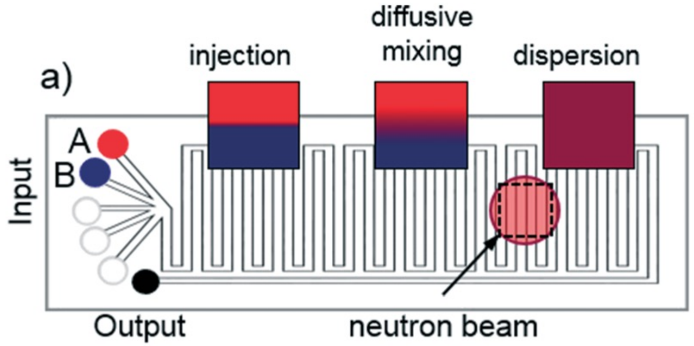
Introduction to small-angle scattering

JUDITH HOUSTON
INSTRUMENT SCIENTIST @ THE ESS

2024-03-11

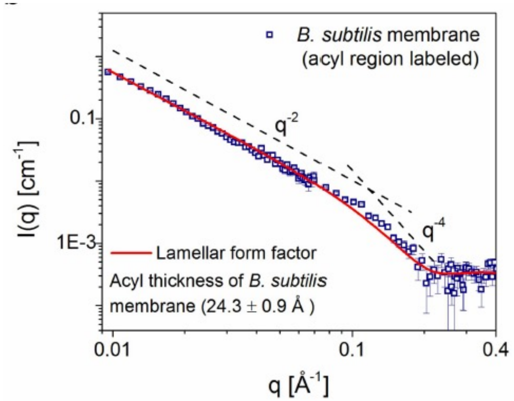
LoKI : SANS for soft matter, materials & bioscience

Microfluidic SANS:
High Throughput Mixing & Tailored Flow Geometry



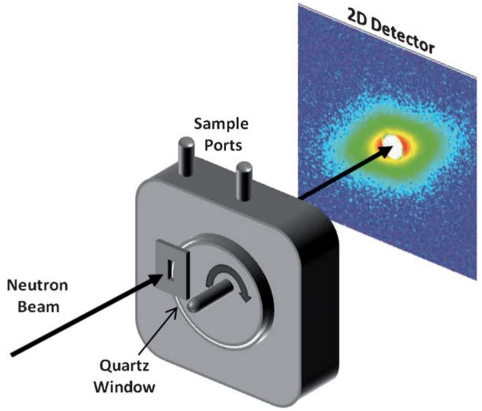
Lab Chip, 2017, **17**, 1559

Biological Samples:
Weak Scatterers & Dilute Solutions



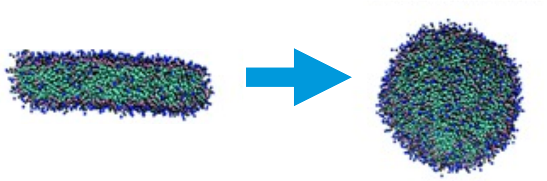
PLoS Bio, 2017, **15**, e2002214

Rheo-SANS:
Structures Under Shear



Soft Matter, 2011, **7**, 9992

Non-Equilibrium Studies:
Self-Assembly & Kinetics



Colloid Polym Sci, 2010, **288**, 827

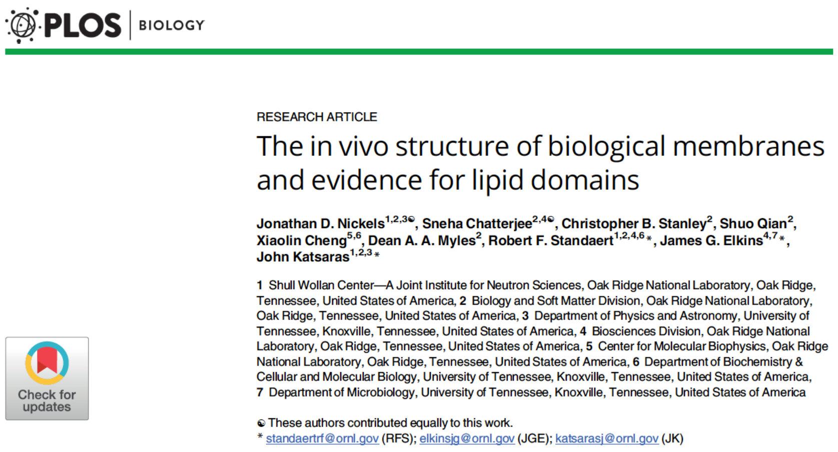
ABILITIES:
- Small beams for flow-through, scanning & microfluidic experiments.
- Perform "single-shot" kinetic measurements on sub-second timescales.
- Investigate multiple length scale systems (simultaneously 0.5-300 nm)
- High throughput of regular SANS measurements

→ **high flux & wide simultaneous size range**.

1

Solving life science problems with neutron tools…

Weakly scattering bio-engineered samples



PLOS | BIOLOGY

RESEARCH ARTICLE

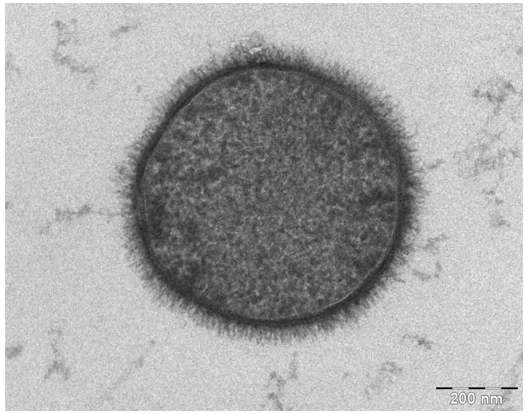
The in vivo structure of biological membranes and evidence for lipid domains

Jonathan D. Nickels[1,2,3⊙], Sneha Chatterjee[2,4⊙], Christopher B. Stanley[2], Shuo Qian[2], Xiaolin Cheng[5,6], Dean A. A. Myles[2], Robert F. Standaert[1,2,4,6]*, James G. Elkins[4,7]*, John Katsaras[1,2,3]*

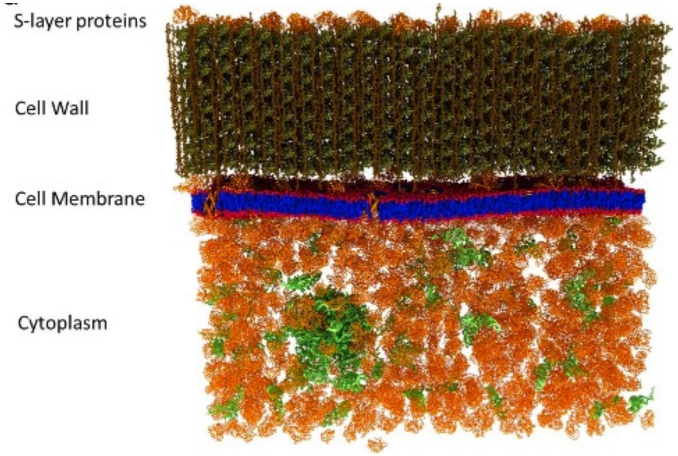
1 Shull Wollan Center—A Joint Institute for Neutron Sciences, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States of America, 2 Biology and Soft Matter Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States of America, 3 Department of Physics and Astronomy, University of Tennessee, Knoxville, Tennessee, United States of America, 4 Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States of America, 5 Center for Molecular Biophysics, Oak Ridge National Laboratory, Oak Ridge, Tennessee, United States of America, 6 Department of Biochemistry & Cellular and Molecular Biology, University of Tennessee, Knoxville, Tennessee, United States of America, 7 Department of Microbiology, University of Tennessee, Knoxville, Tennessee, United States of America

⊙ These authors contributed equally to this work.
* standaertrf@ornl.gov (RFS); elkinsjg@ornl.gov (JGE); katsarasj@ornl.gov (JK)

Check for updates

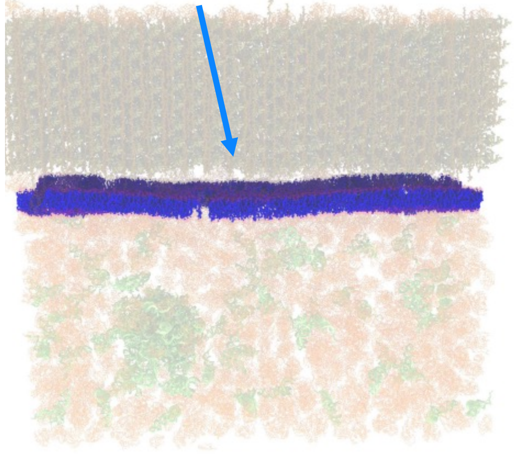


Normal cell components: everything is visible...

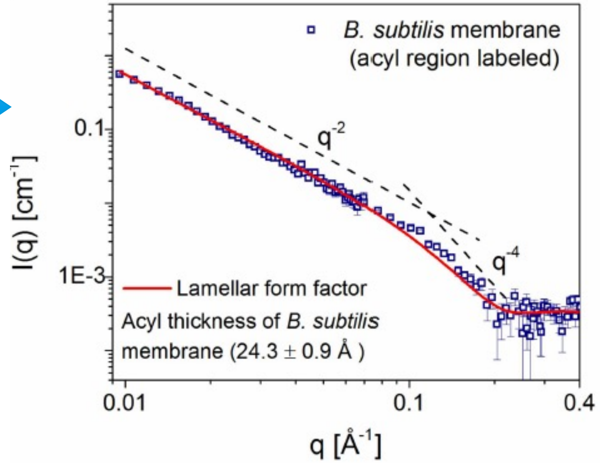


After labelling with specific amounts of H and D

What the neutron beam sees...



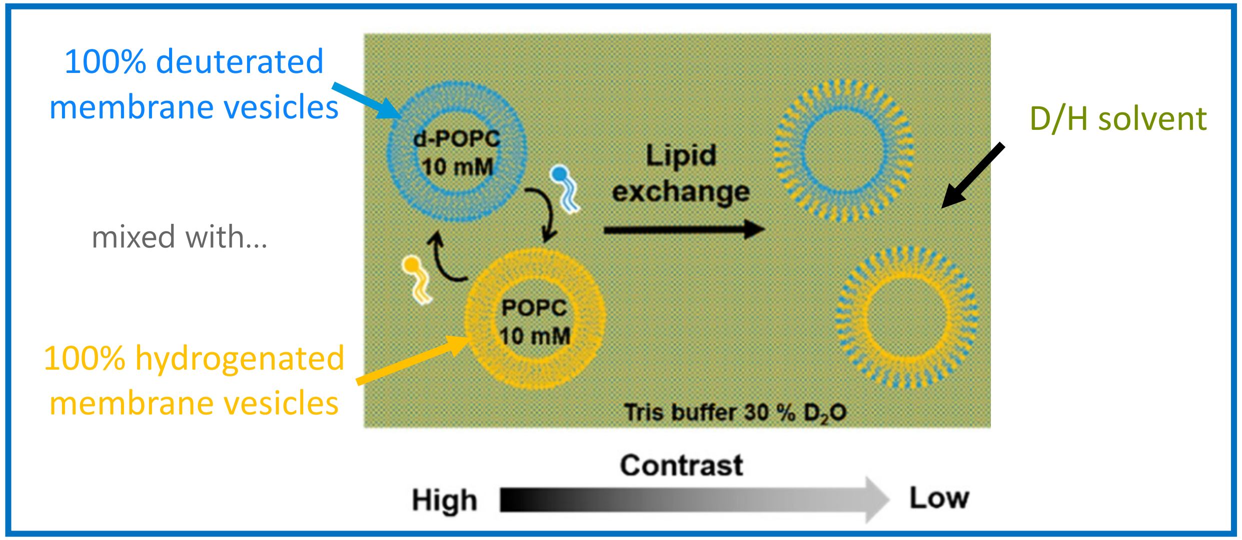
Indirect evidence of cell membrane structure!



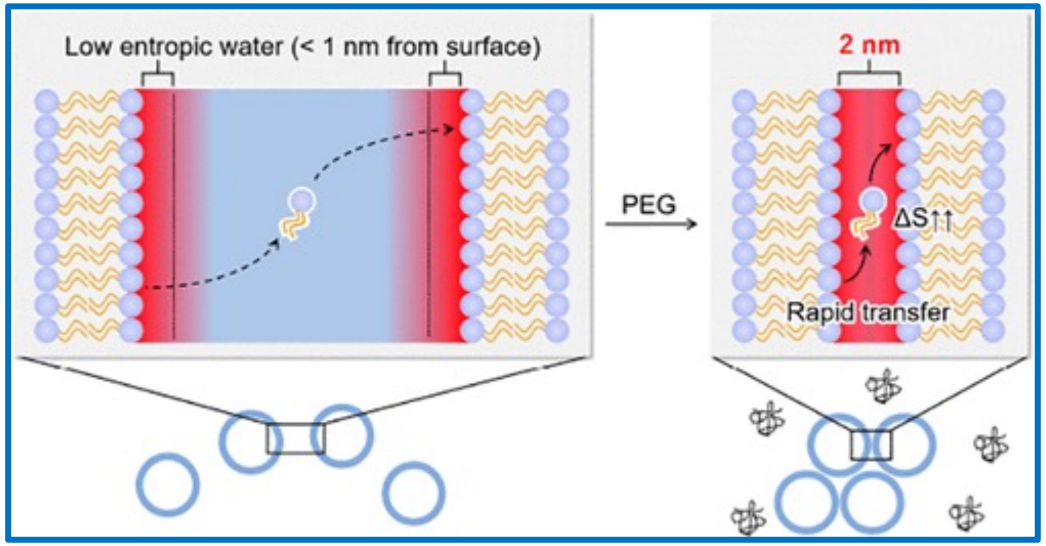
Probing membrane dynamics

Understanding membrane biofusion

The experiment



The result

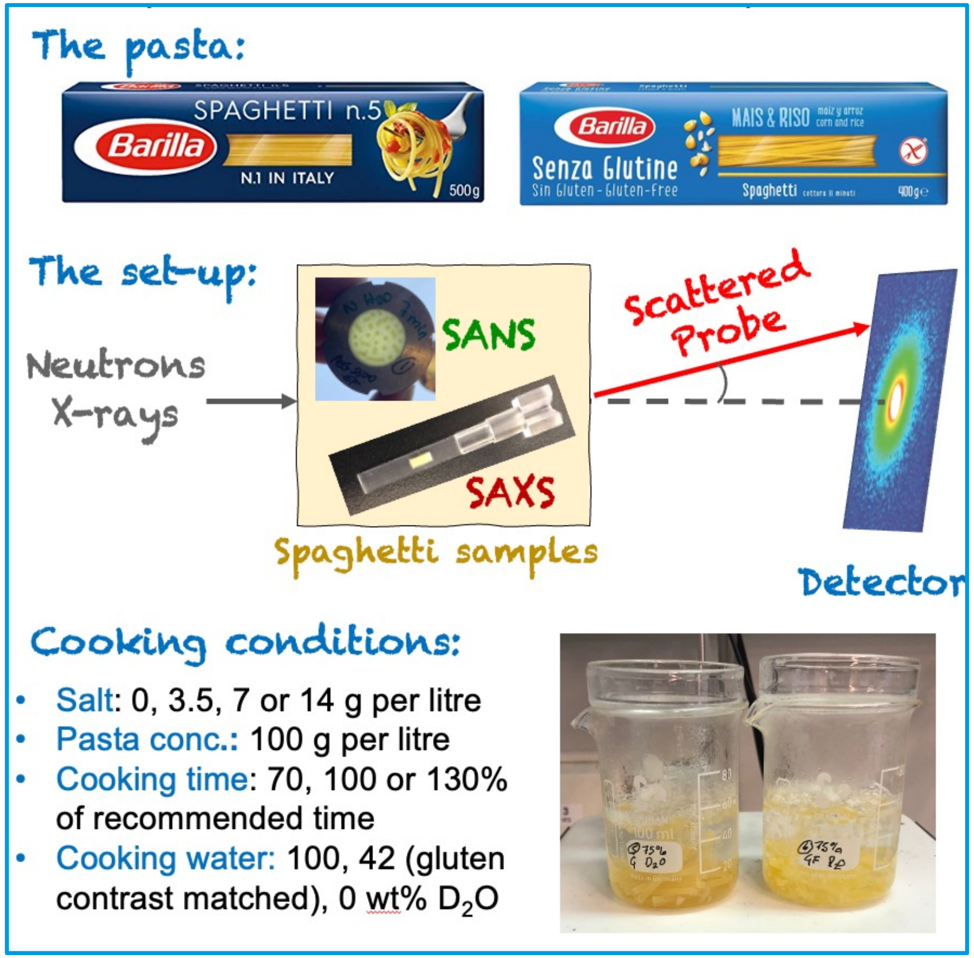


- Lipid exchange between the outer leaflets of the vesicles decreases the difference in contrast
- Rate of decrease in contrast directly related to rate of exchange

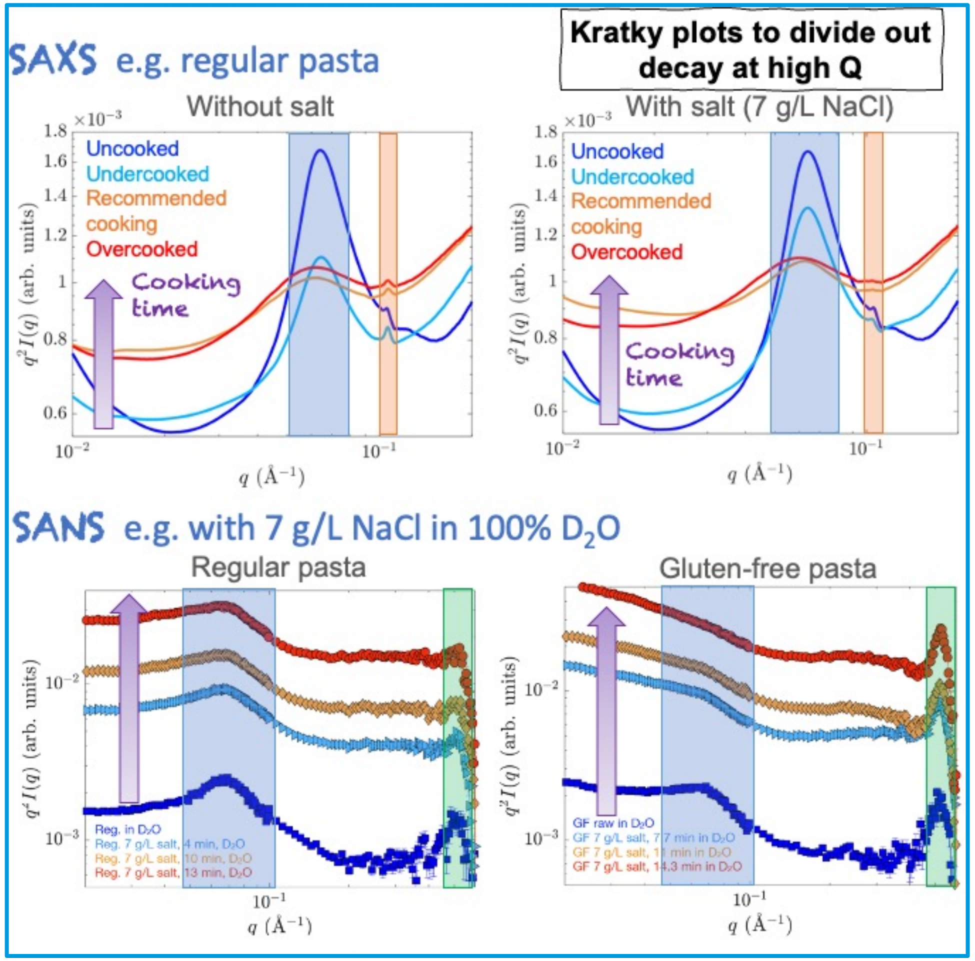
J. Phys. Chem. Lett. 2023, 14, 18, 4172

Gluten vs. gluten-free pasta varieties

The experiment



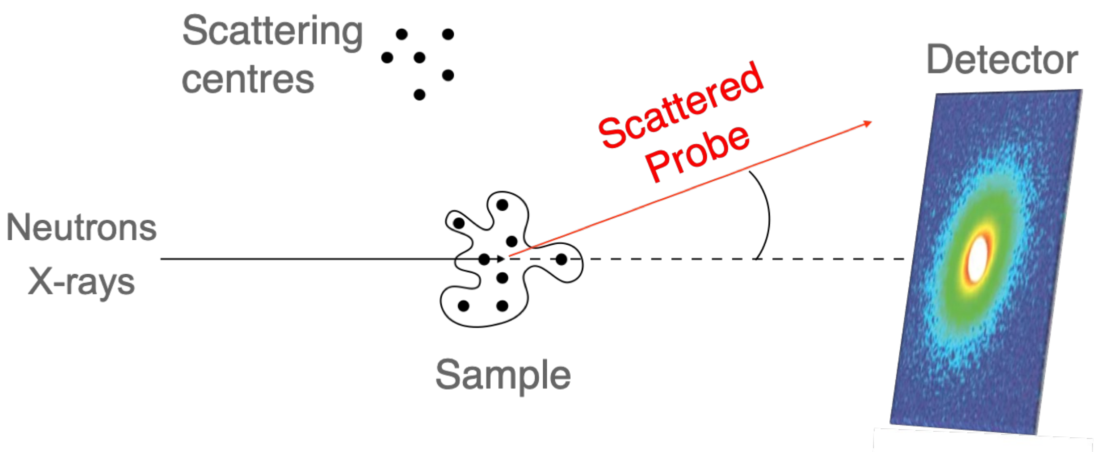
The result



J. E. Houston & A. Scotti, manuscript in preparation

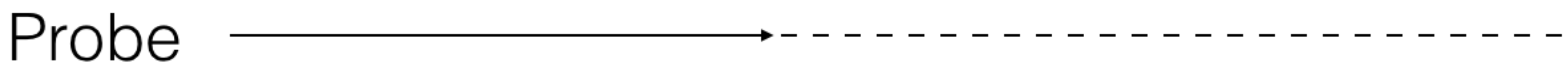
2

Small-angle scattering and How does it work?



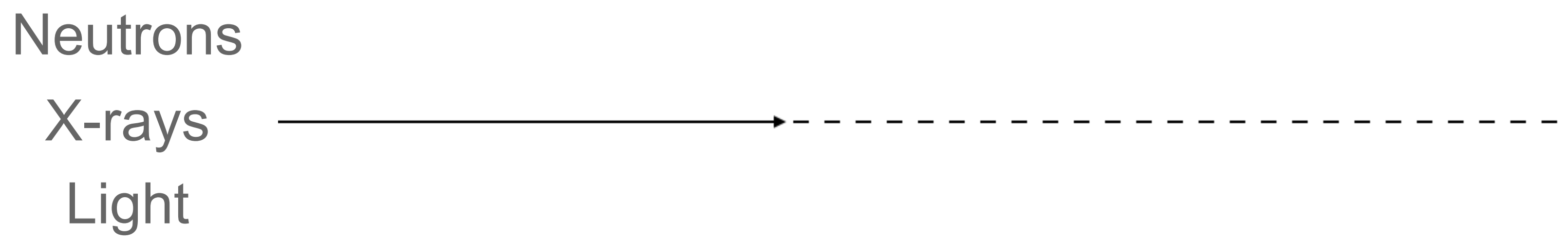
Small-angle scattering

Experimental technique which uses **elastic scattering** at **small angles** to investigate the structure of substances at a mesoscopic scale of ~1–200 nm



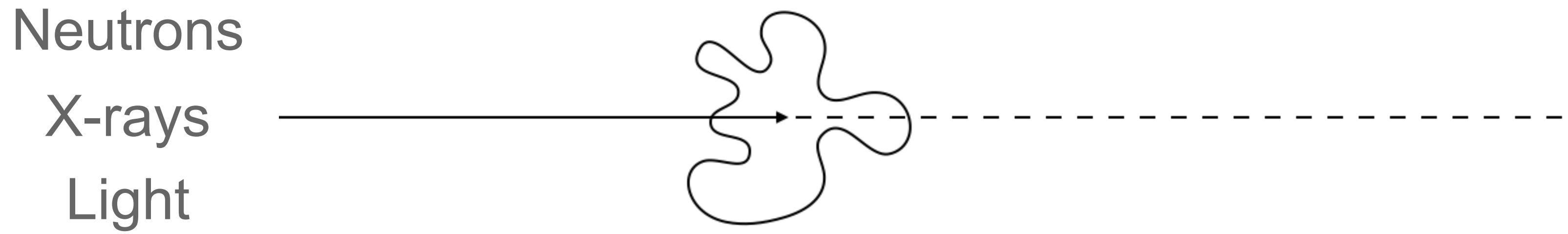
Small-angle scattering

Experimental technique which uses **elastic scattering** at **small angles** to investigate the structure of substances at a mesoscopic scale of ~1–200 nm



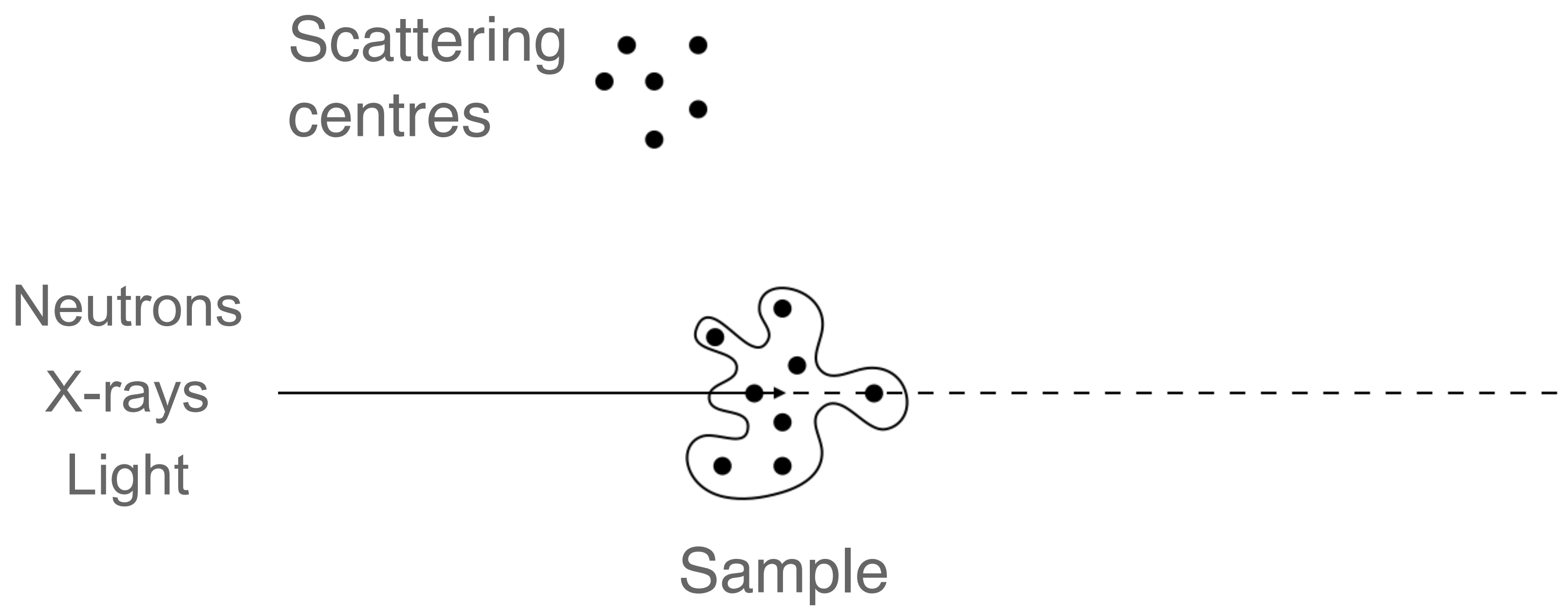
Small-angle scattering

Experimental technique which uses **elastic scattering** at **small angles** to investigate the structure of substances at a mesoscopic scale of ~1–200 nm



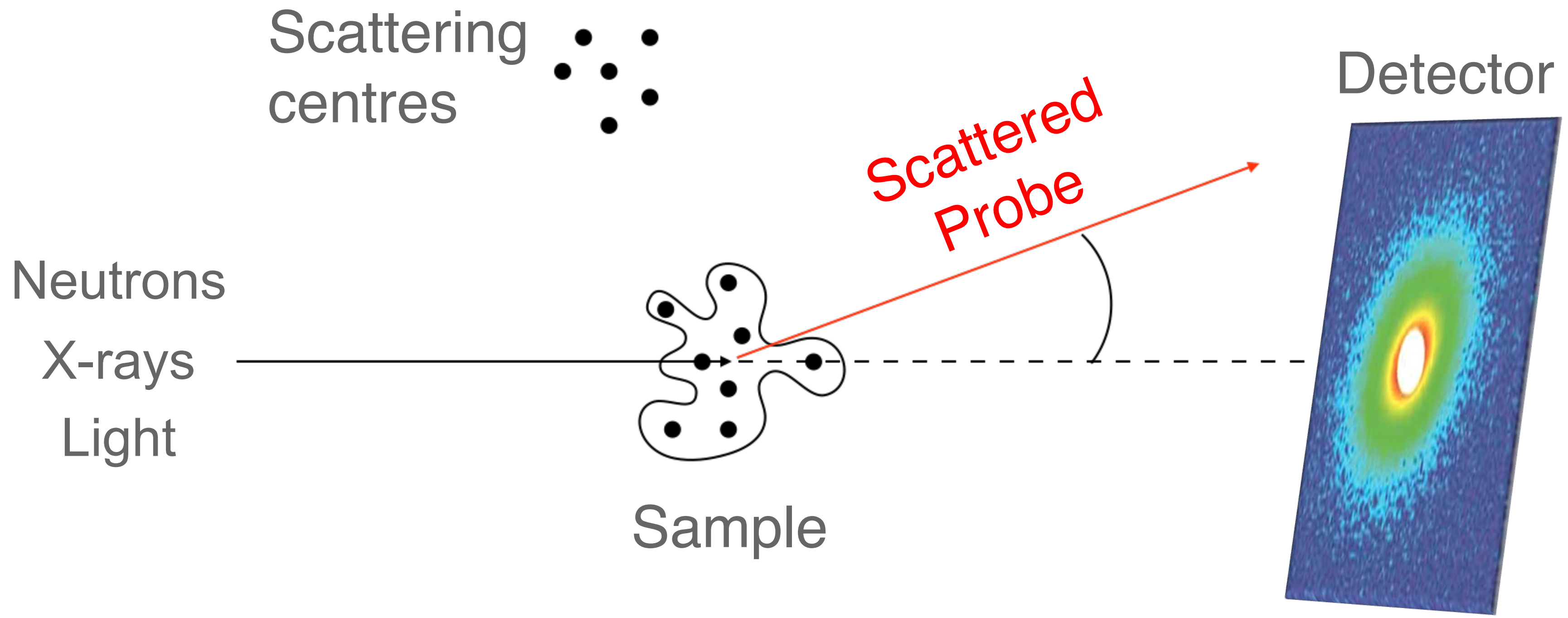
Small-angle scattering

Experimental technique which uses **elastic scattering** at **small angles** to investigate the structure of substances at a mesoscopic scale of ~1–200 nm

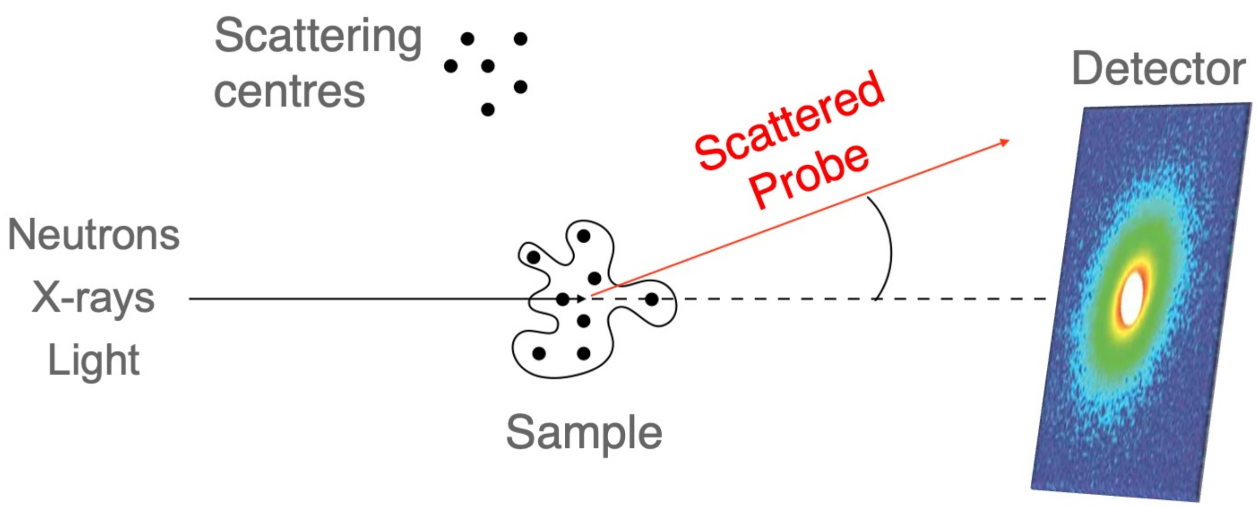


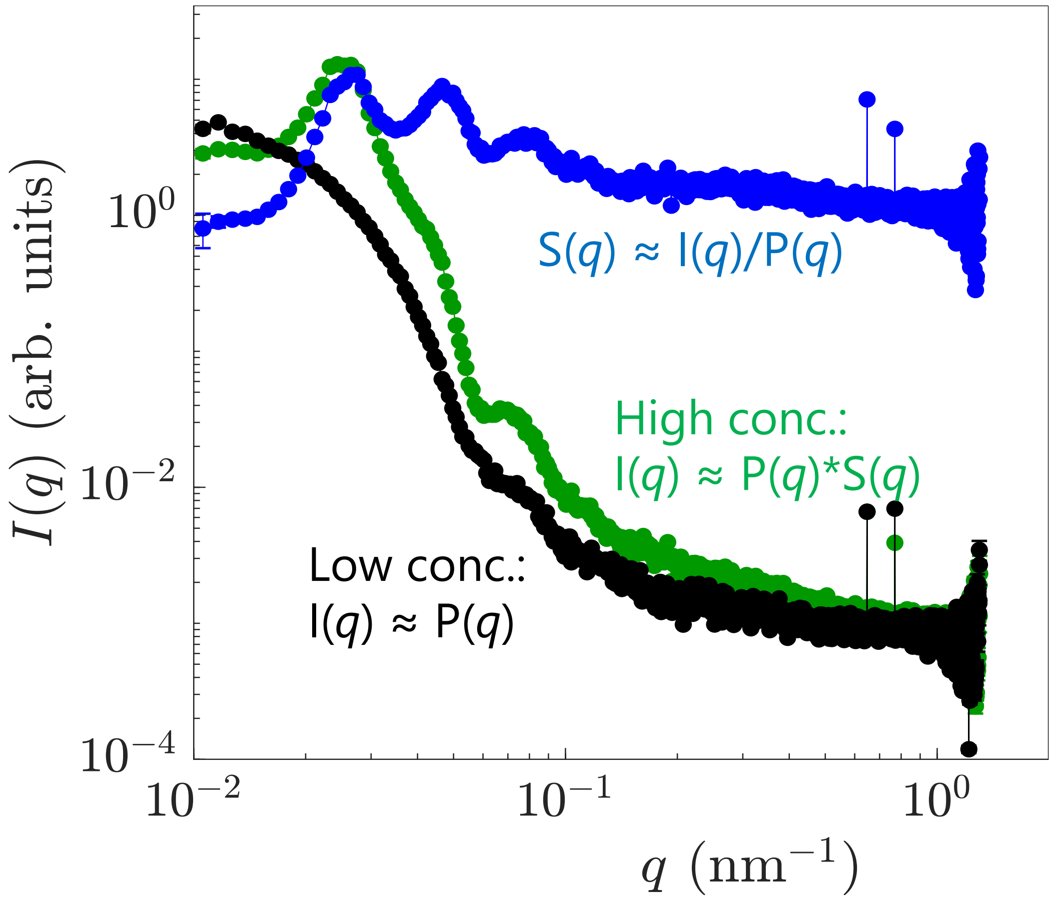
Small-angle scattering

Experimental technique which uses **elastic scattering** at **small angles** to investigate the structure of substances at a mesoscopic scale of ~1–200 nm

