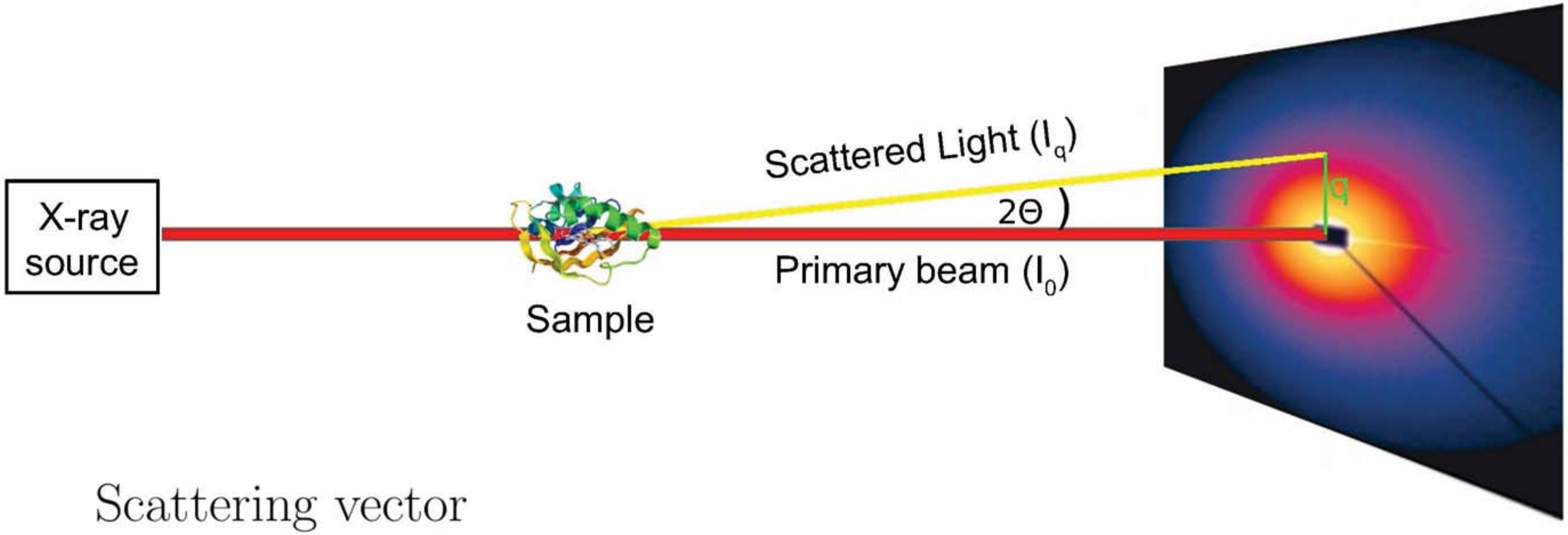
Small Angle X-ray Scattering Introduction, theory and sample preparation

Ralf Stehle
Biomoleculare NMR-Spektroskopie

Small angle scattering



Scattering vector

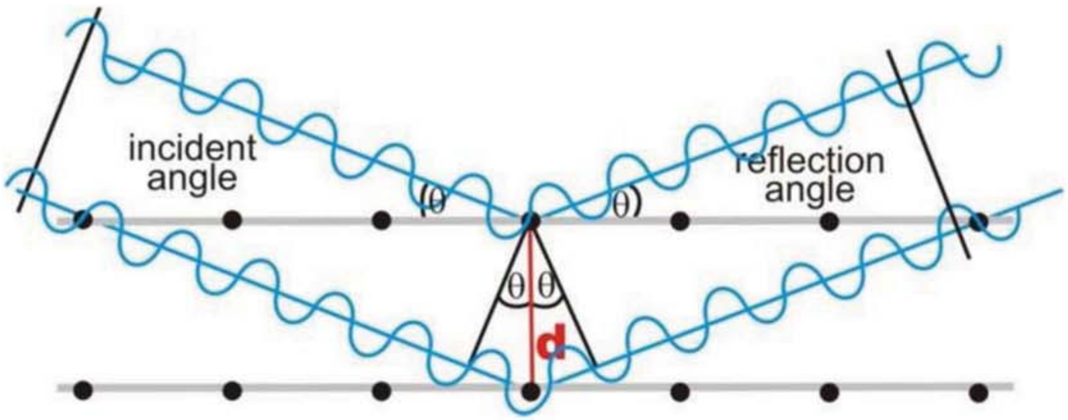
$$|\vec{q}\,| = \frac{4\pi}{\lambda}\, sin 2\theta$$

$$\vec{q} = (\vec{k}_s - \vec{k}_0)$$

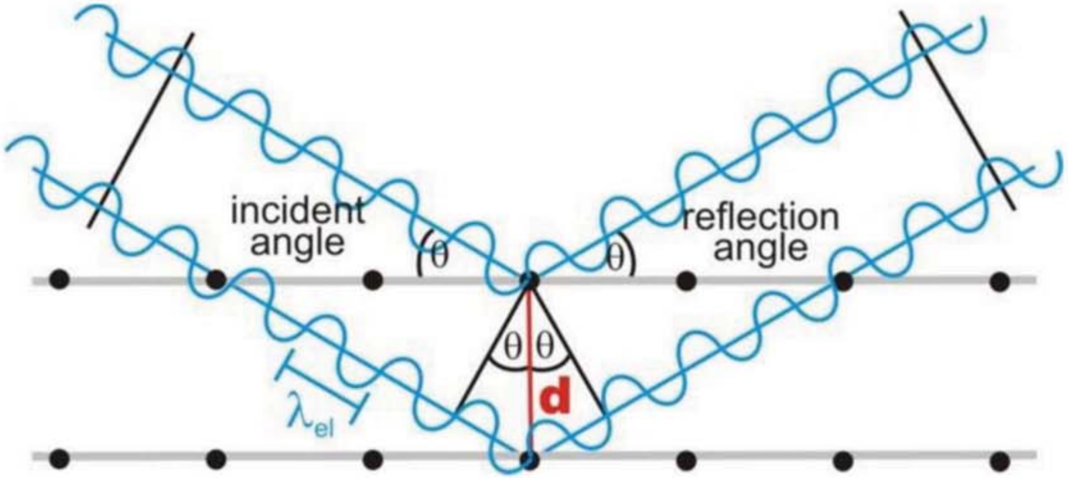
k_0, k_s wave vectors of incident and scattered beam

(In some publications the scattering vector is called *s* instead of *q*)

Bragg's Law



Destructive interference

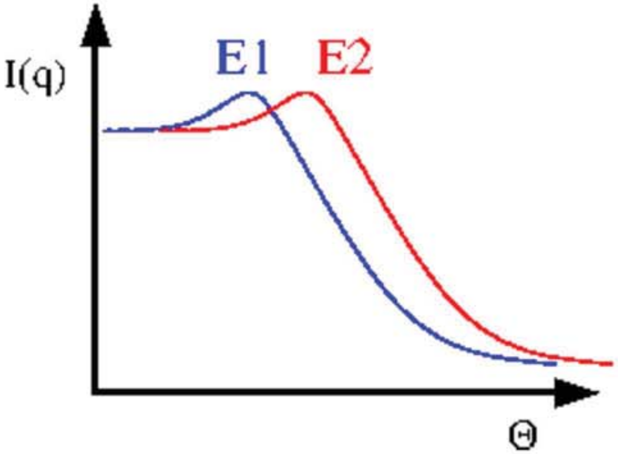


Constructive interference

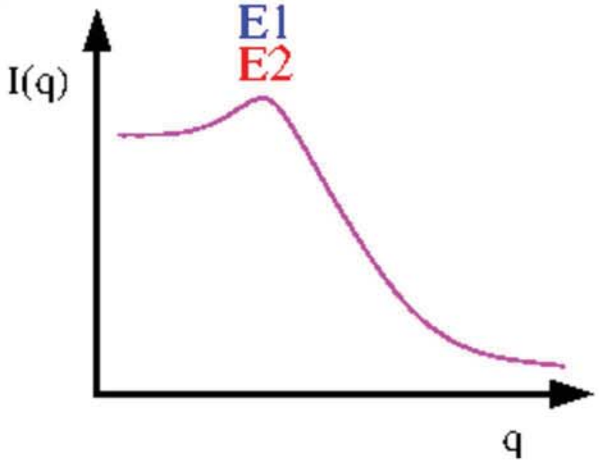
Small angle scattering

Scattering vector

$$|\vec{q}\,| = \frac{4\pi}{\lambda}\, sin2\theta$$

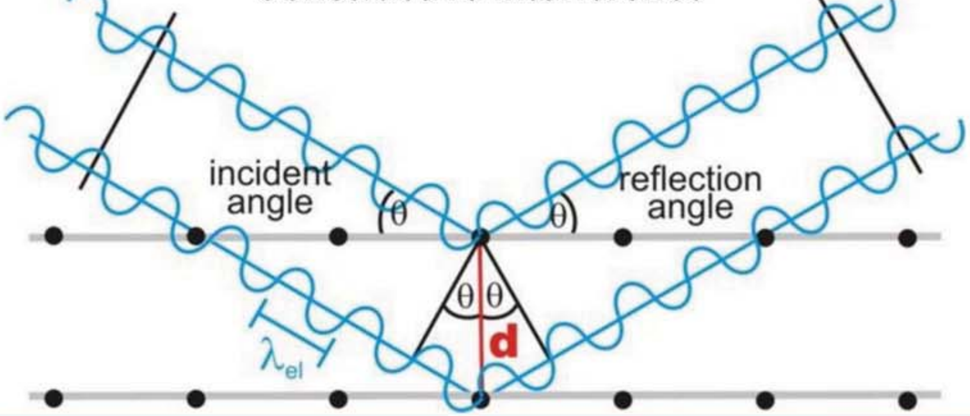


normalisation by
x-ray energy



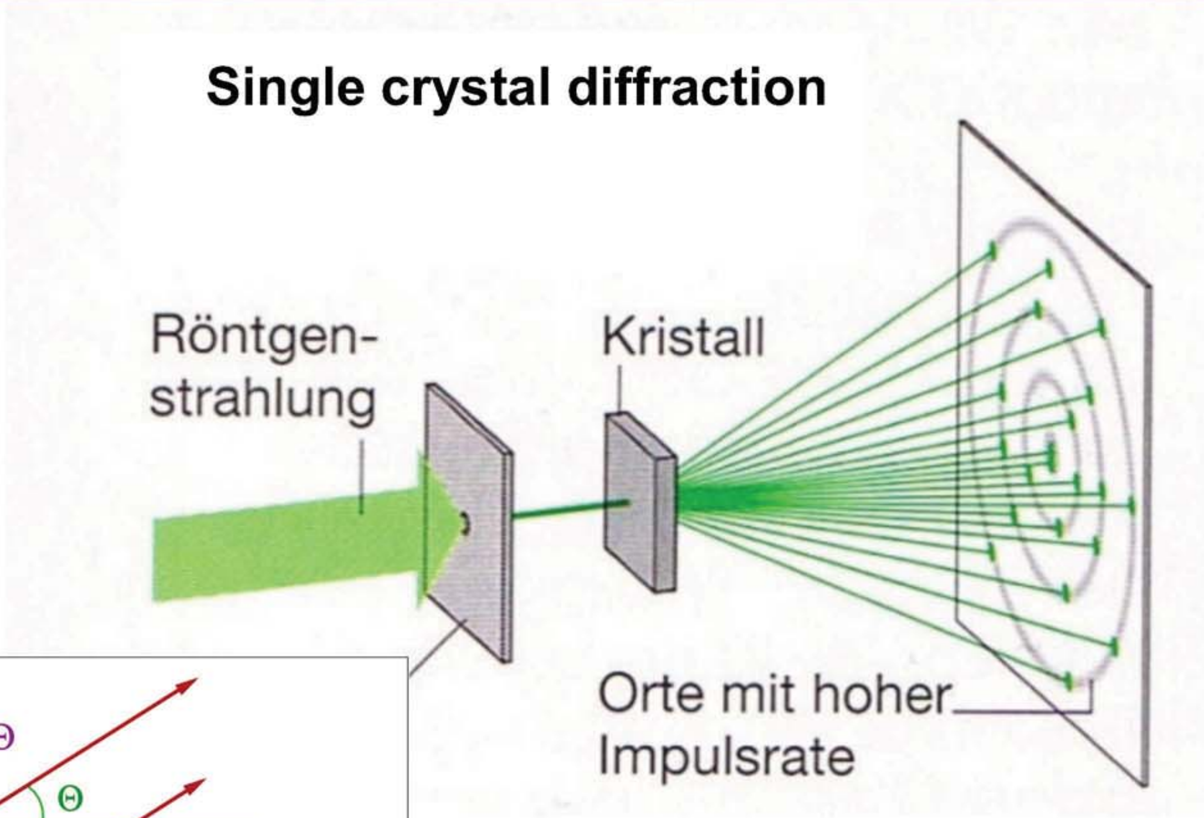
Bragg's Law

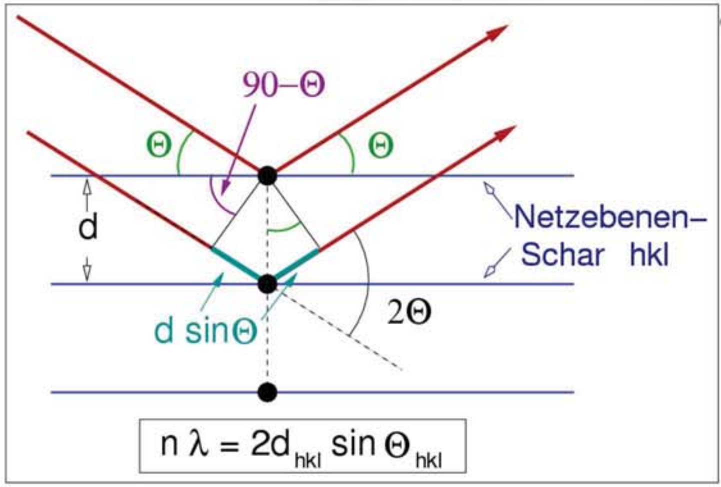
Constructive interference



Small angle scattering

Single crystal diffraction





<u>Orientation of the crystal is known</u>

-Scattering vector q

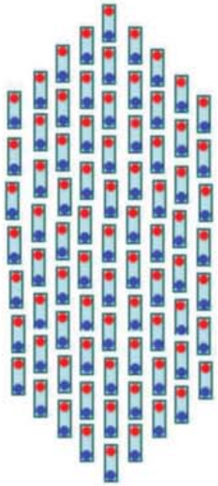
-orientation in reciprocal space hkl

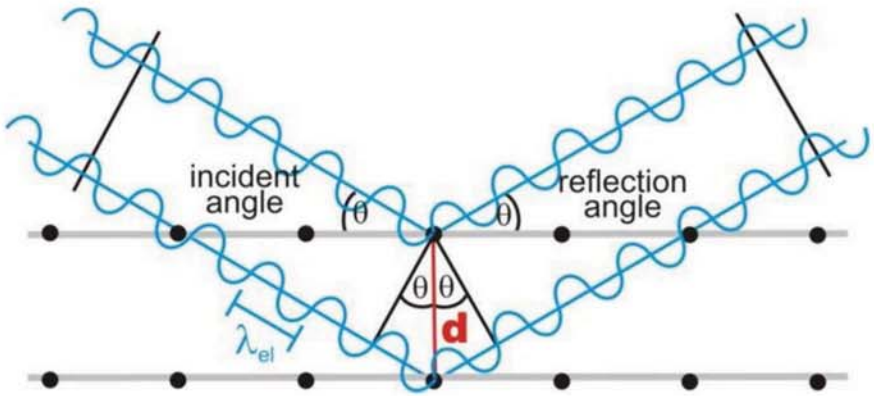
Small angle scattering and Single crystal diffraction

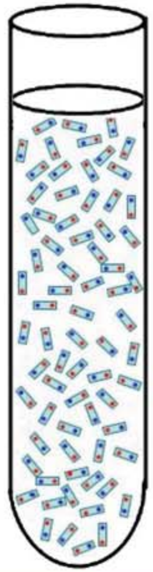
Defined orientation of molecules in a crystal

