Small-Angle X-ray Scattering – Interpretation of the results: “What can be obtained from the scattering curve?”

András Wacha

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Outline

Recapitulation

Self-assembled systems
  Ordered phases
    Multilamellar and hexagonal phases
  Unilamellar vesicles
  Micellar systems

Continuous – hierarchical – systems
  Anisotropy and porosity of activated carbons
  In situ experiments on a gold-cysteine self-assembling nanocomplex

Particulate systems
  Size distribution of SiO₂ nanoparticles
  Proteins – biological macromolecules

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Summary
Pinhole camera

X-ray generator with multilayer optics

Collimation: three pin-hole stages

Incident X-rays

Sample stage with detachable vacuum chamber

Exchangeable flight tube

Scattered X-rays

Two-dimensional position sensitive detector

Beam-stop stage
X-ray tube and synchrotron
Bragg’s law

- Periodic sample ($d$ repeat distance)
- $\theta$ incidence and reflection angle
- Constructive interference at the detector: waves reflected from neighbouring planes meet *in phase*
- $\Delta s = n\lambda$ where $n \in \mathbb{N}$
- From geometry: $\Delta s = 2d \sin \theta$
- $2d \sin \theta = n\lambda$
- $\frac{4\pi}{\lambda} \sin \theta = \frac{2\pi}{d} n$
- $q = \frac{2\pi}{d} n$
Connection between structure and scattering

Electron density → Fourier transform → Scattered amplitude

Autocorrelation → Distance distribution (PDDF)

Inverse Fourier transform → Differential scattering c.s. ("intensity")

Absolute square
Guinier and Porod limits

Intensity (arb. units)

Guinier region

\[ I \propto e^{-\frac{q^2 R_g^2}{3}} \]

\[ R_g = \sqrt{\iiint \rho(\vec{r}) \vec{r}^2 d^3 \vec{r}} \]

Porod region

\[ I \propto q^{-\alpha} \]

Particles with flat surfaces (Porod): \( \alpha = 4 \)

Gauss-chains (Kratky): \( \alpha = 2 \)

Mass fractals: \( \alpha = d_m \)

Surface fractals: \( \alpha = 6 - d_s \)
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**Self-assembled systems**

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Summary
Lipid systems, liposomes

- Amphipatic molecules: hydrophilic headgroups, hydrophobic carbon chains
- Self-assemble in aqueous solution
- Cell membranes of living organisms
- Other similar molecules: surfactants, detergents etc.
- Application in research & industry:
  - Model membranes
  - Drug carrier vehicles
  - Nanoreactors
  - ...
- Phase transitions
  - Thermotropic
  - Lyotropic
Self-assembled structures of phospholipid systems

- The self-assembled structure is determined by:
  - Shape of the lipid molecule
  - Length and flexibility of the carbon chains
  - Electrostatic charge of the headgroups

- Bilayer lipids: approximately cylindrical
- Non-bilayer lipids: conical shape
  - Large headgroup cross-section area: micelle / hexagonal phase
  - Small headgroup cross-section area inverse micelle / inverse hexagonal phase
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Lyotropic phases of lipid/water systems

**Figure 1**

*Lyotropic liquid and liquid crystalline phases*

- **Drug or Solubilizer/Drug**
- **L3 Phase**
- **Microemulsion**
- **Reversed Hexagonal**
- **Reversed Micellar**
- **Normal Hexagonal**
- **Normal Micellar**
- **L3 Phase**
- **Normal Cubic**
- **Lamellar**
- **Reversed Cubic**
- **Microemulsion**

Particle Sciences
Thermotropic phases of DPPC/water mixtures

Gel phase ($L_{\beta}'$)
Rippled gel phase ($P_{\beta}$)
Liquid crystalline phase ($L_{\alpha}$)

Pretransition
Main transition

Temperature (°C)

$T_{\text{pretransition}}$
$T_{\text{main transition}}$

$q$ (1/nm)