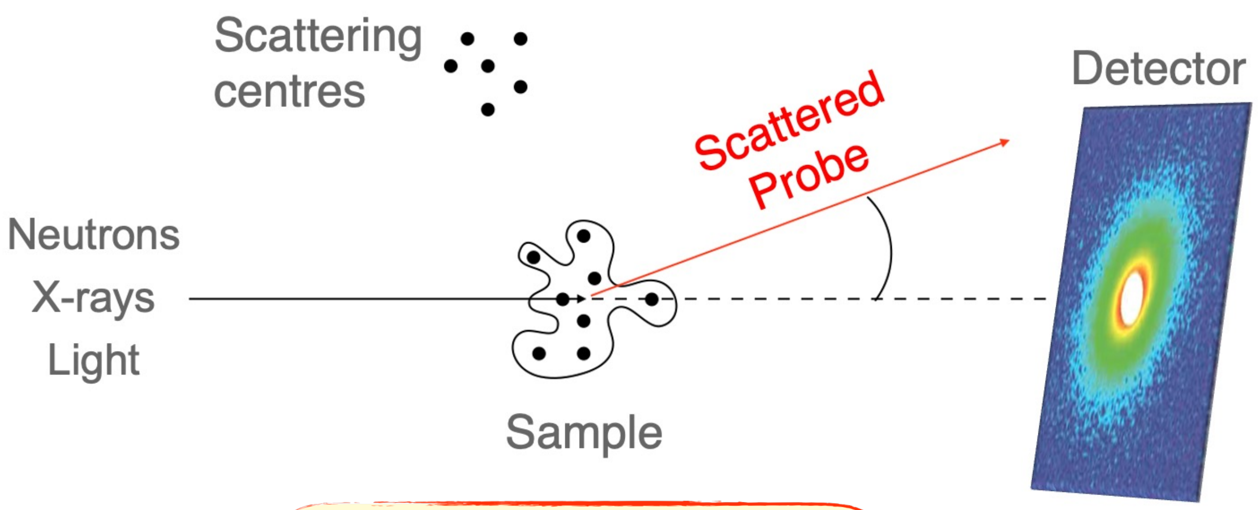


Small-angle scattering





Small-angle scattering



n : particle number density

$\Delta\rho$: **scattering contrast**

V : particle volume

$P(q)$: **particle form factor**

$S(q)$: **structure factor**

$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

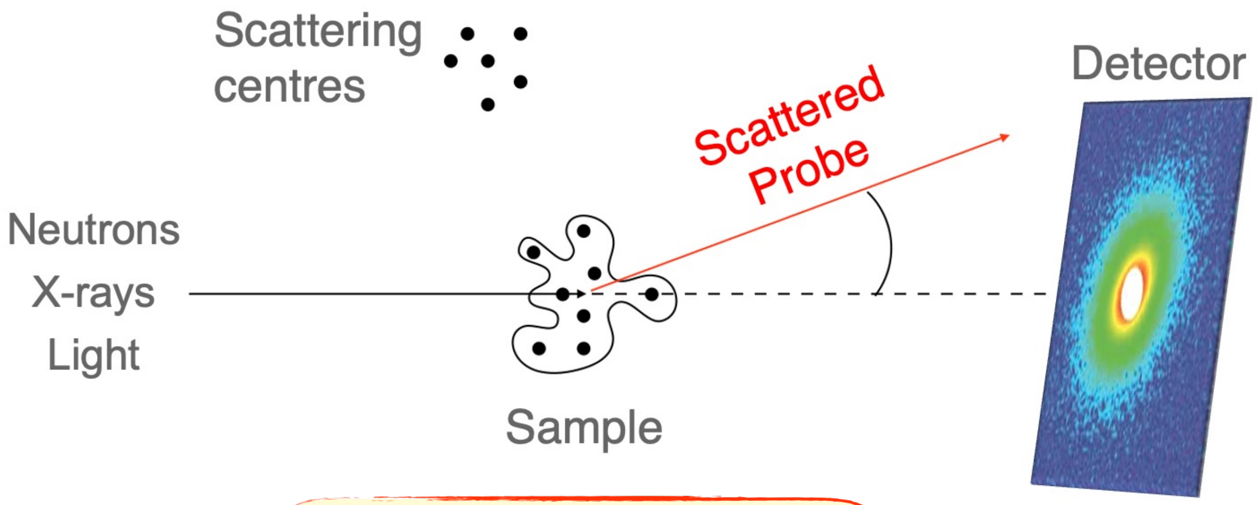
Scattering vector:

$$q = \frac{4\pi}{\lambda}\sin\left(\frac{\theta}{2}\right)$$

θ → Scattering angle

λ → Wavelength of the probe

Small-angle scattering

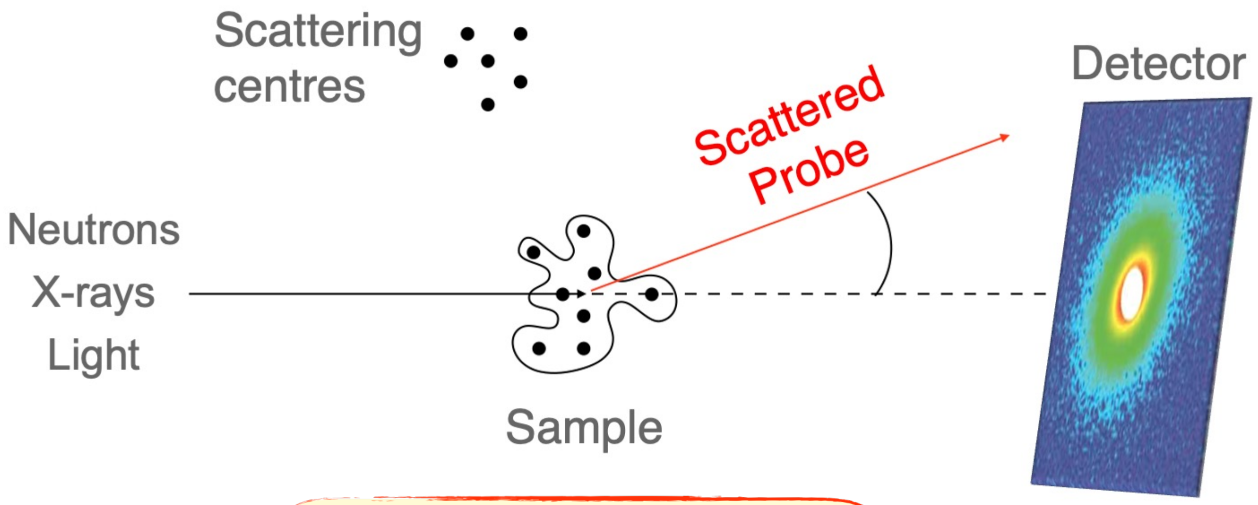


$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

- n : particle number density
- $\Delta\rho$: scattering contrast
- V : particle volume
- $P(q)$: particle form factor
- $S(q)$: structure factor

Form factor P(q): all information on the single scattering object (shape, architecture, size...)

Small-angle scattering



$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

n : particle number density

$\Delta\rho$: **scattering contrast**

V : particle volume

$P(q)$: **particle form factor**

$S(q)$: **structure factor**

Form factor P(q): all information on the single scattering object (shape, architecture, size...)

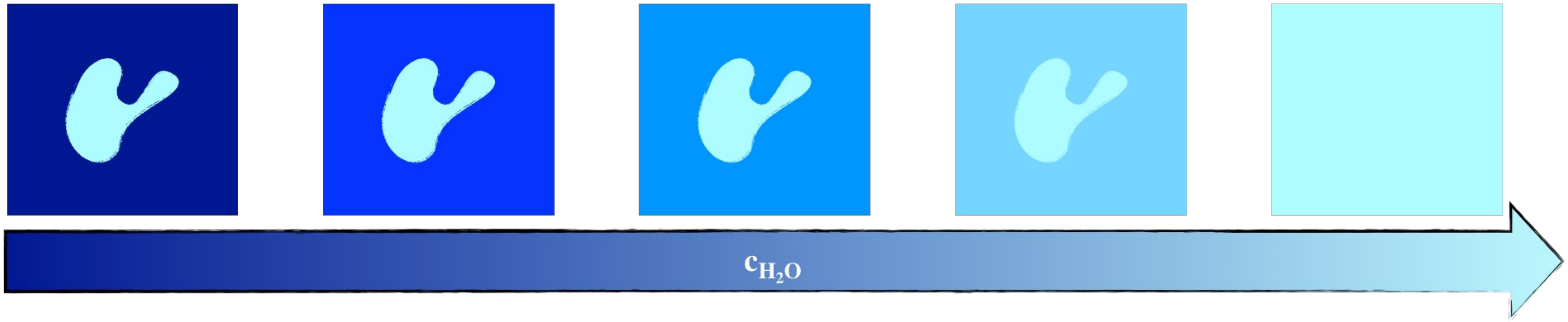
Structure factor S(q): all information on the arrangement in the sample (nearest neighbour distance, phase, crystalline lattice)

Scattering contrast, *Δρ*

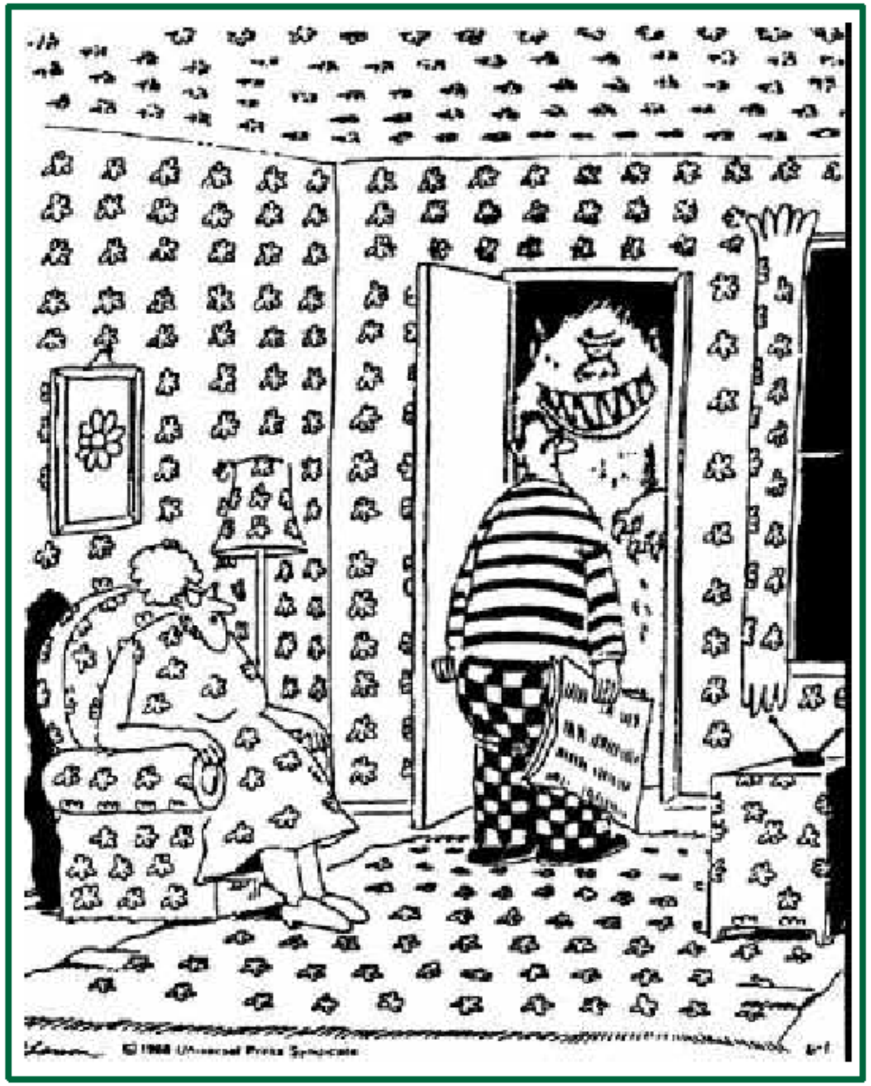
$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

$\Delta\rho$: difference in scattering length density between solvent and sample.

$$\Delta\rho = |\rho_{H_2O} - \rho_{SiO_2}|$$



Contrast matching



When the monster came, Lola remained undetected.

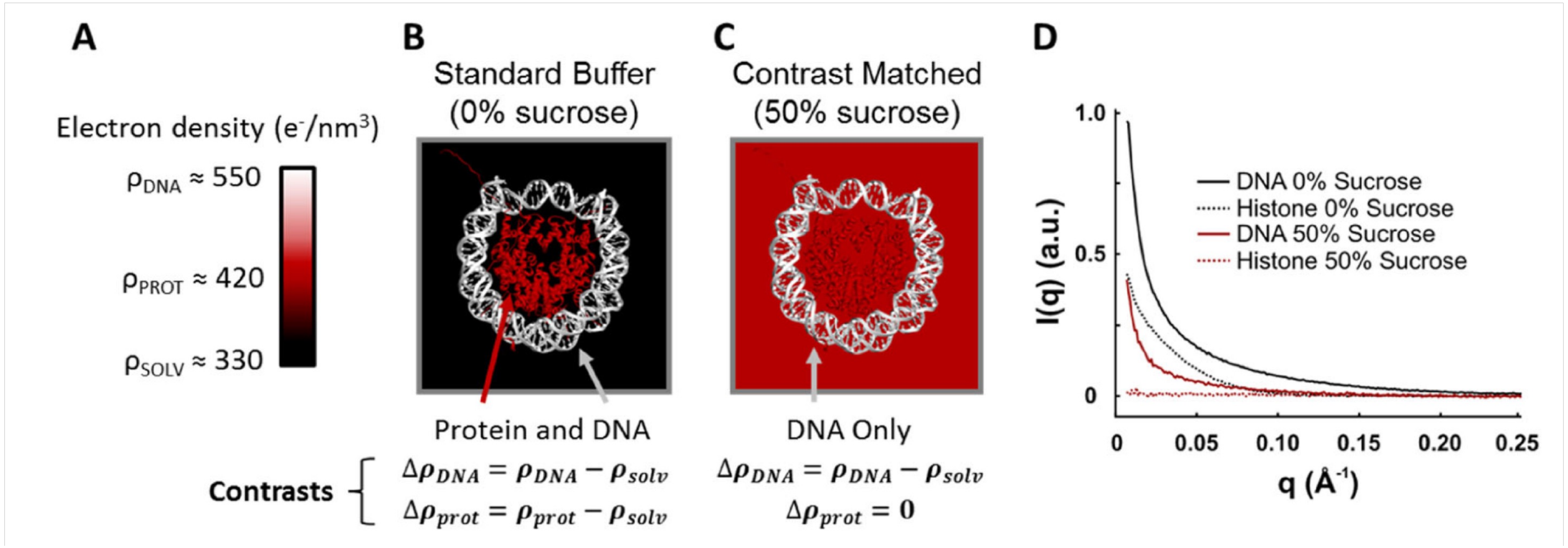
Harold, of course, was immediately devoured.

Contrast matching using light

Laura Waller on youtube.com

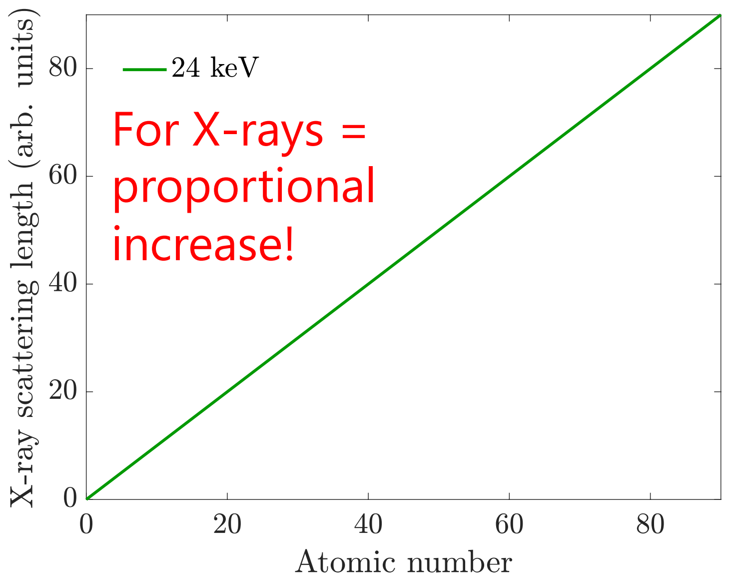
Contrast matching using light and X-rays

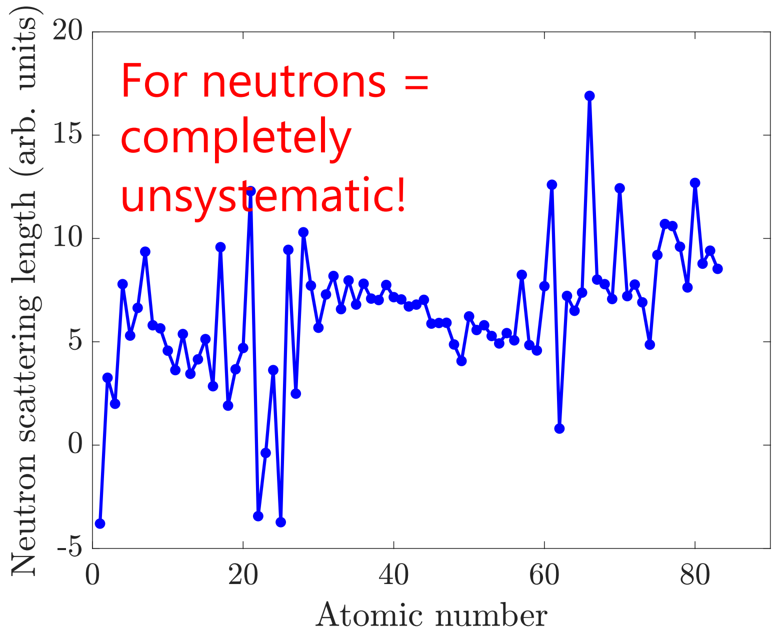
- Contrast matching is possible for all the scattering methods.
- Light: match the between the **refractive index** of the sample and the solvent.
- X-ray: match the **electron density** between the sample and the solvent.

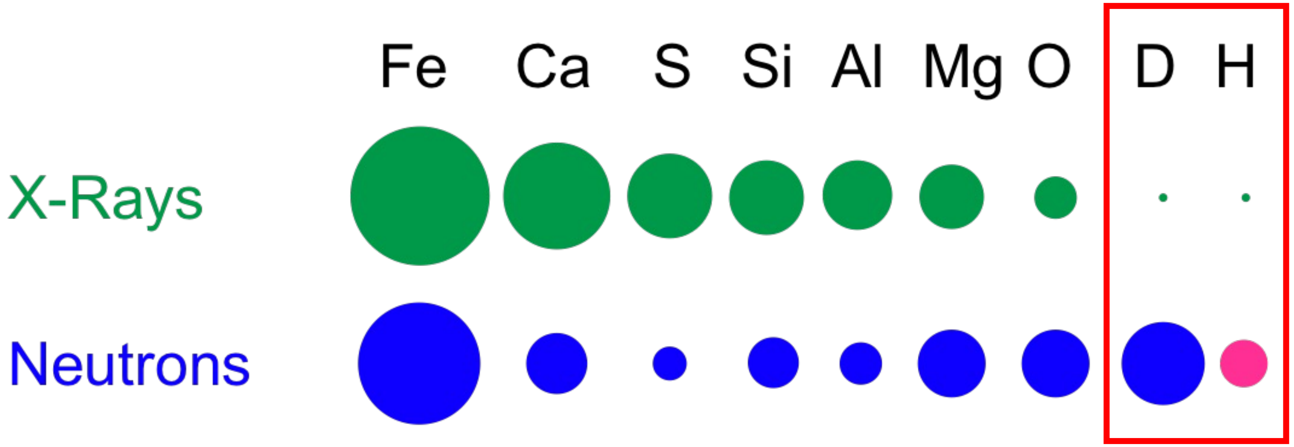


This same idea works for neutrons by playing with H/D substitution!

Recap: X-rays and neutrons see things differently!

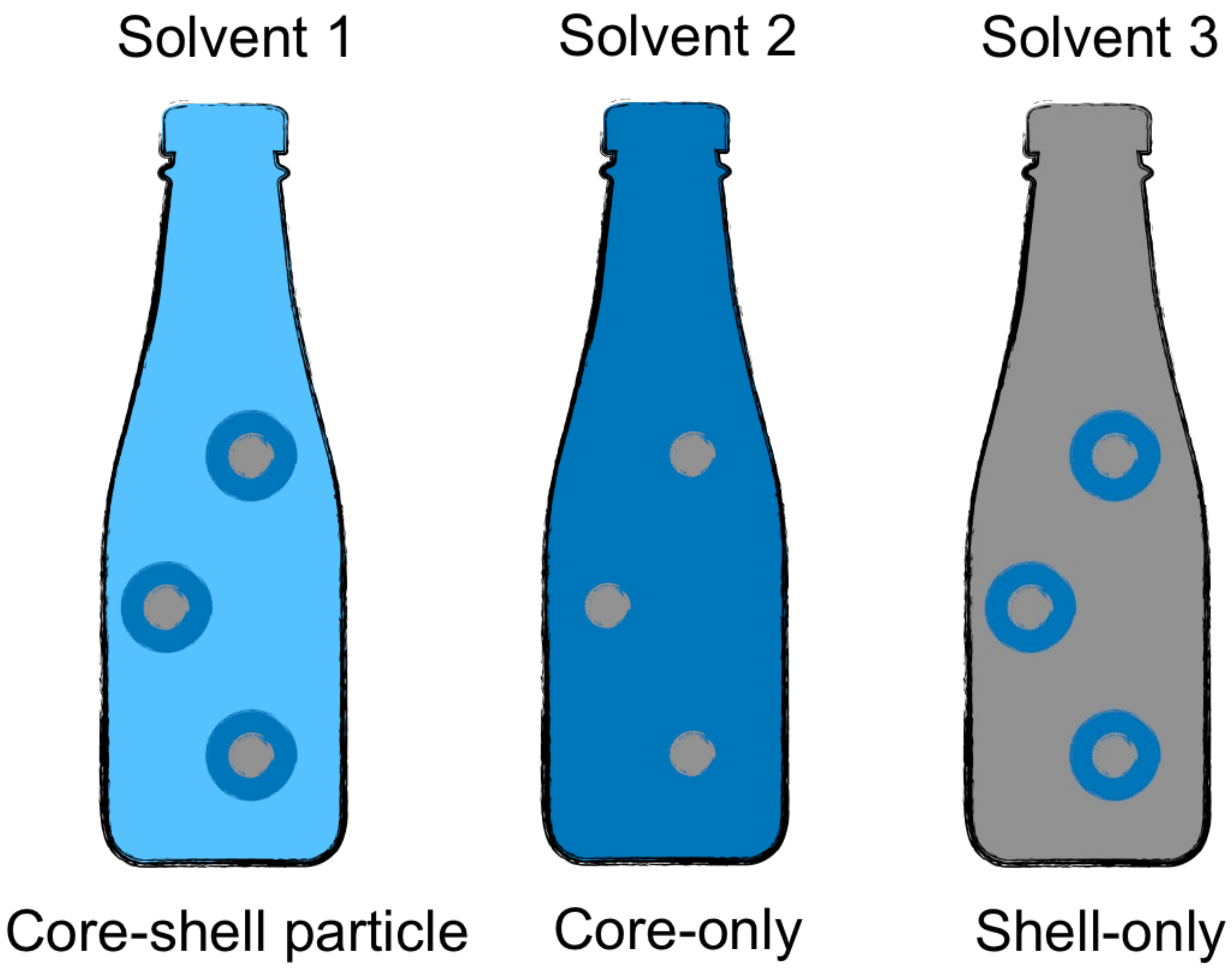






...even for different isotopes of the same element!

Neutrons and contrast matching

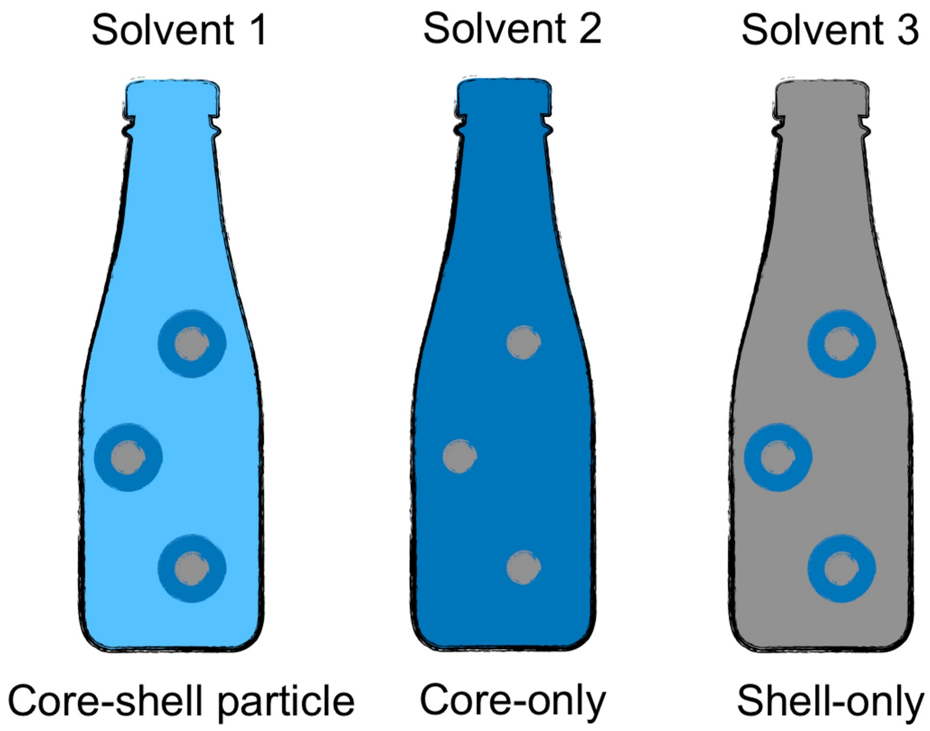


$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

Selective deuteration in combination with neutrons lets us investigate selected parts of complex assemblies.

Combining X-Ray and Neutron measurements provides more information

Neutrons and contrast matching



$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

Selective deuteration in combination with neutrons lets us investigate selected parts of complex assemblies.

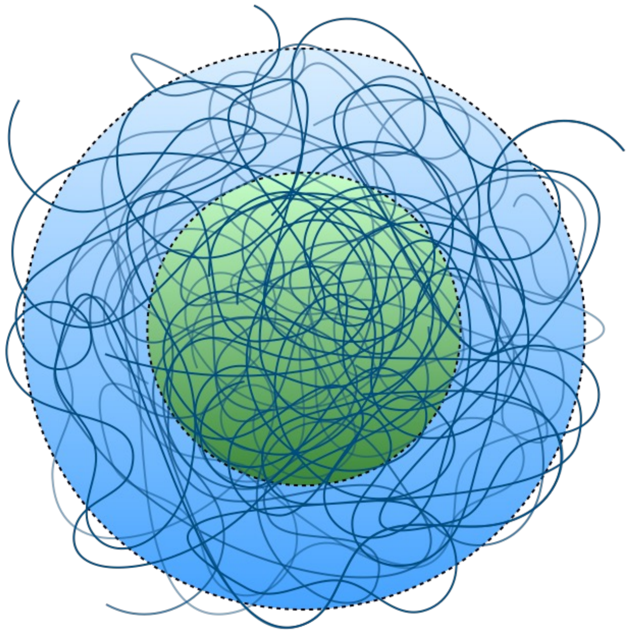
Combining X-Ray and Neutron measurements provides more information

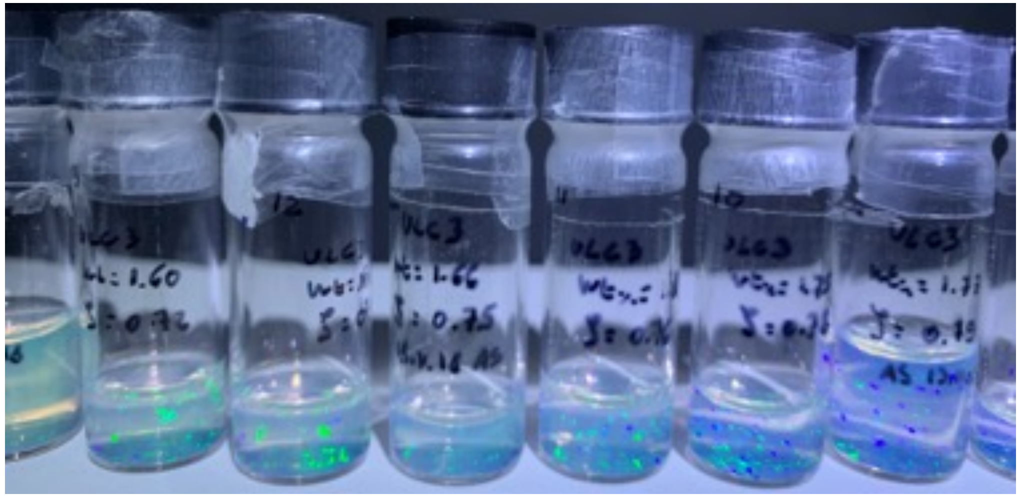
Why is it so important for Life Science?

- one of the easiest way to change the contrast is switching from H → D
- one of the main elements of biological matter is H, thus neutron scattering and CV is natural tool

3

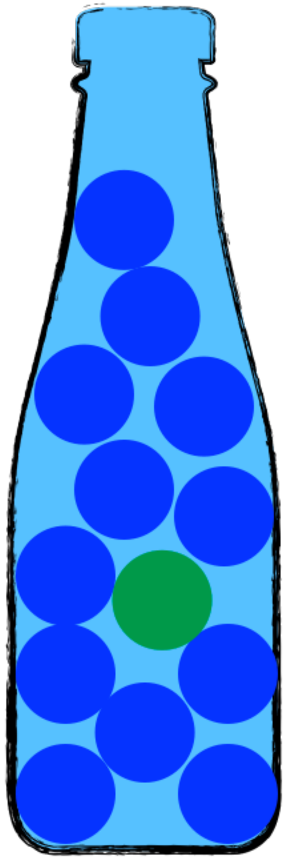
Colloidal particles in a crowded environment





Example: Colloidal particles in crowded environment

Solvent 1



All particles visible

Question:
how does a soft colloid respond to crowding?

