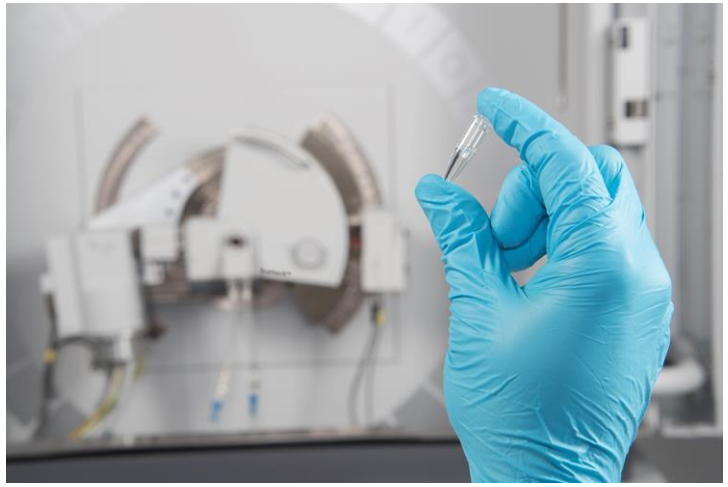
Characterizing Biological Macromolecules by SAXS

Detlef Beckers, Jörg Bolze, Bram Schierbeek, PANalytical B.V., Almelo, The Netherlands



This document was presented at PPXRD - Pharmaceutical Powder X-ray Diffraction Symposium

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Empyrean Nano edition

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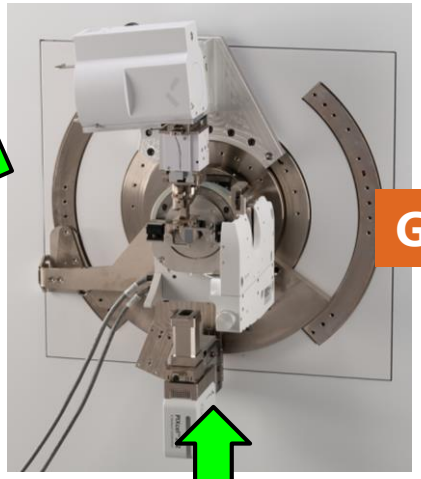
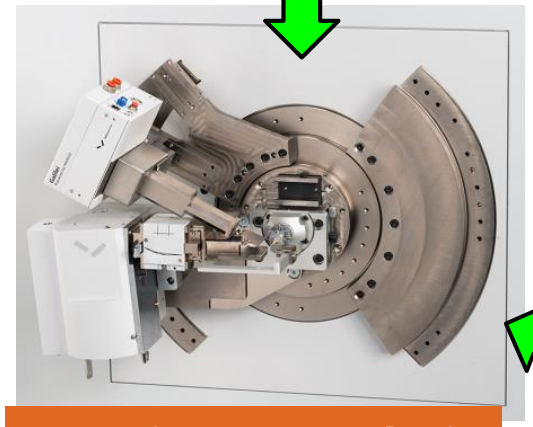
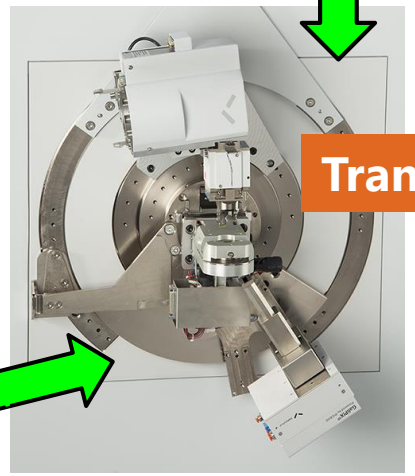
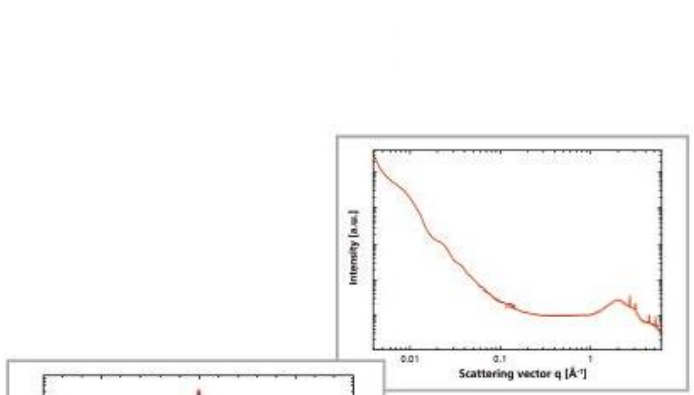
Bio-SAXS

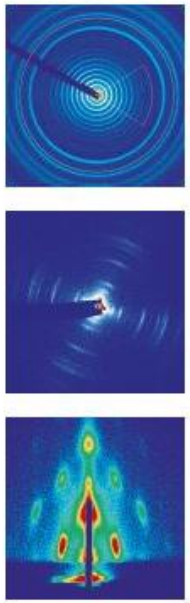






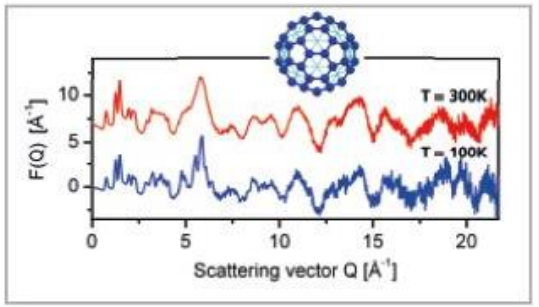
Empyrean Nano edition





Goniometer-based X-ray scattering platform

Offering the widest *q*-range – without any gap



Nanomaterial analysis on multiple length scales

SAXS (1D & 2D), BioSAXS

Total scattering (PDF)

USAXS

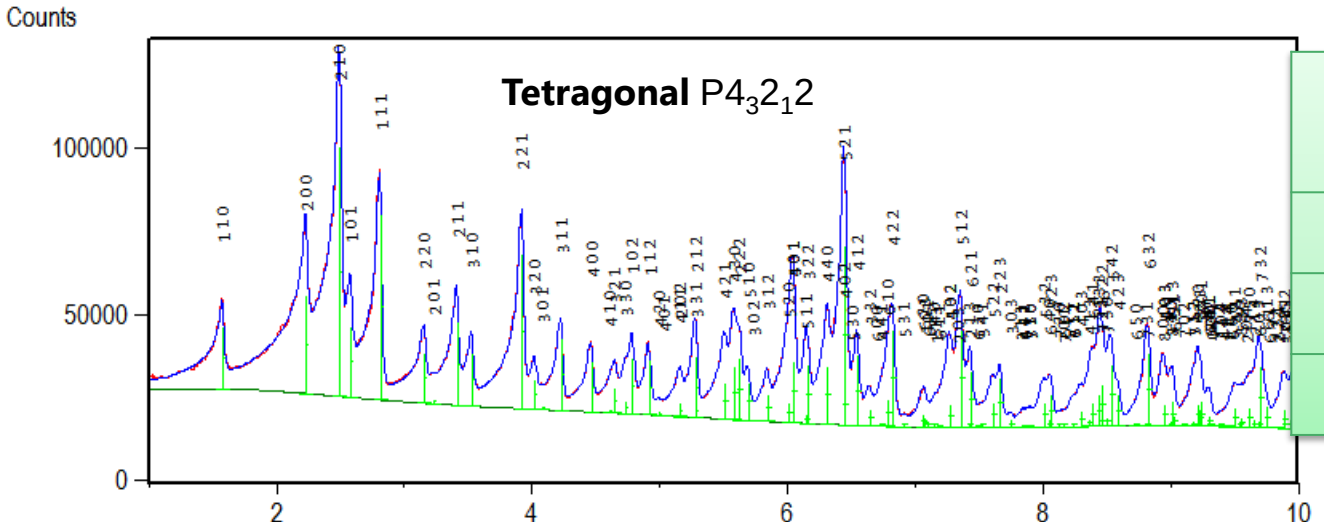
WAXS (1D & 2D)

Cu radiation

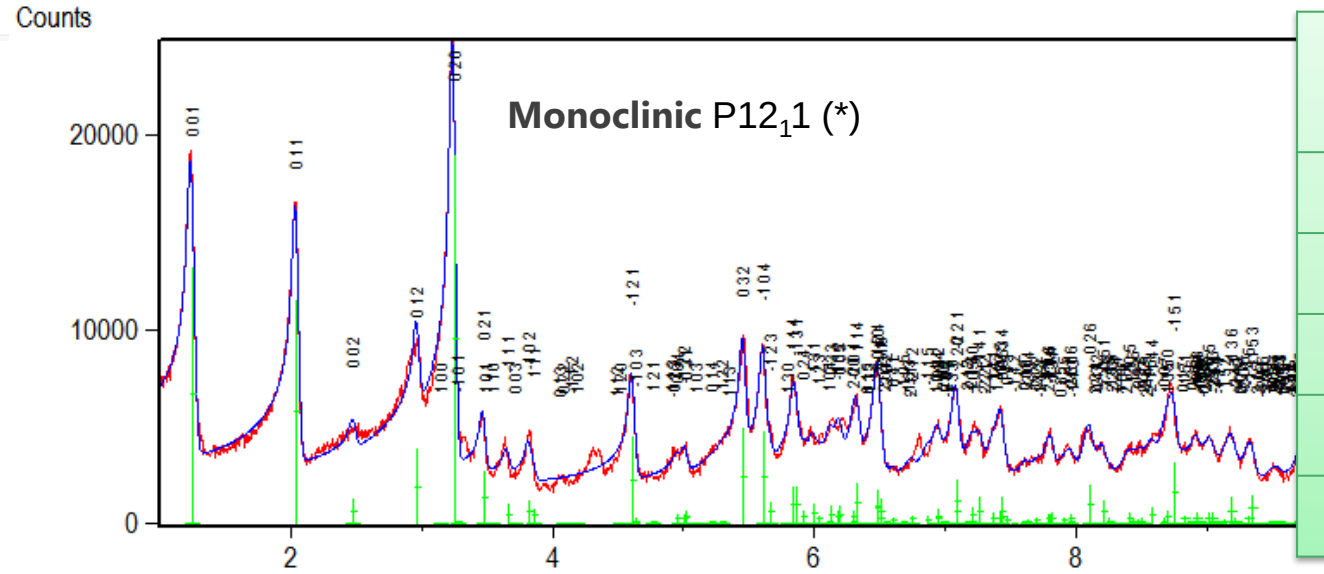
Ag or Mo radiation

0.005	0.1	5	80	160	2θ [deg]
1700	100	2	0.1	0.0	d_{Bragg} [nm]
0.00036	0.0071	0.36		22	q[Å⁻¹]

Protein powder screening and purity



Crystal system:	Tetragonal
a (Å)	79.120(3)
c (Å)	37.935(1)
V (Å³)	237474(6)



Crystal system:	Monoclinic
a (Å)	28.20(2)
b (Å)	54.50(1)
c (Å)	71.68(4)
β (°)	95.495(3)
V (Å³)	109660(60)

(*) Highest probability acc. ExtSym

Pawley fit within HighScore Plus

The scattering process

The X-rays interact with the electrons in the sample.

