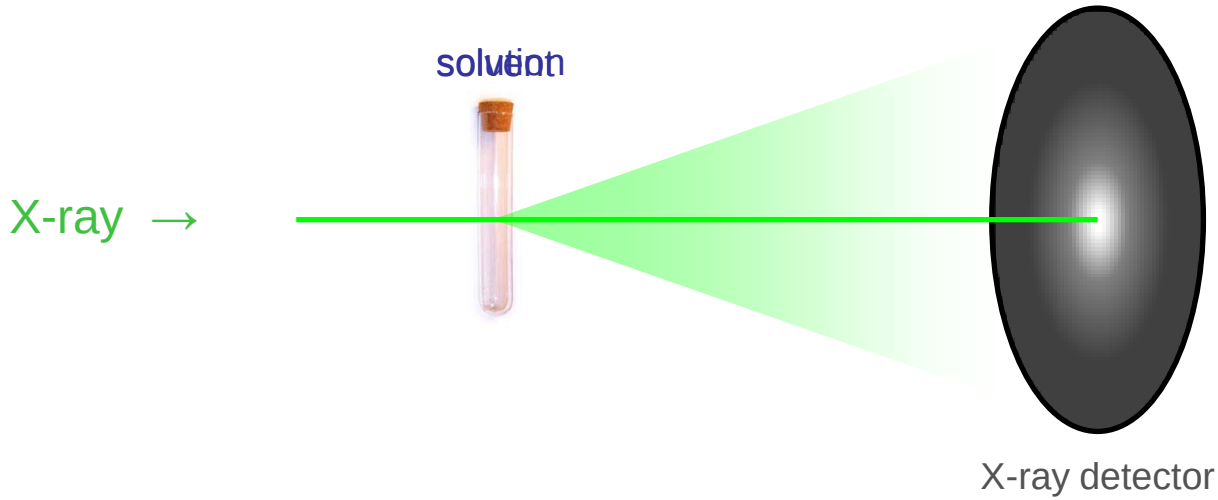
SAXS data reduction and analysis

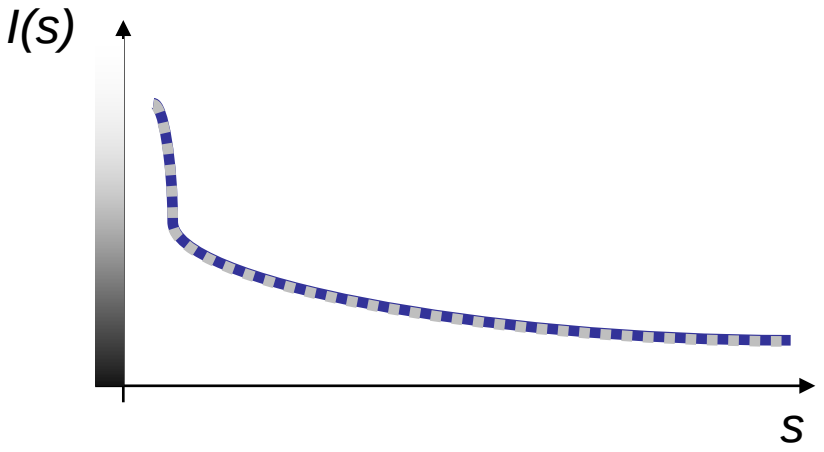
Daniel Franke
EMBL Hamburg

Outline

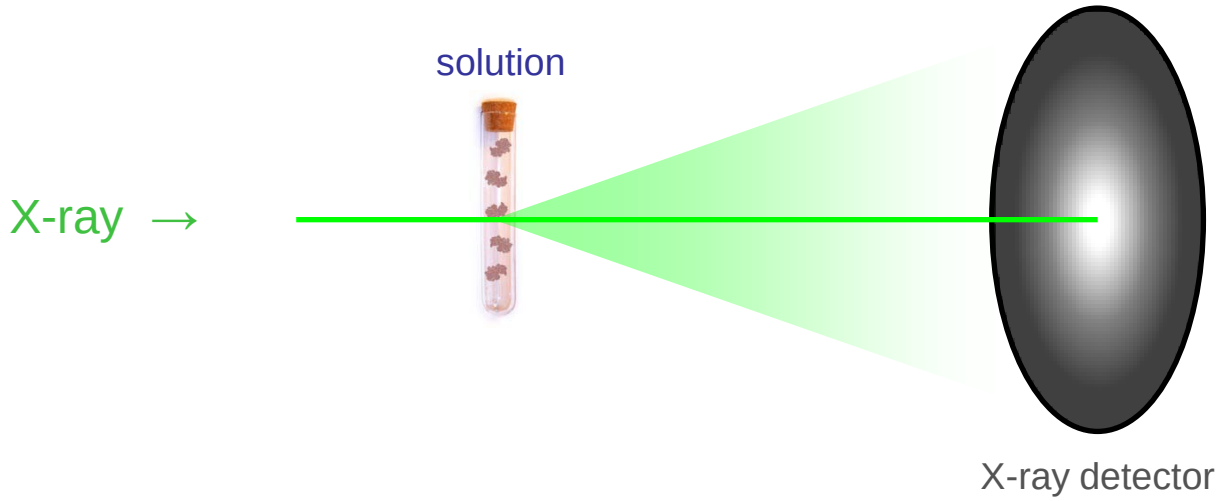
- SAXS experiment setup
- 3D → 2D → 1D
- Background subtraction
- Concentration effects
- R_g, MM
- Volume
- Distance distribution function p(r)

