from a Guinier plot, Or even better, calculated from the whole experimental scattering pattern:

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

R_g is the second moment (standard deviation) of the electron density distribution.

Guinier's formula and the contrast

$$I(s)=\overline{\rho^2}V^2\left(1-\frac{4}{3}\pi^2R^2s^2+\ldots\right)=\overline{\rho^2}V^2\exp\left(-\frac{4}{3}\pi^2R^2s^2\right)\text{ for }\frac{4}{3}\pi^2R^2s^2\leq 1$$

where R is the radius of gyration and I(0) the forward scattering is: $\overline{\rho^2}V^2$

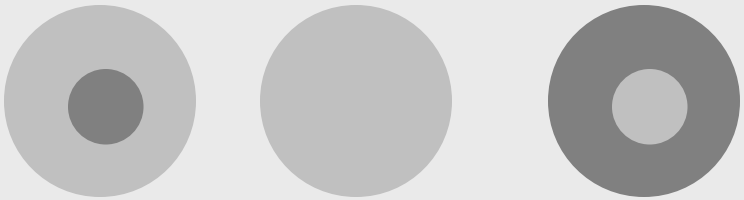
If the contrast changes so do I(0) and R:

$$R^2=R_c^2+\frac{\alpha}{\overline{\rho}}-\frac{\beta}{\overline{\rho^2}}$$

displacement of centre of scattering mass with contrast

radius of gyration at infinite contrast

second moment of internal structure



$\alpha<0$ $\alpha=0$ $\alpha>0$

$\beta=0$ $\beta>0$

Contrast: X-rays

Substance	X-rays
Proteins	2.5
Nucleic acids	6.7
Fatty acids	-1.1
Carbohydrates	4.5

One can change the contrast e.g. by adding salts like CsBr but this has disadvantages like increasing absorption, fluorescence and changing ionic strength.

The alternative is to use anomalous scattering (see e.g Stuhrmann HB. Acta Crystallogr A. 2008, 64:181-91) but beware of radiation damage.

TIP: If you need to change the contrast USE NEUTRONS!

Average contrast (X 10^{10} cm^{-2}) of biological macromolecular assemblies in water.

Typical values of radii of gyration

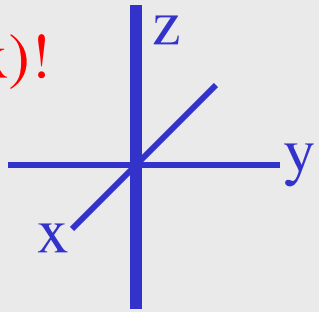
	M_w	R_g(nm)
Ribonuclease	12700	1.48
Lysozyme	14800	1.45
B-lactoglobulin	36700	2.17
Bovine serum albumin	68000	2.95
Myosin	493000	46.8
Brome mosaic virus	$4.6\ 10^6$	13.4
Tobacco mosaic virus	$3.9\ 10^7$	92.4

For a sphere of radius R: $$R_g^2 = \frac{3}{5} R^2$$

For an infinite rod

or a long fiber with its axis along z, the transform is limited to the X,Y plane (i.e. the cross-section)

Remember δ(x)!



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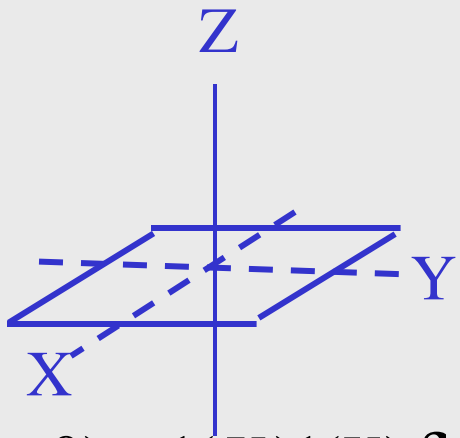


$$f(0,0,\infty) = \delta(x)\delta(y)1(z) \qquad F(\infty,\infty,0) = 1(X)1(Y)\delta(Z)$$

In such a case the radius of gyration of the cross-section and the mass/unit length can be derived using a representation analogous to the Guinier plot with a plot of sI(s) *vs* s^2 to obtain

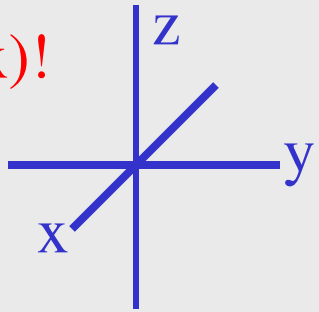
$$sI(s) = \frac{m}{L}\exp(-2\pi^2 R_c^2 s^2)$$

For a circular cross-section
$R_c = R/\sqrt{2}$

For a flat object like a membrane

With a small thickness (T) along z, the transform is limited to the Z-axis (i.e. the thickness)

Remember δ(x)!