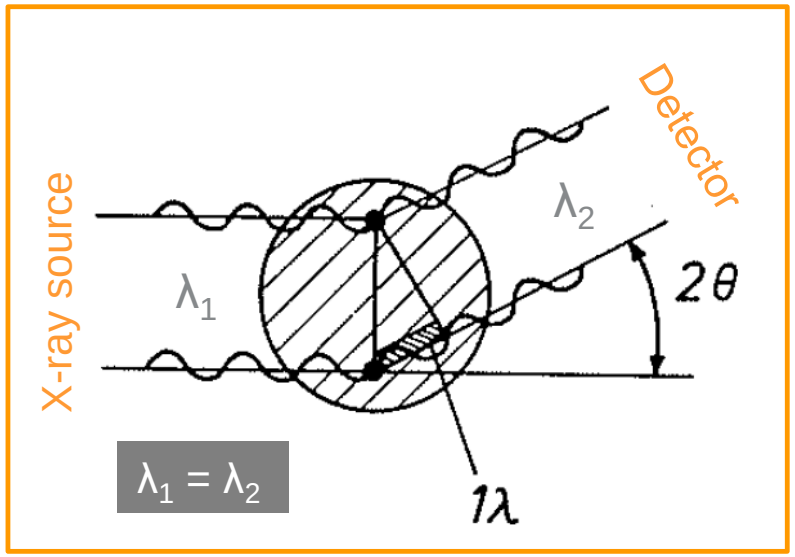


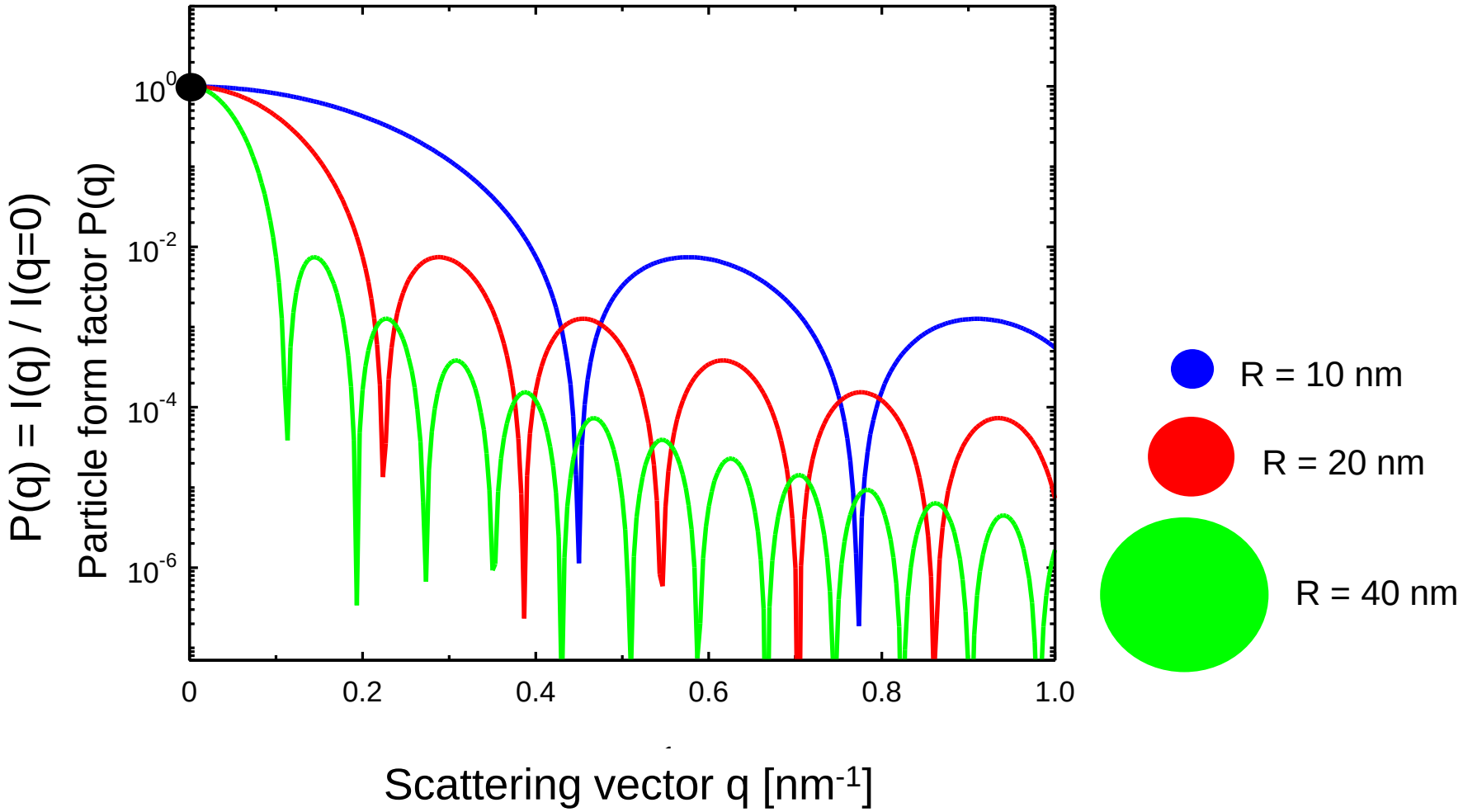
Image taken from Glatter & Kratky, "Small Angle X-ray Scattering", Academic Press, 1982

Thomson scattering

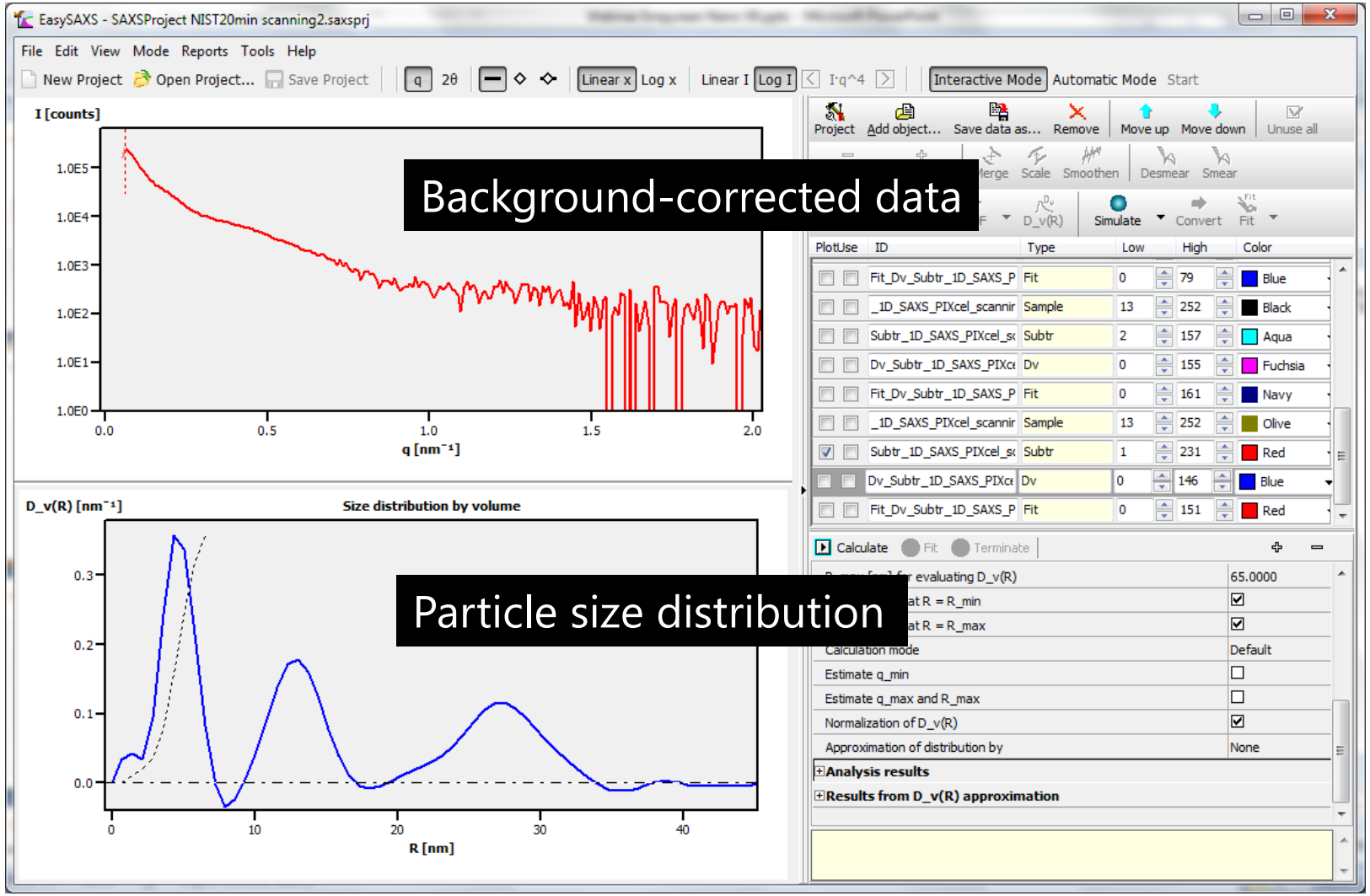
- Electrons oscillate in the electric field of the X-rays
- They emit secondary, coherent waves that interfere with each other
- For X-rays, all electrons can be treated as free electrons (cf. light scattering: only outer electrons scatter).

The interference pattern is measured at small angles *2θ*, very close to the direct beam.

SAXS from spheres of different size



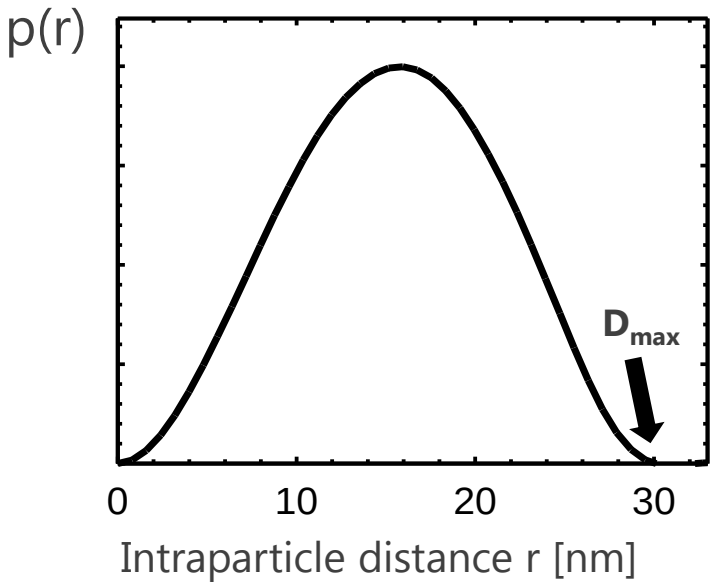
Trimodal particle size distribution

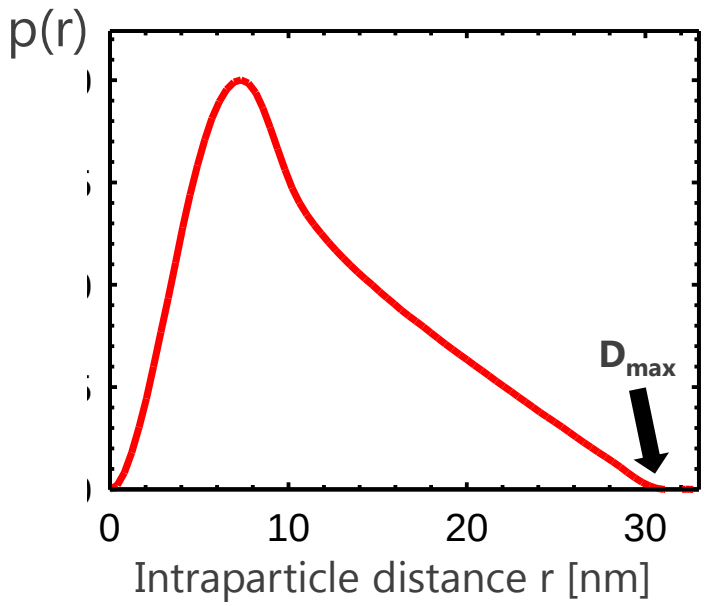


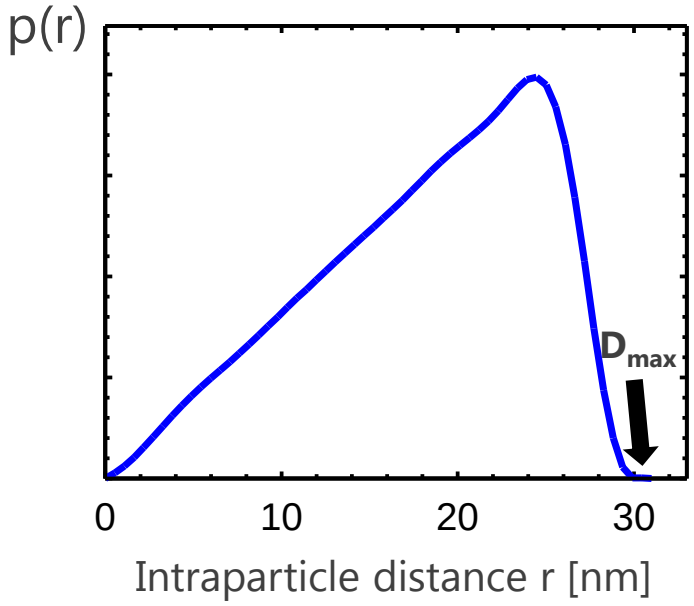
Data analysis was done without any assumptions about the shape or modality of the size distribution curve.