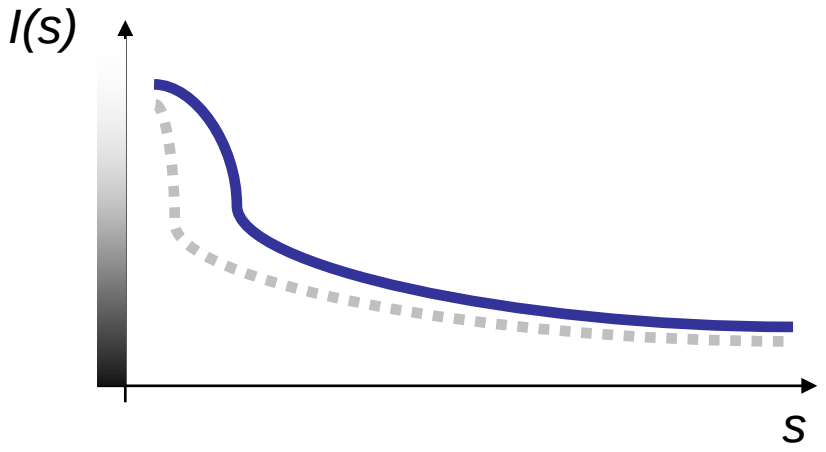
SAXS experiment

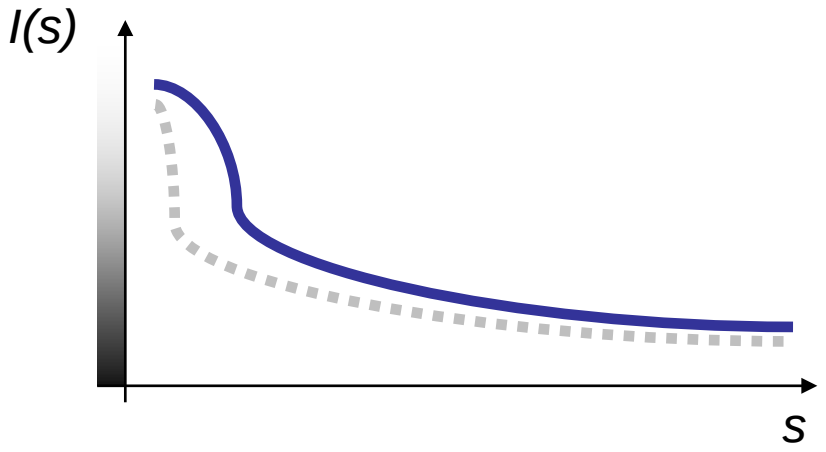




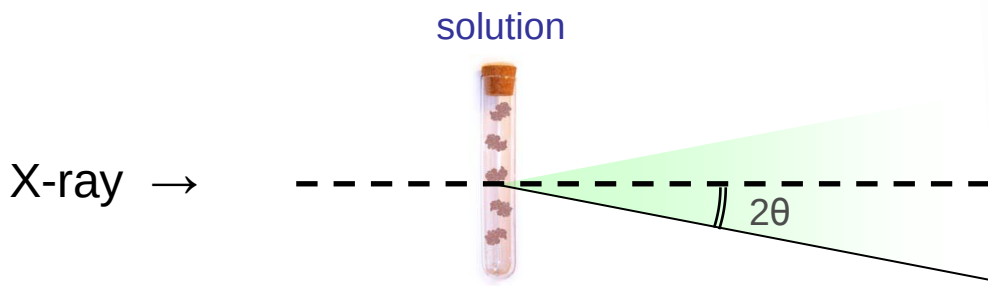
SAXS experiment

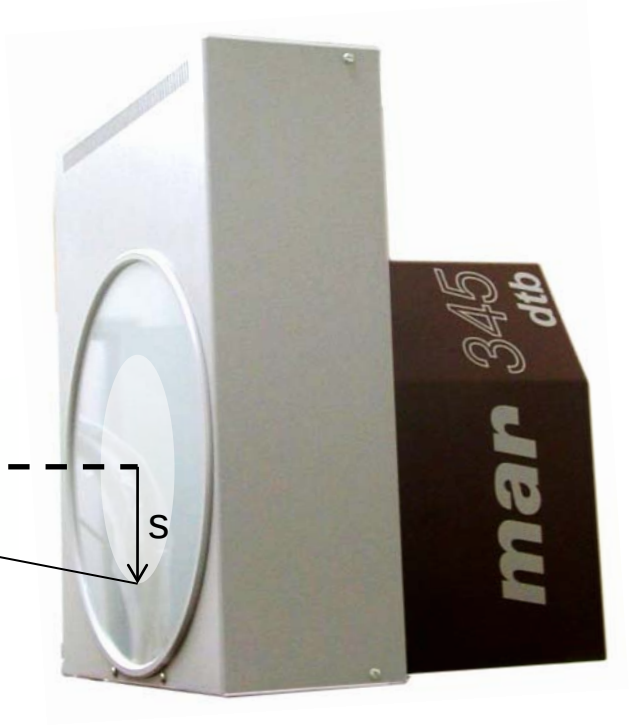




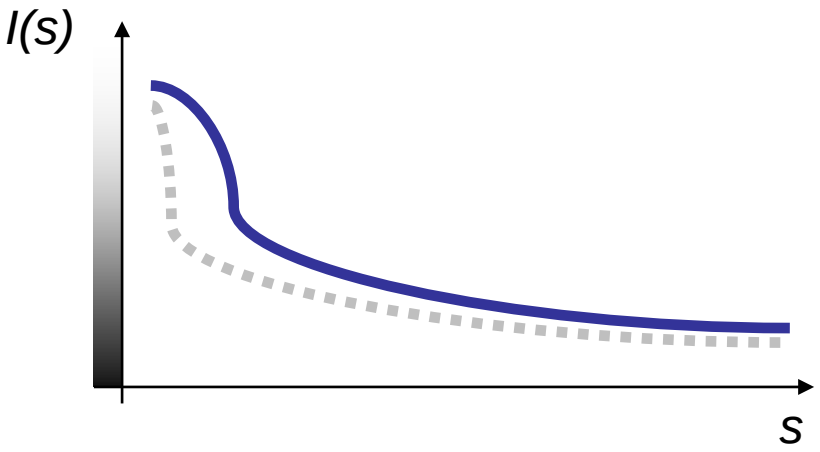

I(s)
s

2D → 1D





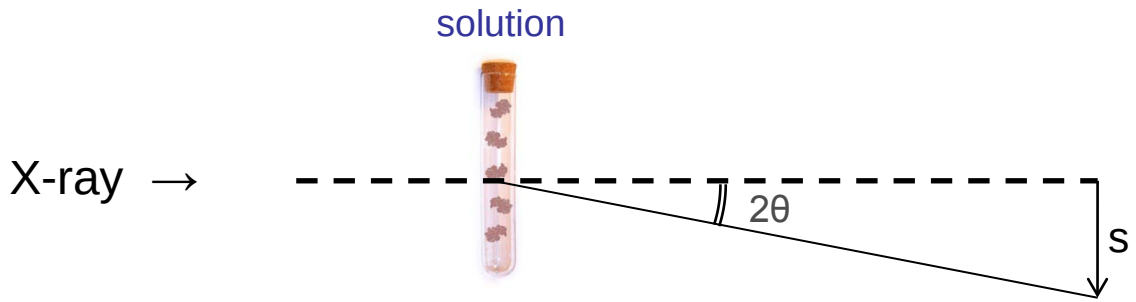
X-ray detector

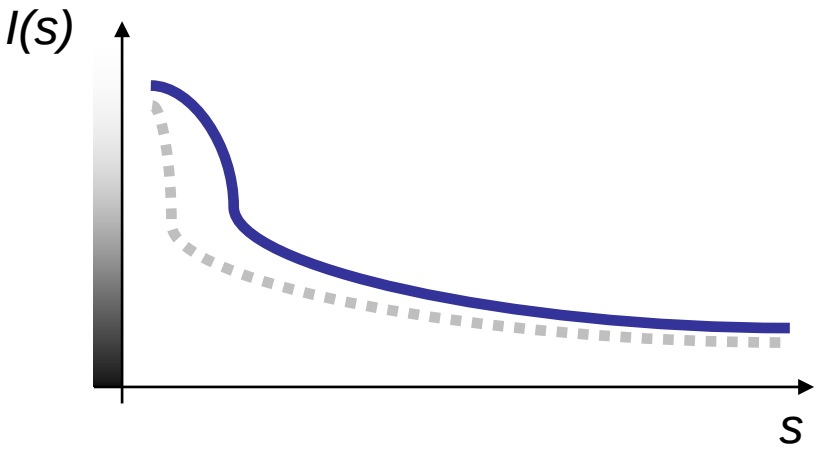


$|s| = 4\pi \sin\theta/\lambda$

s – scattering vector
2θ – scattering angle
λ – wavelength
I(s) – intensity

2D → 1D



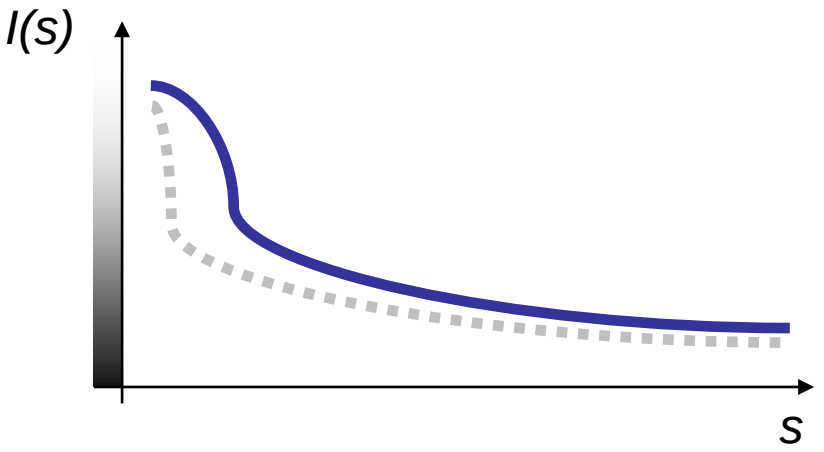


$|s| = 4\pi \sin\theta/\lambda$

s – scattering vector
2θ – scattering angle
λ – wavelength
I(s) – intensity

Normalization

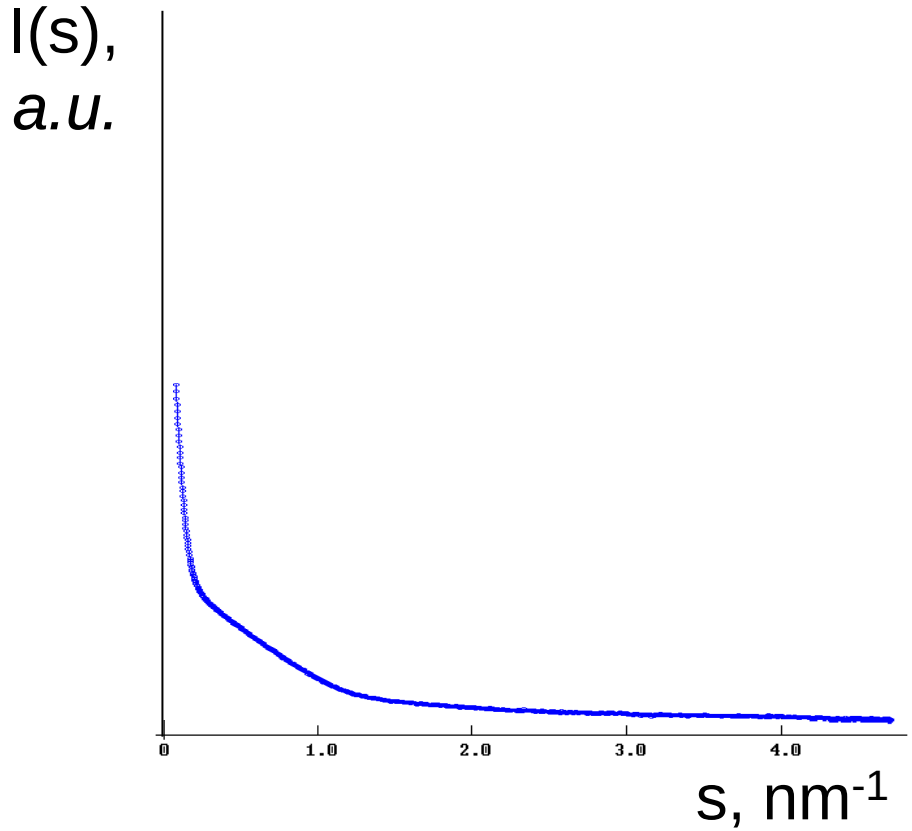
- Transmitted beam
- Exposure time



Notations and units

$|s| = 4\pi \sin\theta/\lambda$

