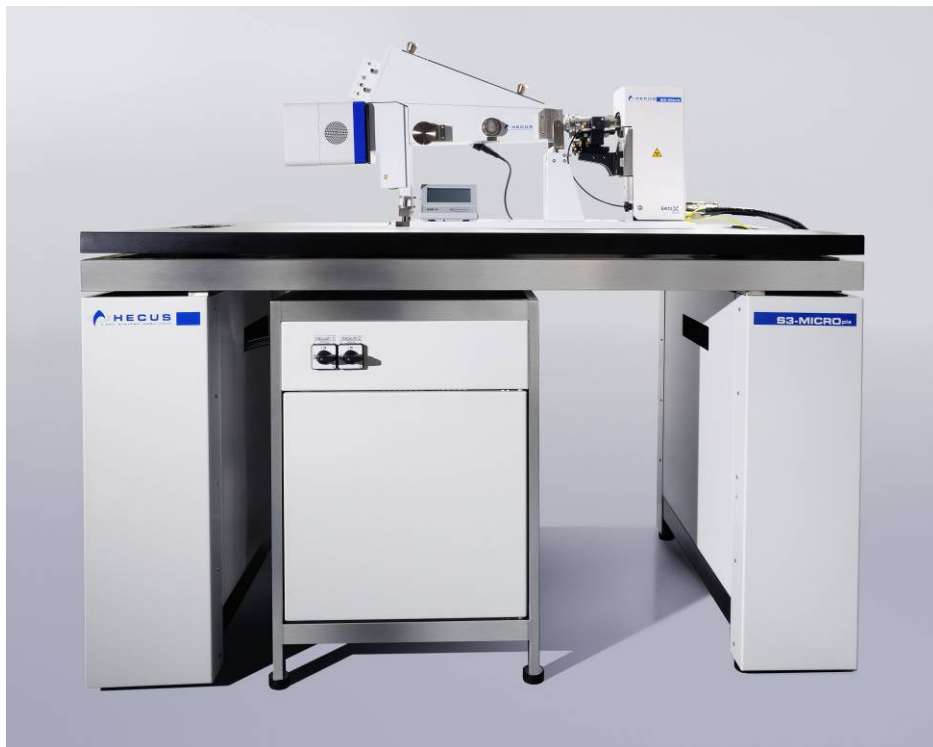


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HIGHLIGHTS OF S3-MICRO

- The point-focussing beam geometry of S3-MICRO has proven highly advantageous by offering the possibility selecting high-flux (MAXS) and high resolution (SAXS) mode, resp.
- Experimental setup for different samples (e.g. for powders, liquids) can be changed and adapted quickly.
- Modular setup allows quick adaptation to use different detectors and/or q-ranges
- Measurements may require less than 10 minutes per sample



(BIO)-NANOPARTICLES IN SOLUTION

Small angle X-ray scattering of proteins – or of nanoparticles in general – in solution has proven to be a valuable method for their (nano)structural characterization and parameterization like size and shape. The most prominent parameter is the particle's radius of gyration R_g , a value which relates to the size of the particle and which can be easily extracted from the inner part of a SAXS curve:

$$I(q) \sim I(0) \cdot \exp(-q^2 \cdot R_g^2 / 3) \quad \text{Guinier's law}$$

with I being the scattered intensity from the sample, q the reciprocal scattering vector (related to the scattering angle 2θ) and $I(0)$ being the extrapolated intensity to the angle zero. The relation between q (reciprocal metric units, nm^{-1} or \AA^{-1}) and 2θ ($^\circ$, angular units) is given by $q = 4\pi (\sin\theta)/\lambda$, with 2θ being the scattering angle with respect to the incident beam and λ the wavelength in nm or \AA of the used X-ray beam.