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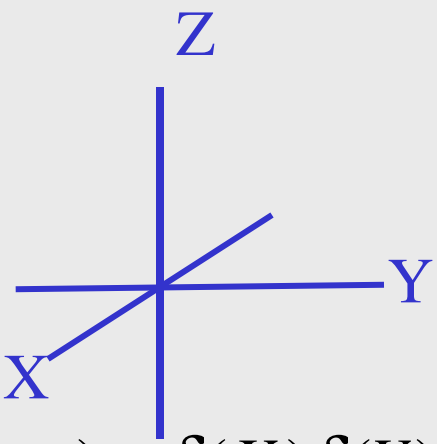


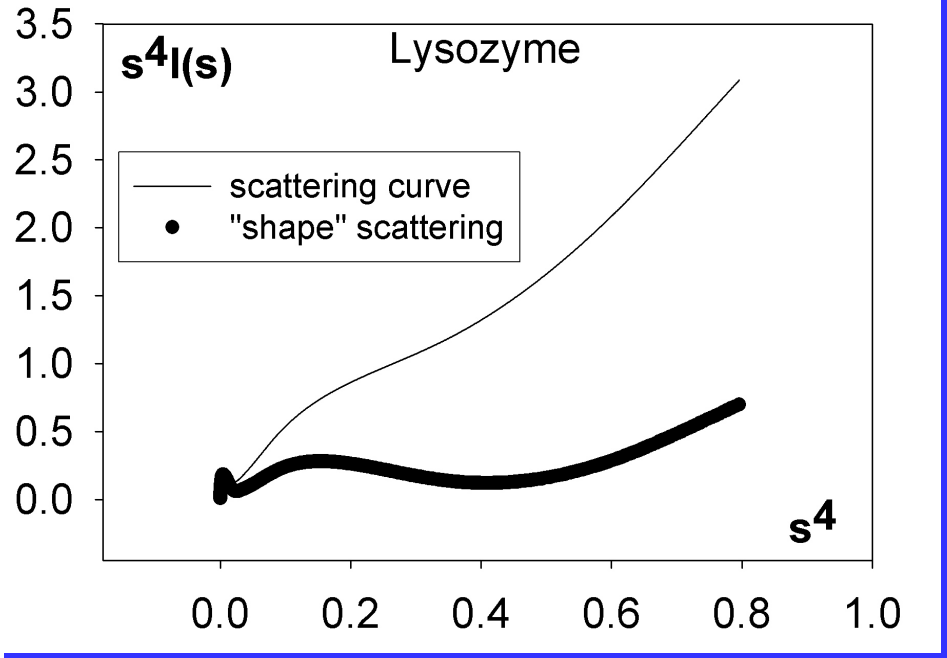
$$f(\infty,\infty,0) = 1(x)1(y)\delta(z) \qquad F(0,0,\infty) = \delta(X)\delta(Y)1(Z)$$

In such a case the radius of gyration of the thickness and the mass/unit area are obtained from a plot of $s^2I(s)$ *vs* s^2 to obtain

$$s^2 I(s) = \frac{m}{A}\exp(-4\pi^2 R_T^2 s^2)$$

with $R_T = T/\sqrt{12}$

Shape scattering

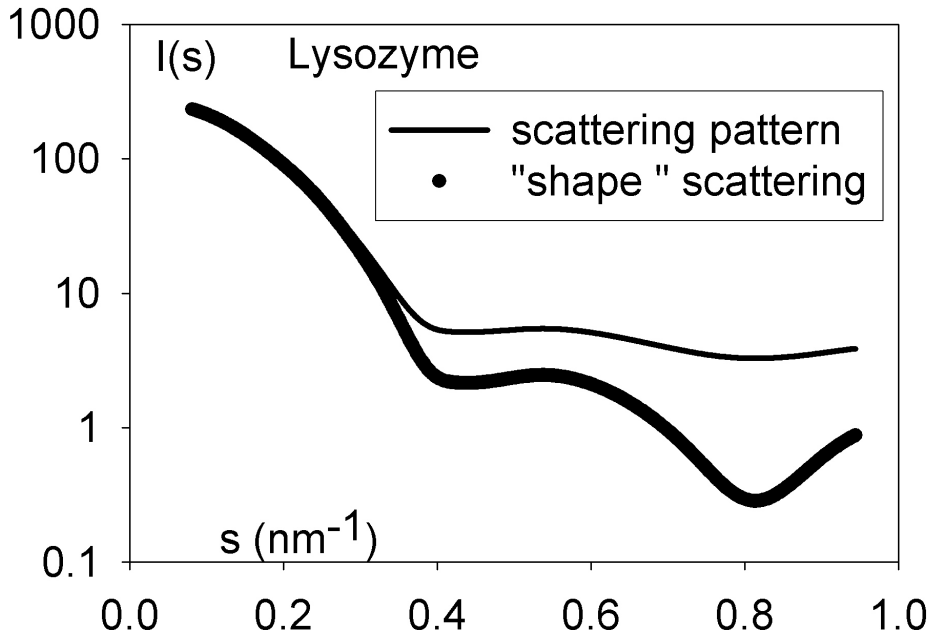


Subtract a constant equal to the slope of the Porod plot to obtain an approximation to the