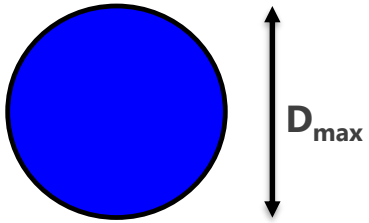
Pair distance distribution function p(r) for different particle shapes

Model calculations

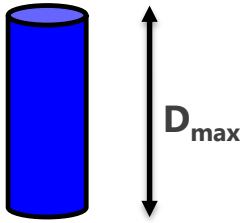




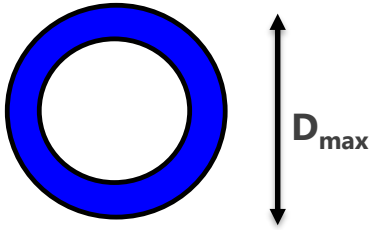




Overall spherical

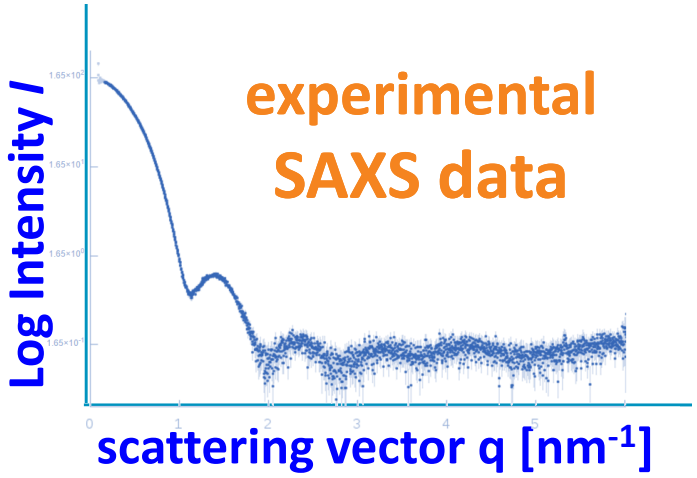


Elongated



Hollow

BioSAXS data analysis

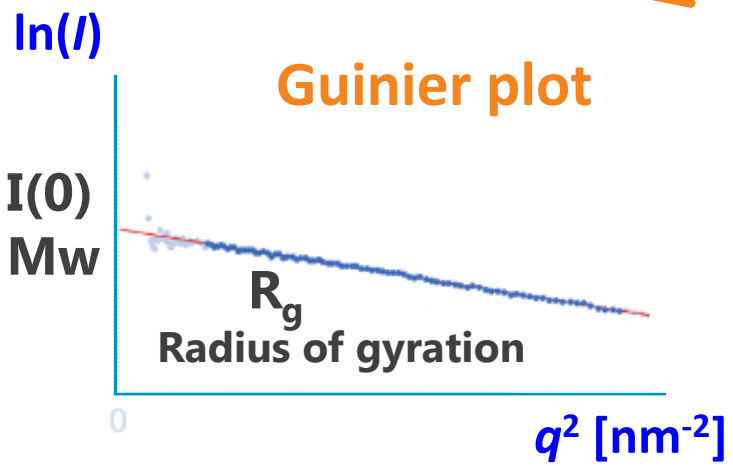


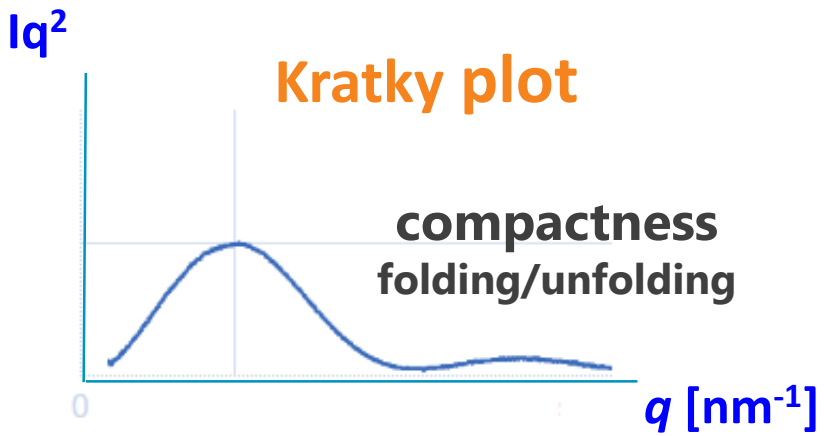
$$|\vec{q}| = \frac{4\pi}{\lambda} \cdot \sin\theta$$

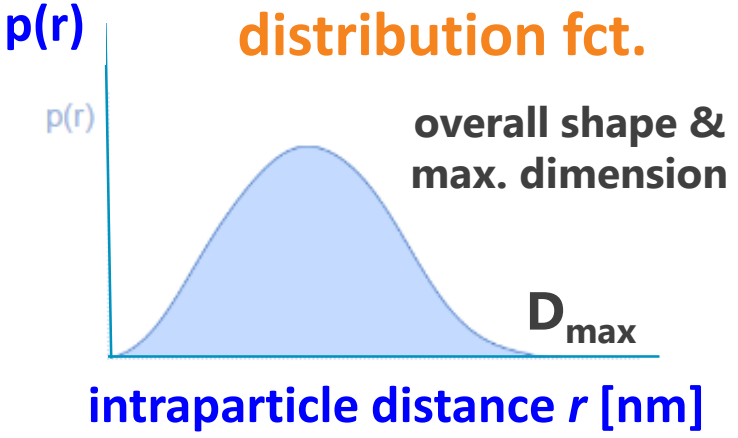
λ wavelength of X-rays
2θ scattering angle

FT

FT^{-1}







Small-Angle Scattering Biological Data Bank, Valentini et al., Nucl Acids Res (2014) 43, D357

SAXS setup

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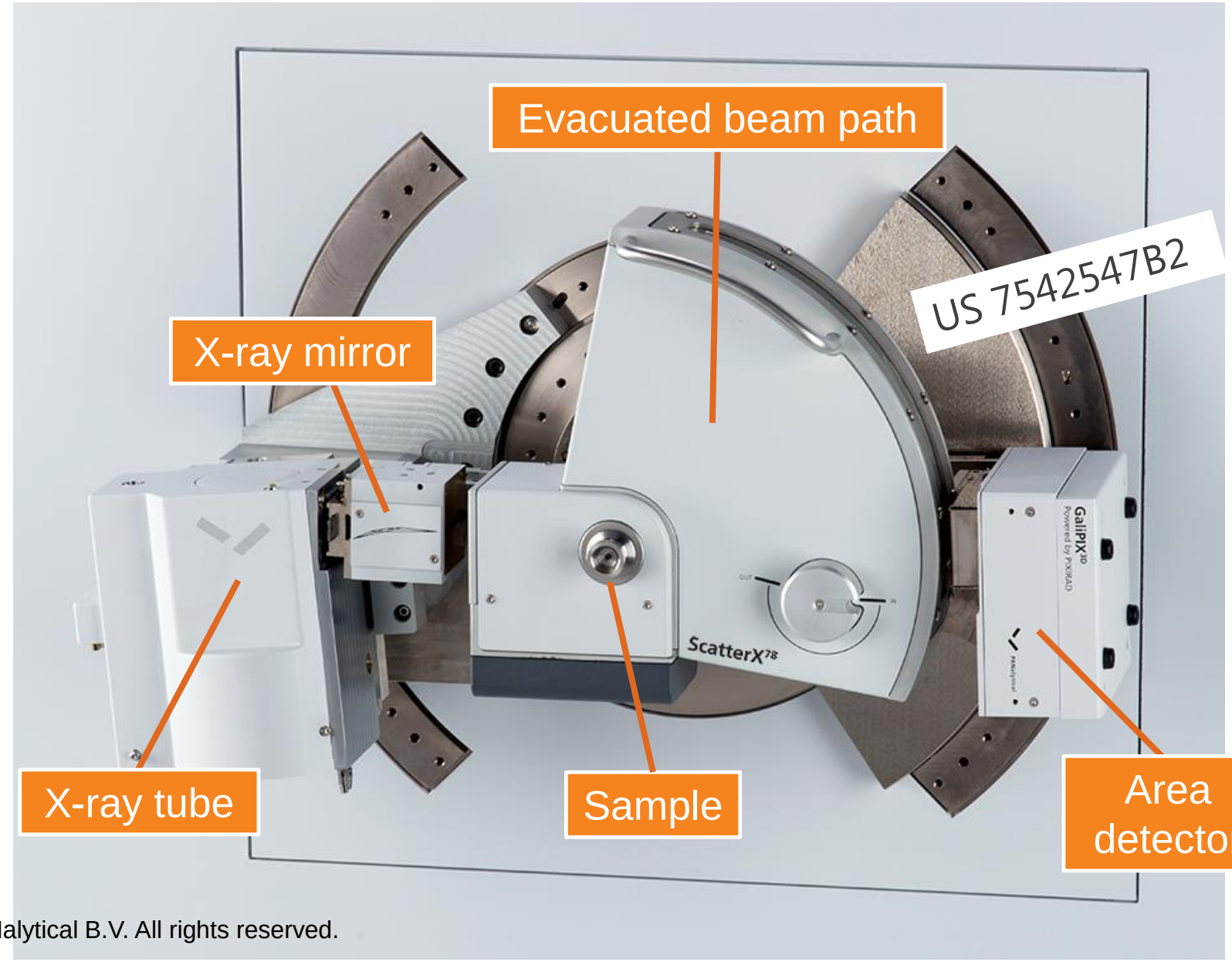


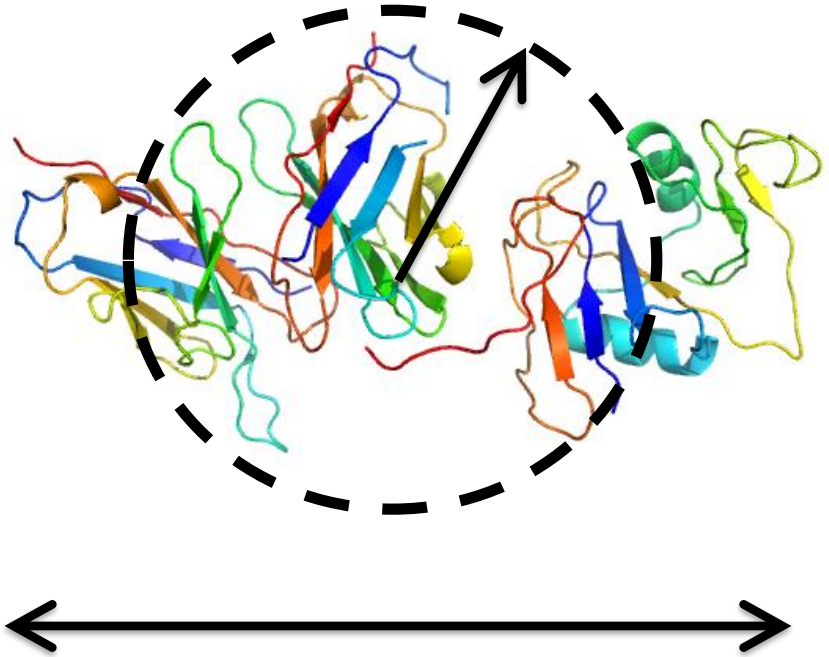
WAXS

Advantages of SAXS on proteins

- Protein characterization can be done *in situ*, with the proteins in solution and under near physiological conditions.
- Effects of e.g. pH, salt concentration, temperature, added ligand can be systematically studied.
- Easy sample preparation as compared to e.g. cryo-EM or SC-XRD.
- Also applicable in case protein doesn’t crystallize!