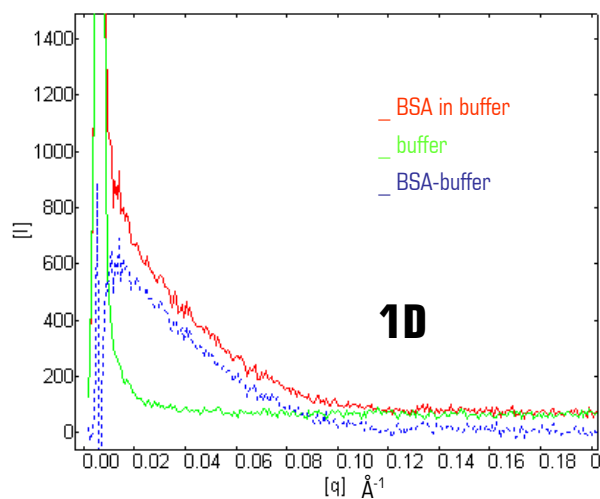
Protein in Solution: BSA

Experimental Setup

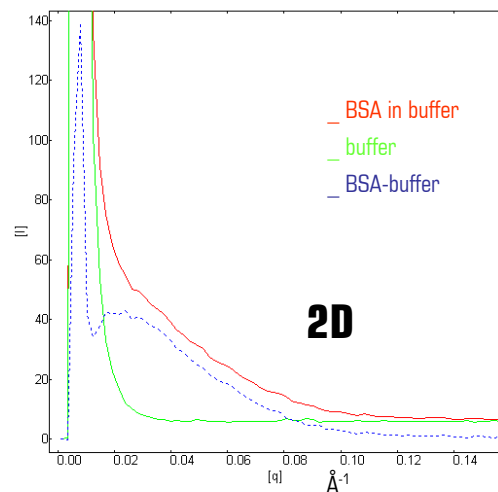
Sample preparation	filled in quartz capillaries of 1 or 2 mm \varnothing
Camera	Hecus S3-MICROpix (point focus)
X-rays	Cu-K α ($\lambda = 1.54 \text{ \AA}$)
Power	50 kV, 1 mA (50 W)
Collimation Setting	Standard-Flux
Detectors	1D-PSD: 1024 channels (54 μm) 2D: Pilatus (Dectris): 487x195 px. (172 μm)
Sample-detector distance	285/304 mm, respectively
q-calibration	by Ag-behenate ($d = 58.38 \text{ \AA}$)
Measured q-range	$0.01 \text{ \AA}^{-1} < q < 0.6 \text{ \AA}^{-1}$
Temperature	20°C
Exposure time	3600 s or 3000 s

Comparison

Comparison of two different measurements of 0.5% BSA (Bovine Serum Albumine [freshly filled each time]), measured with a 1D and a 2D detector respectively. Please note the different exposure times and diameters of the capillaries in the two different measurements.