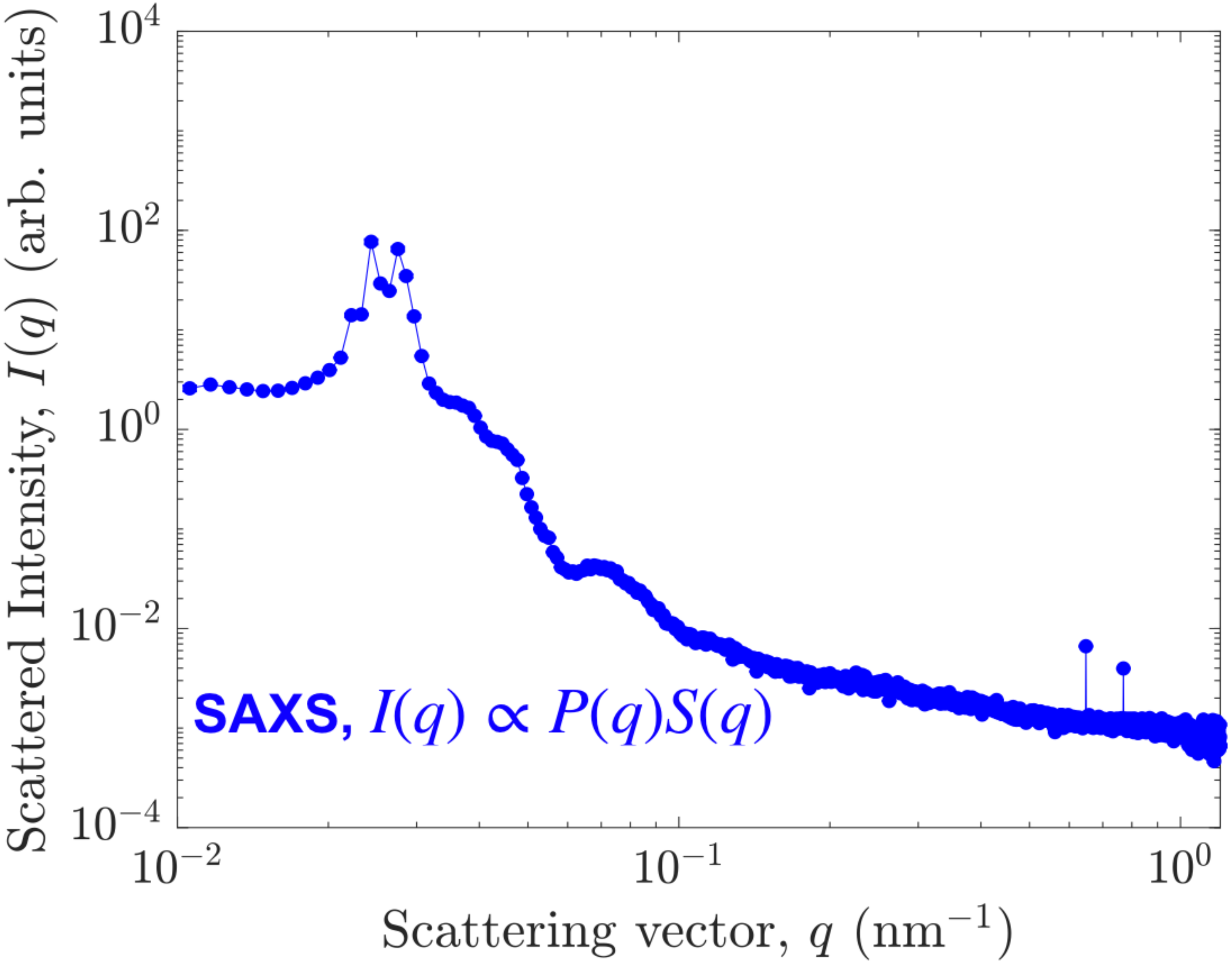
Problem:
in concentrated solutions

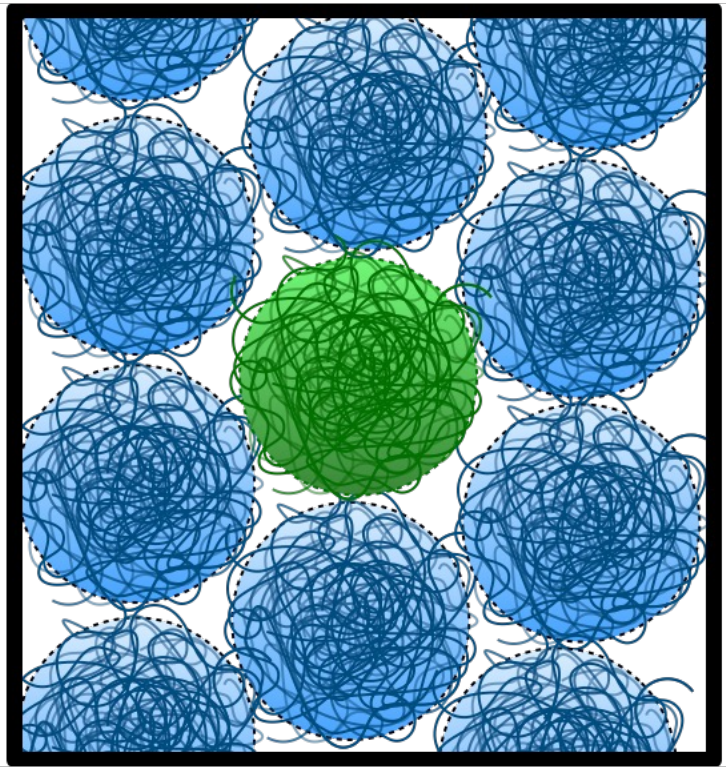
$$I(q) \propto P(q)S(q)$$

Hydrogenated particle

Deuterated particle

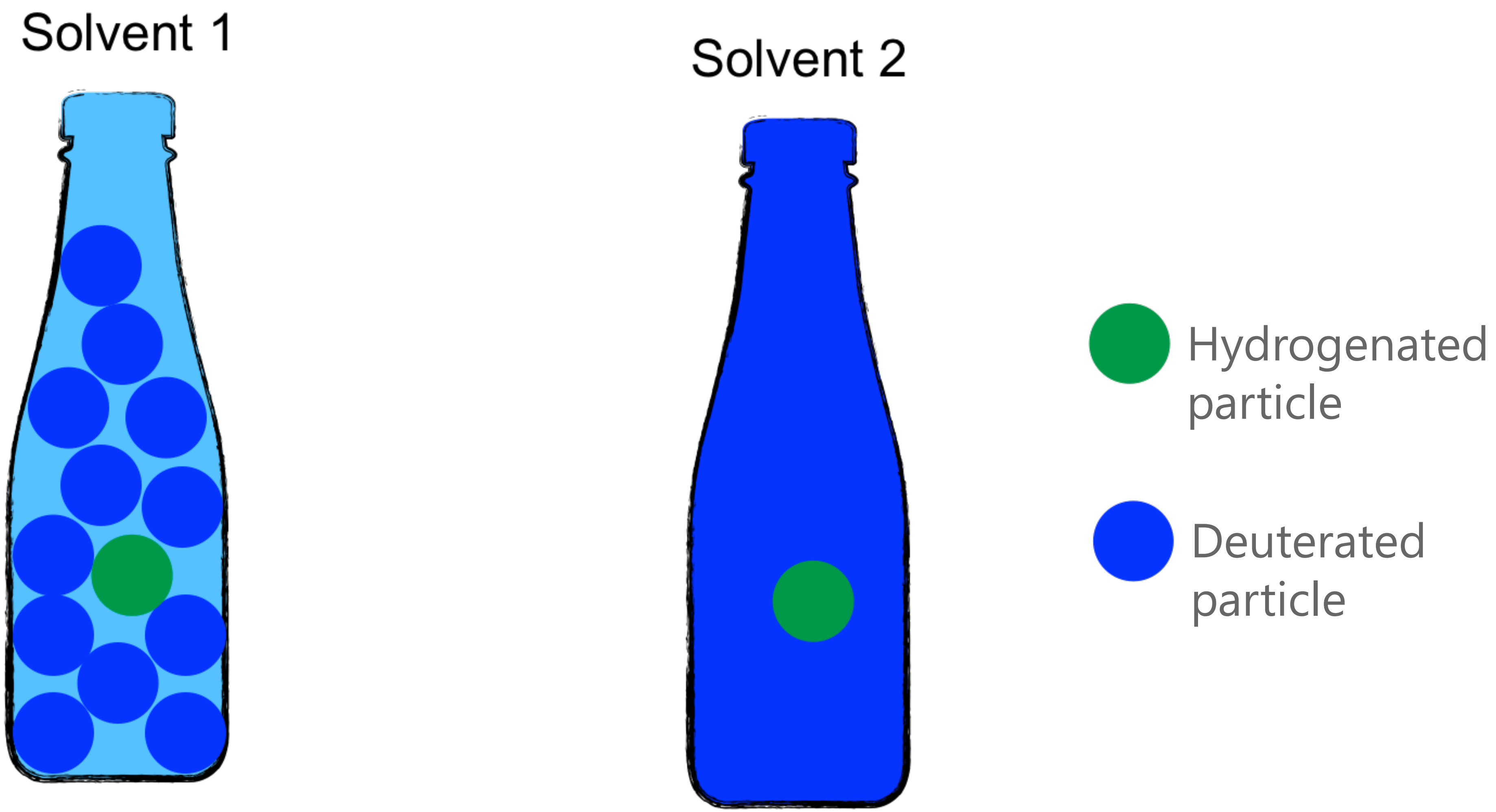
Example: Colloidal particles in crowded environment





Courtesy of Andrea Scotti (RWTH)

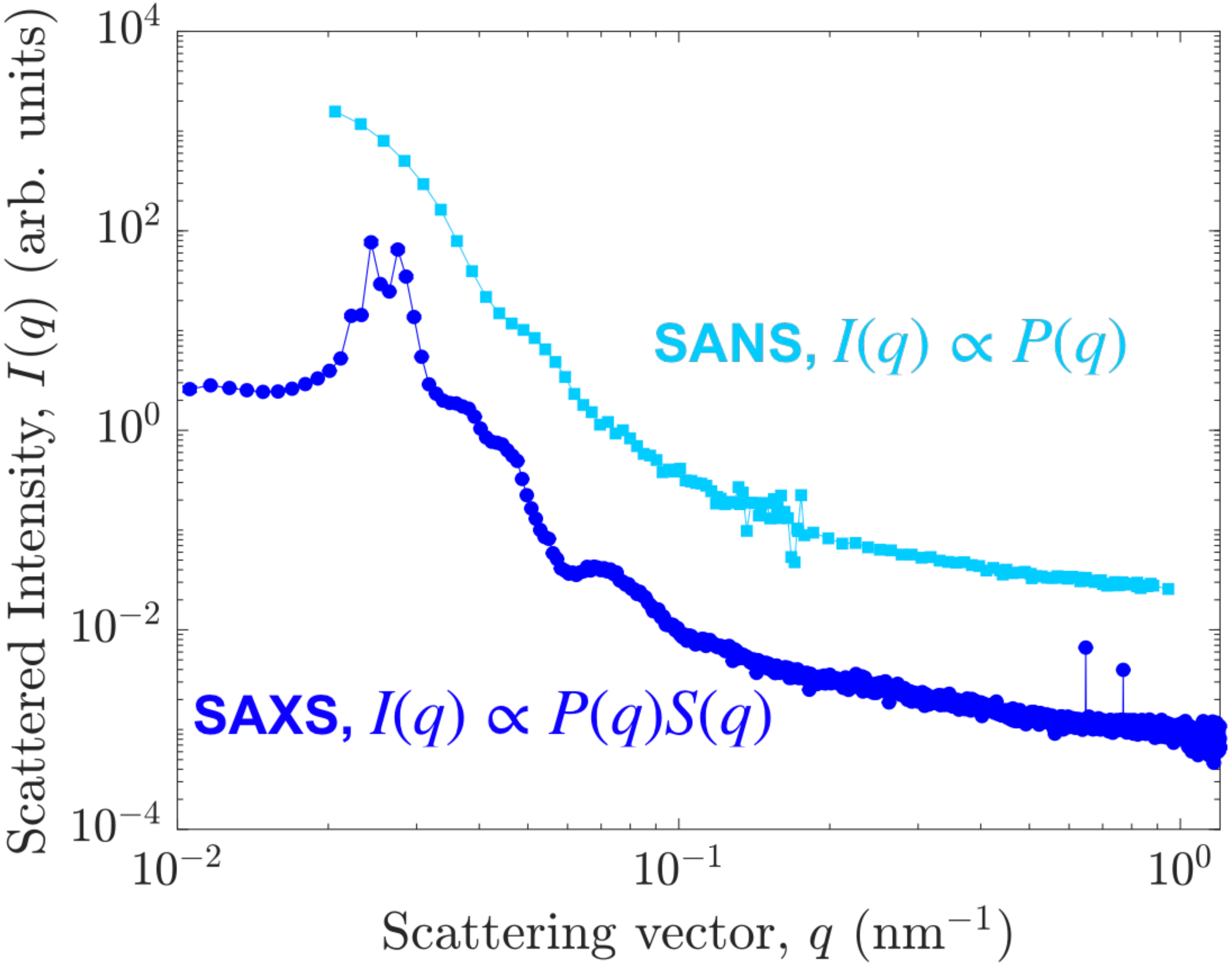
Example: Colloidal particles in crowded environment



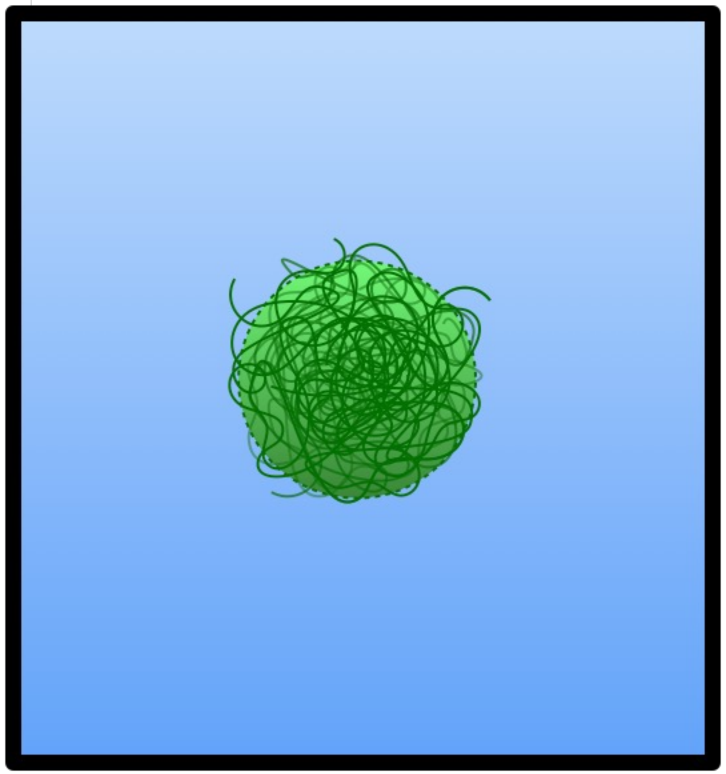
All particles visible

Only the labelled particle is visible

Example: Colloidal particles in crowded environment



SANS, $I(q) \propto P(q)$



Courtesy of Andrea Scotti (RWTH)

How do we experimentally determine the contrast matching point? **Method 1**

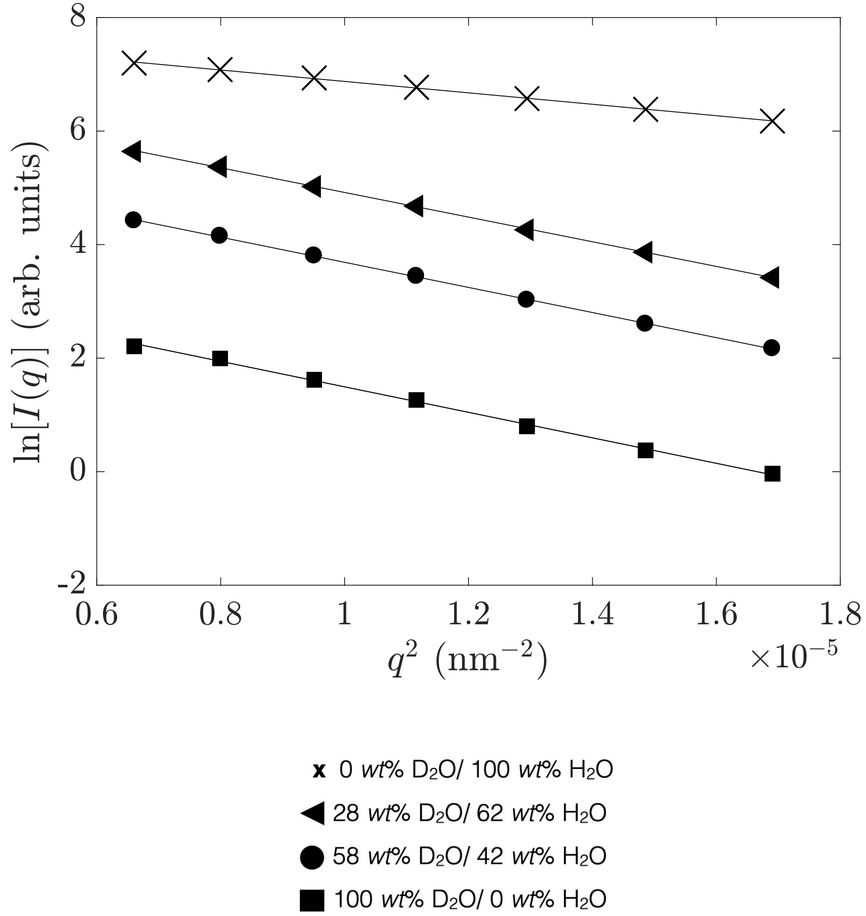
Step 1: prepare a series of the same sample (highly diluted) in different solvents (e.g. water/heavy water mixtures).

Step 2: perform small-angle neutron scattering measurements at low-Q (Guinier regime).

Step 3: fit the data at low-Q with the Guinier approximation for all the different solvents

$$\lim_{q \to 0} P(q) = I(0) \exp\left[-\frac{q^2 \langle S^2 \rangle}{3} \right]$$

Step 4: plot the values of I(0) obtained from the fits versus the solvent composition and look for the intercept with the *x*-axes



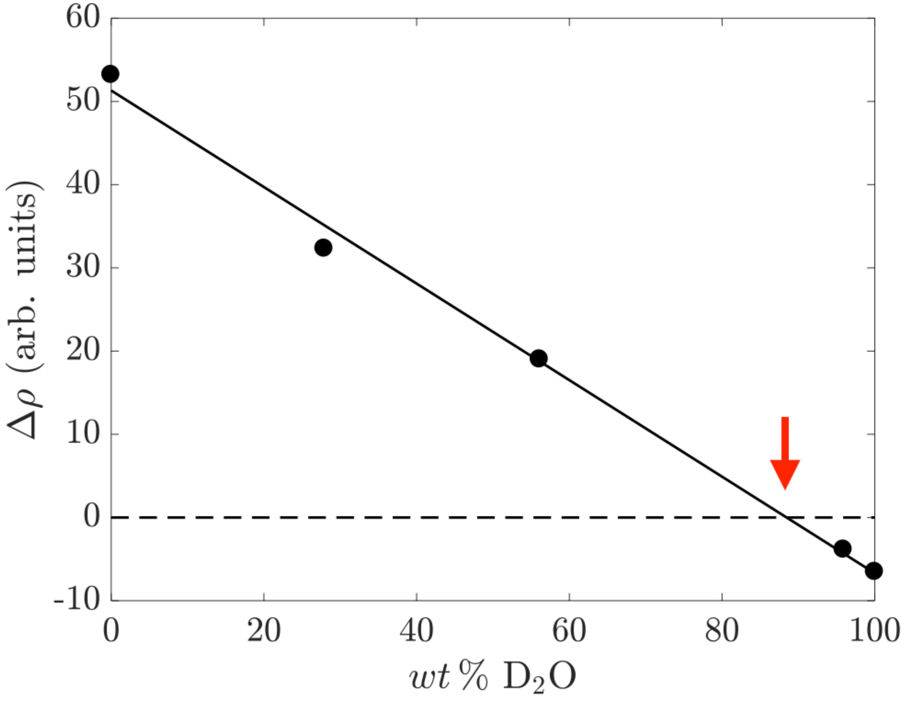
How do we experimentally determine the contrast matching point? **Method 1**

Step 1: prepare a series of the same sample (highly diluted) in different solvents (e.g. water/heavy water mixtures).

Step 2: perform small-angle neutron scattering measurements at low-Q (Guinier regime).

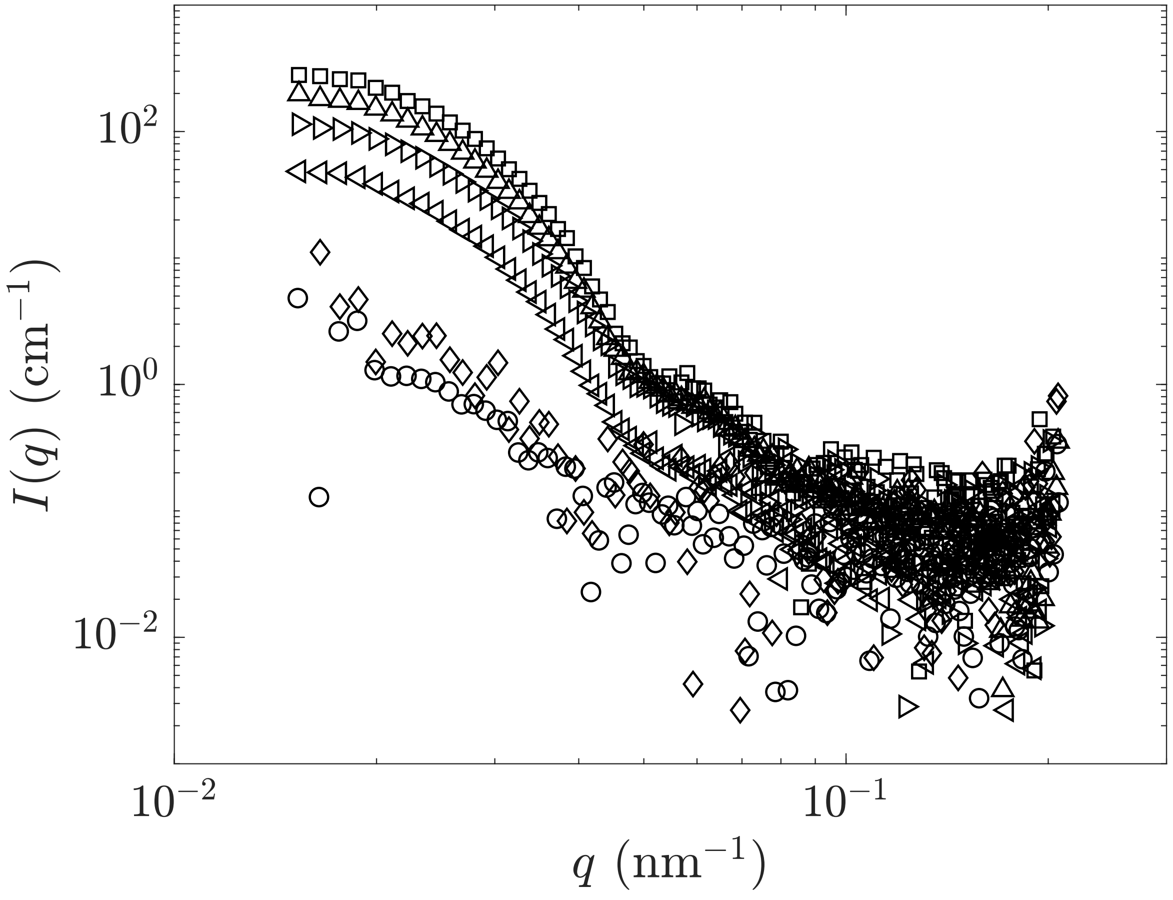
Step 3: fit the data at low-Q with the Guinier approximation for all the different solvents

$$\lim_{q \to 0} P(q) = I(0) \exp\left[-\frac{q^2 \langle S^2 \rangle}{3} \right]$$



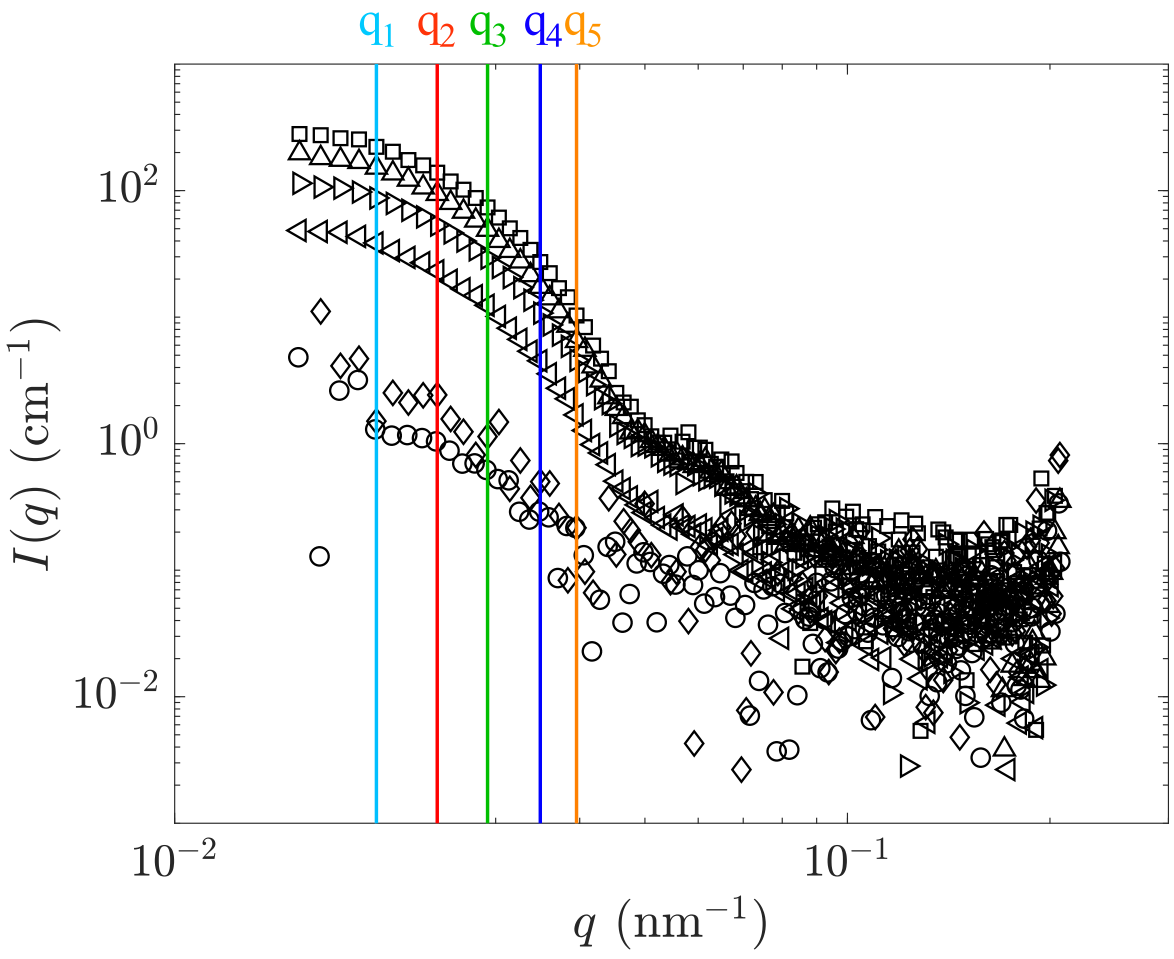
Step 4: plot the values of I(0) obtained from the fits versus the solvent composition and look for the intercept with the *x*-axes

How do we experimentally determine the contrast matching point? **Method 2**



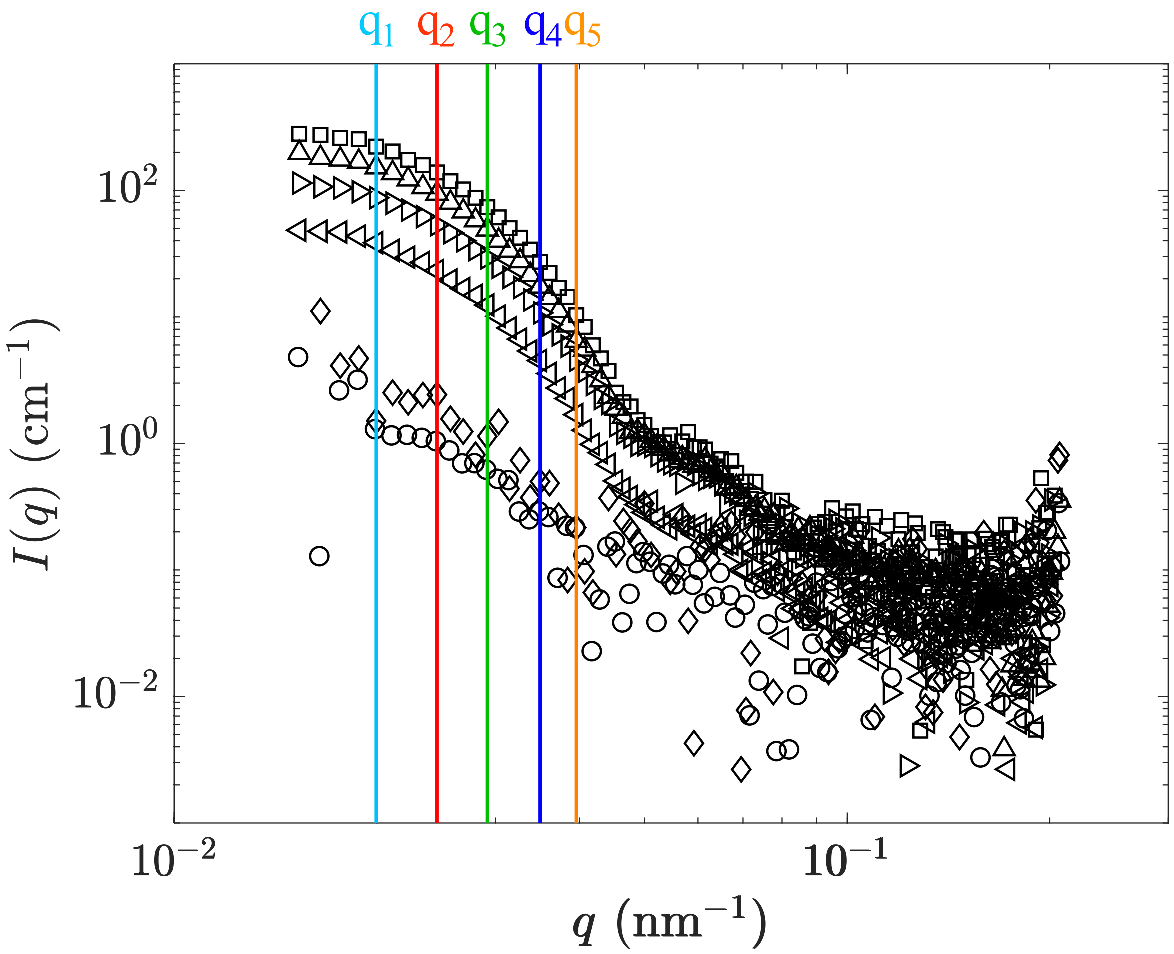
- □ 0 wt% D_2O / 100 wt% H_2O
- ▷ 20 wt% D_2O / 80 wt% H_2O
- ◇ 50 wt% D_2O / 50 wt% H_2O
- ○ 60 wt% D_2O / 40 wt% H_2O
- ◁ 80 wt% D_2O / 20 wt% H_2O
- △ 100 wt% D_2O / 0 wt% H_2O

How do we experimentally determine the contrast matching point? **Method 2**



- □ 0 wt% D_2O / 100 wt% H_2O
- ▷ 20 wt% D_2O / 80 wt% H_2O
- ◇ 50 wt% D_2O / 50 wt% H_2O
- ○ 60 wt% D_2O / 40 wt% H_2O
- ◁ 80 wt% D_2O / 20 wt% H_2O
- △ 100 wt% D_2O / 0 wt% H_2O

How do we experimentally determine the contrast matching point? **Method 2**

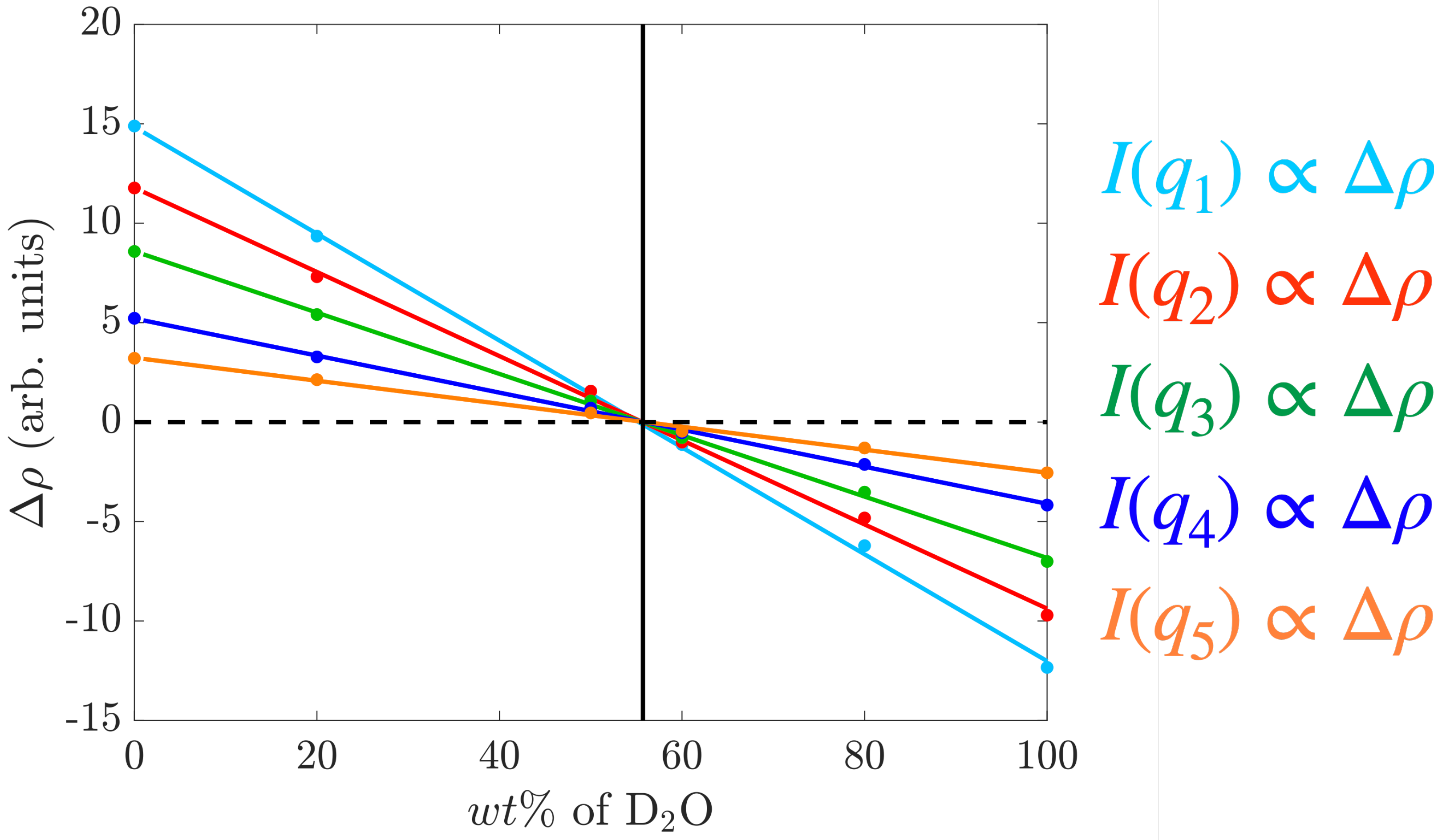


- □ 0 wt% D_2O / 100 wt% H_2O
- ▷ 20 wt% D_2O / 80 wt% H_2O
- ◇ 50 wt% D_2O / 50 wt% H_2O
- ○ 60 wt% D_2O / 40 wt% H_2O
- ◁ 80 wt% D_2O / 20 wt% H_2O
- △ 100 wt% D_2O / 0 wt% H_2O

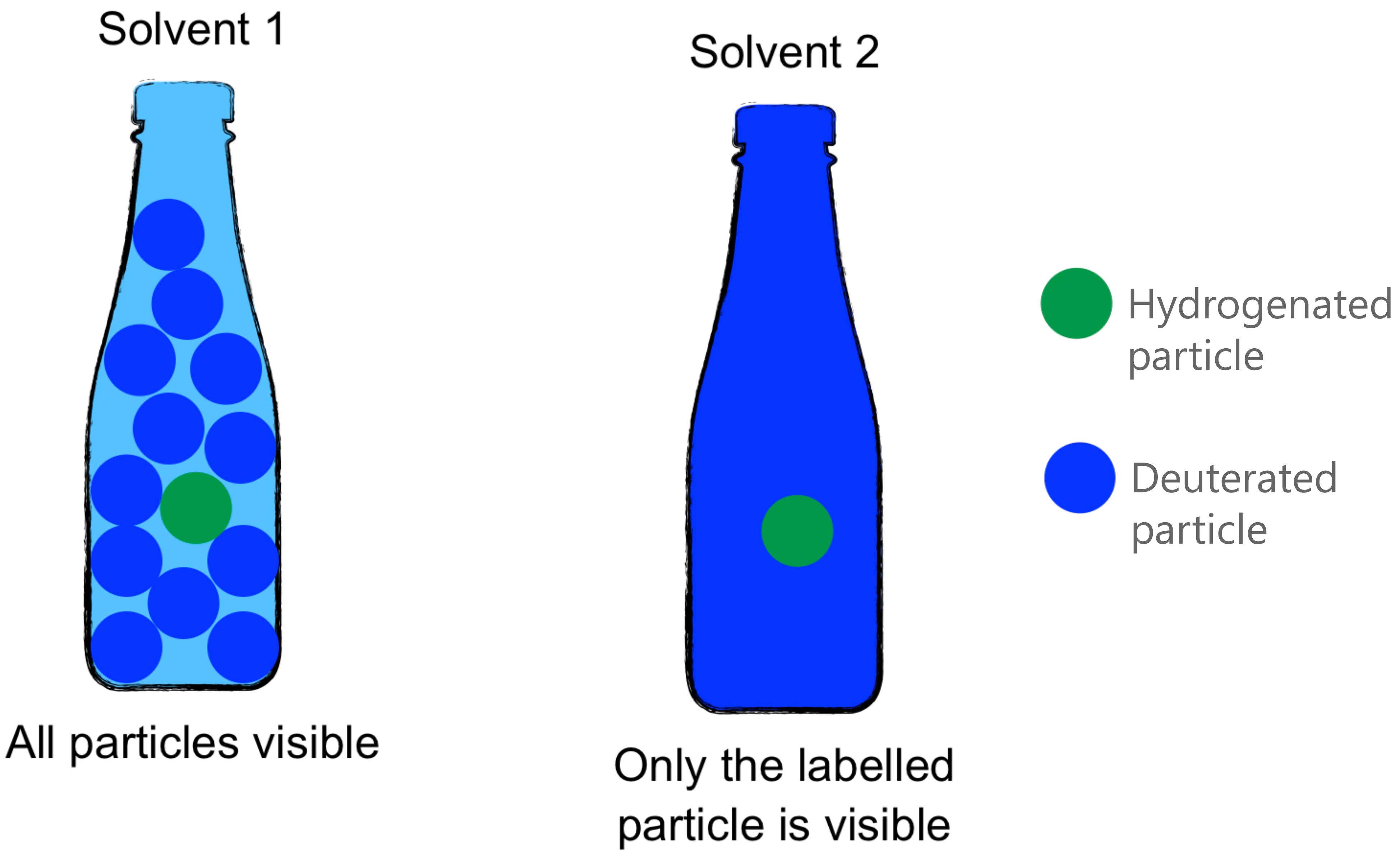
$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

All the samples have the same concentration of the same particles.