Information obtainable from Bio-SAXS experiments (I)

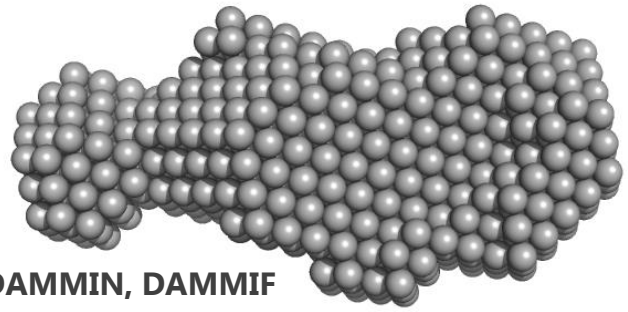
Radius of gyration R_g : overall size parameter



Molecular weight: differentiate between oligomeric forms

Overall shape: e.g. overall spherical vs. elongated

3D envelope shape



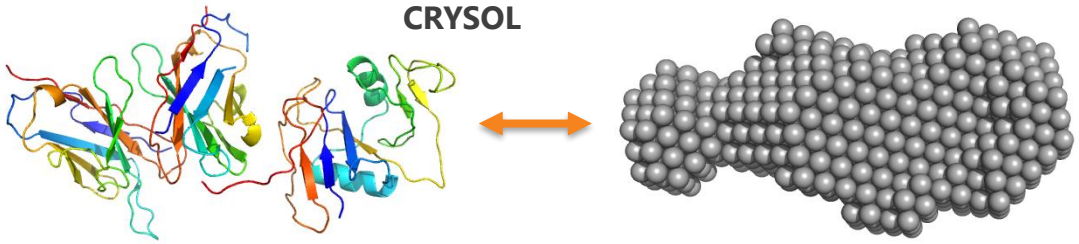
DAMMIN, DAMMIF

D_{max} : maximum dimension

Information obtainable from Bio-SAXS experiments (II)

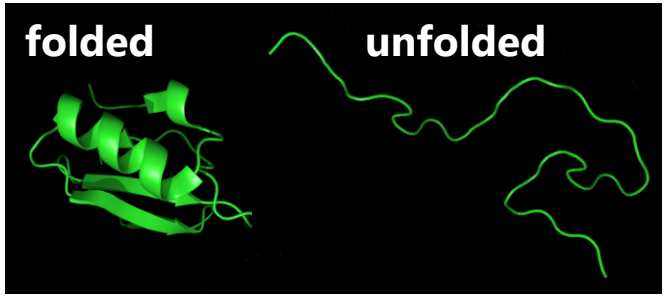
Validation of atomic structures

- Protein in crystal vs. in solution



Degree of compactness / flexibility

- Protein folding / unfolding



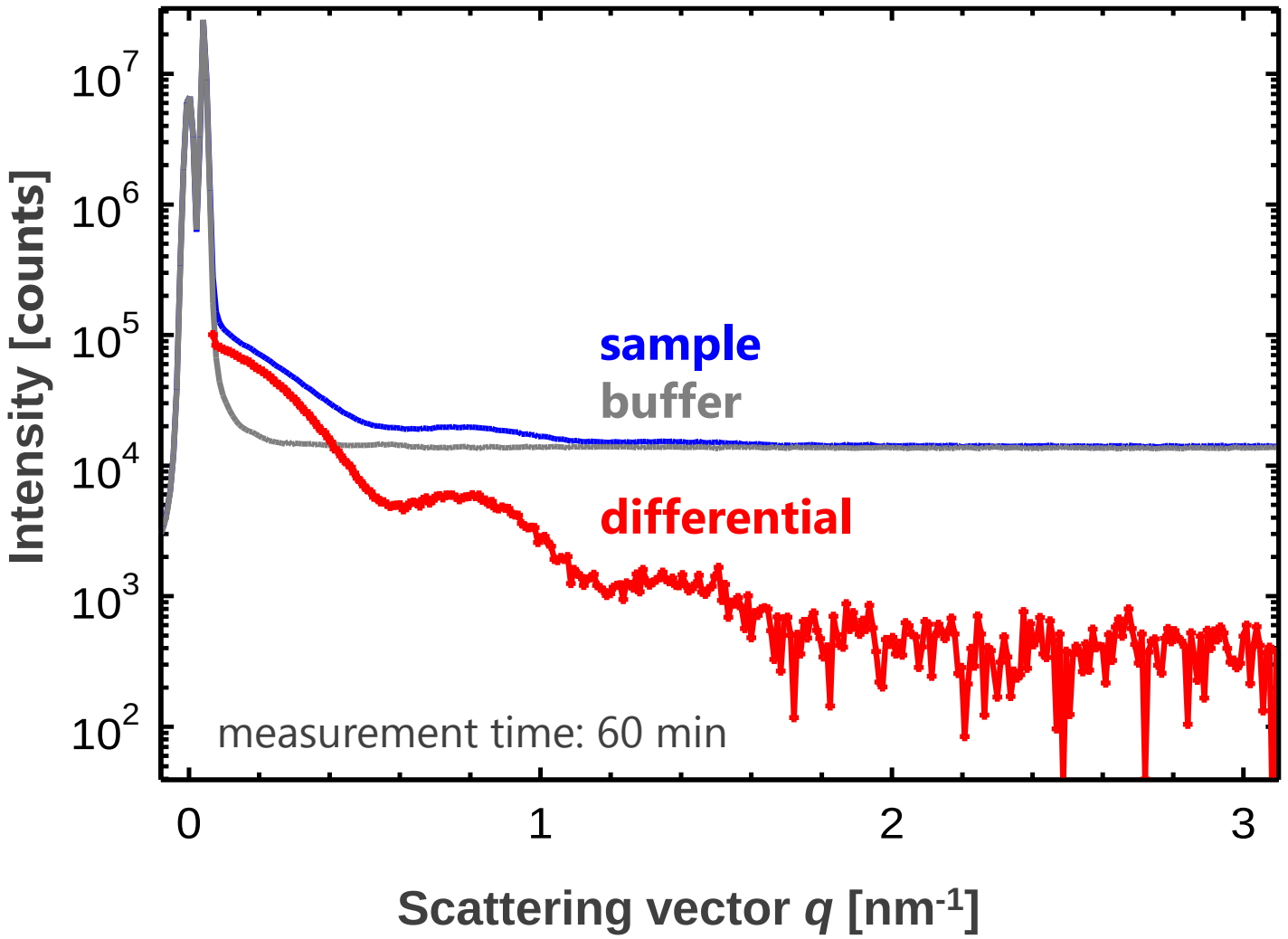
Protein stability

- Detect protein aggregation
- Differentiate repulsive and attractive protein-protein interactions

Protein dynamics

- Time-resolved measurements

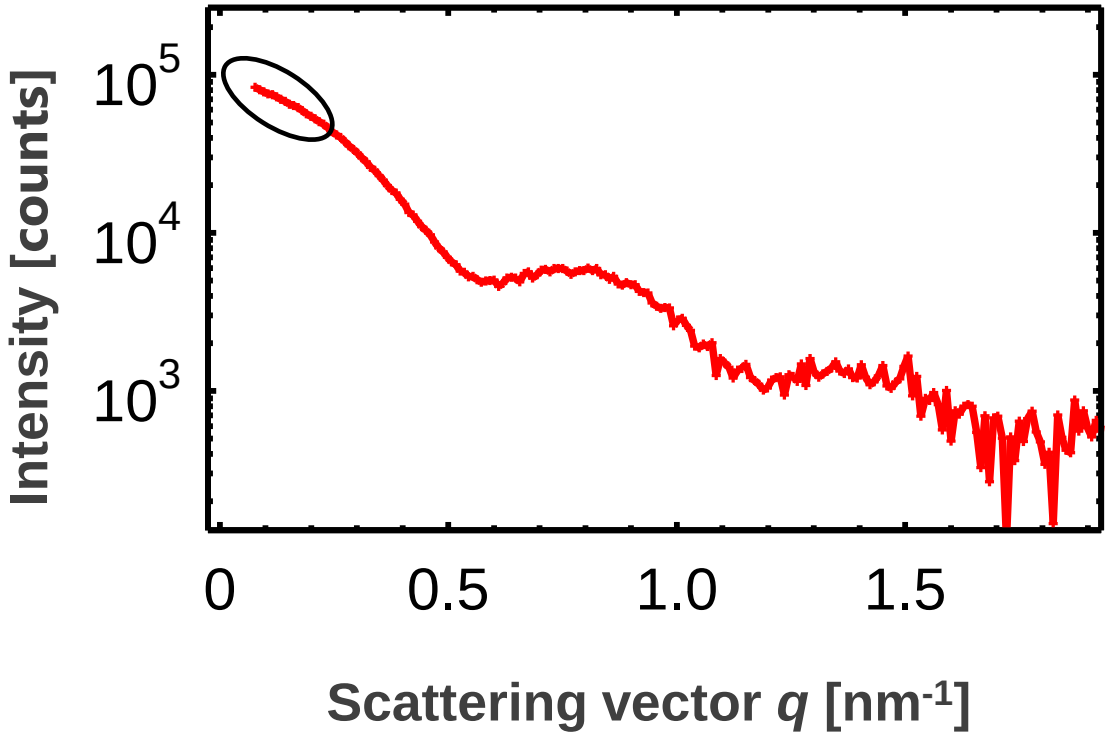
Protein size shape and structure

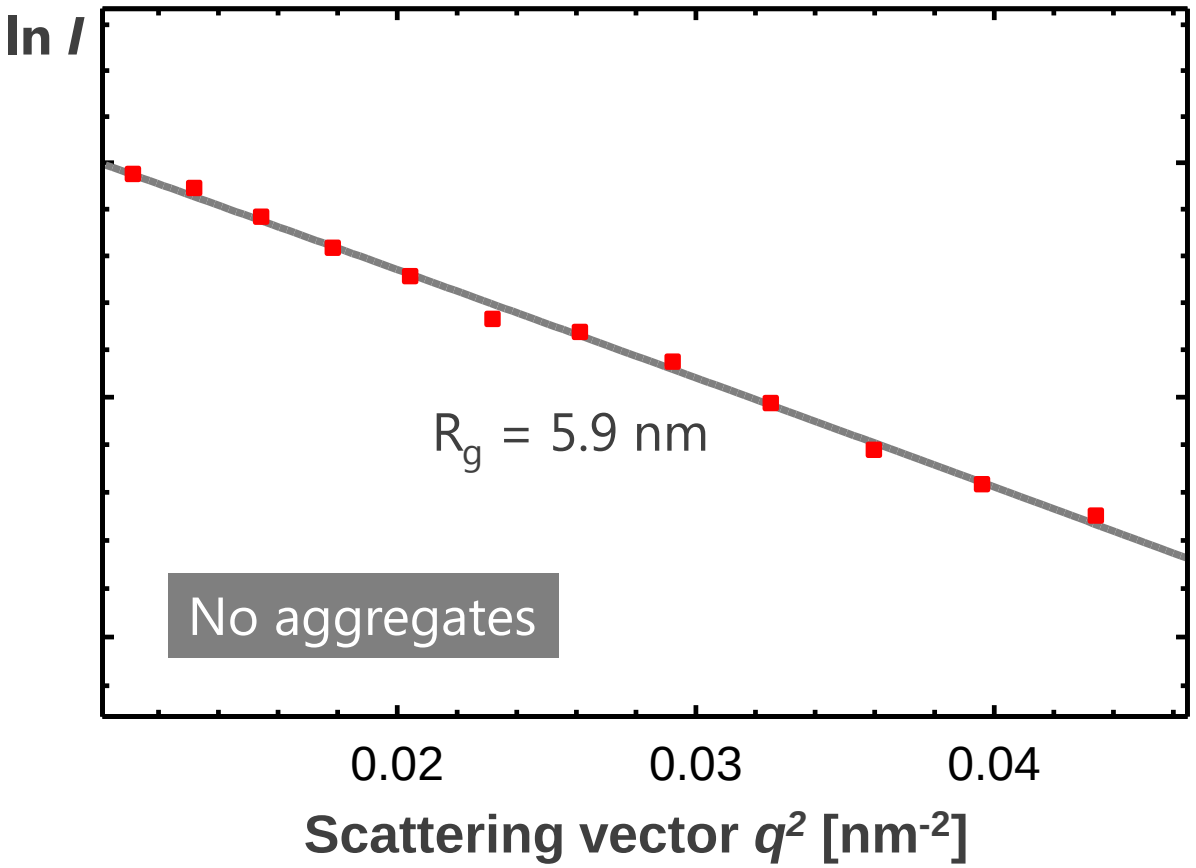


Apoferritin (12 mg/ml)

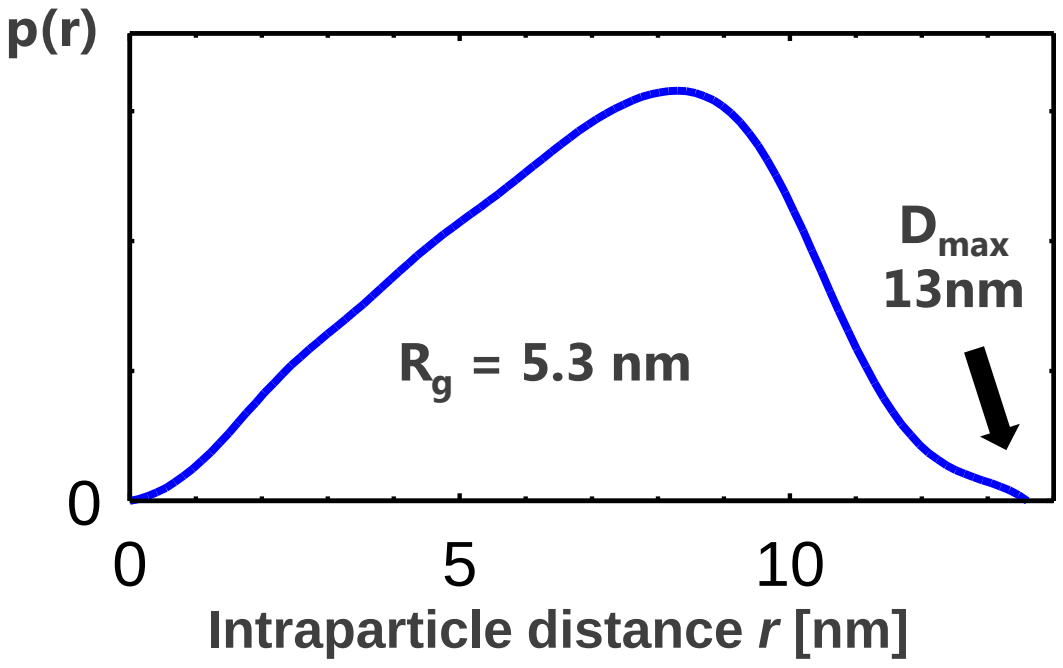
An iron storage protein.