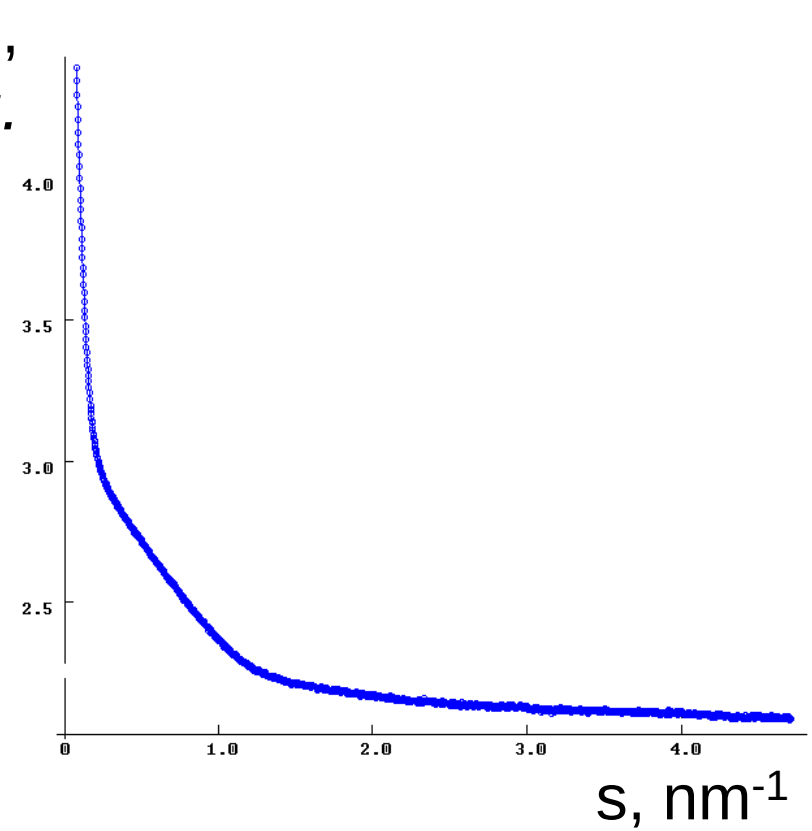
2θ – scattering angle
λ – wavelength



Notations and units

$|s| = 4\pi \sin\theta/\lambda$

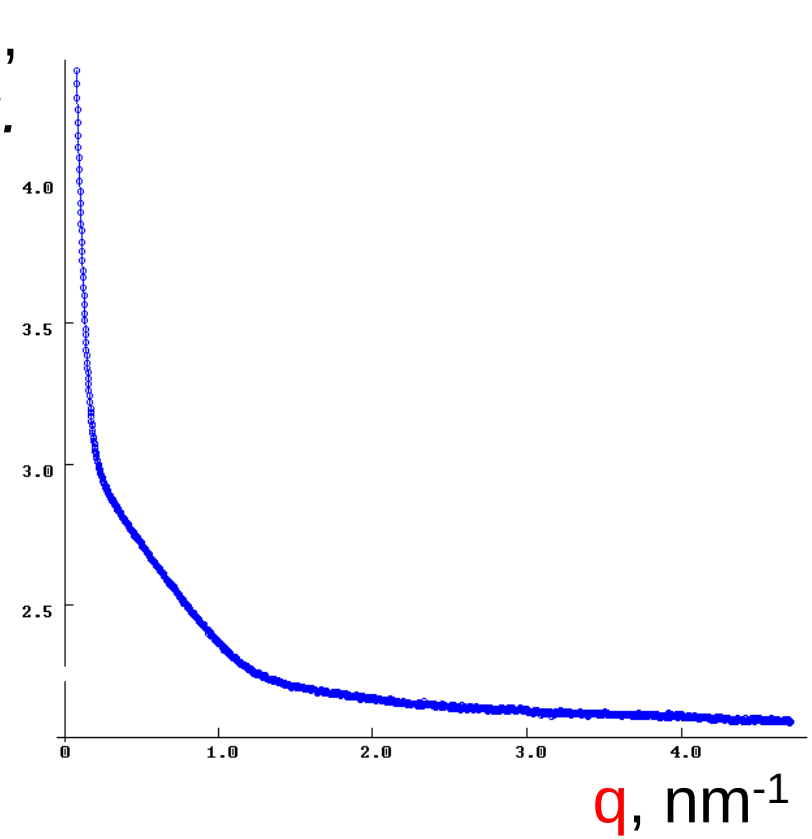
2θ – scattering angle
λ – wavelength



Notations and units

$|q| = 4\pi \sin\theta/\lambda$

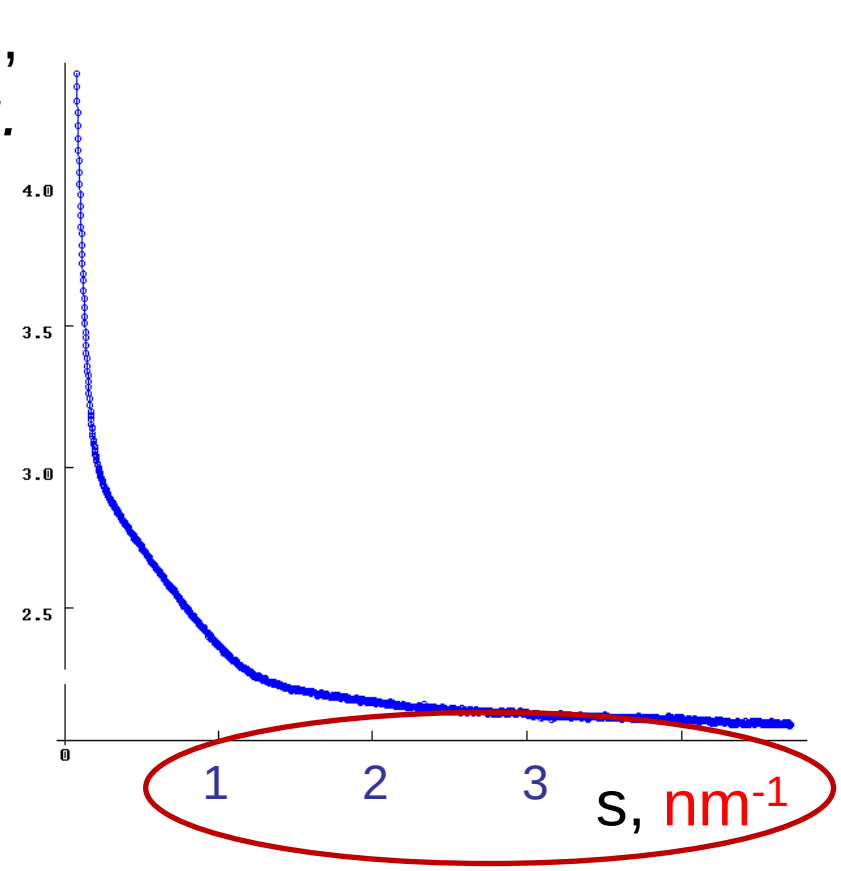
2θ – scattering angle
λ – wavelength



Notations and units

$|s| = 4\pi \sin\theta/\lambda$

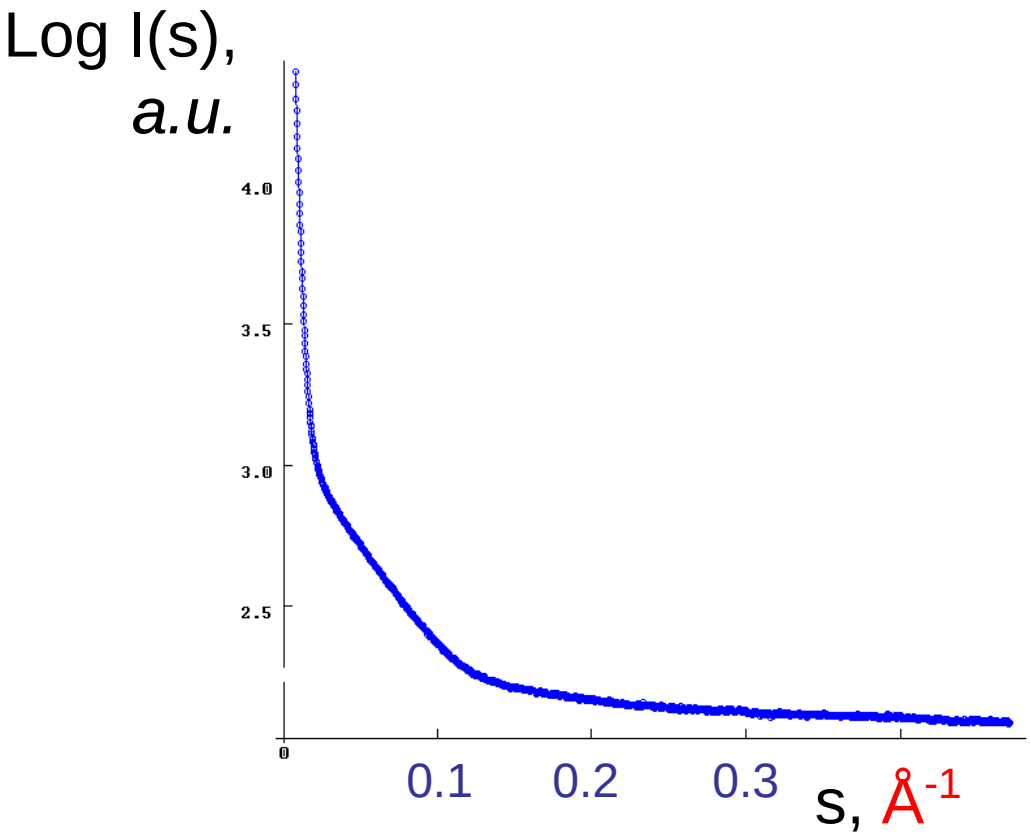
2θ – scattering angle
λ – wavelength



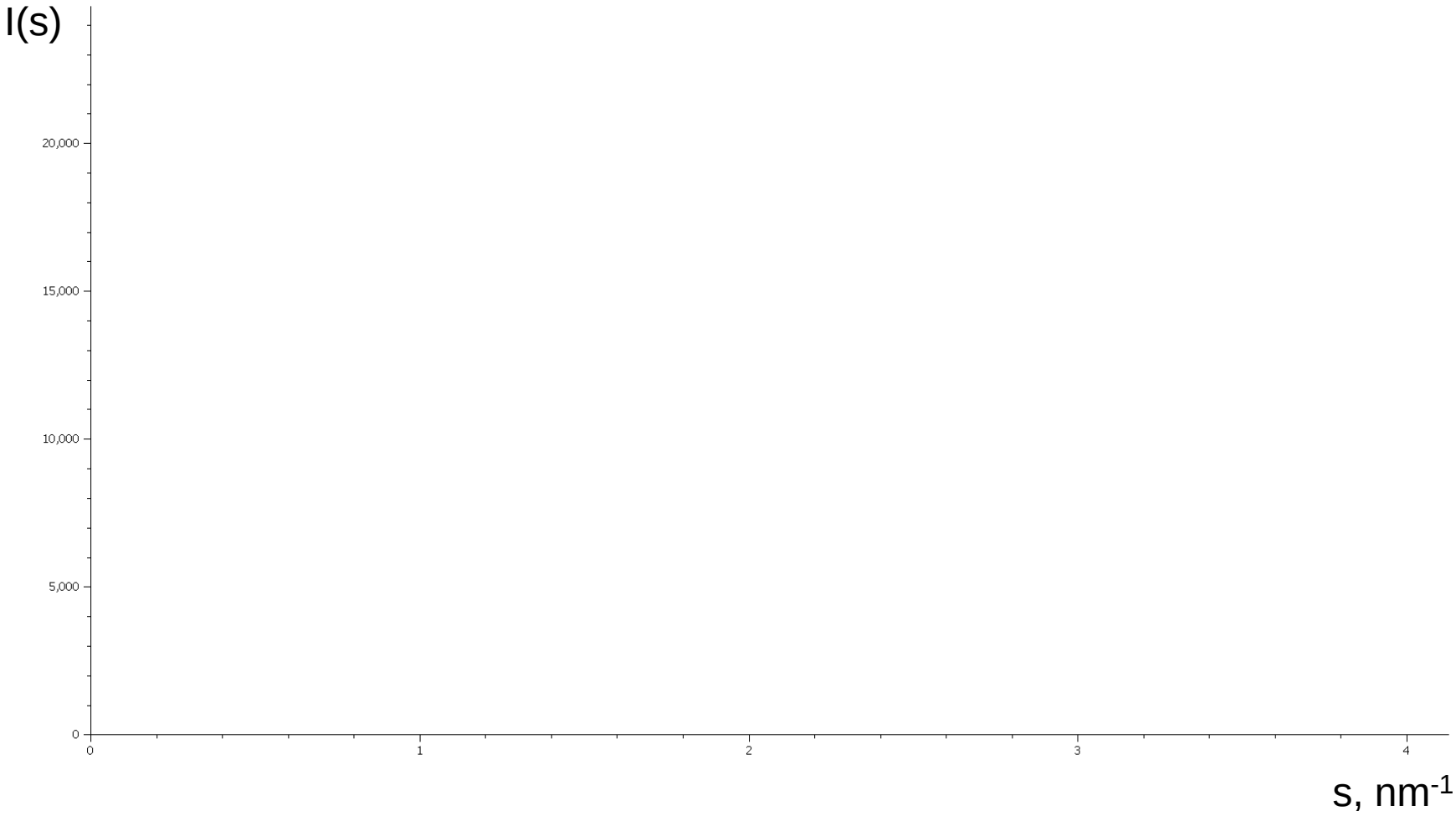
Notations and units

$|s| = 4\pi \sin\theta/\lambda$

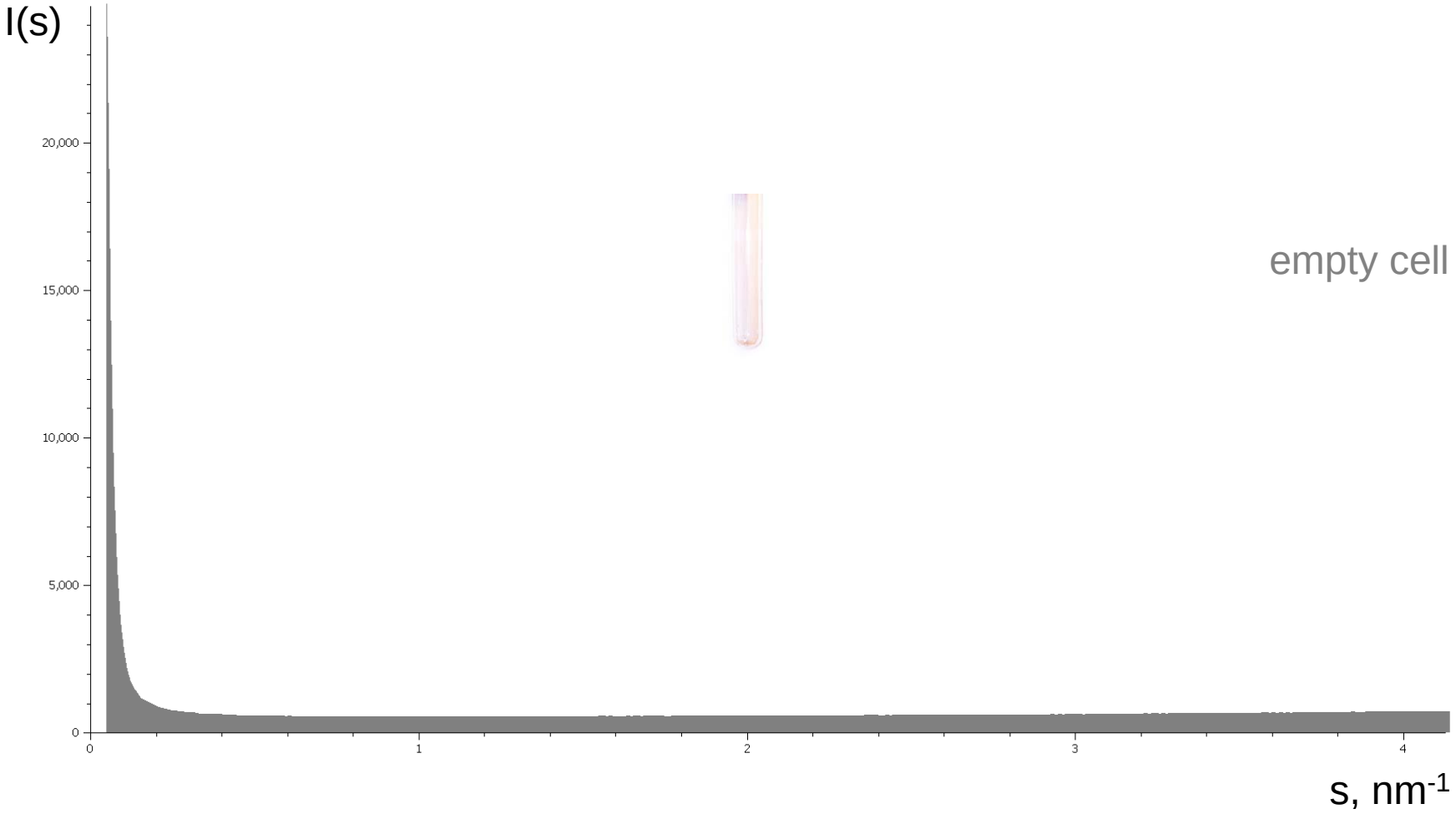
2θ – scattering angle
λ – wavelength



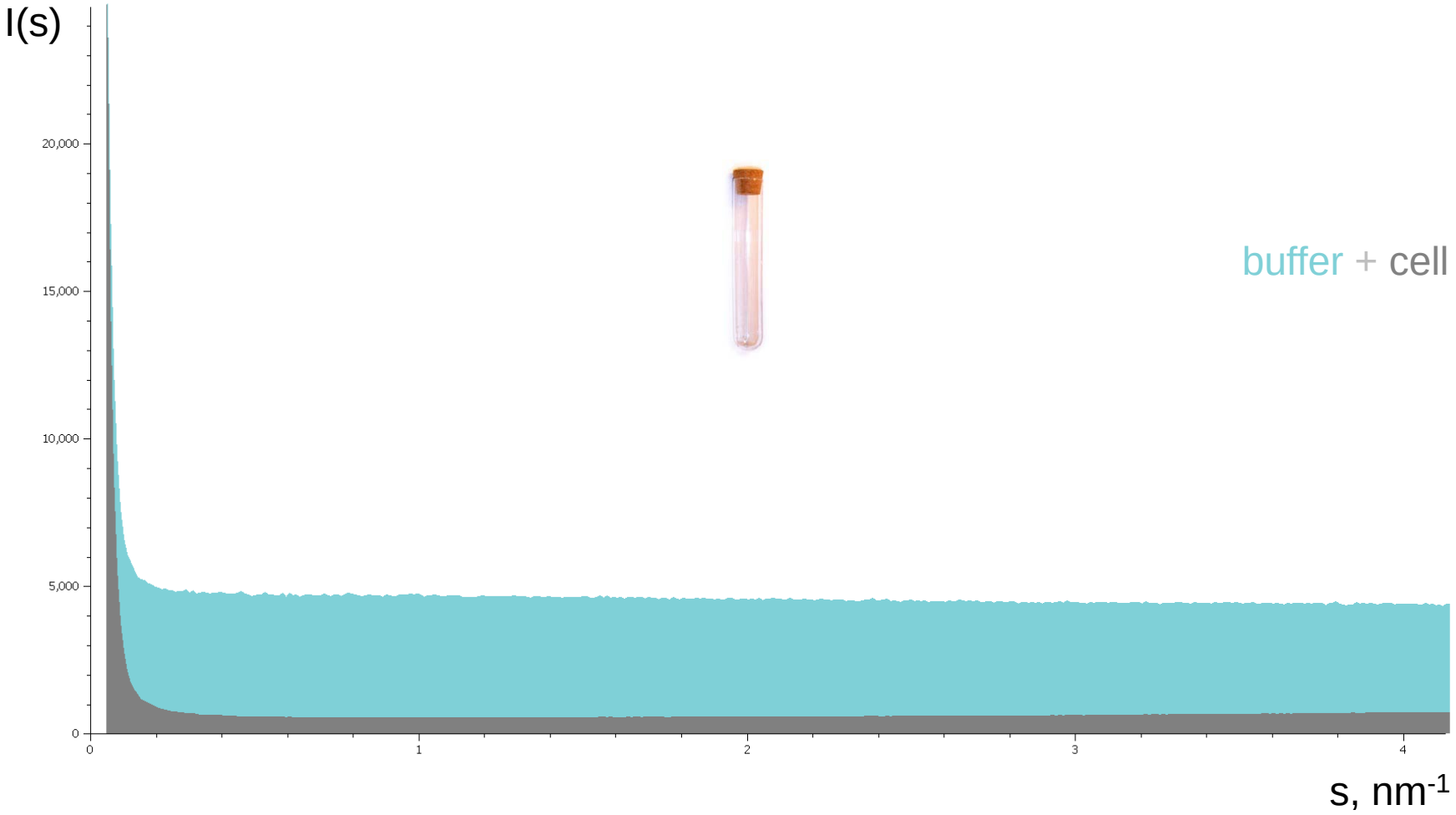
Sample and buffer



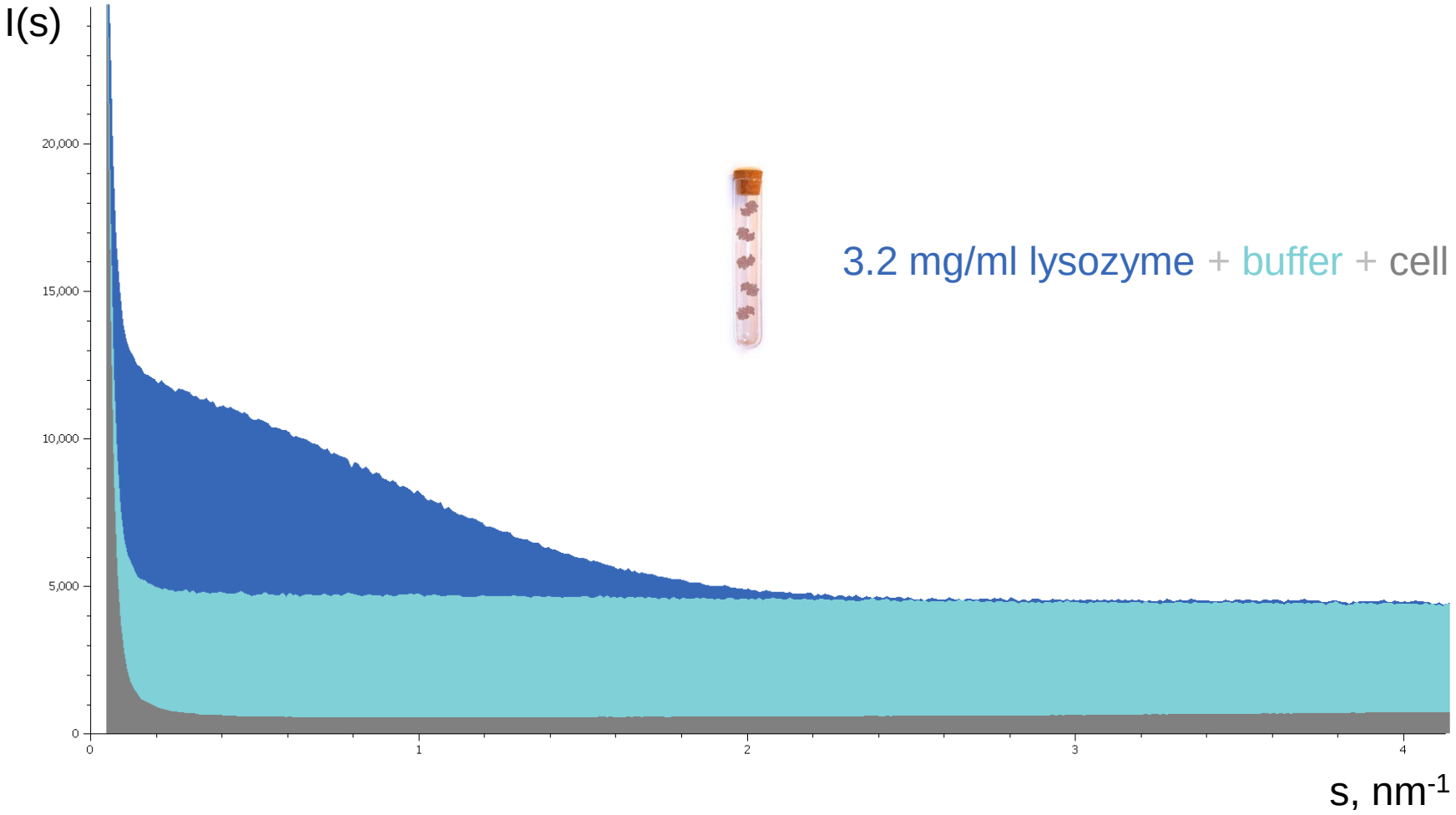
