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Basics of X-ray Scattering

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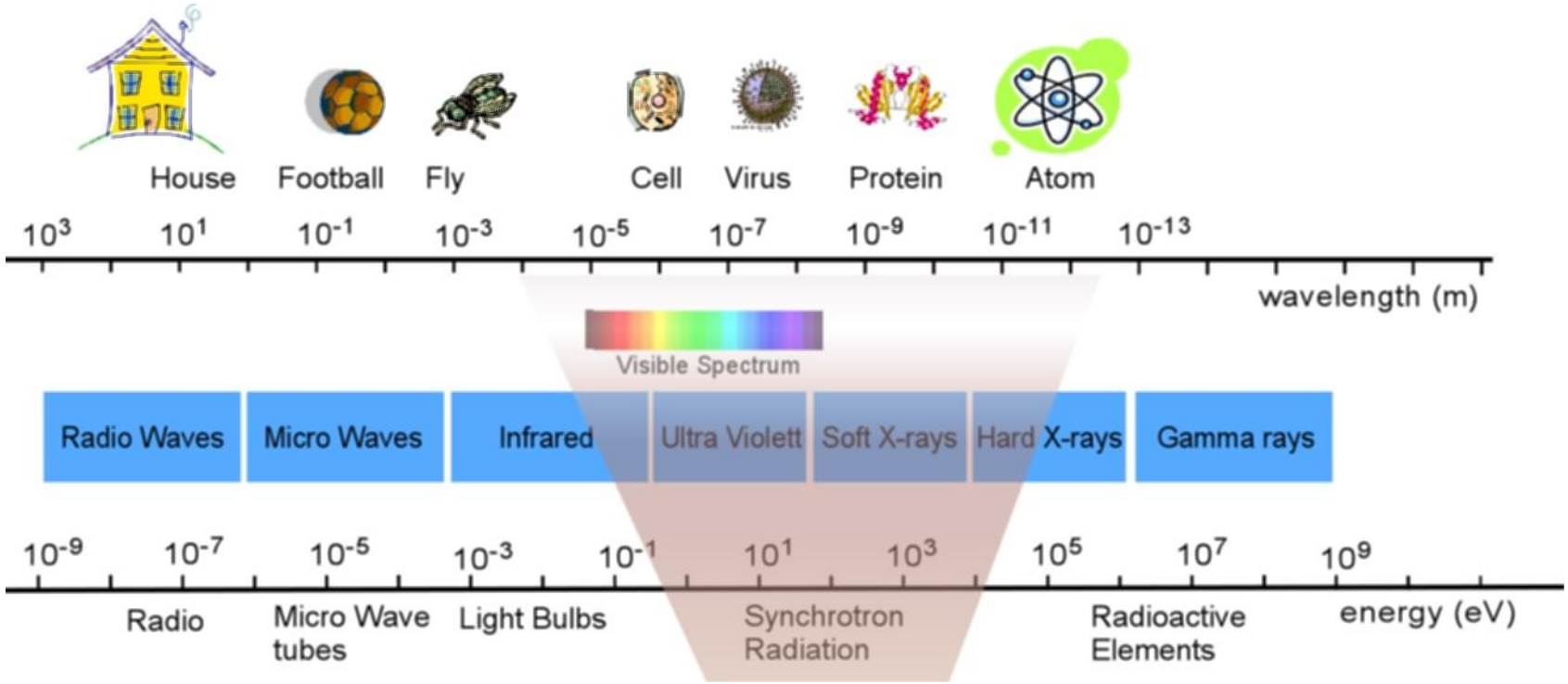
The Electromagnetic Spectrum



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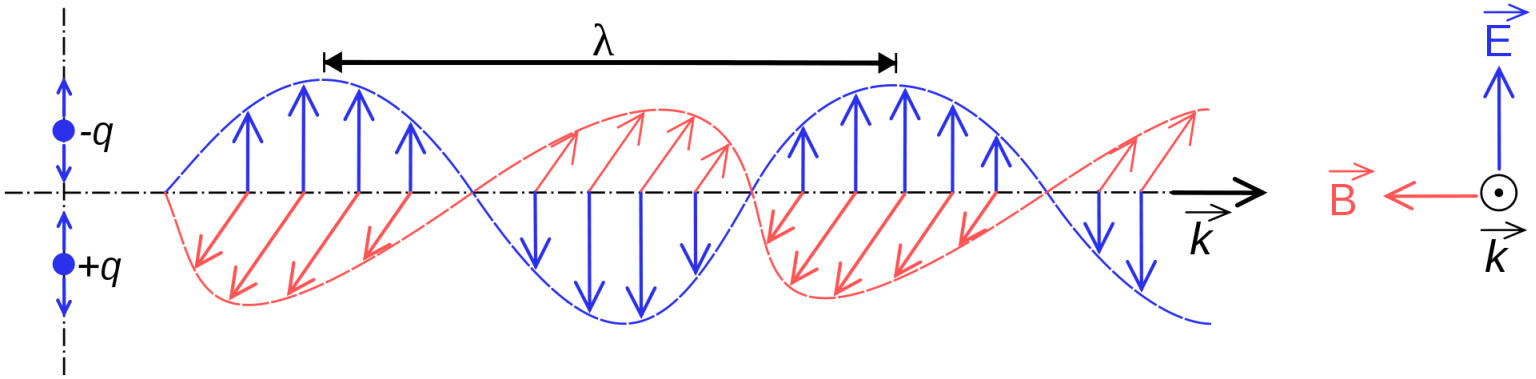
X-ray Scattering

Description of a plane electro-magnetic wave

Electromagnetic waves:



The electromagnetic waves that compose electromagnetic radiation can be imagined as a self-propagating transverse oscillating wave of electric and magnetic fields. This diagram shows a plane linearly polarized EMR wave propagating from left to right. The **electric field** is in a vertical plane and the magnetic field in a horizontal plane. The two types of fields are always in phase with each other, and no matter how powerful, have a ratio of electric to magnetic intensity which is fixed and never varies.

Source: Wikipedia

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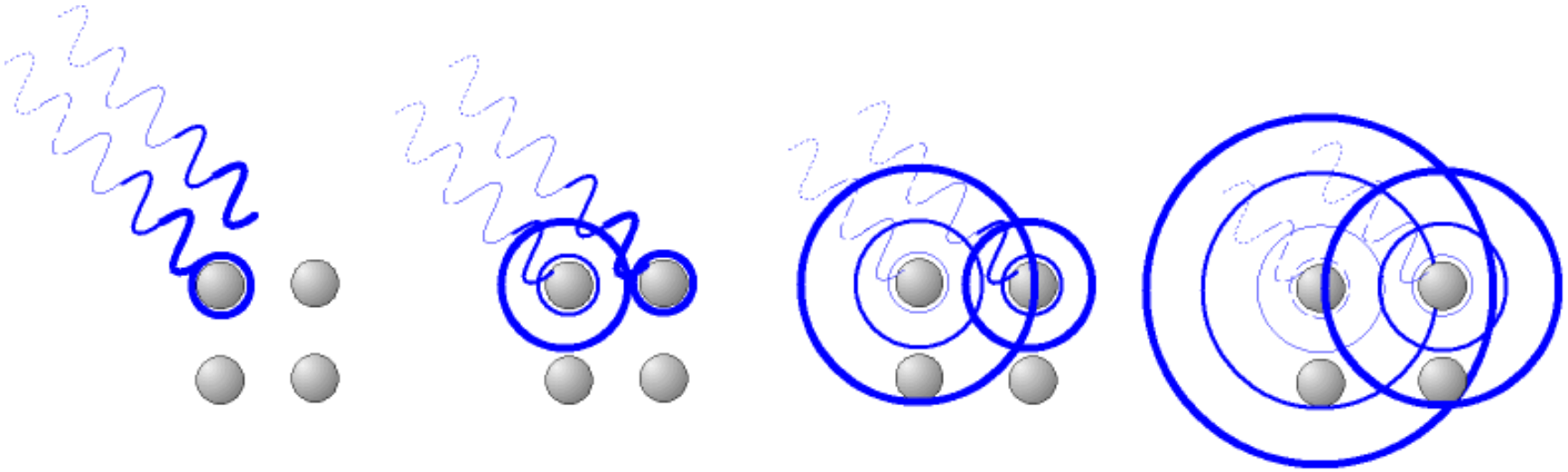
X-ray scattering



X-rays are scattered at the electrons of the atomic shell. During the scattering process the electron is starts oscilating. It becomes a dipol and a spherical wave is sent out. The wavelength and energy of the scattered wave does not change (elastic scattering).

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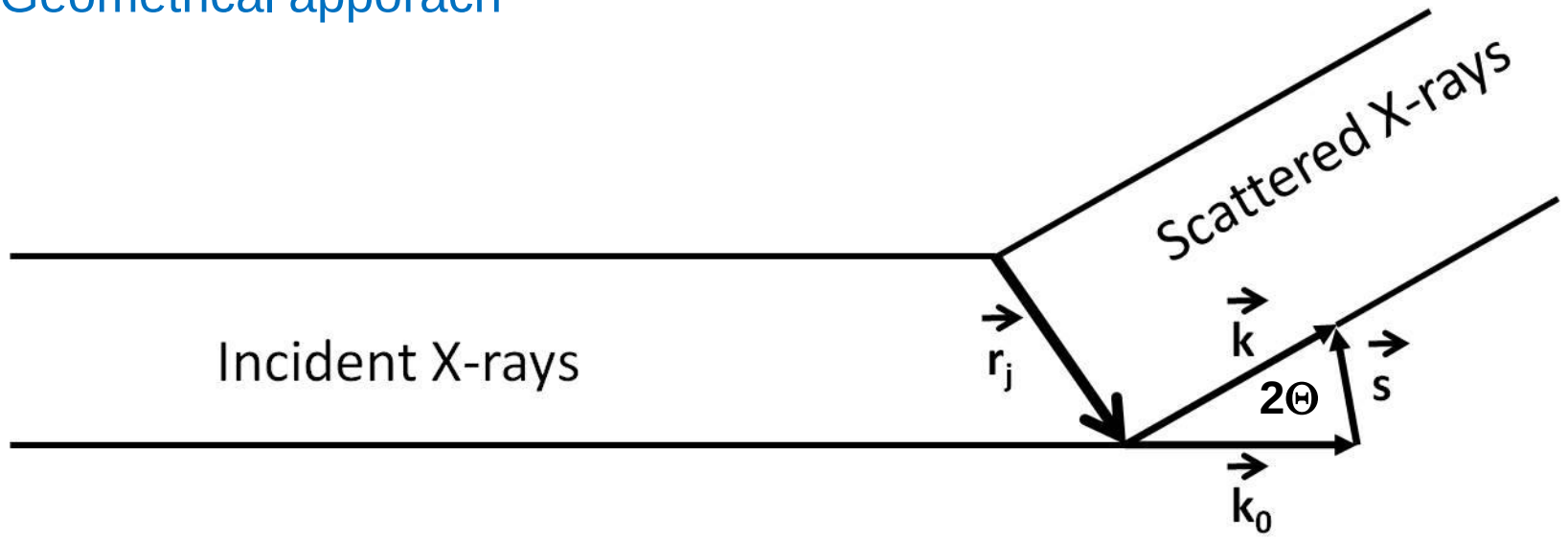
X-ray scattering

Geometrical apporach



Scattering vector $\vec{s} = (\vec{k} - \vec{k}_0)$

Scattering angle 2Θ

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X-ray scattering

Scattering from a single electron

For a single scattering process the amplitude $\boldsymbol{A_j}$ of scattered X-ray photons can be described as a plain wave scattered by an ensemble of atoms:

$$A_j = b_j \, e^{-i\frac{2\pi}{\lambda}\left(\vec{k}_0 - \vec{k}\right)\cdot\vec{r}}$$

With b_j is the scattering cross section, the r_j describes the inner distance vector and the vector k_0 is the wave vektor of the incident wave.