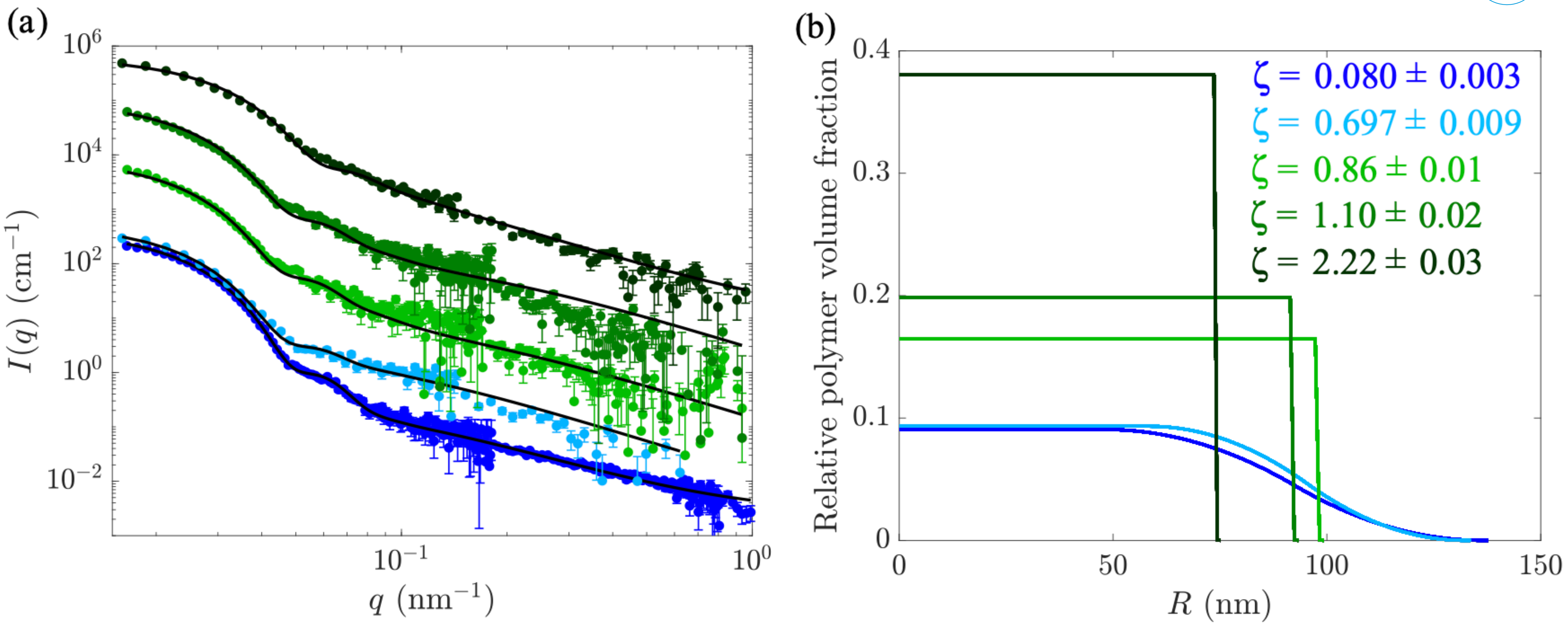
How do we experimentally determine the contrast matching point? **Method 2**



Example: Colloidal particles in crowded environment

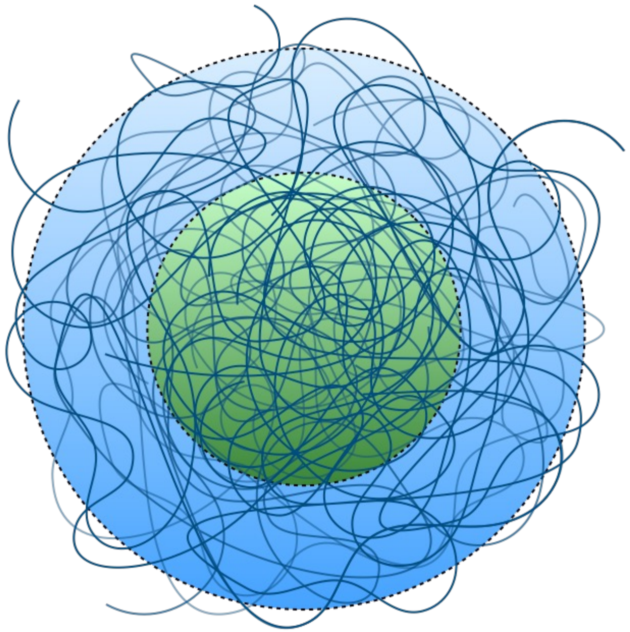


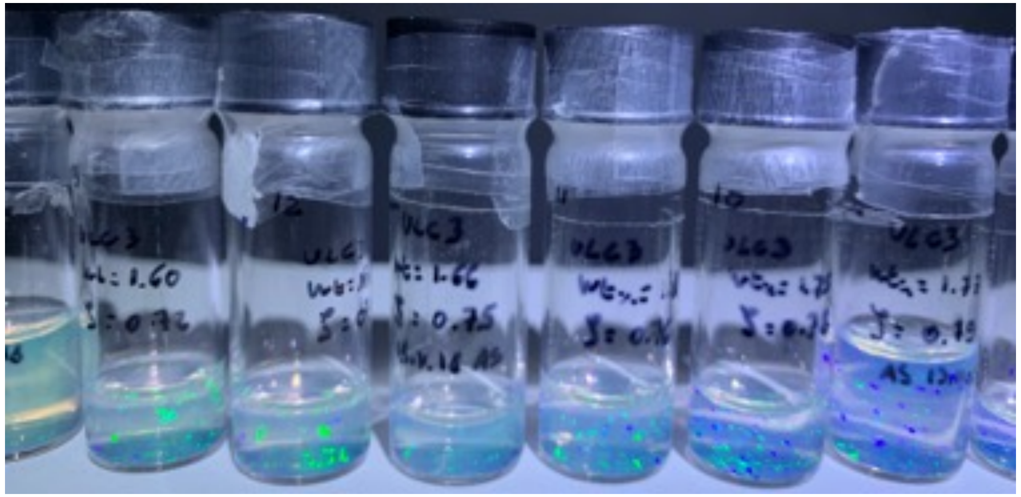
Small-angle neutron scattering with contrast variation



4

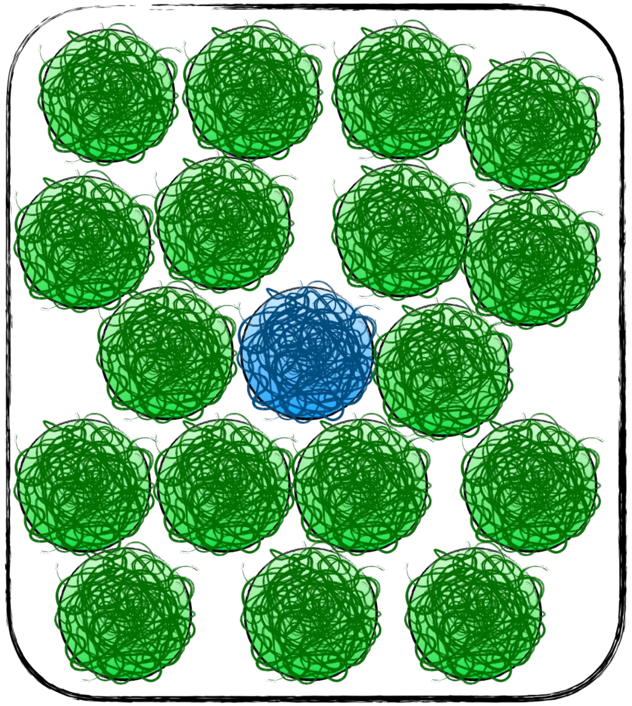
Using SAS to quantify the softness of small colloids



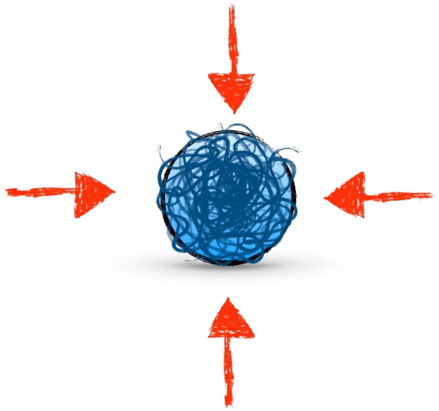


Colloids in crowded suspensions

Deswelling



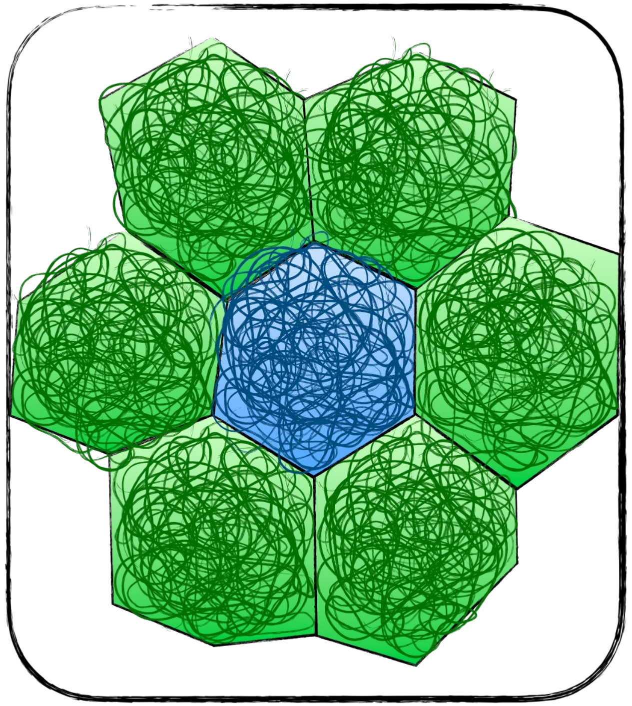
We need
the bulk modulus!



Bulk modulus

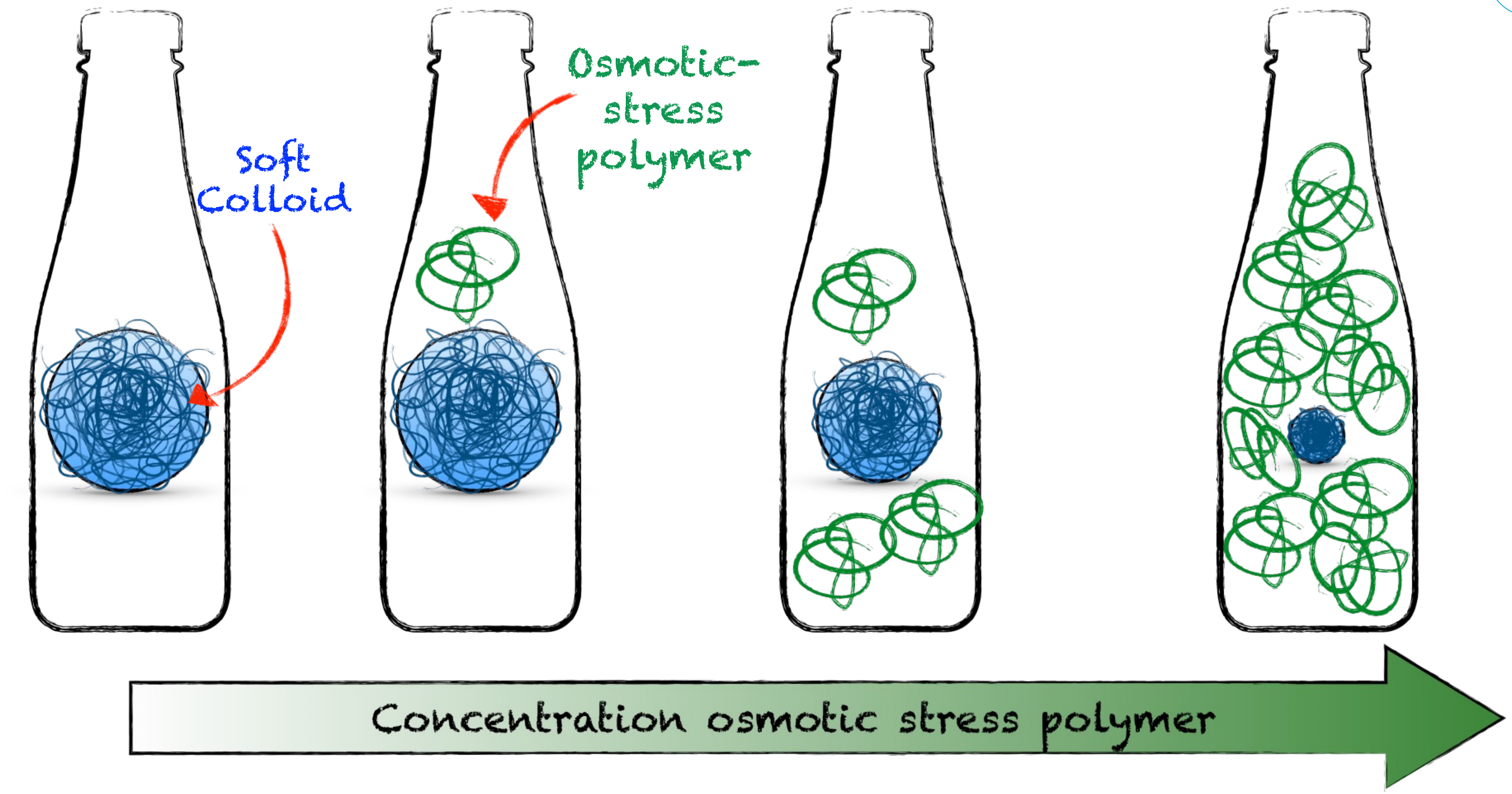
$$K = -v\frac{d\pi}{dv}$$

Faceting

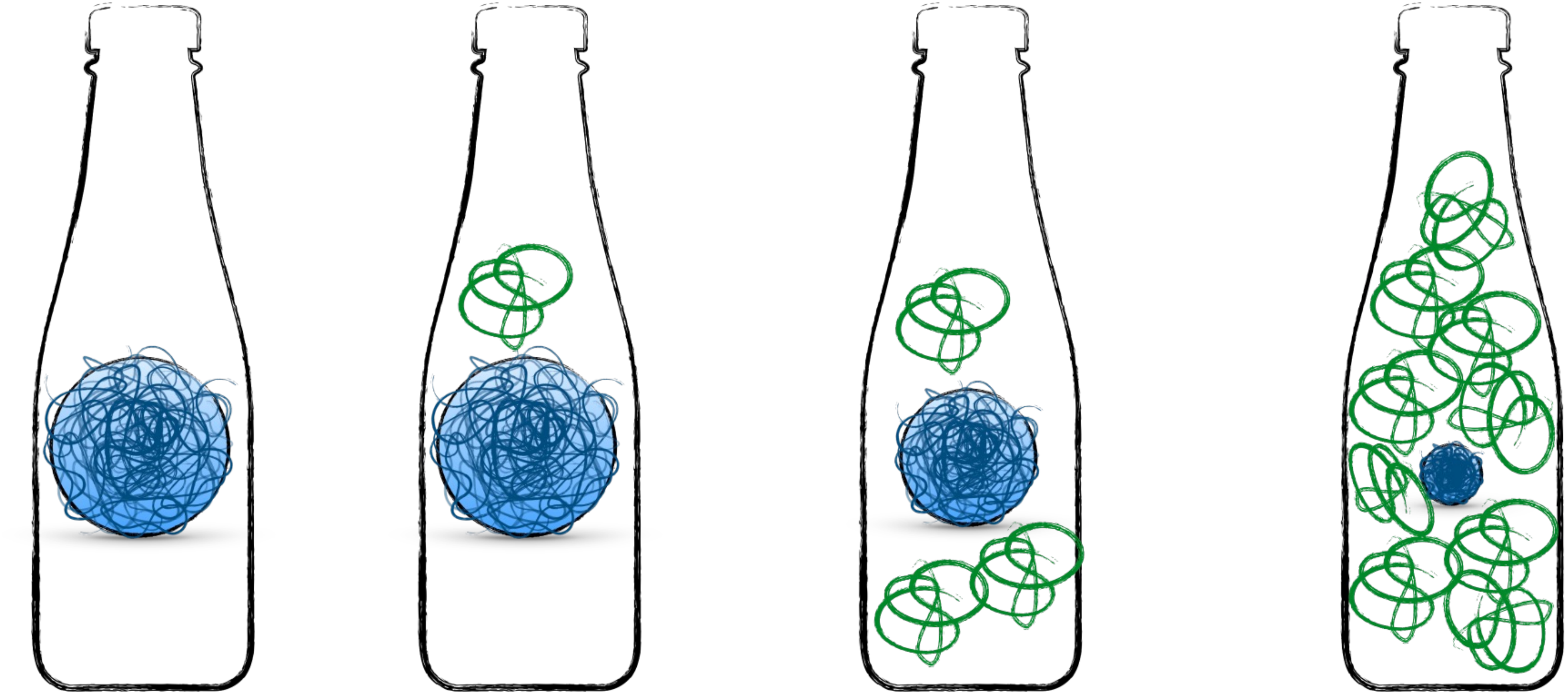


We cannot use microscopy, particles too small!

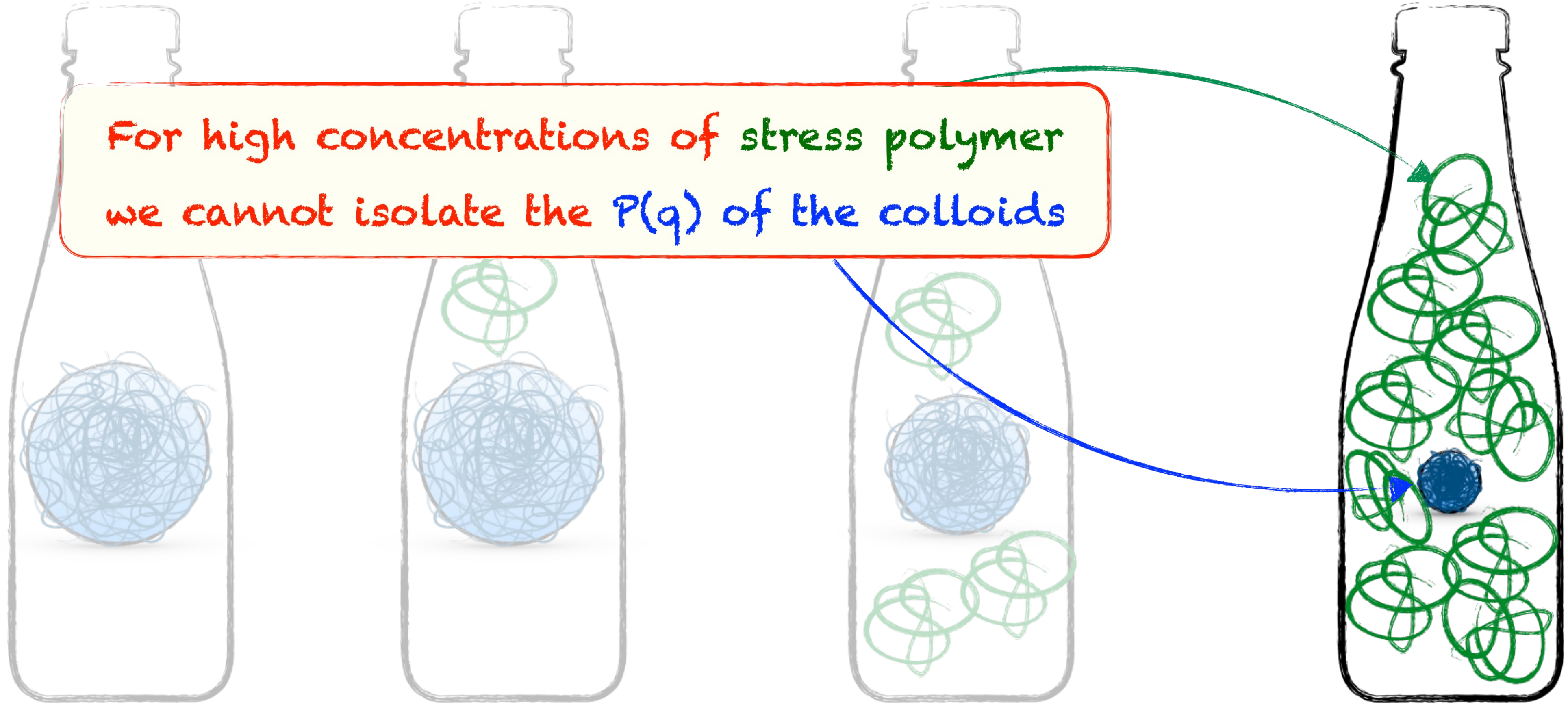
How do we measure K? Osmotic stress solutions



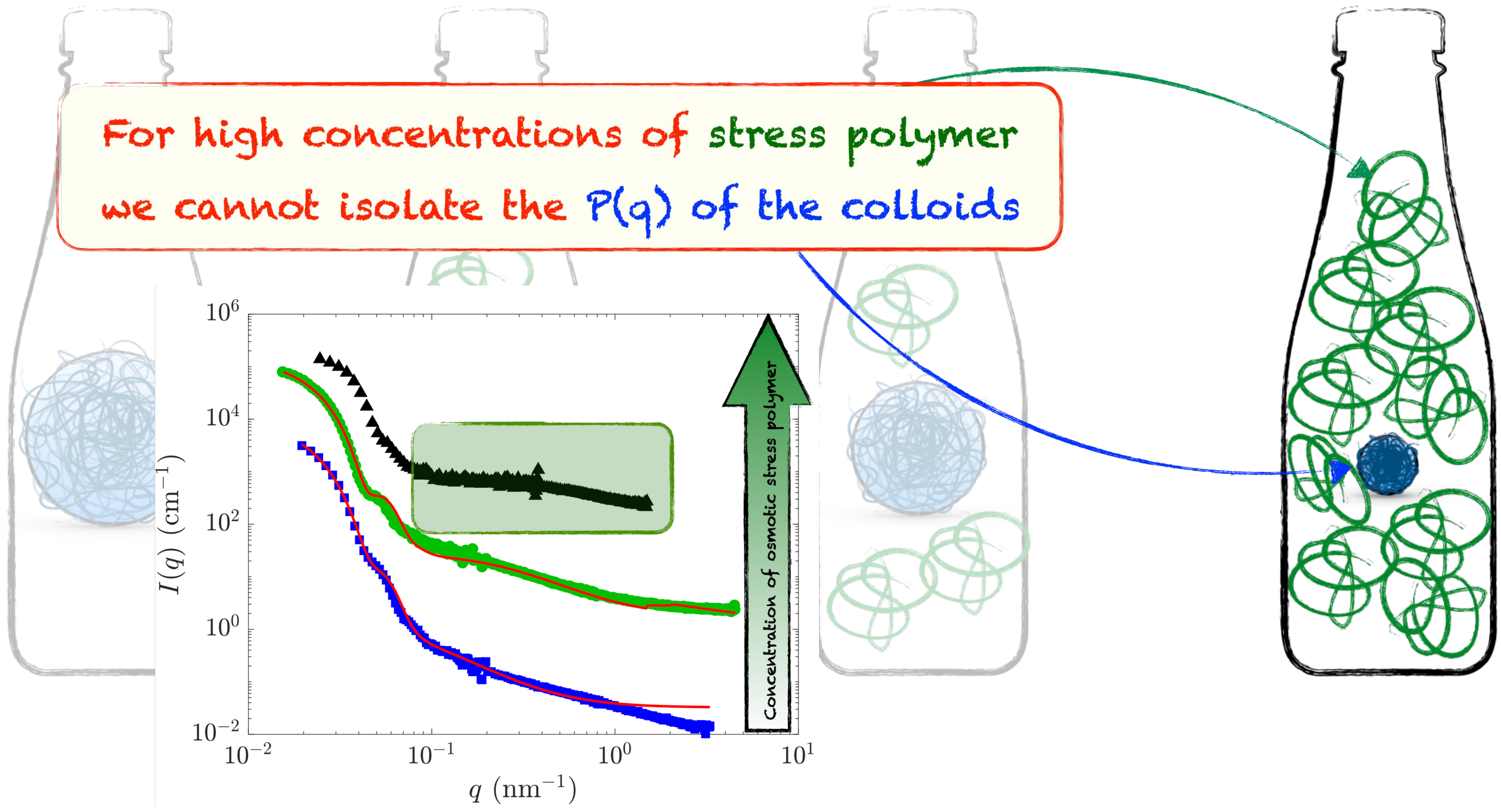
Information not accessible at high pressures



Information not accessible at high pressures



Information not accessible at high pressures

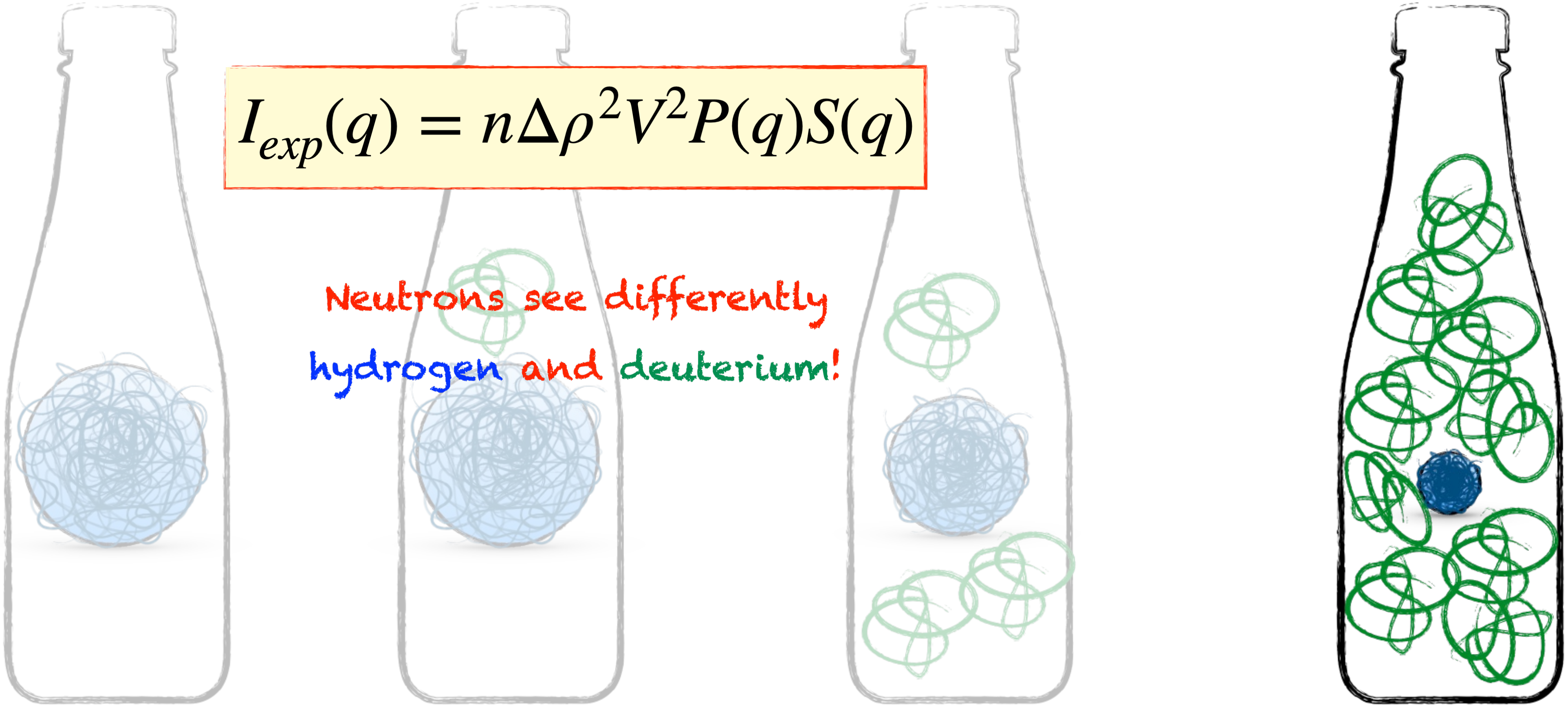


Neutrons and contrast variation

$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

Neutrons see differently

hydrogen and deuterium!



Neutrons and contrast variation

$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$

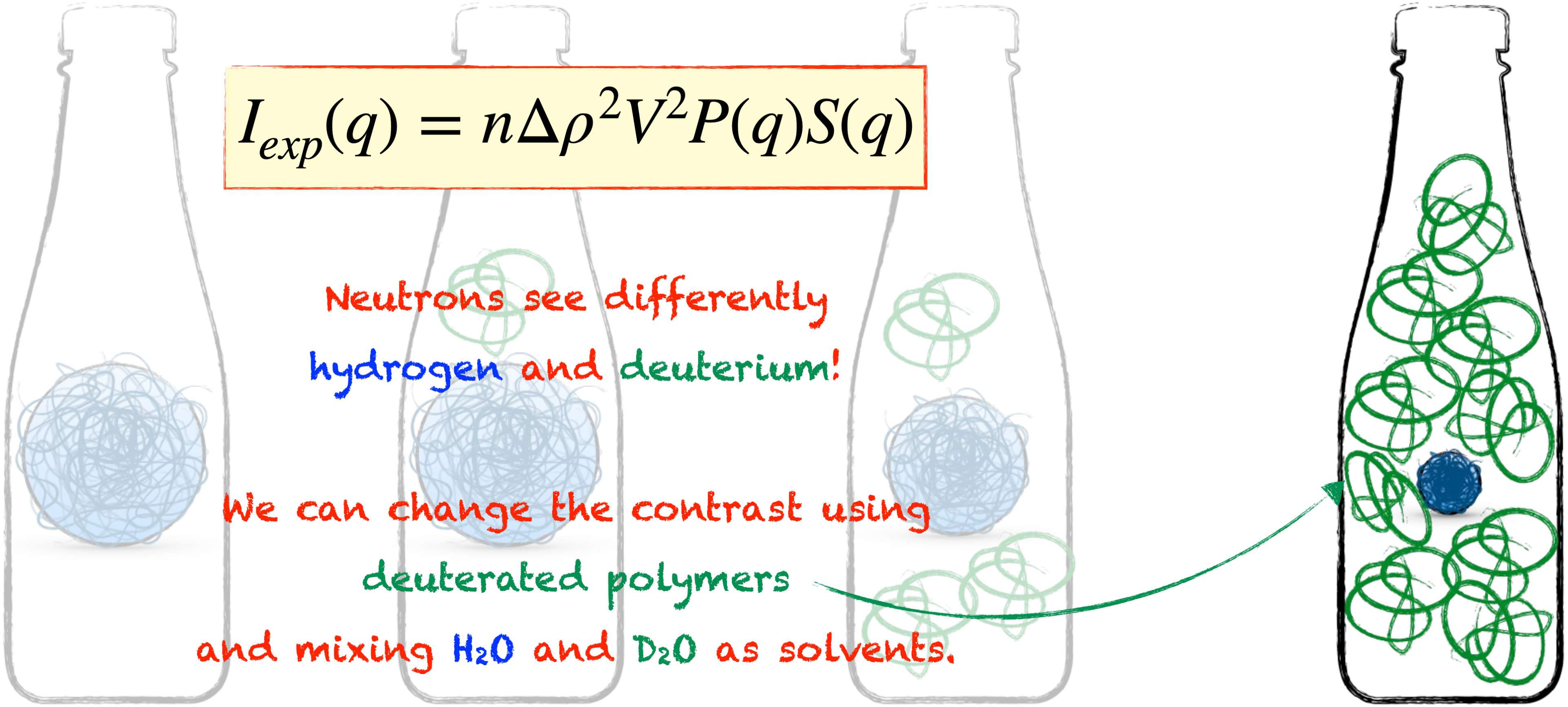
Neutrons see differently

hydrogen and deuterium!