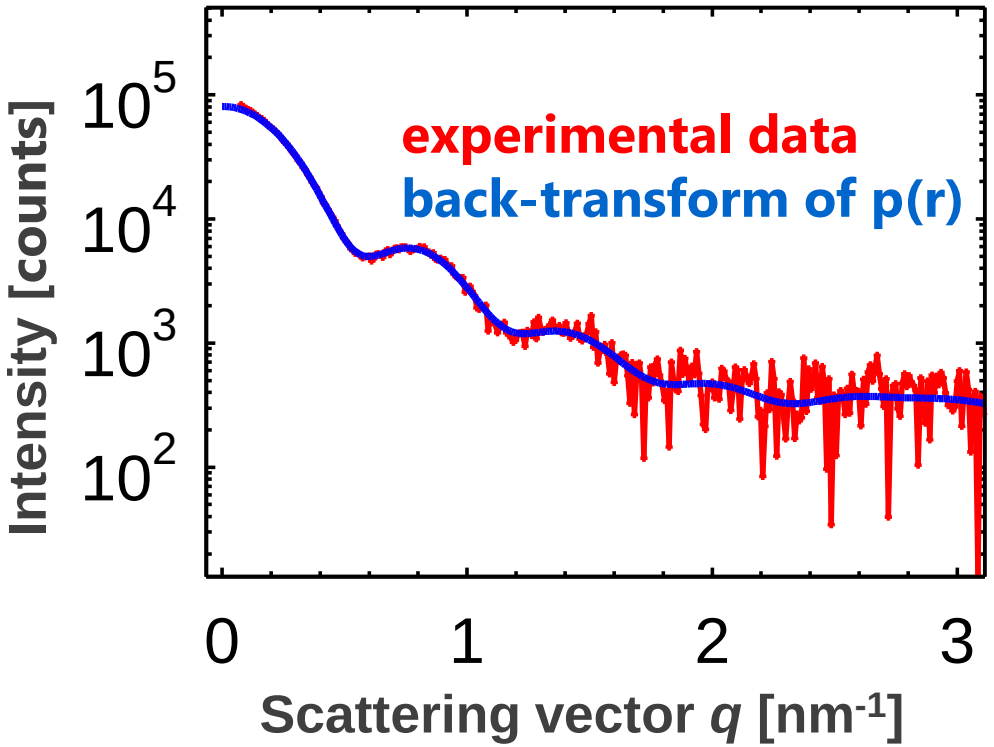
Apoferritin - Guinier plot





Apoferritin - Pair distance distribution function p(r)



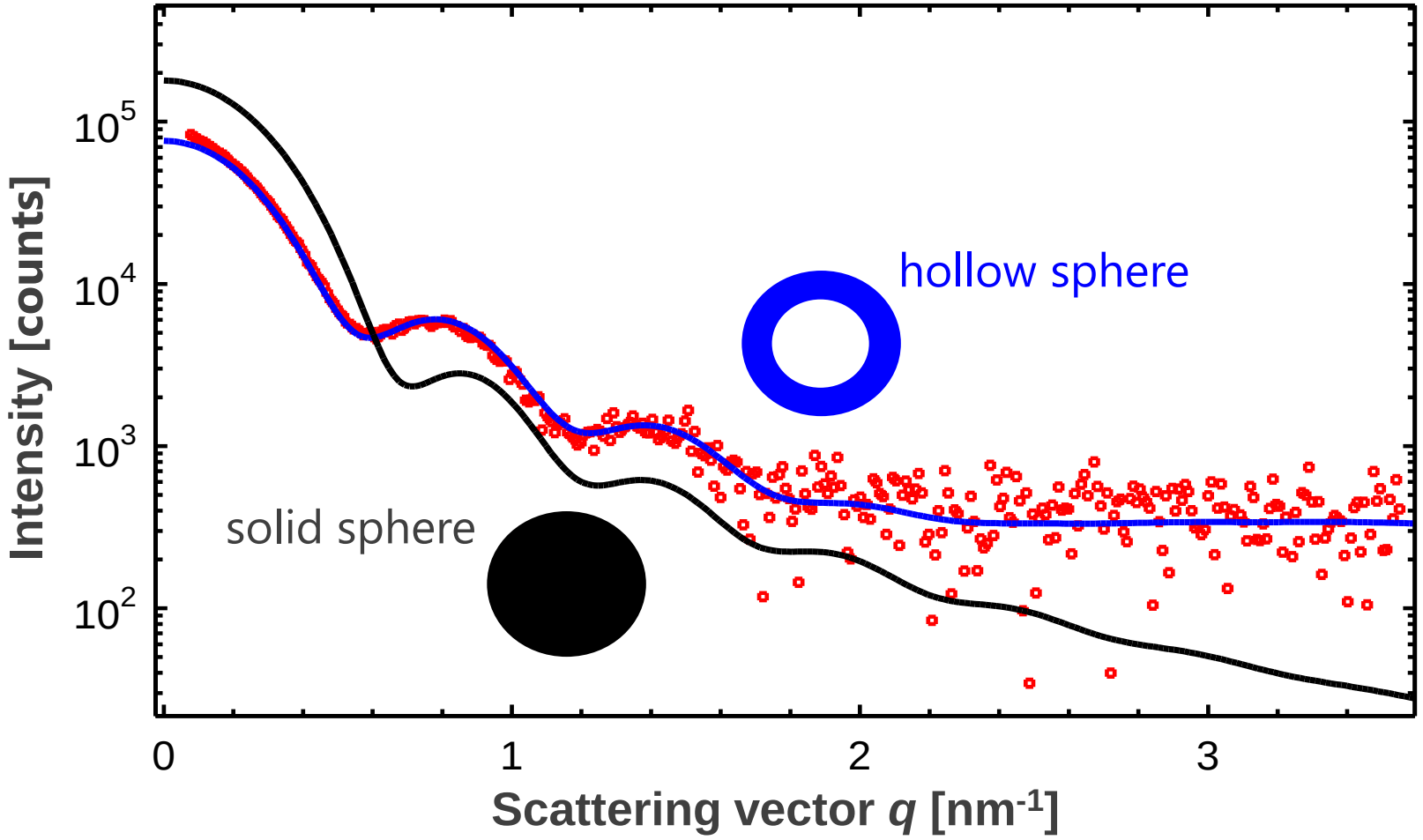


The characteristic shape of the p(r) function points to an object with a hollow structure.

Apoferritin - hollow sphere model

(*) PM Harrison, *The structure of apoferritin: molecular size, shape and symmetry from x-ray data*, J. Molec. Biol. **6** (1963), 404-22

From the X-ray data apoferritin molecules have a molecular weight of 480,000 and a form approximating on the average at a resolution of 26 Å to a spherical shell having an external radius of 61 ± 3 Å and internal to external radius ratio about 0.6.



Hollow sphere model(*):

R_{core} = 37 Å

d_{shell} = 24 Å

3D protein shape reconstruction (*ab initio*)

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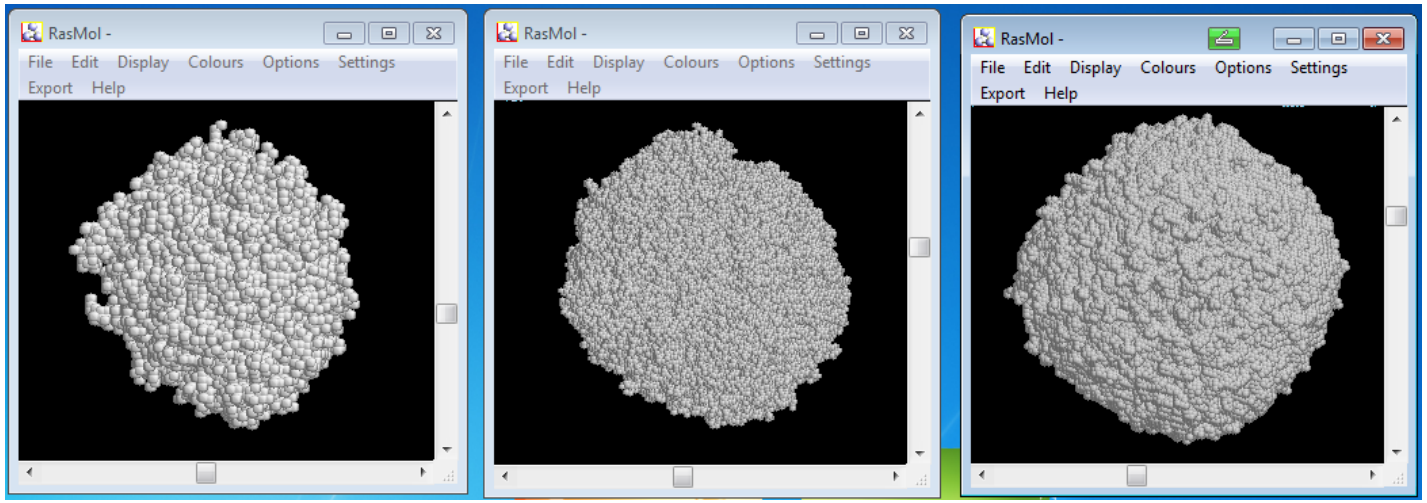
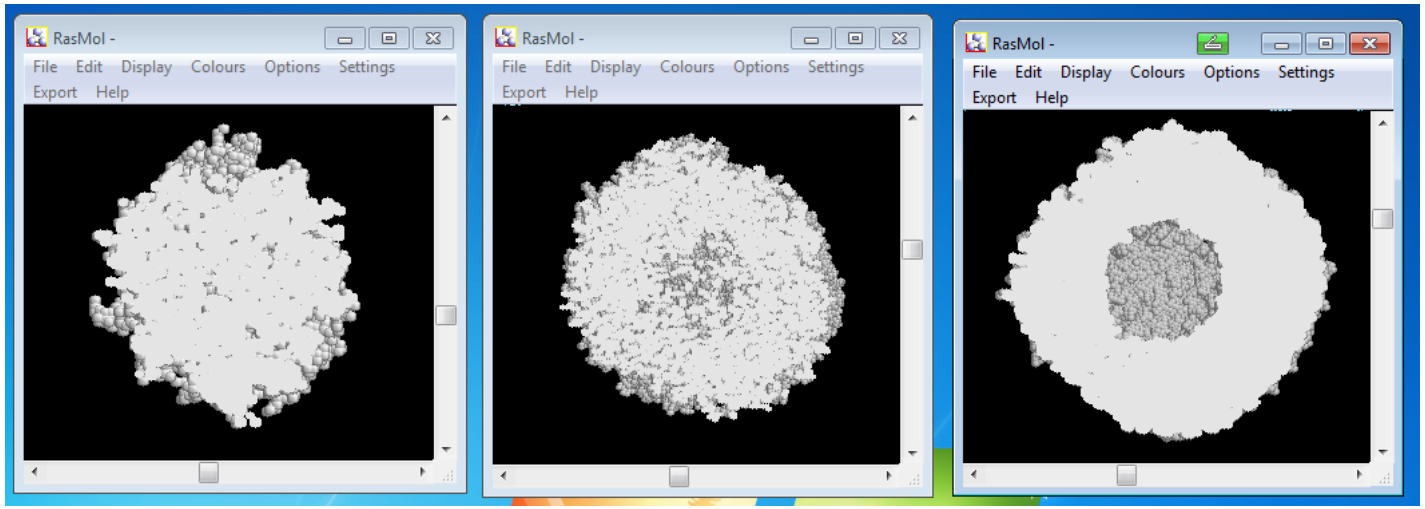
(*) D. Franke, D.I. Svergun et al., J. Appl. Cryst. ***50***, (2017)z

DAMMIF / DAMMIN*

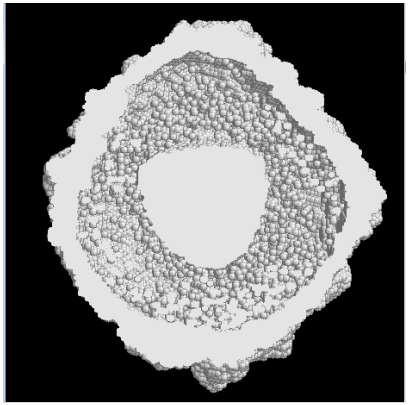
start → **end**



front view



cross-sectional structure



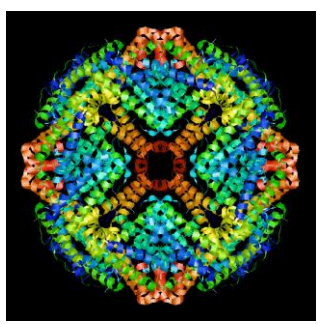
Inverse cross-sectional structure.
Beads indicate the location of the buffer

Protein structure in crystal form vs. in solution

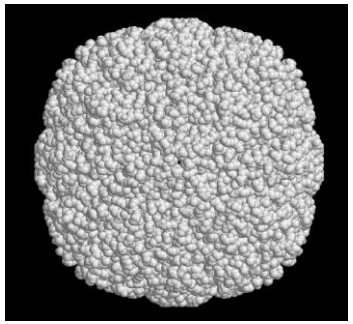
Calculation of SAXS data from published atomic coordinates.