Sample and buffer



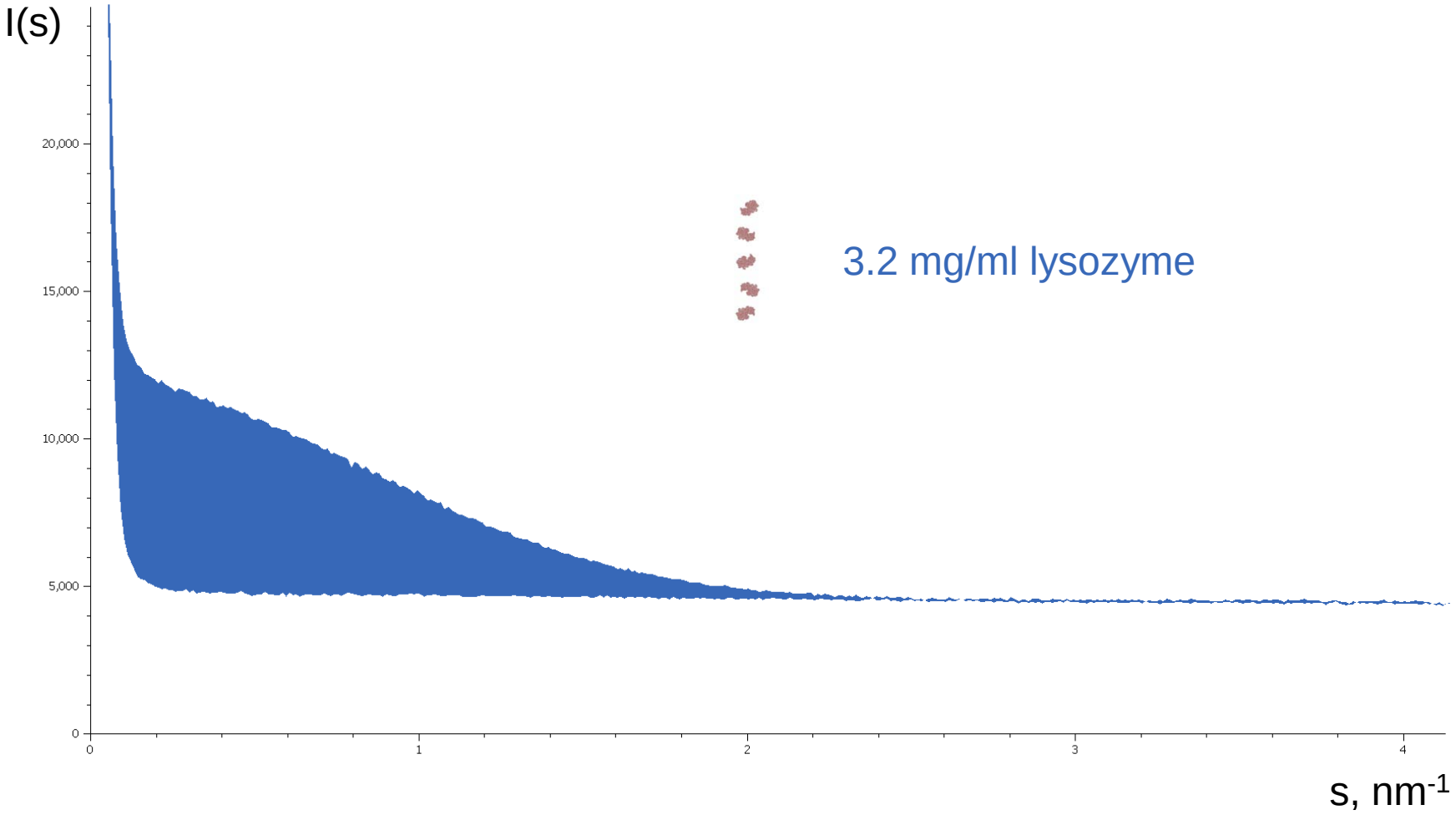
Sample and buffer



Sample and buffer

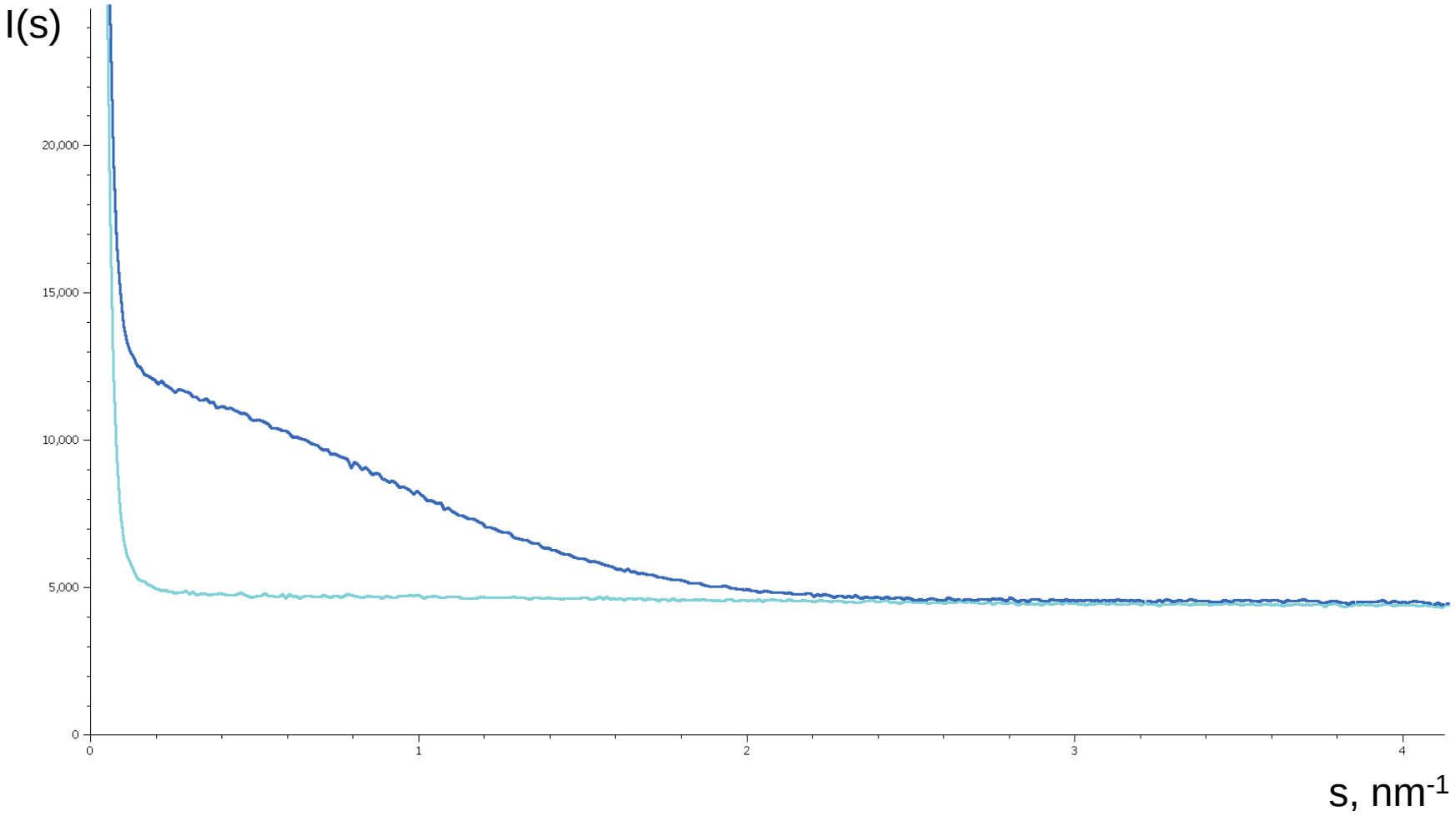


Sample and buffer



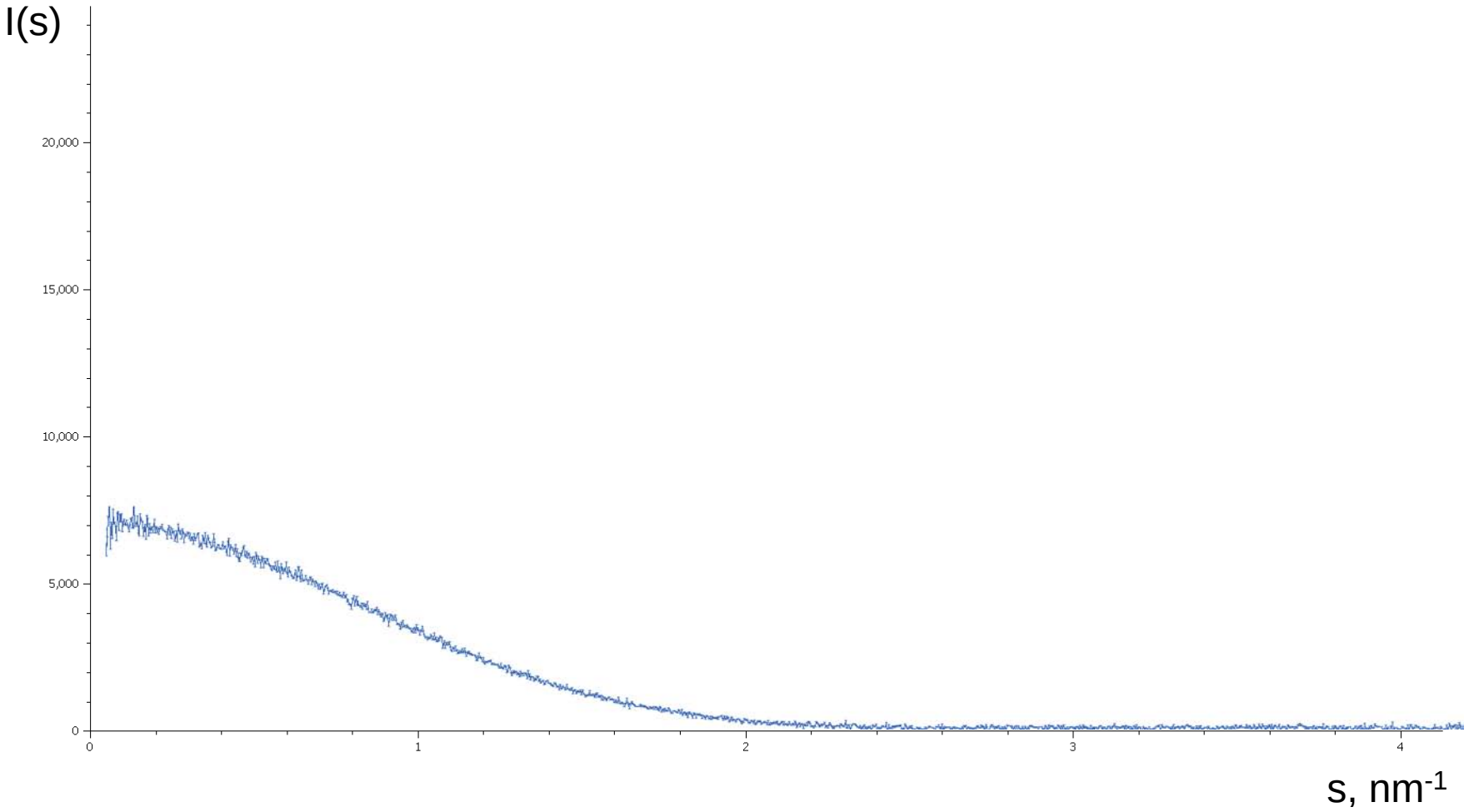
Background subtraction

Solution minus Solvent

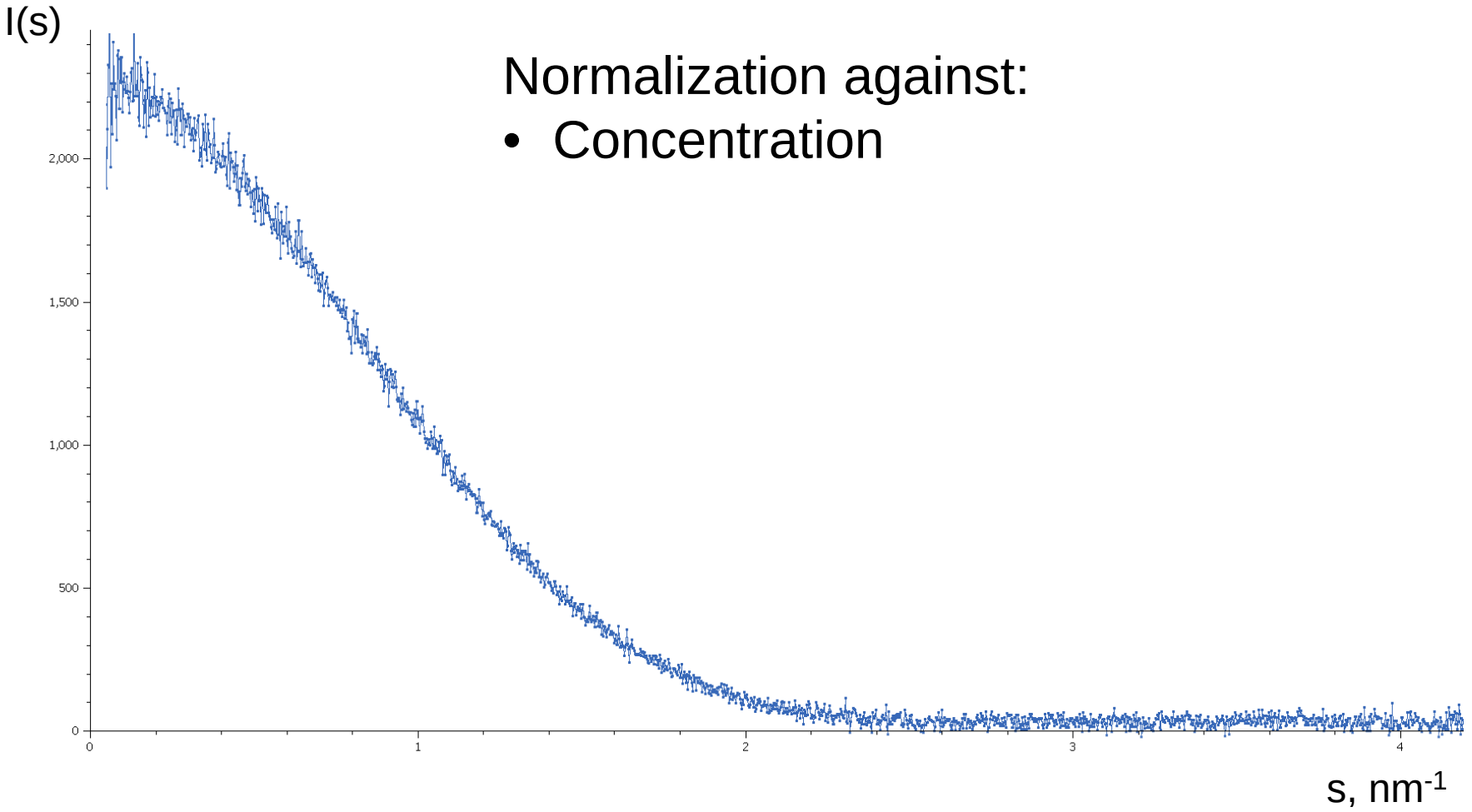


Background subtraction

Solution minus Solvent



Background subtraction

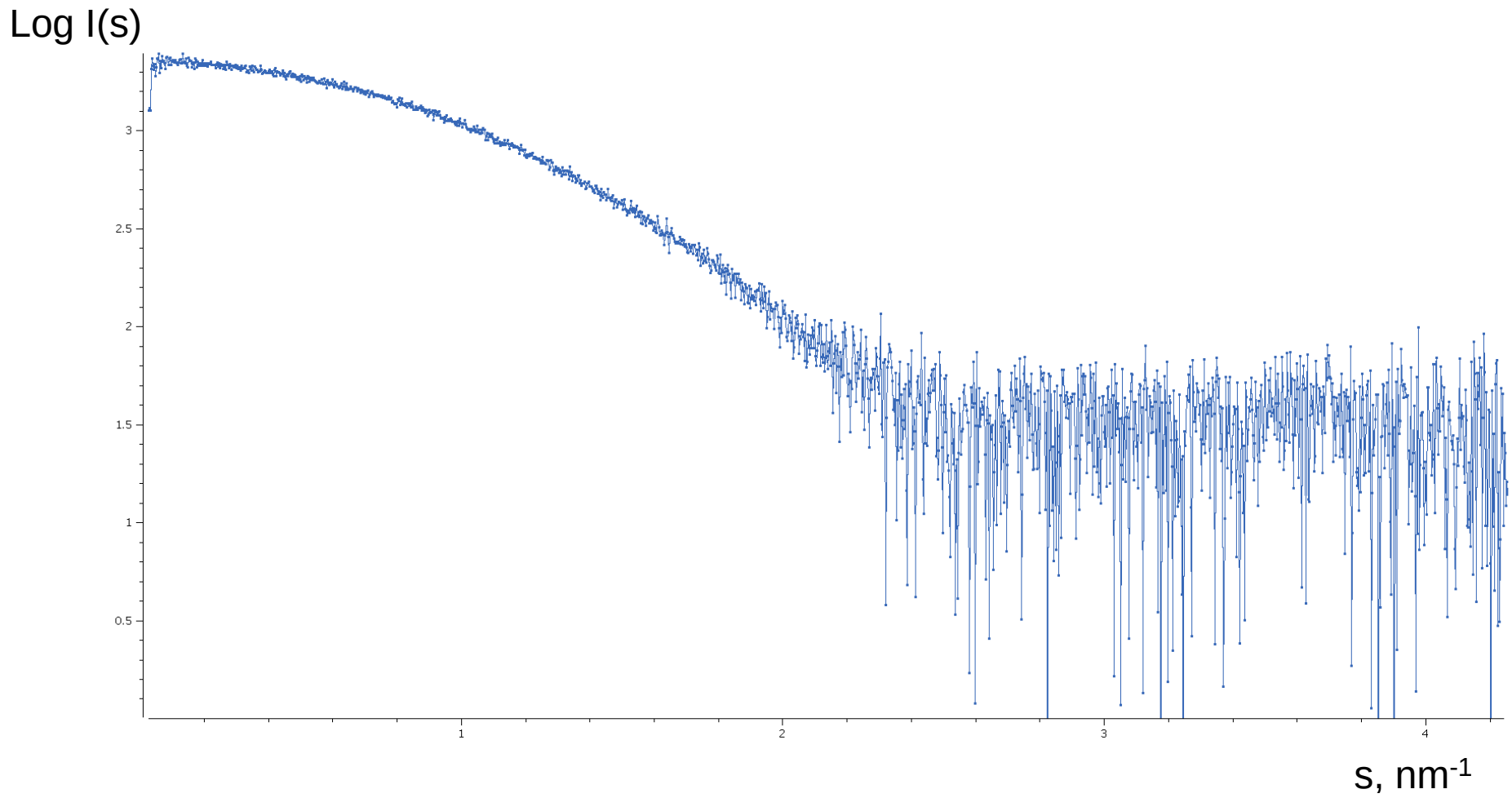


Normalization against:

- Concentration

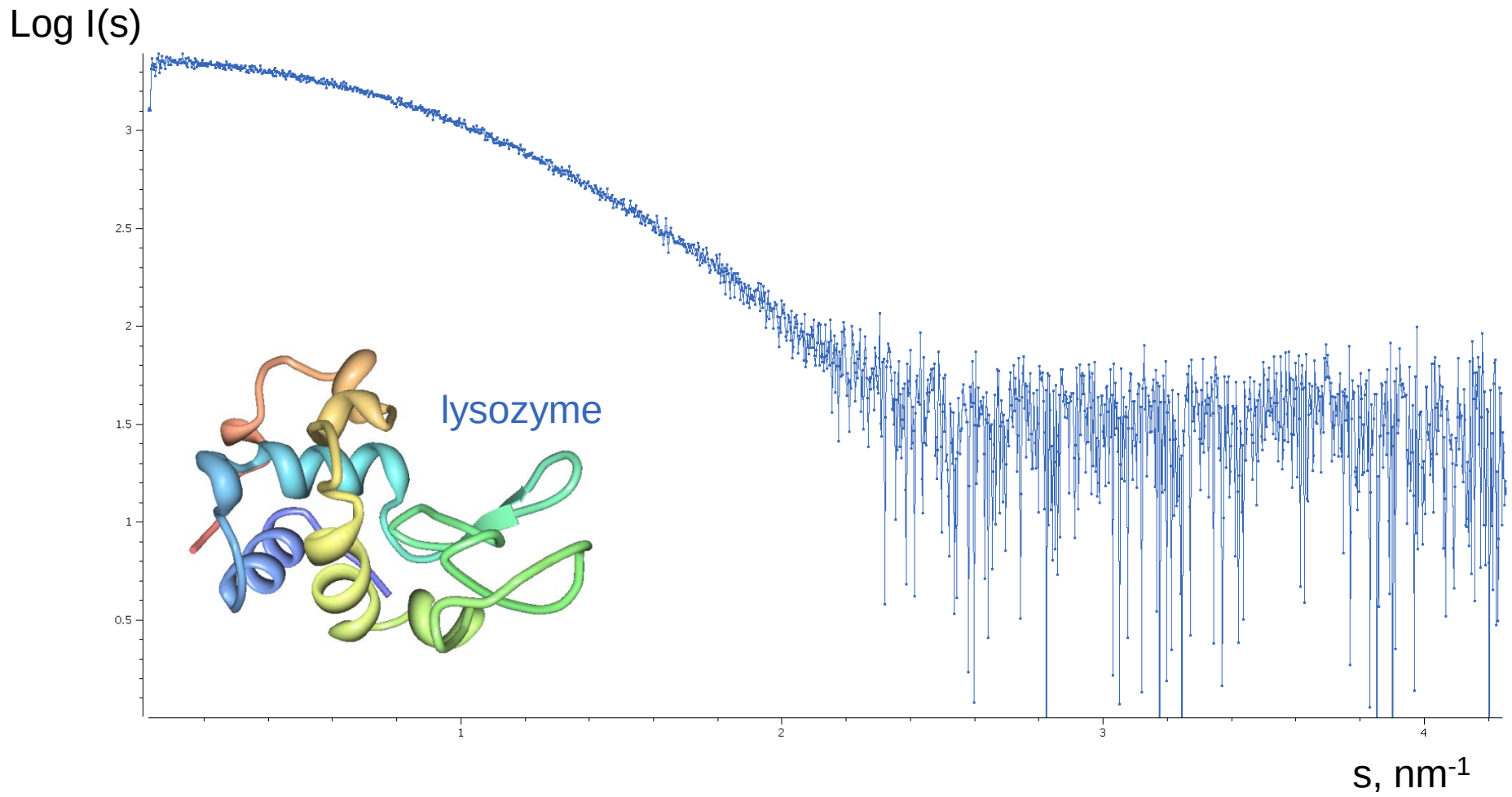
Background subtraction

Solution minus Solvent



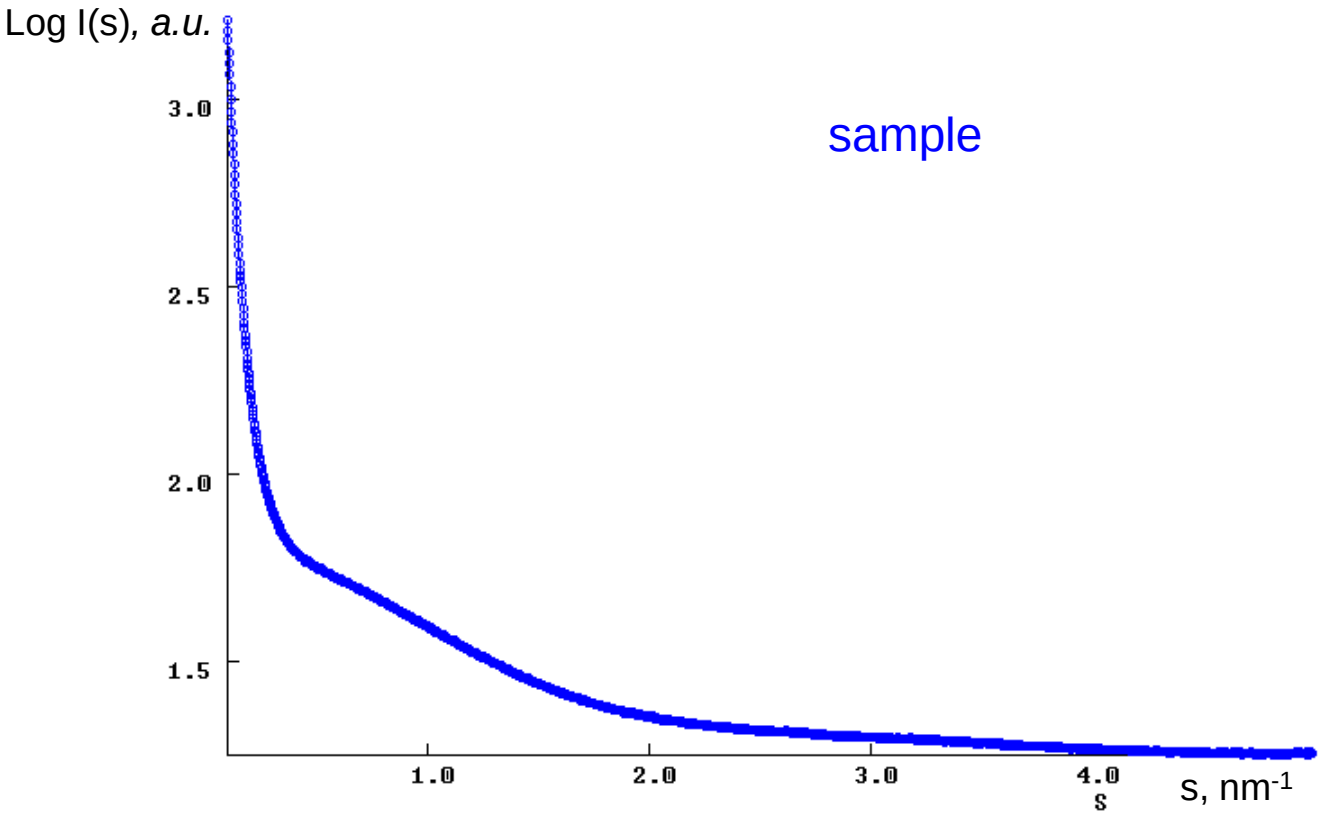
Data quality

"Can I use this data for further analysis?"



Data quality

Radiation damage



Data quality

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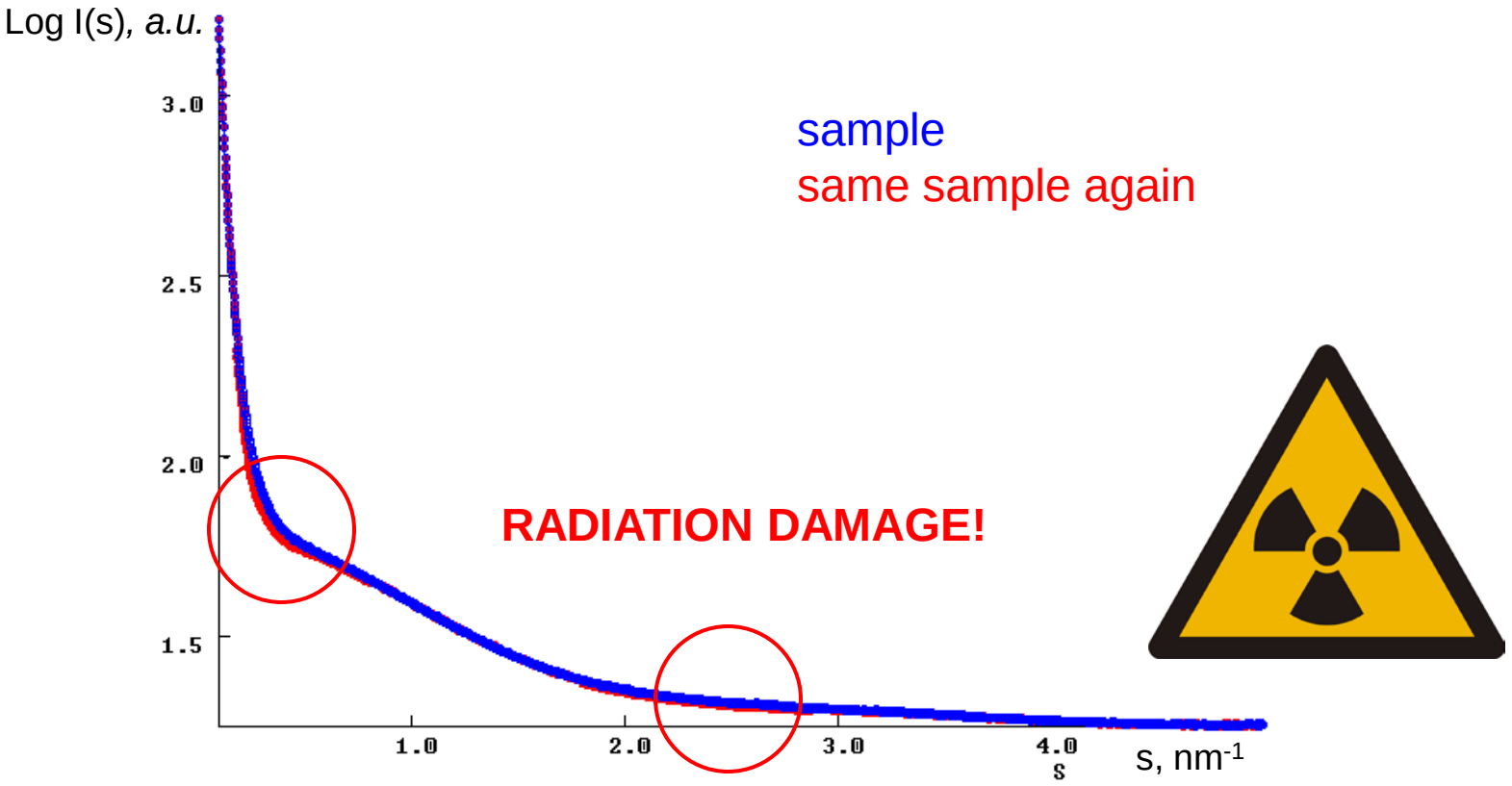