SHAPE scattering

At larger s-values the scattering of a particle with $\rho_s \neq 0$ oscillates around a straight line given by POROD's law:

$$s^4 I(s) = Bs^4 + A$$



Mixtures

$$I(s) = \sum_{i=1}^{N} n_i I_i(s) \qquad R^2 = \sum_{i=1}^{N} n_i I_i(0) R_i^2 / I(0)$$

n_i: number concentration of the i^{th} species, with forward scattering $I_i(0)$ and radius of gyration R_i.

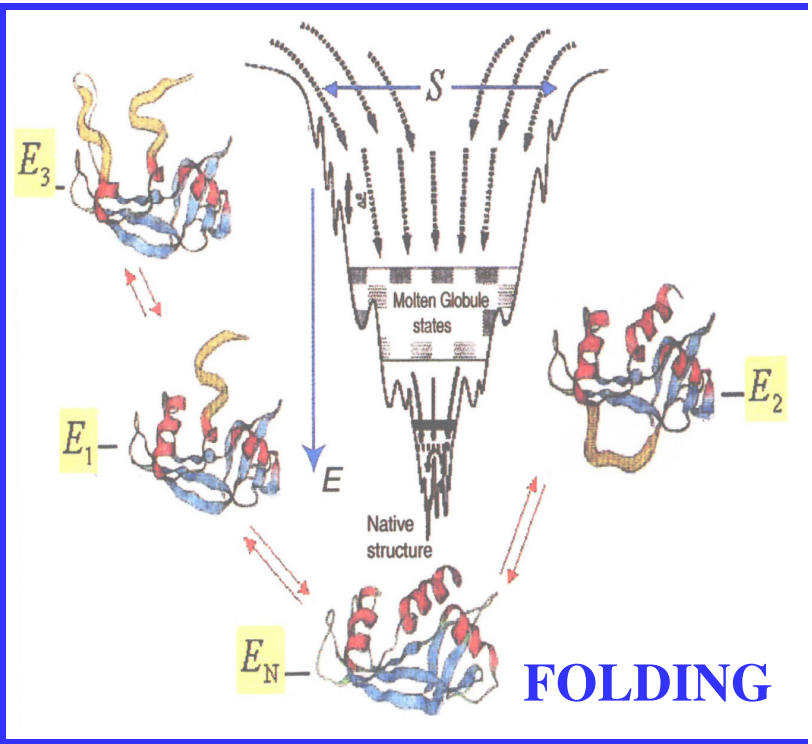
These formula illustrate that a small quantity of high molecular mass or hight contrast particles will have a large influence on the scattering curve.

For modeling it is thus indispensable to
make sure that the solutions do not contain aggregates!

Proteins at low resolution

Hydration shell

solvent



Coupled equilibria
Non-contact interactions

Interactions/ stability/activity modulated by

IONS:
Kosmotropes e.g. Na^+
Chaotropes e.g. K^+

Crowding max. conc.
300-500mg/ml

OSMOLYTES
e.g. free amino acids
polyhydroxy alcohols
methylated ammonium and sulfonium compounds
urea.

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 $x = \left(2\pi R_g s\right)^2$

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).

