# Variations on contrast in scattering experiments

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# Outline

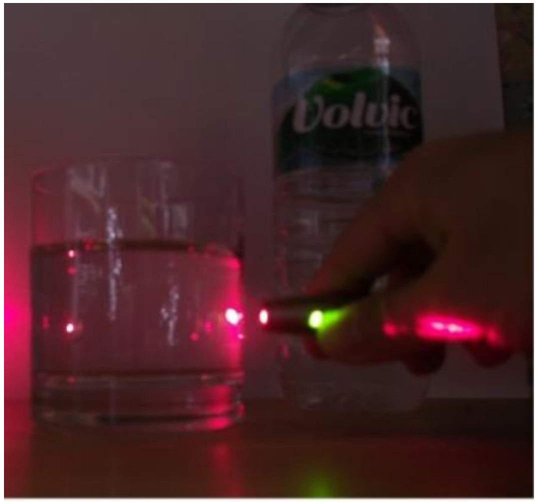
## The basics:

1) Scattering process and scattering length.
2) Contrast with neutron and X-rays.
3) How to calculate contrast?

## Applications and examples:

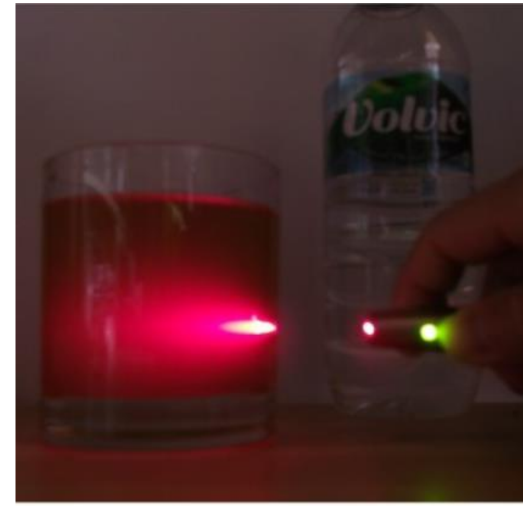
4) Contrast functions describing structure.
5) Contrast variation: *homogeneous* vs. *heterogeneous* structures.
6) Examples of casein micelles, proteins and protein networks.

# Basic observation of scattering: ↗ Martin's talk




Water itself doesn't scatter laser light.


Tea infusion does scatter laser light, even though its color so clear we can see through the wood grain of table as below.




2nd infusion containing denser constituent scatters more light.

**NB: X-ray, neutron, or light scattering**

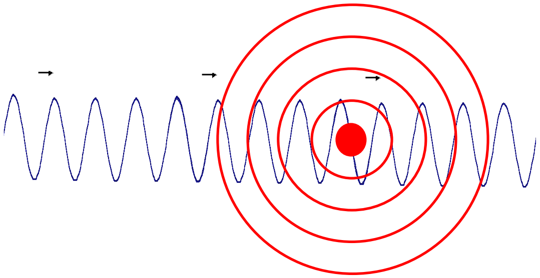
⇒ (Mostly) same mechanism, same story

⇒ But different visibility of objects

1st infusion extracted by cool water is so clear, so we can see through the wood grain of table.

# Scattering mechanism

Elementary scattering process:



Scattering power

What are its units?

**Scattering amplitude:**

- $A_0$ is incoming amplitude
- $A_s$ is scattered amplitude
- **Scattering power** * exp(ikr)/r

⇒ Scattering power of the nucleus or atom is a length, the **scattering length b**

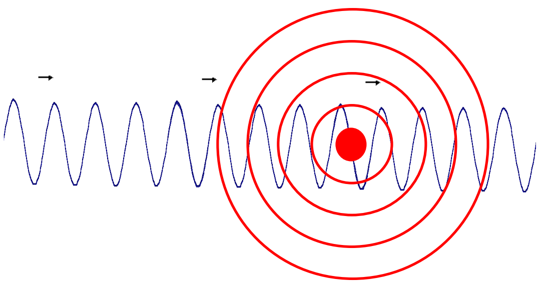
⇒ b can be positive or negative! $e^{i\pi} = -1$

⇒ Different for neutrons and for X-rays, for n it depends on isotopes !

⇒ Measuring scattering tells you which nucleus/atom you see!