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X-ray scattering

Scattering from an ensemble

Within an particle, the scattering amplitudes of all atoms have to be summed up:

$$A(\vec{k}) = \sum_j b_j \, e^{-i\frac{2\pi}{\lambda}(\vec{k}_0 - \vec{k})\cdot\vec{r}_j}$$

Or, using $\vec{s} = (\vec{k} - \vec{k}_0)$ as **scattering vector**

$$A(\vec{s}) = \sum_j b_j \, e^{-i\frac{2\pi}{\lambda}\vec{s}\cdot\vec{r}_j}$$

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X-ray scattering

Scattering intensity

The scattering amplitude is experimentally not accessible, but the scattering intensity. The intensity is the product of the scattering amplitude with ist complex conjugate and results to:

$$AA^* = I(\vec{s}) = \sum_j \sum_k b_j \, b_k \, e^{\vec{s} \cdot \vec{r}_{jk}}$$

With the vector r_{jk} as inner distance vector, pointing from the j^{th} scatting center to the k^{th} scattering center.
The b_j are the atomic scattering densities. They depend on the number of electrons of the atom and the heavier an atom the higher is b_j.

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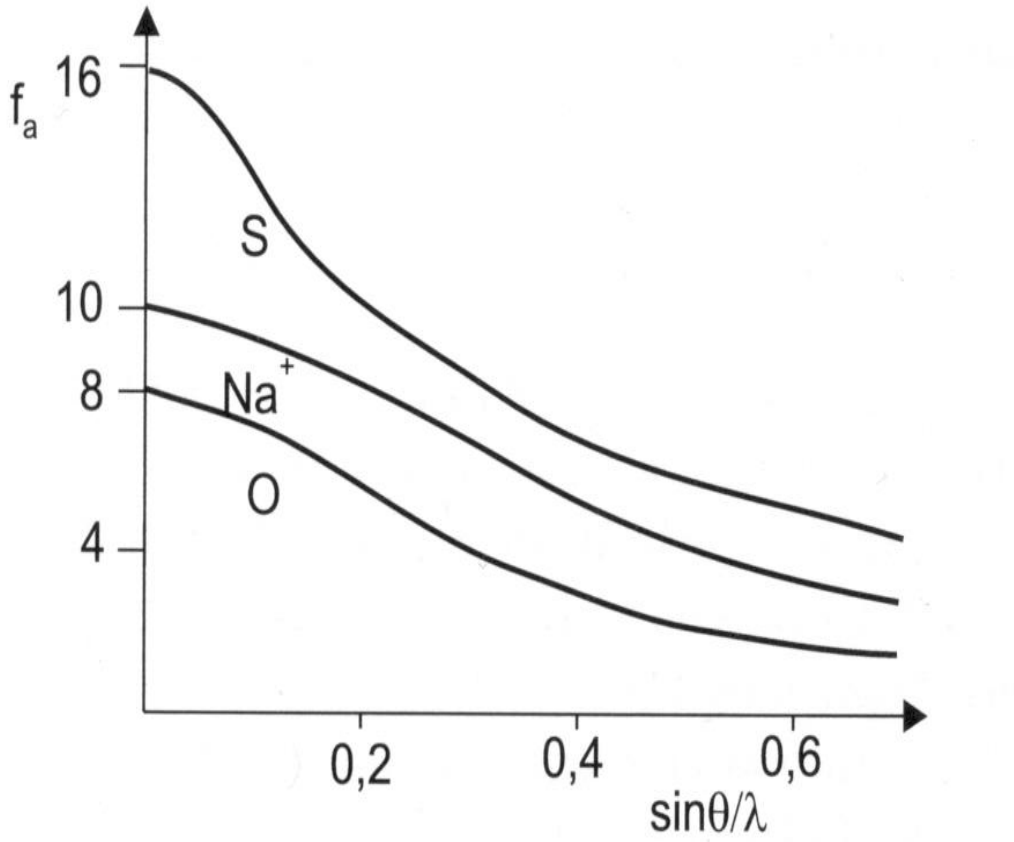
X-ray scattering

Atomic scattering factors

The atomic scattering factor is the Fourier Transform of the atomic electron density.

It can be calculated by quantum mechanics and can be found at International Tables of Crystallography, Volume C



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X-ray scattering

Electron density

As seen on the atomic scattering factors, the scattering density of the depends not only on the number of electrons, but as well on the distribution of these electrons around the atom.

This electron density ρ can be defind as follows:

$$b_j = \rho_j \, dV_j$$

With the dV_j as the volume element surrounding the scattering center.

The reconstitution of the electron density $\rho(r)$ and its distribution in the particle is the aim of every X-ray scattering or diffraction experiment.

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X-ray scattering

Electron density

The effective scattering density depends on the electron density of the surrounding of the atom.
A net electron density can be defined by subtracting the electron density of the enironment ρ_S:

$$\Delta\rho(\vec{r}) = \rho(\vec{r}) - \rho_S$$

The average value of the excess density is called contrast, which is typically very small for biological objects containing light atoms only.

This is not true for neutron scattering where the variation of the contrast is a very powerfull technique.

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X-ray scattering

Scattering from dilute samples

Scattering from an ideal solution:

- no interation between the particles
- only on species of particle
- particles are free to move

Dilute monodisperse solution fulfill these paramters!

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X-ray scattering

Scattering from dilute samples

In the ideal dilute solutions the scattering intensity from the entire sample will be isotropic and proportional to the scattering from the single particle averaged over all orientations Ω.

Averaging of the term $\left\langle e^{\vec{s}\cdot\vec{r}_{jk}} \right\rangle_{\Omega} = \frac{\sin sr_{jk}}{sr_{jk}}$ lead to:

$$I(s) = \sum_j \sum_k \rho(r_j)\rho(r_k)\frac{\sin sr_{jk}}{sr_{jk}}$$

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X-ray scattering

Scattering from dilute samples

The vectors s and r are now reduced to their absolut value, which lead to a significant loss of information in the SAXS pattern compared to e.g. crystallographic data

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

Debye 1915

$$s = |\vec{s}| = \frac{2\pi}{\lambda} \sin 2\Theta$$

The p(r) function is called the pair distance distribution function.

The I(s) is the Fourier-Transform of the p(r) function.

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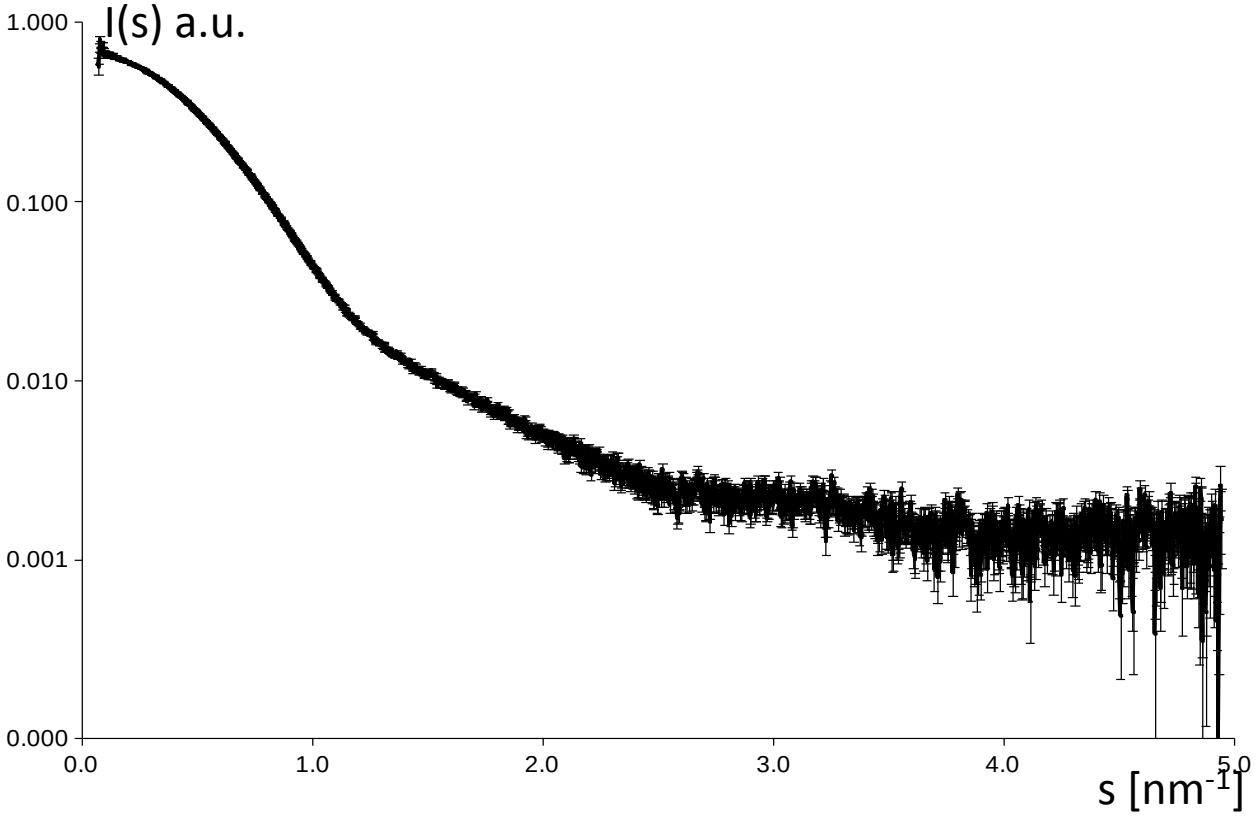
X-ray scattering

The scattering function I(s)



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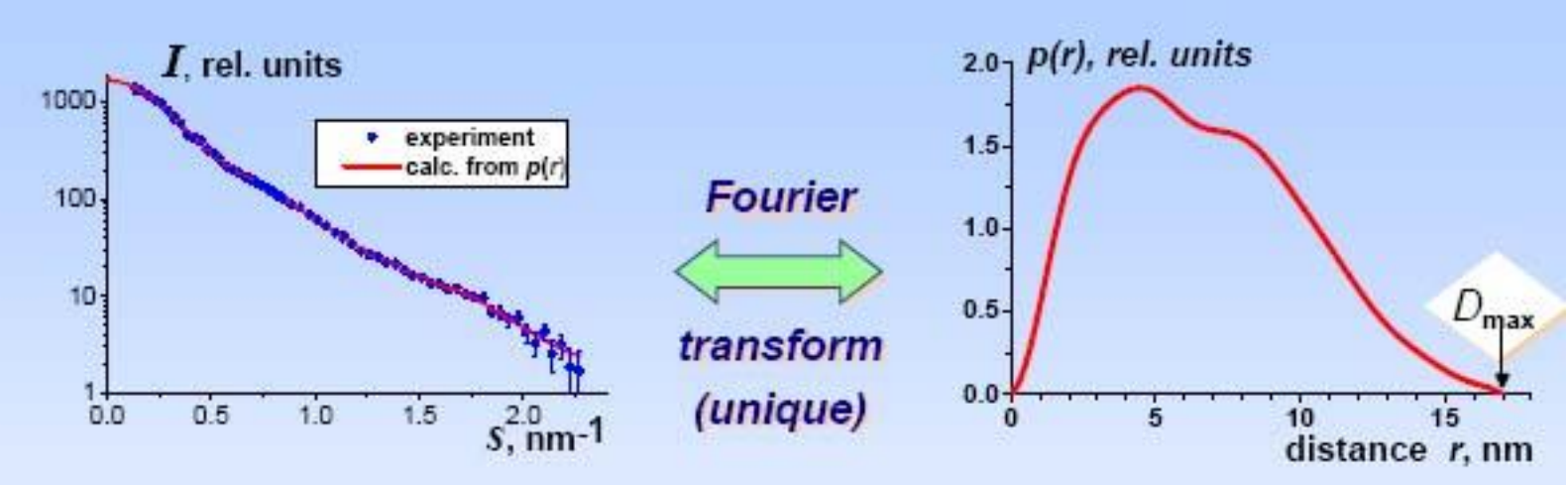
X-ray scattering