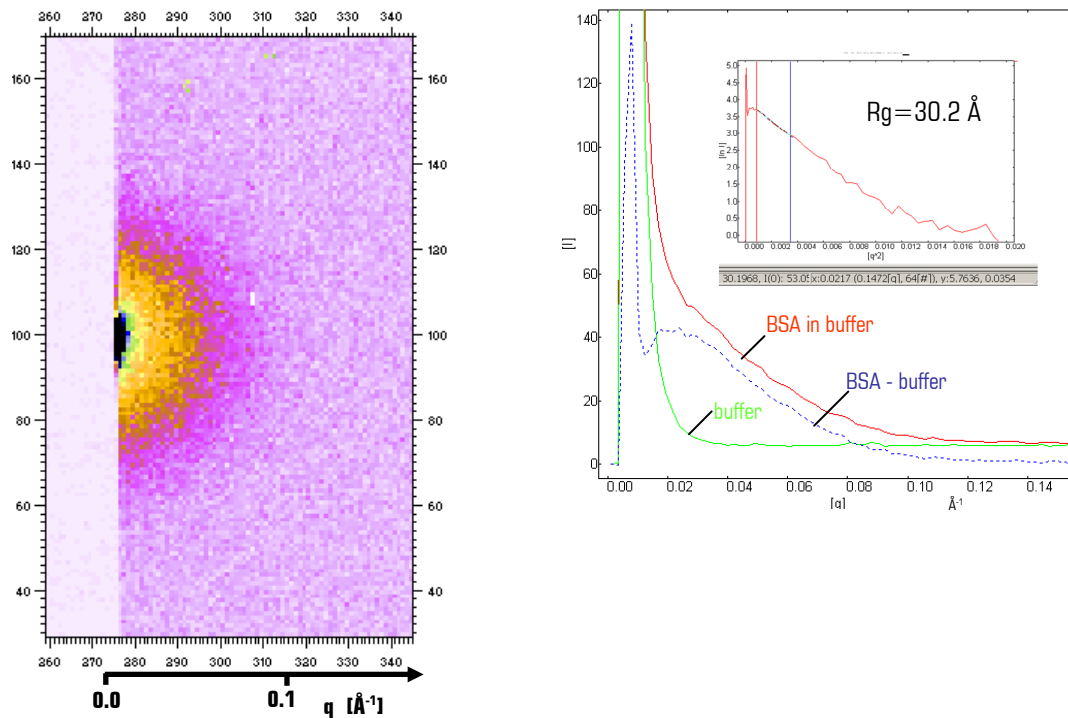


*BSA in 2 mm quartz-capillaries measured with 1D-PSD.
Exposure time 3600 s.*



BSA in 1 mm quartz-capillaries measured with Pilatus. Exposure time 3000 s. Data were azimuthally averaged using FIT2D.

SAXS solution measurement of BSA with 2D-SAXS-detector



Left: 2D-SAXS pattern of a albumin (BSA) solution (5 mg/ml) in buffer zoomed into the inner part ($q < 0.17 \text{ \AA}^{-1}$). The beam center is at pixel $x=272$, $y=99$. Exposure time was 3000 s. Note, that the background ($q > 0.15 \text{ \AA}^{-1}$) has only 0.002 counts/s/pixel.

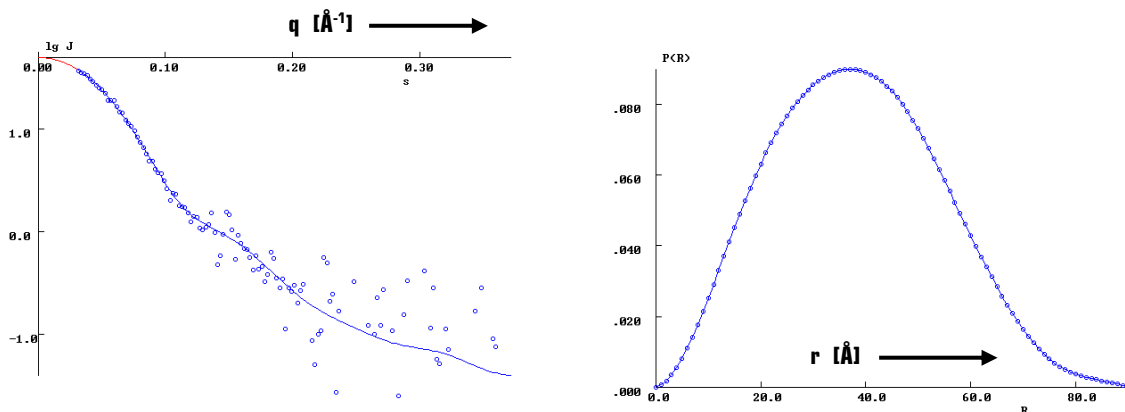
Right: Azimuthally averaged (using FIT2D, A. Hammersley, ESRF, Grenoble) scattering curve of BSA solution (red), the respective buffer (green) and back-ground-subtracted scattering curve (blue). The insert shows the Guinier-plot yielding a R_g of $30.2 \pm 0.5 \text{ \AA}$.

Fourier-transformation & calculation of distance-distribution function

Fourier-transformation and calculation of the distance-distribution function $p(r)$ (right) using the program GNOM (ATSAS 2.3 package by D. Svergun EMBL-Hamburg). Data were measured with the 2D detector and then azimuthally averaged in order to obtain the 1D-scattering curves. The SAXS-curve of the buffer was subtracted from the BSA SAXS-curve after proper normalization (left).