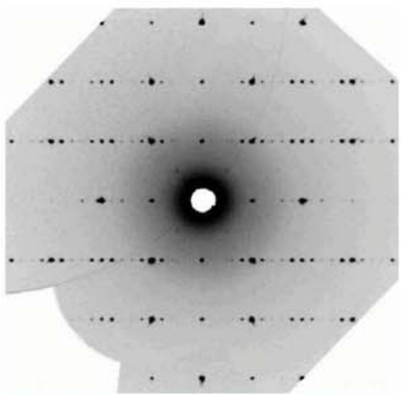
Braggs Law

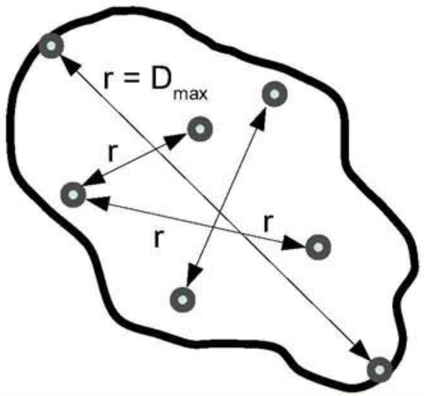
Random orientation of molecules in solution

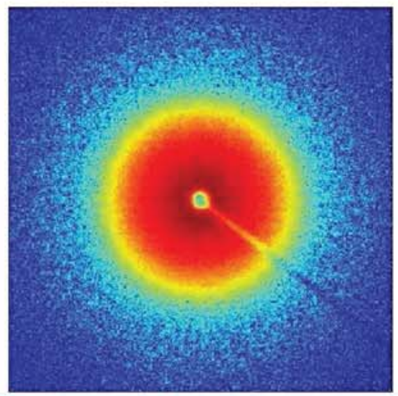












Small angle scattering

Scattering centers

-Light: outer electrons
-X-rays: all electrons
-Neutrons: atomic cores

Substance	e^-	Volume [nm^3]	e^- density [e/nm^3]
H2O	10	0.029	334
Proteins	-	-	~430
Rb^+	36	0.014	2571
Au	79	0.011	7181
Tl^+	80	0.016	5000

Scattering length density

$$\rho(r)=\sum \rho_i(r)\,b_i$$

b = scattering length
i = scattering center

$$b=\frac{e^2}{4\pi\varepsilon_0 mc^2}=0.282*10^{-14}[m]$$

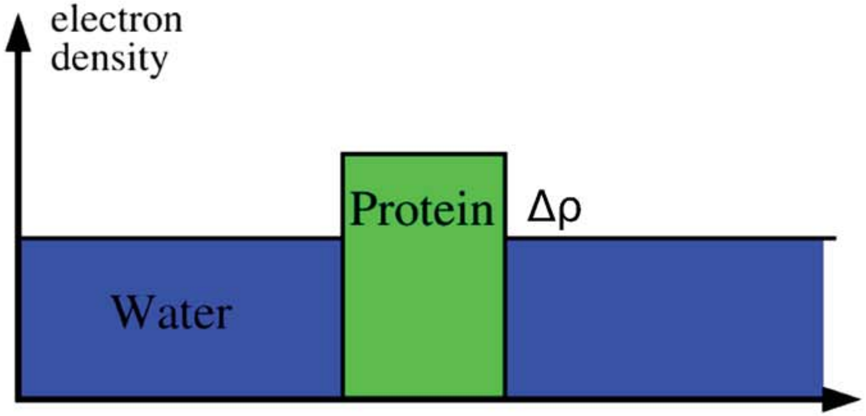
Scattering length of electrons

$$A(q)=\int_V \rho(r)\,e^{-iq*r}\,dr$$

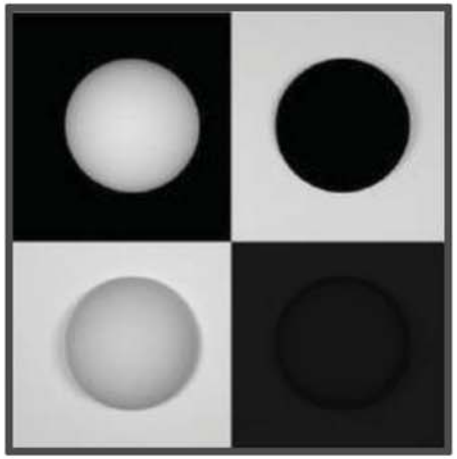
Scattering Amplitude

Scattering contrast

Electron density contrast



Optical contrast



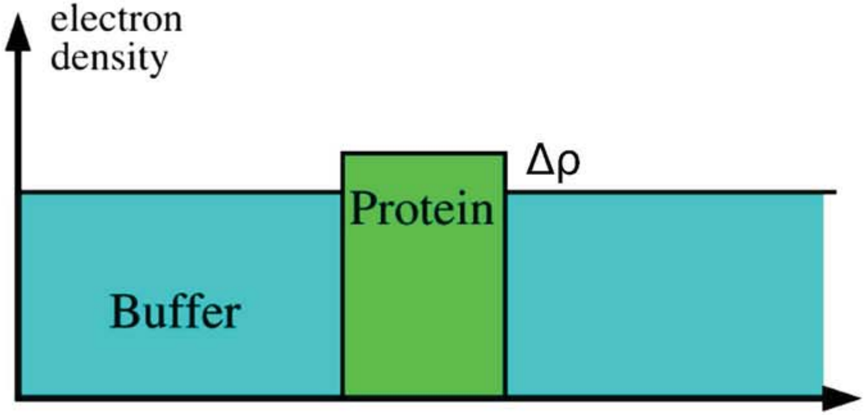
Excess electron density

$$\Delta\rho_{Ala}=\rho_{Ala}-\rho_{H_2O}V_{Ala}$$

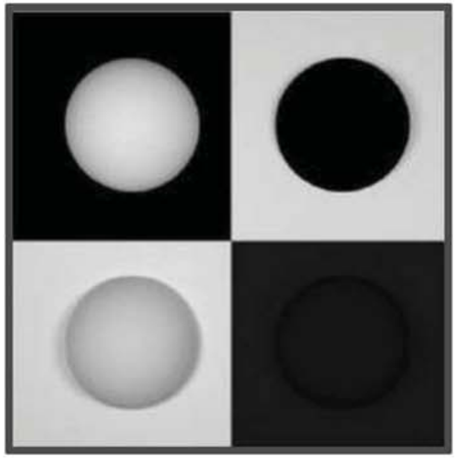
Substance	e^-	Vol. [nm^3]	SLD [cm^{-2}]
H_2O	10	0.029	$9.5 *10^{10}$
Ala	48	0.104	$1.3 *10^{11}$
Au	79	0.011	$1.4 *10^{12}$
NaCl	28	0.044	$1.8 *10^{11}$

Scattering contrast

Electron density contrast



Optical contrast



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Small angle scattering

Two Phase problem and contrast

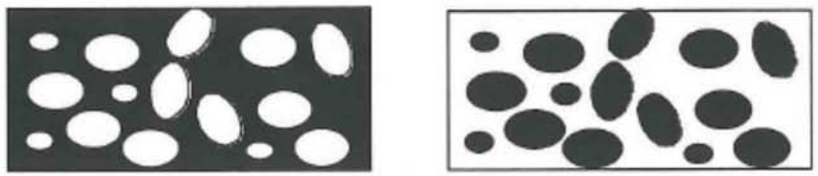


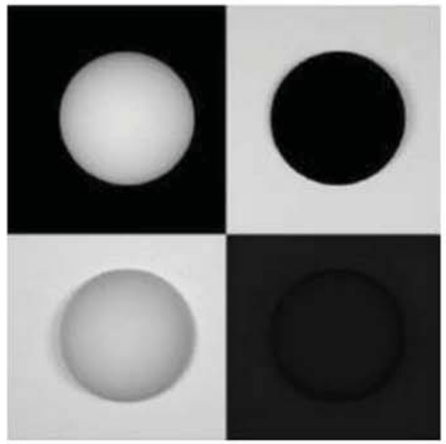
Fig. 1. (a)"White" holes in "black" matrix; (b) "Black" grains in a" white" solvent.

$$A(q)=\int_{\Phi V} \rho_1 e^{-iq*r} dr + \int_{(1-\Phi)V} \rho_2 e^{-iq*r} dr$$

Φ = Volume Fraction

$$A(q)=\int_{\Phi V} (\rho_1-\rho_2)e^{-iq*r} dr + \rho_2\int_{V} e^{-iq*r} dr$$

$$A(q)=\int_{\Phi V} \Delta\rho_1 e^{-iq*r} dr + \rho_2\delta(q)$$



- $\Delta\rho$ is equal to $(\rho_1 - \rho_2)$ in Φ and zero elsewhere
- For $(q \neq 0)$ The scattered intensity depends only on the contrast between both phases

Scattering Intensity

Scattering Vector

$$q = (4\pi/\lambda)\sin(2\theta) = (\vec{k}_s - \vec{k}_i)$$

k_i, k_s wave vectors of incident and scattered beam

$$I(q) = \frac{A(q)A^*(q)}{V}$$

Scattering Intensity

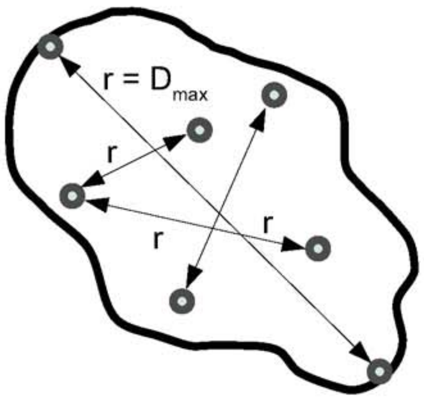
$$I(q) = \frac{1}{V}\int_V \int_V \rho(r)\rho(r')e^{-iq*(r-r')}\,dr\,dr'$$

Correlation function $\gamma(r) = \frac{1}{V}\int_V \rho(r')\rho(r+r')\,dr' = \frac{1}{V}P(r)$ Patterson Function

$$I(q) = \int_V \gamma(r)e^{-iq*r}\,dr$$

Small angle scattering

Form Factor



$$a(q) = \int_{V_{Part}} \rho(r) e^{-iq*r}\, dr$$ Amplitude

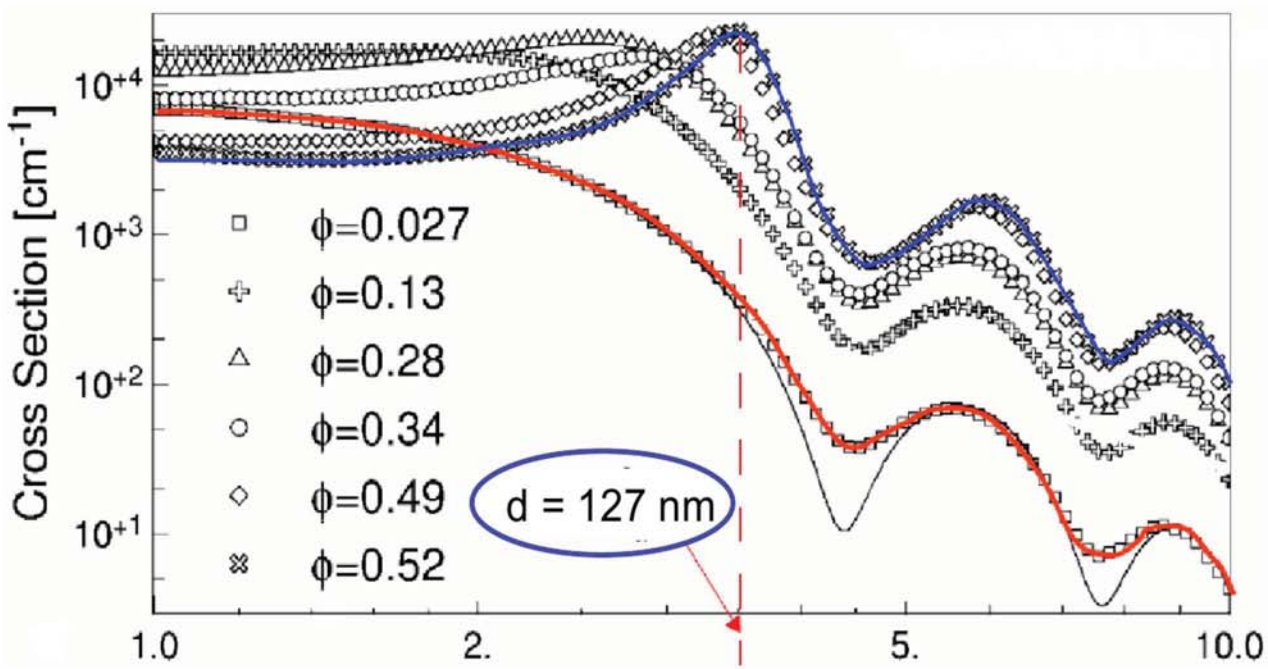
$$I_{Part} = a(q) a^*(q) = V_{Part}^2 P(q)$$ Intensity

$$P(q) = \frac{1}{V_{Part}^2} \int_{V_{Part}} \int \rho(u) \rho(v) e^{-iq*(u-v)}\, du\, dv$$ Form Factor

$$I_a(q) = \frac{N}{V} V_{Part}^2 P(q) = \Phi V_{Part} P(q)$$ Intensity per unit volume

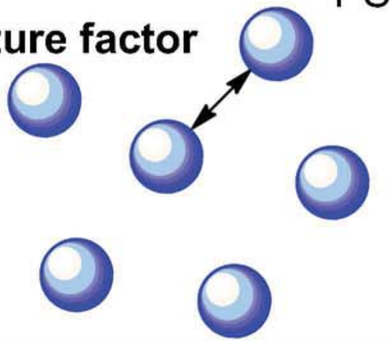
N = Number of particles

Structure Factor and Form Factor



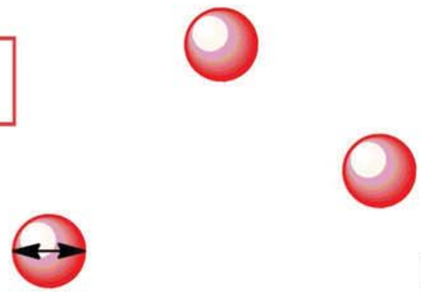
PS nano particles R=66.5 nm. Φ=volume fraction.

structure factor



$$I_a(q) = \Phi V_{Part} P(q) \; S(q)$$

form factor



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L. B. Lurio, *et. al.*, *Phys. Rev. Lett.* **2000**, 84, 785.

Small angle scattering

Form Factor Averaged over all Orientations

$$P(q)=\frac{1}{V_{Part}}\int_{V_{Part}} \gamma(r)\, e^{-iq*r}\, dr$$

Form factor with auto correlation function

Averaging over solid angle

$$\gamma_{Part}(r)=\langle \gamma_{Part}(r)\rangle_{\Omega} \qquad P(q)=\langle P(q)\rangle_{\Omega}$$

Ω = solid angle

$$P(q)=\frac{1}{V_{Part}}\int_{V_{Part}} \left(\frac{1}{4\pi}\int_{\Omega} \gamma(r)\, d\Omega\right) e^{-iq*r}\, dr$$

$$P(q)=\frac{1}{V_{Part}}\int_{0}^{D} 4\pi r^{2} \gamma_{Part}(r)\, \frac{\sin(qr)}{qr}\, dr$$

$D = D_{max}$

Scattering intensity Averaged over time and Absolute Calibration

Local scattering length density fluctuations $\eta(r) = \rho(r) - \langle\rho\rangle$

$\langle\rho\rangle =$ Density fluctuations averaged over time

Normalised correlation functtion $\gamma_0(r) = \dfrac{\gamma(r) - \langle\rho\rangle^2}{\langle\eta^2\rangle}$

$$I(q) = \langle\eta^2\rangle \int_V \gamma_0(r) e^{-iq*r}\, dr$$

SAXS Absolut Calibration

$$\sigma = \frac{\text{Scattered energy}}{\text{Incident energy per unit area}}.$$