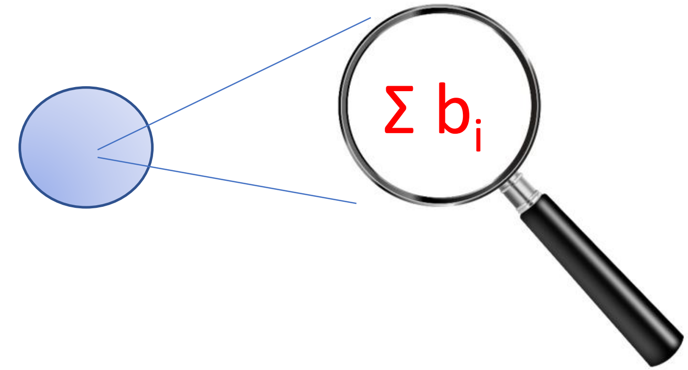
# Mechanism: Atoms making up materials

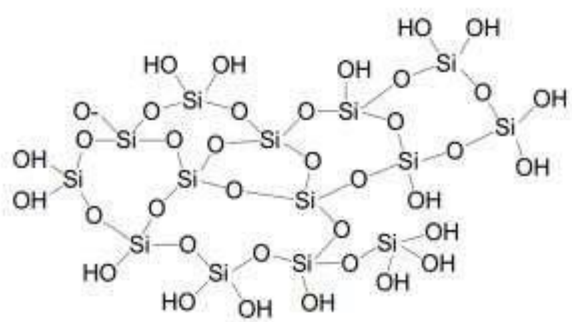
Elementary scattering process:

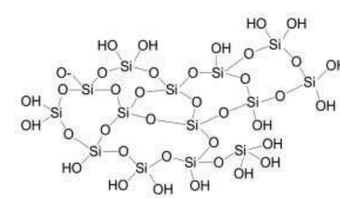


Scattering length b

Example:
silica NP



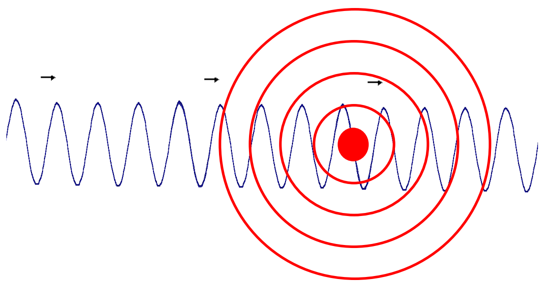


→ **S**cattering **l**ength **d**ensity

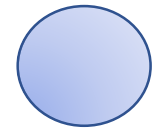
$\rho = \Sigma\, b_i / V$

# Calculate scattering length density

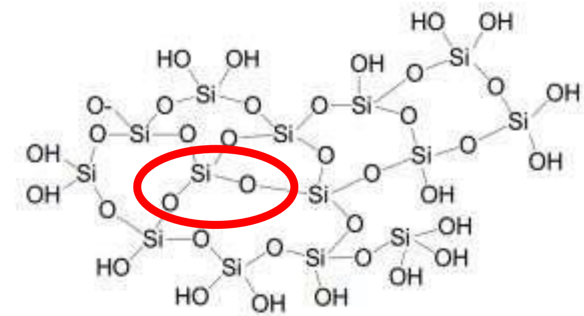
Elementary scattering process:



Scattering length b



Example silica NP



→ **S**cattering **l**ength **d**ensity

$\rho = \Sigma\, b_i/V$

In practice, how to calculate the ”sld” $\rho$ :

e.g. $SiO_2$ : Si and 2O, $\Sigma\, b_i = b_{si} + 2b_O = (4.15 + 2*5.8)$ fm

Molecular volume V = ? $V = M/d\, N_A = 60$ g/mol **/** (2.2 g/cm$^3$ $N_A$)

**homogeneous** $V = 4.55\ 10^{-23}$ cm$^3$ => $\rho = 3.5\ 10^{10}$ cm$^{-2}$

# Take-home message

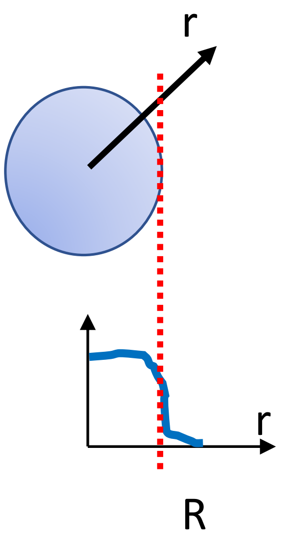
⇒ **Scattering length** = scattering power of a nucleus (n) or atom (X-rays).