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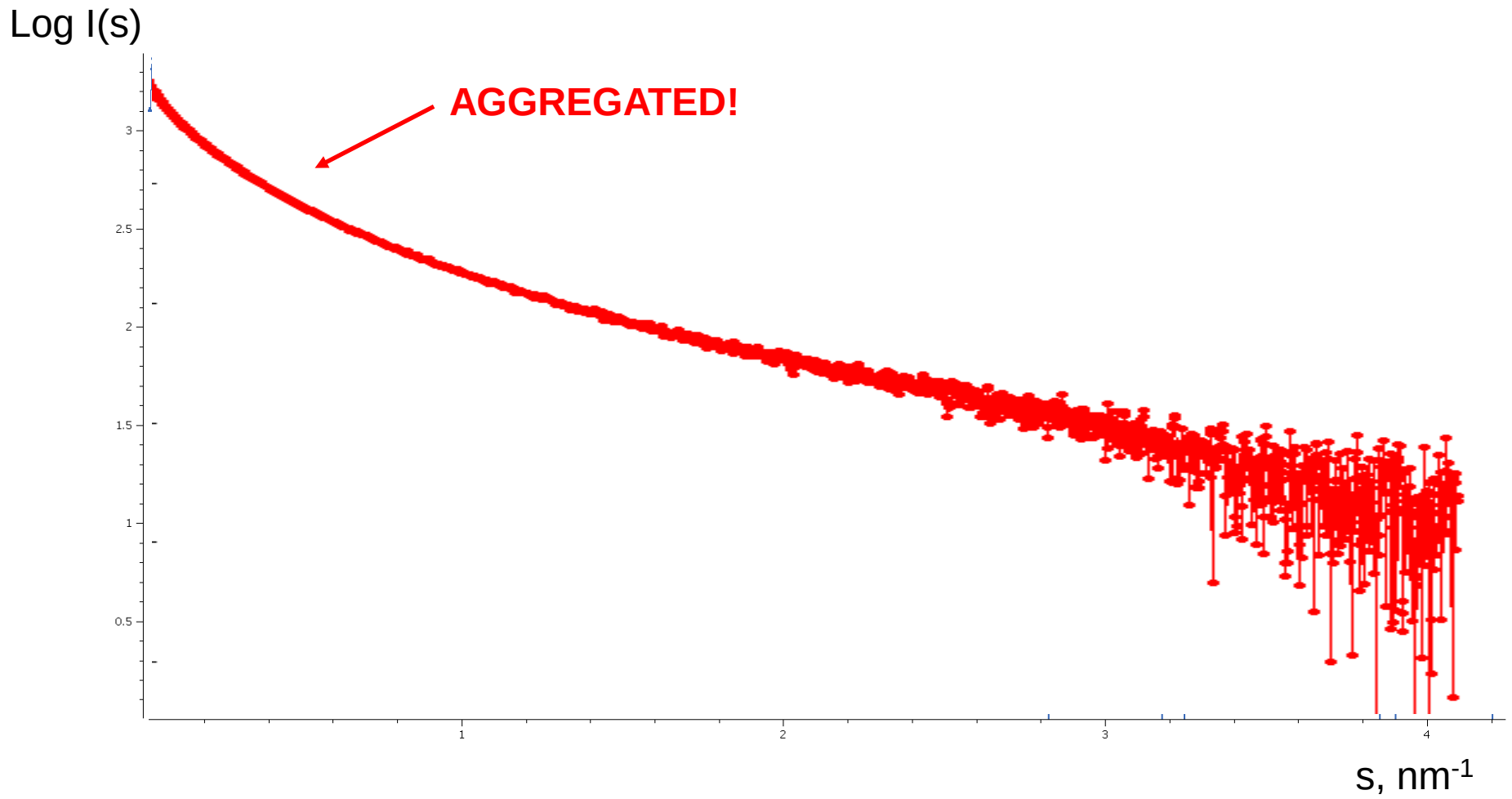
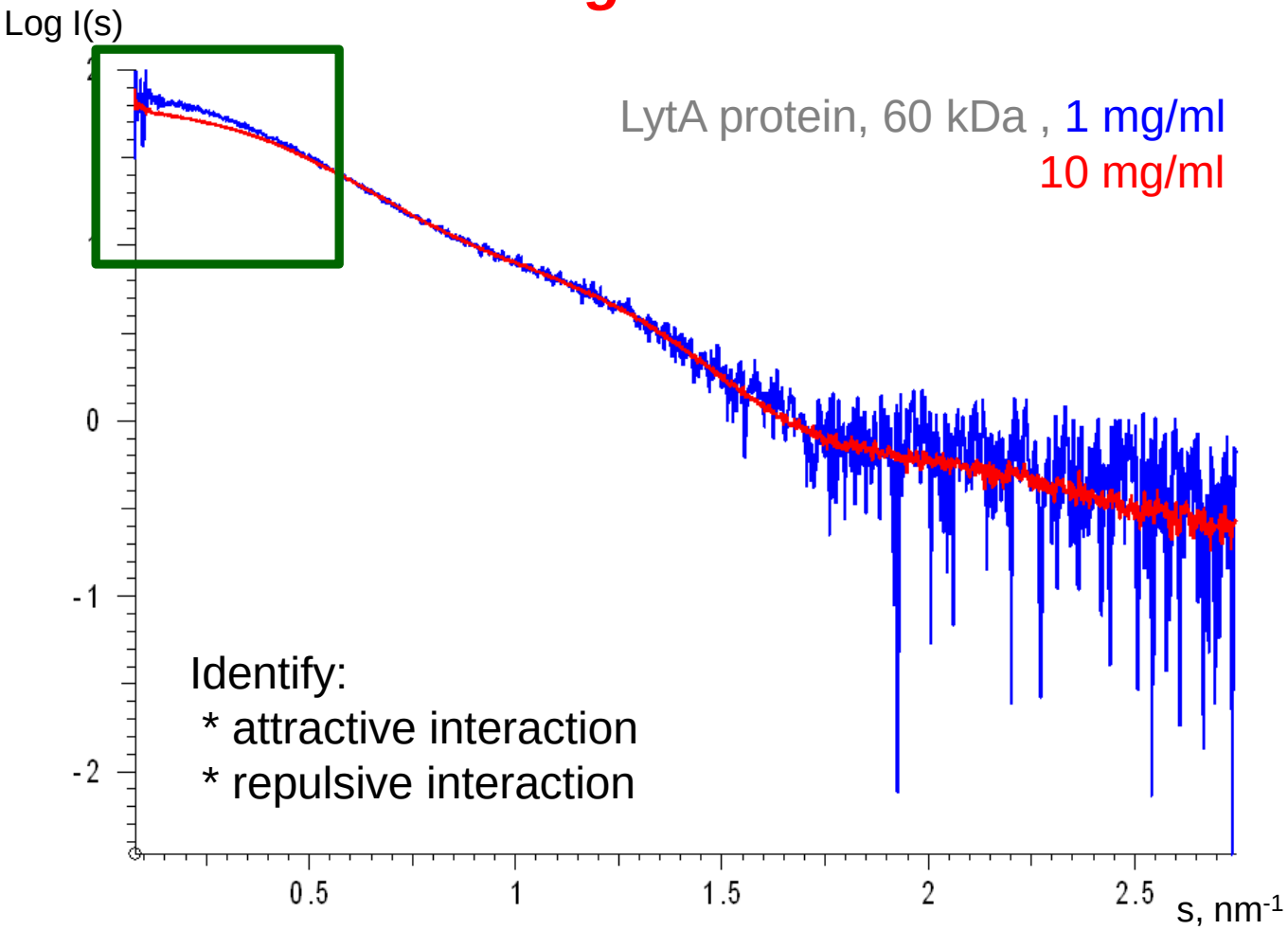