Scattering cross section

$$\frac{d\Sigma}{d\Omega} = n \frac{d\sigma}{d\Omega}.$$

Macroscopic Scattering cross section

$$\frac{d\Sigma(q)}{d\Omega} = c\,\rho^2\,\bar{v}^2\,M_1\,P(q).$$

Molecular Weight

ρ Scattering contrast
$\bar{v}$ Specific volume
$P(q)$ Form factor

Absolut Scattering of water

$$\frac{d\Sigma}{d\Omega} = \rho^2\,kT\,\chi_T,$$

Isothermal compressibility
Scattering lengt density

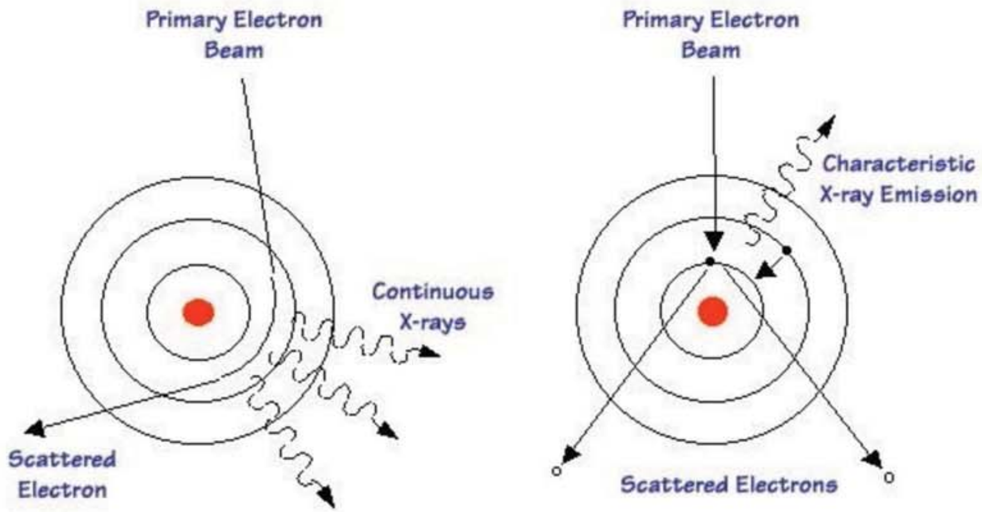
Absolut calibration of Sample

$$\left(\frac{\partial\Sigma}{\partial\Omega}\right)_S(q) = \left(\frac{\partial\Sigma}{\partial\Omega}\right)_{st}(q)\,\frac{[I_S(q) - BG_S]}{d_S T_{S+CAP}}\,\frac{d_{st} T_{st+CAP}}{[I_{st}(q) - BG_{st}]}$$

Thickness
Transmission
Background

X-Rays, instrumental setup and measurement

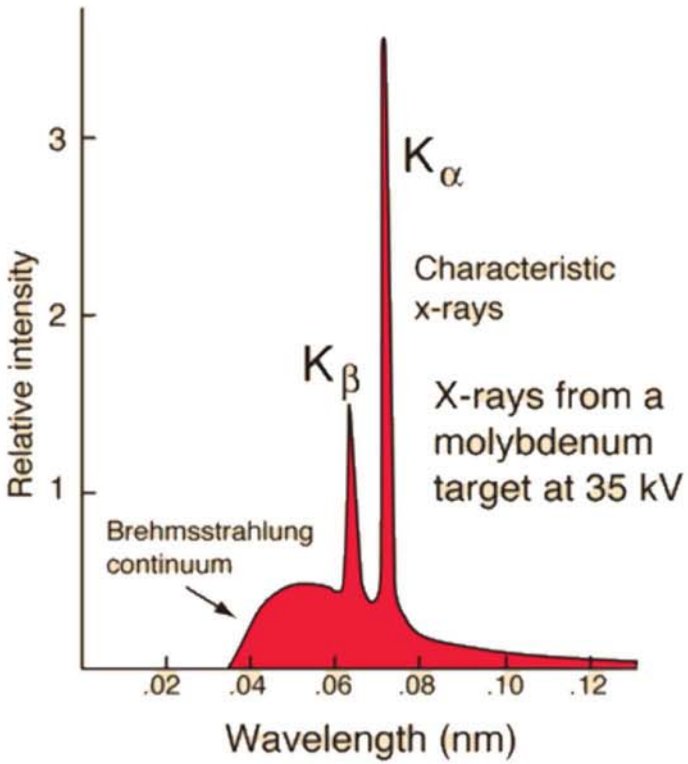
X-Ray production



www2.rgu.ac.uk/life_semweb/xrayfig1.gif

Accelerated electrons hit atoms inside of the anode.
-The electron can be scattered by the atom looses energy which is emitted as irradiation (Bremsstrahlung)
-The fast electron kicks an electron out of the K-shell. A third electron is falling from the L or M shell into the K shell. The energy difference is emitted as x-ray irradiation ($K_{\alpha/\beta}$irradiation)

Energy spectrum of a Cu Laboratory source

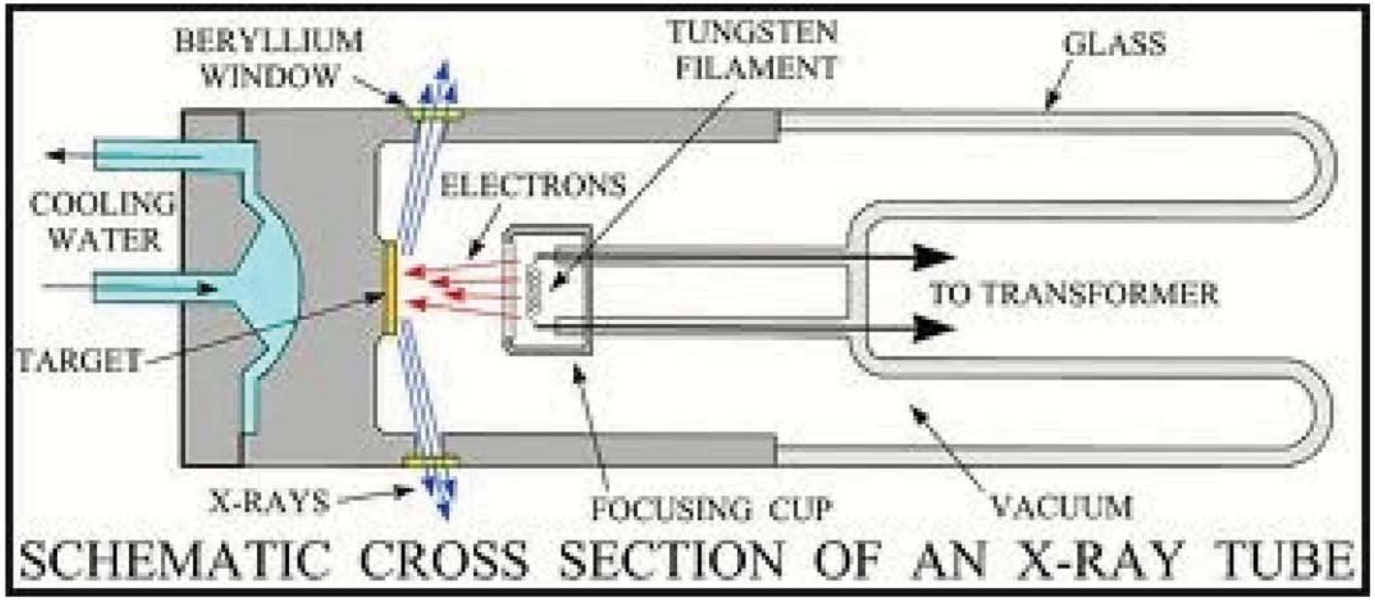


K_α irradiation is emitted from an L – K transition
K_β irradiation is emitted from an M – K transition

Sealed Tube

Acceleration of electrons of high voltage Inside an evacuated tube between filament and Anode

Target: Cu, Mo, W (anode)
40 – 60 kV, 10 – 30 mA
10^6 Photons/s



Simple design very stable but relatively low amount of photons

SFB1035 conformational switches

Instrumental setup

Technische Universität München

Sealed

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between filament and Anode

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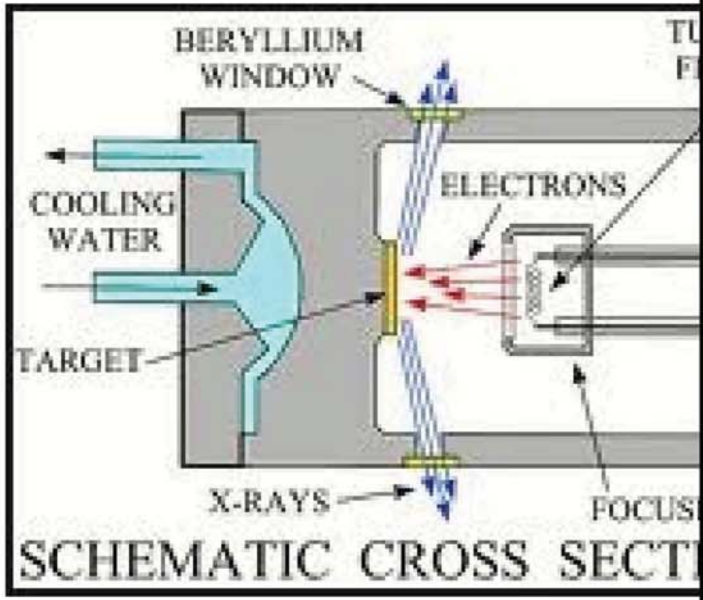
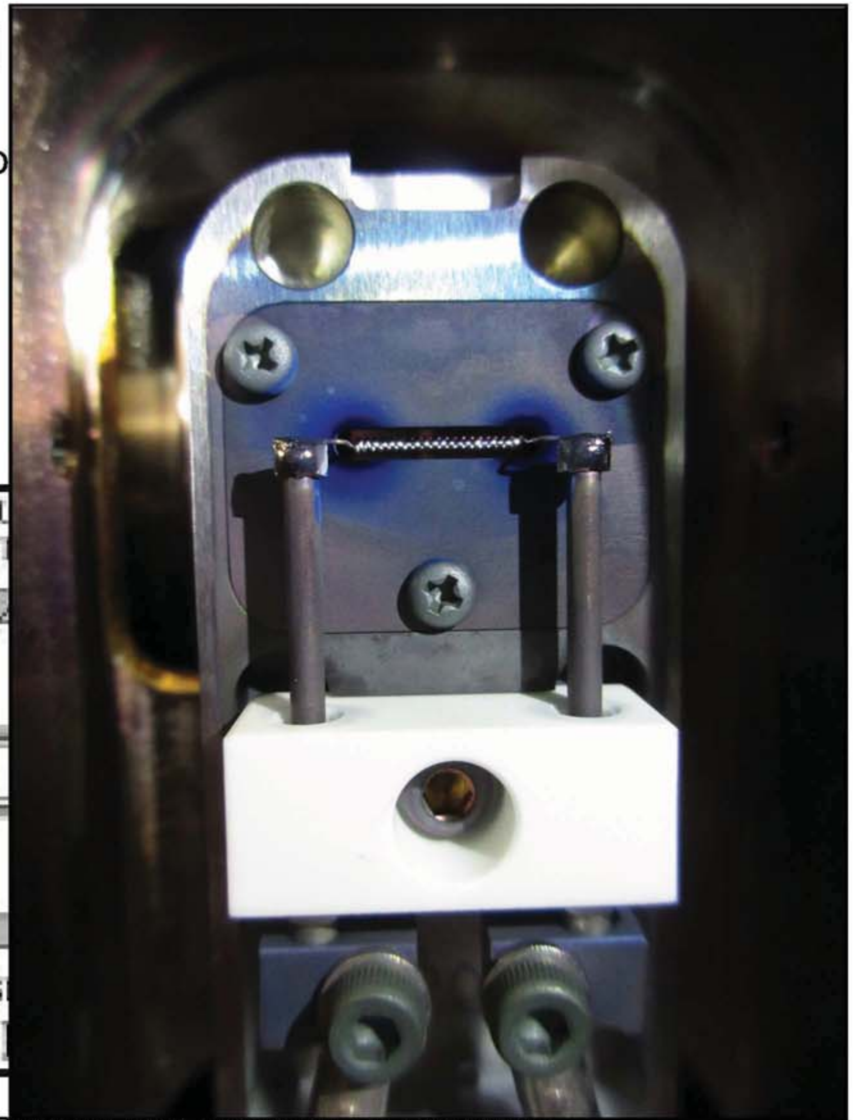
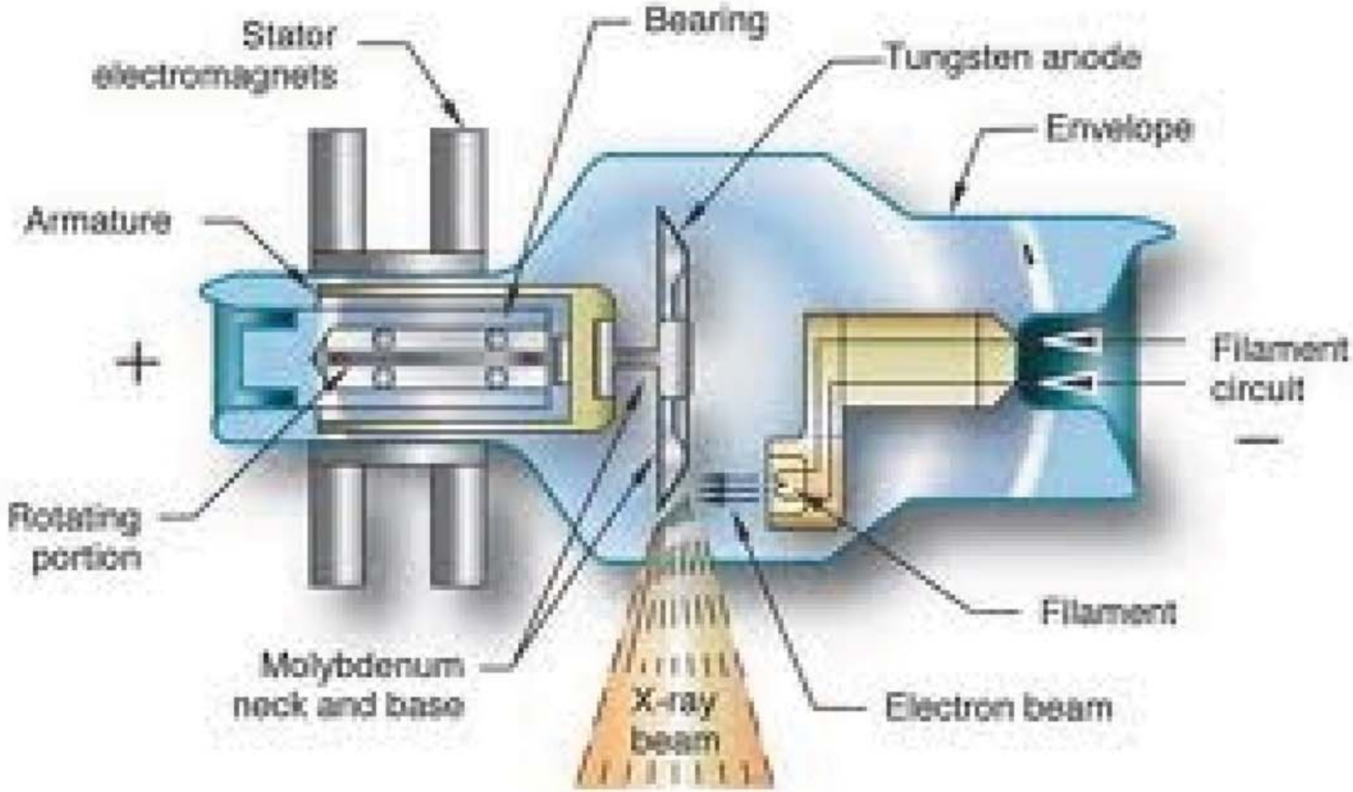