Input data: scattering curve: $0.03 < q < 0.37 \text{ \AA}^{-1}$

Radius of gyration yielding from Guinier-plot: $30.2 \pm 0.5 \text{ \AA}$



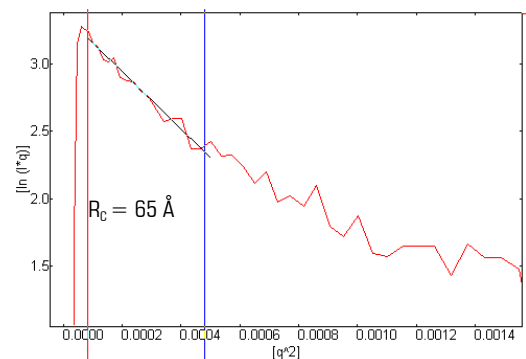
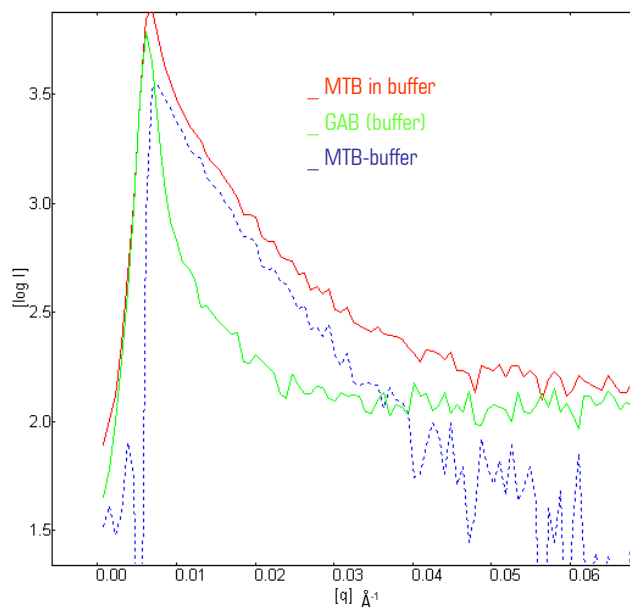
Stabilized Microtubules (MTB)

Experimental Setup

Samples	stabilized microtubules (MTB), 7 mg/mL, drop-frozen in glycerol-containing buffer (GAB), filled in quartz capillaries of 1 mm \emptyset
Camera	Hecus S3-MICROpix (point focus)
X-rays	Cu-K α ($\lambda = 1.54 \text{ \AA}$)
Power	50 kV, 1 mA (50 W)
Collimation Aperture	Standard-Flux
Detector	1D-PSD: 1024 channels (54 μm)
Sample-detector distance	285 mm
Calibration	by Ag-behenate ($d = 58.38 \text{ \AA}$)
Measured q-range	$0.008 \text{ \AA}^{-1} < q < 0.6 \text{ \AA}^{-1}$
Temperature	20°C
Exposure Time	3600 s

SAXS of a stabilized microtubules (MTB) solution, 7 mg/mL, in GAB

Zoom into the inner part of the SAXS-curves of MTB-tubules in GAB (buffer), of the buffer and of the buffer-subtracted sample. The difference between the scattering of the MTB solution and the GAB (buffer) is practically zero for $q \sim > 0.15 \text{ \AA}^{-1}$. In a normal Guinier-plot no reliable linear Guinier-range could be found and a radius of gyration R_g of $\sim > 150 \text{ \AA}$ could be estimated with this camera-setup. However, with a Guinier plot for rods ($\ln(I^*q)$ vs q^2), an R_C (radius of gyration of the cross-section) of $\sim 65 \text{ \AA}$ was obtained.