Dilution series

***Low* and *High* Concentration**

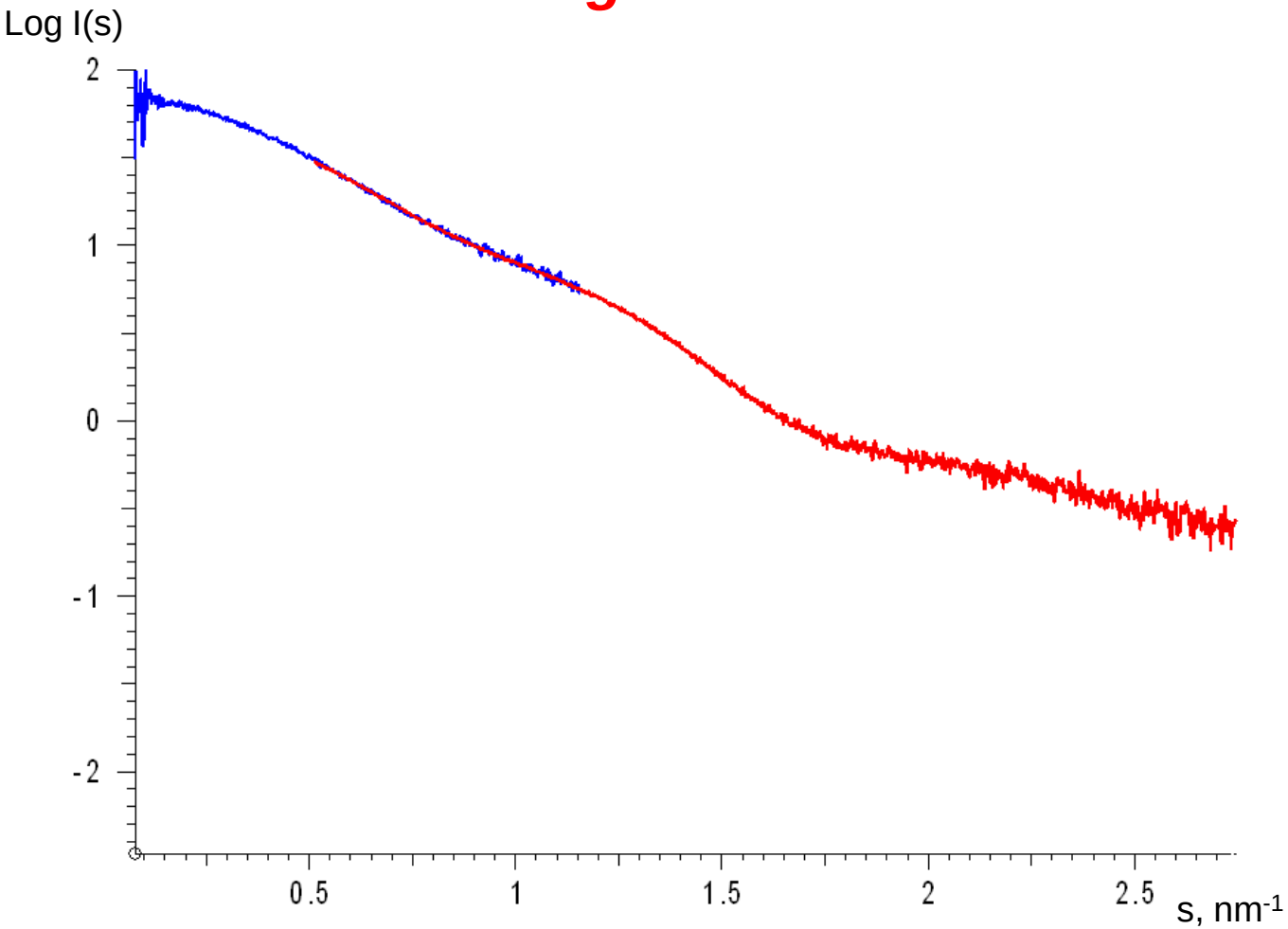
Dilution series

***Low* and *High* Concentration**

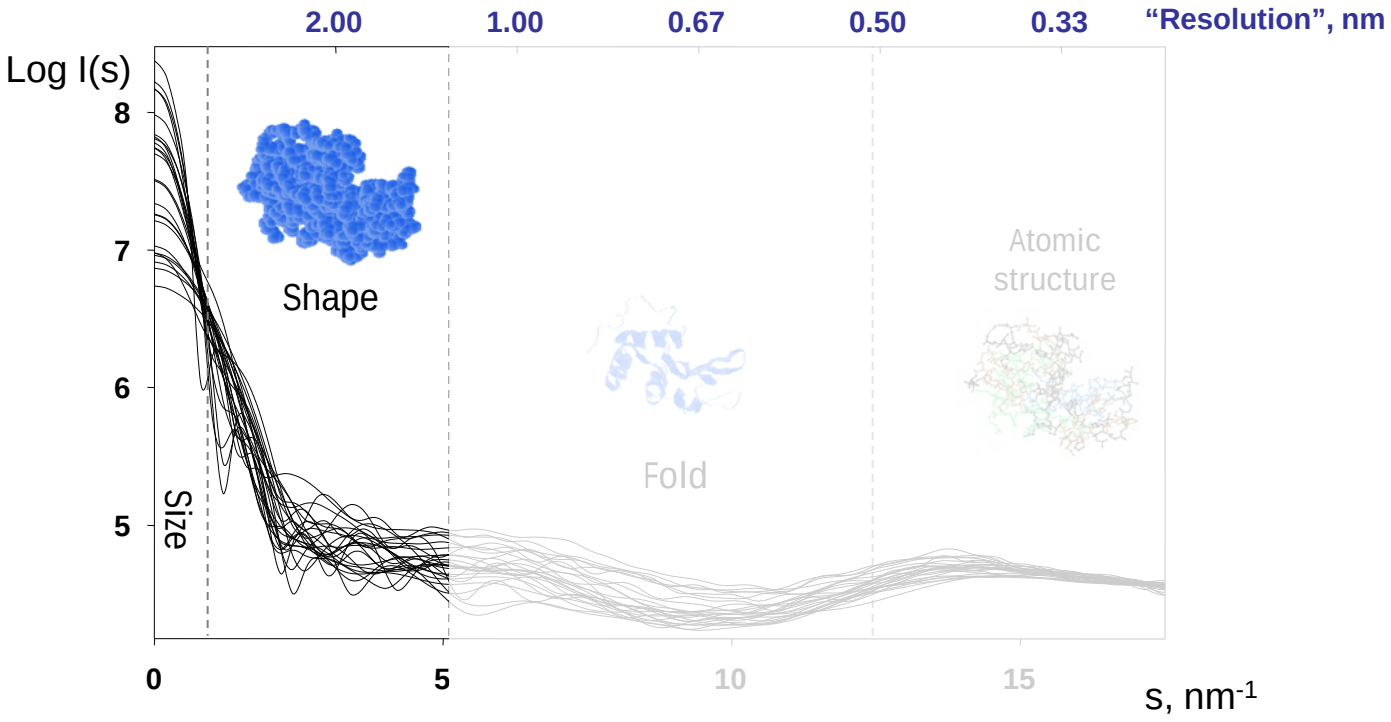


Merging data

***Low* and *High* Concentration**

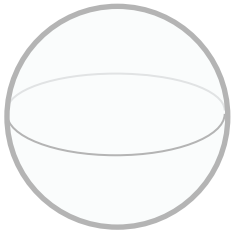


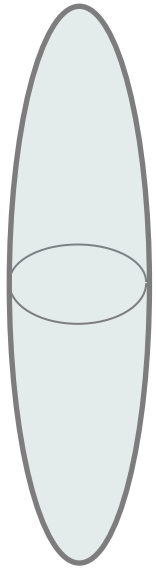
Data range

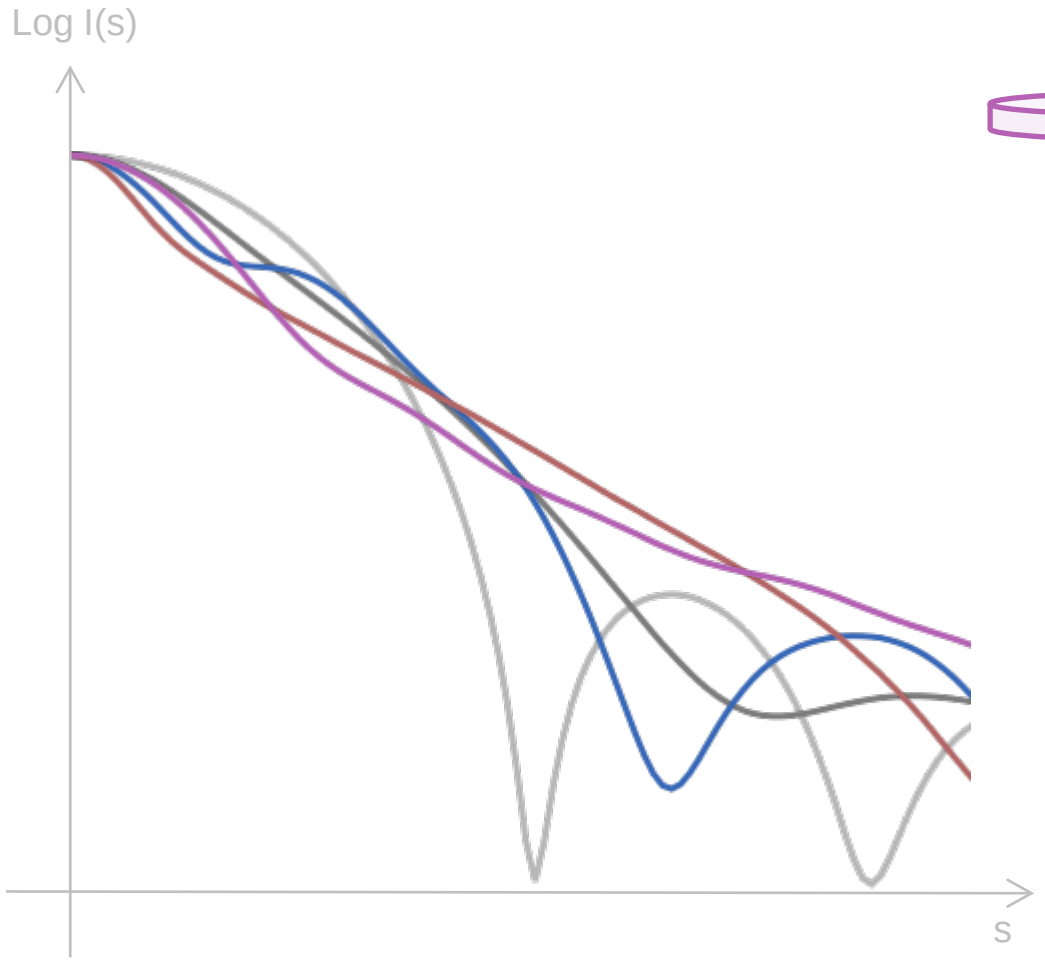


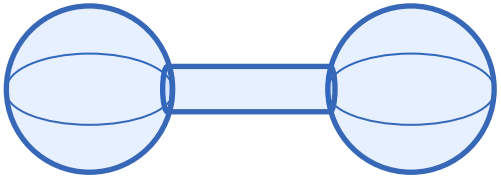
$s_{min} = \pi/D_{max}$

Shape

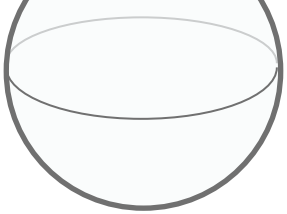




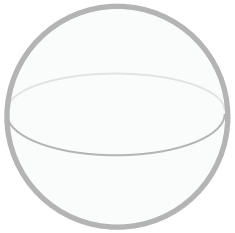




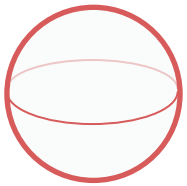
Size



200 nm^3

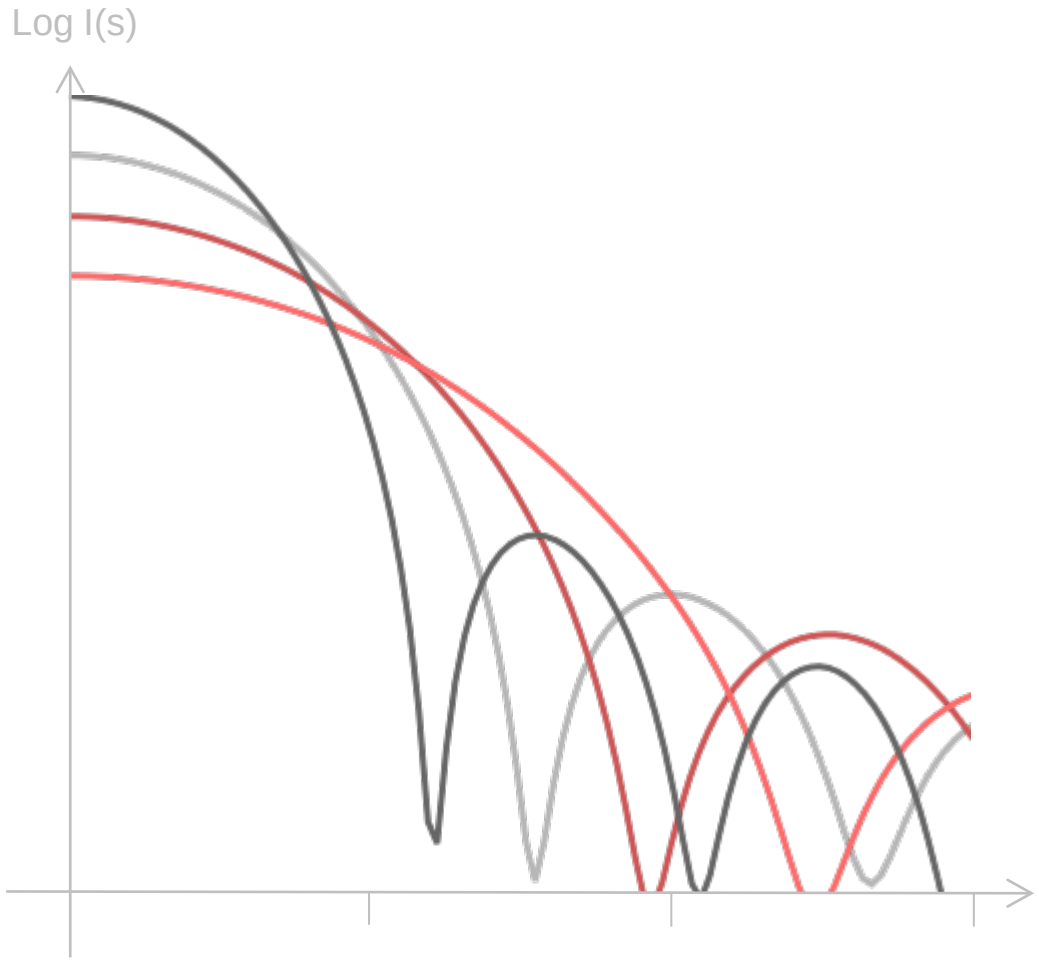


100 nm^3



50 nm^3

25 nm^3



Radius of gyration (R_g)

Definition

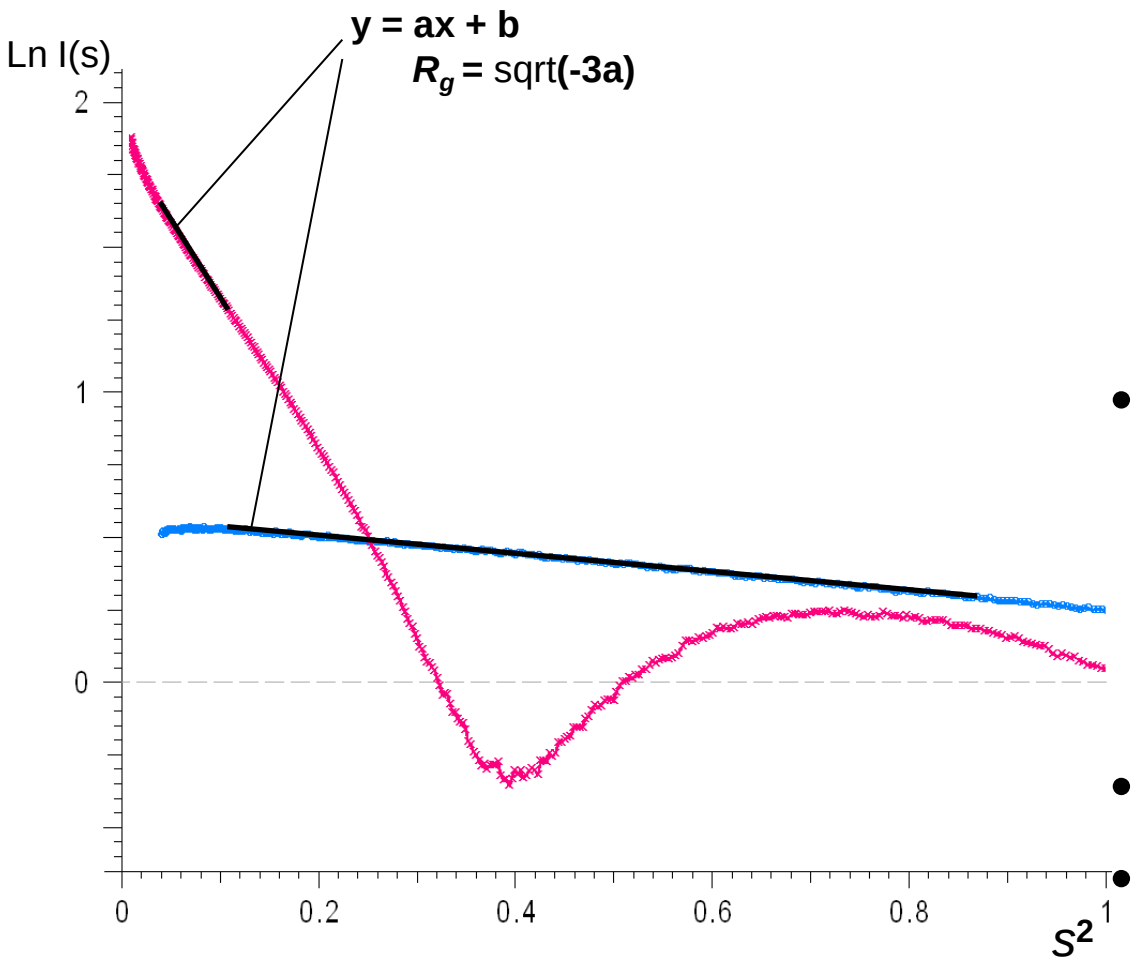
Measure for the overall size of a macromolecule

Average of square center-of-mass distances in the molecule

weighted by the scattering length density

Radius of gyration (R_g)

Guinier plot



- Estimate of the overall size of the particles

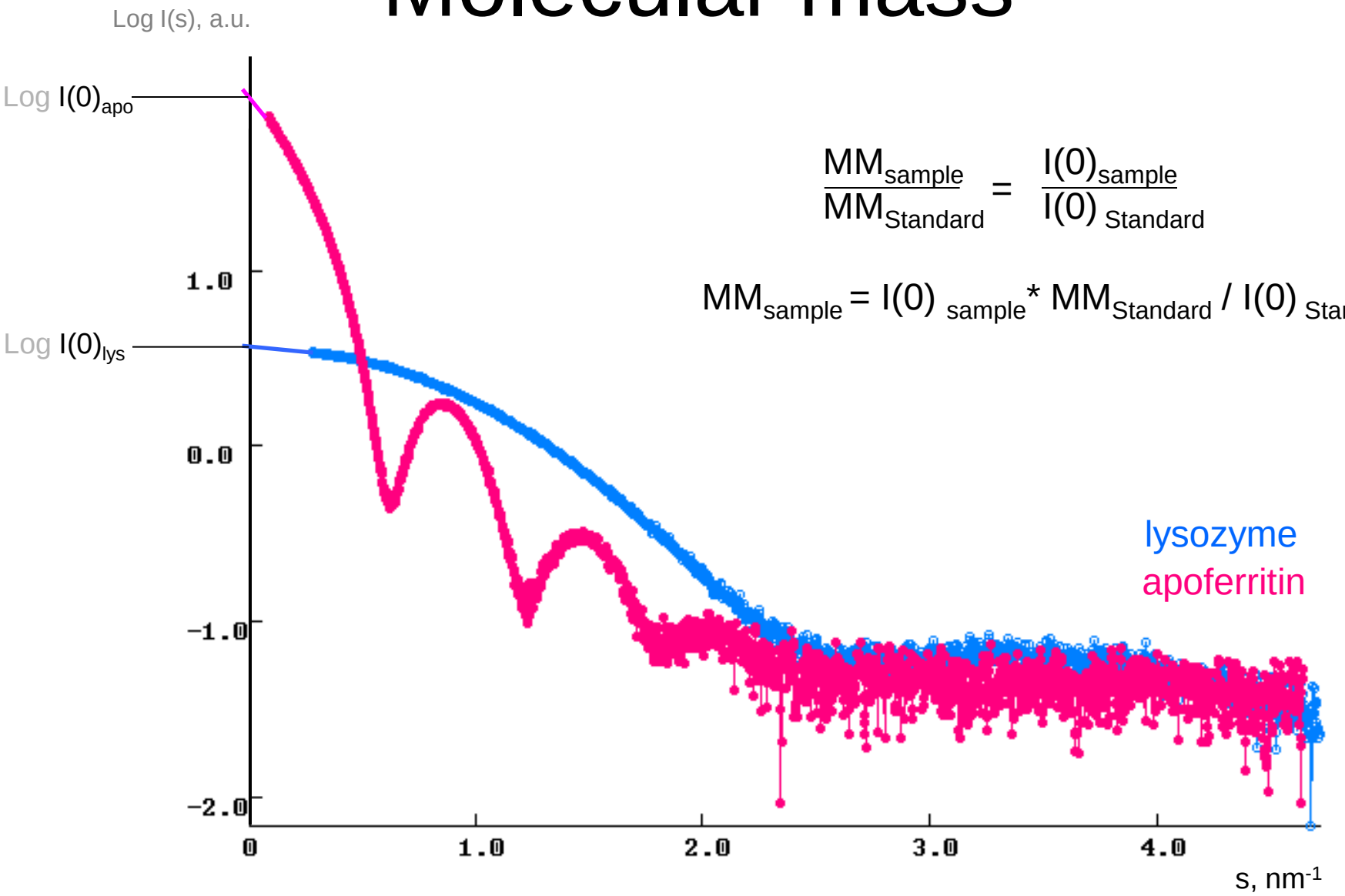
  Guinier approximation:

  $$I(s) = I(0)\exp(-s^2 R_g^2/3)$$

  $$sR_g \lesssim 1.3$$

- Quality of the data
  - aggregation
  - polydispersity
  - improper background substraction
- Zero angle intensity *I(0)*
- First point to use

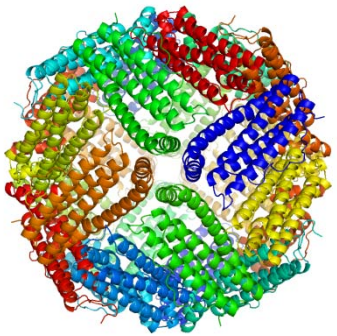
Molecular mass



$$\frac{MM_{sample}}{MM_{Standard}} = \frac{I(0)_{sample}}{I(0)_{Standard}}$$

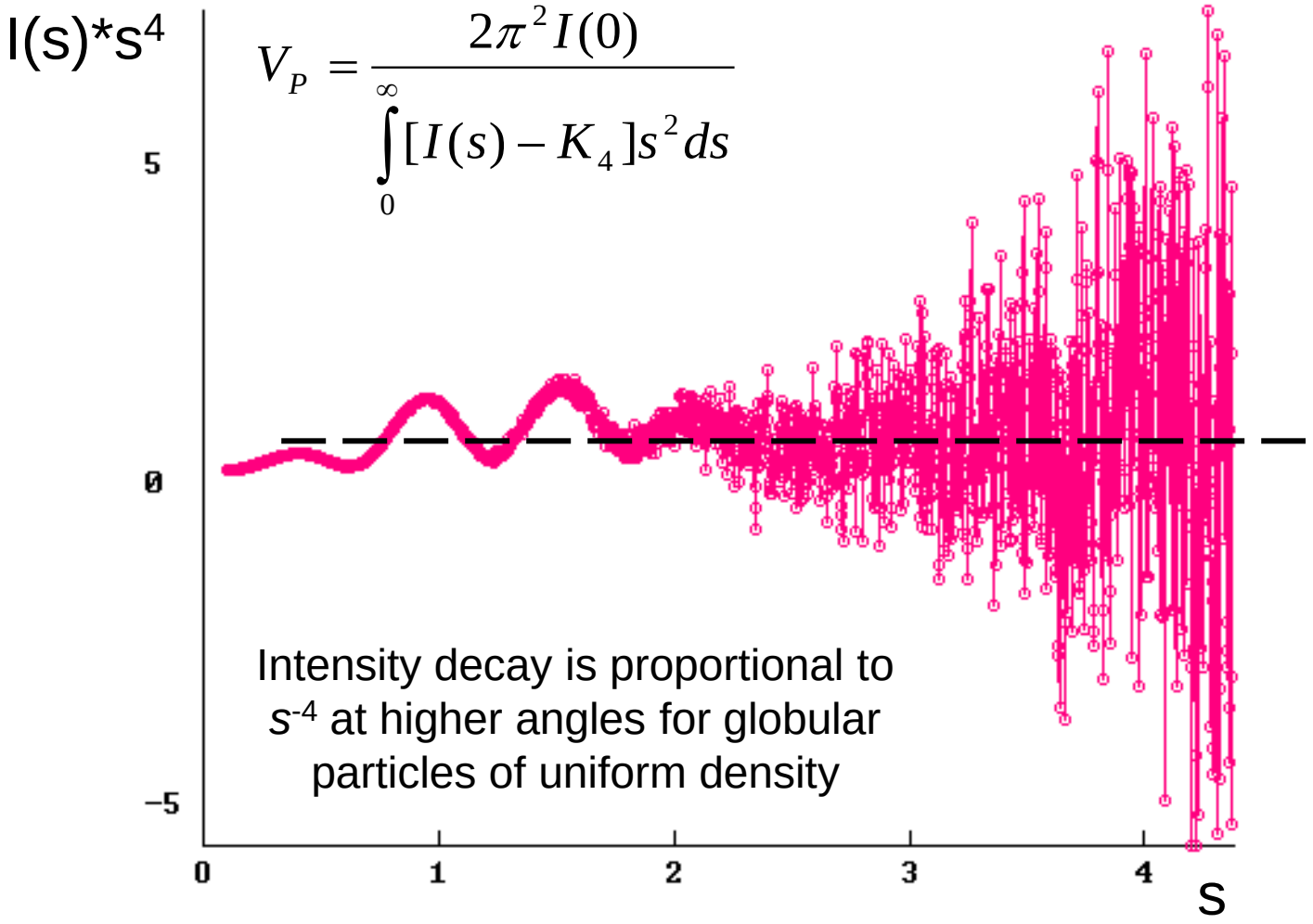
$$MM_{sample} = I(0)_{sample} * MM_{Standard} / I(0)_{Standard}$$

Porod plot, volume



Excluded volume of
the hydrated particle:

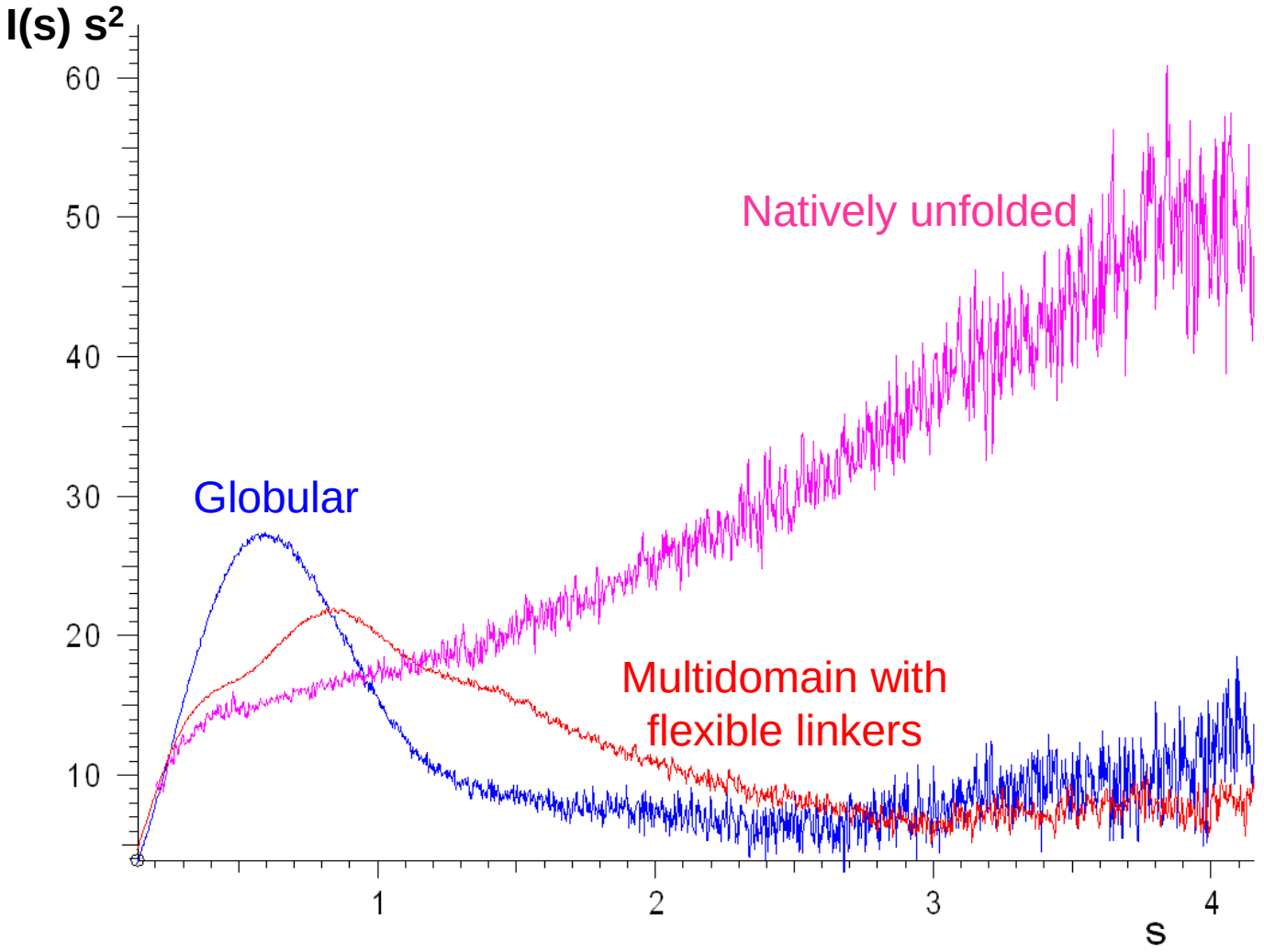
$$V_P = \frac{2\pi^2 I(0)}{\int_0^\infty [I(s) - K_4]s^2 ds}$$