$0.98$ $1.97$
Thermotropic phases of DPPC: SAXS

Relative peak positions: 1, 2, 3, 4 → lamellar
Thermotropic phases of DPPC: SAXS

Relative peak positions: 1, 2, 3, 4 → lamellar

<table>
<thead>
<tr>
<th>Temperature</th>
<th>25°C</th>
<th>38°C</th>
<th>46°C</th>
<th>55°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase</td>
<td>$L_\beta$</td>
<td>$P_\beta'$</td>
<td>$L_\alpha$</td>
<td>$L_\alpha$</td>
</tr>
<tr>
<td>Repeat distance</td>
<td>6.373 nm</td>
<td>&quot;7.193 nm*&quot;</td>
<td>6.657 nm</td>
<td>6.569 nm</td>
</tr>
</tbody>
</table>
DOPC

Relative peak positions: 1, 2, (3)

<table>
<thead>
<tr>
<th>Temperature</th>
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<th>38°C</th>
<th>46°C</th>
<th>55°C</th>
</tr>
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<tr>
<td>Phase</td>
<td>$L_\alpha$</td>
<td>$L_\alpha$</td>
<td>$L_\alpha$</td>
<td>$L_\alpha$</td>
</tr>
<tr>
<td>Repeat distance</td>
<td>6.323 nm</td>
<td>6.370 nm</td>
<td>6.440 nm</td>
<td>6.642 nm</td>
</tr>
</tbody>
</table>
DOPE: hexagonal phase

Relative peak positions: $1, \sqrt{3}, 2, \sqrt{7}, 3, \sqrt{12}, \sqrt{13}$

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Phase</th>
<th>Lattice parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>25°C</td>
<td>H$_{\text{II}}$</td>
<td>6.458 nm</td>
</tr>
<tr>
<td>38°C</td>
<td>H$_{\text{II}}$</td>
<td>6.244 nm</td>
</tr>
<tr>
<td>46°C</td>
<td>H$_{\text{II}}$</td>
<td>6.119 nm</td>
</tr>
<tr>
<td>55°C</td>
<td>H$_{\text{II}}$</td>
<td>5.989 nm</td>
</tr>
</tbody>
</table>
Coexistence of phases

- Room temperature: lamellar phase ($L_\alpha$)
- 38°C: appearance of the inverse hexagonal phase ($H_{\|}$)
- 46°C: the cubic phase ($Q_{\|}$) appears, three phases coexist
- 55°C: the lamellar phase vanishes
- after cooling: the cubic phase remains, the lamellar phase is not recovered: memory effect!
Sterically stabilized unilamellar vesicles

- Unilamellar vesicle: a single phospholipid bilayer
- Hydration of lipids: multilamellar vesicles are formed *spontaneously*
- “Unilamellarization”: ultrasound treatment / extrusion
- Avoiding spontaneous fusion to multilamellar vesicles:
  - Charged lipids
  - Sterical stabilization: e.g. with PEG-conjugated lipids
- Primary application: drug carrier and targeting agents ⇒ size is critical!
Sterically stabilized vesicles

- Less electrons in the object ⇒ weaker scattering
- No layer-layer correlation ⇒ no peaks
- What we see is the *phospholipid bilayer form factor*
Scattering of a phospholipid bilayer

\[ I_{SSL}(q) = \left[ F_{\text{PEG},\text{in}}(q) + F_{\text{head},\text{in}}(q) + F_{CH}(q) + F_{\text{head},\text{out}}(q) + F_{\text{PEG},\text{out}}(q) \right]^2 \]
Scattering of a bilayer

\[ l_{SSL}(q) = \left[ F_{\text{PEG, in}}(q) + F_{\text{head, in}}(q) + F_{\text{CH}}(q) + F_{\text{head, out}}(q) + F_{\text{PEG, out}}(q) \right]^2 \]