CRYSOL

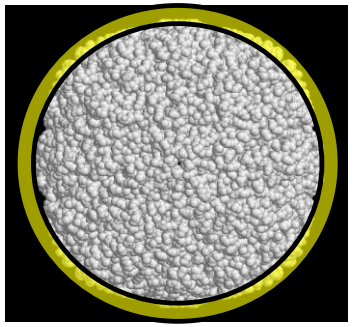
PDB 1IER



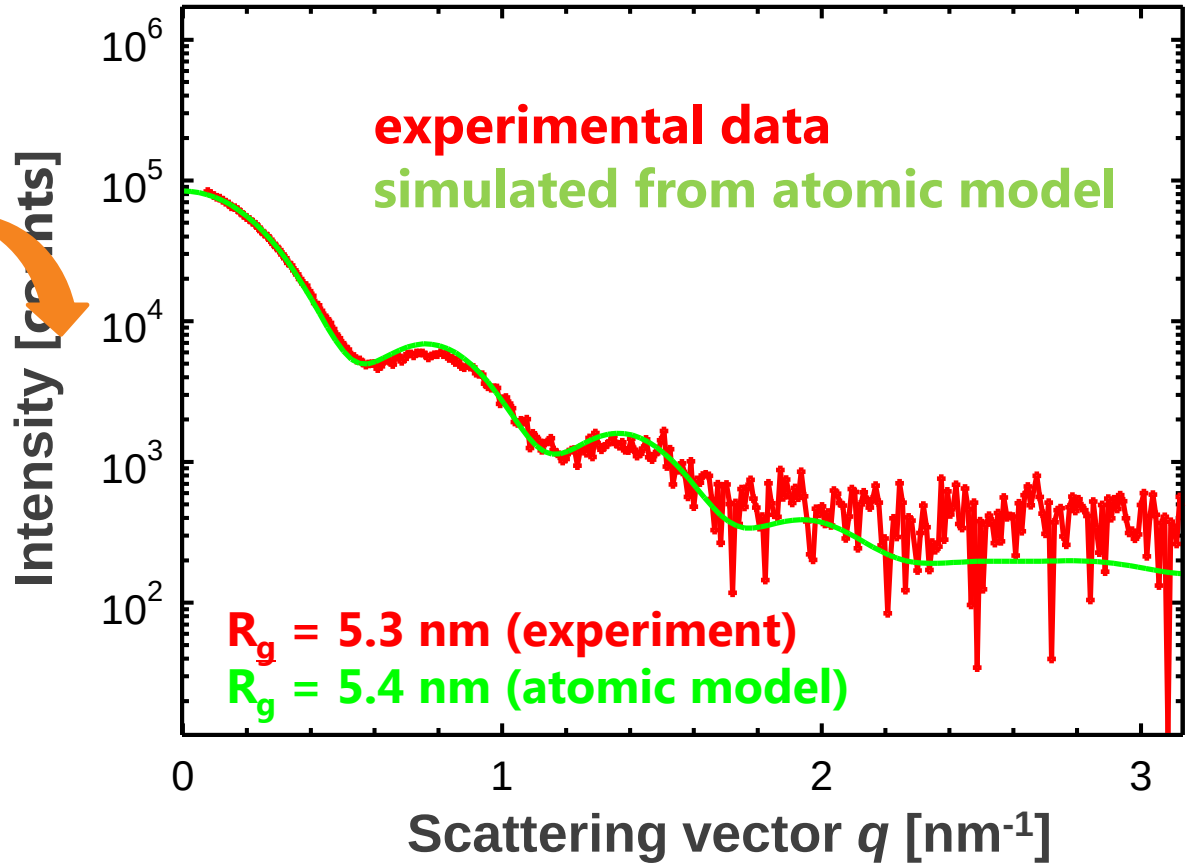
atomic structure



low-resolution structure

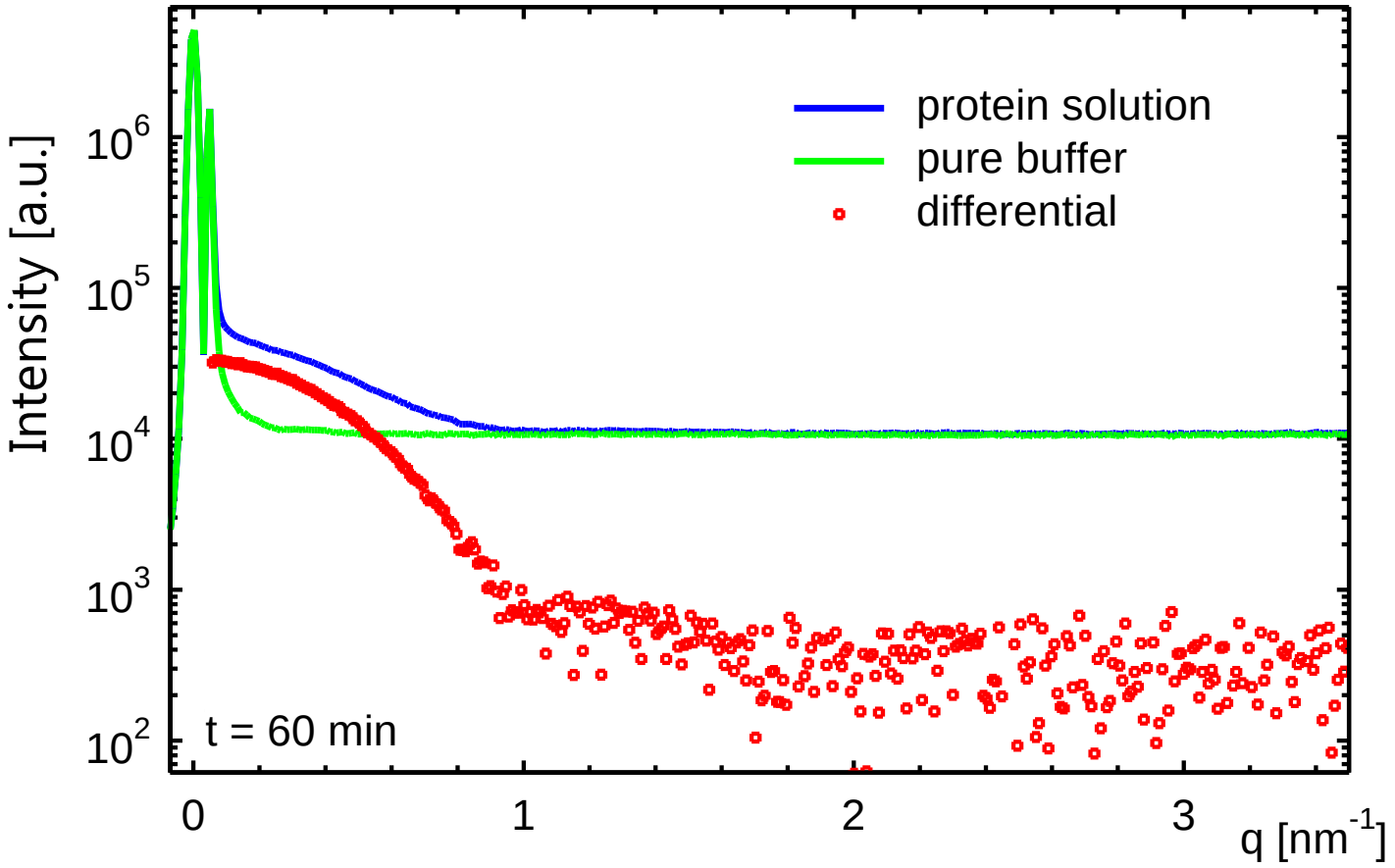


hydration layer added



The crystal structure of the protein is similar to its structure in solution.

Glucose isomerase (11 mg/ml)

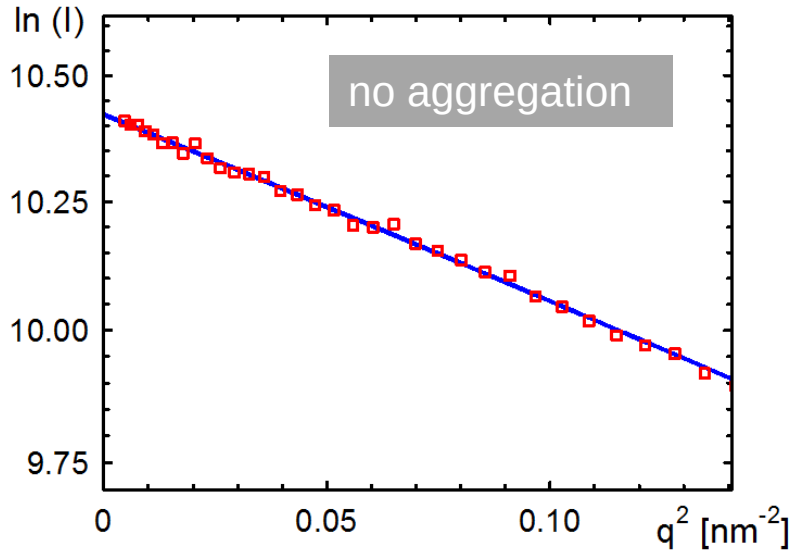


- Enzyme produced by microorganisms (bacteria)
- Catalyzes the conversion of glucose to fructose

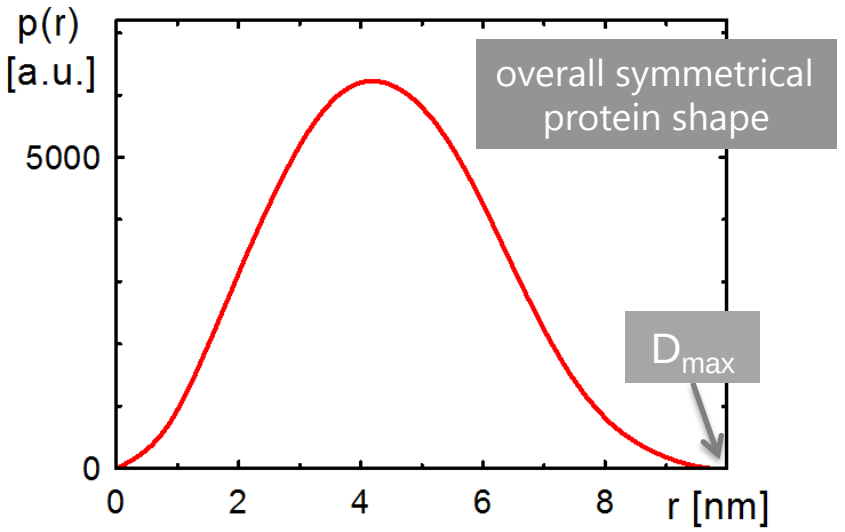
100 mM Tris, 1 mM $MgCl_2$, pH = 8

Glucose isomerase

Guinier plot



Pair distance distribution function



	R_g from Guinier plot	R_g from $p(r)$	D_{max} from $p(r)$
Empyrean Nano (60 min)	3.31 nm	3.32 nm	9.7 nm
Empyrean Nano (10 min)	3.33 nm	3.27 nm	9.5 nm
Synchrotron	3.25 nm [1]	n.a.	9.7 nm [2]