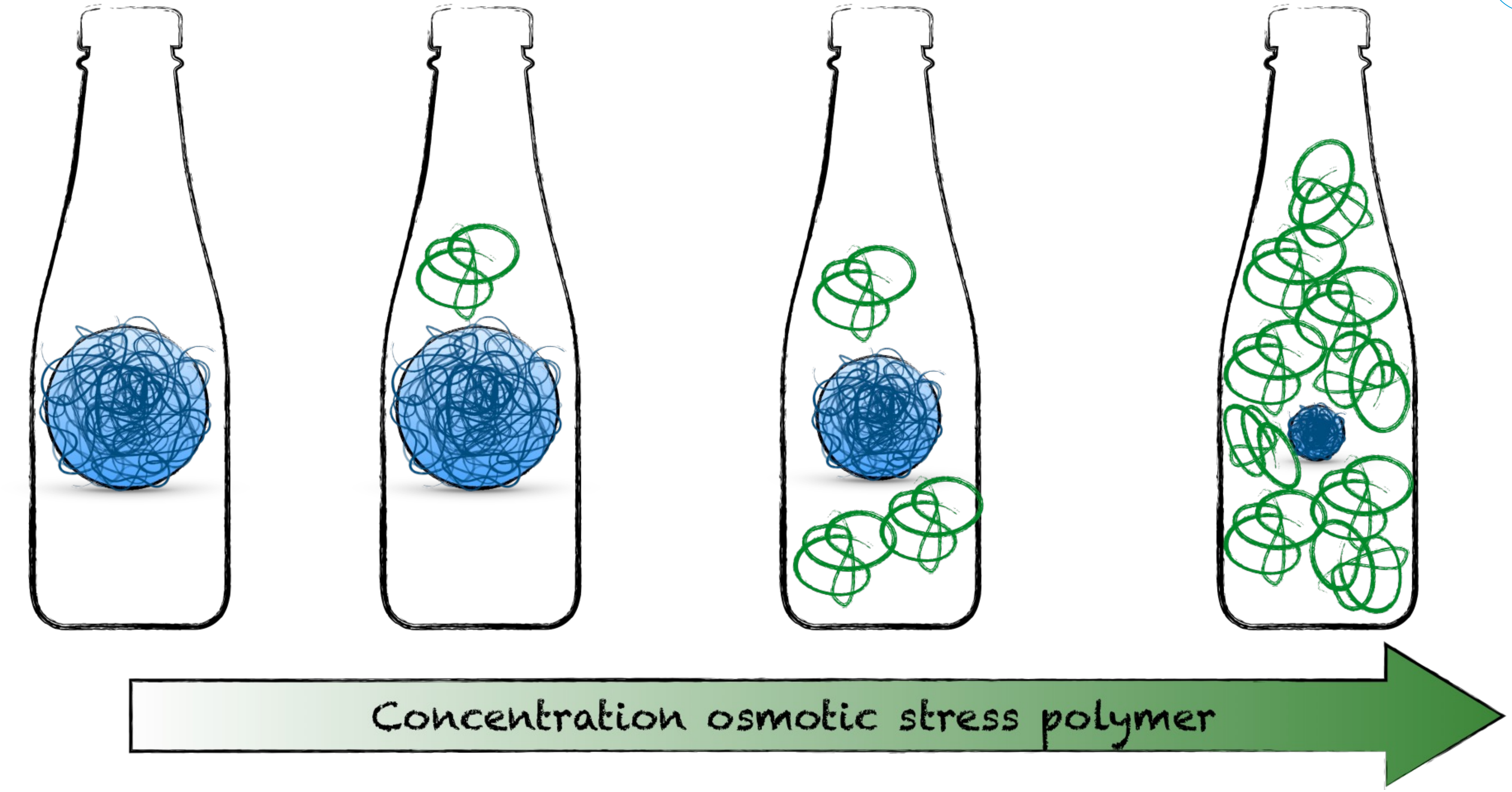
We can change the contrast using

deuterated polymers

and mixing H_2O and D_2O as solvents.

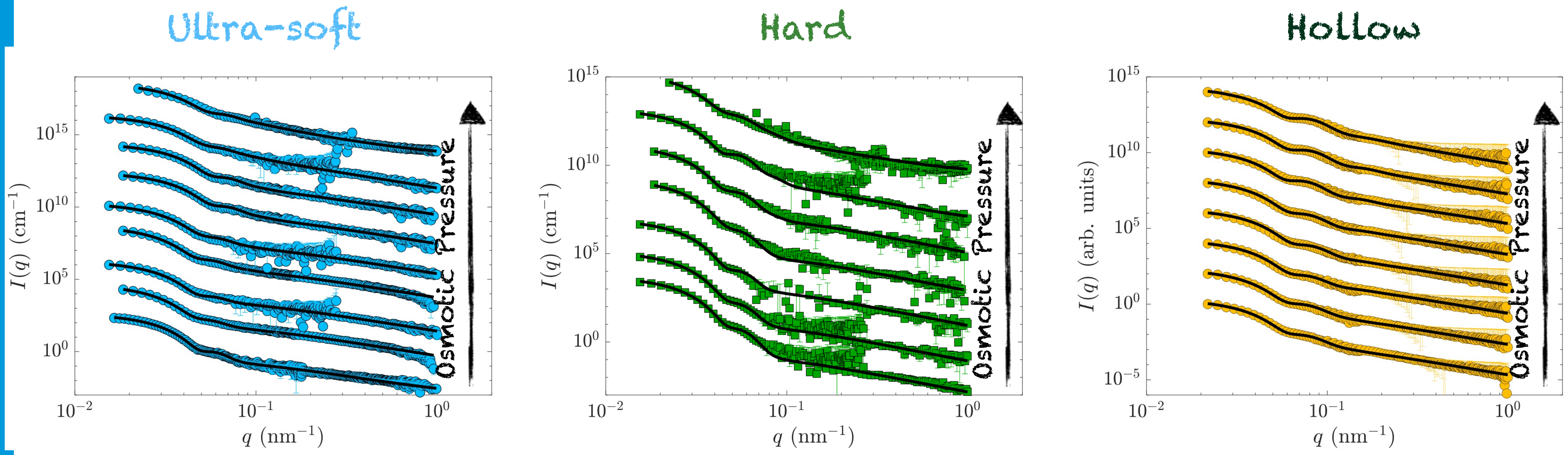


Osmotic stress solutions for small colloids



Now we see only the colloids!

How do we measure the elastic moduli?



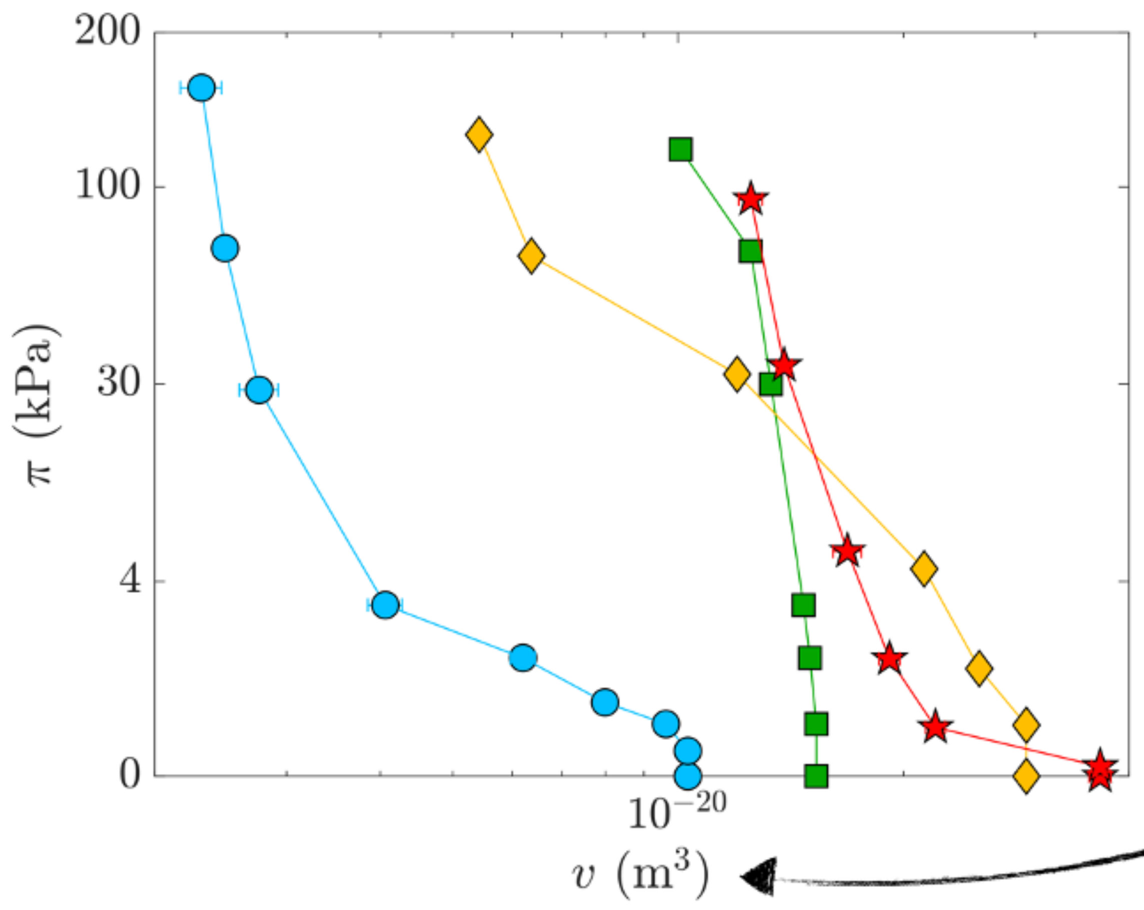
We probe a larger range of pressures (contrast-variation)

and we can obtain the characteristic lengths.

J. E. Houston, L. Fruhner, A. de la Cotte, J. Rojo González, A. Petrunin, U. Gasser, R. Schweins, J. Allgaier, W. Richtering, A. Fernandez-Nieves, and **A. Scotti**. *Science Advances* **8**: eabn6129 (2022); **A. Scotti**, U. Gasser, A. V. Petrunin, L. Fruhner, W. Richtering and J. E. Houston. *Soft Matter* **18**: 5750 (2022).

An unusual representation of the results

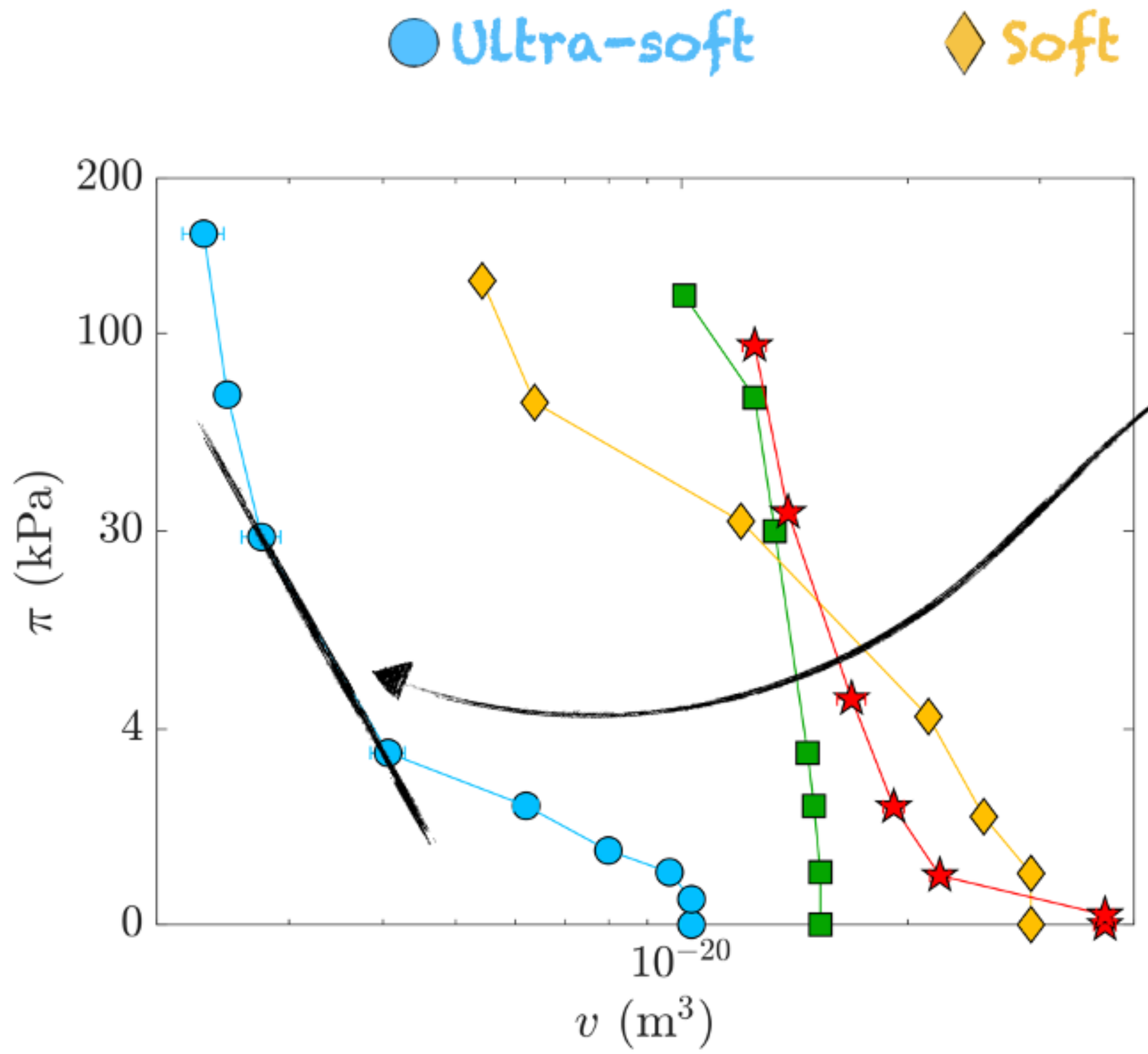
Ultra-soft Soft Hard Hollow



We use the particle radius to compute the volume variations.

J. E. Houston, L. Fruhner, A. de la Cotte, J. Rojo González, A. Petrunin, U. Gasser, R. Schweins, J. Allgaier, W. Richtering, A. Fernandez-Nieves, and A. Scotti. *Science Advances* 8: eabn6129 (2022); A. Scotti, U. Gasser, A. V. Petrunin, L. Fruhner, W. Richtering and J. E. Houston. *Soft Matter* 18: 5750 (2022).

An unusual representation of the results

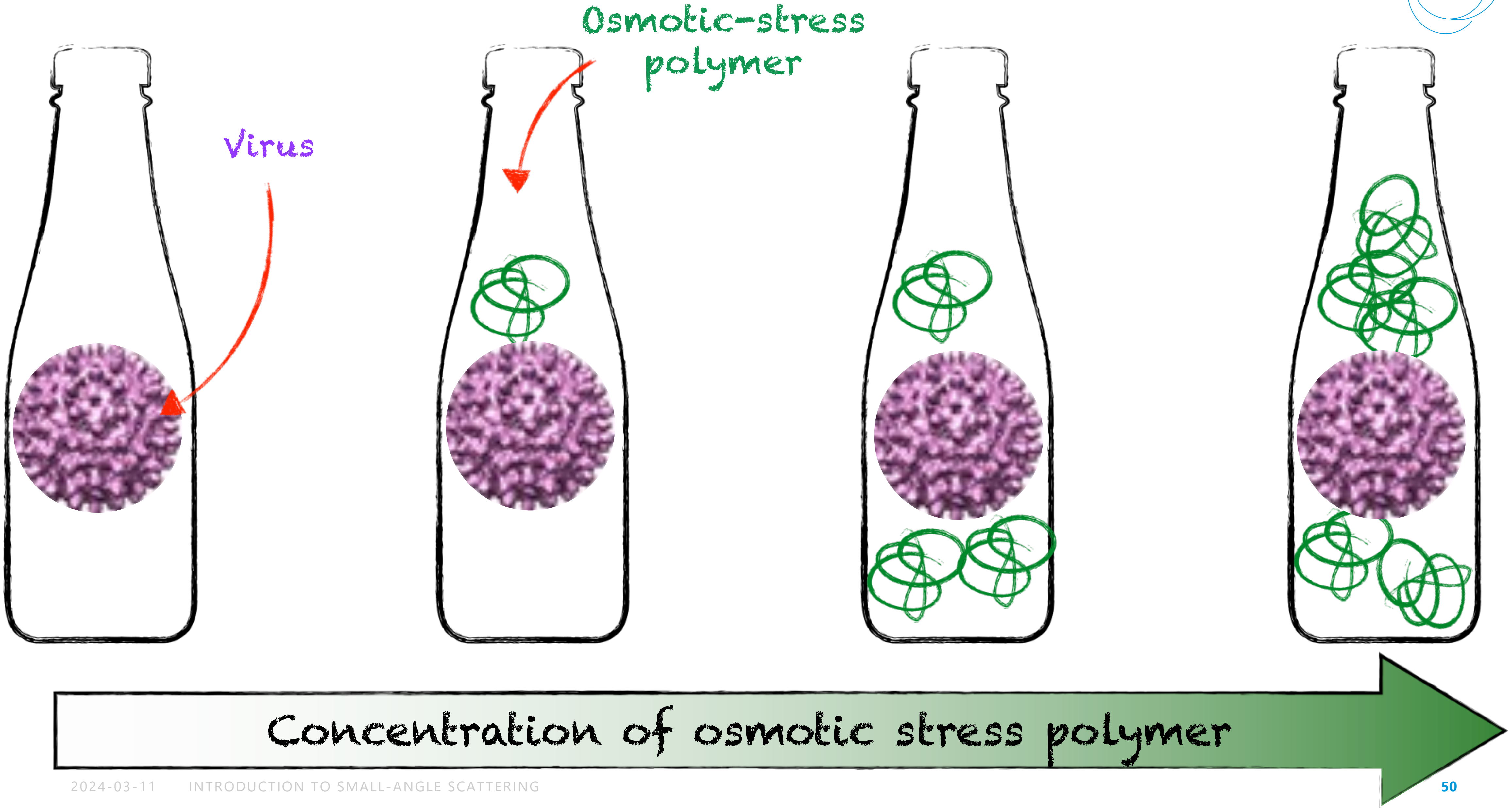


The (local) slope gives the bulk modulus:

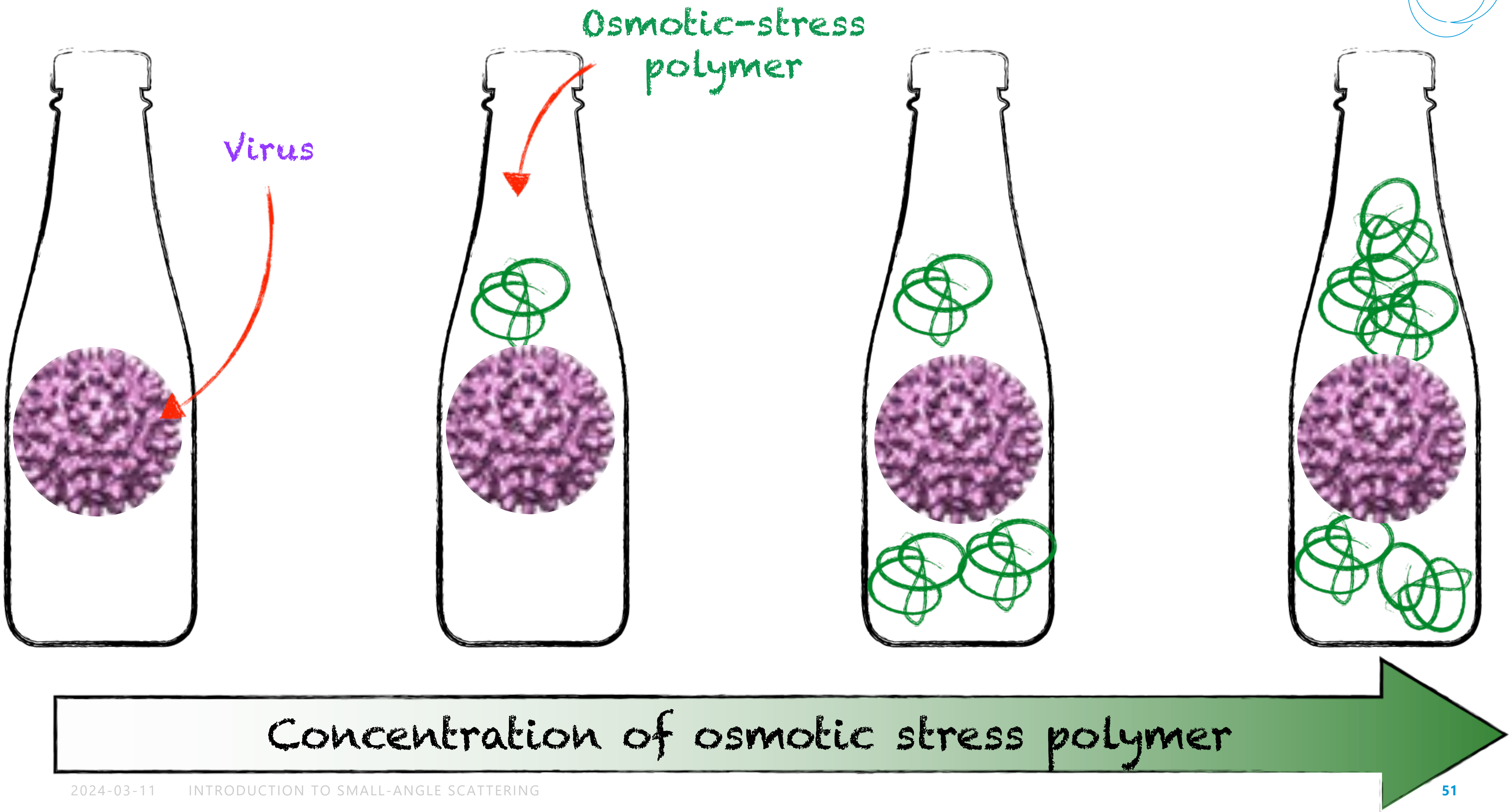
$$K = -v\frac{d\pi}{dv}$$

J. E. Houston, L. Fruhner, A. de la Cotte, J. Rojo González, A. Petrunin, U. Gasser, R. Schweins, J. Allgaier, W. Richtering, A. Fernandez-Nieves, and A. Scotti. *Science Advances* **8**: eabn6129 (2022); A. Scotti, U. Gasser, A. V. Petrunin, L. Fruhner, W. Richtering and J. E. Houston. *Soft Matter* **18**: 5750 (2022).

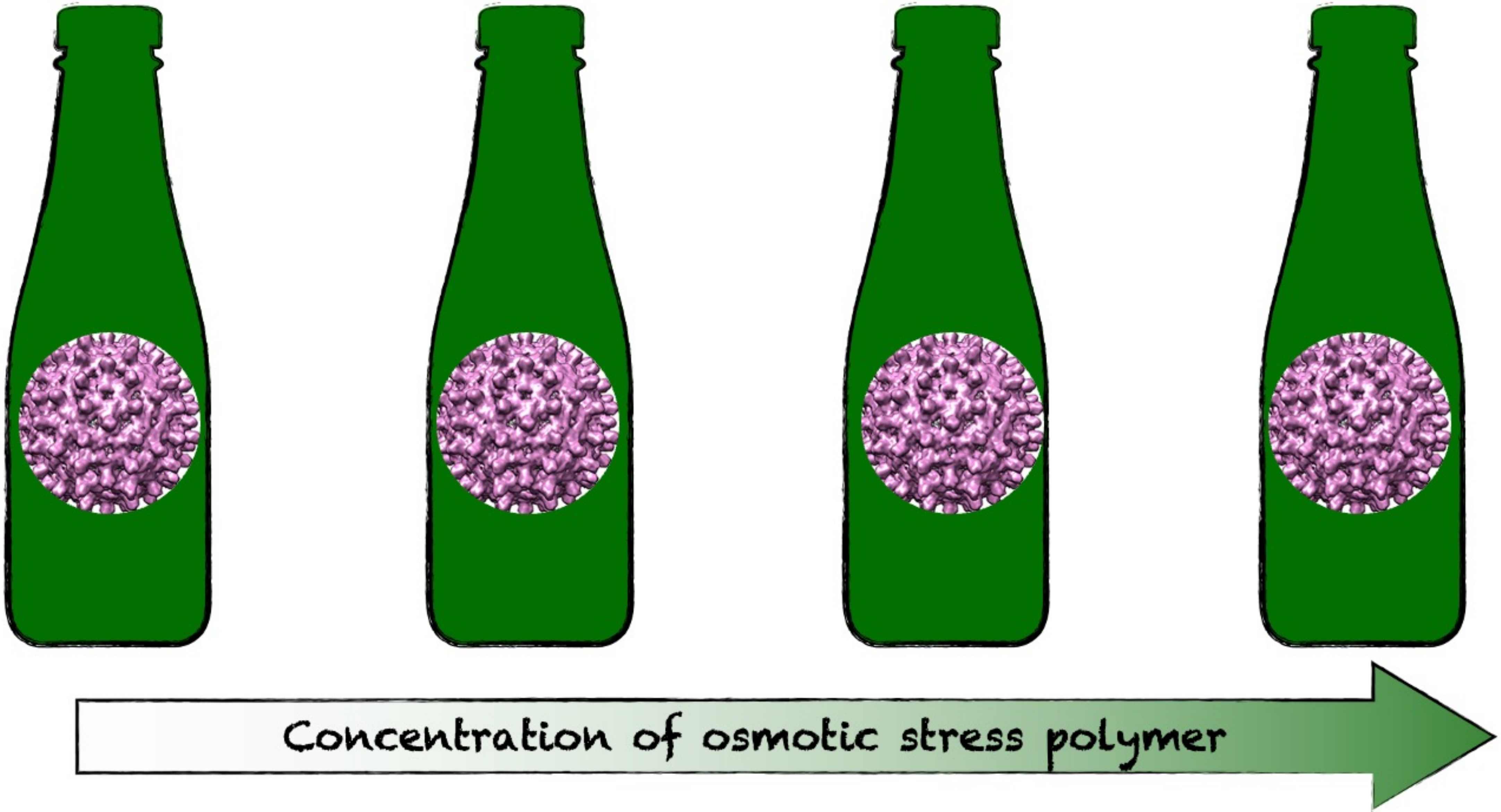
Bio-colloids and osmotic stress



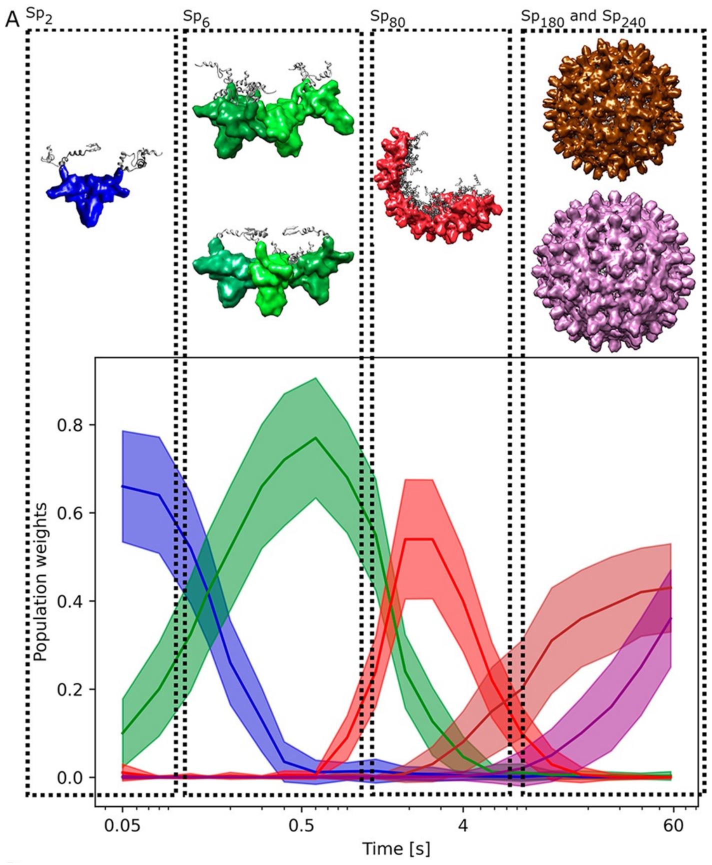
Bio-colloids and osmotic stress

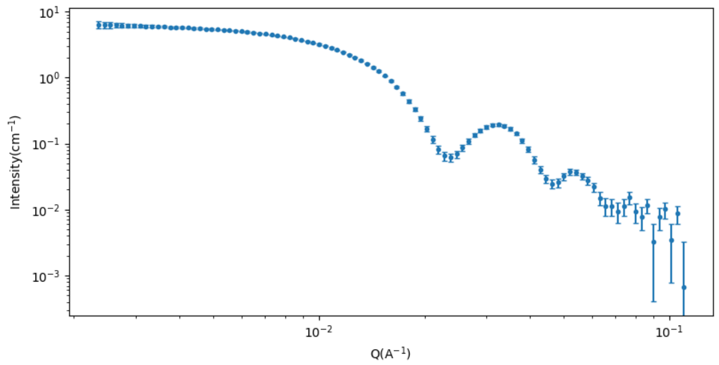


Bio-colloids and osmotic stress, contrast



Interesting also for bio-relevant colloids





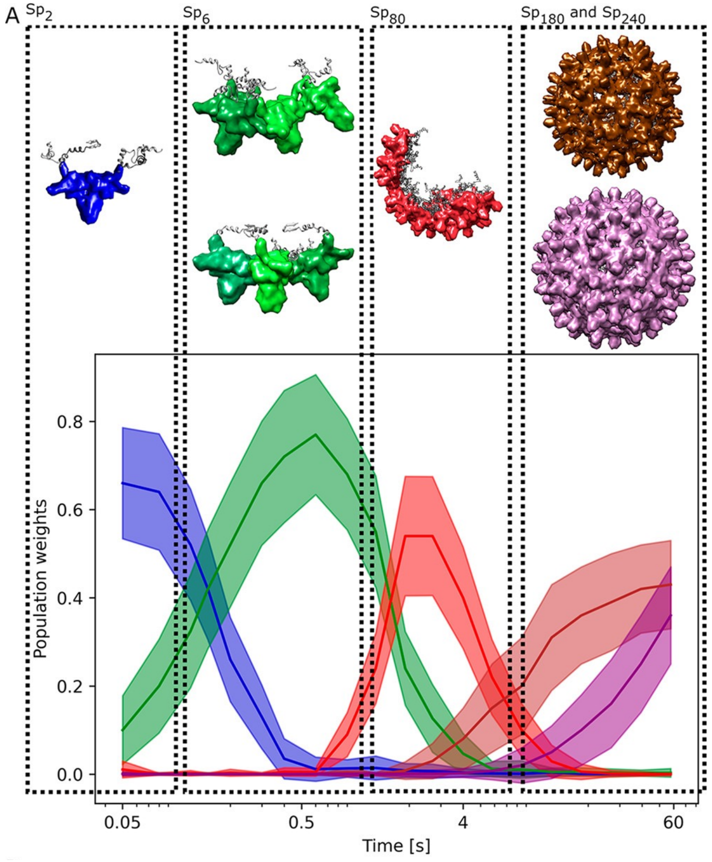
Explore the effects of different osmotic stress on the final architecture of the viral capsid and the self-assembly.

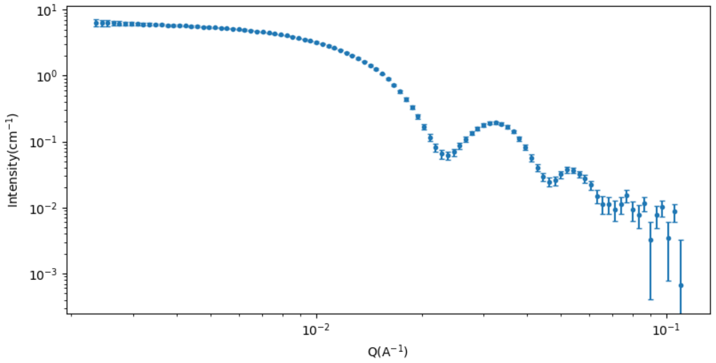
I. André (LU, Sweden)

W. Potrzebowski (ESS, Sweden)

A. Scotti (Lund/Malmö, Sweden)

Interesting also for bio-relevant colloids

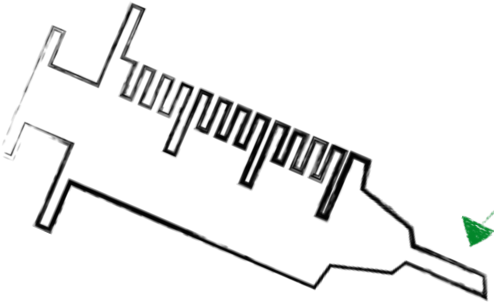


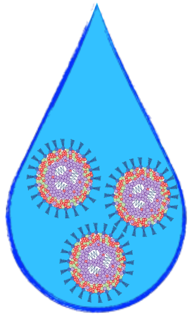


Explore the effects of different osmotic stress on the final architecture of the viral capsid and the self-assembly.