Simple design very stable but rela

Rotating Anode

Target: Cu, Mo, W (anode)
40 – 60 kV, up to 200 mA
10^8 Photons/s

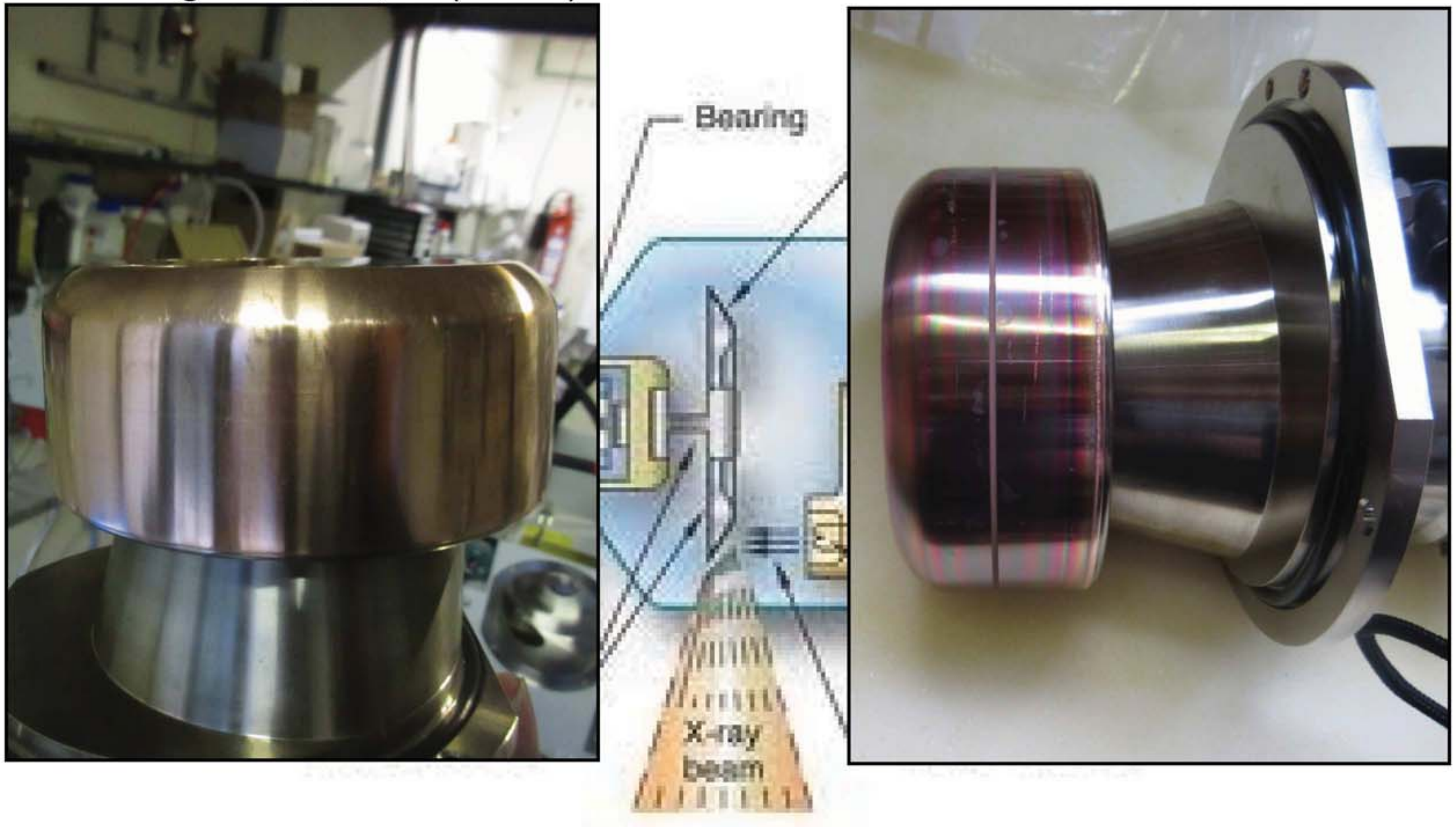


high amount of photons, due to moving parts high maintenance time and effort

Instrumental setup

Rotating Anode

Target: Cu, Mo, W (anode)

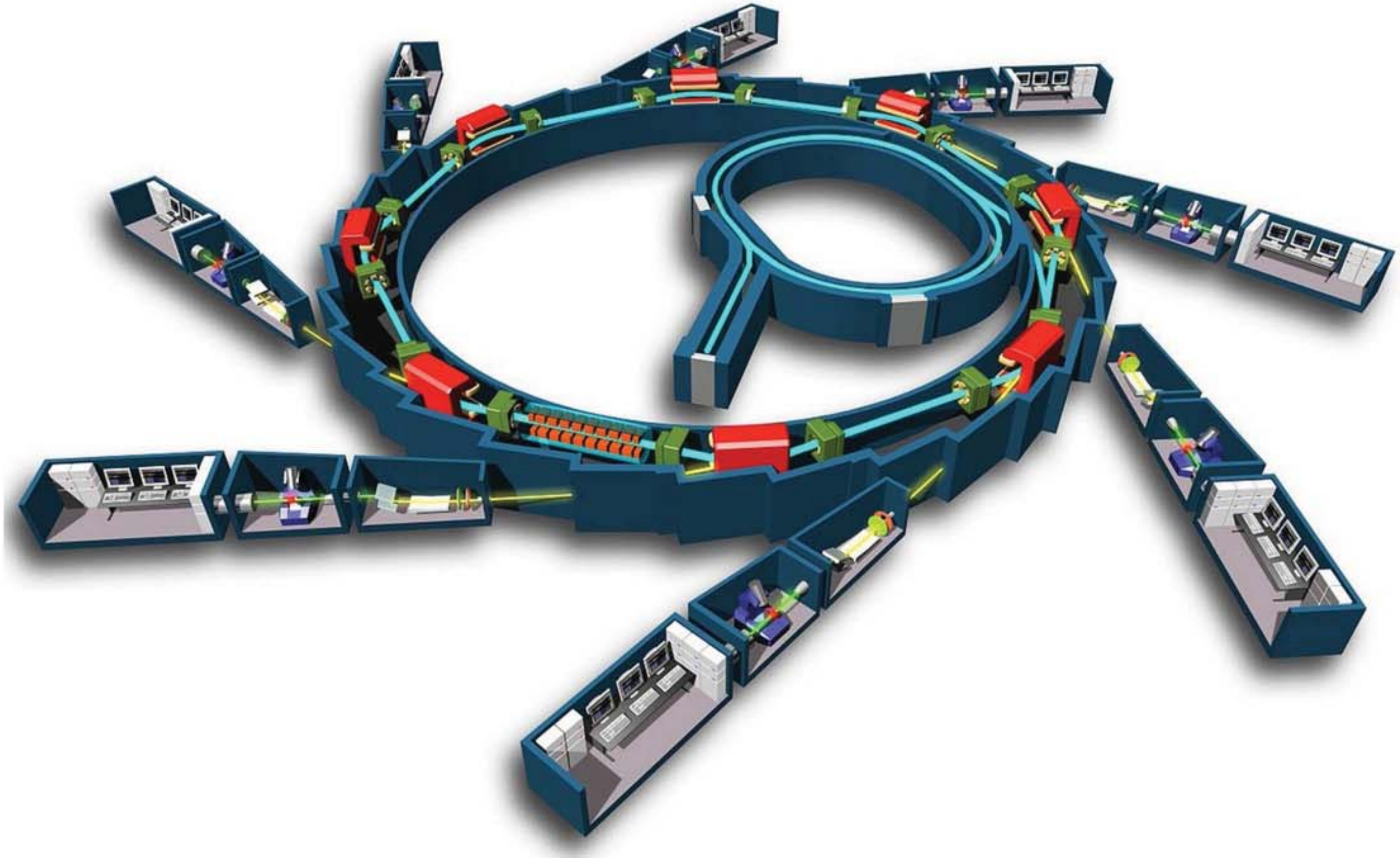


high amount of photons, due to moving parts high maintenance time and effort

Instrumental setup

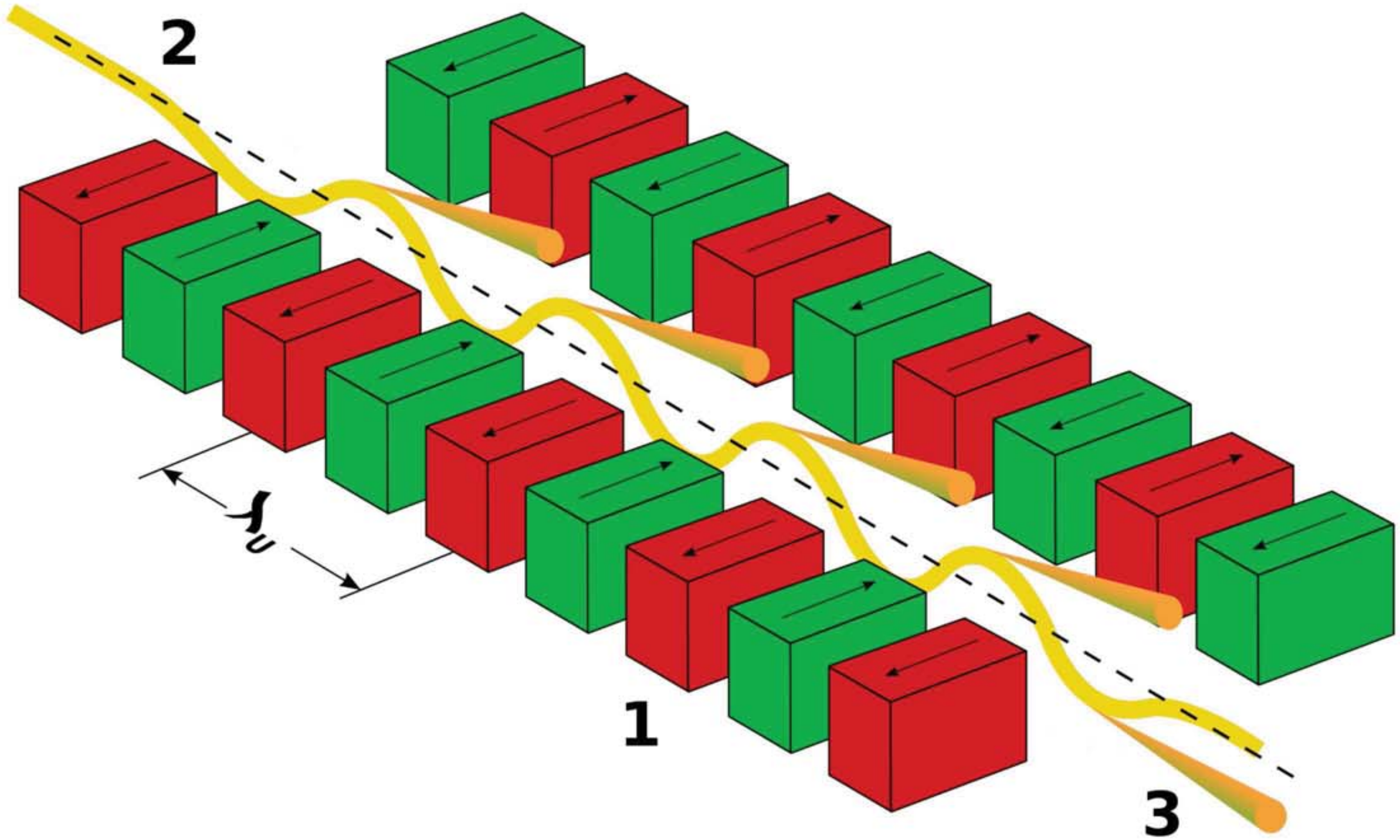
Syncrotron

Evacuated ring tube
4.5 - 6 GeV, 20 - 300 mA single or multi bunch
Up to 10^{14} Photons/s



Instrumental setup

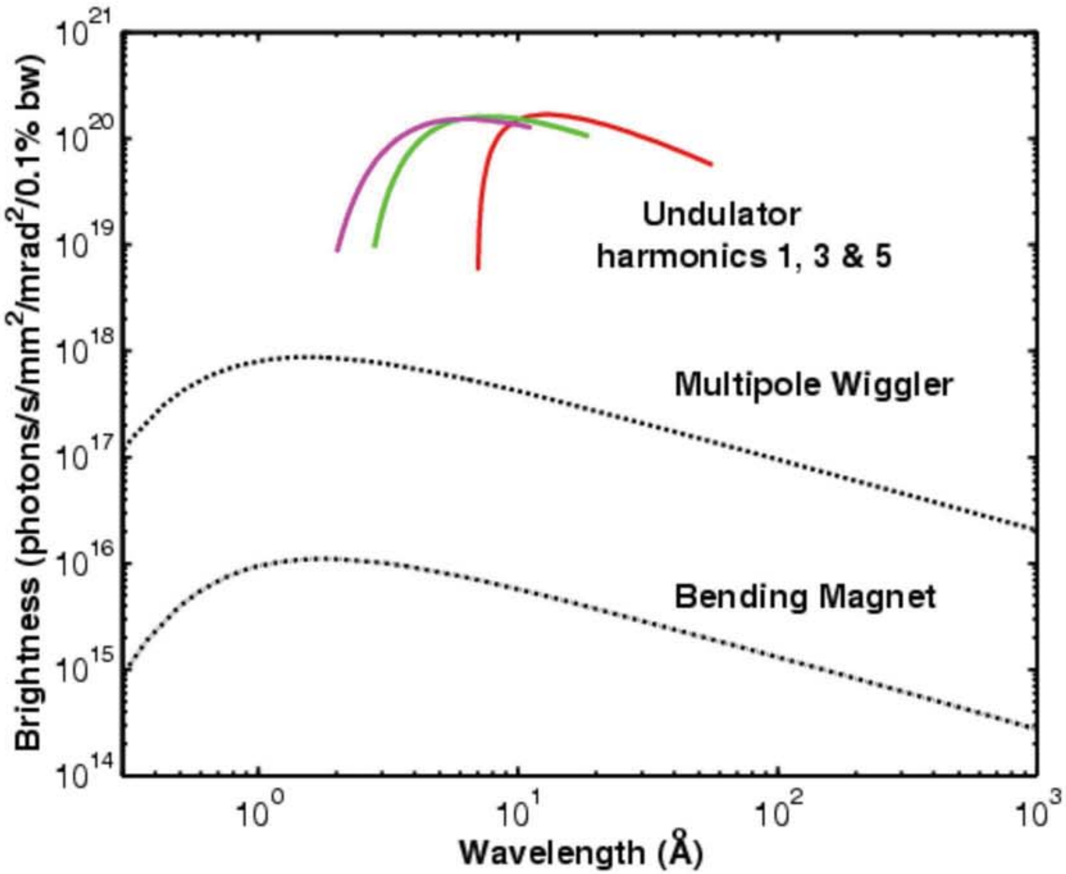
Undulator/Wiggler at a syncrotron



Alternating angular acceleration of the electron beam leads to a tangential emission of irradiation. The overlaying beams from top and bottom are used for experiments.

Instrumental setup

Energy spectrum at syncrotrons

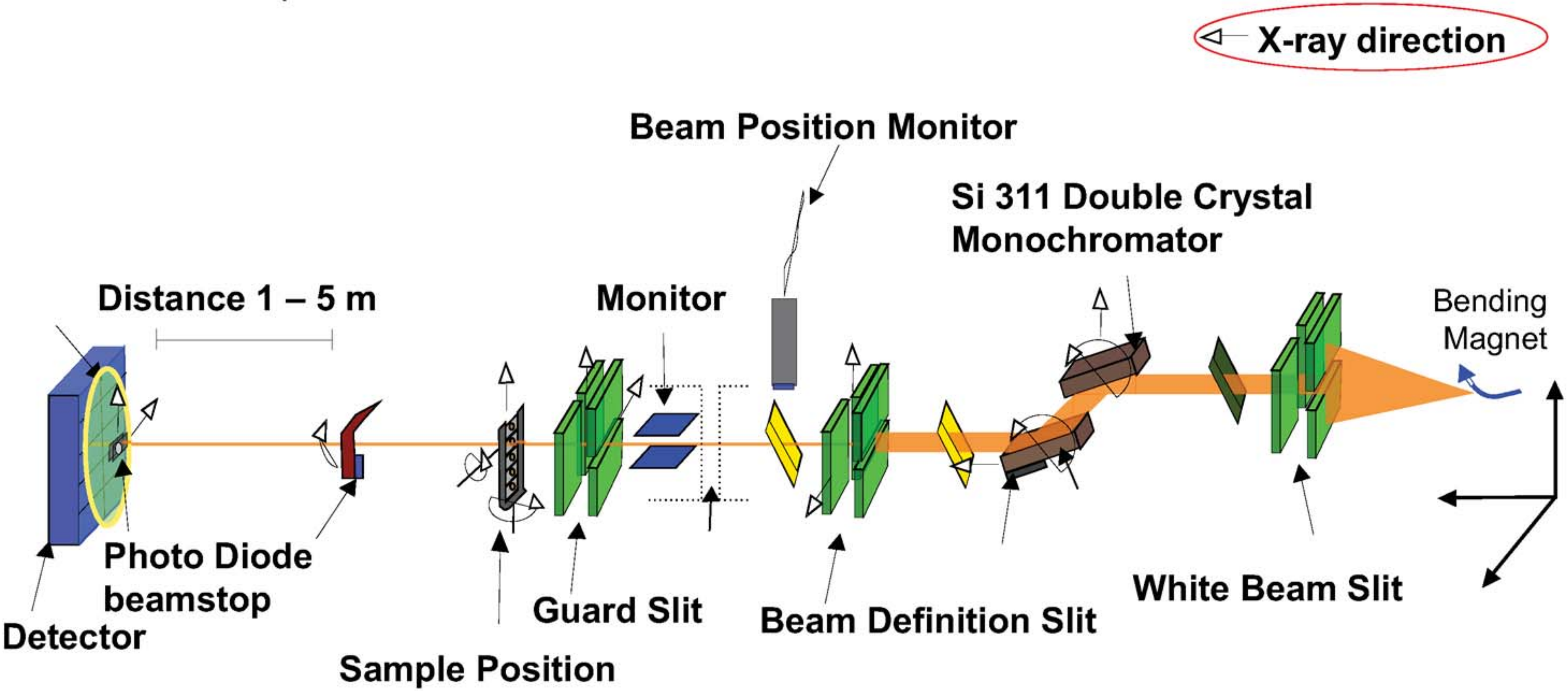


- Bending magnets have a low intensity but a wide energy range.
- Wigglers have a spectrum like a bending magnet with much more intensity.
- With Undulators a much higher energy is possible however the energy range is very limited.