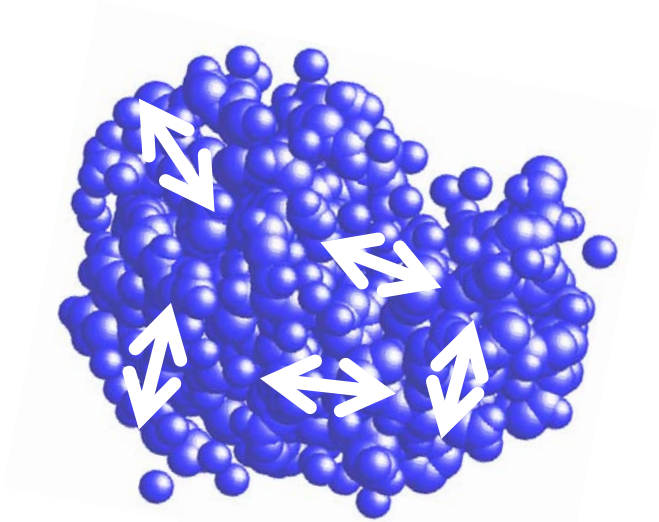
Intensity decay is proportional to s^{-4} at higher angles for globular particles of uniform density

Kratky plot

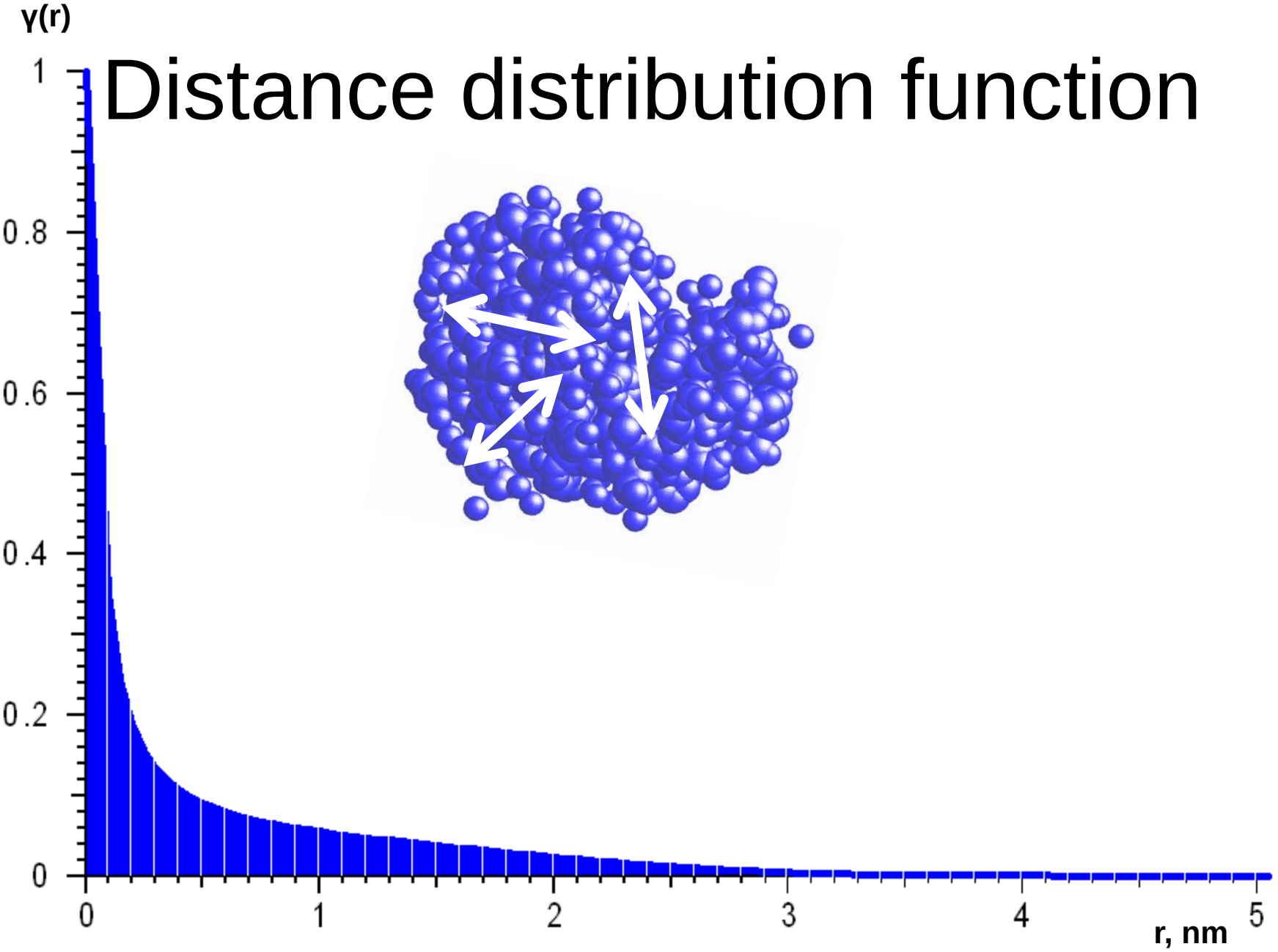
Patterns of globular and flexible proteins