Pair distance distribution function p(r)



$$I(s) = 4\pi \int_{r=0}^{\infty} p(r) \frac{\sin(sr)}{sr} dr$$

$$p(r) = \frac{1}{2\pi^2} \int_{s=0}^{\infty} s^2 I(s) \frac{\sin(sr)}{sr} ds$$

Distance distribution function
"**how often which distance**" appears in the particle

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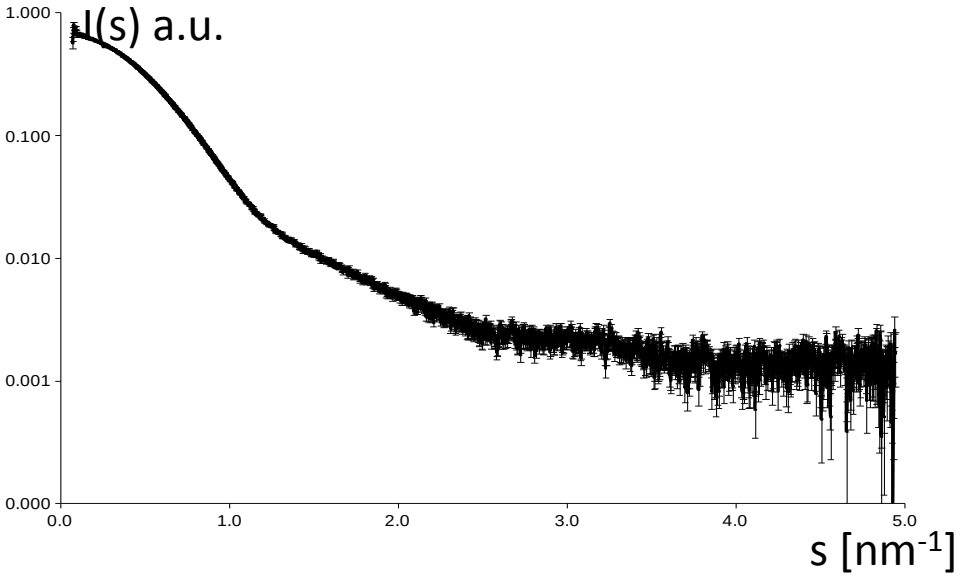
X-ray scattering

Analysis of the scattering intensity



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

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X-ray scattering

Radius of gyration – Guinier plot

Expanding the sin-function in the scattering intensity I(s) in a series

$$\left(\frac{\sin sr}{sr} = 1 - \frac{s^2 r^2}{6} + \frac{s^4 r^4}{120} - \cdots\right)$$

, the Debye-Function can be expressed as

$$I(s) \cong \int_0^\infty p(r)\left(1 - \frac{s^2 r^2}{6} + \cdots\right) dr = \int_0^\infty p(r)\, dr - \frac{s^2}{6}\int_0^\infty p(r)\, r^2 dr + \cdots$$

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X-ray scattering

Radius of gyration – Guinier plot

The second moment of the distance distribution function p(r) is given by:

$$\int_0^\infty p(r)\, r^2\, dr$$

and the radius of gyration Rg can be introduced in analogy with classical mechanics:

$$Rg^2 = \frac{1}{2} \frac{\int_0^\infty p(r)\, r^2\, dr}{\int_0^\infty p(r)\, dr}$$

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X-ray scattering

Radius of gyration – Guinier plot

Putting the things together one gets:

$$I(s) \cong \int_0^\infty p(r)\left(1 - \frac{Rg^2}{6}s^2 + \cdots\right)dr$$

And finally the Guinier approximation:

$$I(s) \cong I_0\, e^{-\frac{Rg^2}{3}s^2}$$

Guinier and Fournet 1955

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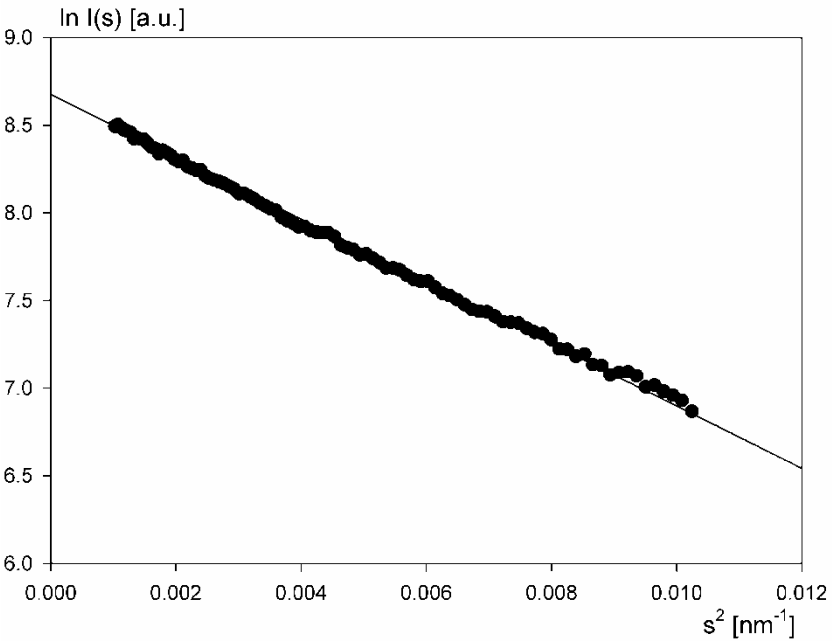
X-ray scattering

Radius of gyration – Guinier plot



Guinier-Approximation:

- every scattering curve is for sRg < 1.2 a Gauss function
- In the so called Guinier-Plot (ln I versus s^2) the Rg can be calculated from the slope of the plot
- The Rg is directly related to the form and the mass distribution of the particle

$$\ln I(s) = -\frac{Rg^2}{3} s^2 + \ln I_0$$

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X-ray scattering

Radius of gyration – Guinier plot

The Rg is historically the first structural parameter determined from the SAXS data (Guinier and Fournet 1955), yielding direct information about the particle size and shape.

The value of I_0 is proportional to the squared number of electrons in the particle and to the particle concentration, so that absolute measurements allow one to determine the molecular mass of the solute.

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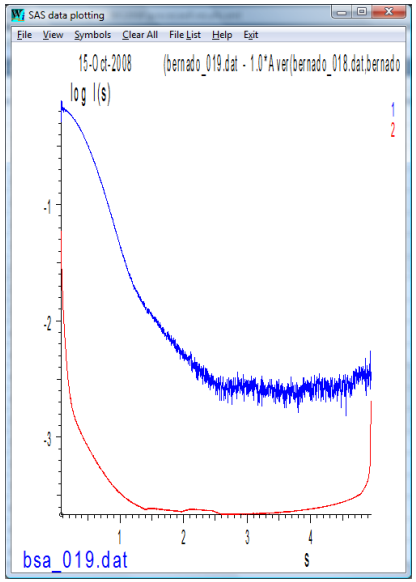
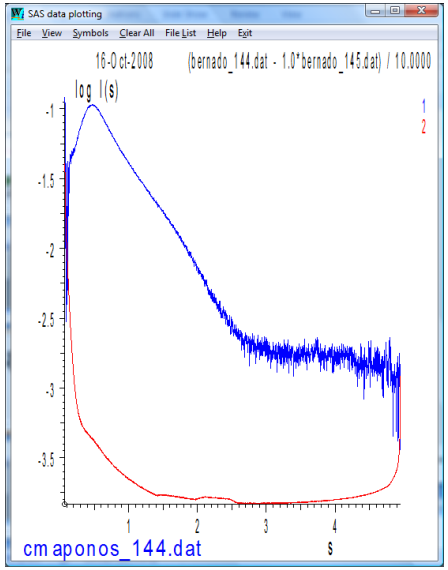
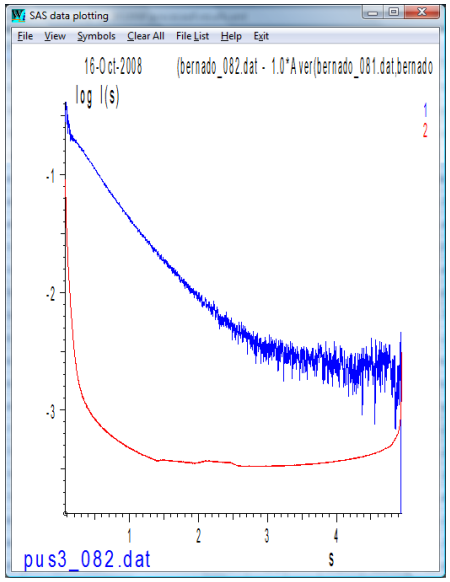
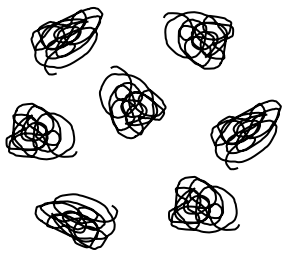
X-ray scattering
Guinier approximation



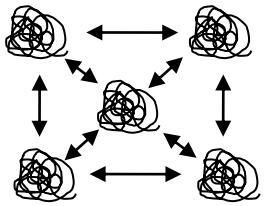




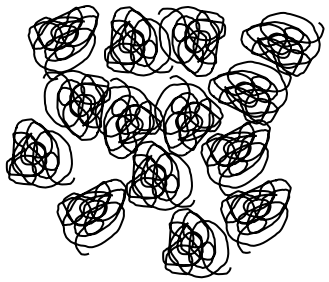
Deviations from the ideal protein solution are easily visible in the Guinier plot!



Ideal solution of particles



Repulsive particle interactions



Attractive particle interactions

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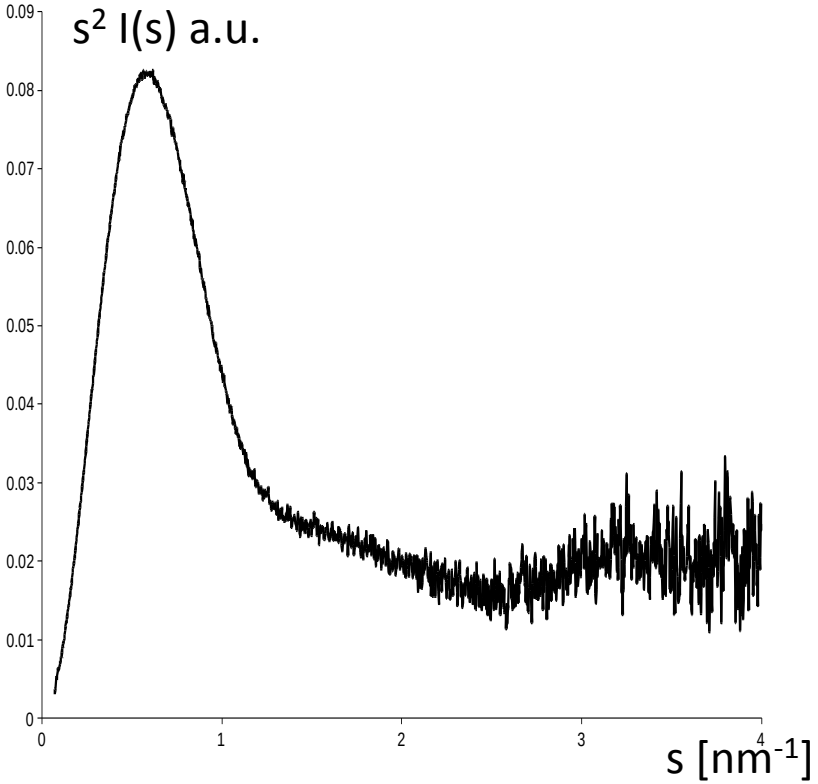
X-ray scattering

Kratky plot

Kratky plots ($I(s)\cdot s^2$ versus s) can be used to identify disordered states and distinguish them from globular particles.