- Every term is a step function or a Gaussian curve

\[ \rho(q) = \begin{cases} 
\rho_0 & \text{if } |r - r_0| < \sigma \\
0 & \text{otherwise} 
\end{cases} \\
\rho(q) = \frac{\rho_0}{\sqrt{2\pi\sigma^2}} e^{-\frac{(r-r_0)^2}{2\sigma^2}} \]

- Model parameters:

<table>
<thead>
<tr>
<th>Inner PEG</th>
<th>Inner headgroup</th>
<th>Carbon chain</th>
<th>Outer headgroup</th>
<th>Outer PEG</th>
</tr>
</thead>
<tbody>
<tr>
<td>\rho_{\text{PEG, in}}</td>
<td>\rho_{\text{head}}</td>
<td>-1</td>
<td>\rho_{\text{head}}</td>
<td>\rho_{\text{PEG, out}}</td>
</tr>
<tr>
<td>\text{r}_{\text{PEG, in}}</td>
<td>\text{r}_{\text{head}}</td>
<td>0</td>
<td>\text{r}_{\text{head}}</td>
<td>\text{r}_{\text{PEG, out}}</td>
</tr>
<tr>
<td>\sigma_{\text{PEG, in}}</td>
<td>\sigma_{\text{head}}</td>
<td>\sigma_{\text{tail}}</td>
<td>\sigma_{\text{head}}</td>
<td>\sigma_{\text{PEG, out}}</td>
</tr>
</tbody>
</table>

+ global intensity scaling factor (A) + constant background (C) + mean vesicle radius (R_0) + spread of the vesicle radius (\delta R)

- Asymmetric model (PEGs are different): 14 parameters
- Symmetric model (PEGs are equivalent): 11 parameters
Sterically stabilized vesicles

A: SSL
Fit (asymmetric)

B: SSL-2
Fit (asymmetric)

C: SSL-0.5
Fit (symmetric)

D: SSL-PEG1k
Fit (symmetric)

- SSL: HSPC + DSPE-PEG2000
- SSL-2: HSPC + 2×DSPE-PEG2000
- SSL-0.5: HSPC + 0.5×DSPE-PEG2000
- SSL-PEG1k: HSPC + DSPE-PEG1000
Micelles

- Self-assembling systems composed of amphipatic molecules
- Conical shape: large hydrophilic head, narrow hydrophobic tail
- Critical micelle concentration (CMC)
- Not only spherical (even when only one component!)
Bicelles

- Two components: long-chained bilayer lipid and short-chained detergent
- The shape is controlled by:
  \[ q = \frac{c_{\text{lipid}}}{c_{\text{detergent}}} \]
  - \( q = 0 \): detergent micelle
  - \( q \to \infty \): bilayer
- Importance: small carriers for membrane proteins
- Typical example: DHPC-DMPC bicelle
  - DHPC: 1,2-Dihexanoyl-sn-Glycero-3-Phosphocholine
  - DMPC: 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine
Scattering of a DHPC micelle

- Scattering: similar to the lipid bilayers
- Guinier region
- Fitting: micelle shape
Peptide-carrying DHPC-DMPC bicelles
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Particulate systems
- Size distribution of SiO₂ nanoparticles
- Proteins – biological macromolecules

Summary
Activated carbons

- Adsorbent, substrate, structural material
- Hierarchical structure
- Preparation:
  1. Pyrolysis: organic $\rightarrow$ C
  2. Activation: pore formation
- Tailorable
  - choice of the precursor
  - parameters of the activation
- Anisotropy: not utilized (but could be...)