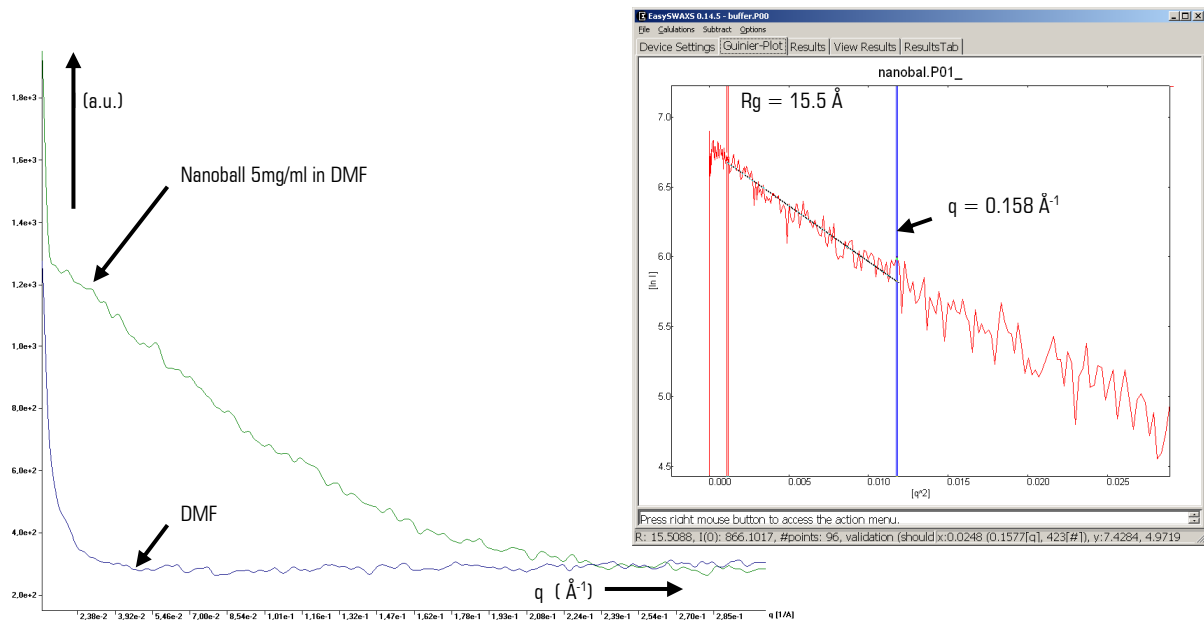
Nanoball

Experimental Setup

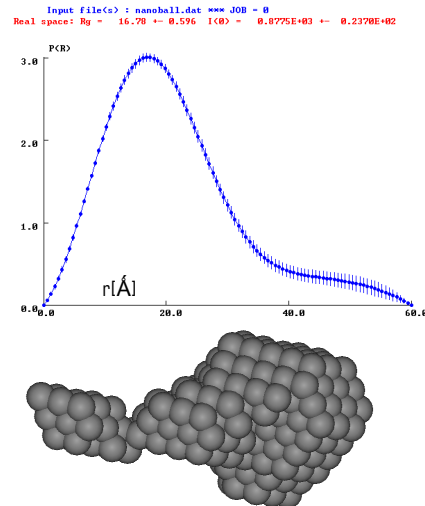
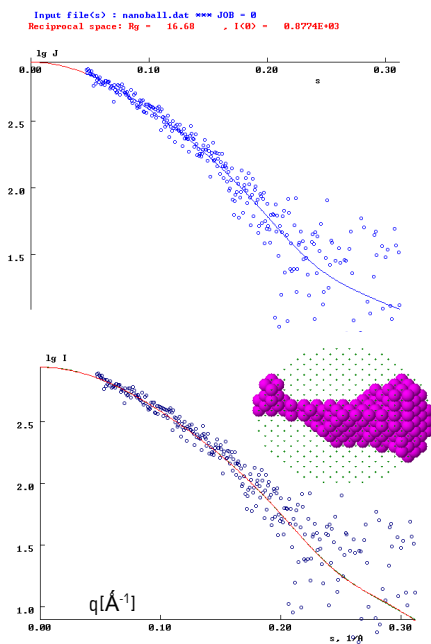
Camera	Hecus S3-MICRO (point focus)
X-rays	Cu-K α ($\lambda = 1.54 \text{ \AA}$)
Power	50 kV, 1 mA (50 W)
Collimation Setting	standard
Detectors	1D: Hecus PSD-50M: 1024 channels (54 μm)
Sample-detector distance	288 mm,
Calibration	by Ag-stearate ($d = 48.68 \text{ \AA}$)
q-ranges	0.6 \AA^{-1} (SAXS)
Temperature	20°C
Exposure time	5000 s

Nanoball in solution: SAXS - Guinier Analysis

SAXS raw-data (nanoball solution and respective solvent DMF) zoomed into the inner part of the scattering curve ($q < 0.3 \text{ \AA}^{-1}$) and respective Guinier-plot (insert) of the background subtracted SAXS-curve, yielding a radius of gyration of $15.5 \pm 0.5 \text{ \AA}$ (which corresponds to a radius of 20 \AA of an ideal sphere). Exposure time 5000 s, 1mm capillary.