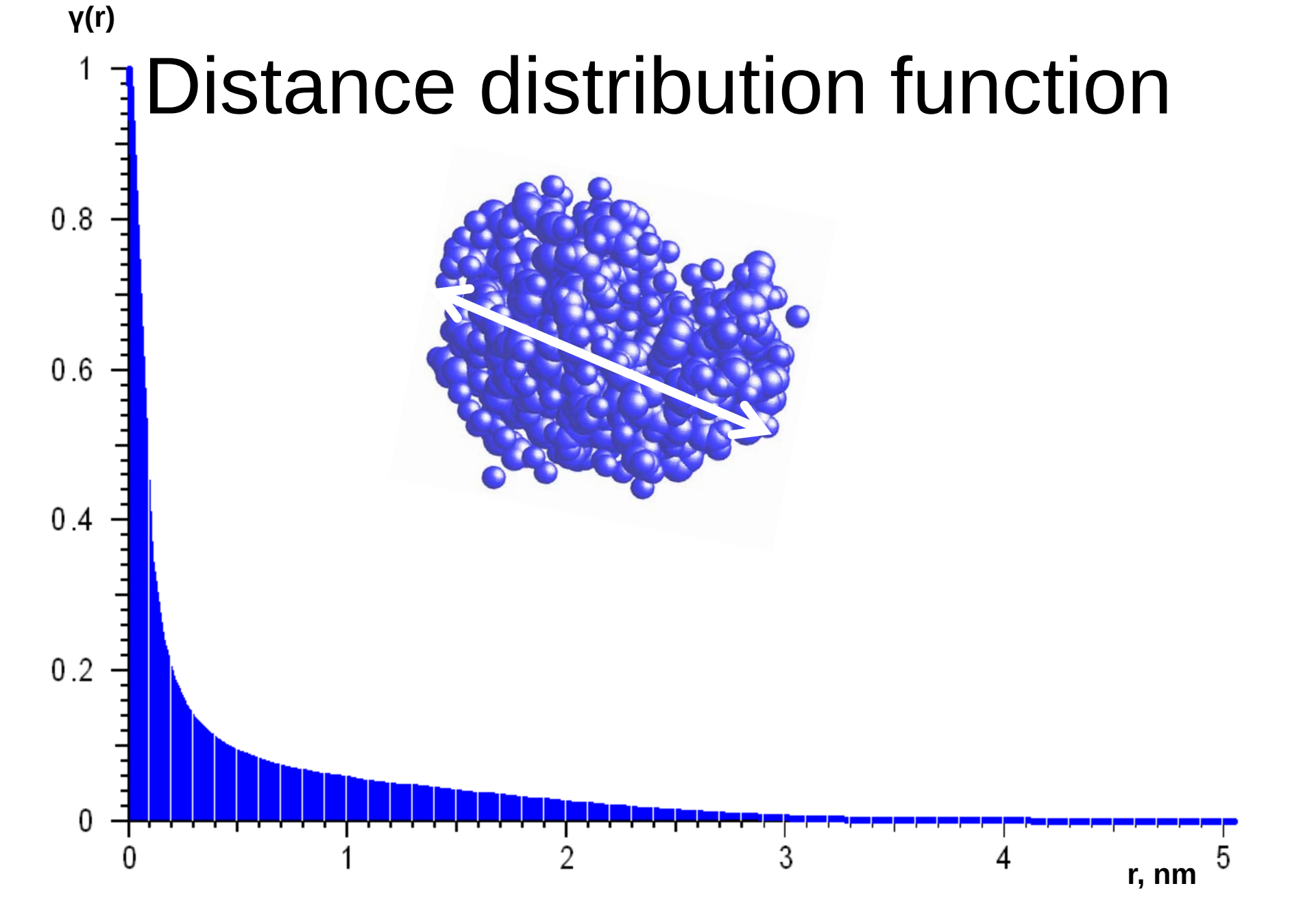
Distance distribution function



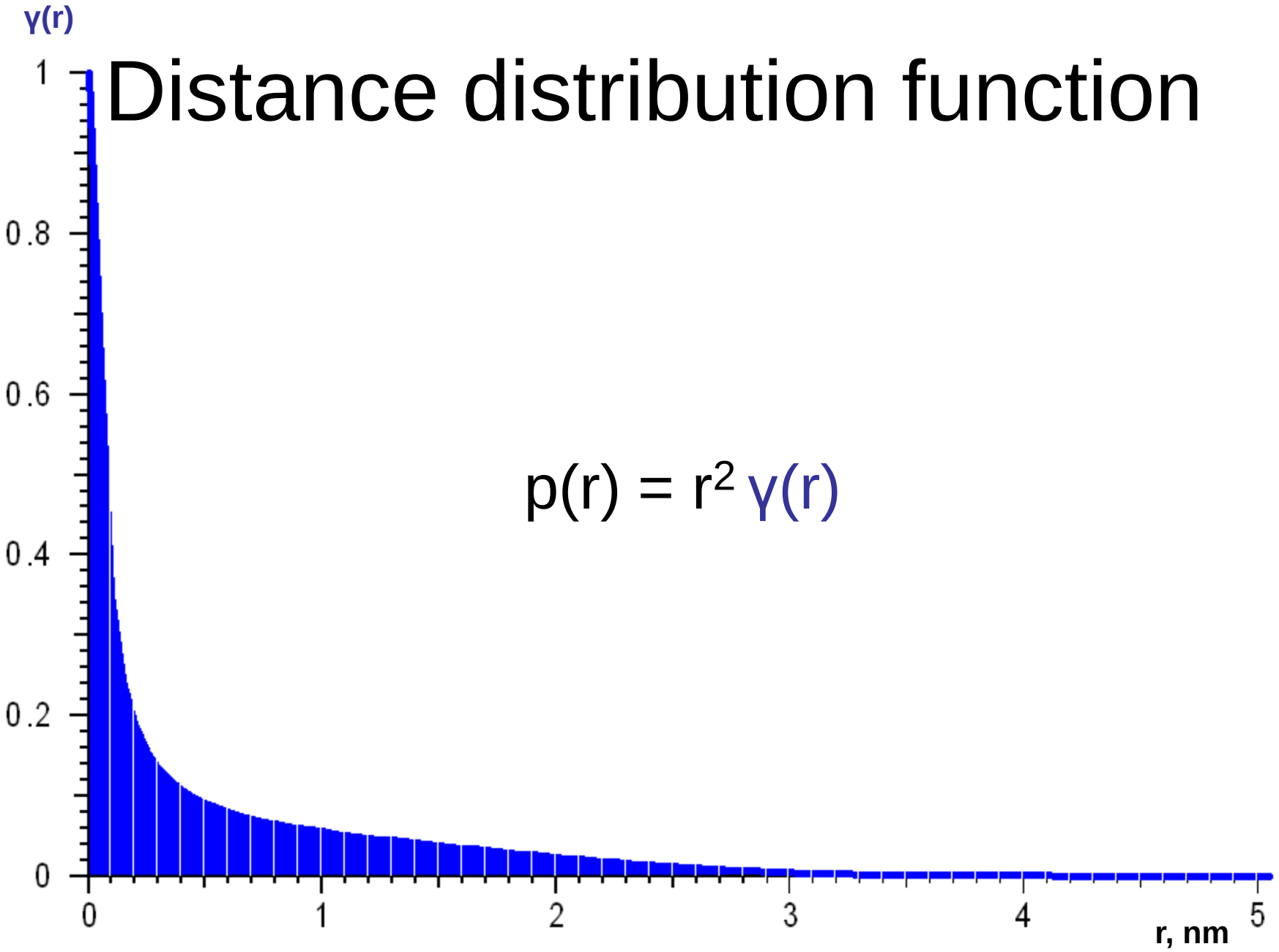
Distance distribution function



Distance distribution function

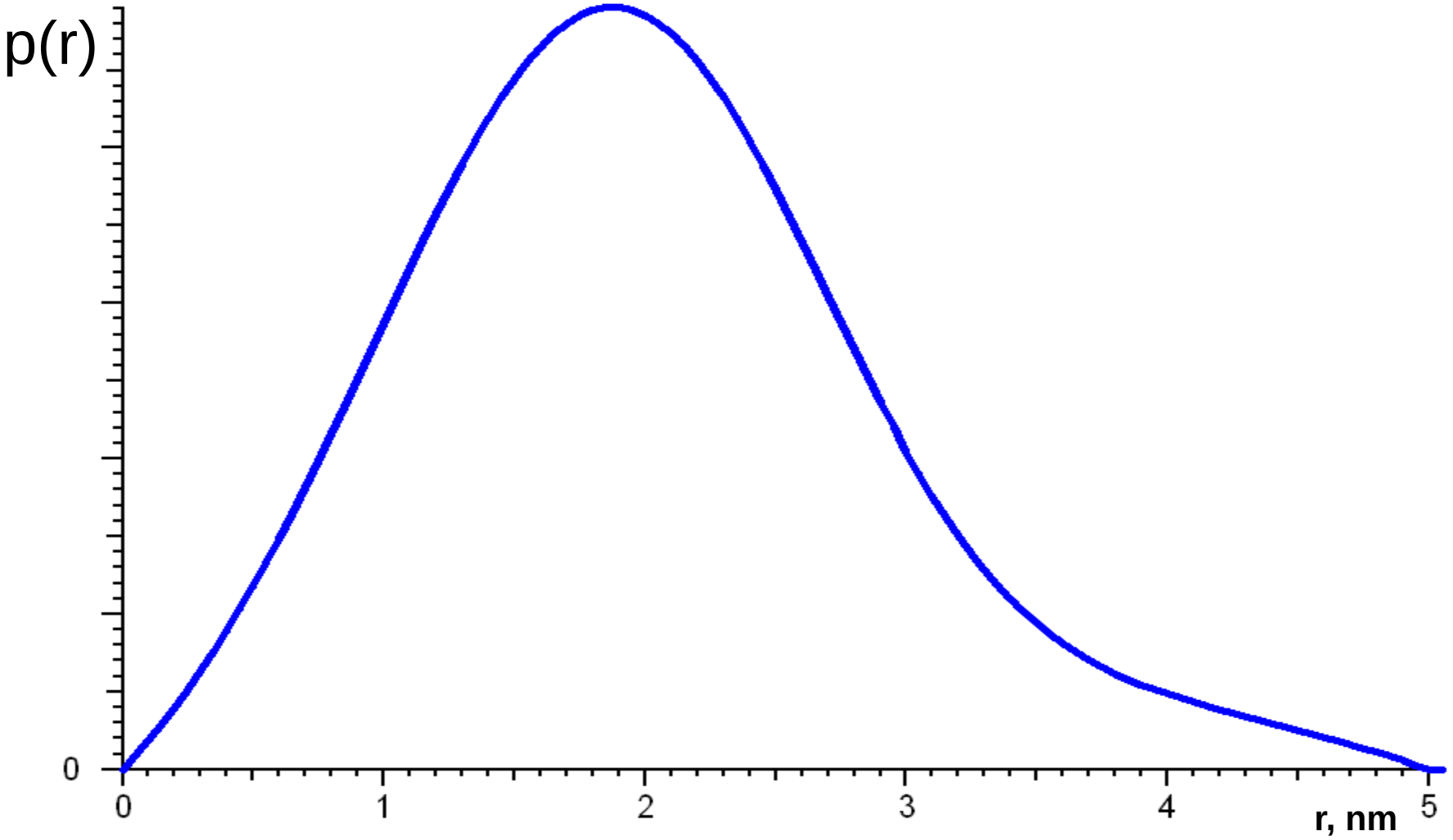


Distance distribution function

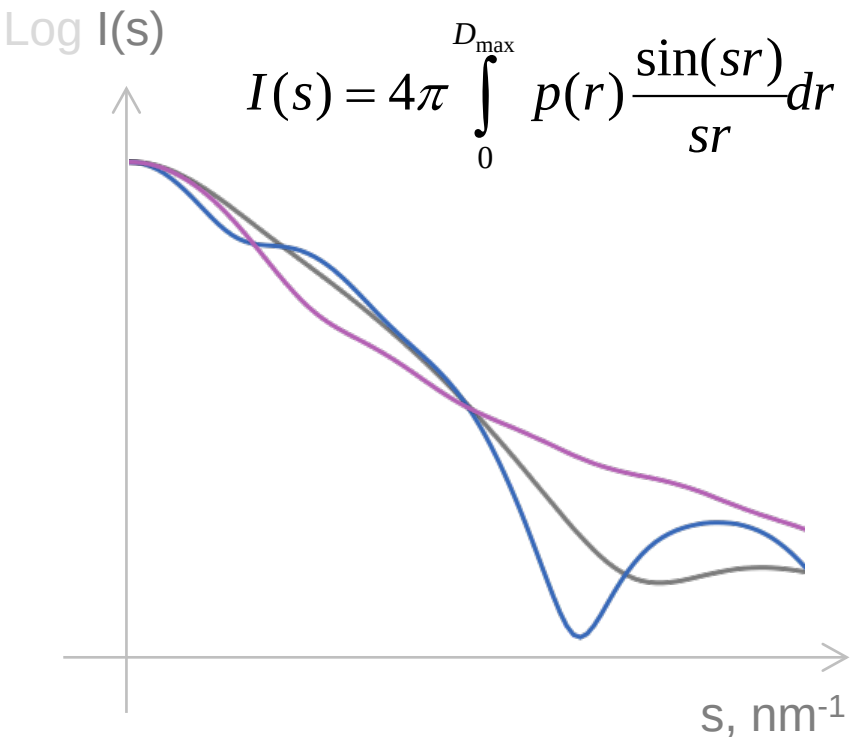


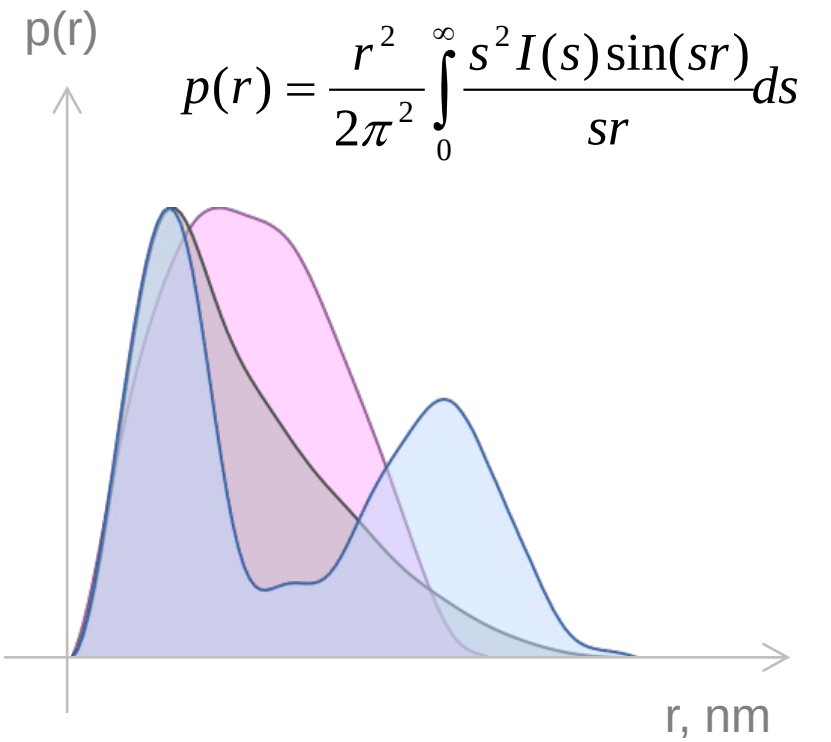
$$p(r) = r^2 \gamma(r)$$

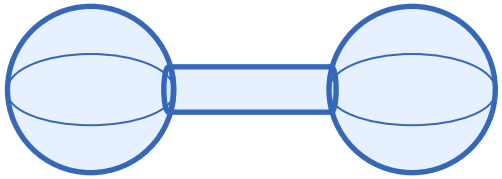
Distance distribution function

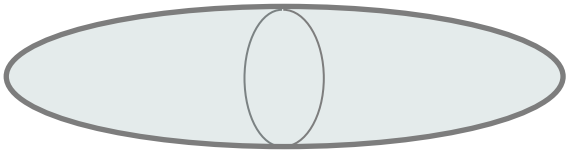


Distance distribution function



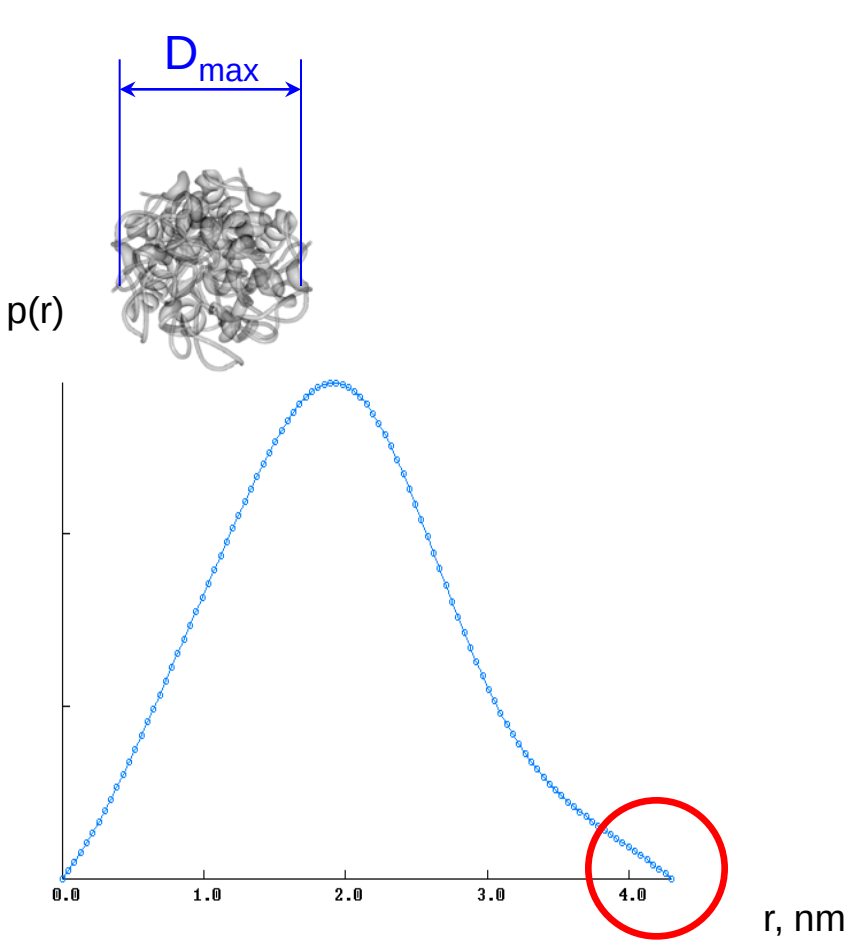


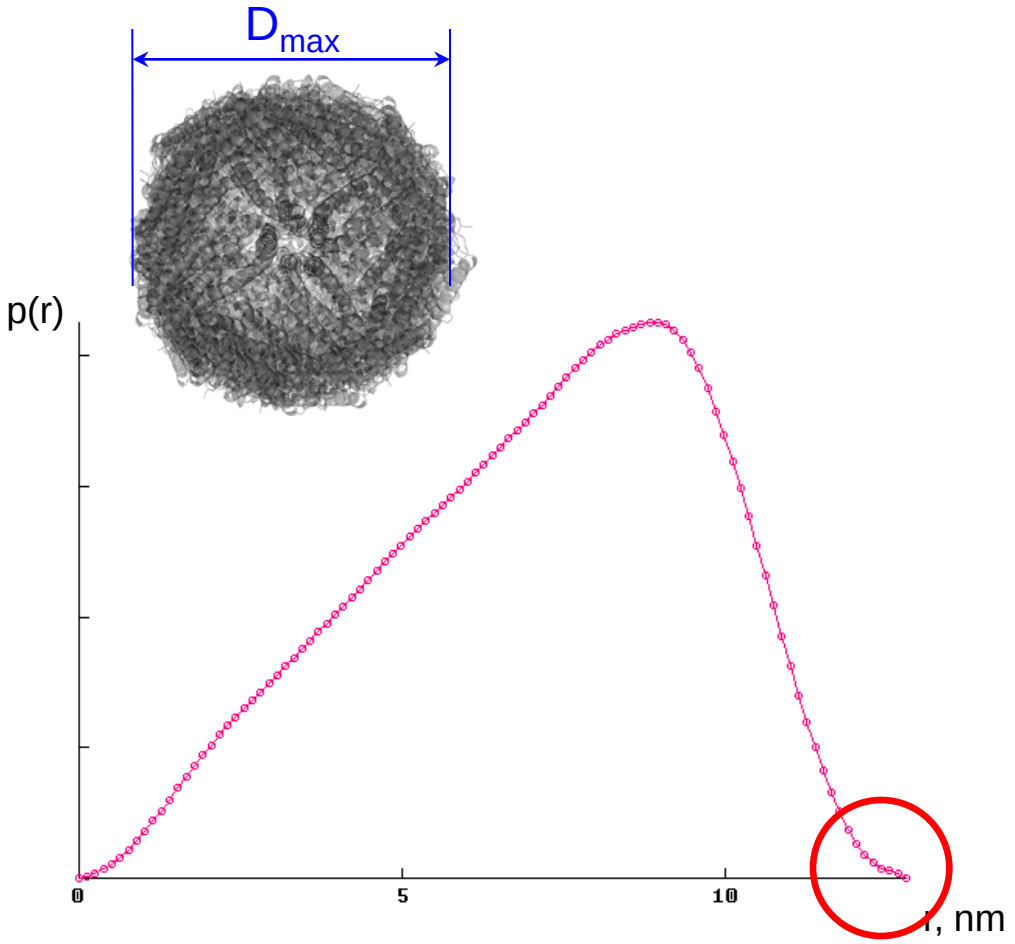




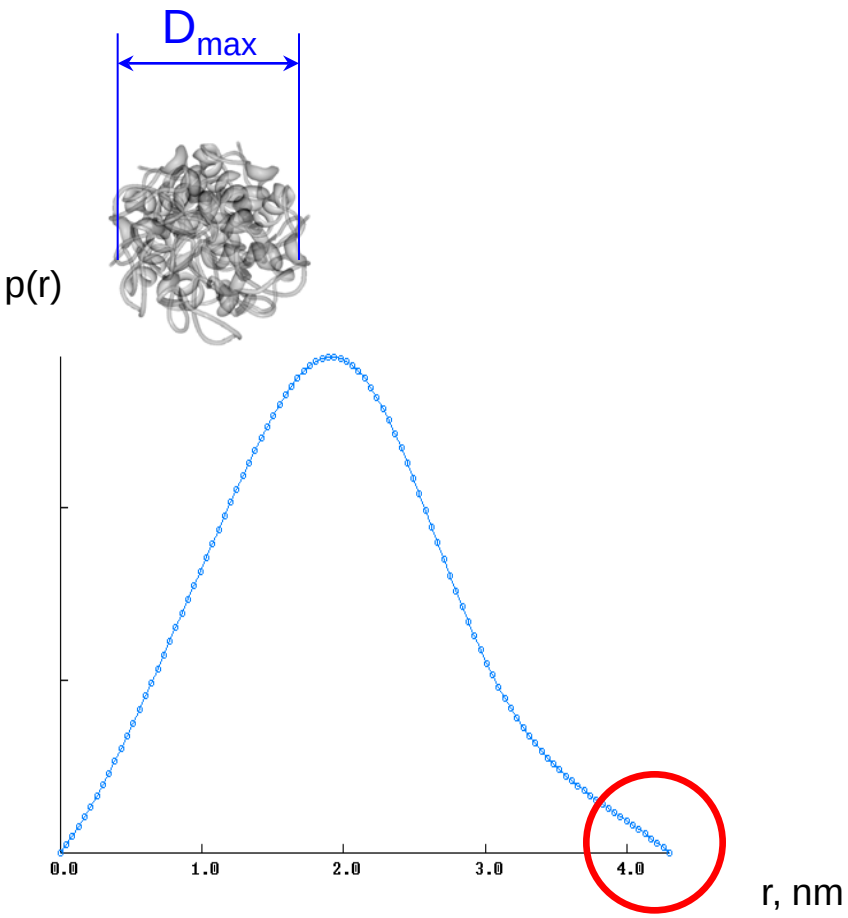
p(r) plot

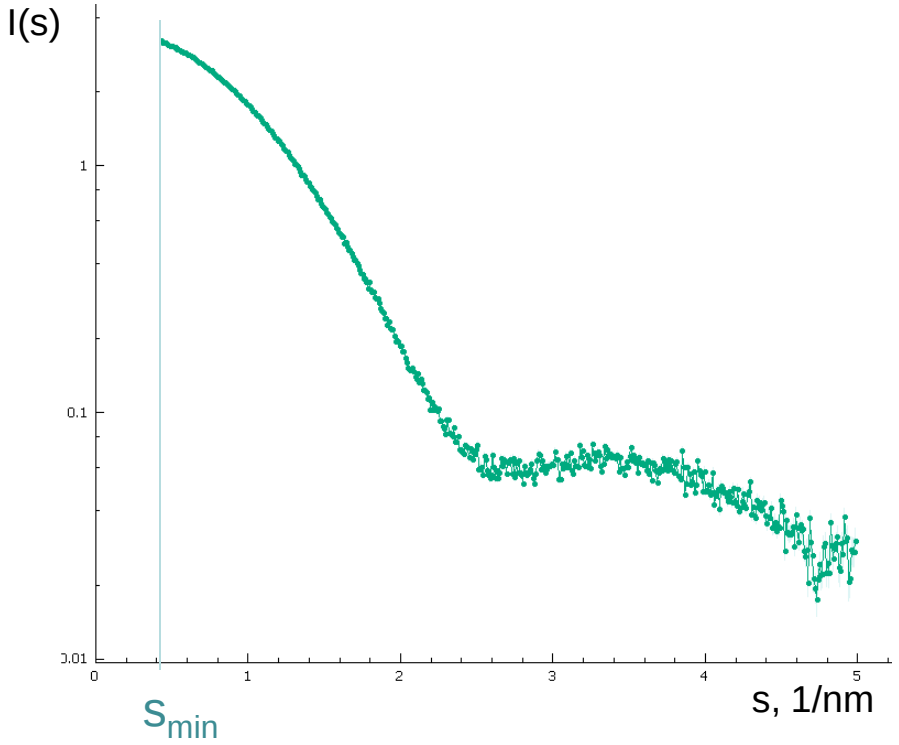
Distance distribution function



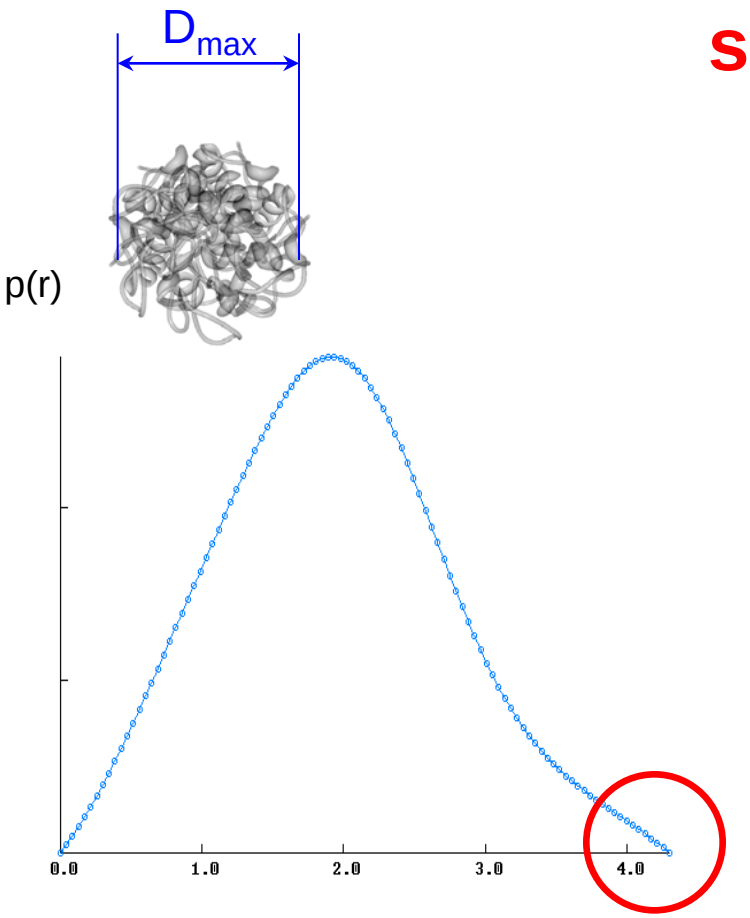


Data quality

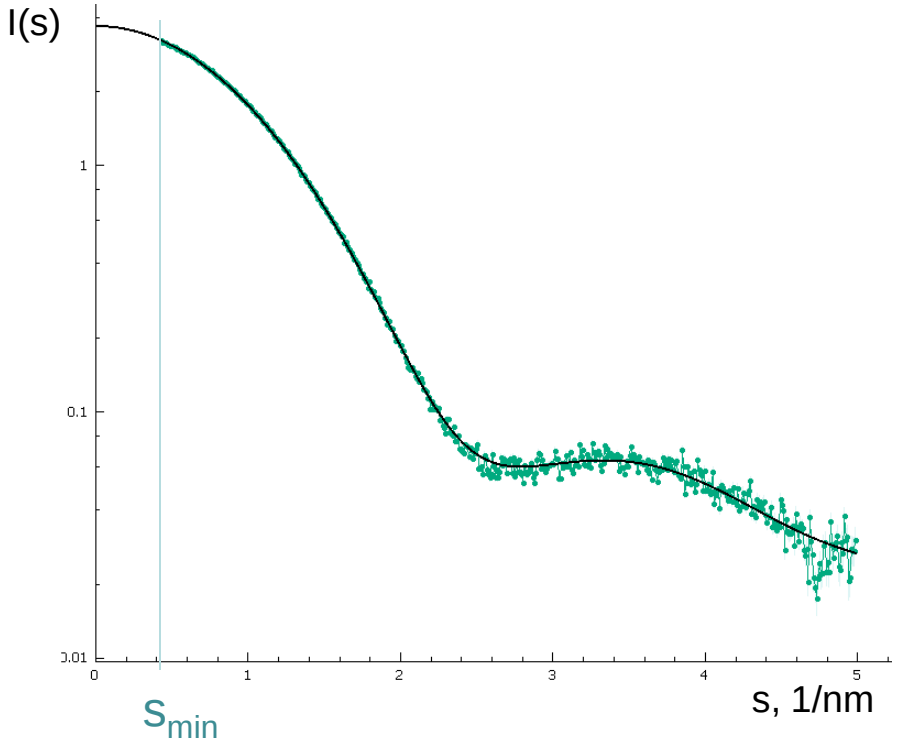




Data quality



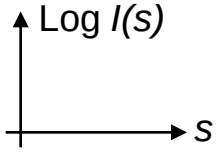
$$s_{min} \leq \pi / D_{max}$$



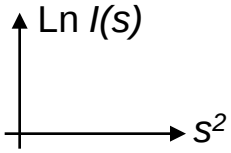
Summary

- Exposure 3D → 2D
- Radial averaging → 1D
- Normalization
- Background subtraction
- Analysis

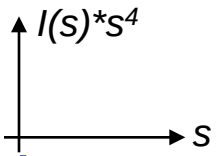
- Log plot



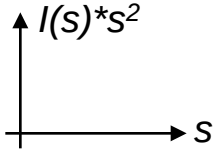
- Guinier plot (R_g, MM)



- Porod plot



- Kratky plot (flexibility)



- p(r) plot