Model of the hierarchical structure

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Model of the hierarchical structure

Sample preparation for SAXS measurements

- Pyrolysis of 1 cm$^3$ wooden cubes (700 $^\circ$C) $\rightarrow$ 6 x 6 x 6 mm$^3$ carbon cubes
- Physical activation:
  \[ \text{C}_\text{(s)} + \text{H}_2\text{O}(\text{g}) \xrightarrow{900^\circ\text{C}} \text{C}_\text{(g)} \]
- Mass decrease (conversion) with increasing activation time:

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Fagus sylvatica (beech)</th>
<th>Quercus robur (oak)</th>
<th>Picea abies (spruce)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0%</td>
<td>0 %</td>
<td>0 %</td>
</tr>
<tr>
<td>15</td>
<td>9 %</td>
<td>10 %</td>
<td>10 %</td>
</tr>
<tr>
<td>45</td>
<td>26 %</td>
<td>26 %</td>
<td>27 %</td>
</tr>
<tr>
<td>90</td>
<td>54 %</td>
<td>55 %</td>
<td>49 %</td>
</tr>
</tbody>
</table>

- SAXS measurements: synchrotron beamlines (Hamburg, Berlin)

SAXS on activated carbons

Tangential cut

- Horizontal scattering pattern: vertical fibrils
- Decrease in anisotropy: breaking of the fibrils, pore formation
- Characterization of anisotropy: azimuthal scattering curves, sector averaging
Extent of the anisotropy in real space

Radial sector averages

- Radial scattering curves from the scattering patterns
  - Averaging over the full $2\pi$ (— — —)
  - Narrowed to the region of the most intensive azimuthal peak (— —)
  - Perpendicular to the previous direction (···)

- Anisotropy does not appear at small sizes ($q > 2 \text{ nm}^{-1} \rightarrow d < 1.5 \text{ nm}$)

- Anisotropy decreases with activation

- Power-law functions ($\rightarrow$ fractal dimension) and Guinier regions ($\rightarrow$ radius of gyration)

- Two Guinier regions
  - Small conversion (short activation time): micropores
  - Large conversion (long activation time): mesopores

- Mass fractal $\rightarrow$ surface fractal transition
  - Spruce: surface fractal appears after 49 % burn-off: microcracks
  - Beech: no surface fractal: inherently porous?
Photoluminescent gold-cystein nanocomplexes

- Protein-stabilized supramolecular gold clusters: photoluminescence
- Au-Cys nanocomplex: a simple model for uncovering the stabilizing mechanism

\[
\text{HAuCl}_4 + \text{Cys} \rightarrow (\text{AuCys})_n^\alpha
\]

- Yellow precipitate
- Water soluble at pH > 12

\[
(\text{AuCys})_n^\beta
\]

- Stable, opalescent suspension
- Orange photoluminescence on UV-excitation

- The speed of transition strongly depends on the temperature of incubation, ranging from a few hours to a day ⇒ time-resolved SAXS on CREDO

TRSAXS on the Au-Cys nanocomplex

Incubation at 20 °C

Curvature at small $q \rightarrow$ Guinier
- Objects with well-defined sizes
- Moves left $\rightarrow$ increase in size
- Increasing intensity $\rightarrow$ their number increases
- Starts with $I \propto q^{-2} \rightarrow$ thin lamellae (generalized Guinier)

Peak at the high-$q$ limit
- Periodic structure
- Increasing intensity $\rightarrow$ more perfect periodicity
Automated model fitting

Number of layers and periodicity

- Guinier approximation for extended lamellae:
  \[ I_{\text{thickness}} \approx G \cdot q^{-2} e^{-q^2 R_T^2} \]
  \( R_T \) → thickness of the homogeneous lamella:
  \[ T = \sqrt{12R_T} \]
- Final periodic distance: 1.29 nm
- Fine structure of the lamellae: Au layers above each other with \( \approx 1.3 \) nm distance, the Cys molecules acting as spacers
- Well-correlated with the increase of photoluminescence intensity: 0.9208
Automated model fitting

Lamellae as seen by TEM

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- FF-TEM measurements: a few nm thick lamellae
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Size distribution of SiO$_2$ nanoparticles

Institute for Reference Materials and Measurements, Joint Research Centre of the European Commission: introducing a new SiO$_2$ particle size standard. Certification of the new material with several SAXS instruments

ERM FD-101b: candidate reference material (CRM)

Methods of size determination:

1. Guinier fit: $I(q \ll 1/R) \approx I_0 e^{-q^2R^2/5}$
2. Fitting of the sphere form factor:
   
   \[ I(q) = \Phi_{\text{sphere}}(q, R) \equiv \frac{V_R^2}{R} \left[ \frac{3}{(qR)^3} (\sin(qR) - qR \cos(qR)) \right]^2 \]
3. Fitting of a sphere distribution:

   \[ I(q) = \int_0^\infty p(R)\Phi_{\text{sphere}}(q, R) dR \]
4. Monte Carlo method: $R_i$ population with $w_i$ statistical weights where $|I(q) - \sum_i w_i \Phi_{\text{sphere}}(q, R_i)|$ is minimized
Size distribution of SiO₂ nanoparticles

Certification of the new material with several SAXS instruments

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Size distribution of SiO$_2$ nanoparticles


Monte Carlo size determination

Methods of size determination:

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4. Monte Carlo method: $R_i$ population with $w_i$ statistical weights where

   $|I(q) - \sum_i w_i \Phi_{\text{sphere}}(q, R_i)|$ is minimized

A favourable side-effect: CREDO has been certified by IRMM for nanoparticle size distribution determination.
Biological Small-Angle X-ray Scattering

BioSAXS

- Biorelevant macromolecules
- Mainly size- and shape determination assuming particles of homogeneous electron density
- Key parameters: \( R_g, I_0 \equiv \lim_{q \to 0} I(q) \)
- Information to be obtained:
  - Size, (low resolution) shape, volume and molecular mass of the protein
  - Flexibility/folding state (folded/disordered)
  - Validation of crystal structures
  - Aligning the relative positions of known domains

Drawbacks / caveats

- Low scattering contrast \( \Rightarrow \) bad signal/noise ratio
- Dilute sample (otherwise Guinier approximation breaks down)
- Purified sample (esp. contaminating large molecules)
- Monodisperse sample (avoid oligomerization, aggregation)
- Featureless scattering curve: danger of “overfitting”
- Uncertainties of background subtraction (solvent scattering)
- Phase problem \( \Rightarrow \) the uniqueness of the determined shape
The BioSAXS method

- Well-established and validated algorithms and methods available
- Basic assumptions: the protein solution is a *monodisperse* population of *independent*, *homogeneous* nanoparticles
  - **independent**: no interparticle interference, Guinier approximation holds
  - **monodisperse**: no oligomerization, no aggregation
  - **homogeneous**: simple shape fitting; SAXS is blind on the atomic length-scale!
Interpretation of BioSAXS measurements

- Guinier approximation: \( I(q \ll R_g) \propto I_0 e^{-\frac{q^2R_g^2}{3}} \); \( I_0 = (\Delta \rho)^2 V^2 \).

- Porod invariant: \( Q \equiv \frac{1}{2\pi^2} \int_0^{\infty} q^2 I(q) dq = 2\pi^2 (\Delta \rho)^2 V \)

- Porod volume: \( V_{\text{Porod}} = \frac{2\pi^2 I_0}{Q} \)

- First steps:
  1. Subtraction of the solvent background (corrected by the volume fraction of the protein)
  2. Guinier fit \( \rightarrow I_0, R_g \)
  3. Porod invariant \( \rightarrow V_{\text{Porod}} \)
  4. Inverse Fourier: \( I(q) \rightarrow p(r) \) pair distance distribution function (PDDF)
  5. \( I_0, R_g \) can be obtained from \( p(r) \):

\[
I_0 = \int_0^{\infty} p(r) dr; \quad R_g^2 = \frac{\int_0^{\infty} p(r) r^2 dr}{2 \int_0^{\infty} p(r) dr}
\]

6. Compare the \( I_0 \) and \( R_g \) obtained from the two methods
7. Further interpretation . . .

- ATSAS: software suite for BioSAXS data processing and interpretation (EMBL Hamburg, Research Group of Dmitri Svergun)
The Kratky plot

- High-\(q\) part of the scattering of a polymer chain following Gaussian statistics: \(I(q \to \infty) \propto \frac{2}{q^2 R_g^2}\)