Distance-distribution function (top right) calculated from the solvent-subtracted SAXS-data (top left) using the program GNOM (D. Svergun, EMBL-Hamburg) and low-resolution shape simulation using DAMMIN (bottom). The maximum in the $p(r)$ -function is centred around 18 Å, the maximum size (diameter) is approximately 60 +/- 2 Å. The shape of the $p(r)$ -function suggests a possible major component of compact particles with sizes of ~40 Å and additional components consisting of higher aggregates or oligomers (dimers).



This model is obtained assuming a monodisperse solution of nanoparticles. If that is not the case (like in polydisperse systems or systems with oligomers or strong associations between the particles), this model will not represent the real shape.

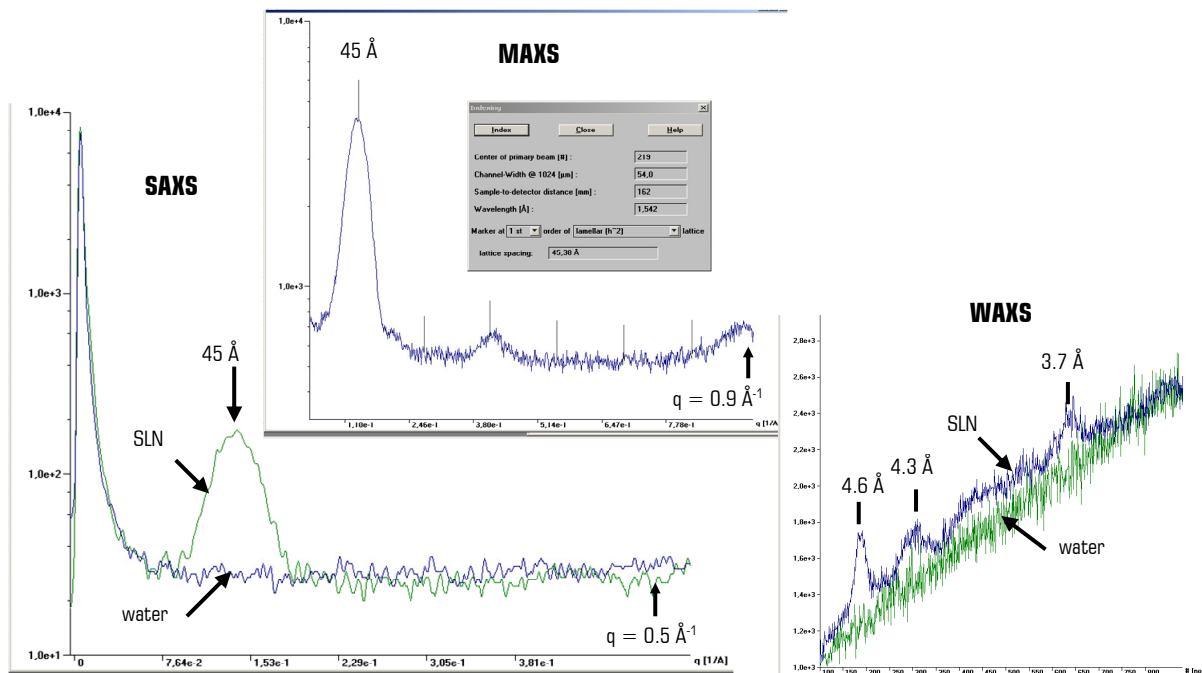
SLN - Solid Lipid Nanoparticle, SDS, BaTiO₃

Experimental Setup

Samples	in quartz capillaries of 1 mm \varnothing
Camera	Hecus S3-MICRO (point focus)
X-rays	Cu-K α ($\lambda = 1.54 \text{ \AA}$)
Power	50 kV, 1 mA (50 W)
Collimation Setting	High-flux
Detectors	2 1D-PSD: 1024 channels (54 μm)
Sample-detector distance	285 mm (SAXS), 127 mm (MAXS), respectively
Calibration	by Ag-stearate ($d = 48.68 \text{ \AA}$)
q-ranges	0.6 \AA^{-1} (SAXS); 0.04 - 1.5 \AA^{-1} (MAXS)
WAXS d-range	3.2 - 4.9 \AA
Temperature	20°C
Exposure time	variable 1000-3600 s

SLN - Solid Lipid Nanoparticle

SWAXS, simultaneous SAXS and WAXS, of SLN in water,
 3600 s exposure, SAXS up to $q \sim 0.5 \text{ \AA}^{-1}$, MAXS (middle-angle scattering) up to $q \sim 0.9 \text{ \AA}^{-1}$
 Bars in MAXS frame indicate expected peak positions for lamellar lattice of 45.4 \AA .