I. André (LU, Sweden)

W. Potrzebowski (ESS, Sweden)





F. Sebastiani (Copenhagen Uni, Denmark)

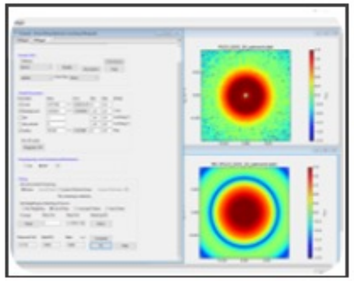
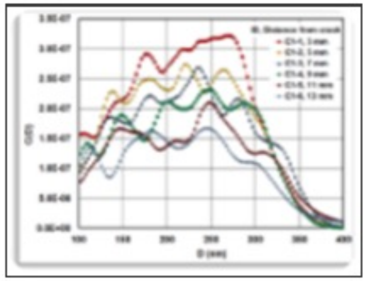
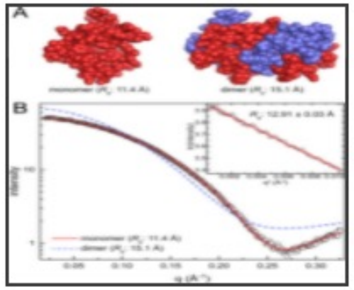
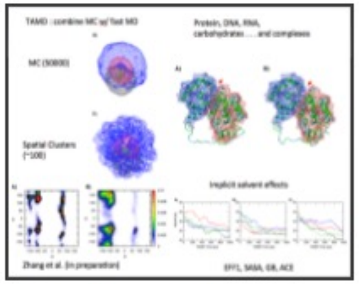
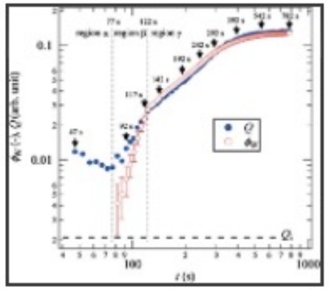
Probe the shape and internal structure of lipid nanoparticles under different osmotic stress.

5

Small-Angle Scattering Data Analysis

Data analysis

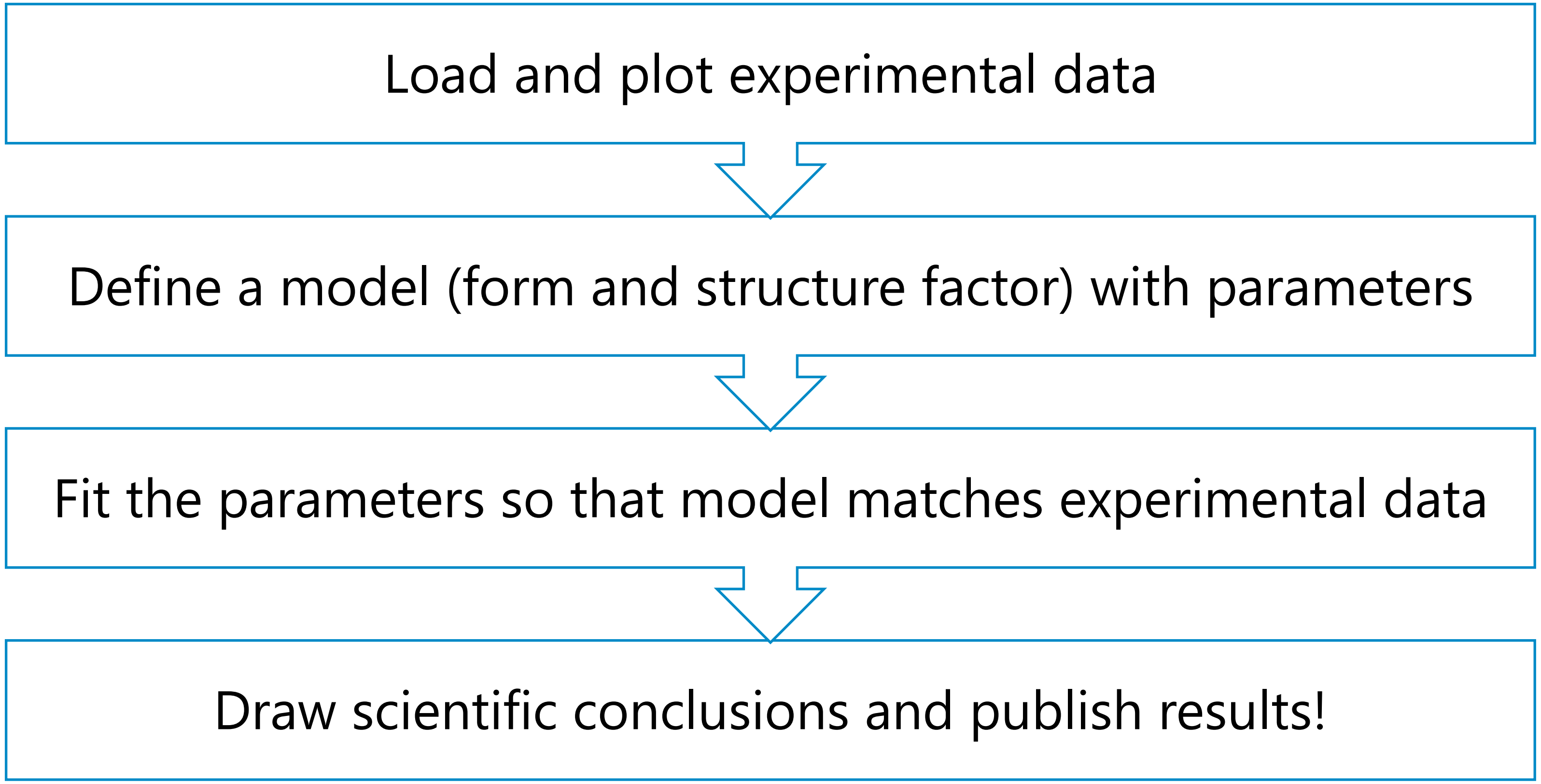
Few different options for SAS data

Model-Fitting Methods	Real-Space Methods	*Ab-Initio* Methods	MC/MD Methods	Other Methods
 *Example of 2D model-fitting using the SasView application*	 *Cavity size distributions in a steel weldment as derived from SANS 10.1179/1743284714Y.0000000577*	 *Ab-initio modelling of polcalcin constrained by SAXS 10.1002/pro.3376*	 *MC & TAMD modelling of proteins constrained by SANS 10.1016/j.jmgm.2017.02.010*	 *Time evolution of the invariant during crystallisation of P4MP1 10.1038/pj.2012.204*

https://www.isis.stfc.ac.uk/Pages/SANSdataanalysisOverview.aspx

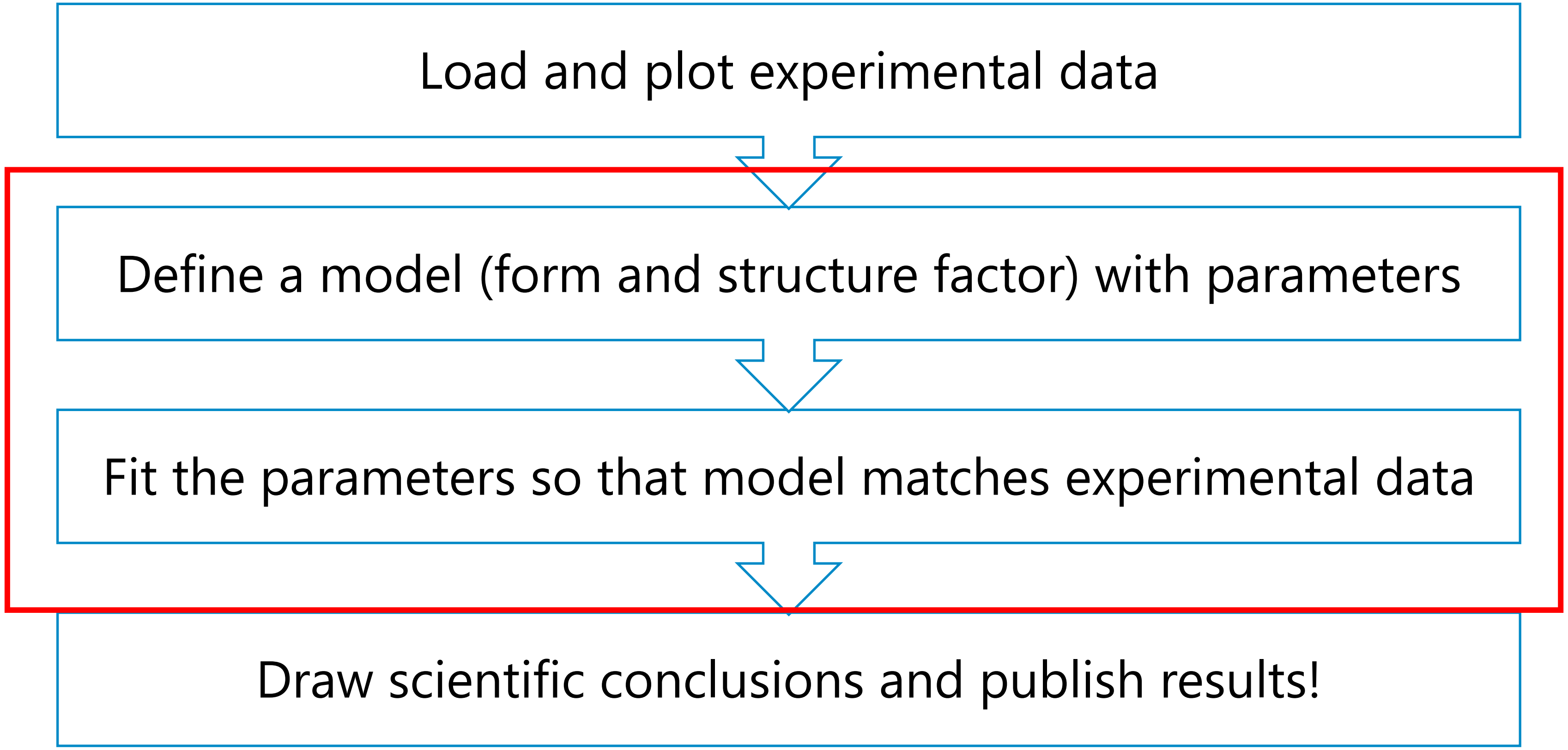
Typical data fitting workflow

For reduced data:

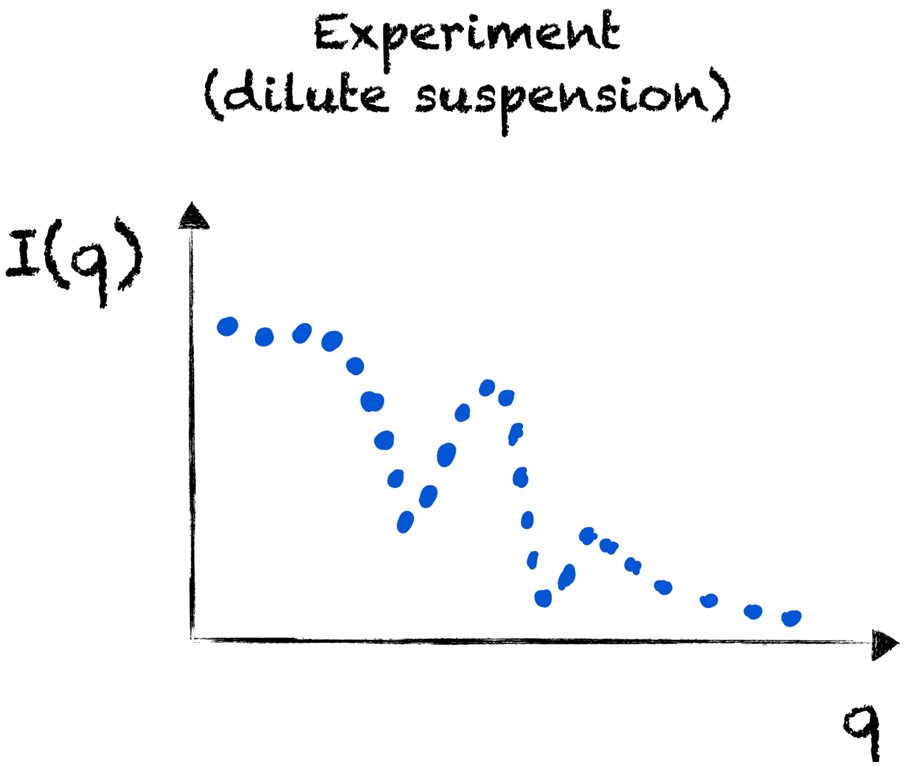


Typical data fitting workflow

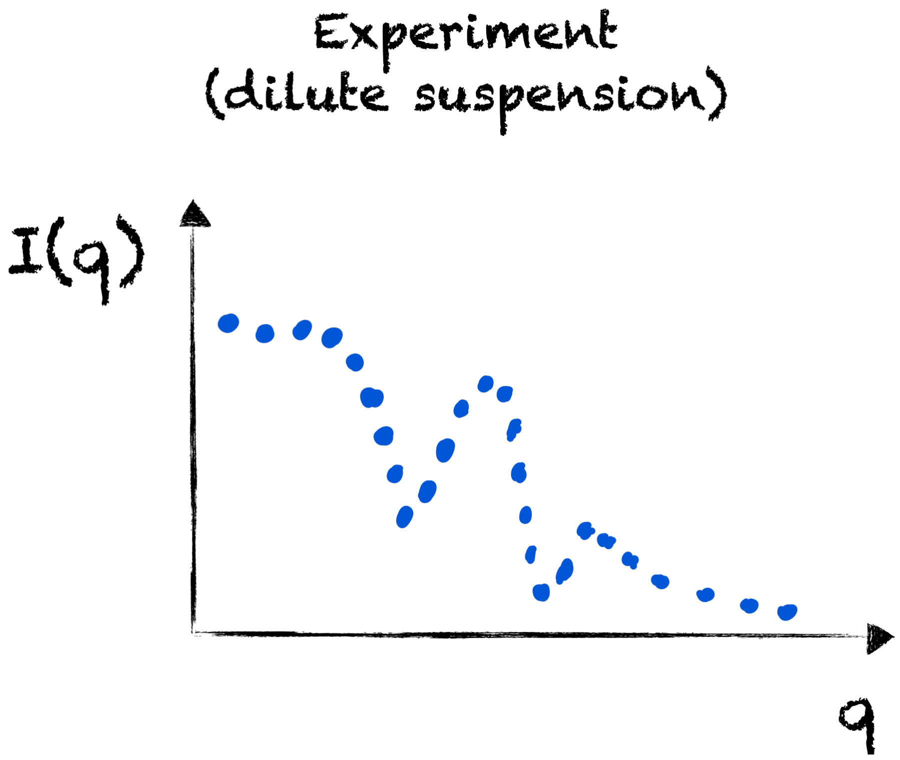
For reduced data:

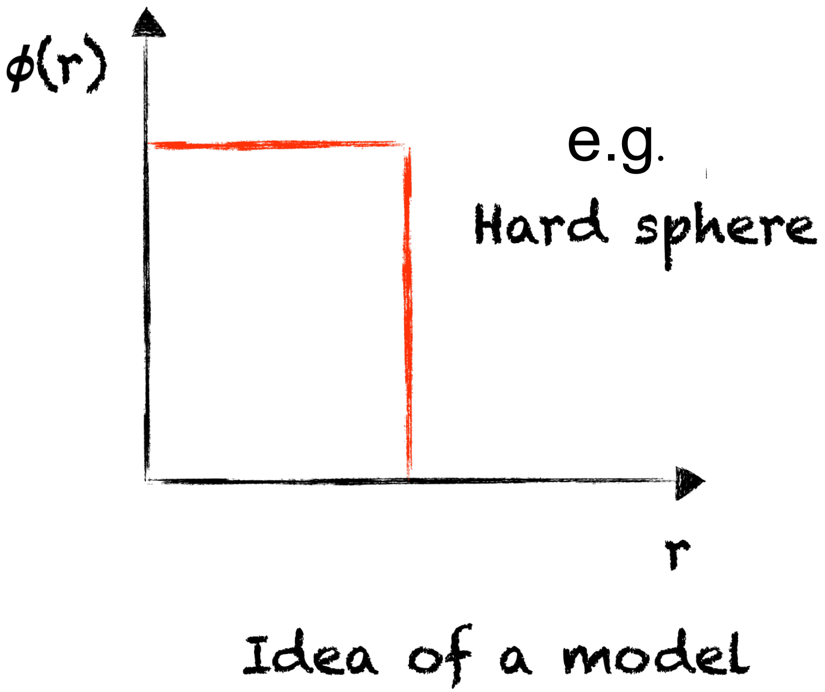


What is data fitting for SAS?

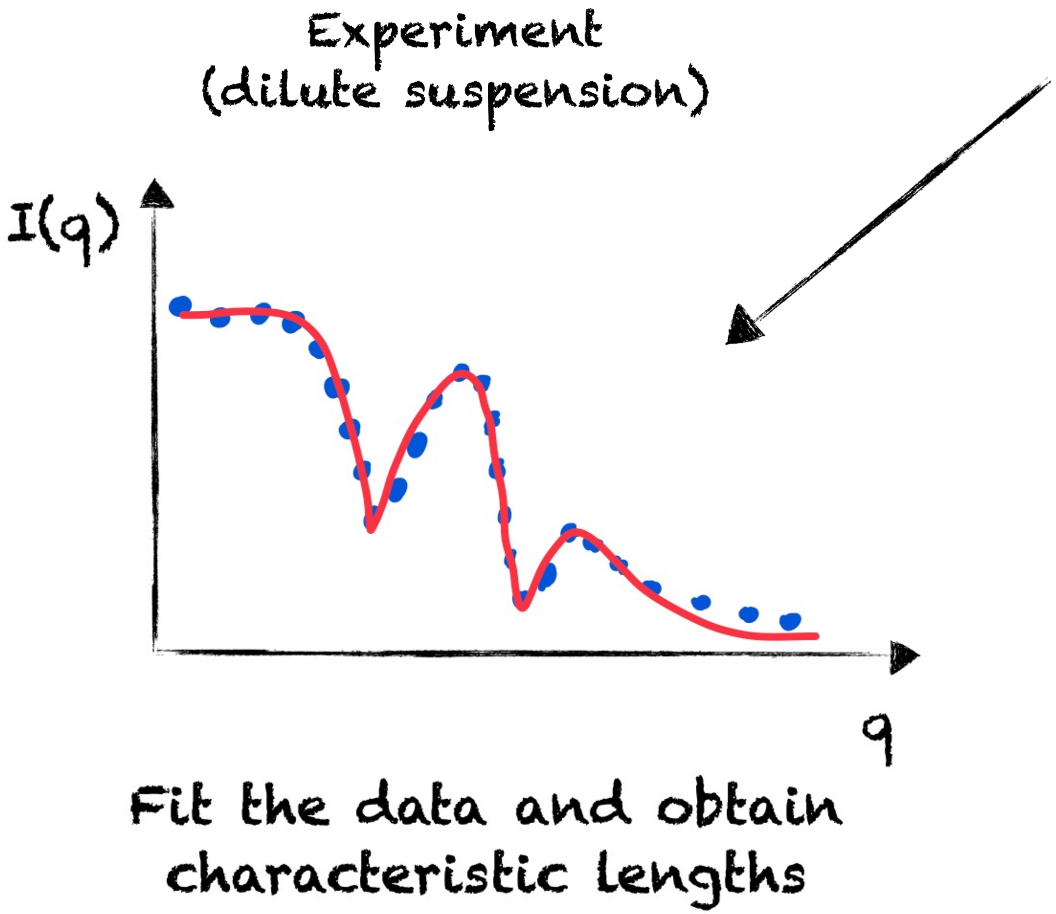


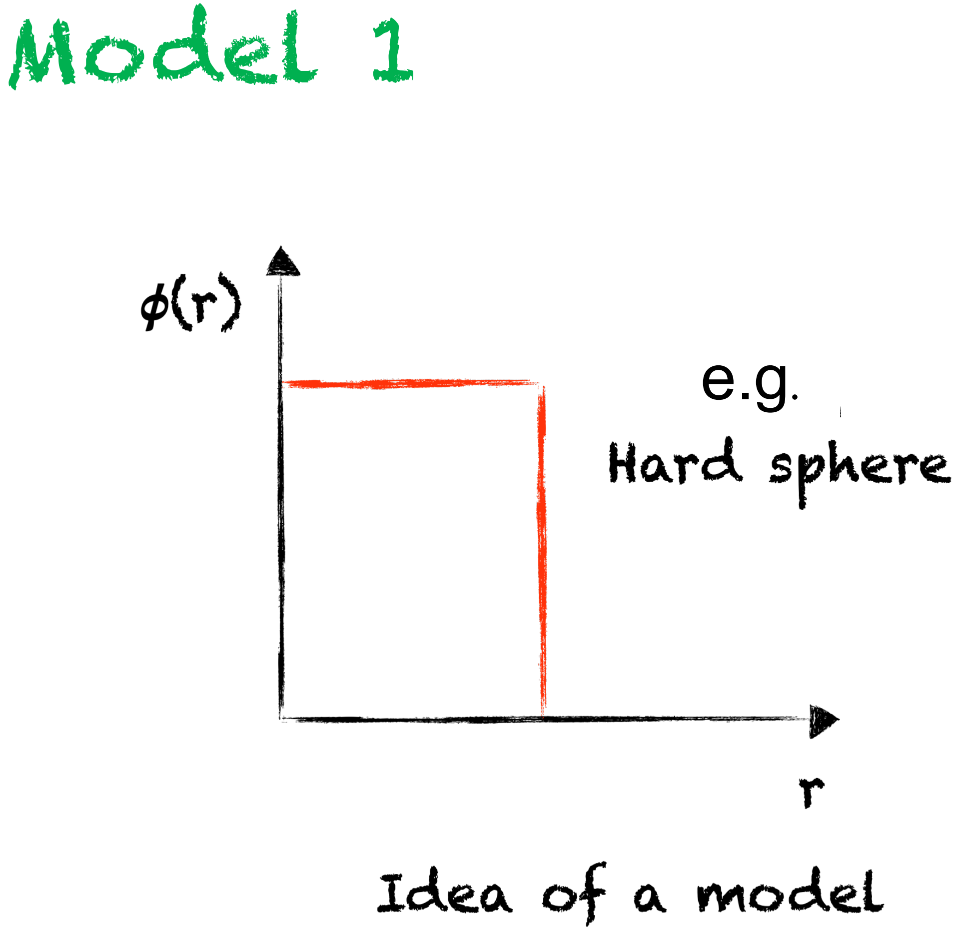
What is data fitting for SAS?





What is data fitting for SAS?

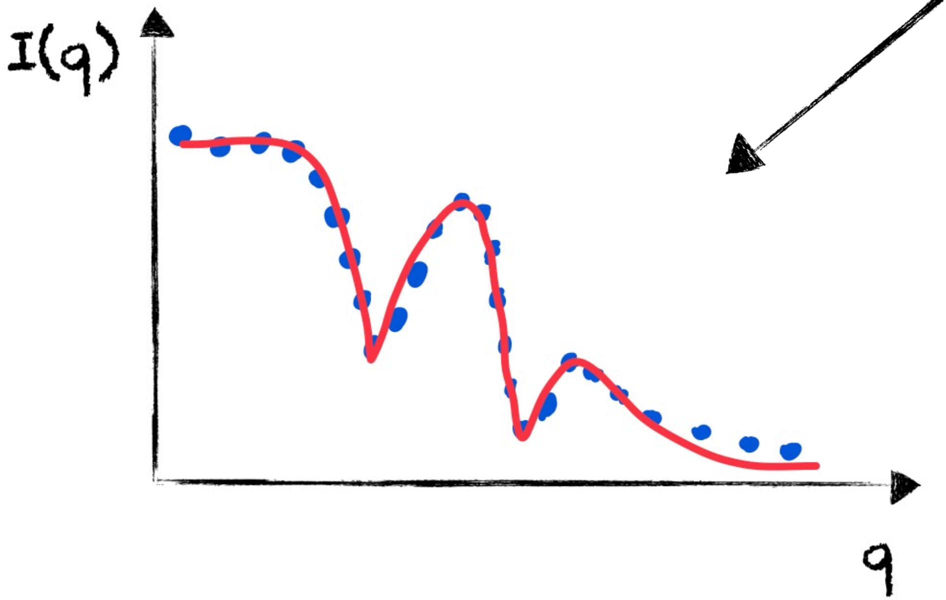




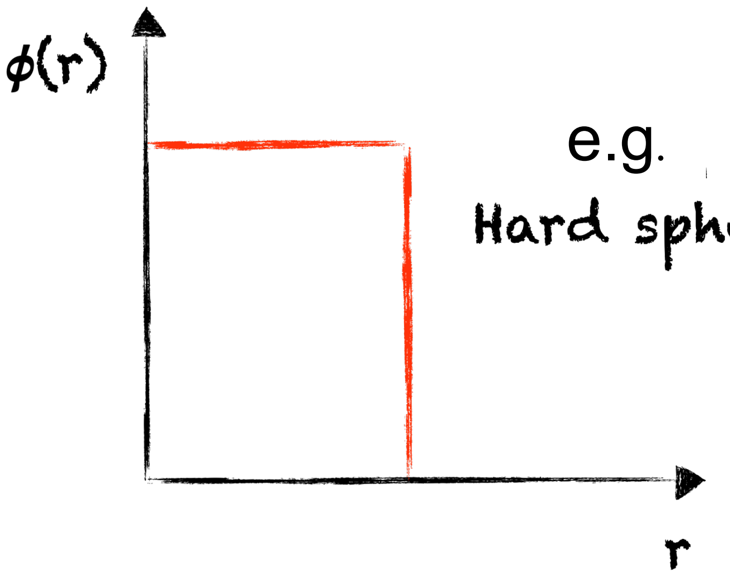
What is data fitting for SAS?

Model 1

Experiment
(dilute suspension)



Fit the data and obtain characteristic lengths

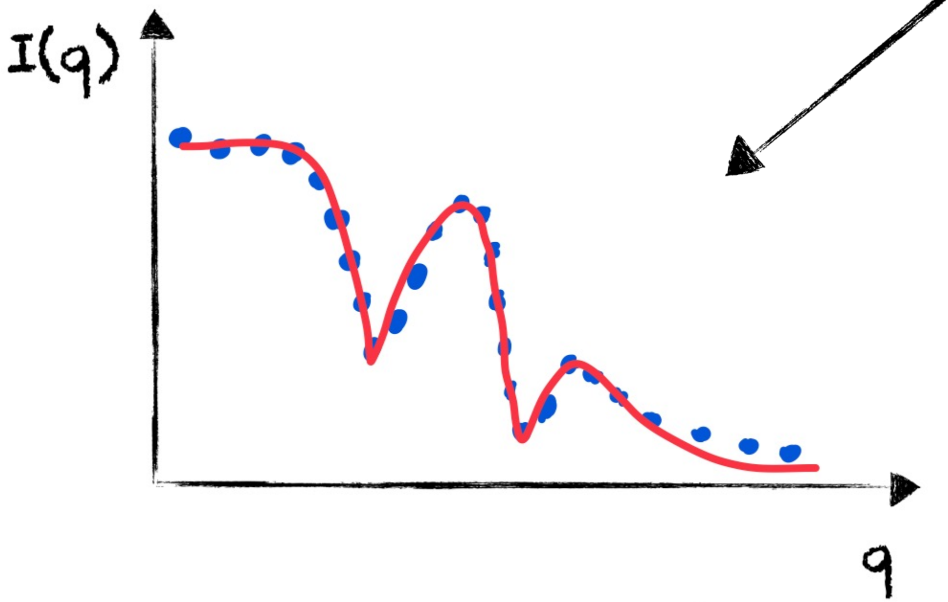


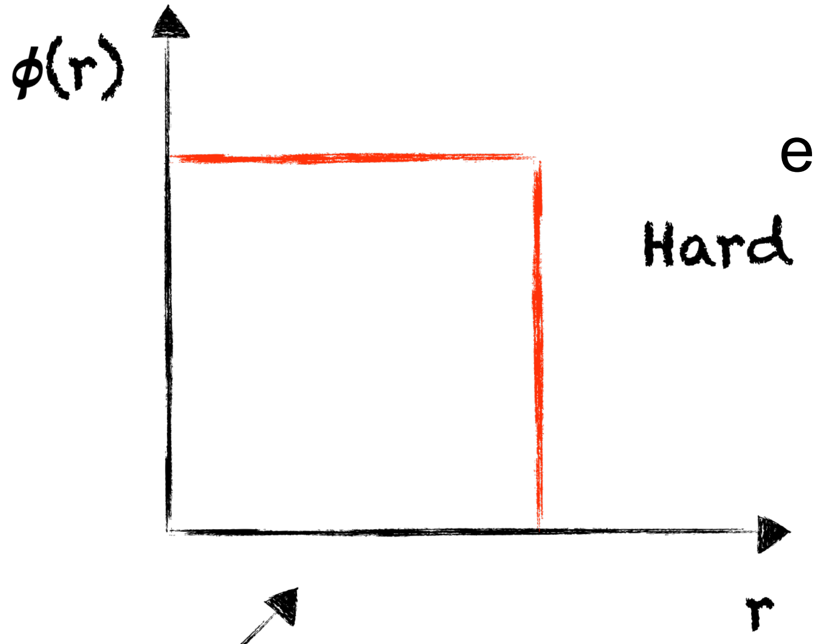
Idea of a model

What is data fitting for SAS?

Model 1

Experiment
(dilute suspension)





e.g.
Hard sphere

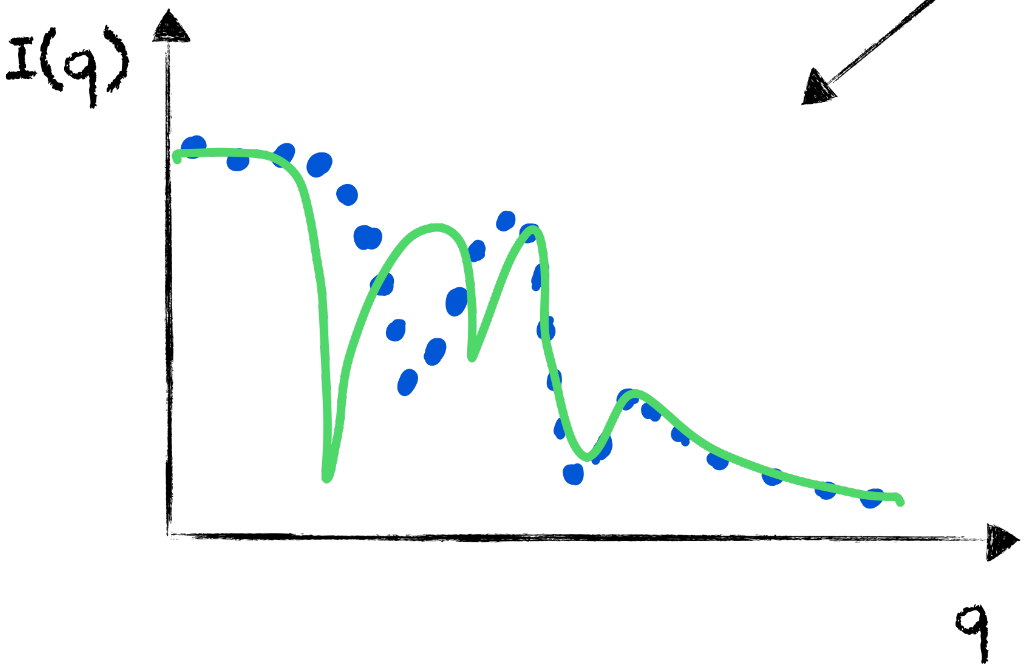
Fit the data and obtain characteristic lengths

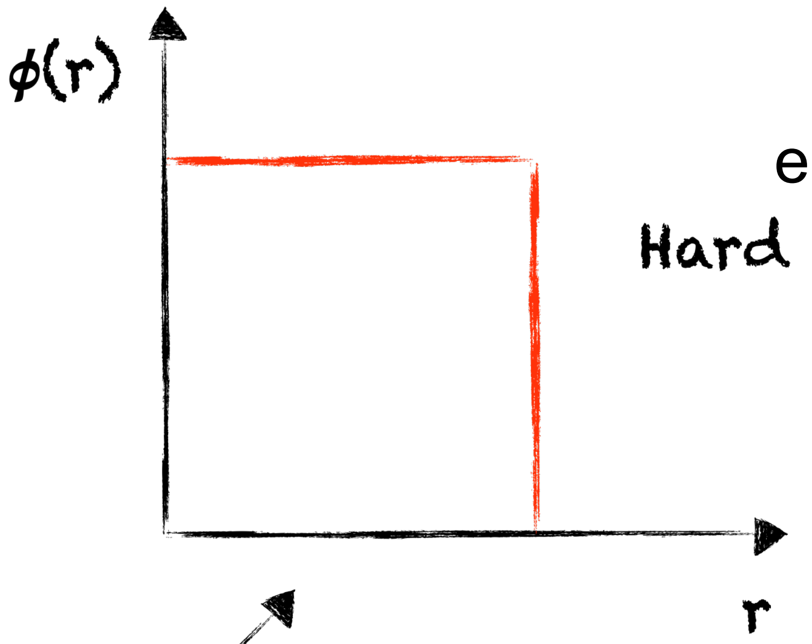
Model is OK!

What is data fitting for SAS?