- Kratky plot: \(q^2 I - q\). Behaviour in the \(q \to \infty\) limit:
  - Folded proteins \((I \propto q^{-4})\): tends to 0
  - Disordered proteins \((I \propto q^{-2})\): constant or divergent
Protein shape fitting from small-angle scattering

Fitting of geometrical shapes to scattering curves or PDDFs

- BODIES program (part of ATSAS)
- Ball, hollow sphere, ellipsoid, dumbbell etc.
- Very few parameters

Dummy atom model (DAM)

- Constructing the shape from tightly packed (fcc or hcp lattice) spherical building blocks
- Monte Carlo algorithm
  1. Random configuration
  2. Small, random modification of the configuration (add/remove a unit)
  3. Calculate scattering
  4. Compare the measured and calculated scattering
     - Better fit: keep the change
     - Worse fit: drop the change (or keep it with a low probability)
  5. Repeat from step #2 until needed
- Many parameters: possible ambiguity of the results
Protein shape fitting from small-angle scattering

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Lysozyme – A “typical” BioSAXS experiment

Crystal structure

- Well-known protein ("veterinary horse")
Lysozyme – A “typical” BioSAXS experiment

Effect of concentration

- Well-known protein (“veterinary horse”)
- Correlation peak
  - Caused by el.stat. repulsion
  - Radius of gyration cannot be determined
- How to get rid of it?
  - Dilution

![Graph showing effect of concentration on Lysozyme](image-url)
Lysozyme – A “typical” BioSAXS experiment

Effect of ionic strength

- Well-known protein (“veterinary horse”)
- Correlation peak
  - Caused by el.stat. repulsion
  - Radius of gyration cannot be determined
- How to get rid of it?
  - Dilution
  - Salting (screening the repulsion)
Lysozyme – A “typical” BioSAXS experiment

Guinier plot

- Well-known protein ("veterinary horse")
- Correlation peak
  - Caused by el.stat. repulsion
  - Radius of gyration cannot be determined
  - How to get rid of it?
    - Dilution
    - Salting (screening the repulsion)
- Guinier plot (log I vs. $q^2$): assessing the $I \propto \exp(-q^2 R_g^2/3)$ shape
Lysozyme – A “typical” BioSAXS experiment

- Well-known protein (“veterinary horse”)
- Correlation peak
  - Caused by el.stat. repulsion
  - Radius of gyration cannot be determined
- How to get rid of it?
  - Dilution
  - Salting (screening the repulsion)
- Guinier plot ($\log I$ vs. $q^2$): assessing the $I \propto \exp\left( -q^2 R_g^2 / 3 \right)$ shape
- Kratky plot ($q^2 I$ vs. $q$): folded protein
The shape of lysozyme

„Dummy atom model“ – coarse-grained description
The shape of lysozyme
„Dummy atom model” – coarse-grained description

Good agreement with the crystal structure!
Calmodulin

- Highly abundant plasma protein of eukaryotic cells ($\approx 1\%$)
- Key element of Ca$^{2+}$-induced signal pathways

Apo (Ca$^{2+}$-free) conformation (MX)

Envelope: Van der Waals surface
Calmodulin

- Highly abundant plasma protein of eukaryotic cells (≈ 1 %)
- Key element of Ca\(^{2+}\)-induced signal pathways
- Changes shape on Ca\(^{2+}\) binding
  - The “EF-hand” motifs open in both end-domains: hydrophobic pockets open up
  - End domains are displaced
  - Secondary structure of the linker part: loop → helix (known crystallization artefact!)