Instrumental setup

B1 beamline at Doris III Hasylab Hamburg

Dedicated ASAXS Beamline
5- 35 keV, 10^6 photons/s

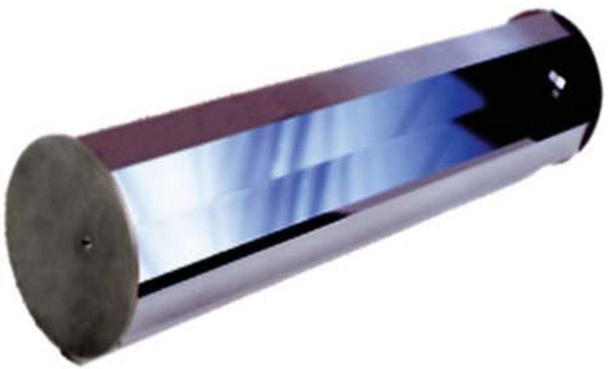


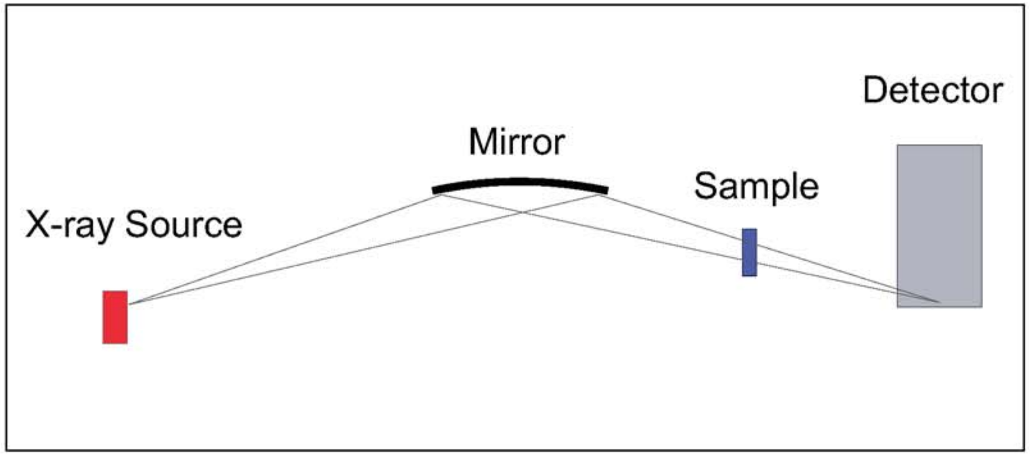
X-ray optics

Due to weak interaction of x-rays with matter there are no normal lenses for x-rays.
- To get a fine beam the primary beam is normally cut by slits.
- Plane mirrors are used under total reflection conditions.
- Multilayered planar mirrors are used for monocromatisation.
- Since 1995 parabolic or ellipsoid mirrors are available to focus x-rays (Göbel mirrors).

For SAXS experiments those mirrors have to be very smooth.

Contrary to single crystal diffraction in SAXS experiments the beam is focused at the detector and not at the sample position.

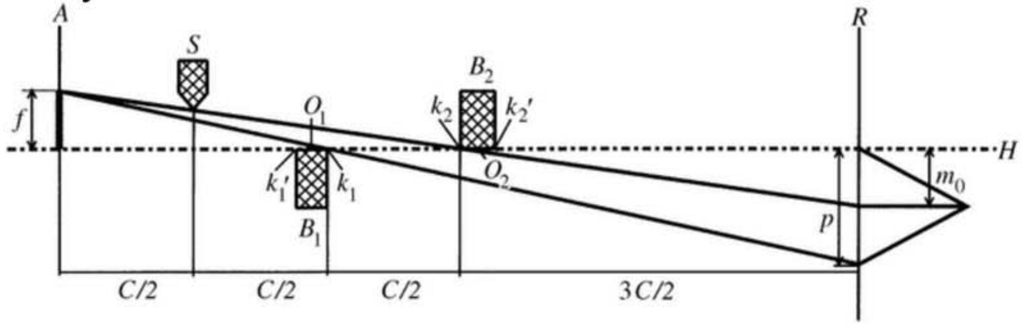




Instrumental setup

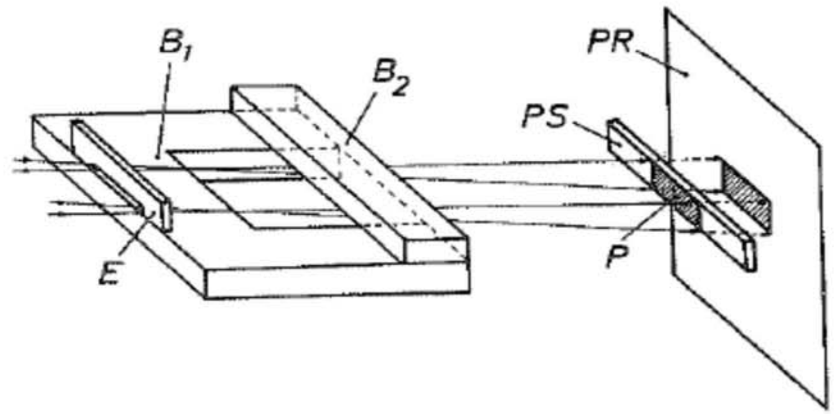
Laboratory Instruments Kratky Camera

X-ray source



Detector

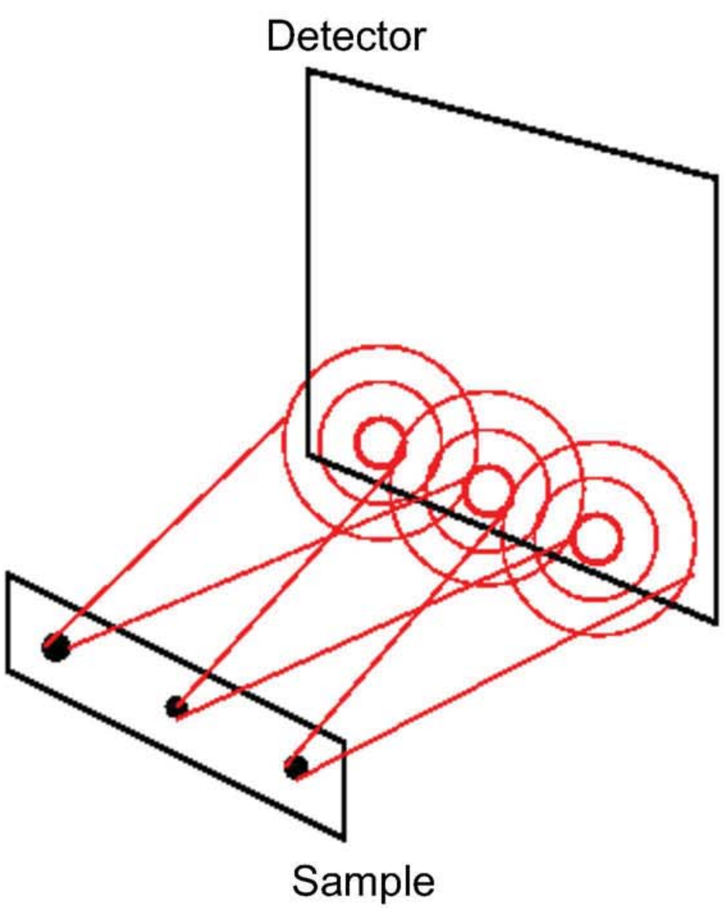
Side view of the collimation system of a Kratky camera. The beam is Cut to its size by the metal blocks B1 and B2. To avoid reflections on the metal surface both surfaces have to be on the same plane (H).



Side top view of the collimation system. To increase the resulting scattering intensity the sample (PS) is illuminated with a bar shaped beam.

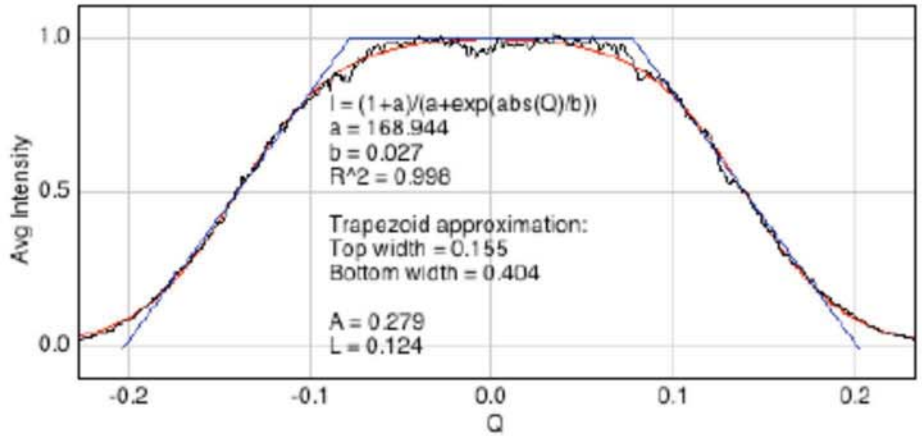
Instrumental setup

Kratky Camera smeared beam profile

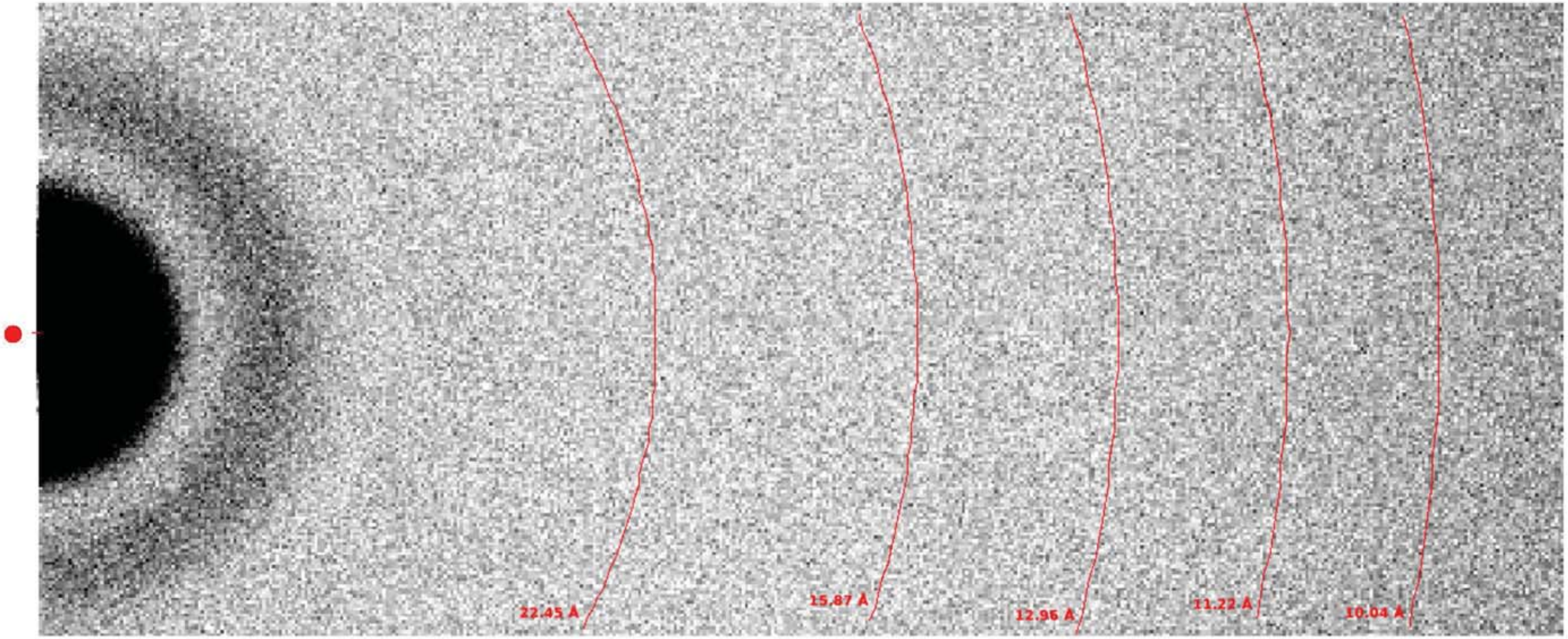


Due to slit collimation the scattering pattern gets smeared. Scattering of different beam centers is overlaying each other.

Empty beam profile of a Kratky camera. Sigmoidal fit to the data to get the trapezoidal primary beam profile.
With this beam profile the desmearing has to be done iteratively.