The scattering intensity of a globular protein has a Gaussian-like shape at small s and decays approximately as $1/s^4$ at high s yielding a bell-shaped Kratky plot with a well defined maximum.

In the case of an unfolded protein, the Kratky plot also presents a plateau over a specific range of s, which is followed by a monotonic increase.



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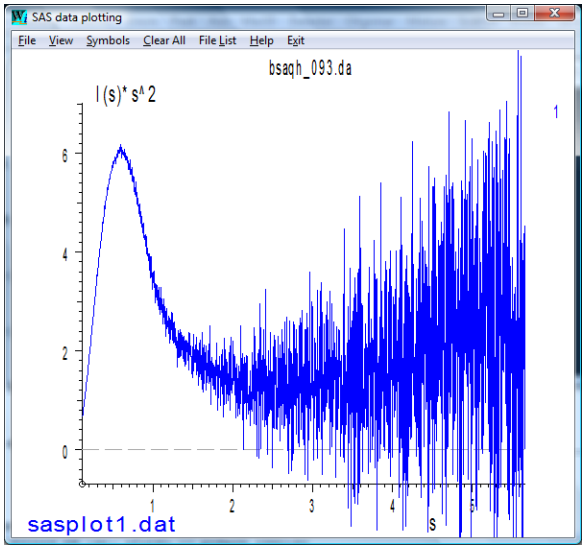
X-ray scattering

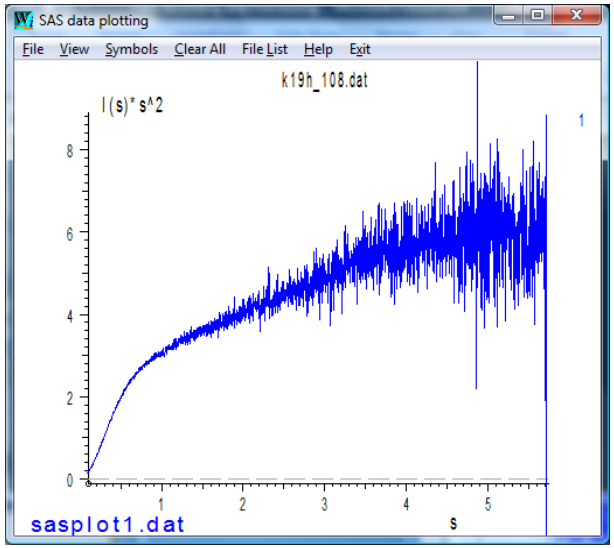
Kratky plot

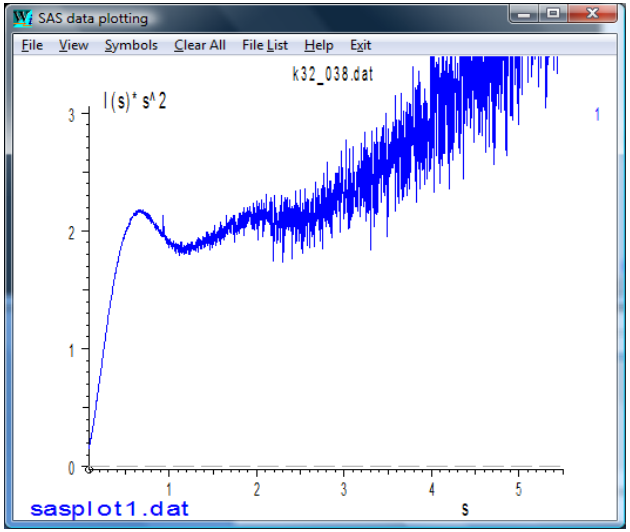
The Kratky plot is typically used to analyze the conformation of proteins, but can be used to analyze the random walk model of polymers.

A Kratky plot can be made by plotting:

$I(s)*s^2$ versus s.







Folded globular protein **Completely unfolded protein** **Partially folded protein**

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X-ray scattering
Porod invariant

Another important overall value computed from the SAXS data is the so-called Porod invariant Q:

$$\int_0^\infty s^2 I(s)\, ds = Q$$

Porod 1951

Contrary to R_g, the Porod invariant depends only on the particle volume and not on its form.

For the Porod analysis, the behavior of the scattering intensity at higher scattering vectors plays a significant role.

This higher angle part of the *I(s)* corresponds to the small interparticle distances.

Therefore in the high *s* regime the scattering from the internal structure and in particular near the particle surface S is dominating the signal.

X-ray scattering
Porod volume

For compact particles, the asymptotic behavior at large *s* is described as

$$I(s) \approx \frac{K}{s^4}$$

introducing K as a constant depended on surface of the scattering particle.

This specific surface value can be estimated directly from the asymptotic behavior of the plot

s^4 I(s) versus s

(Porod plot) at high s.

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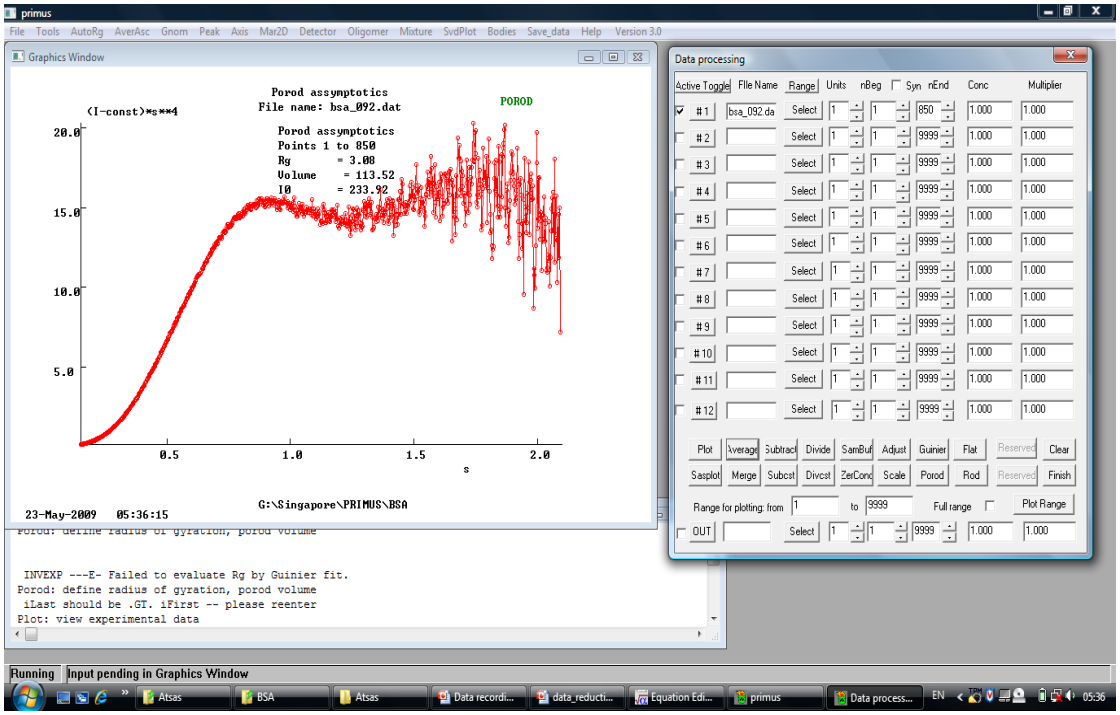
X-ray scattering

Porod plot



Porod approximation:

$I(s) = K*s^{-4}$

$$\frac{K}{Q} \propto \frac{S}{V}$$

$$Q = \int_{s=0}^{\infty} s^2 I(s)\, ds$$

Q = Porod invariant

$\frac{S}{V}$ shape to volume ratio

Rule of thumb: Porod volume is approximately two times molecular weight

Here BSA: MW 66 kDa; Porod volume 115

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Hardware and instrumentation part

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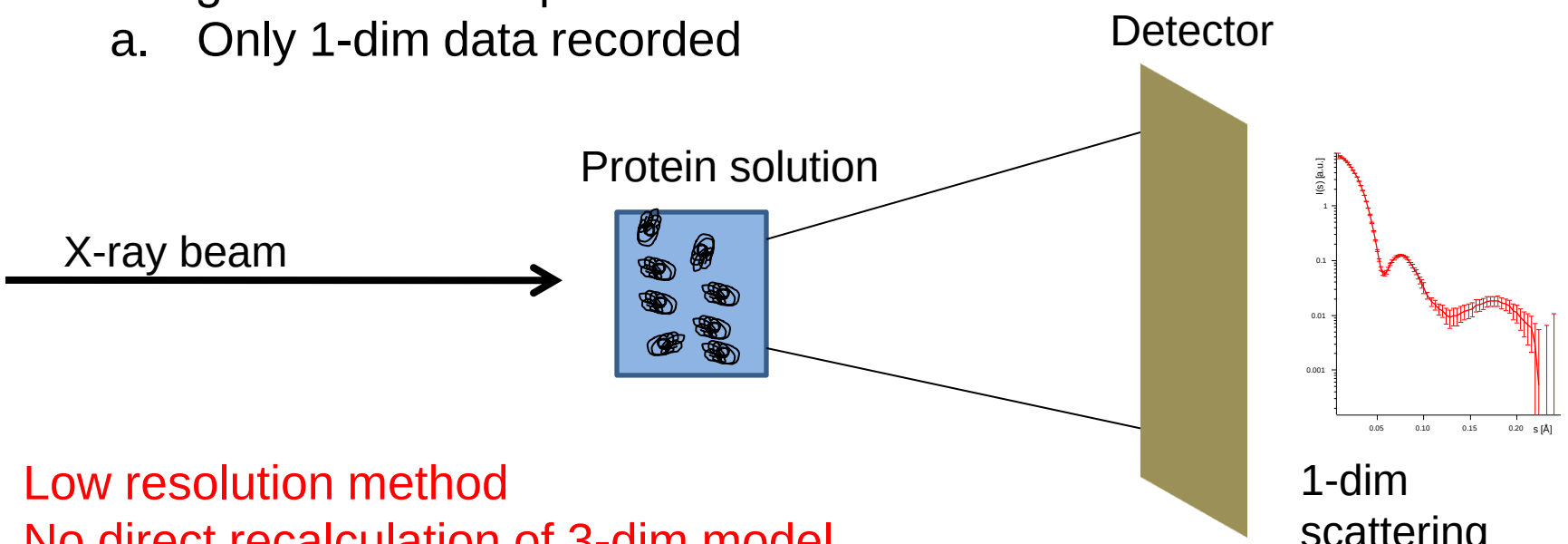
Biological Small Angle X-ray Scattering:

1. Biological SAXS is a solution scattering technique
   a. Change of environmental parameters such as temperature, pH, salt is possible
   b. No protein crystals necessary
2. "Single shot" technique
   a. Only 1-dim data recorded



1-dim scattering curve

Low resolution method
No direct recalculation of 3-dim model

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SAXS Synchrotron based

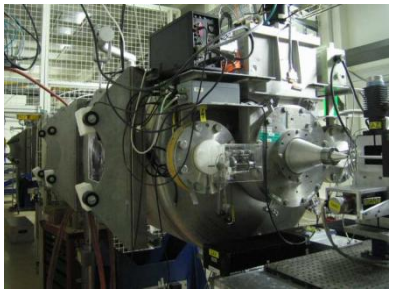
At all running synchrotron source are SAXS beamlines available!

At all new constructed synchrotrons SAXS beamlines are planned!

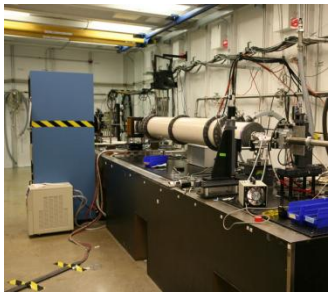
All SAXS beamlines are highly oversubscribed and “working horses” on their facilities!

The scientific applications ranging from soft condensed matter, nano-science and fiber diffraction on ordered biological systems up to structural biology in solution.