R_g: radius of gyration

D_{max}: maximum dimension of protein

Simulation of SAXS data from SC-XRD data and comparison with experimental data

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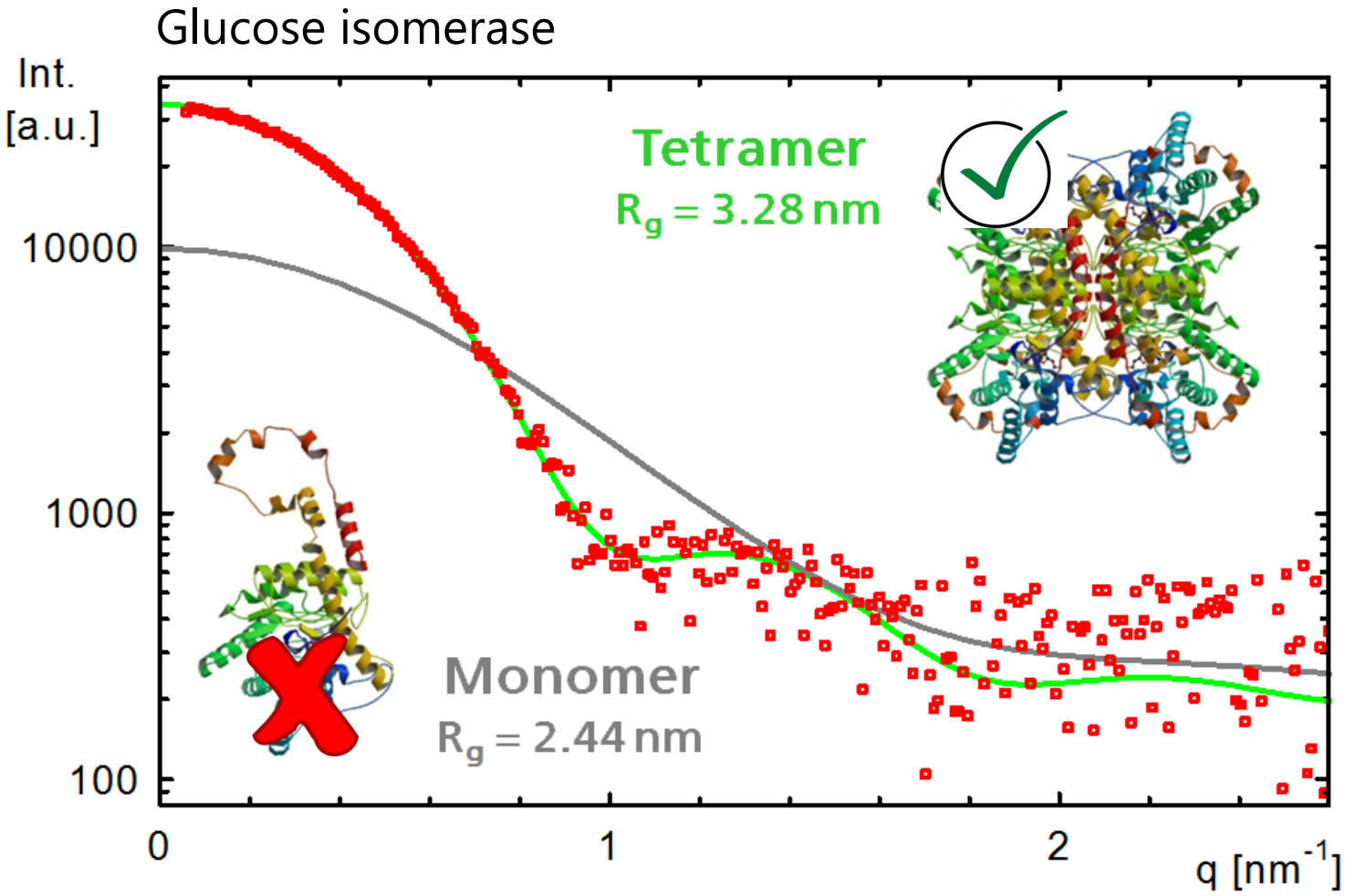


CRYSOL software[1]

Conclusion:

In solution the protein is forming a tetramer.

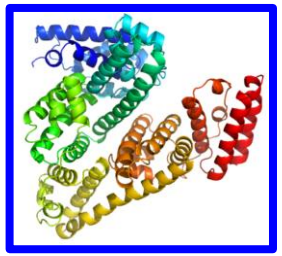
The structures in the crystal and in solution are similar.

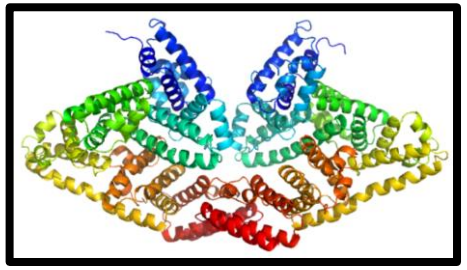
[1]Svergun D.I., Barberato C. and Koch M.H.J. (1995) CRYSOL - a Program to Evaluate X-ray Solution Scattering of Biological Macromolecules from Atomic Coordinates *J. Appl. Cryst.* , ***28, 768-773.***

Oligomeric mixtures – Bovine Serum Albumin (BSA)

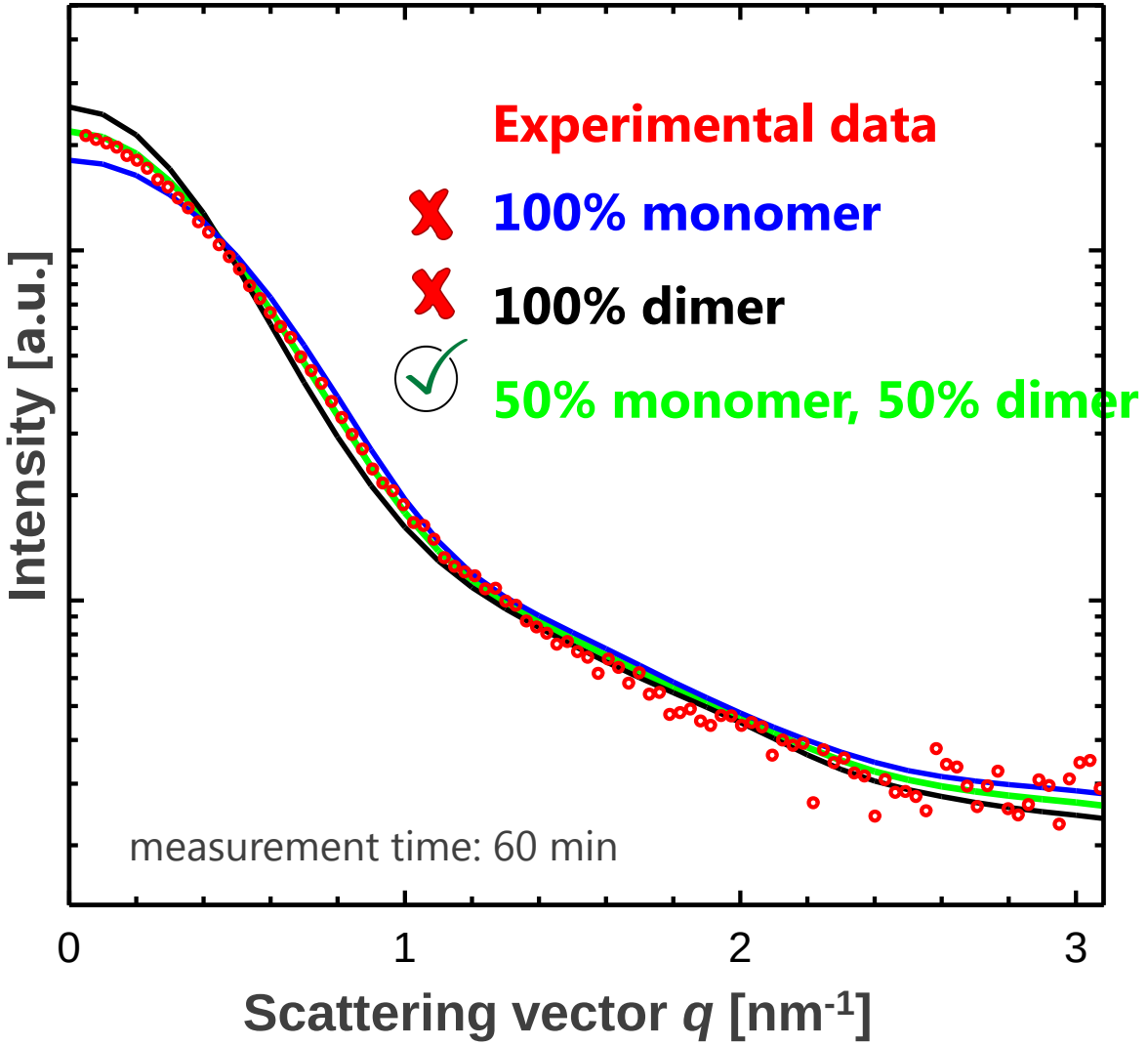
Bovine serum albumin (BSA), 10 mg/ml in 50 mM Hepes, 50 mM NaCl, pH = 7.5, T = 20 °C

SAXS data for monomer and dimer were simulated from the published atomic structures (using CRYSOL).



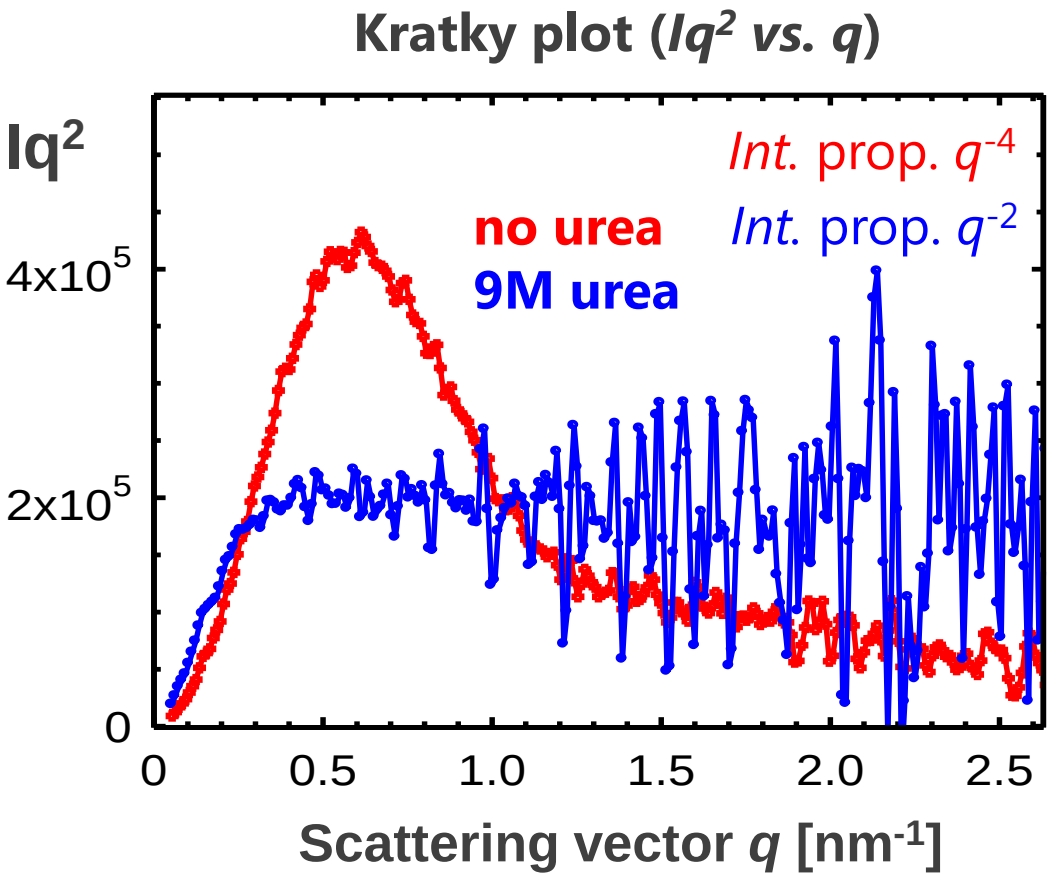


Protein Data Bank PDB 4F5S



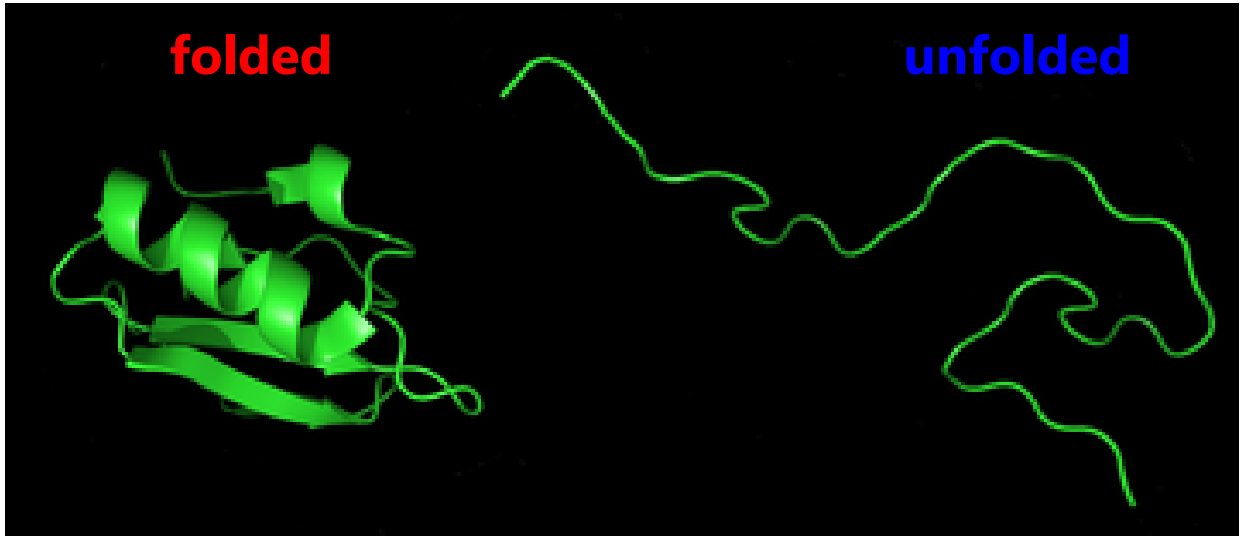
Protein folding / unfolding - BSA in Hepes buffer

SAXS measurements were done without and with added urea (known to be a denaturant).