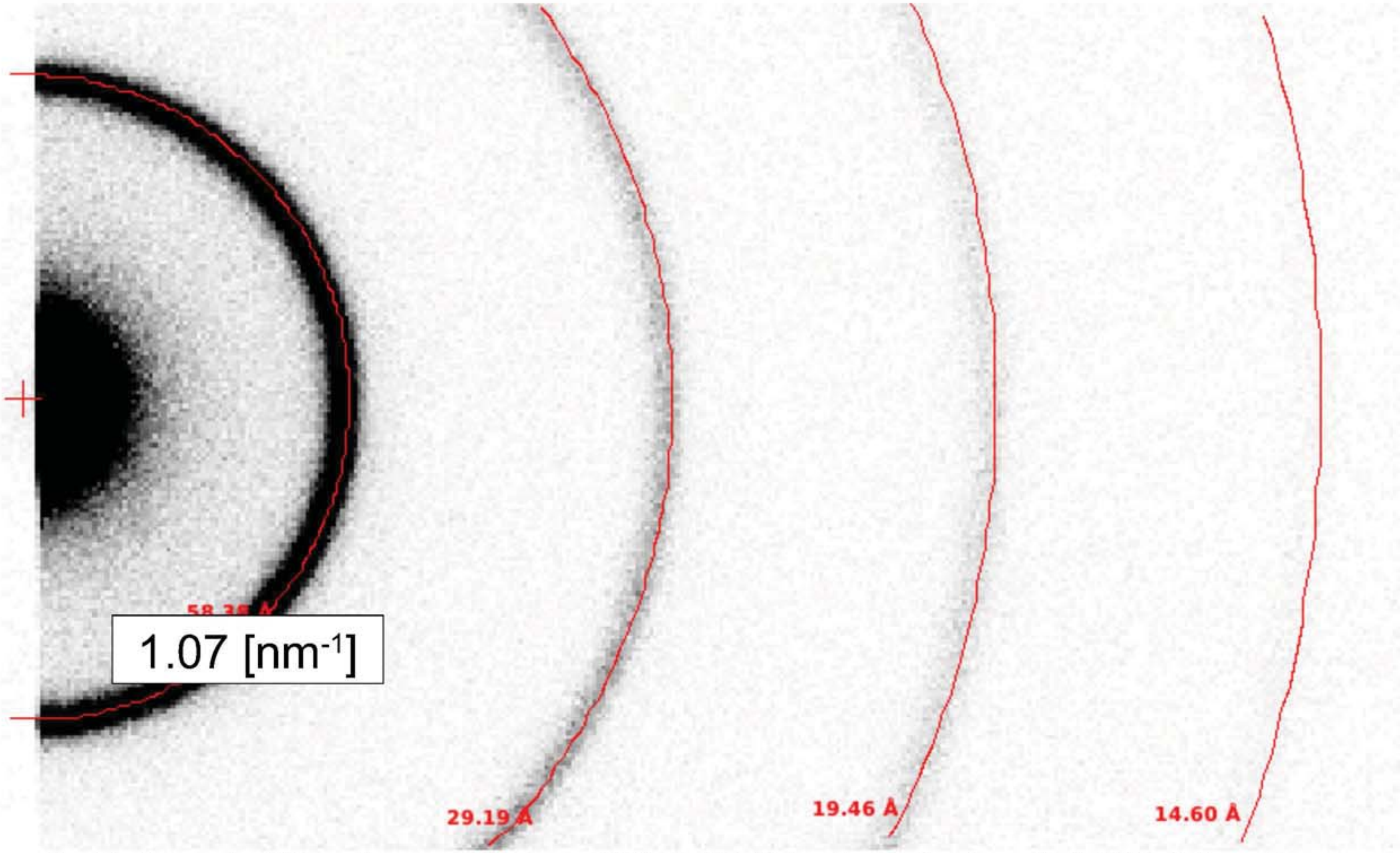


SAXS measurement



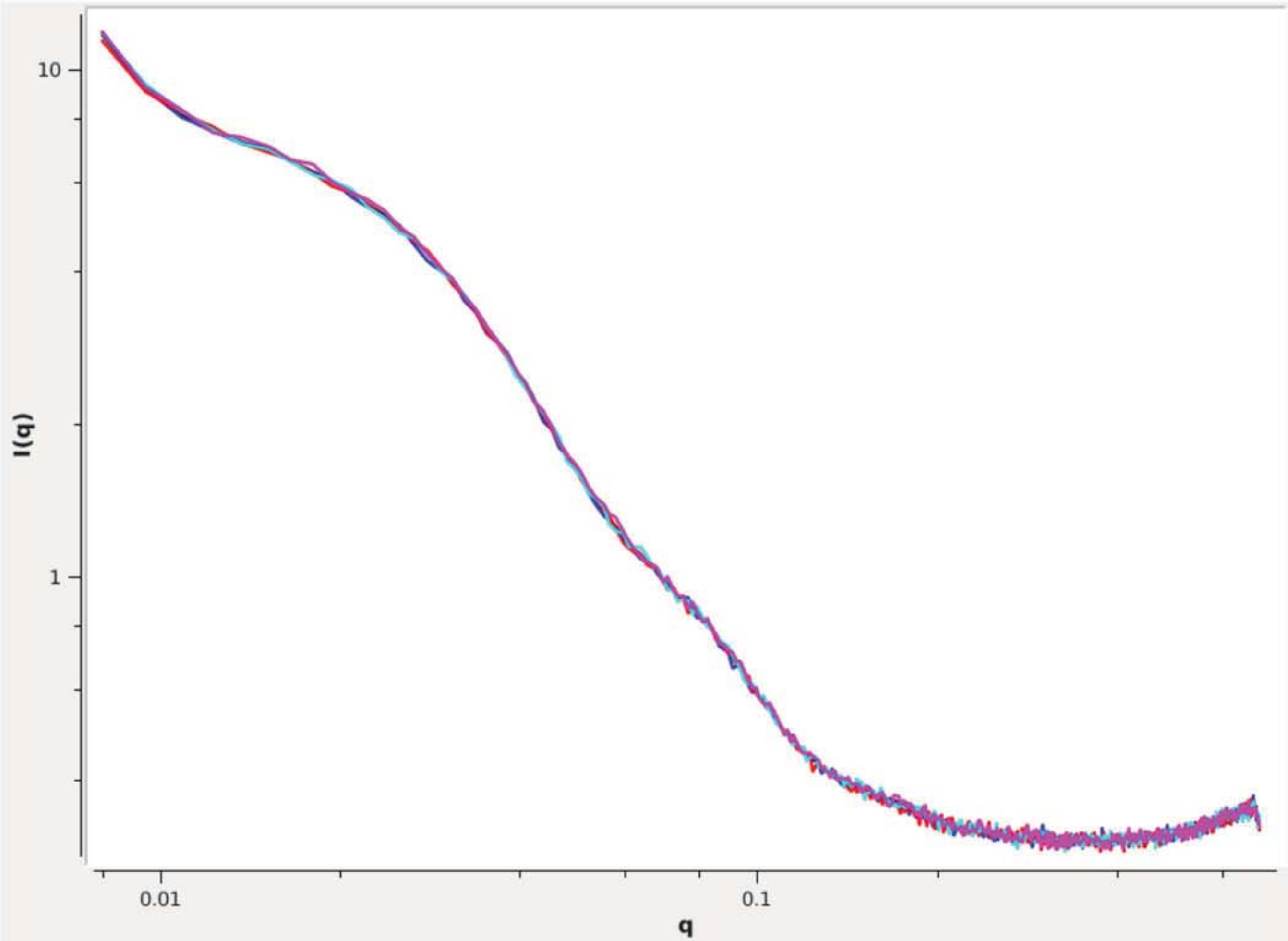
hclpp 5 mg/ml 1 hour average of 4 frames

q-calibration and beam center



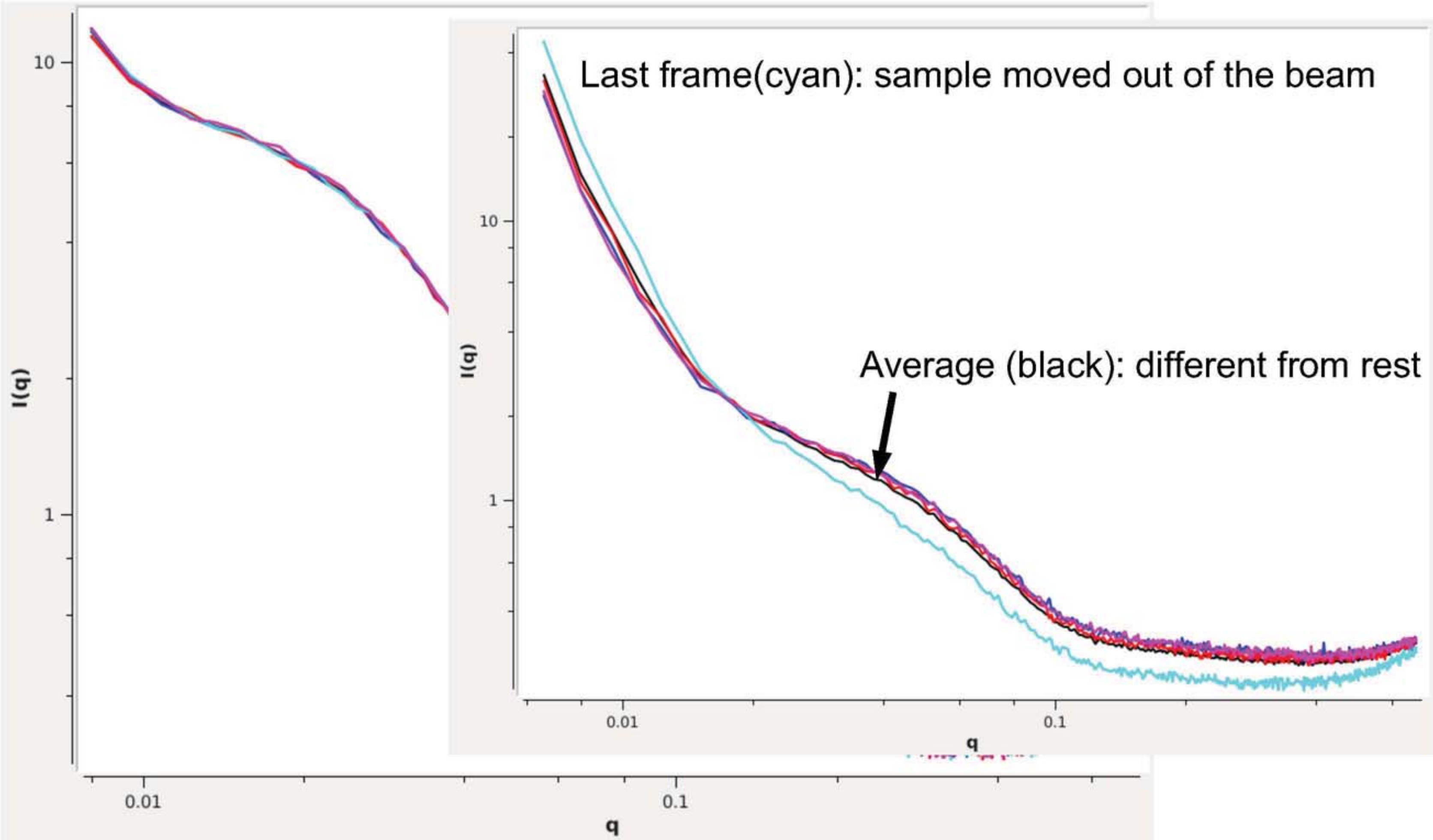
Ag Behenate 30 s beam center: 482.7x90.9 [px], detector dist 482,1 [mm] pixel size 79 [µm]

Raw scattering curves



IgG 5 mg/ml 4 frames of 900 s each and the average. They have to fall upon each other.

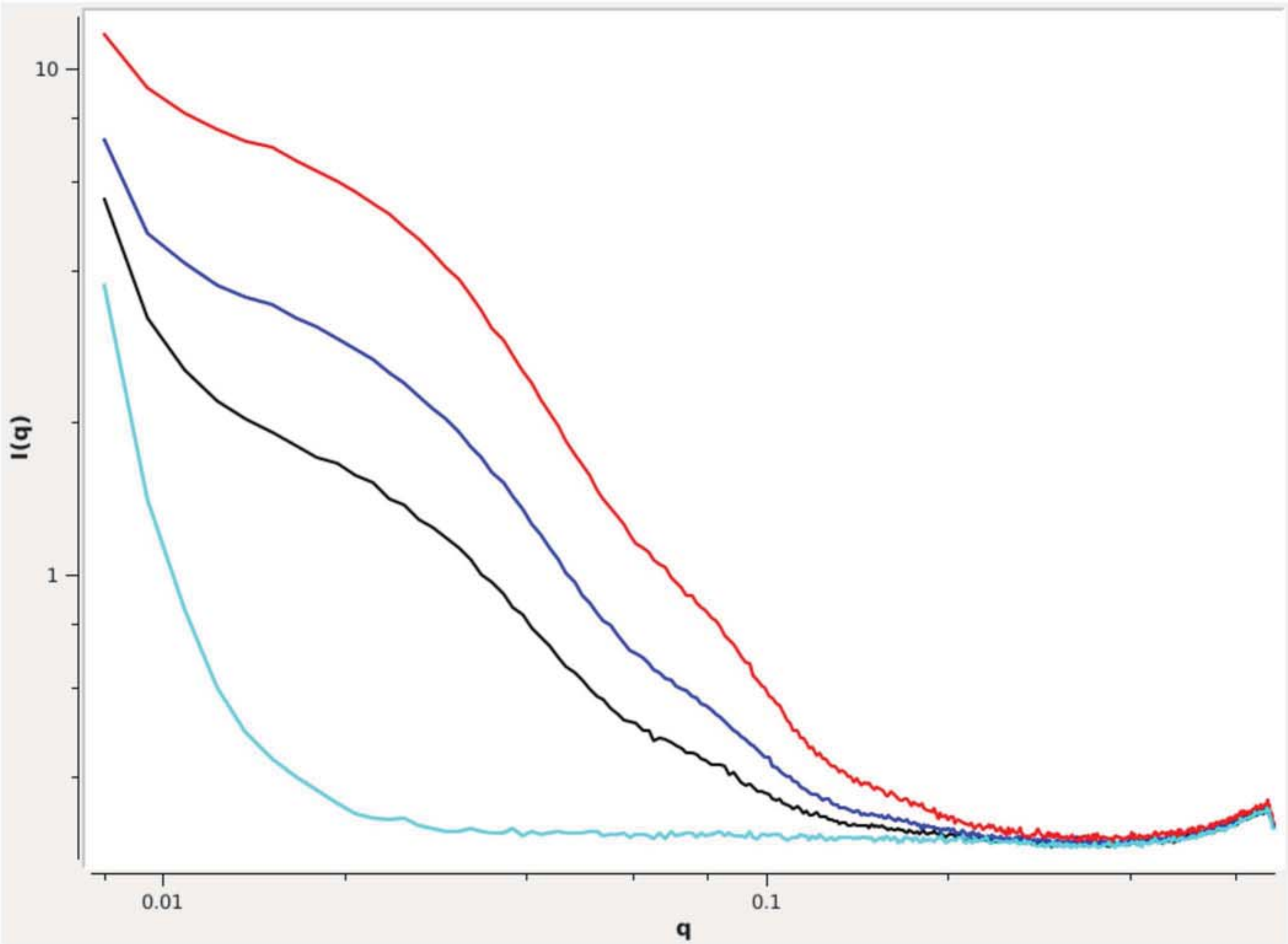
Raw scattering curves



IgG 5 mg/ml 4 frames of 900 s each and the average. They have to fall upon each other.

Instrumental setup

Raw scattering curves from IgG and solvent



IgG red 5 mg/ml blue; 2.5 mg/ml; black 1.25 mg/ml; Buffer cyan (5mg 1h, rest 2 h)

Solvent subtraction

Experimental Data

$$I_{Sample} = (I_{Protein} + I_{Water} + I_{Salt} + I_{Capillary} + I_{Background})$$

$$I_{Solvent} = (I_{Water} + I_{Salt} + I_{Capillary} + I_{Background})$$

Transmission $Tr = \frac{I_s}{I_0}$

I_s Sample Intensity

I_0 Empty Beam Intensity

(both measured with a photo diode behind the sample position)

Typical transmissions for SAXS experiments are between 0.2 - 0.3

Solvent subtraction simple case $Tr_{Sample} \approx Tr_{Solvent}$

$$I_{Protein} = \frac{I_{Sample}}{Tr_{Sample} * d} - \frac{I_{Solvent}}{Tr_{Solvent} * d} \qquad Tr_{Sample} \approx Tr_{Solvent}$$

d capillary thickness

Solvent subtraction

Experimental Data

$$I_{Sample} = (I_{Protein} + I_{Water} + I_{Salt} + I_{Capillary} + I_{Background})$$

$$I_{Solvent} = (I_{Water} + I_{Salt} + I_{Capillary} + I_{Background})$$

$$I_{Capillary} = (I_{Empty\ Capillary} + I_{Background})$$

$$I_{Background} = (I_{Empty\ beam})$$

Transmission $$Tr = \frac{I_s}{I_0}$$

I_s Sample Intensity

I_0 Empty Beam Intensity

(both measured with a photo diode behind the sample position)

Data treatment with different transmission or thickness $Tr_{Sample} \neq Tr_{Solvent}$

$$I_S = \left(\frac{(I_{Sample} - I_{Background})}{Tr_{SamplewithoutTc} * d} - \frac{(I_{Capillary} - I_{Background})}{Tr_{Capillary} * d} \right)$$

$$I_L = \left(\frac{(I_{Solvent} - I_{Background})}{Tr_{SolventwithoutTc} * d} - \frac{(I_{Capillary} - I_{Background})}{Tr_{Capillary} * d} \right)$$

$$Tr_{SamplewithoutTc} = \frac{Tr_{Sample}}{Tr_{Capillary}}$$

d capillary thickness

$$I_{Protein} = I_S - I_L$$

Solvent subtraction

E

$I_{Sample} = (I_{Protein}$

$I_{Solvent} = (I_{Water}$

$I_{Capillary} = (I_{Empty}$

Transmission $Tr = \frac{I_s}{I_0}$

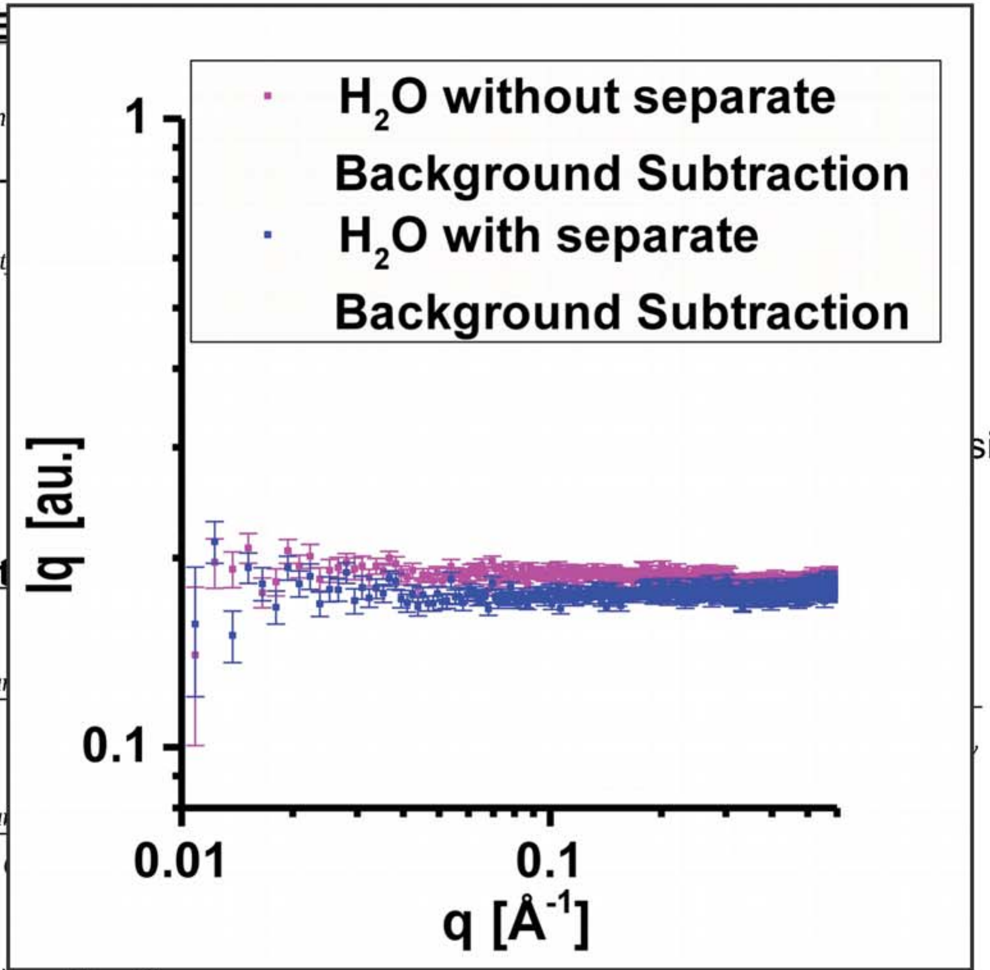
sition)