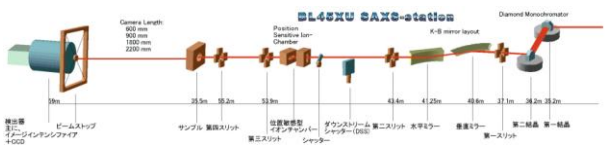
HASYLab, Hamburg



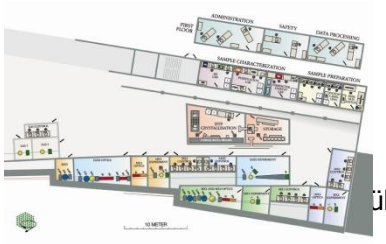
ESRF, Grenoble



APS, Chicago



SPring 8, Hyogo



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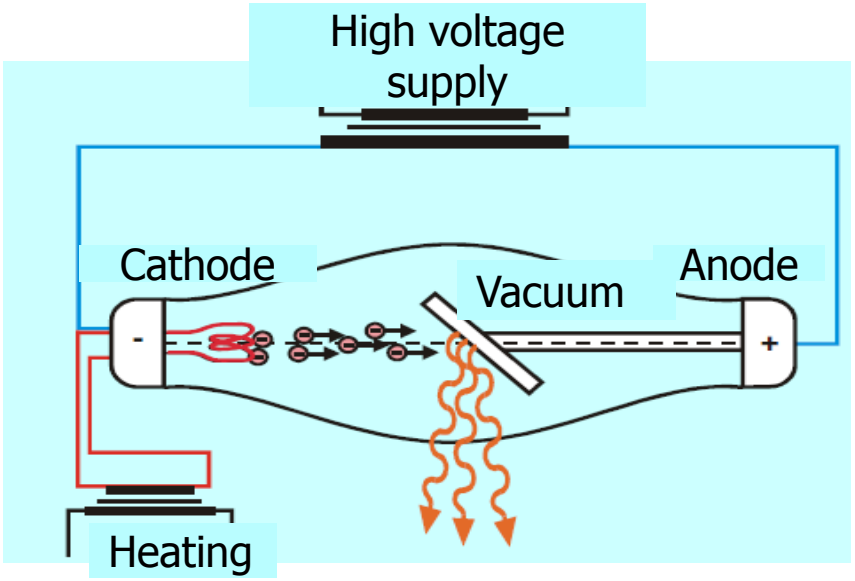
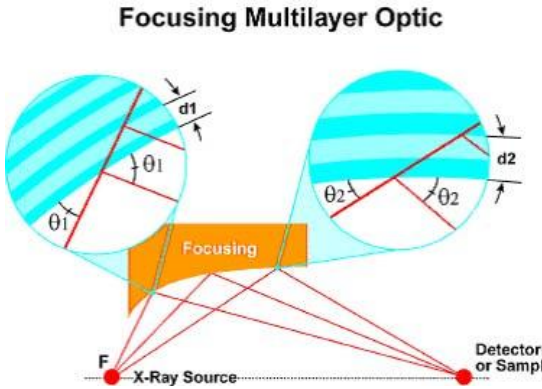
SAXS Lab sources

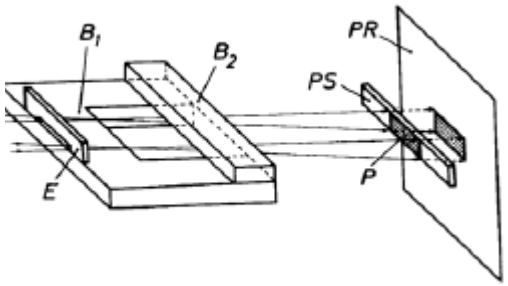

Bruker NanoStar





Bruker NanoStar
Anton Paar SAXSess
Rigaku BioSAXS 1000

Rigaku BioSAXS 1000



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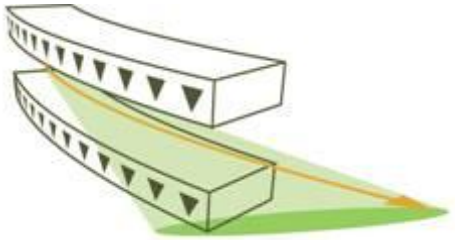
Radiation from Synchrotron Storage Rings

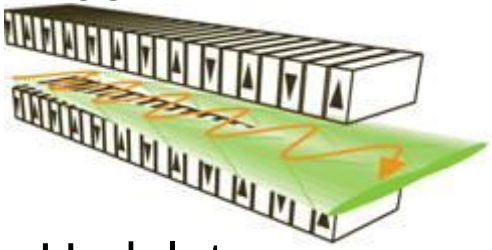
Production of X-rays

Bending magnet



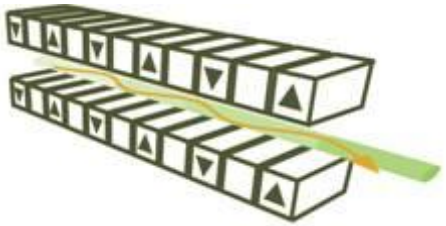
Necessary part of a synchrotron. Many of these dipole magnets form the synchrotron storage ring. The electrons (positrons) are deflected and accelerated in the magnetic field. This acceleration generates the synchrotron light. The light is emitted tangential to the electron beam.

Wiggler



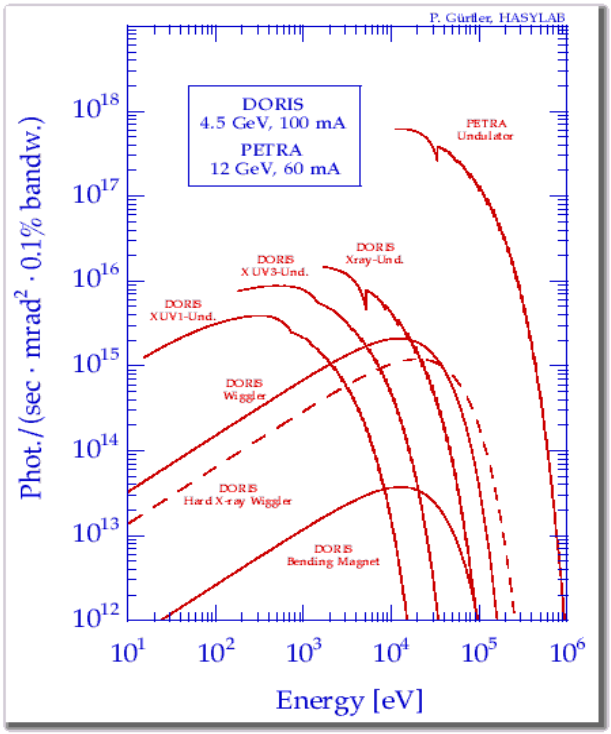
Stack of magnetic dipoles. Put into the straight sections of the storage ring. Wigglers produces more light than bending magnets in a smaller source size.

Undulator



Most powerfull insertion device! A stack of magnetic dipoles generate a high flux of photons in a very small source size. The specific arrangement of the dipoles ($d=n*\lambda$) produces a discrete spectrum with coherent properties.

http://www.physics.uwa.edu.au



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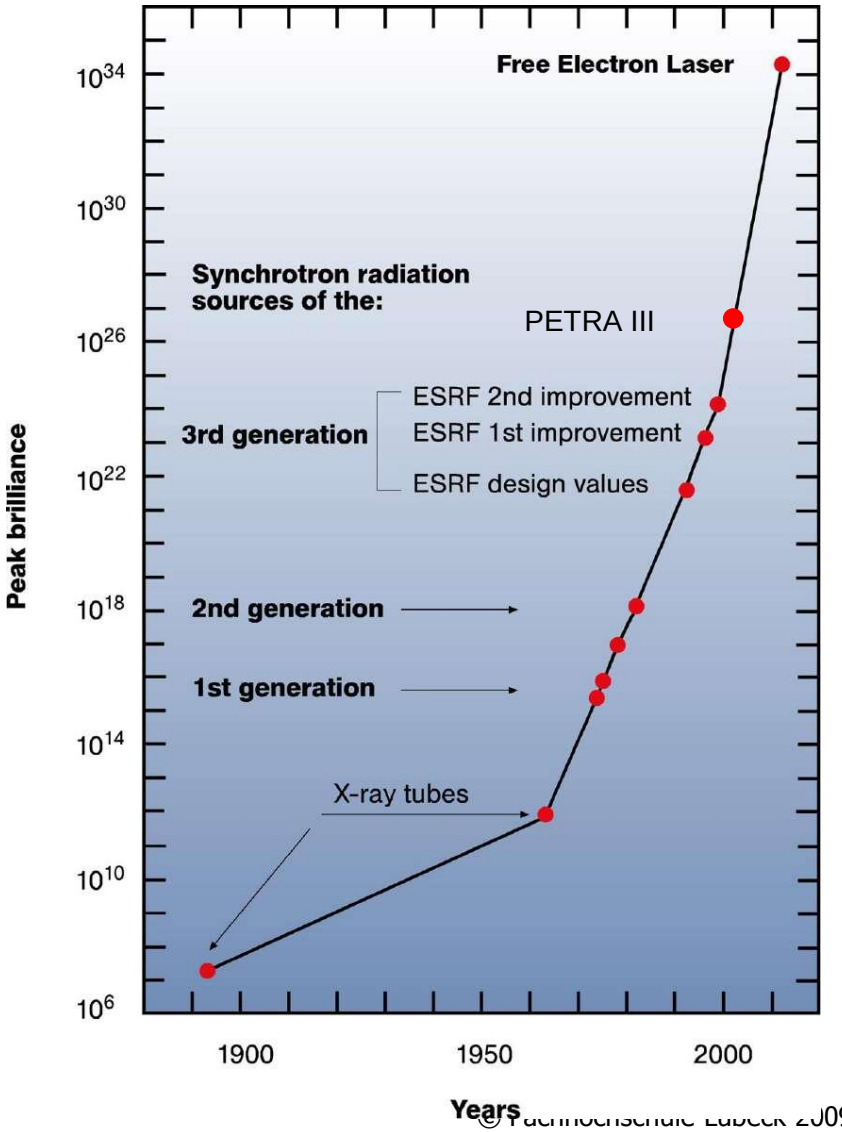
X-ray sources
Brilliance

Increase of the beam brilliance from X-ray tubes to third generation sychrotron sources to free electron lasers (forth generation)