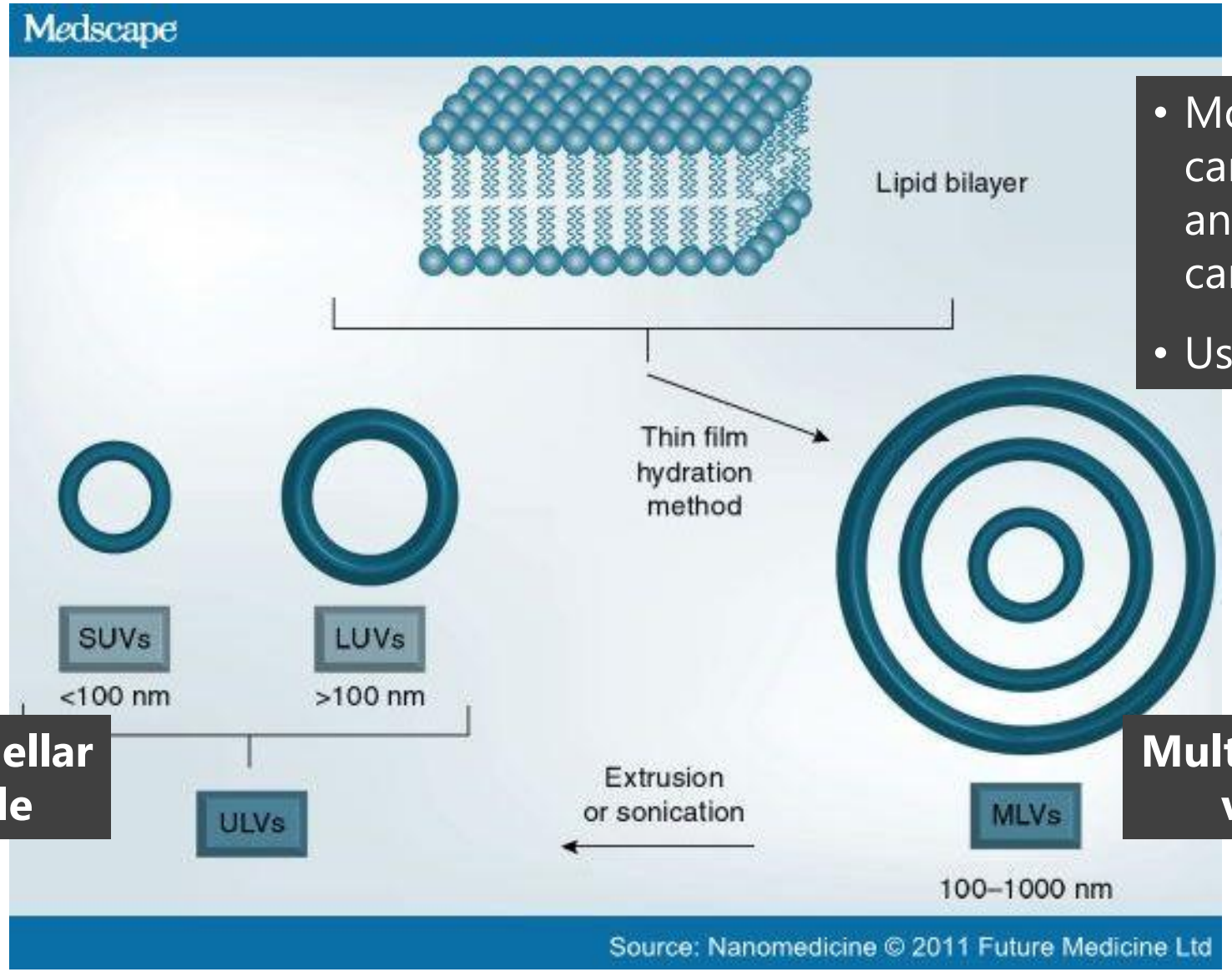
In pure buffer (no urea):
folded,
globular particle

In buffer with 9M urea:
unfolded,
dissolved polymer chain



Confirmed by R_g determination

Liposomes



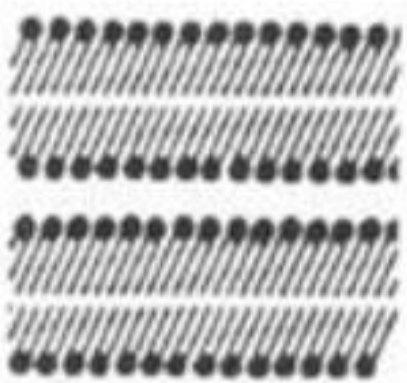
- Model biomembrane, can be further functionalized and structure / properties can systematically be studied
- Use as drug carrier

Uni-lamellar vesicle

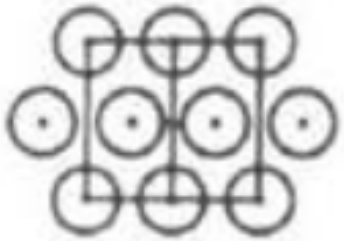
Multi-lamellar vesicle

Lipid phase transition temperature

Lipid bilayer structure



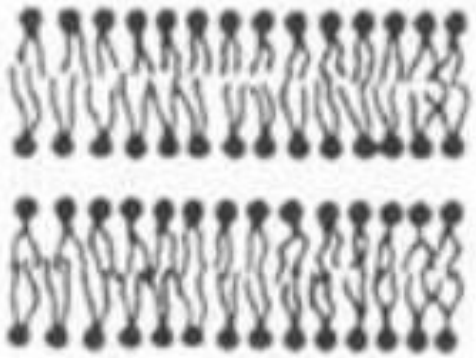
fully extended, closely packed, slightly tilted alkyl chains



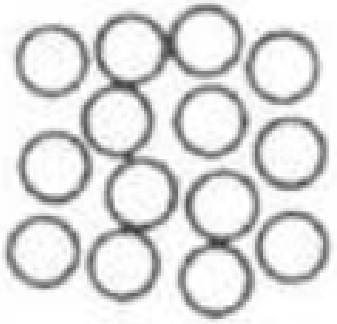
solid phase ("gel phase")

temperature

phase transition temperature T_m

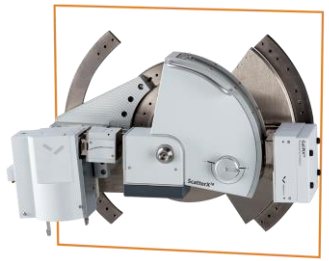


randomly oriented, fluid alkyl chains



fluid phase

SAXS / WAXS on liposomes



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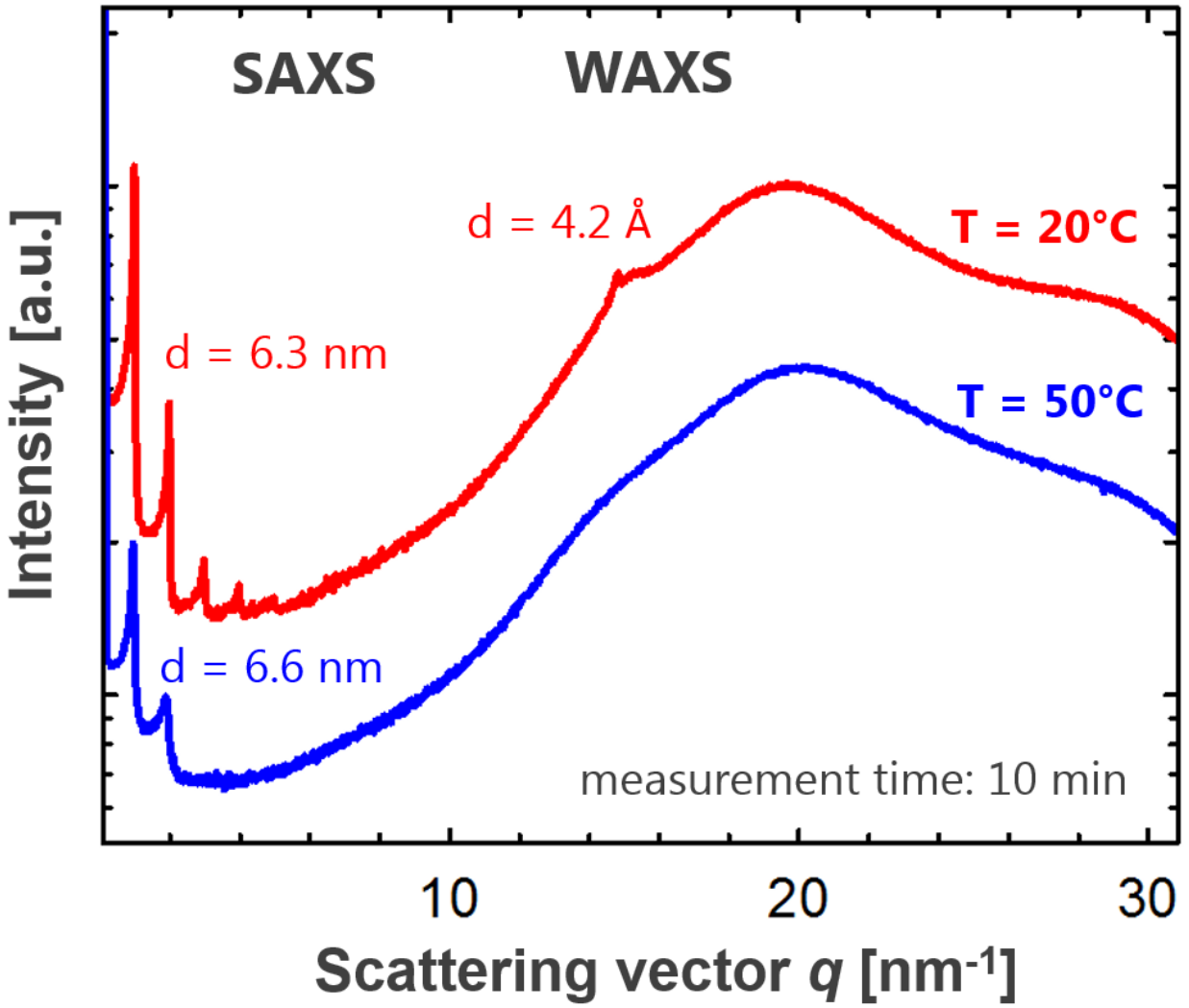
Test sample:
Phospholipid DPPC (25 mg/ml in a PBS buffer), before extrusion.
- Forming multilamellar vesicles.

The **SAXS** signal contains information about the lipid bilayer structure and stacking.

From the **WAXS** signal information about the alkyl chain packing can be deduced.

Melting temperature of DPPC = 41°C



Summary

- Structural studies on proteins were performed to determine:
  - Overall size and shape (simulation and ab-initio determination)
  - Folding / unfolding (tertiary structure)
  - Stability and complex formation (quaternary structure)
  - Oligomeric state and oligomeric mixture
  - Molecular weight


Malvern
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