Experiment
(dilute suspension)

Model 1



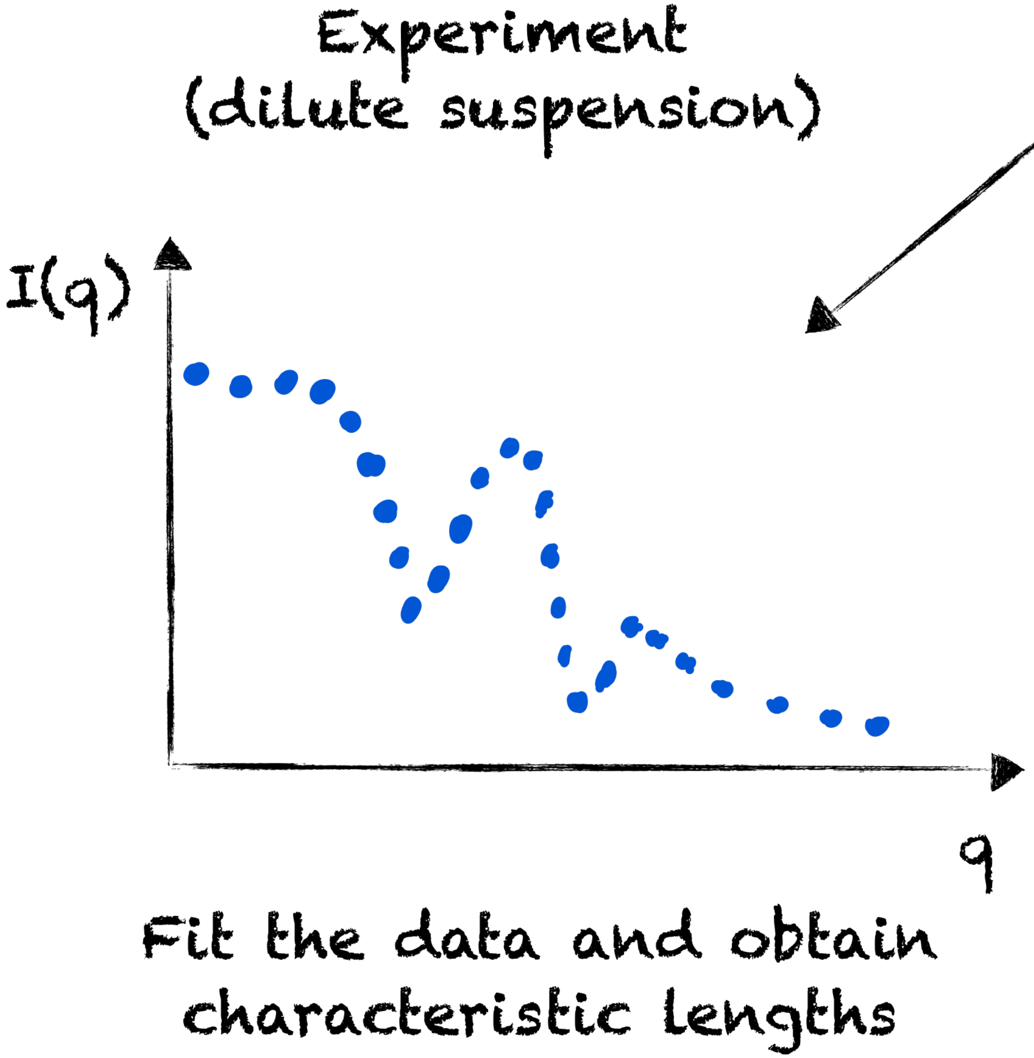


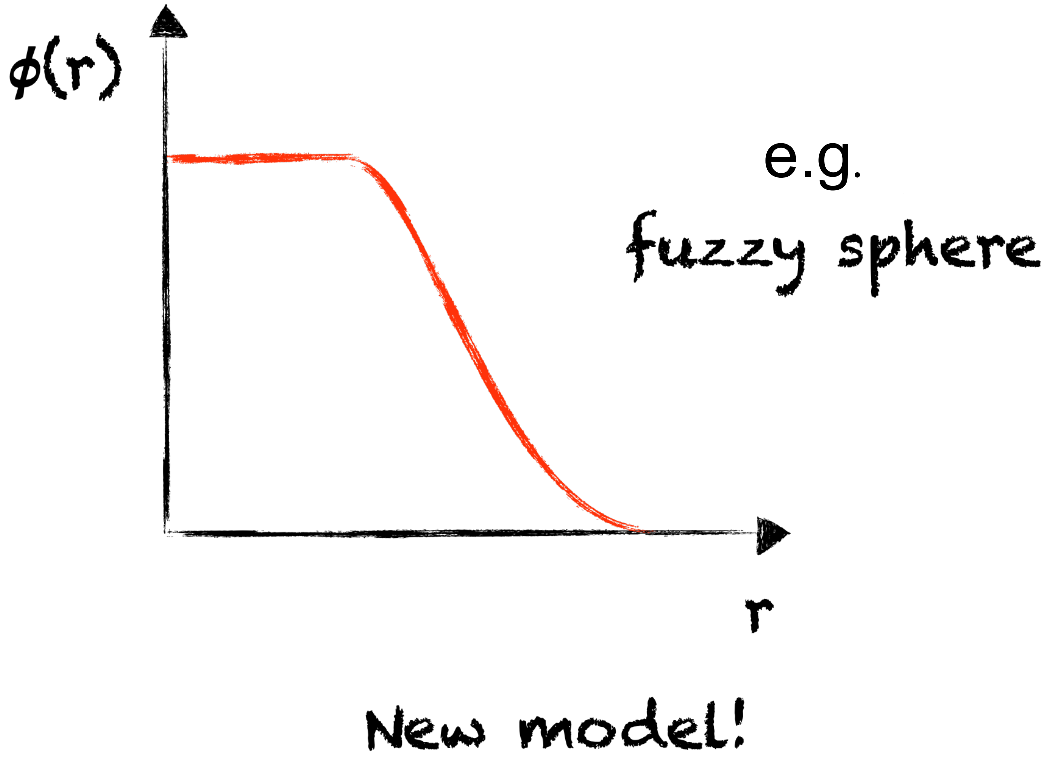
e.g.
Hard sphere

Fit the data but it does not work

Model is wrong!

What is data fitting for SAS?

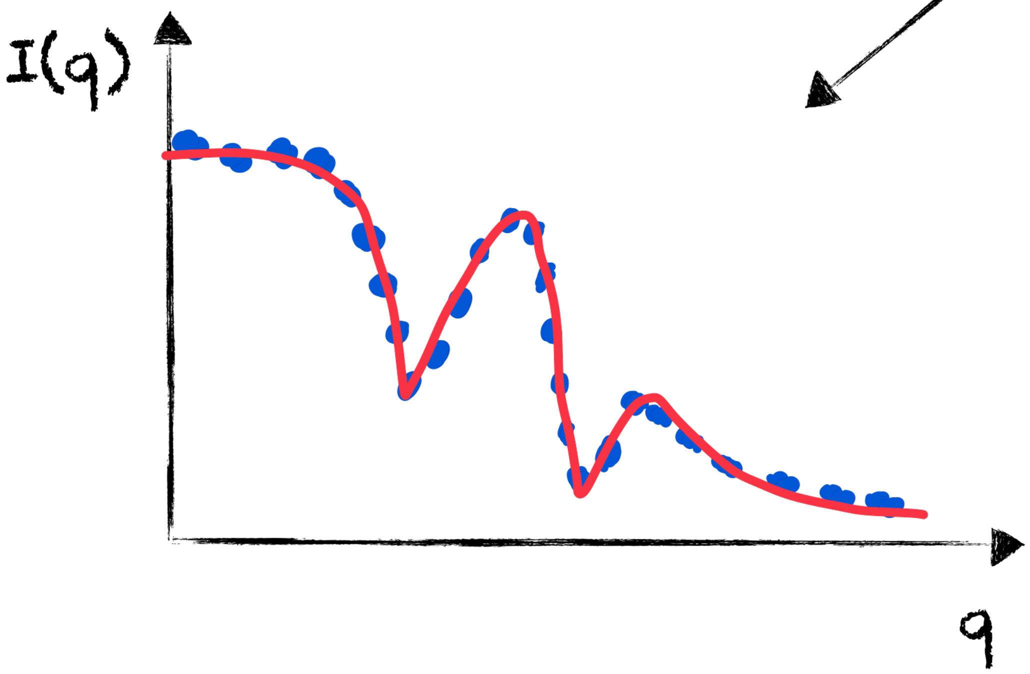




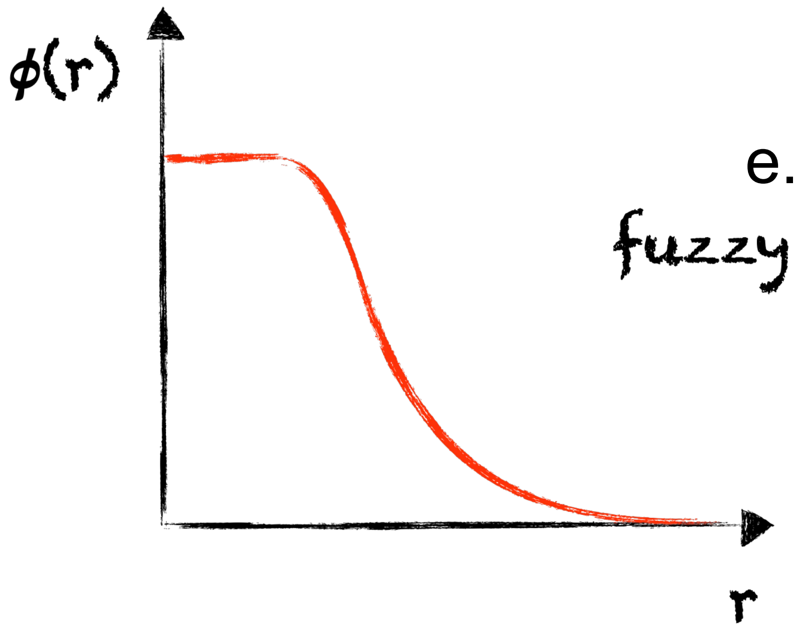
What is data fitting for SAS?

Experiment
(dilute suspension)

Model 2



Fit the data and obtain characteristic lengths



e.g.
fuzzy sphere

Model is OK!

Fitting in SasView

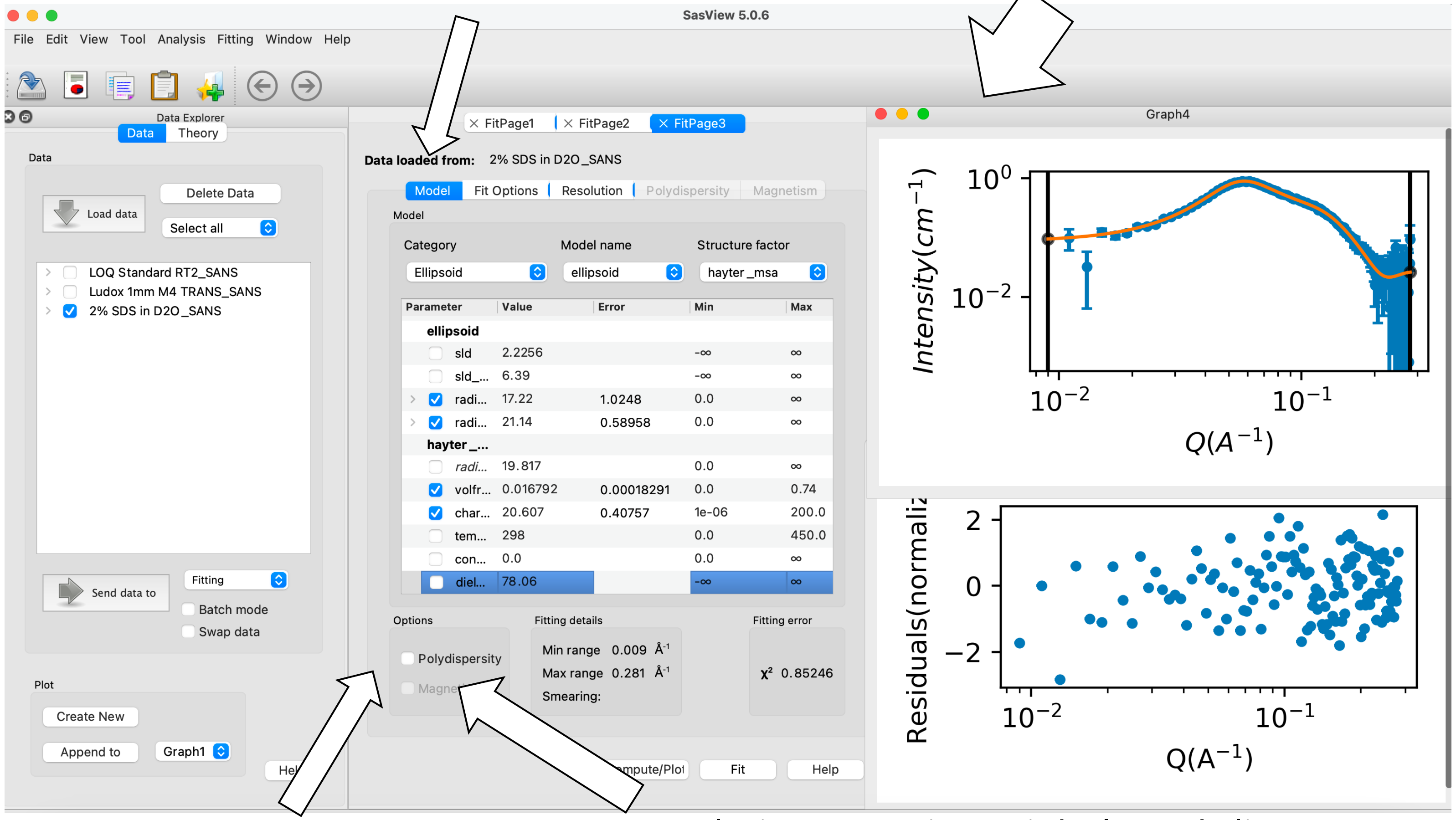
70+ models to explain data

Wide choice of built-in models (> 70)
F(Q), S(Q) & F(Q)*S(Q)

Single, batch and simultaneous
1D and 2D fitting

Data management
Common data formats supported, including NXCansas & *cansas1D*

Analysis Tool Choice
&
Plotting



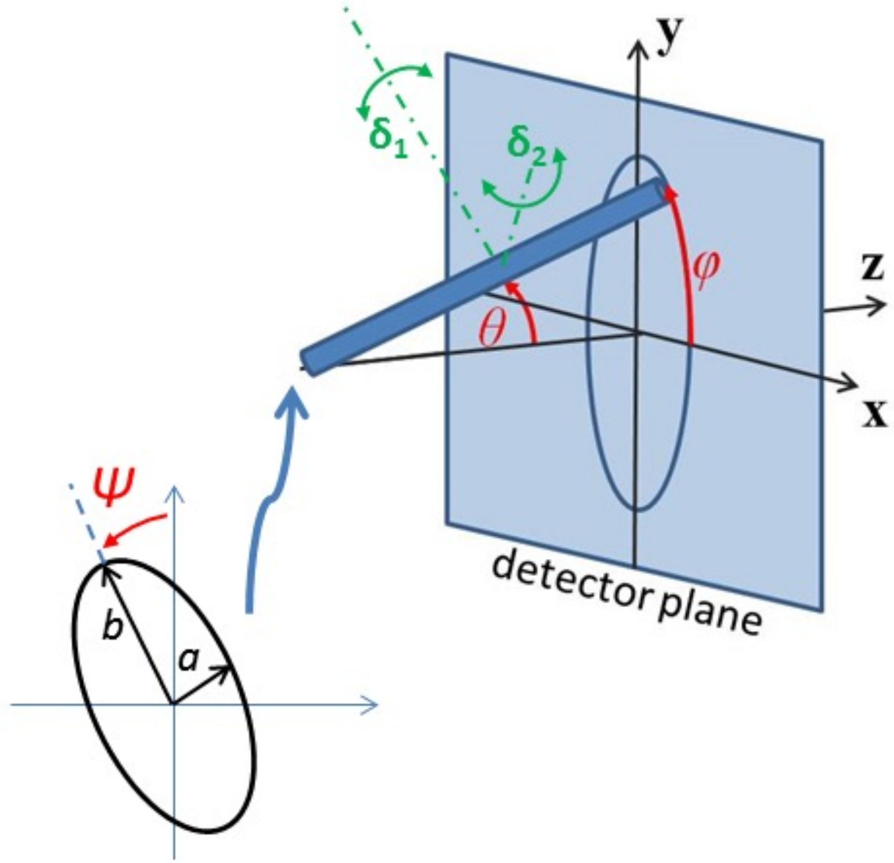
Polydispersity (choice of distribution and distribution parameters)

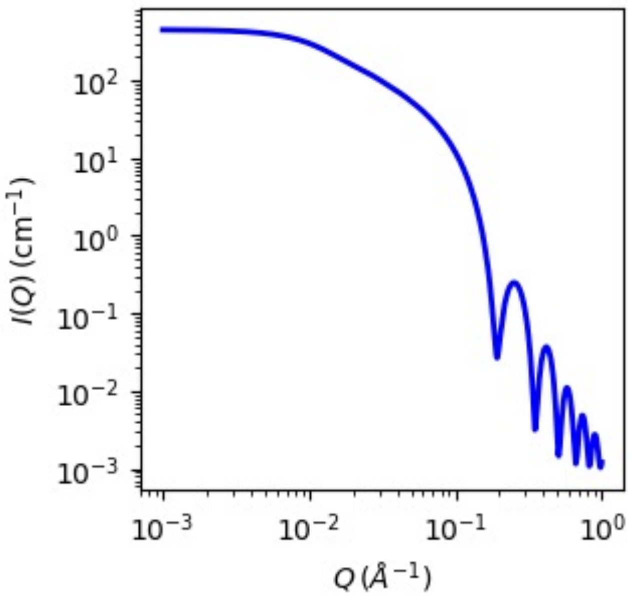
Resolution smearing (pinhole and slit)
Automatically from data or provide parameters

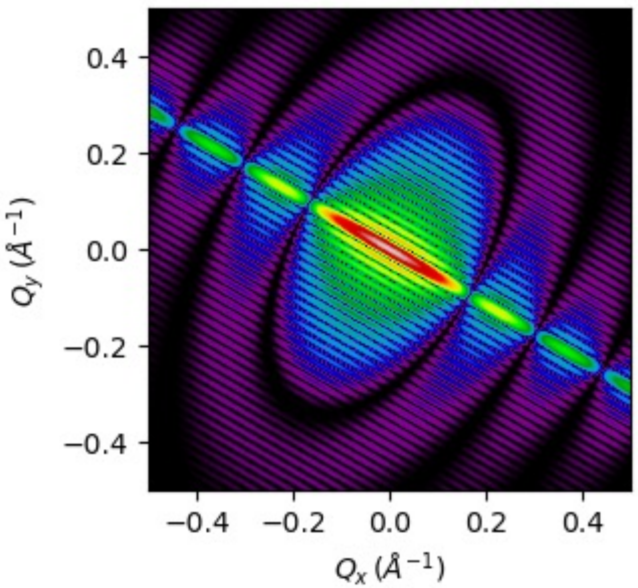
Courtesy of Wojciech Potrzebowski

2D fitting

For oriented or magnetic particles





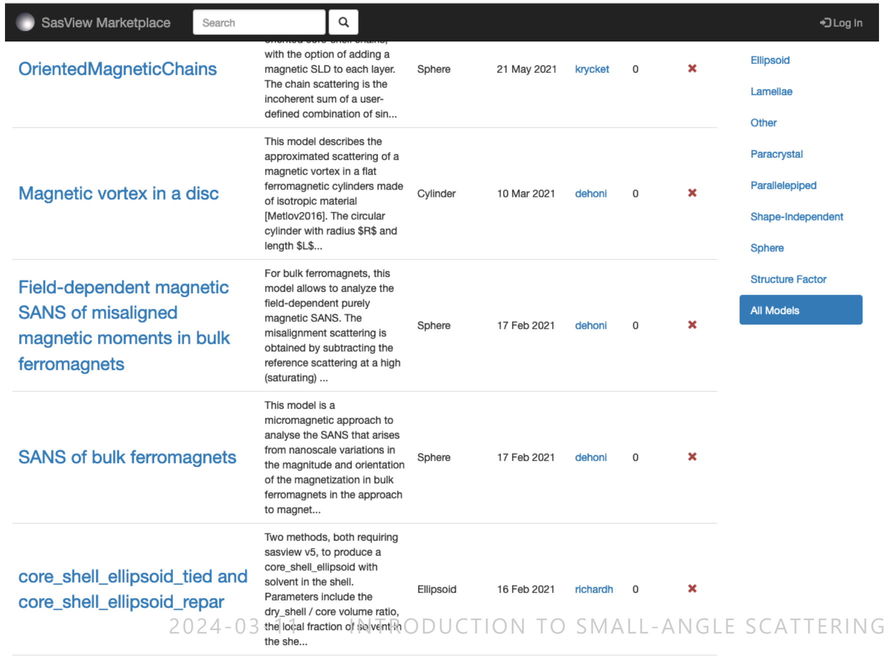


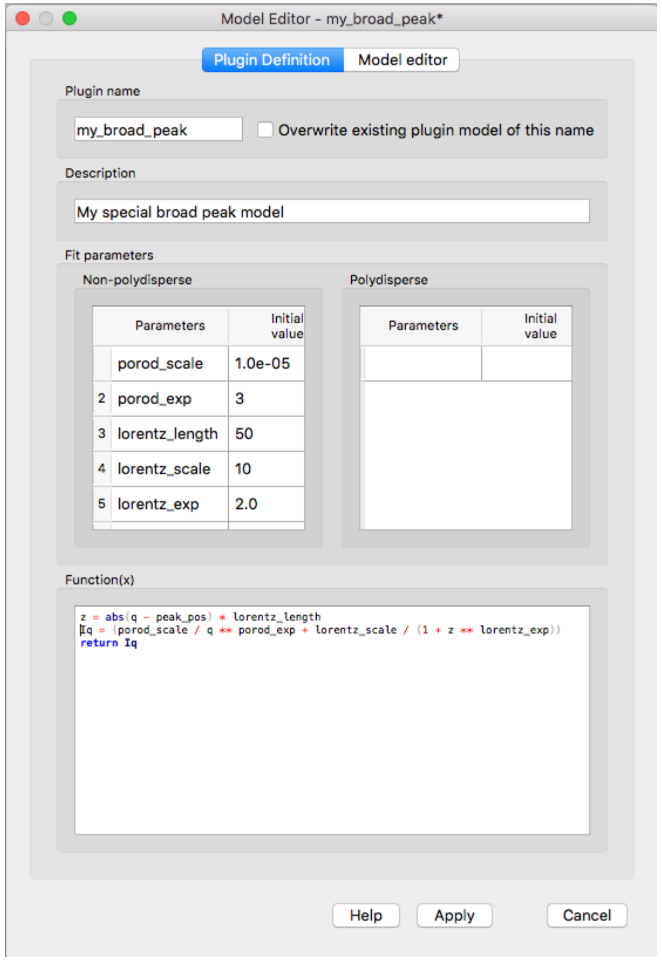
1D and 2D cylinder model

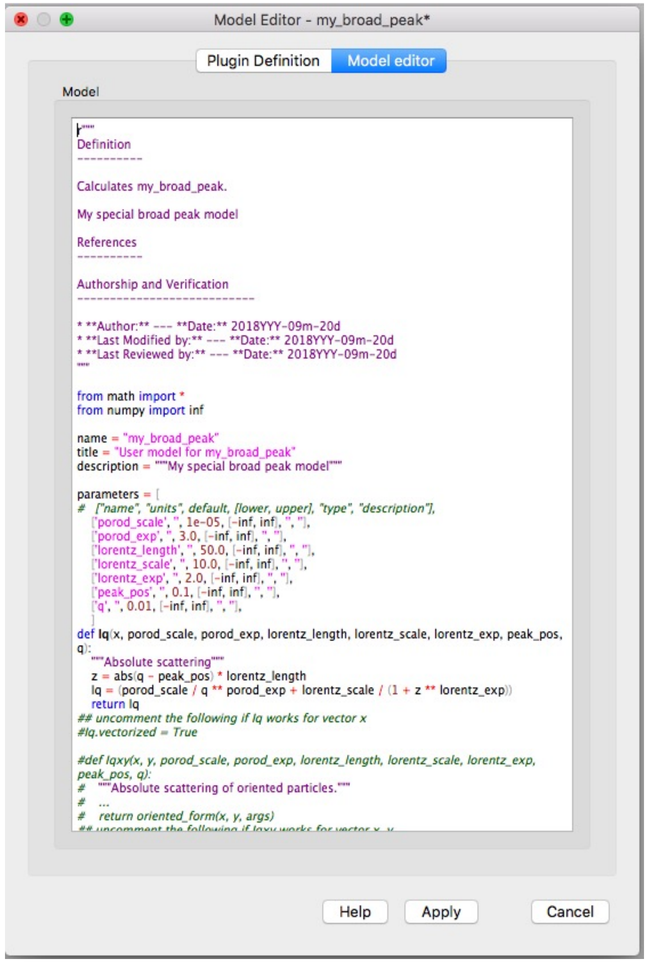
Plugin models

SasView provides tools and infrastructure for custom/plugin models

- Dedicated editor
- Syntax and performance testing
- Directly available in SasView ecosystem
- Community developed models can be deposited to marketplace: https://marketplace.sasview.org/

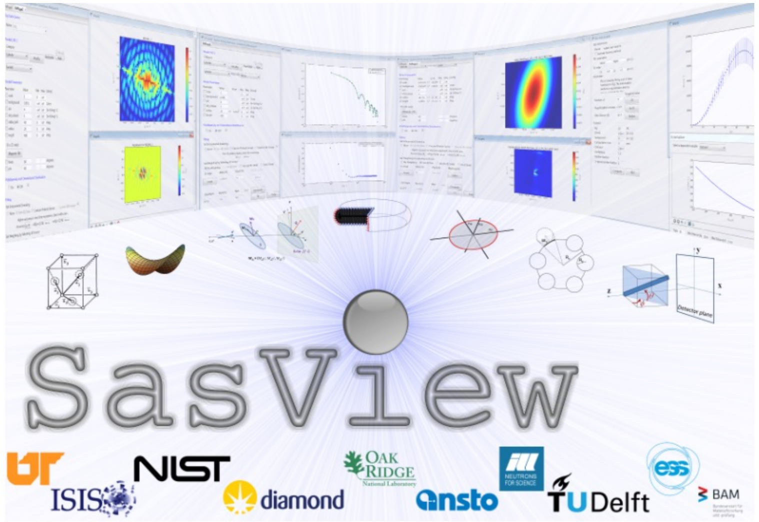






Plugin models tutorial

Available at https://www.sasview.org/download/



SasView Tutorials

Creating Custom Fitting Models in SasView Version 5.x

www.sasview.org

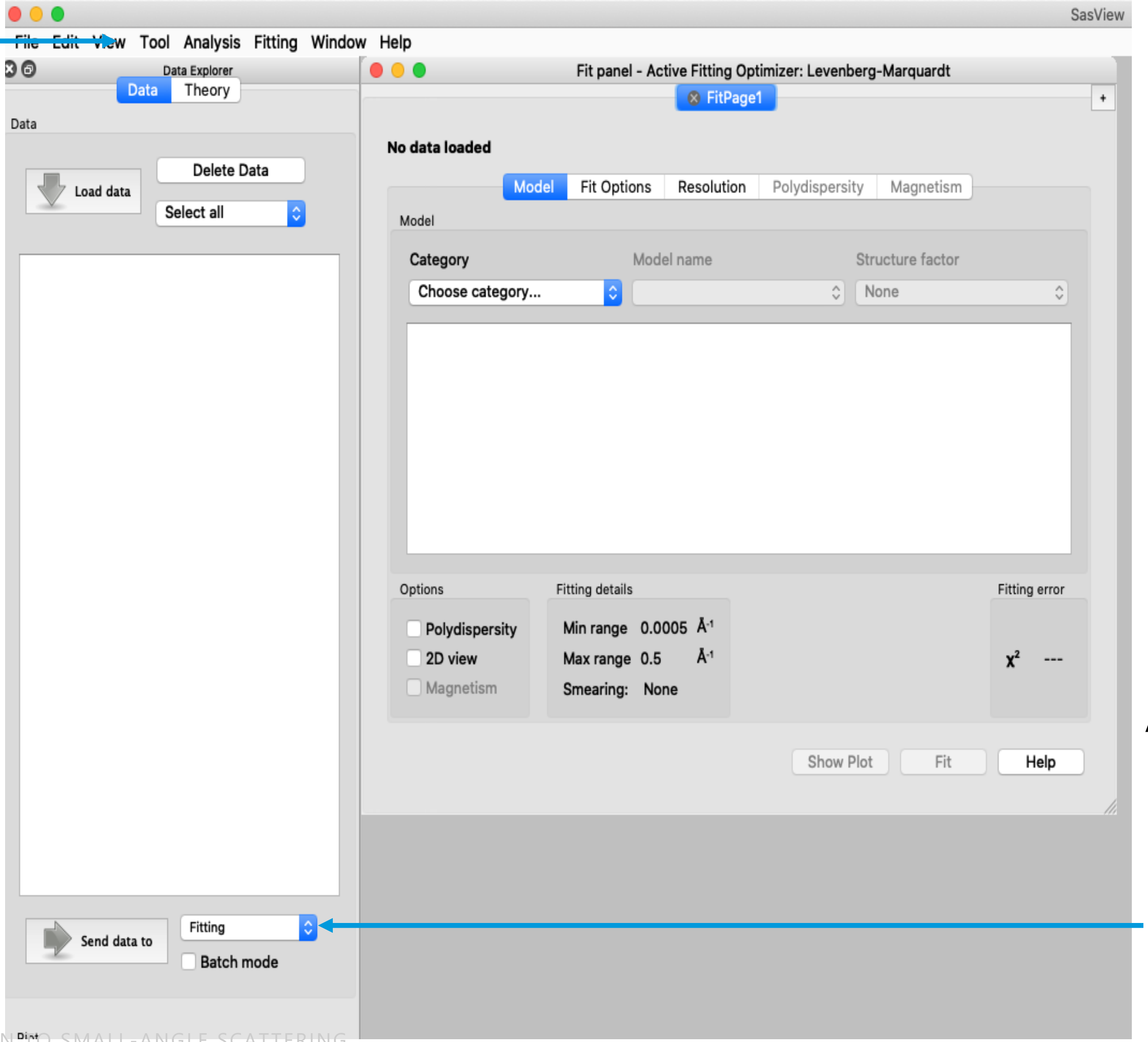
Contents

Other SasView functionality

Various utility tools and calculators

Tools

- Data Operation
- SLD calculator
- Density/Volume calculator
- Slit Size Calculator
- Kiessig Thickness Calculator
- Q Resolution Estimator
- Generic Scattering calculator
- Orientation Viewer
- Python Shell/Editor
- Image Viewer
- File Converter

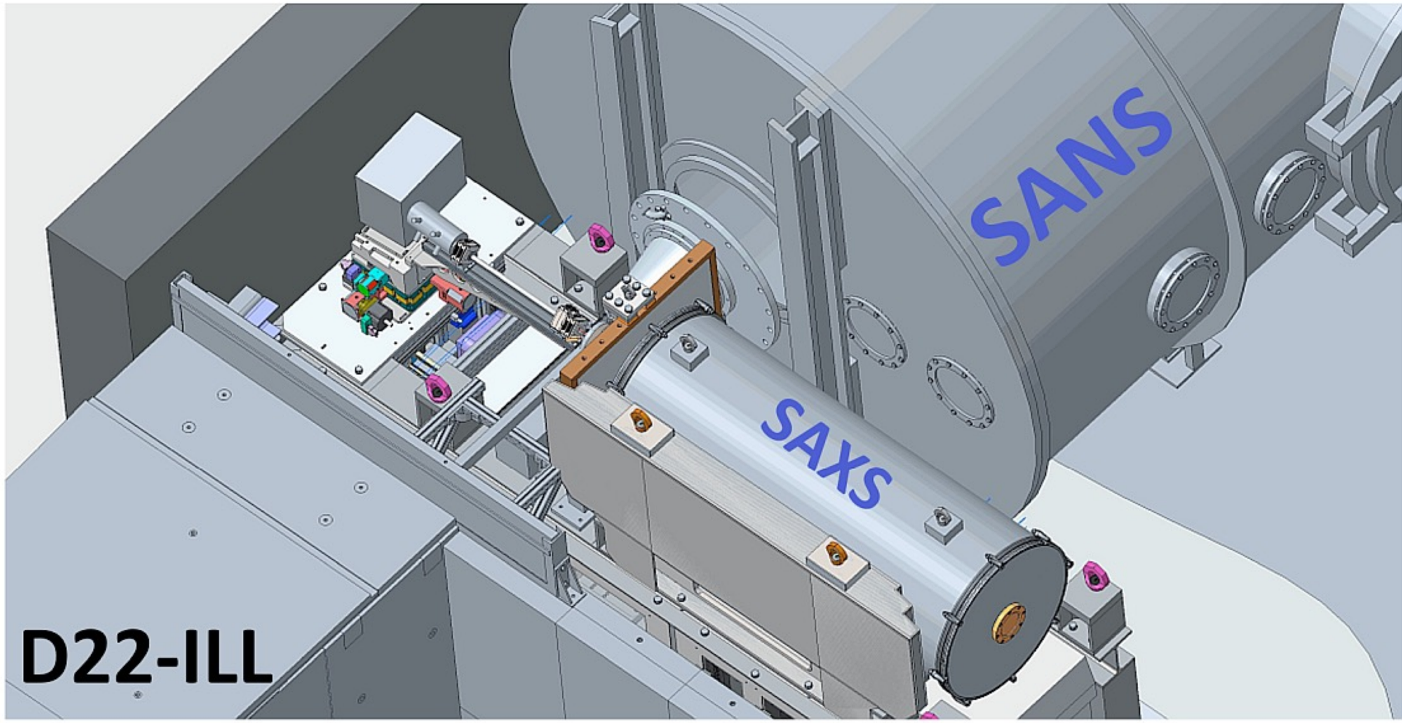


Analysis

- Fitting
- Invariant
- Pr Inversion
- Correlation Function

Combined SAXS and SANS

Simultaneous measurements on the same sample

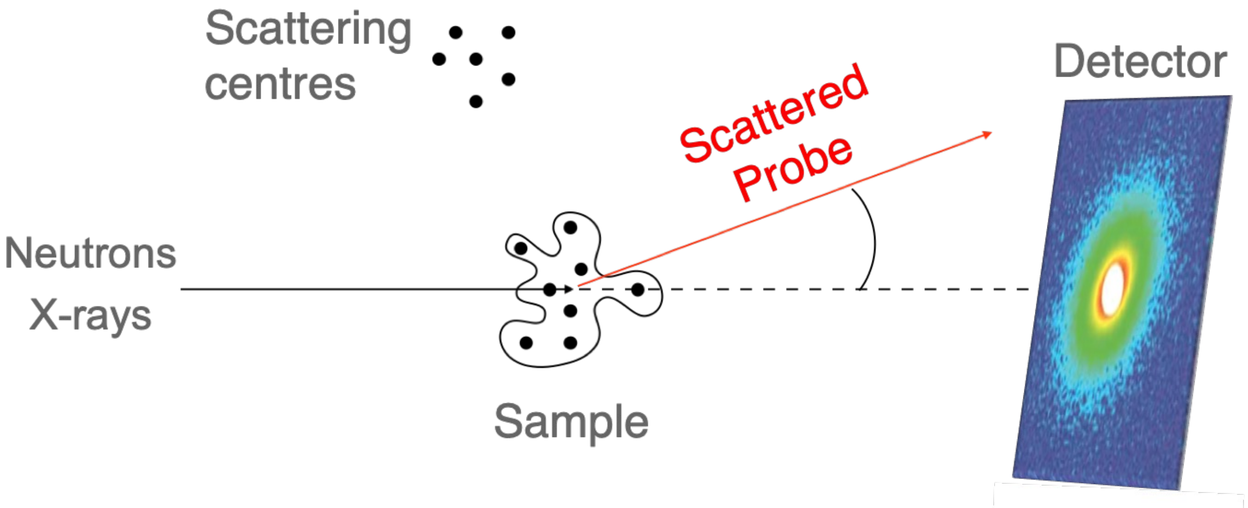


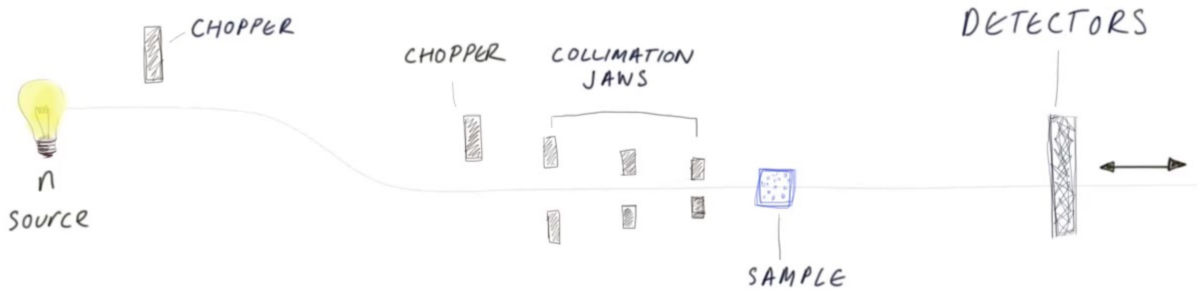
- Running at D22 instrument at ILL
- Low-flux SAXS source
- Specialized sample environment
- Non-trivial data analysis
- SasView (6.0.0) provides a weighting mechanism in simultaneous fitting