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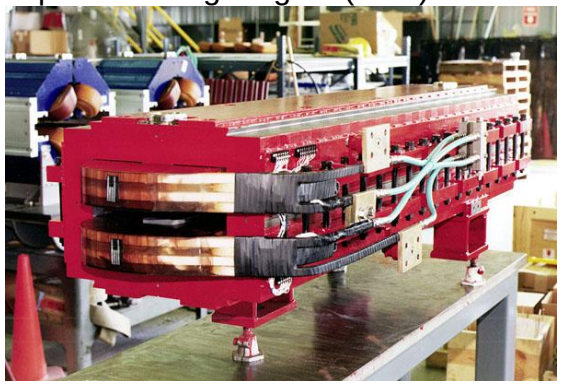
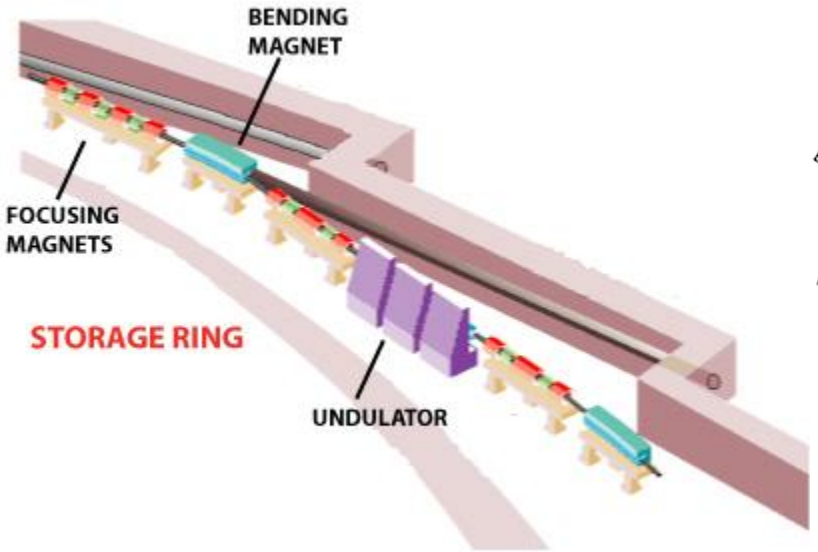
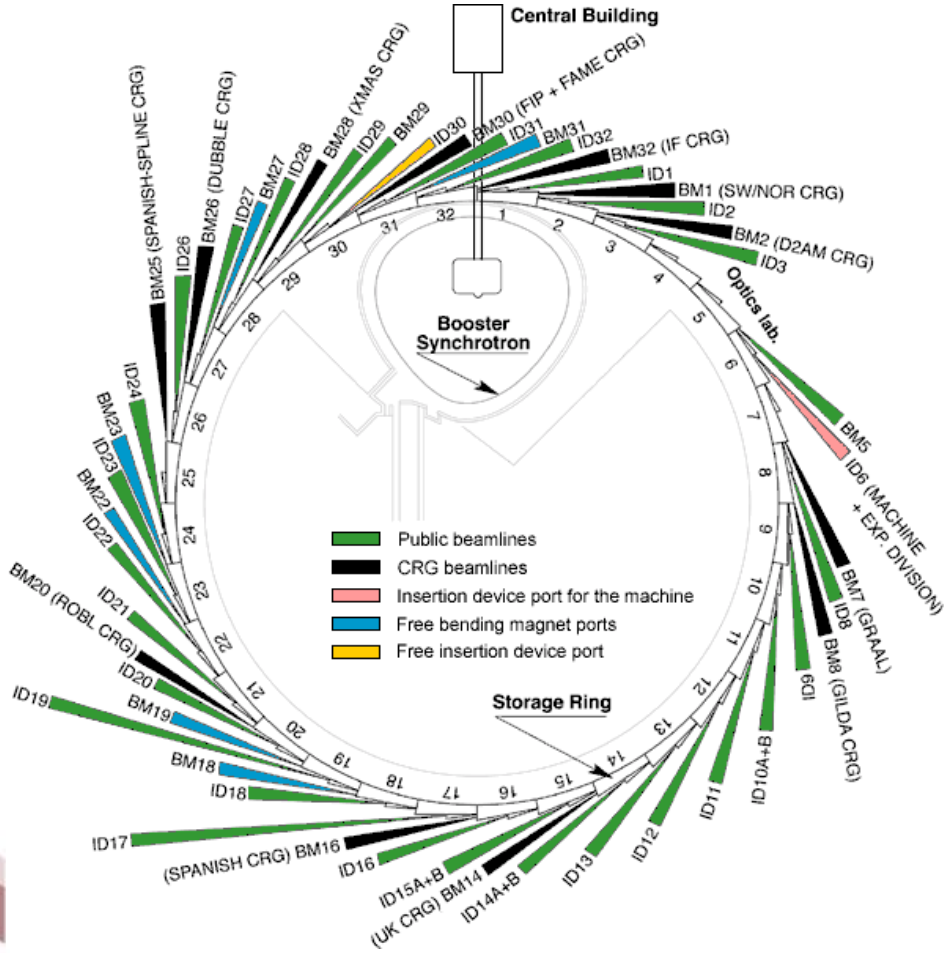
Radiation from Synchrotron Storage Rings

Dipole bending magnet (APS)







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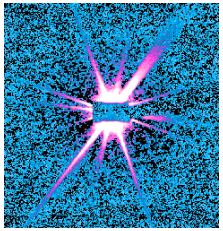
X-ray optics
Beam defining



Beam defining by slit pairs:

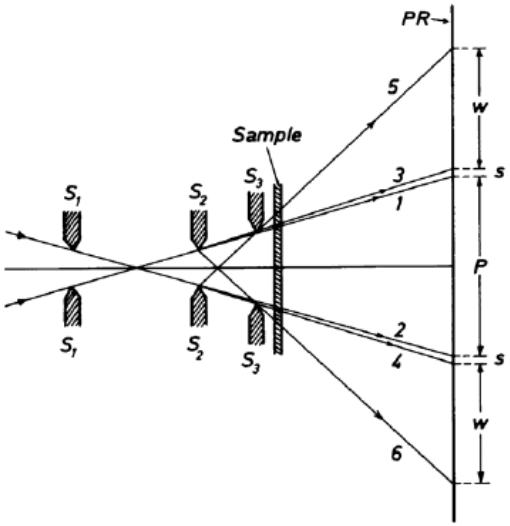
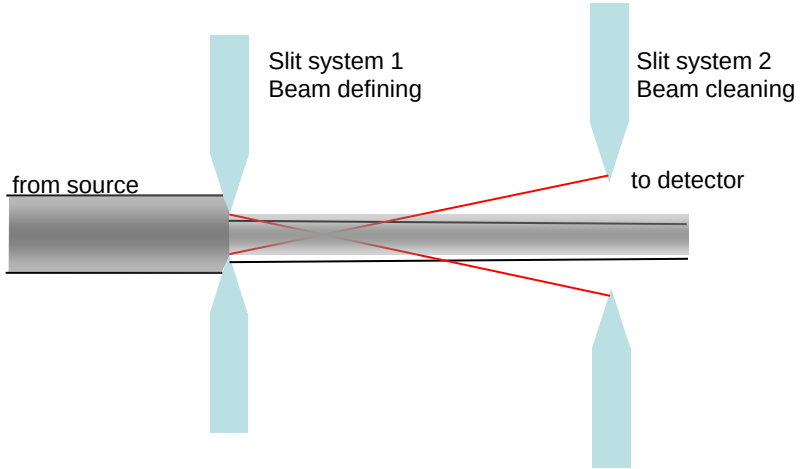


FIG. 2. Slit camera with three slits S_1, S_2 and S_3, each consisting of a pair of edges running perpendicular to the plane of paper. The dimensions in the vertical direction are greatly enlarged.

From Glatter & Kratky 1982
Small Angle Scattering
Book online available!
http://physchem.kfunigraz.ac.at/sm/Index.html
Or google "Otto Glatter"

Problem:
Every edge cutting the beam produces parasitic scattering! Refractive streaks at low angles impedes SAXS experiments!

Solution:
Successive slit systems. First slits cutting the beam and second system cuts out the undesired parasitic scattering



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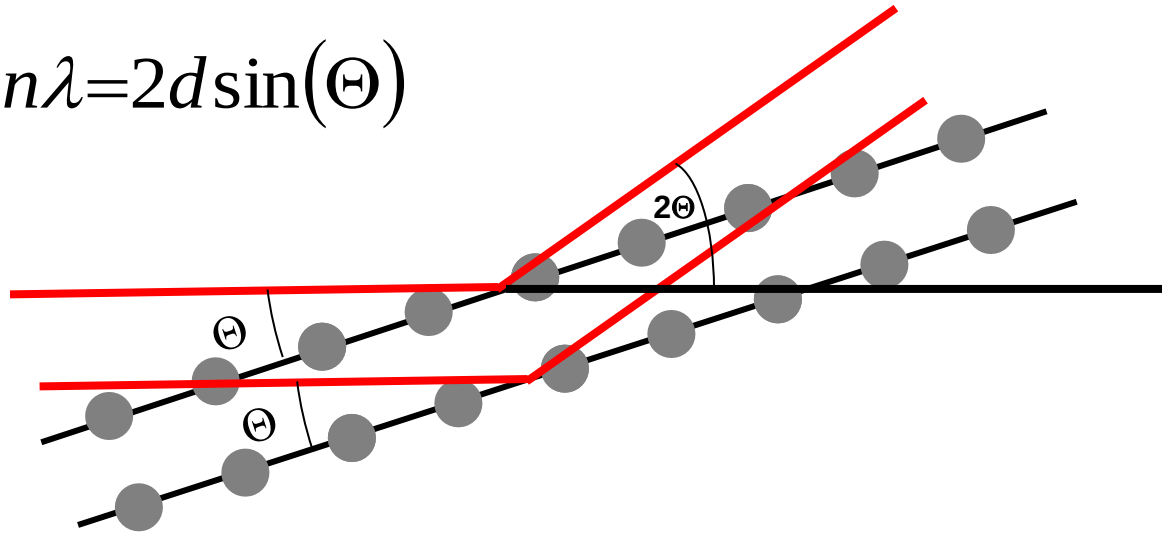
X-ray Optics
Monochromator

Monochromatization of the X-ray is achieved by using single crystals in Bragg diffraction geometry

$$n\lambda = 2d\sin(\Theta)$$



For instance a Si 111 crystal with d-spacing (=distance between the crystal lattice) of d=3.14 Å deflects the beam for a wavelength of λ=1 Å to Θ ~ 10° (9.16°).

Problem:
The Bragg condition is also fulfilled for multiple orders of the wavelength n. These higher order have to be filtered by the X-ray mirror

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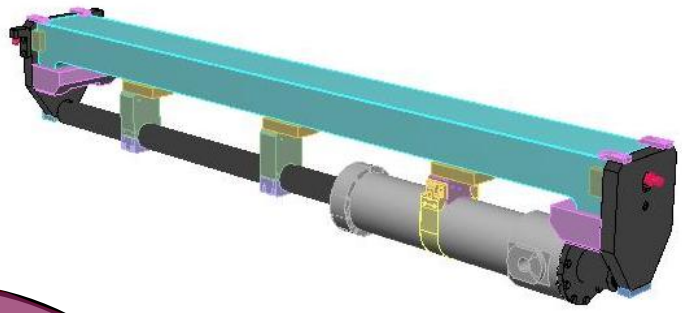
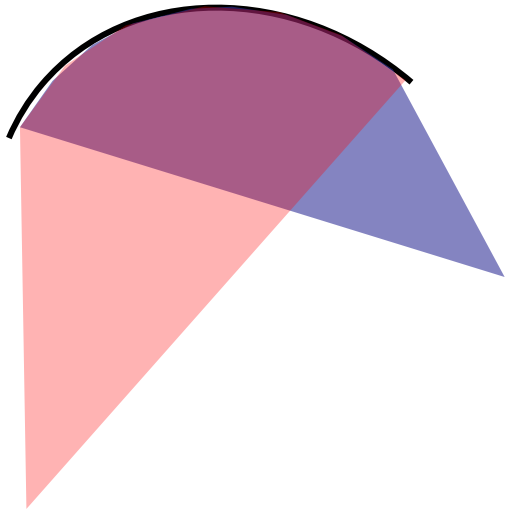
X-ray optics

Focusing

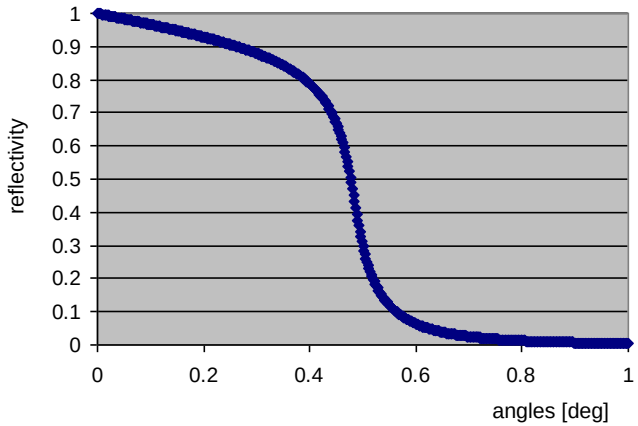
The glancing angle for X-rays on surfaces are very small. Only under grazing conditions X-ray mirrors can be used for focusing.