Debye law for extended chains: NCS unfolding

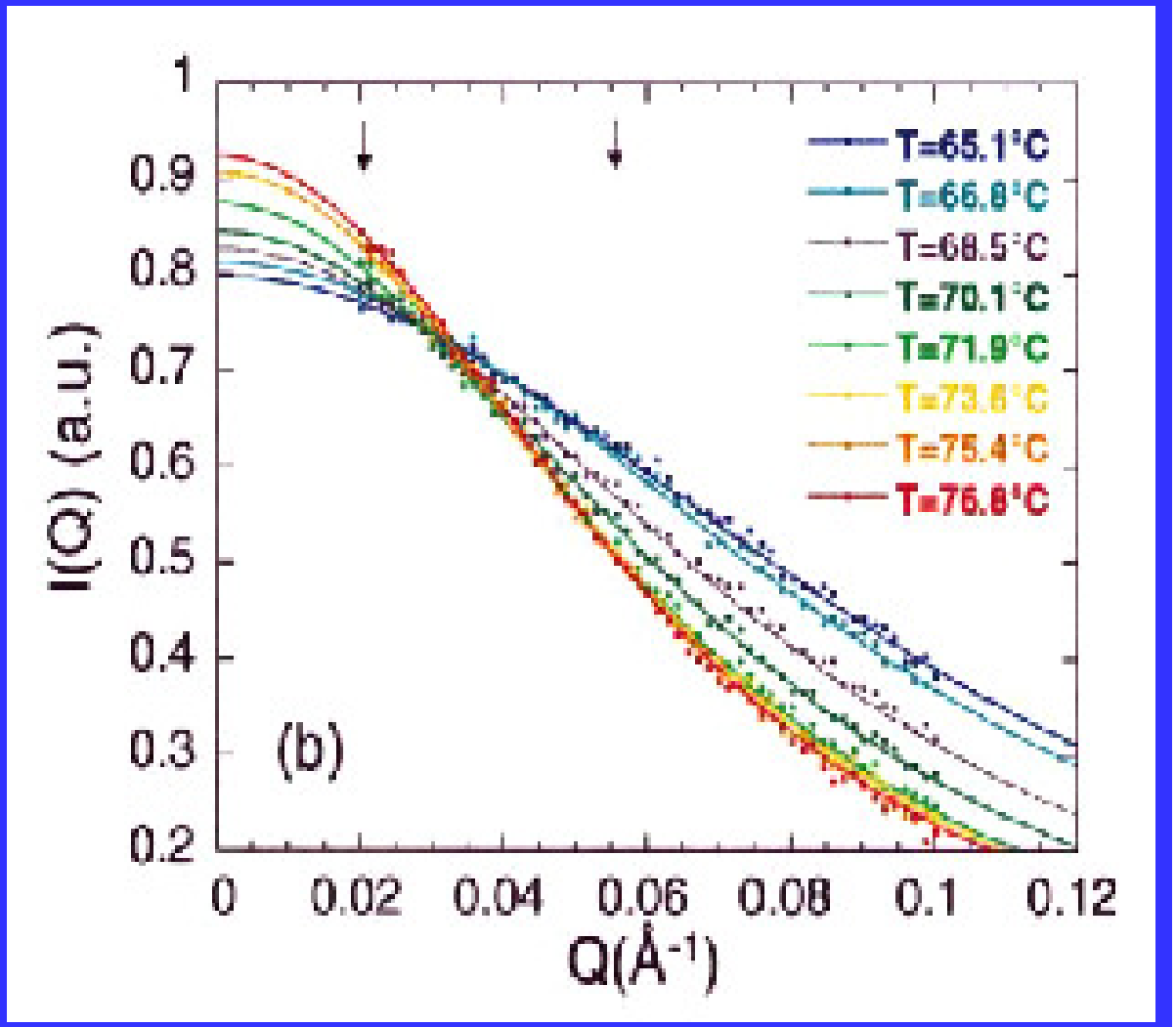
Neocarzinostatine : small (113 residue long) all-β protein.