⇒ The scattering of an *object* depends on its **scattering length density.**

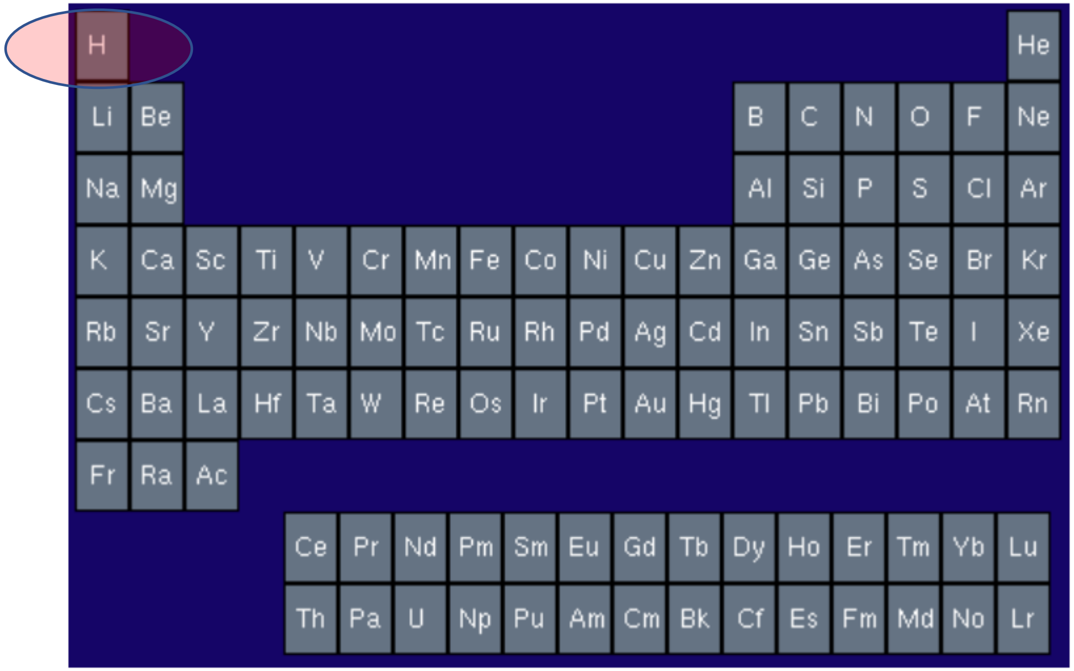
⇒ **SLD =** function in space **describing the structure.**

⇒ Measuring **sld means** measuring **density** ($g/cm^3$) - if you know the chemistry.

⇒ Local densities can be tricky (here homogeneous substances!).



# Neutron scattering lengths and cross sections




*Structure* shows up in a coherent scattering process

**Neutron scattering lengths and cross sections**

| Isotope | conc | Coh b | Inc b | Coh xs | Inc xs | Scatt xs | Abs xs |
|---|---|---|---|---|---|---|---|
| H | --- | -3.7390 | --- | 1.7568 | 80.26 | 82.02 | 0.3326 |
| 1H | 99.985 | -3.7406 | 25.274 | 1.7583 | 80.27 | 82.03 | 0.3326 |
| 2H | 0.015 | 6.671 | 4.04 | 5.592 | 2.05 | 7.64 | 0.000519 |
| 3H | (12.32 a) | 4.792 | -1.04 | 2.89 | 0.14 | 3.03 | 0 |

1 fm = $10^{-15}$ m

https://www.ncnr.nist.gov/resources/n-lengths/

NIST Center for Neutron Research

Home | Live Data

**Material**

H2O

**Neutron Activation**

For rabbit system [Calculate]

| Thermal flux | Cd ratio | Thermal/fast ratio |
|---|---|---|
| 1e8 | 0 | 0 |
| **Mass** | **Exposure** | **Decay** |
| | 10 | 1 y |

**Absorption and Scattering**

[Calculate]

| Density | Thickness |
|---|---|
| | 1 |
| **Source neutrons** | **Source X-rays** |
| 10 Ang | Cu Ka |