Ca\(^{2+}\)-bound conformation (MX)

Envelope: Van der Waals surface
Calmodulin – SAXS results

Scattering curves

- Very similar scattering curves
- Scattering curves: dumbbell shape

![Scattering curves graph]

- Simila radii of gyration
- Partially disordered (linker part?)
- Dumbbell shape
- Apo conformation more "loose"
- Ca\(^{2+}\) binding makes the structure more rigid
- Differences from the crystal structure: crystallization artefacts?
Calmodulin – SAXS results

Guinier plot

- Very similar scattering curves
- Scattering curves: dumbbell shape
- Similar radii of gyration

![Graph showing Guinier plots for Ca²⁺-bound and apo hCaM, with similar trends.]
Calmodulin – SAXS results

Kratky plot

- Very similar scattering curves
- Scattering curves: dumbbell shape
- Similar radii of gyration
- Partially disordered (linker part?)
Calmodulin – SAXS results

DAM: apo conformation

- Very similar scattering curves
- Scattering curves: dumbbell shape
- Similar radii of gyration
- Partially disordered (linker part?)
- Dummy atom model:
  - Dumbbell shape
  - Apo conformation more “loose”
Calmodulin – SAXS results

DAM: $\text{Ca}^{2+}$-bound conformation

- Very similar scattering curves
- Scattering curves: dumbbell shape
- Similar radii of gyration
- Partially disordered (linker part?)
- Dummy atom model:
  - Dumbbell shape
  - Apo conformation more “loose”
  - $\text{Ca}^{2+}$ binding makes the structure more rigid
  - Differences from the crystal structure: crystallization artefacts?
Reliability of dummy atom models

- Phase problem!
- Methods to improve reliability
  - Several candidate shapes from multiple runs of DAMMIF
  - Screening the candidates with DAMSEL
  - Average the remaining shapes with DAMAVER
  - Refine the average shape with DAMMIN

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- Quantification of the ambiguity (AMBIMETER)

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- Quantification of the ambiguity (AMBIMETER)
  - A library has been made from all possible shapes
  - Dimensionless scattering curves for the library elements: $I(q)/I_0$ vs. $qR_g$

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Reliability of dummy atom models

- **Phase problem!**
- **Methods to improve reliability**
  - Several candidate shapes from multiple runs of DAMMIF
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- **Quantification of the ambiguity (AMBIMETER)**
  - A library has been made from *all possible shapes*
  - Dimensionless scattering curves for the library elements: \( I(q)/I_0 \) vs. \( qR_g \)
  - Find number of those library elements where the curve is compatible with the measured one

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- Lysozyme: 1; apo calmodulin: 422; Ca\(^{2+}\)-bound calmodulin: 417

Petoukhov & Svergun, Acta Crystallographica D 2015, 71(5), 1051-1058
Outline

Recapitulation

Self-assembled systems
  Ordered phases
    Multilamellar and hexagonal phases
  Unilamellar vesicles
  Micellar systems

Continuous – hierarchical – systems
  Anisotropy and porosity of activated carbons
  In situ experiments on a gold-cysteine self-assembling nanocomplex

Particulate systems
  Size distribution of SiO$_2$ nanoparticles
  Proteins – biological macromolecules

Summary
Literature and software

Software

- SASFit: model fitting
- ATSAS: BioSAXS data handling, \( R_G \), PDDF calculation, dummy atom fitting etc.
- SANSView: plotting, model fitting

Literature

- Boualem Hammouda: *Probing Nanoscale Structures: The SANS Toolbox* ([link](http://www.ncnr.nist.gov/staff/hammouda/the_SANS_toolbox.pdf))
- J. Kohlbrecher, I. Breßler: *SASFit manual* ([link](http://kur.web.psi.ch/sans1/SANSSoft/sasfit.html))
Summary

Interpretation of SAXS results

- Multilamellar vesicles and ordered lipid systems: determination of the periodic repeat distance
- Sterically stabilized vesicles: the radial electron density distribution of the phospholipid bilayer
- Micelles and bicelles: shape, core-shell model parameters
- Activated carbons: anisotropy, fractal properties
- Gold-cysteine nanocomplex: the time evolution of the photoluminescent nanostructure
- SiO$_2$ nanoparticles (repeated): size, size distribution
- BioSAXS: determination of the size, shape and flexibility of proteins in solution
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Thank you for your attention!