Bending of the highly polished mirror surface permits focusing on the parabolic mirror profile





Reflectivity for Rh@8keV



In order to use the full beam size X-ray mirrors on synchrotrons are typically in the range of 30cm to 1m. The grazing angle can be increased by using high Z-elements (e.g. Rhodium, Platin, Nickel) as reflecting surface. These mirrors are acting as well as high energy filter; important for monochromatization

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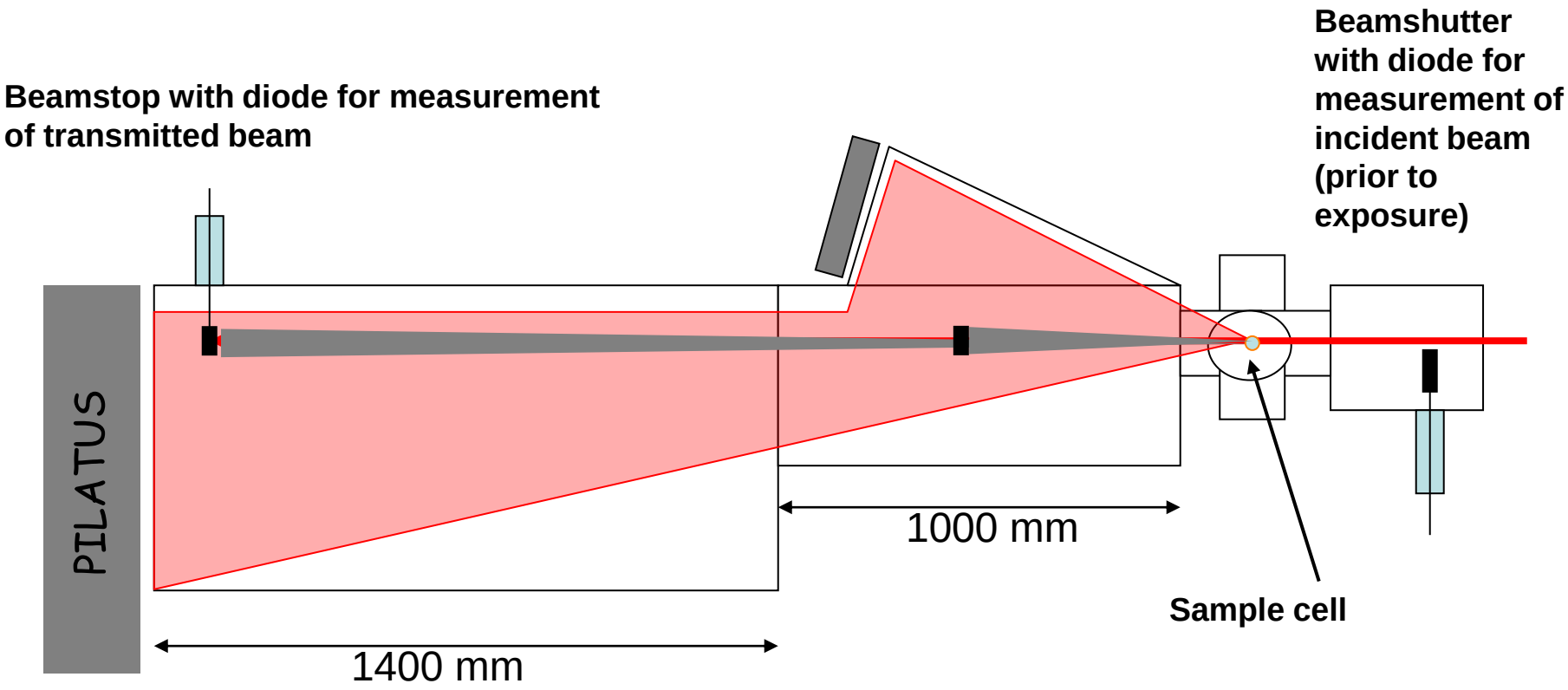
Schematic X33 SAXS setup



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The PETRA-III BioSAXS beamline P12

Detector tube and automated sample changer

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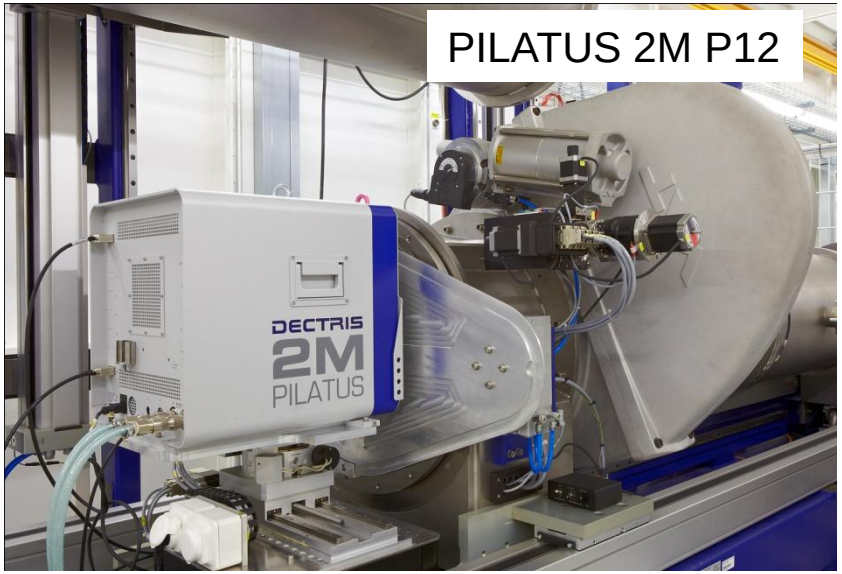
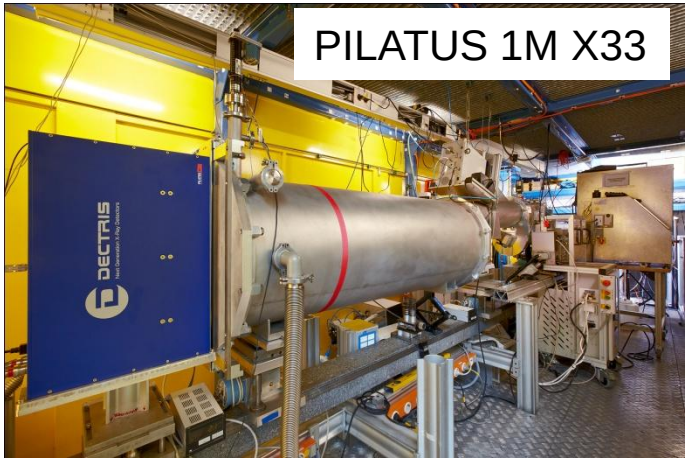
X-ray detectors

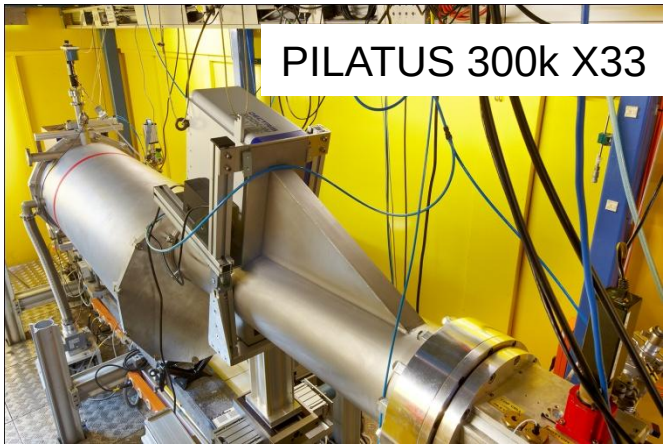
Single photon counting detectors



PILATUS 2M P12



PILATUS 1M X33



PILATUS 300k X33

The PILATUS pixel detectors are at the moment the best detectors for solution SAXS.

Data aqusition integrated into the beamline control software (BMS). Advanced data storage concept for fast operation of a combined SAXS/WAXS detector setup.

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X-ray detectors
Single photon counting detectors

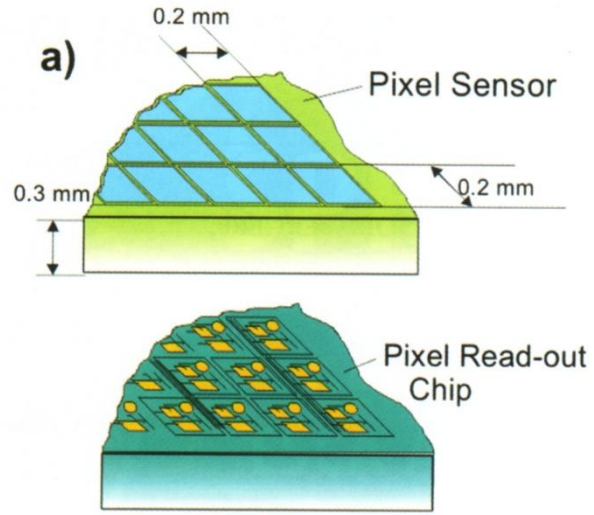
Bump bonding over small indium metal balls.

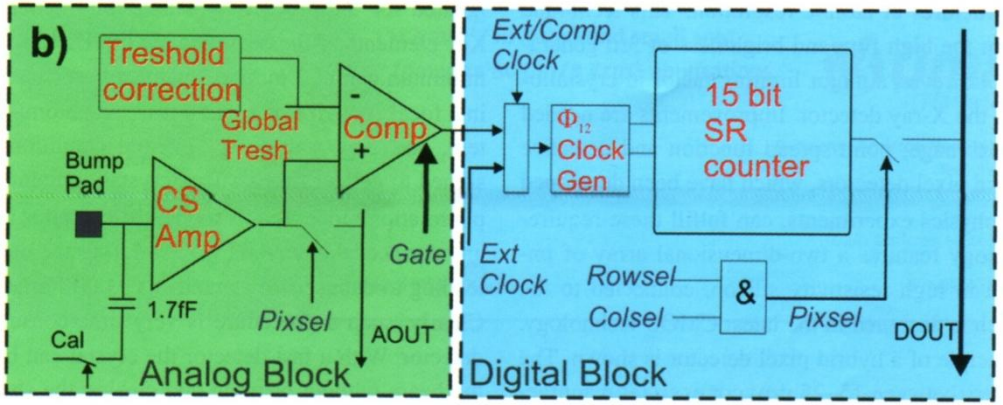
Advanced microtechnology for production needed.

Every pixel is readout separately.

Single photon counting device.

Expensive technology!