arrows : angular range

used for R_g determination

$$\frac{I(s)}{I(0)} = \frac{2}{x^2}(x - 1 + e^{-x})$$

$$x = \left(2\pi R_g s\right)^2$$



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

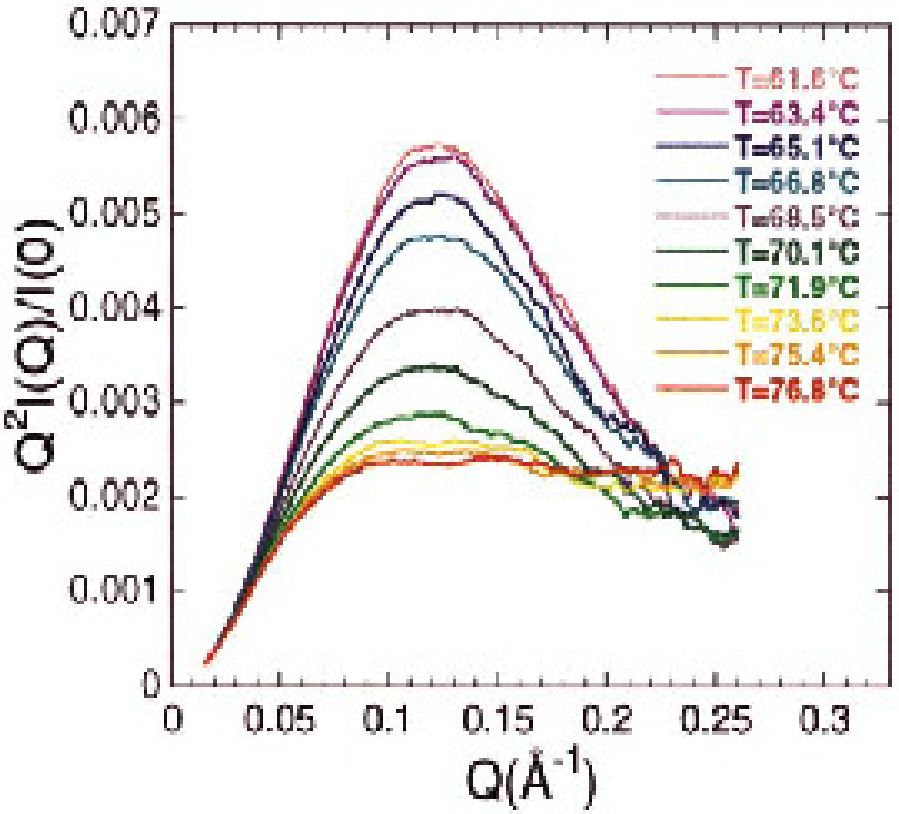
Kratky plot: $s^2I(s)$ vs s

Is sensitive to the degree of compactness of a protein.

Globular particle : bell-shaped curve

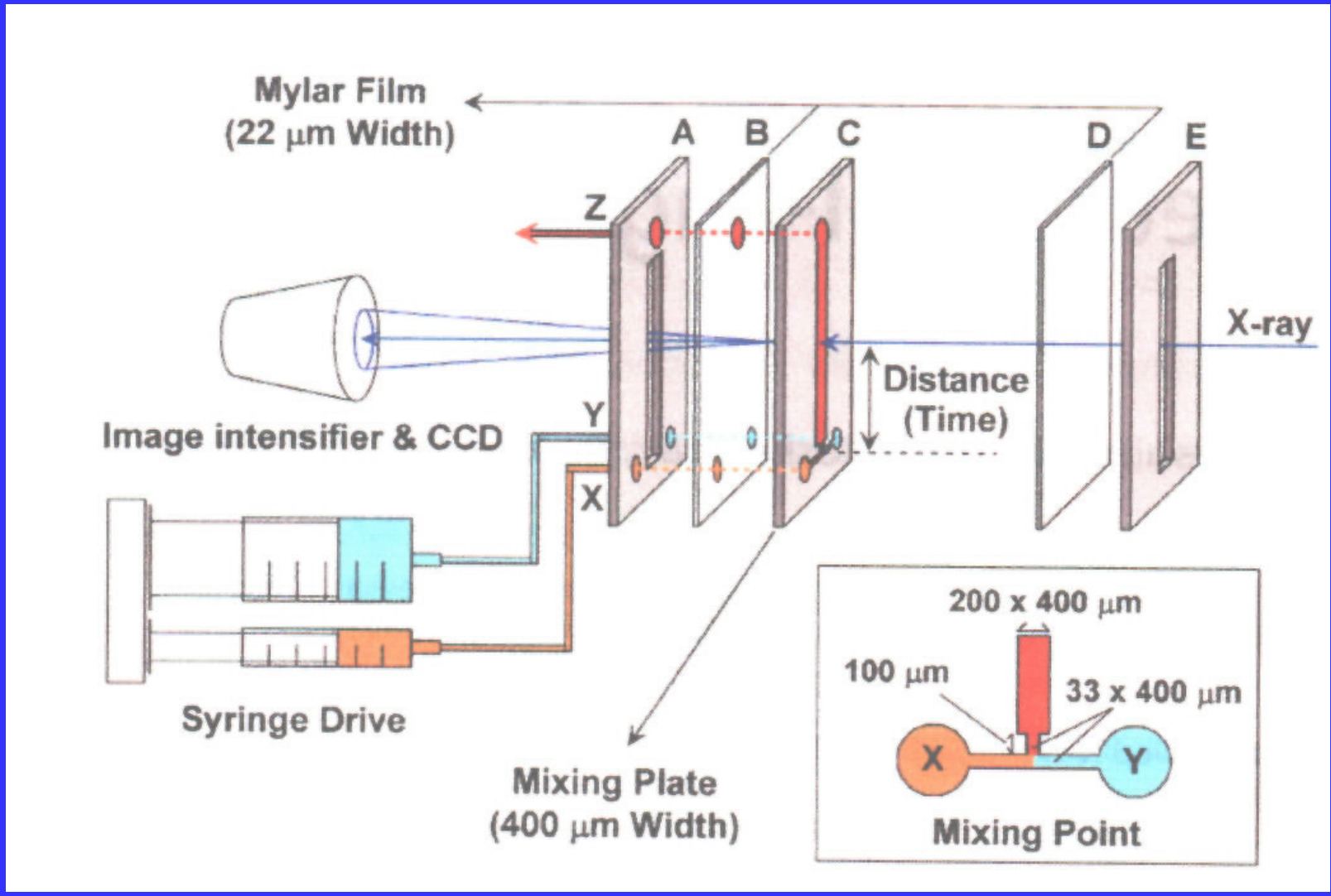
Gaussian chain : plateau at large s-values but a plateau does not imply a Gaussian chain!