# Scattering length density

$\Sigma\ b_i/V$

## Scattering from H2O

Source neutrons: 10.000 Å = 0.82 meV = 396 m/s

Source X-rays: 1.542 Å = 8.042 keV

Sample in beam: H2O at 1.00 g/cm$^3$

| 1/e penetration depth (cm) | | Scattering length density ($10^{-6}$/Å$^2$) | | Scattering cross section (1/cm) | | X-ray SLD ($10^{-6}$/Å$^2$) | |
|---|---|---|---|---|---|---|---|
| **abs** | 8.084 | **real** | -0.561 | **coh** | 0.004 | **real** | 9.469 |
| **abs+incoh** | 0.174 | **imag** | -0.000 | **abs** | 0.124 | **imag** | -0.032 |
| **abs+incoh+coh** | 0.174 | **incoh** | 21.180 | **incoh** | 5.621 | | |

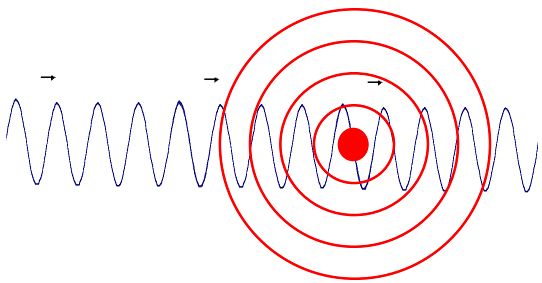
Neutron transmission is 0.320% for 1 cm of sample (after absorption and incoherent scattering).

Transmitted flux is 3.199e+5 n/cm$^2$/s for a 1e8 n/cm$^2$/s beam.

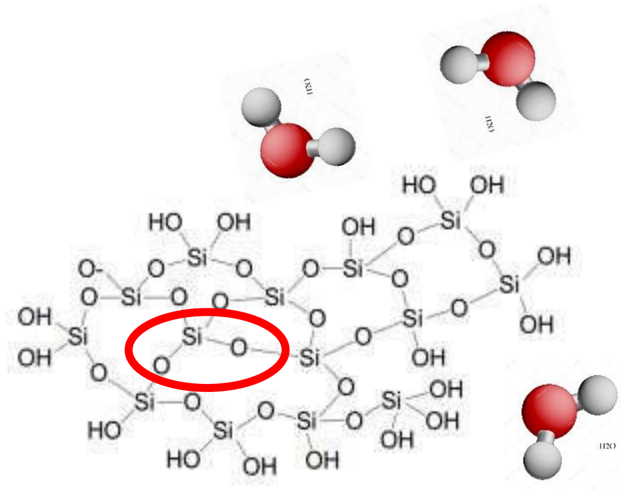
Contrast match point: < 0% $D_2O$

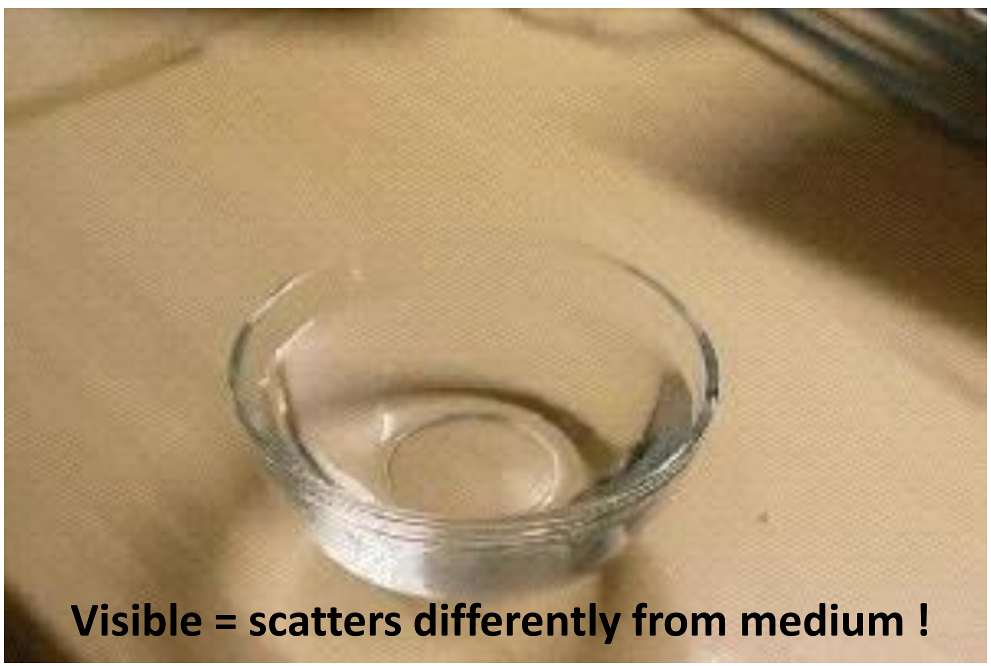
# Particle/molecule in an environment (solvent, matrix…)