X-Ray scattering

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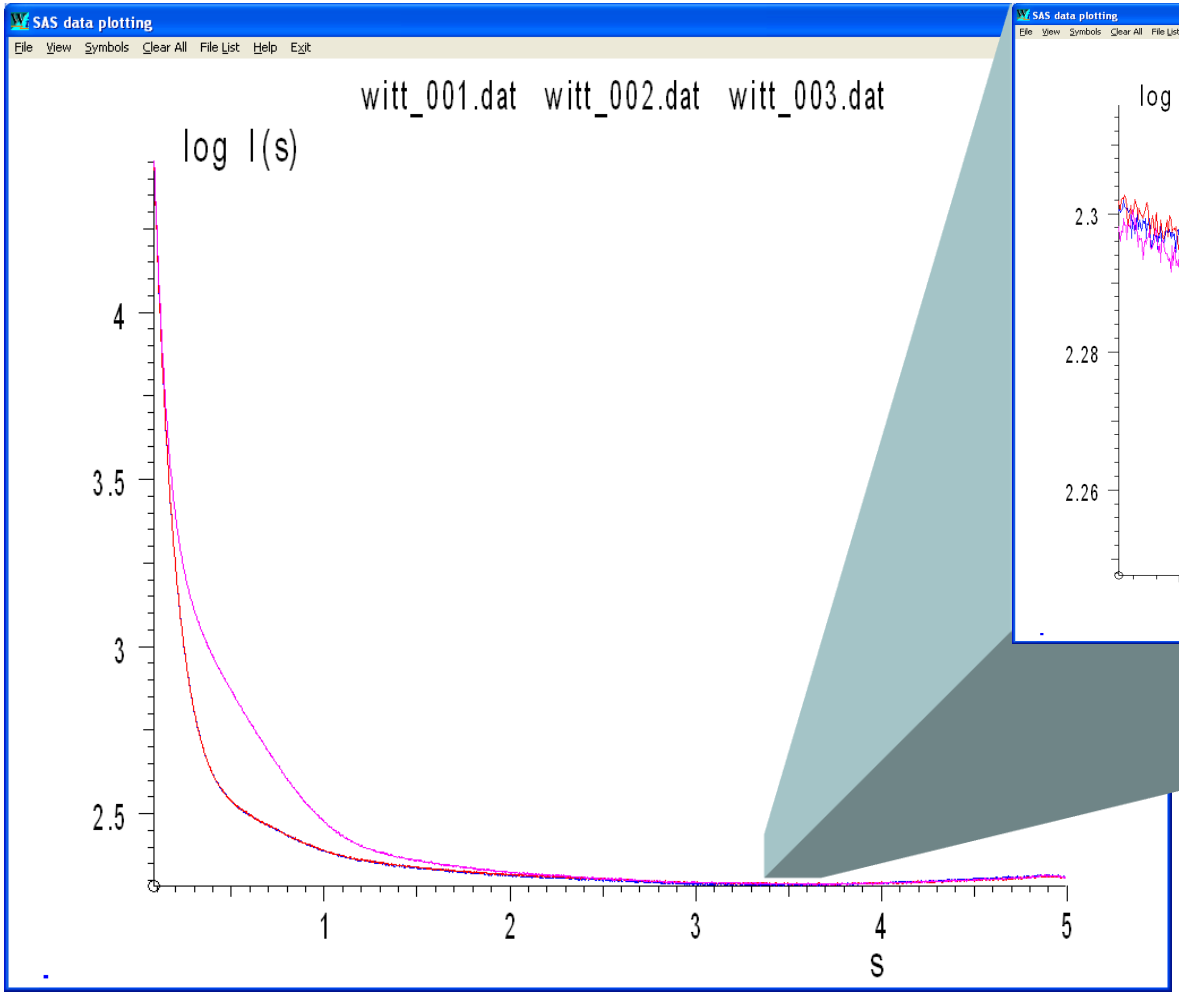
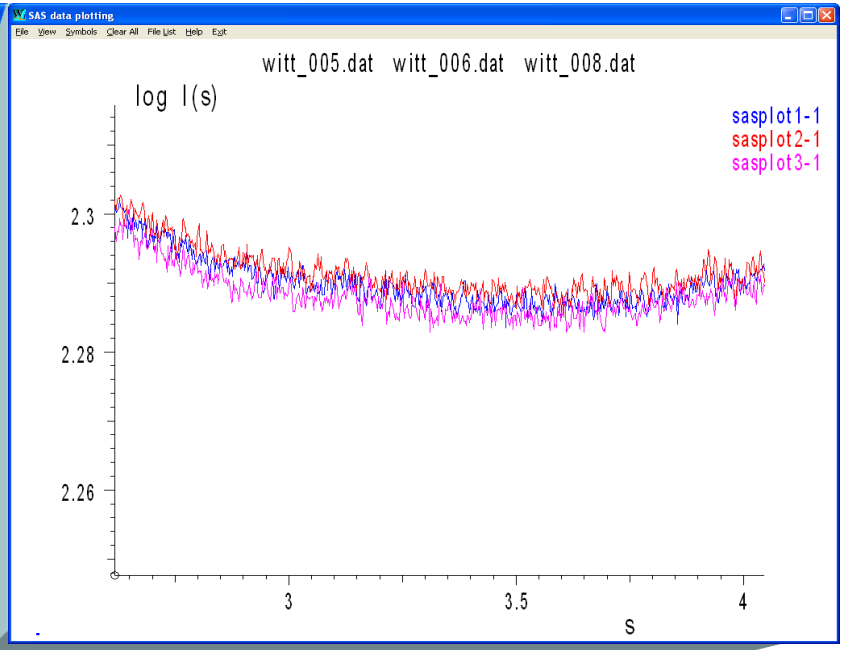
X-ray detectors
Low background noise recording





We are looking to protein signals of less than 0.5% above the back- ground level!

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Summary and Conclusion

X-rays are scattered at the electrons of the atomic shell.

The heavier an atom the better it scatters! Proteins consist mostly of hydrogen, carbon and oxygen ….

For solution scattering the particles have to be in an ideal solution.

Due to the random orientation of the particles in solution, no atomic resolution is possible!

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Summary and Conclusion

The Guinier approximation is valid for small *s* values, i.e. large interparticle distances. The radius of gyration is related to the shape of the particle

From the shape of the scattering function interparticle interactions can be estimated.

The Porod and Kratky analysis is valid at the high *s* regime and the scattering occurs from the internal structure. The Kratky plot gives information of the folding state of a protein.

From the Porod analysis the specific volume of a protein can be derived.

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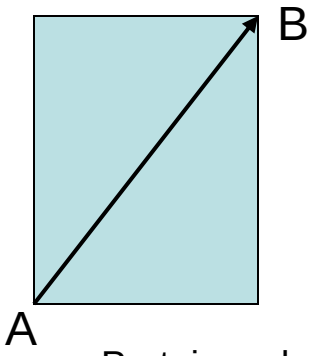
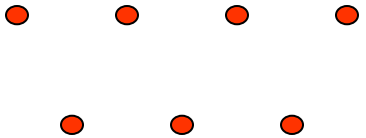
X-ray scattering vs crystallography

Protein crystallography: Molecules (proteins) are fixed in a discrete crystal lattice



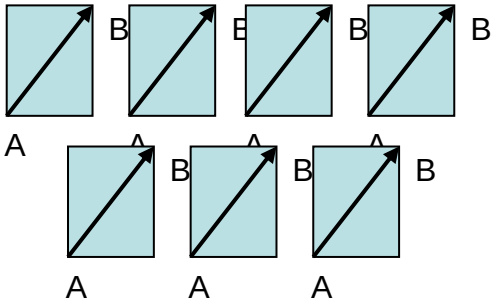
Protein molecule

X



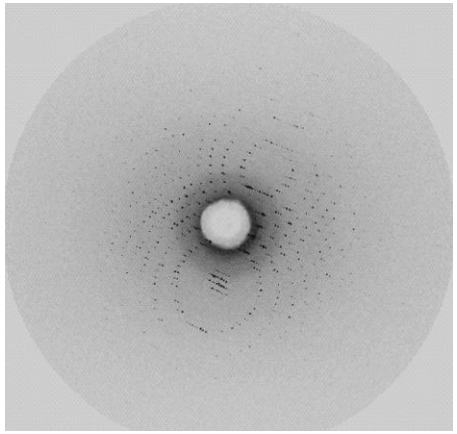
Lattice

=



Protein crystal with fixed positions

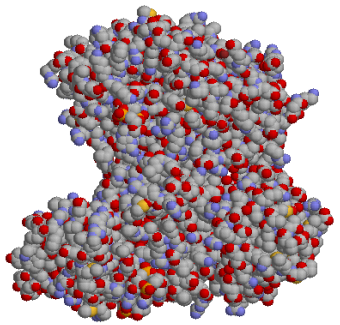
$$I(\vec{s}) = \int_V \rho^*(\vec{r})^2 e^{-i\vec{s}\vec{r}}\, dV$$



<u>3-dim information</u> about:
Length of the vector **AB**
Orientation of vector **AB**

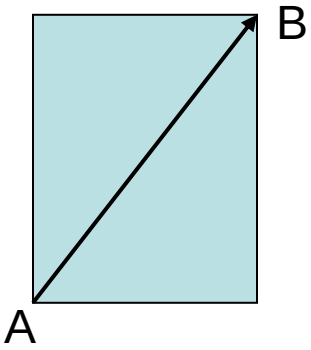
Fourier Transformation

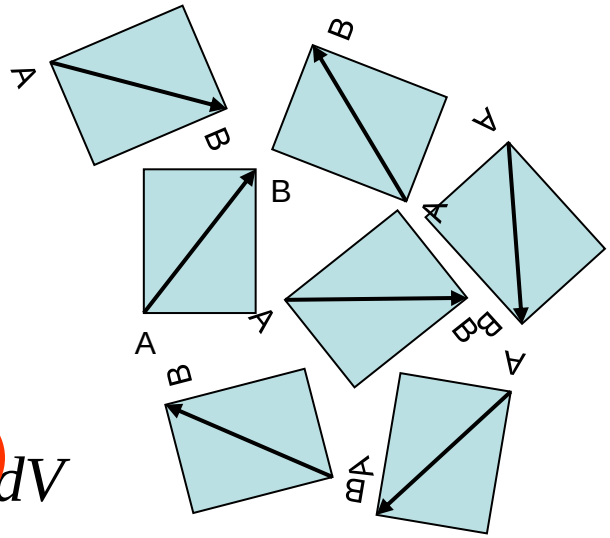
<u>3-dim protein structure</u>
with atomic resolution



X-ray scattering vs crystallography

Small angle scattering investigates protein solutions!





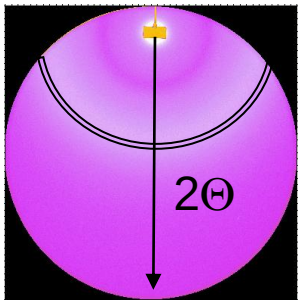
$$I(s)=4\pi\int_0^\infty p(r)\frac{\sin(sr)}{sr}dr$$

$$s=\frac{1}{d}=\frac{2\pi}{\lambda}\sin(2\Theta)$$

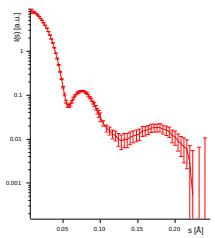
$$I(\vec{s})=\int_V \rho^*(\vec{r})\,e^{-i\vec{s}\vec{r}}\,dV$$

$$\left\langle e^{-i\vec{s}\vec{r}}\right\rangle_\Omega=\frac{\sin(sr)}{sr}$$

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



Radial avarage



1-dim data

X-Ray scattering

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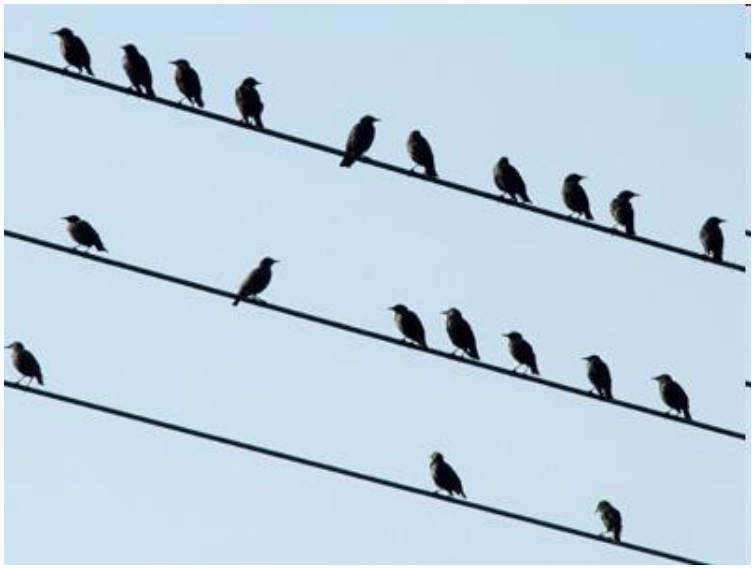
Protein crystallography



Protein SAXS solution scattering

X-Ray scattering

SAXS is a cool technique!

You can use a lot of expensive and funny machines!

Let´s meet at the beamline(s)!