In thermal unfolding of NCS the Kratky plot has a plateau although unfolded NCS is not a Gaussian chain when unfolded.
A thick persistence chain is a better model in this case.



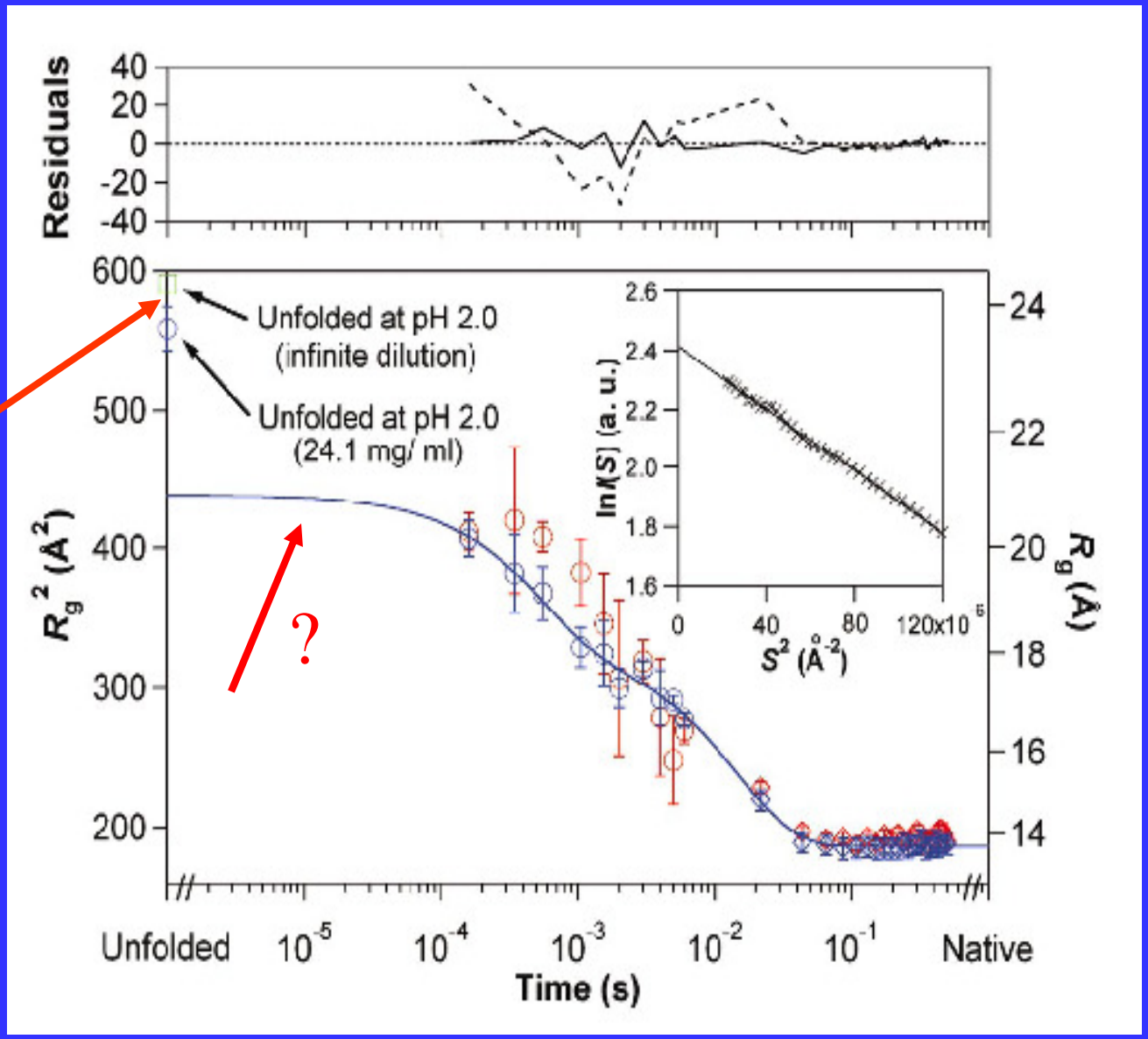
Pérez et al., *J. Mol. Biol.*(2001), 308, 721-743