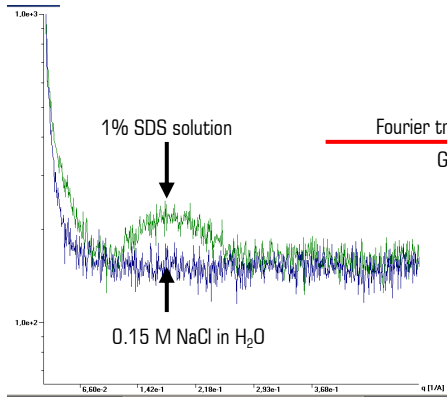


SDS - Sodium Dodecyl Sulfate

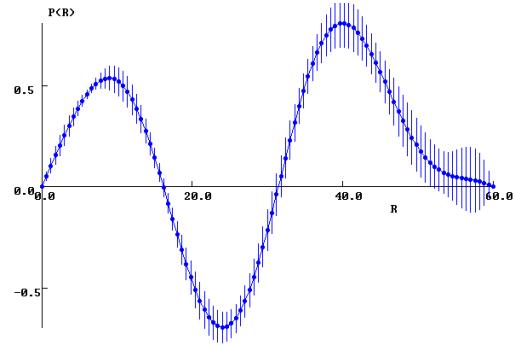
SAXS of 1% SDS in an 0.15M aqueous NaCl solution

Exposure time 3000 s

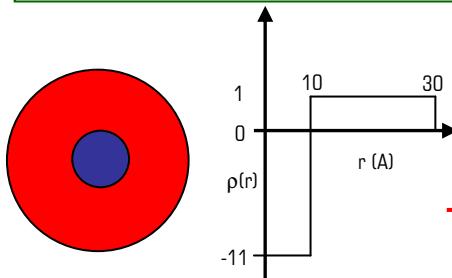
Particle size and modelling of inner structure



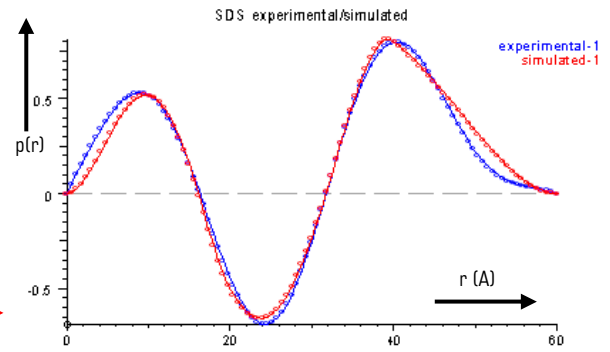
Fourier transformation
GNOM



Simulation of the experimental $p(r)$ function by a simple 2 step spherical electron-density model with an outer diameter of 60 Å ($\Delta\rho = 1$) and an inner core-diameter of 20 Å ($\Delta\rho = -11$).