Data treatment with different t

$I_S = \left(\frac{(I_{Sample} - I_{Backgrou}}{Tr_{SamplewithoutTc} *} \right.$

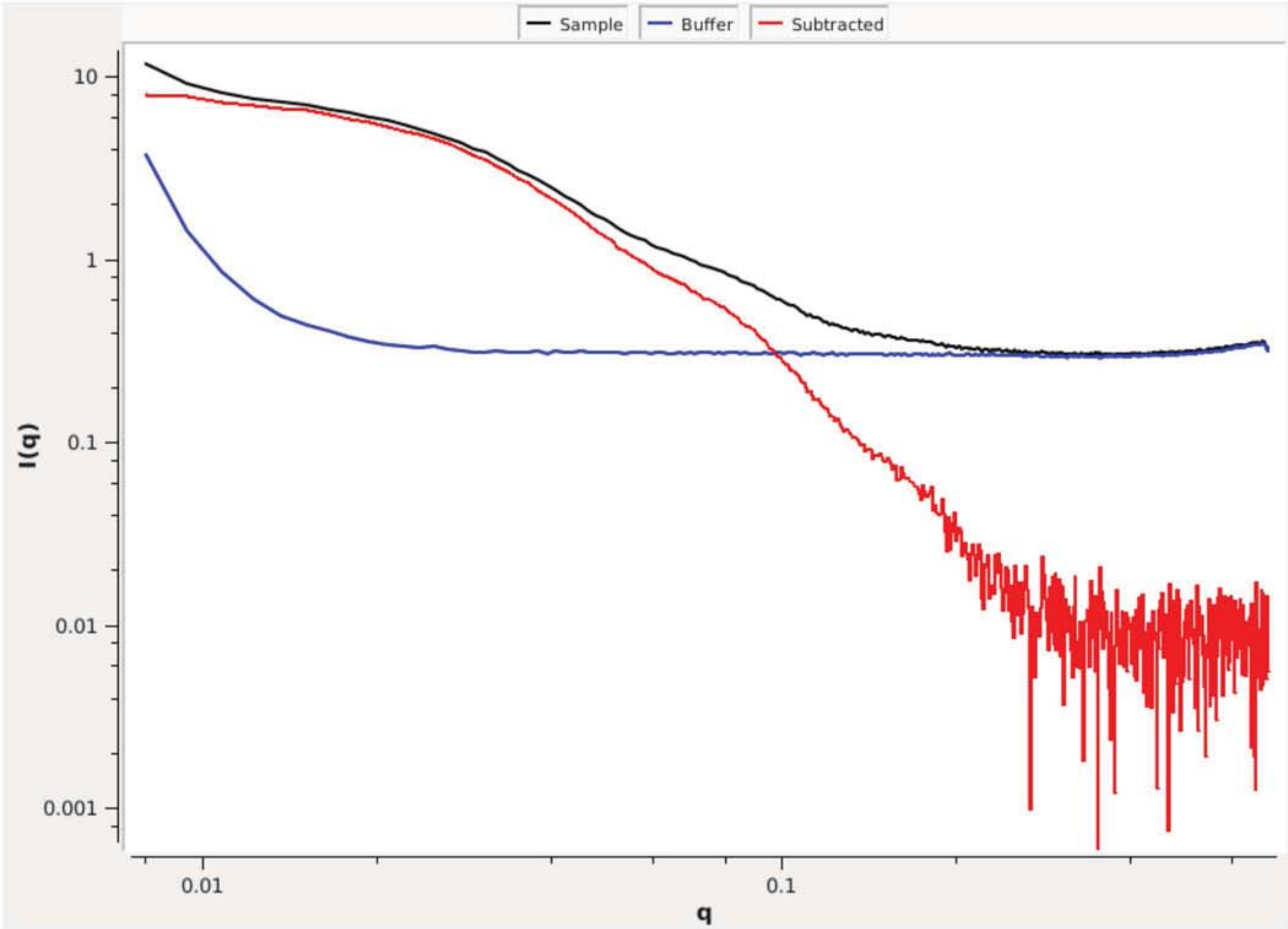
$I_L = \left(\frac{(I_{Solvent} - I_{Backgrou}}{Tr_{SolventwithoutTc} *} \right.$

$I_{Protein} = I_S - I_L$



Instrumental setup

Subtracted scattering curve of IgG



IgG 5 mg/ml 1h raw (black), subtracted (red), Buffer 2h (blue)

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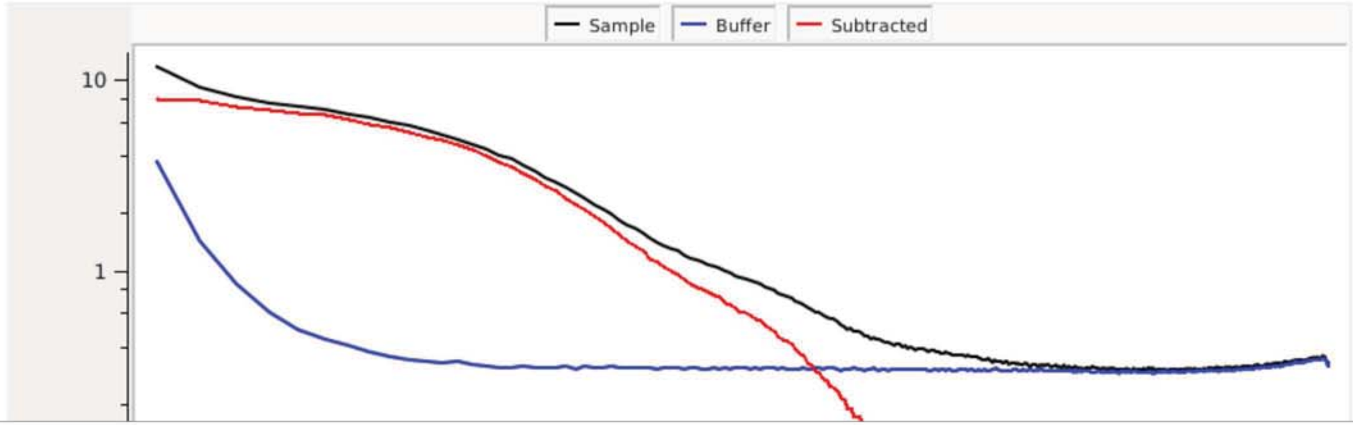
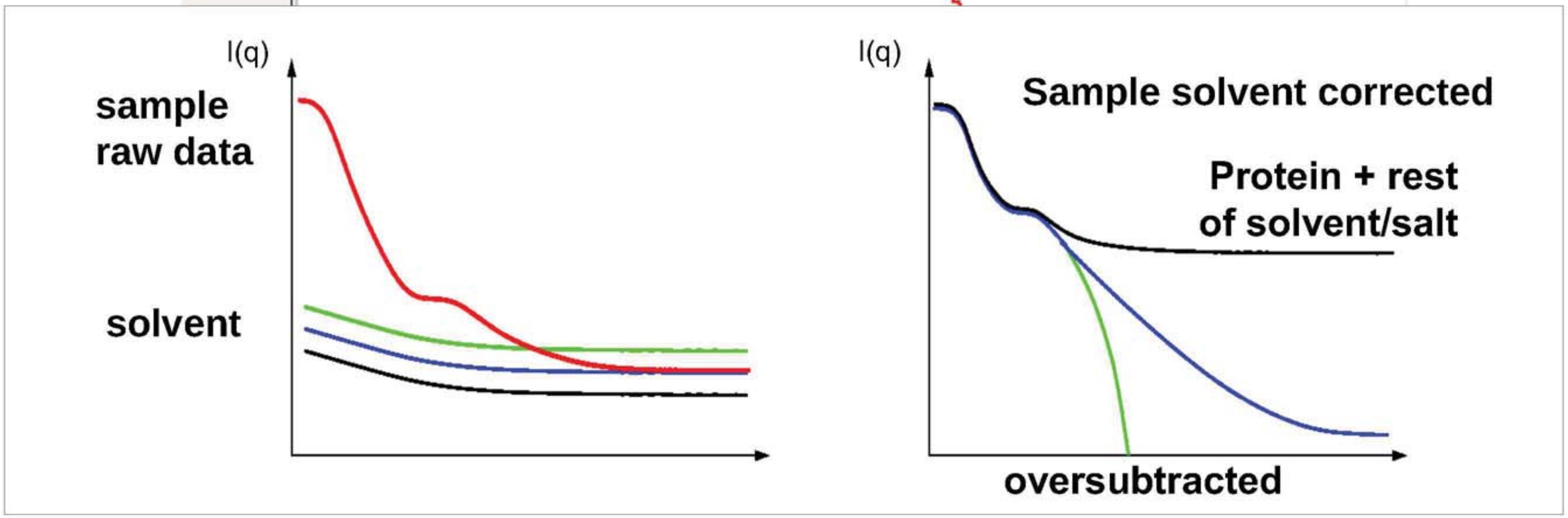
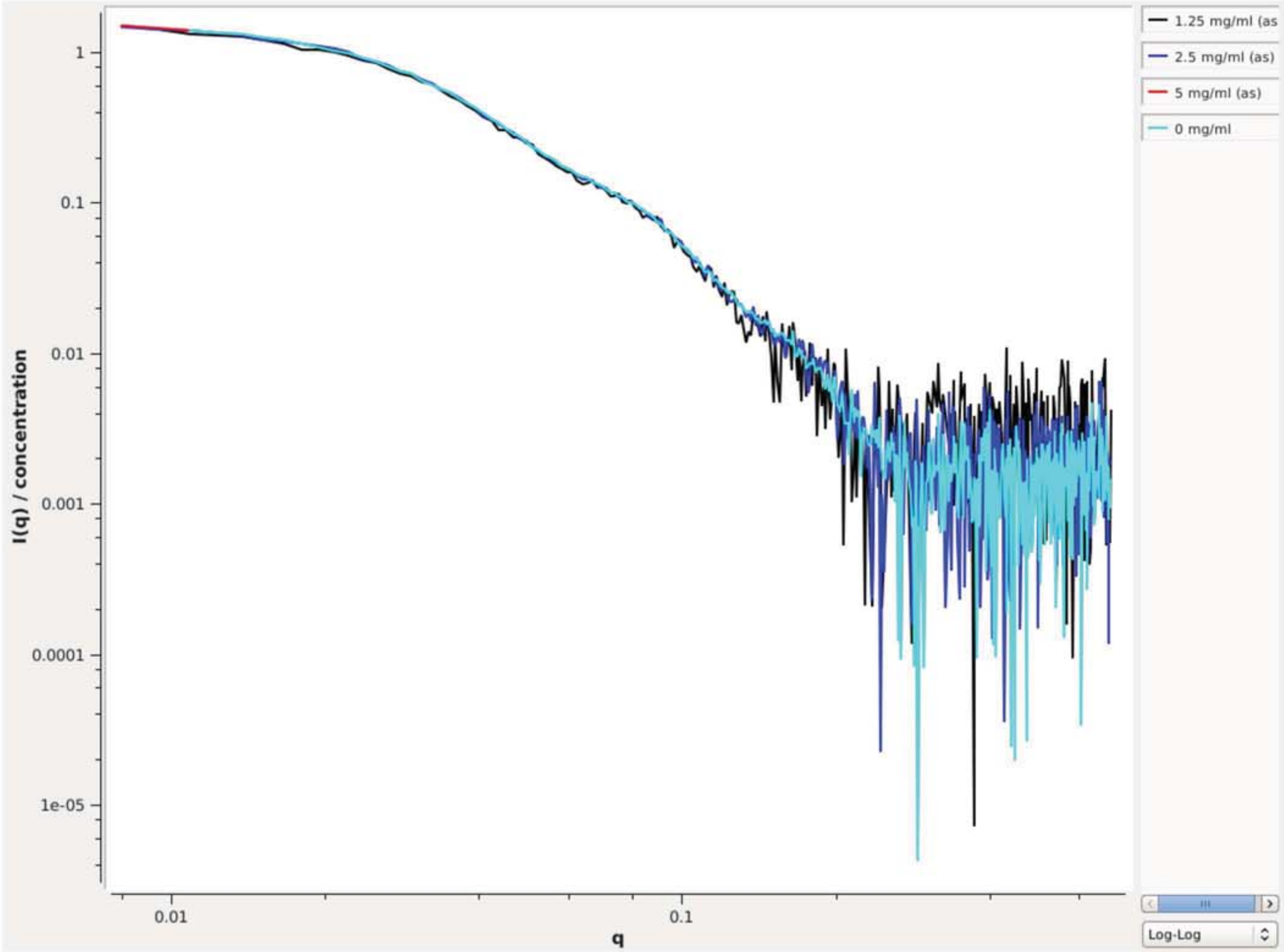




IgG 5 mg/ml 1h raw (black), subtracted (red), Buffer 2h (blue)

Scattering curves divided by concentration

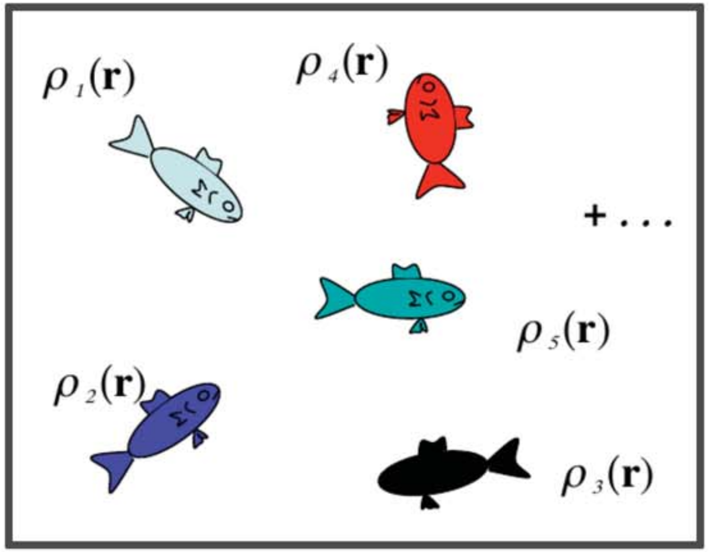


IgG curves are falling upon each other, no concentration dependent oligomerisation...

Sample preparation

Sample preparation

What are we looking at?



$$I(\mathbf{Q}) = |\mathbf{F}_1(\mathbf{Q})|^2 + |\mathbf{F}_2(\mathbf{Q})|^2 + \ldots$$

Dirty sample

Sample preparation

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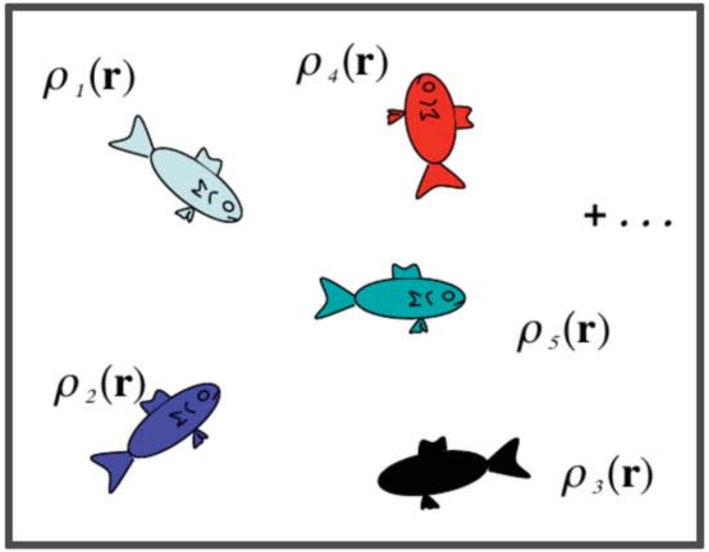

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Dirty sample

What do we get?