Protein folding: cytochrome c



Akiyama, S. et al. (2002) Proc. Natl. Acad. Sci. USA, 99, 1329-34

Protein folding: cytochrome c

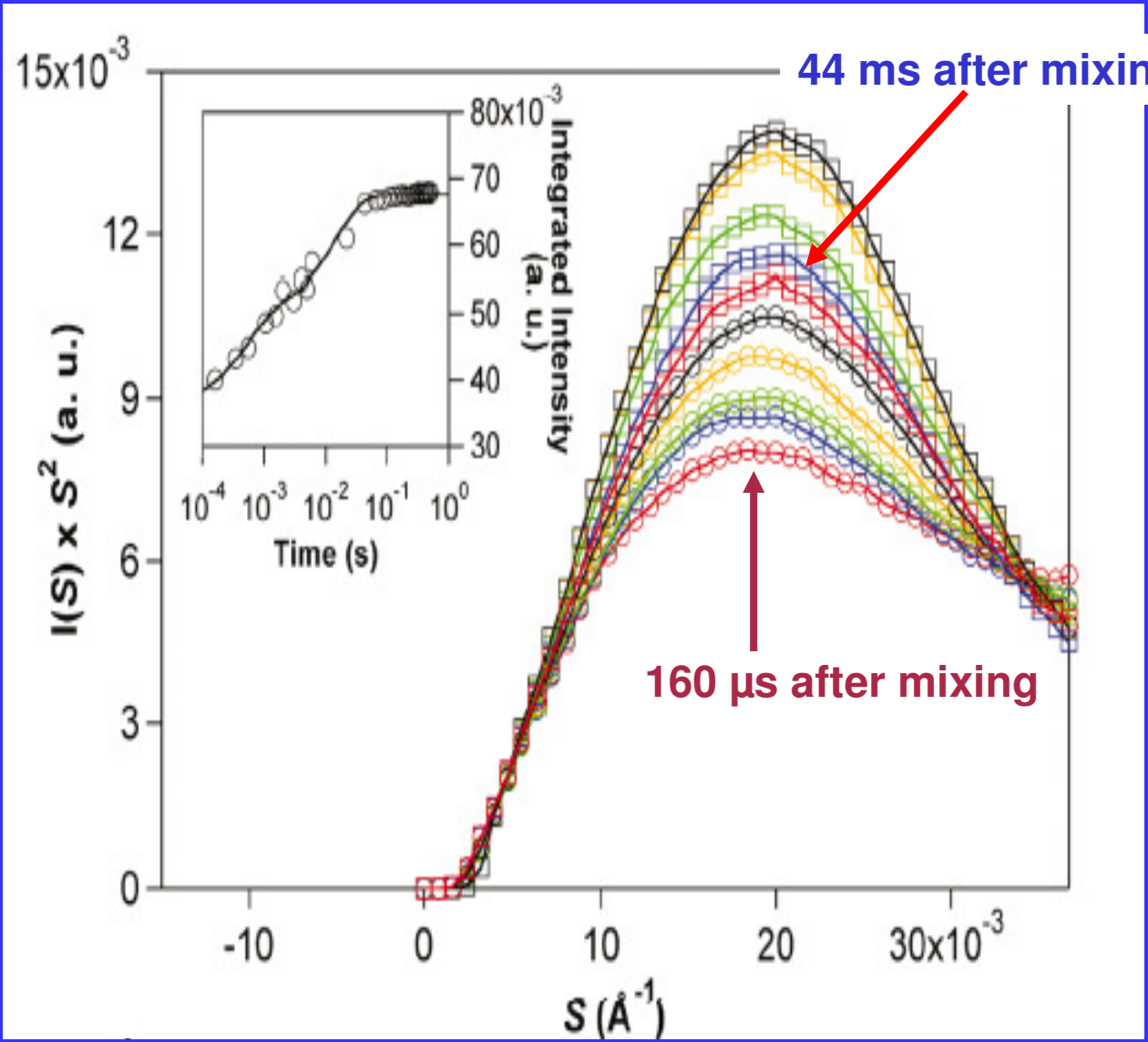


Initial Ensemble ?

expect >900 Å^2 for random chain !