Simulation

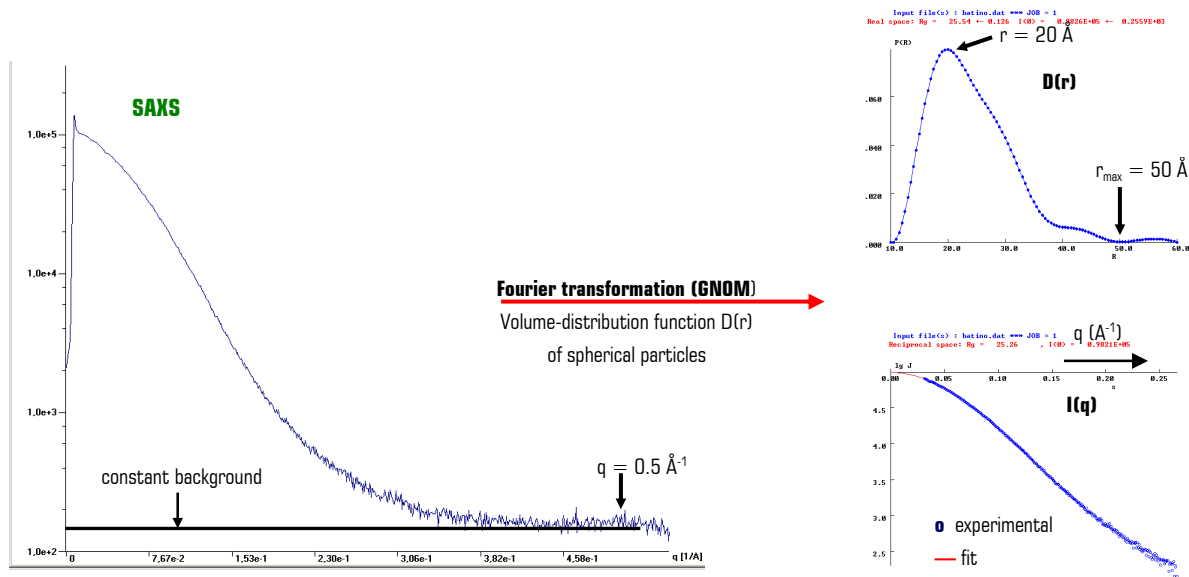


BaTiO₃ Nanoparticle Dispersion

SAXS/MAXS/ of nanoparticle dispersion in PGMEA (No solvent background measurement –constant background fitted); Exposure time: 3600s / Real-space modelling

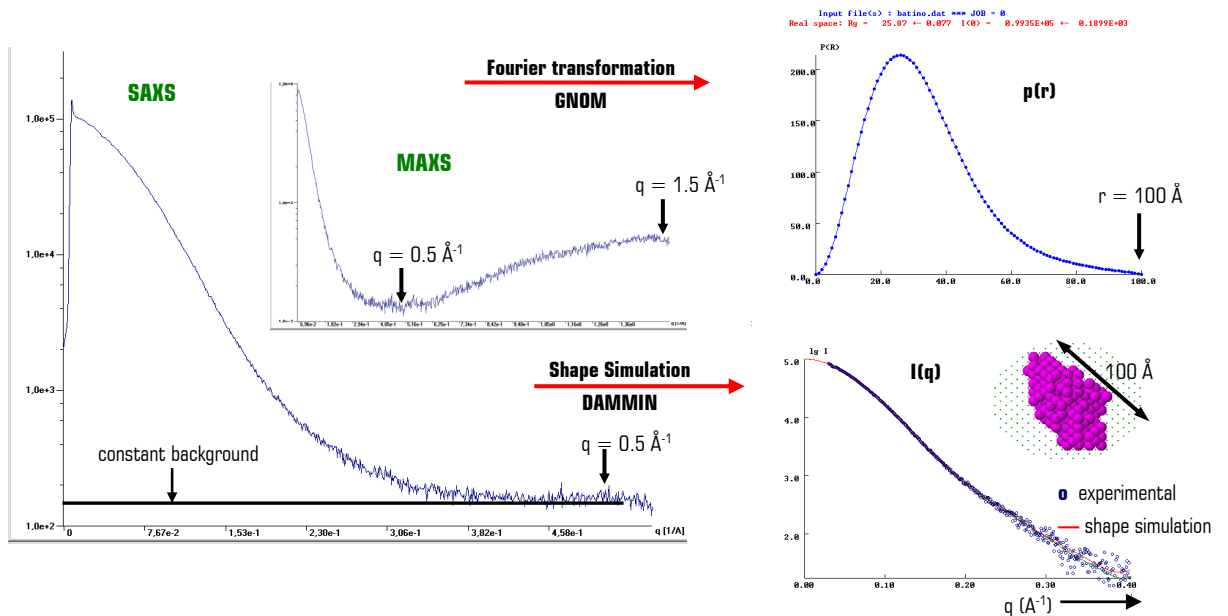
SAXS modelling in terms of polydisperse spherical particles

The function volume-distribution function $D(r)$ has a maximum at $r \sim 20 \text{ \AA}$ (most frequent radius); a radius of gyration (R_g) of 26 \AA (z-average), and a maximum radius (r_{\max}) of $\sim 50 \text{ \AA}$ (diameter 100 \AA) were obtained.



SAXS modelling in terms of monodisperse particles with asymmetric shape

Low-resolution shape simulation: maximum particle radius 100 \AA



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