A statistical average of the scattering of all species in the illuminated volume

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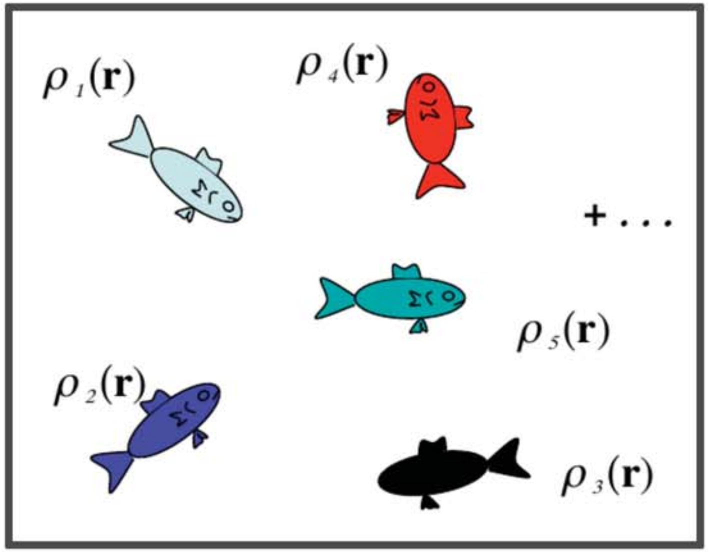

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Dirty sample

What do we get?

A statistical average of the scattering of all species in the illuminated volume

What do we get out of this?

A statistically averaged model of all species in the illuminated volume

Sample preparation

$+ |\mathbf{F}_2(\mathbf{Q})|^2 + \ldots$

Dirty sample

A statistical av inated volume

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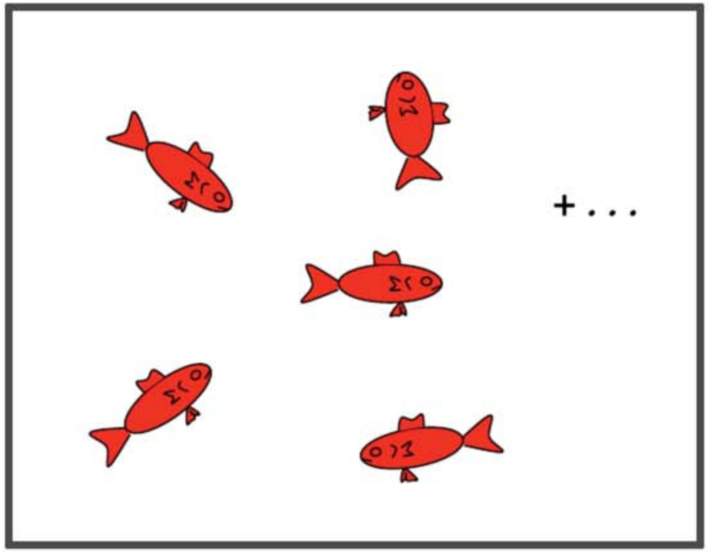
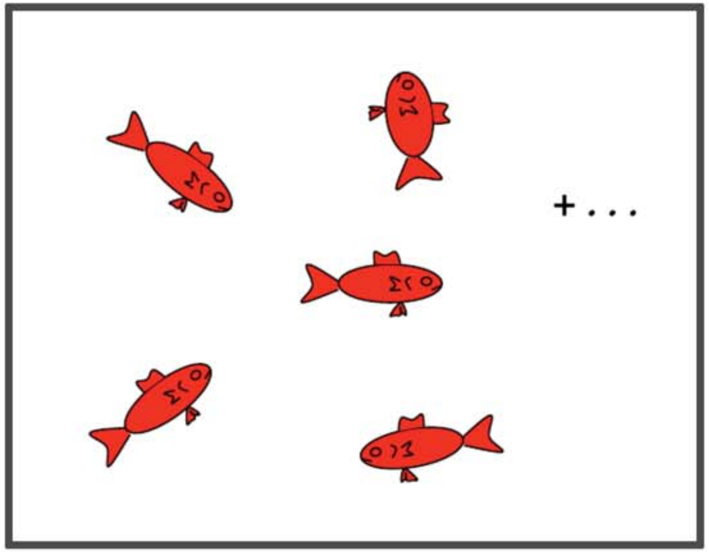


$$I\,(\mathrm{Q}) = N < |\mathbf{F}\,(\mathbf{Q})|^2 >$$

Clean sample

Sample preparation

What are we looking at?



$$I(Q) = N < |\mathbf{F}(\mathbf{Q})|^2 >$$

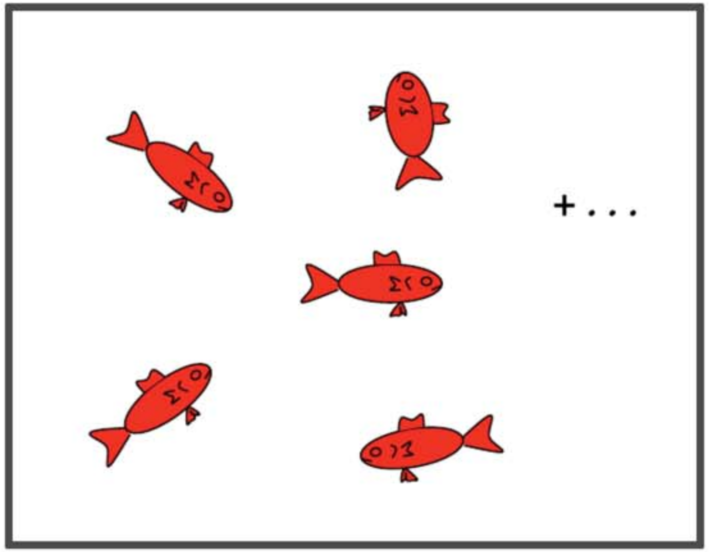
Clean sample

What do we get?

A scattering profile of all particles in the illuminated volume

Sample preparation

What are we looking at?



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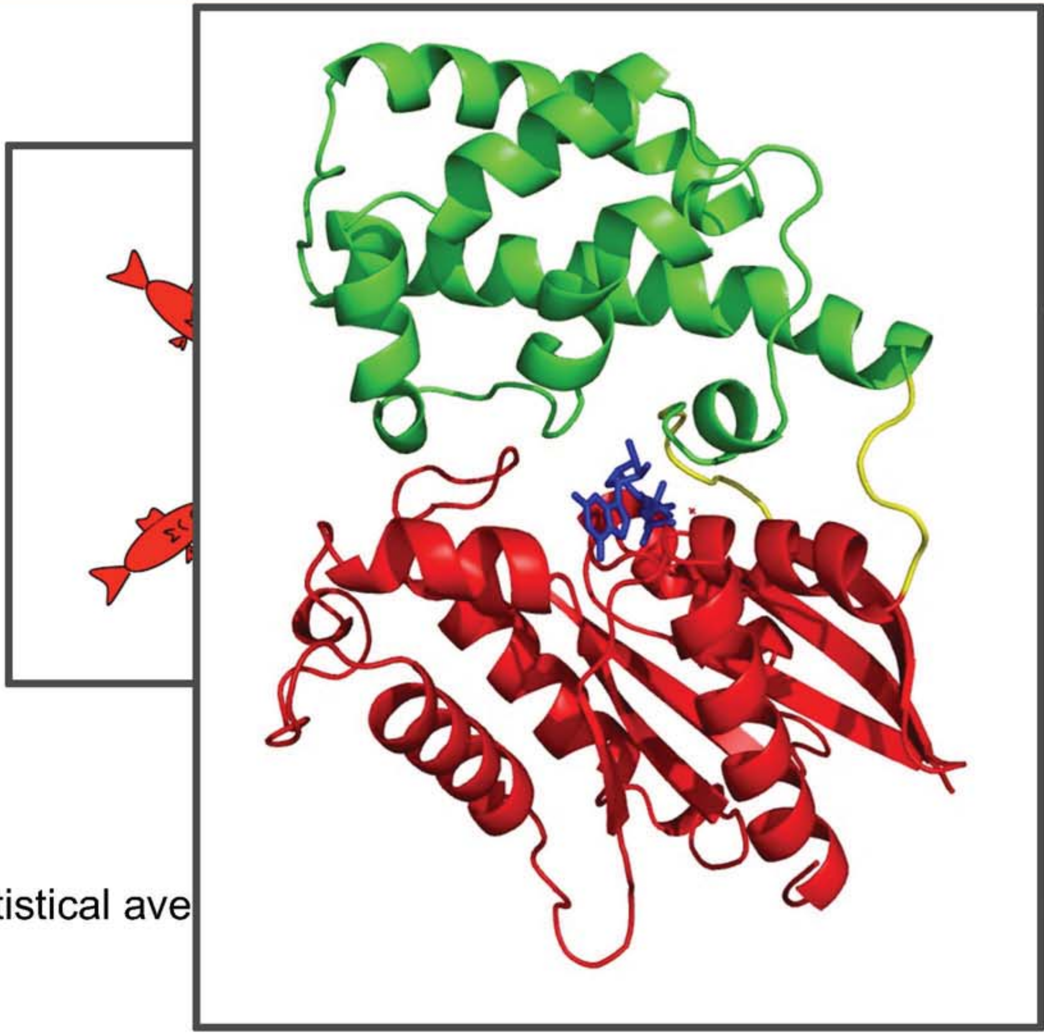
What do we get?

A scattering profile of all particles in the illuminated volume

What do we get out of this?

An averaged model of all conformations of the protein in the illuminated volume

Sample preparation



$< |F(Q)|^2>$

Clean sample

A statistical ave

minated volume

<u>What do we get out of this?</u>

An averaged model of all conformations of the protein in the illuminated volume

Characterisation of samples

SDS gel	unfolded AS-chain, no Agglomerates visible	NO
DLS	nativ conditions, sensitive to impurities and Agglomerates	OK
SLS	nativ conditions, sensitive to Agglomerates	OK
GPC - (SLS)	nativ conditions,semiquantitive analysis of impurities, Agglomerates	OK

Small angle scattering

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Agglomerates

$$V_{Part} \approx r^3, \quad I_{Part} = V_{Part}^2 P(q) \longrightarrow I_{Part} \propto r^6$$

Example

Stock solutions
(1) Protein 10mg/ml,
100mM NaCl, 10mM Tris
(2) RNA 100mg/ml, H_2O

Samples

Protein	(1) 70 µl
RNA	(2) 7 µl, 63 µl H_2O
Complex	(1) 63 µl, (2) 7 µl
Buffer	
Water	

Small angle scattering

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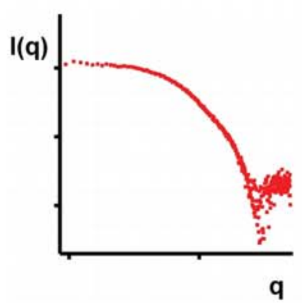
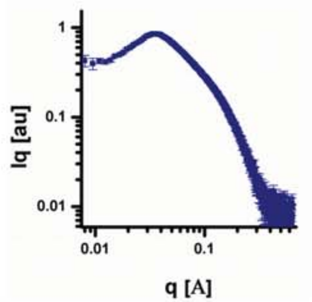


RNA



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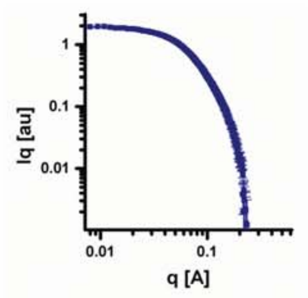

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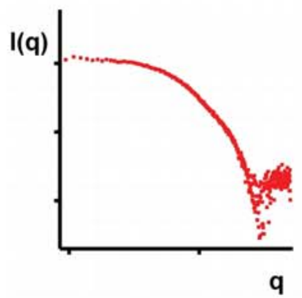

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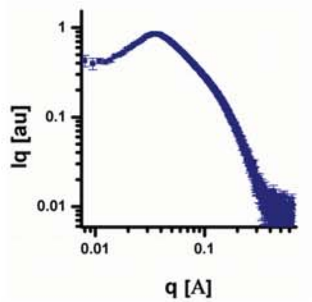

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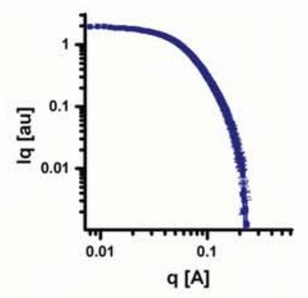

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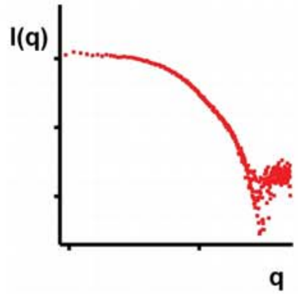
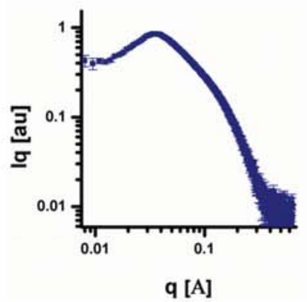


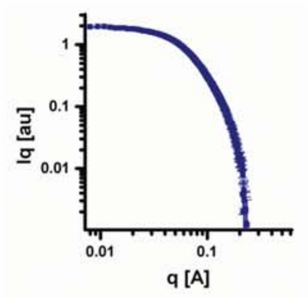
OK

RNA



Structure factor due to polyioic repulsion

Complex



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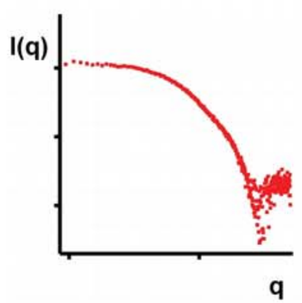

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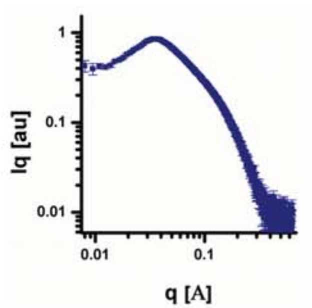

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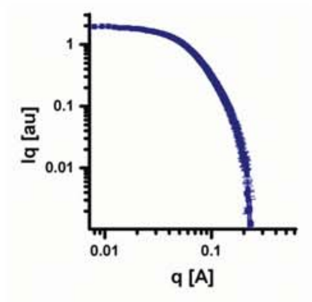

Oversubtraction sample and buffer do not have the same salt concentration

Small angle scattering

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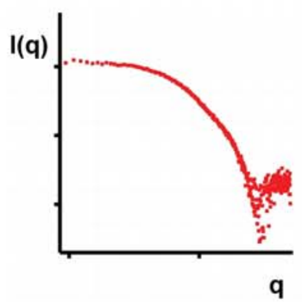
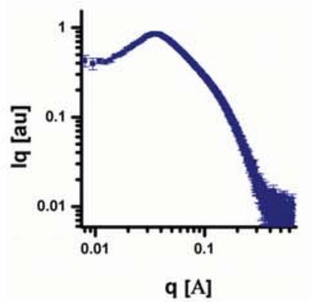


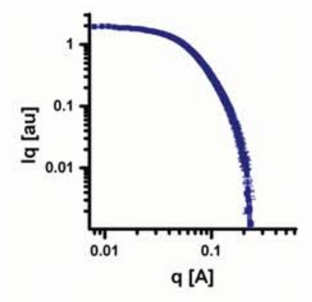
OK

RNA



Structure factor due to polyioic repulsion
The repulsing electric field of polyions has to be screened out with 20 – 50 mM salt.

Complex



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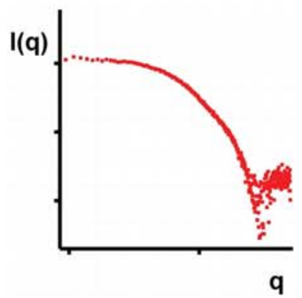
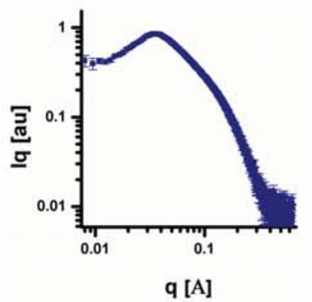


OK

RNA



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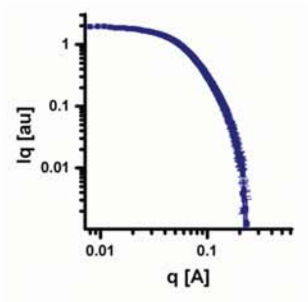

Oversubtraction sample and buffer do not have the same salt concentration
Preparation of both components in the same buffer or buffer exchange after mixing

Thank You