Akiyama, S. et al. (2002) Proc. Natl. Acad. Sci. USA 99, 1329-34

cytochrome c folding



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

Labeling

Matthew-Fenn R.S. et al. (2008)
Science 232, 446-9

(| | D | L1 | L2 | | + | | D | L1 | L2 | |) - (| | D | L1 | L2 | | + | | D | L1 | L2 | |

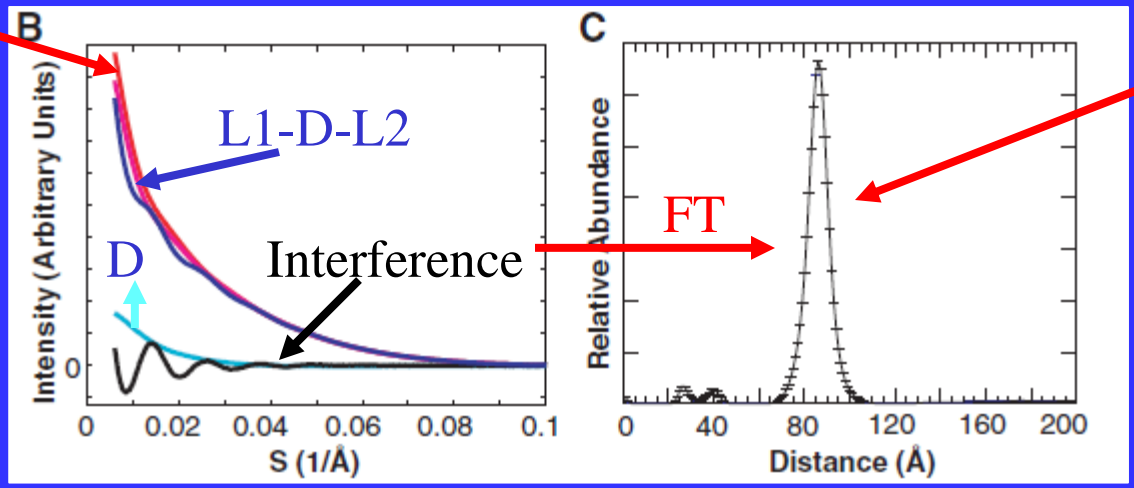
	D	L1	L2
D	X	X	X
L1	X	X	X
L2	X	X	X

	D	L1	L2
D	X	0	0
L1	0	0	0
L2	0	0	0

	D	L1	L2
D	X	X	0
L1	X	X	0
L2	0	0	0

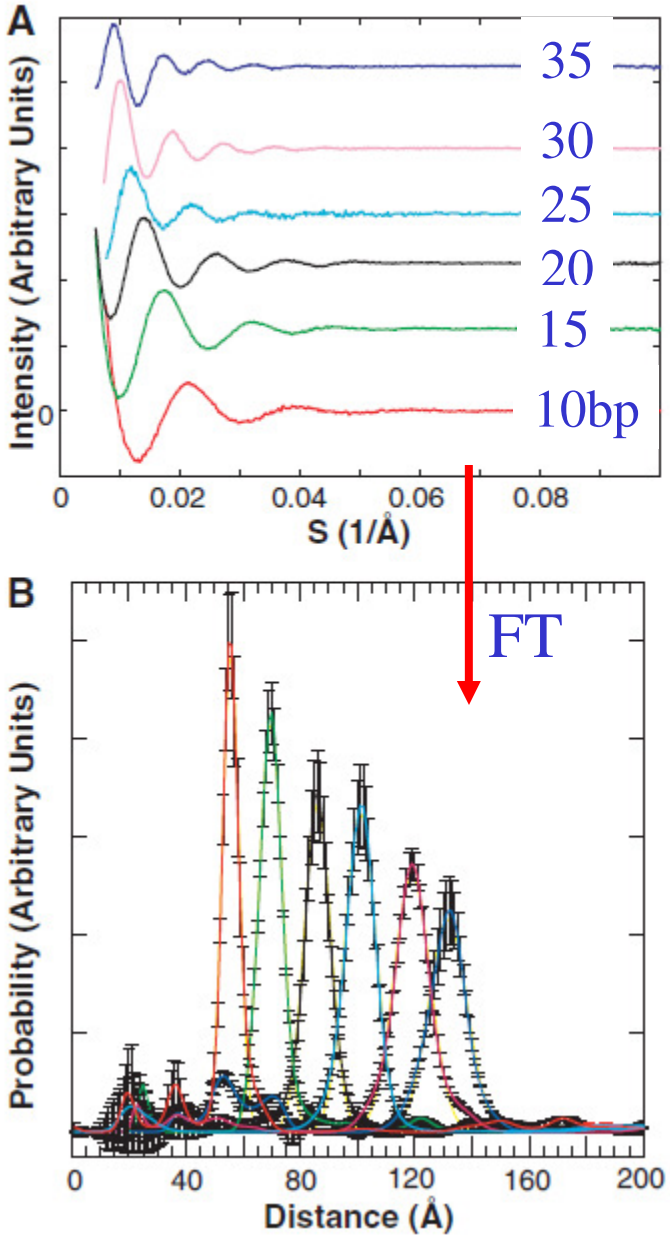
	D	L1	L2
D	X	0	X
L1	0	0	0
L2	X	0	X

L1-D or L2-D



Distance between L1 and L2

Interference



Note the difference in position and width (variance) of the distribution of end to end distances as a function of the number of base pairs between labels.

In absence of applied force DNA is at least 1000 softer than in single molecule stretching experiments. Stretching is cooperative over more than two turns of the double helix. DNA is not an elastic rod.

What SAXS/SANS have to offer