6

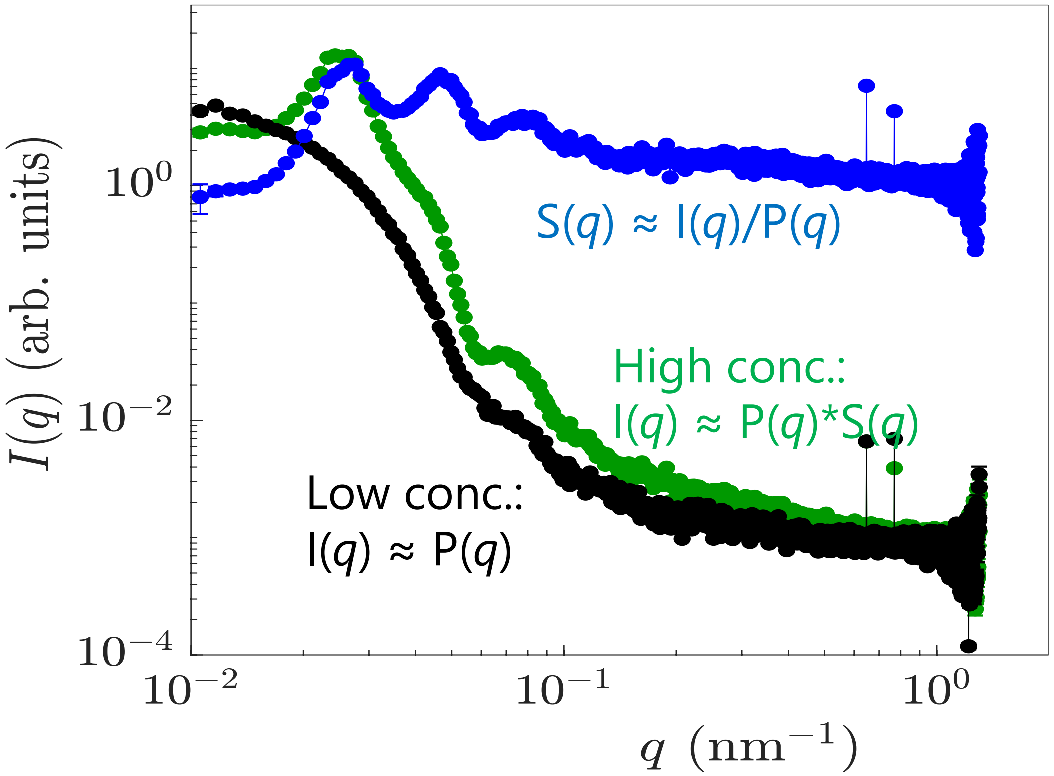
Small-angle scattering instruments

Small-angle scattering





$$I_{exp}(q) = n\Delta\rho^2 V^2 P(q) S(q)$$



SAXS: synchrotron vs. benchtop

synchrotron
High brightness
High time resolutions: measurements in ms
Risk of damage to biomaterials
Large dynamic q-range often due to multiple detectors

SAXS: synchrotron vs. benchtop

synchrotron	Benchtop
High brightness	Low brightness – low risk of damage to bio materials, but poor time resolution
High time resolutions: measurements in ms	Poor time resolution: measurements in min-h
Risk of damage to biomaterials	Low risk of damage to biomaterials
Large dynamic q-range often due to multiple detectors	Limited dynamic q-range at one detector

SANS: Continuous vs. time-of flight

We need to probe the wavevector, Q with neutrons....

$$q = \frac{4\pi}{\lambda} sin(\frac{\theta}{2})$$

Continuous
Fixed wavelength (monochromatic)
Fixed λ, varying θ
Need several measurements at different detector distances to cover adequate q-range
Typically reactor sources (exceptions: TOF instruments in monochromatic mode)

SANS: Continuous vs. time-of flight

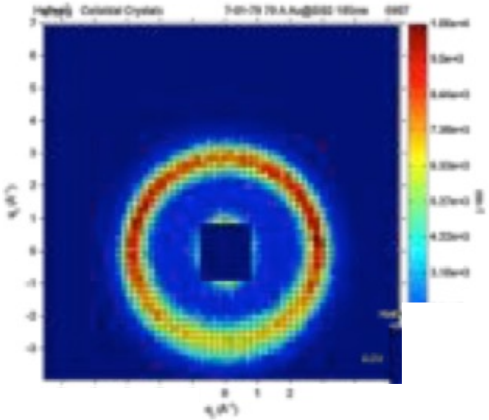
We need to probe the wavevector, Q with neutrons….

$$q = \frac{4\pi}{\lambda} sin(\frac{\theta}{2})$$

Continuous	Time-of-flight
Fixed wavelength (monochromatic)	Wavelength band
Fixed λ, varying θ	Varying θ, varying λ
Need several measurements at different detector distances to cover adequate q-range	Large dynamic q-range at one detector distance, q_{max}/q_{min}
Typically reactor sources (exceptions: TOF instruments in monochromatic mode)	Typically spallation sources (exceptions: ILL D33 and ANSTO Bilby)

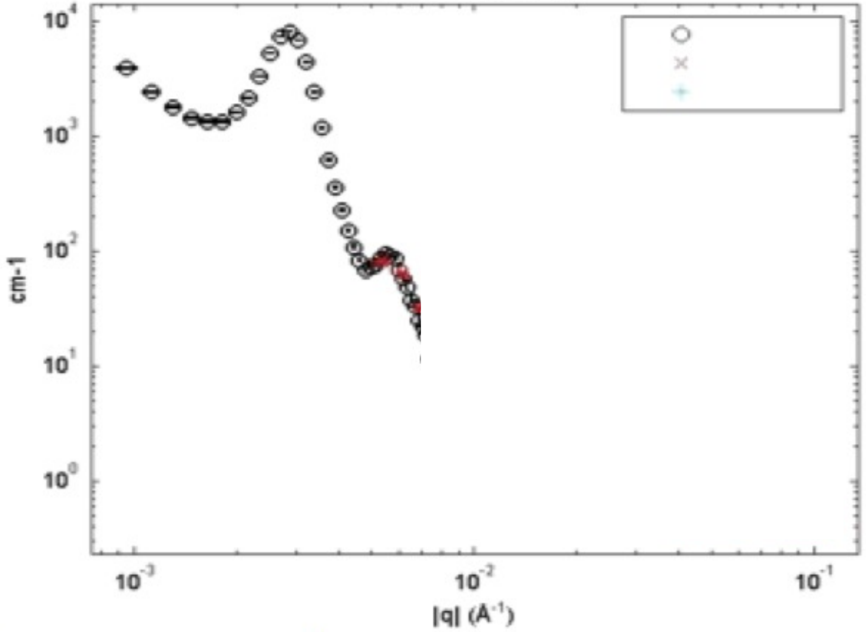
SANS: Continuous vs. time-of flight

High flux but often need several measurements at different distances to cover an adequate *Q*-range



Low q:
Largest sample-to-detector distance

Monochromatic beam



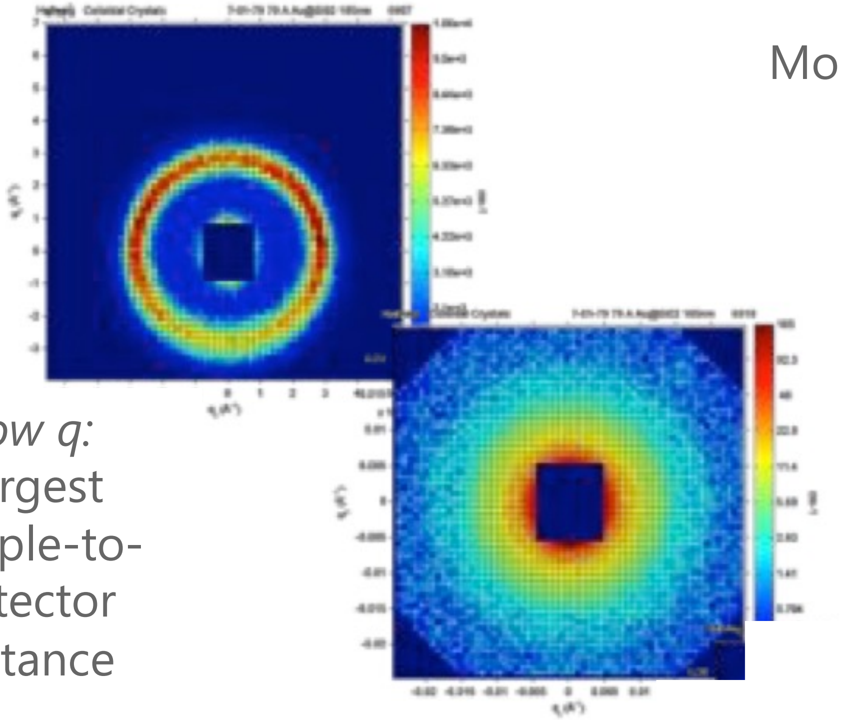
Figures from ILL

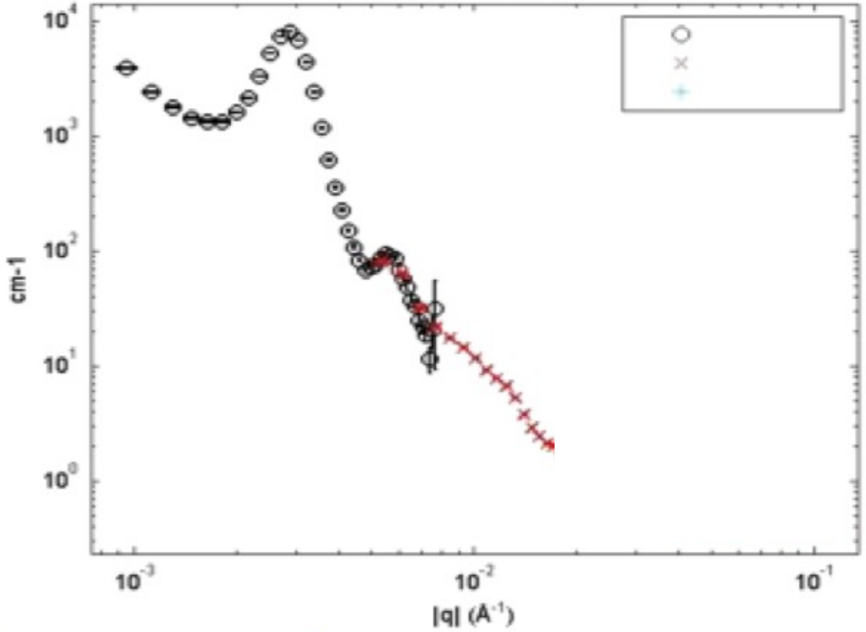
SANS: Continuous vs. time-of flight

Need several measurements at different distances to cover an adequate *Q*-range

Monochromatic beam

Low q:
Largest sample-to-detector distance

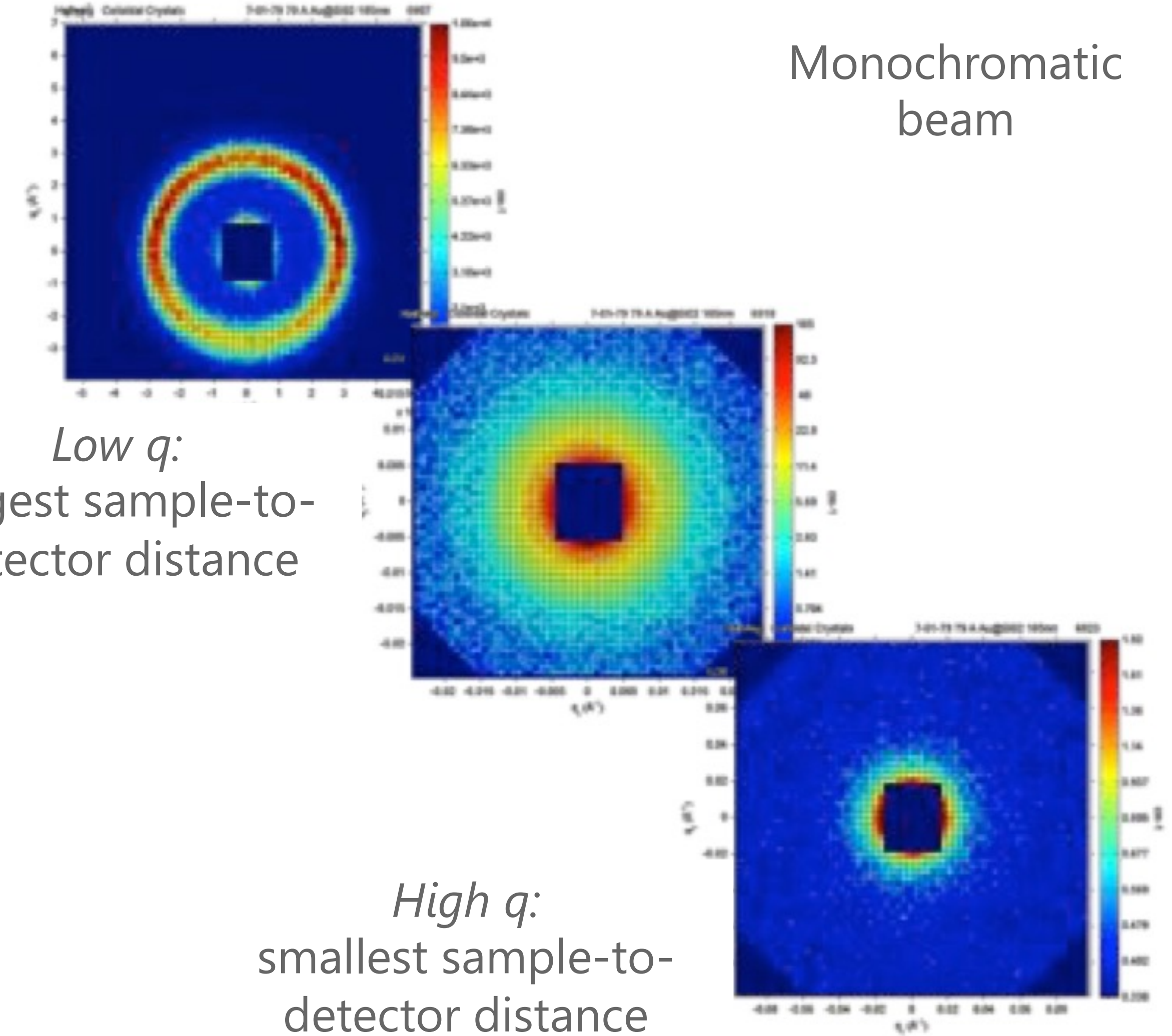




Figures from ILL

SANS: Continuous vs. time-of flight

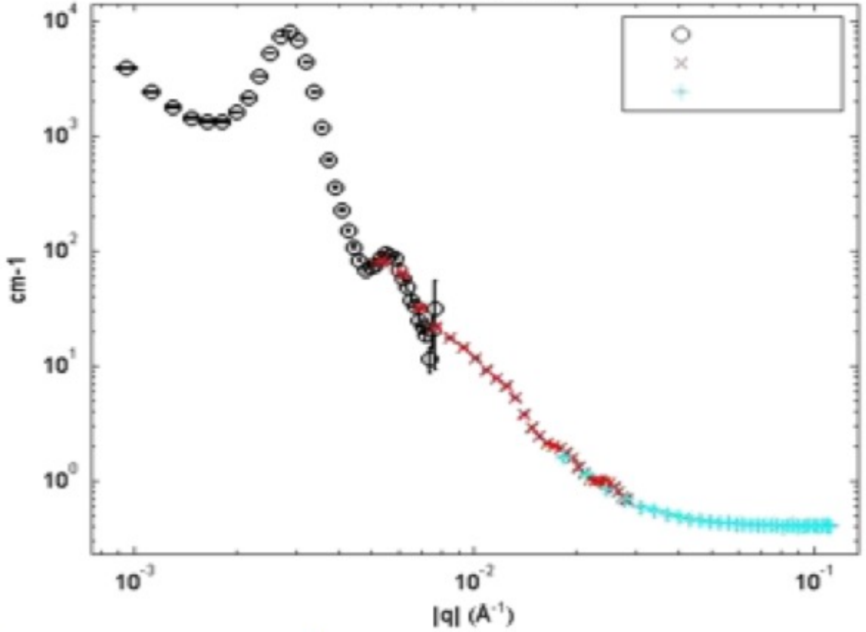
Need several measurements at different distances to cover an adequate *Q*-range



Monochromatic beam

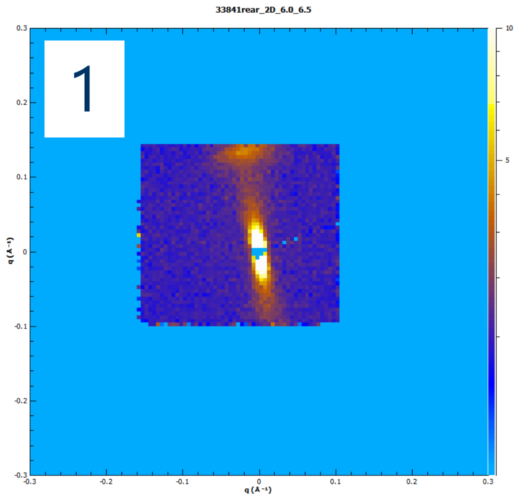
Low q:
Largest sample-to-detector distance

High q:
smallest sample-to-detector distance

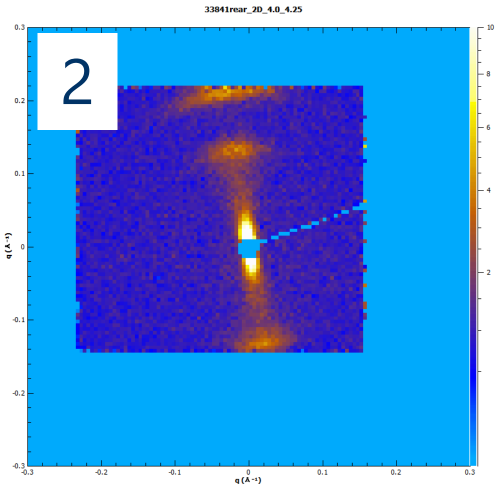


Figures from ILL

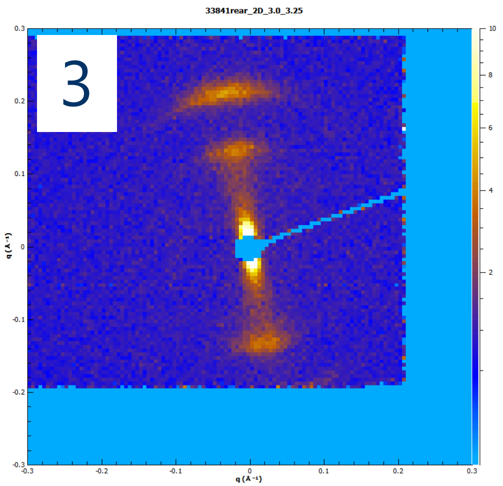
SANS: Continuous vs. **time-of flight**



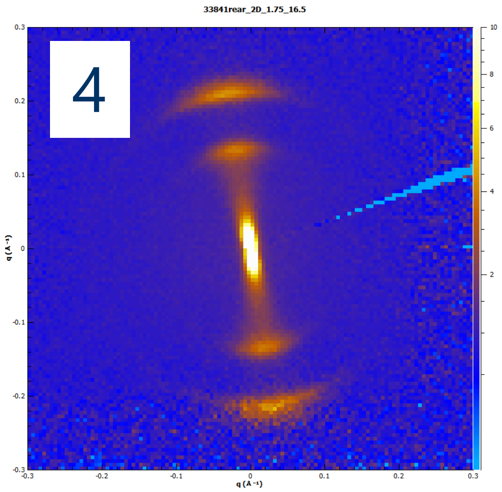
"fixed wavelength"
~6.25 Å



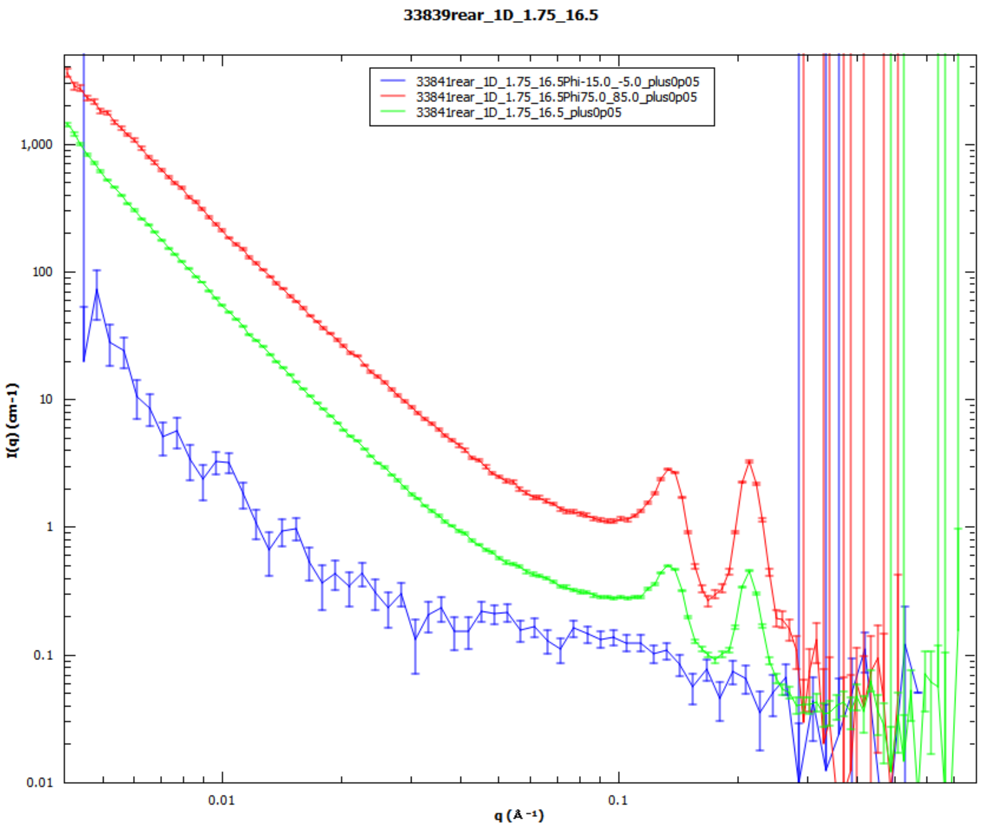
Shorter wavelengths
expand Qmax



Even shorter wavelengths
expand Qmax further



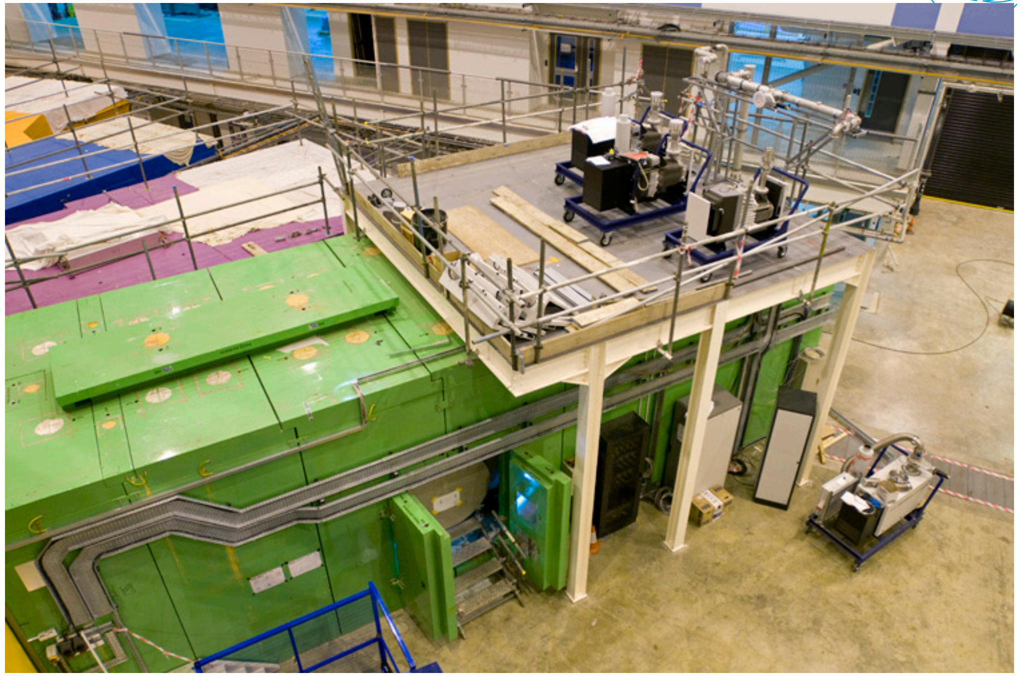
Full 1.75 to 16.5 Å gives a
wide simultaneous Q range.



Courtesy of R. K. Heenan and M. Hollamby

SANS: So you have a choice reactor *vs.* spallation…

e.g. D11 or D33 @ ILL, France (reactor source)

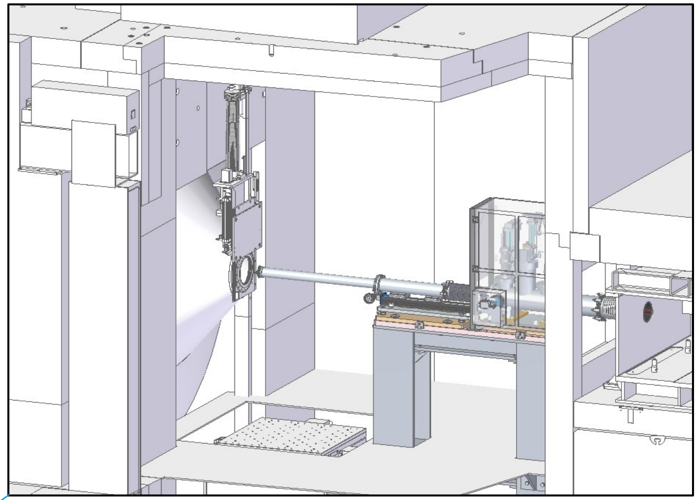


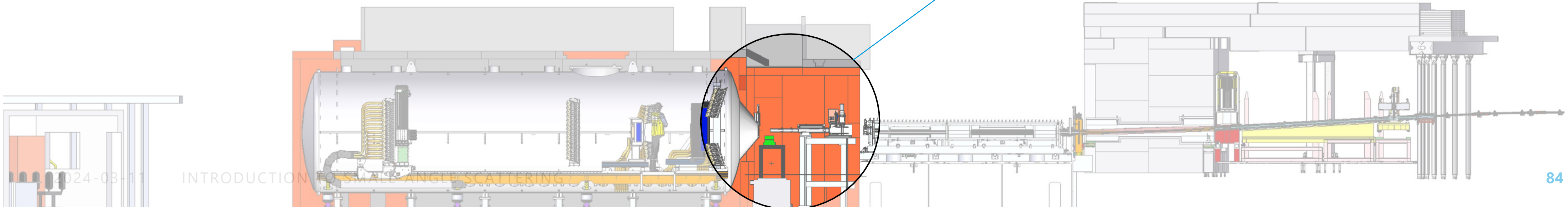
e.g. SANS2D @ ISIS, UK (spallation source)

Need to also consider the sample environment!

Sample environment

How do we control the positioning and conditions of our samples in the neutron beam?





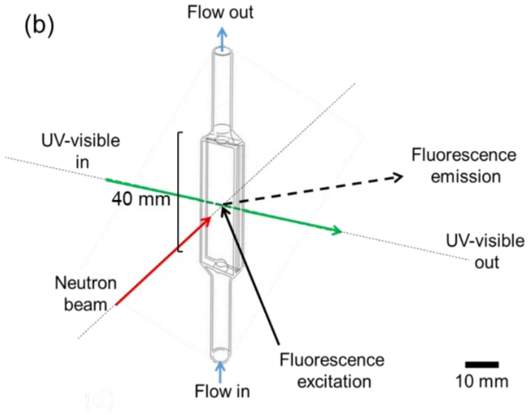
Sample environment

The "off-the-shelf" variety

- **Thermostated cell/capillary holder**
- Rheometer
- Flow cell with HPLC pumps
- Rotating cell holder
- Couette shear (higher shear rates)
- Plate-plate shear (for e.g. polymers)
- 2.5T electromagnet
- Humidity chamber
- Stopped-flow equipment
- Stress/strain rig (load capacity for stretching polymers)
- Cryostats

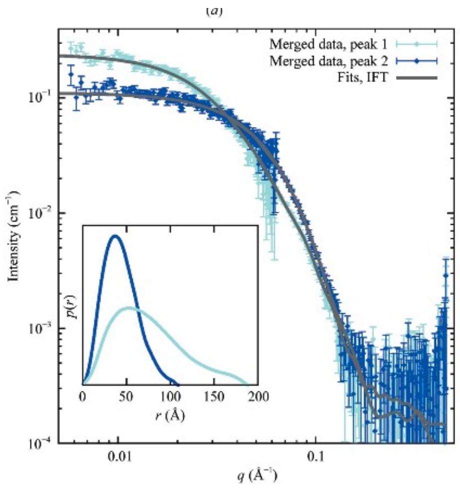
Custom-built sample environments

NuRF set-up

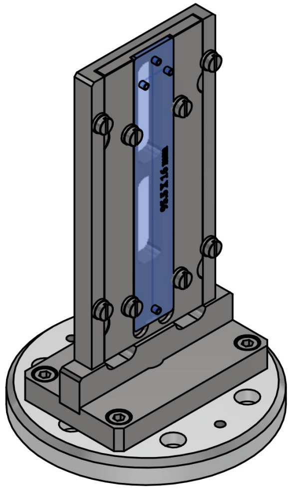


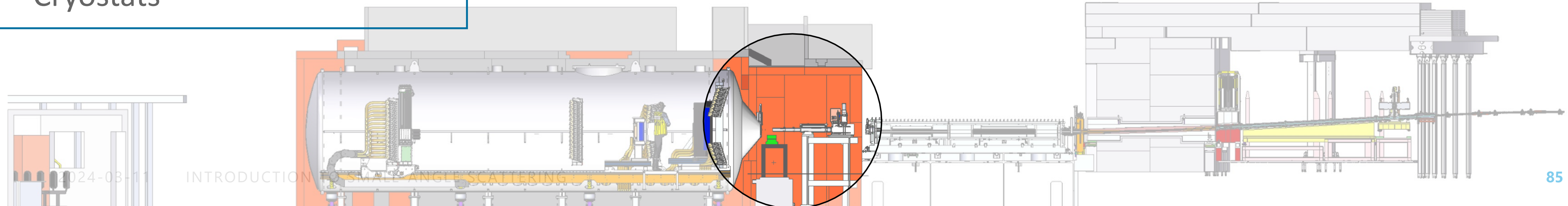
in situ fluorescence, UV/vis absorption, densiometry on a continuous flow cell

Size-Exclusion Chromatography-SANS



Microfluidics





Sample cells for biological solutions

Things to consider before selecting a cell:

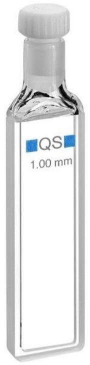
1. How much hydrogen is in my sample? How concentrated is my sample?
2. Is my sample easy to pipette? super viscous? a film? solid?
3. How much sample do I have?
4. What cells are available and/or used at the beamline?

Sample cells

- Quartz cells - **no SAS signal** and low background
- **Cell thickness** may depend on the H content of the sample
  - 1 mm for samples with more than 50% H
  - 2 or 5 mm for predominantly deuterated samples
- **Stopper or no stopper?**
- **Cell shape**:
  - 10 mm width rectangular cell
  - Cylindrical cell (banjo)
  - 20 mm width rectangular cell (tank)
  - Sandwich cell
- **Sample volume** for standard cells: 200 uL to 1 mL
- Some sample environments require specific cells (Al, TiZr…)

Capillaries

Quartz cuvettes



Sandwich cells

- ✓ Highly reproducible
- ✓ Low scattering
- ✓ Low background

Conclusions

- Small-angle scattering is an experimental technique which uses elastic scattering at small angles to investigate the structure of substances at a mesoscopic scale of ~1–200 nm
- Neutrons are a non-destructive, penetrating probe of structure on the atomic to macroscopic scale.
- Neutrons provide chemical sensitivity being especially sensitive to light elements.
- Neutron scattering can be isotope dependent, so contrast variation using H/D substitution allows complex structures to be more easily understood

Thanks for listening!

Any questions please ask!

(or email judith.houston@ess.eu)