Elementary scattering process:



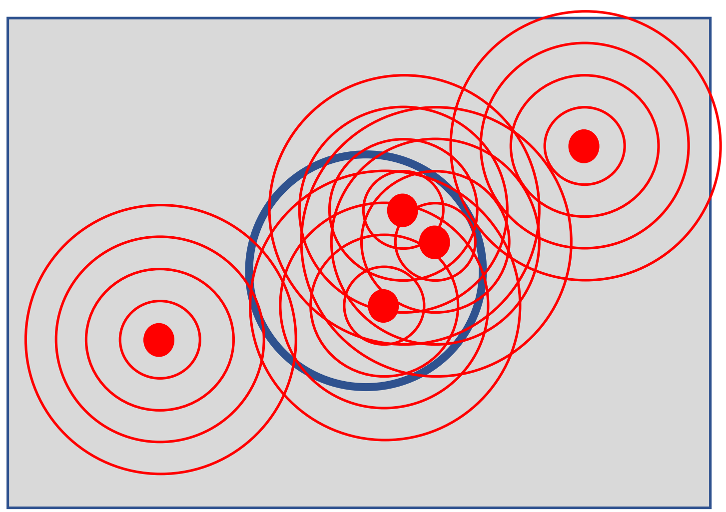
Scattering length b



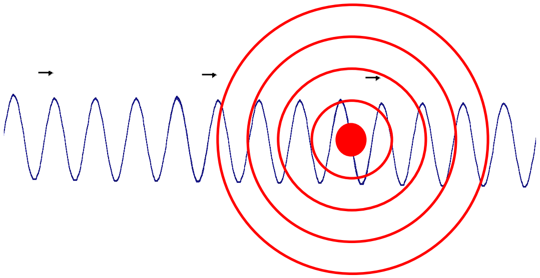

→ Scattering length density

Σ b/V

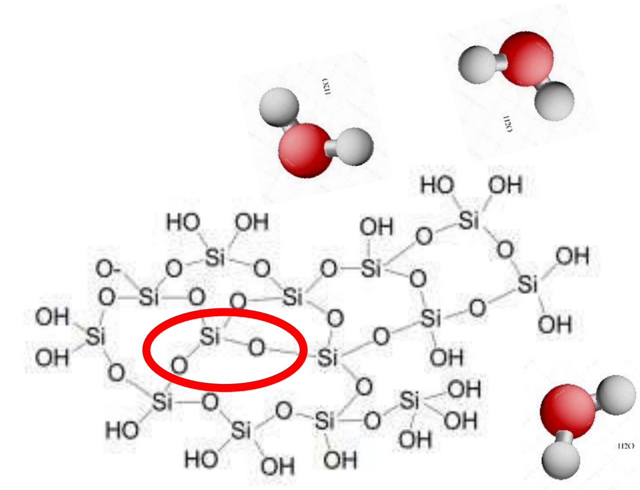


# Scattering contrast

Elementary scattering process:



Scattering length b

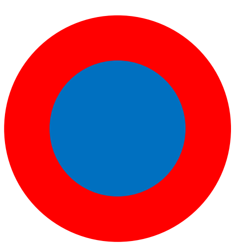


→ Scattering length density

$\Sigma\, b/V$

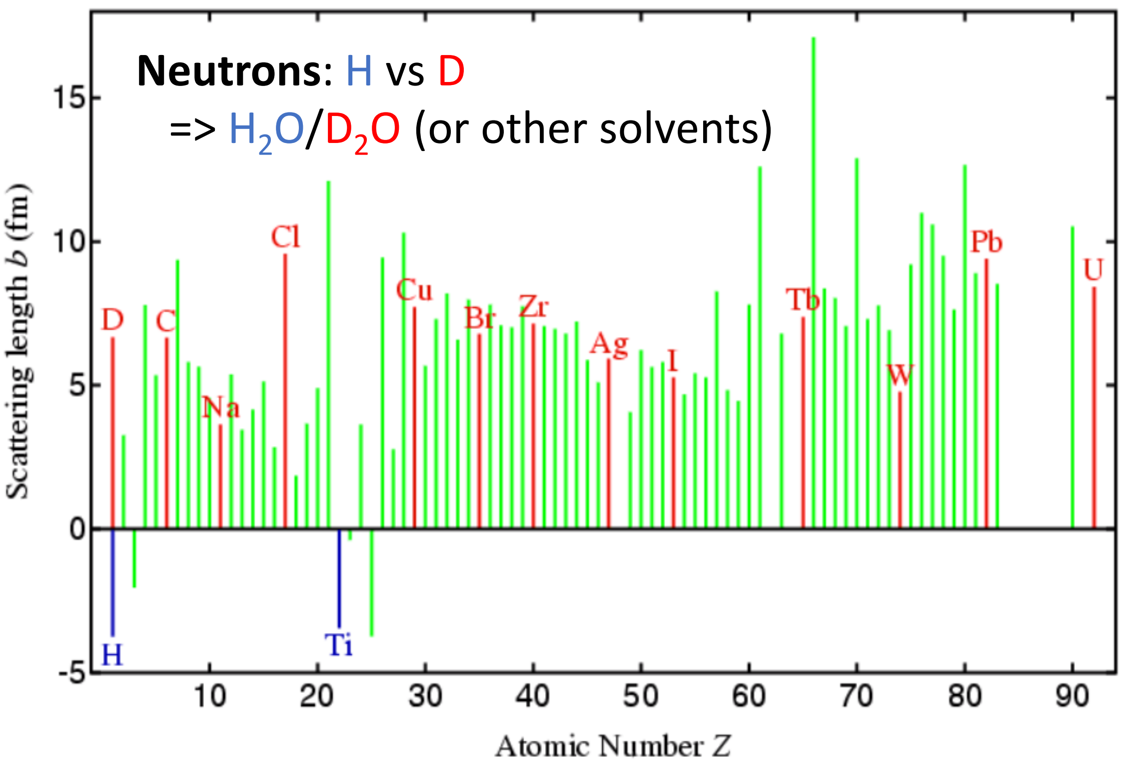
→ CONTRAST = difference in scattering length density

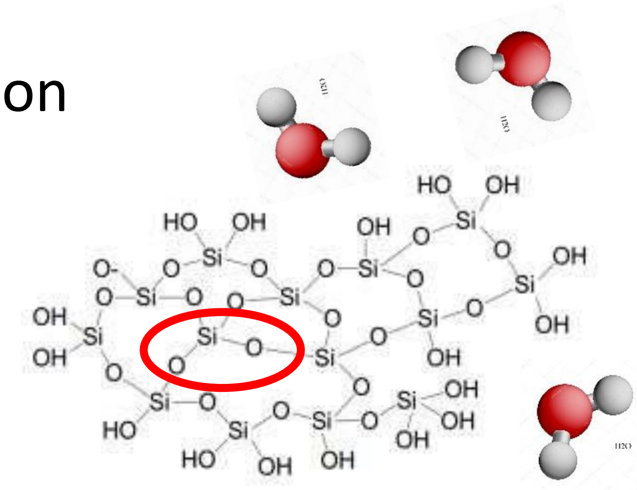
$$\Delta\rho = \rho_{object} - \rho_{medium}$$

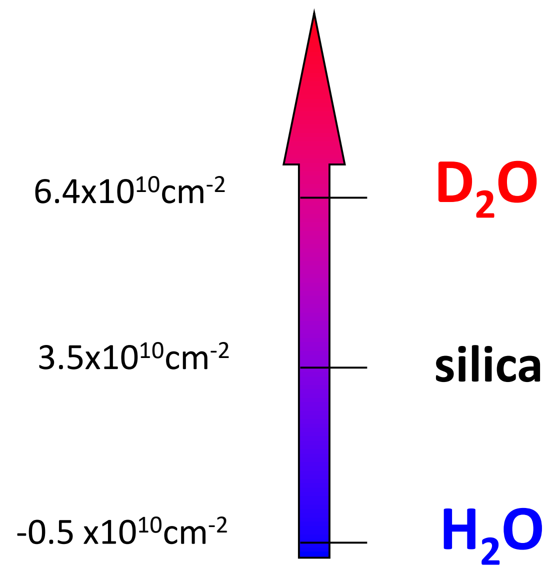


# Scattering lengths with **neutrons**

→ Scattering length density Σ b/V depends on radiation



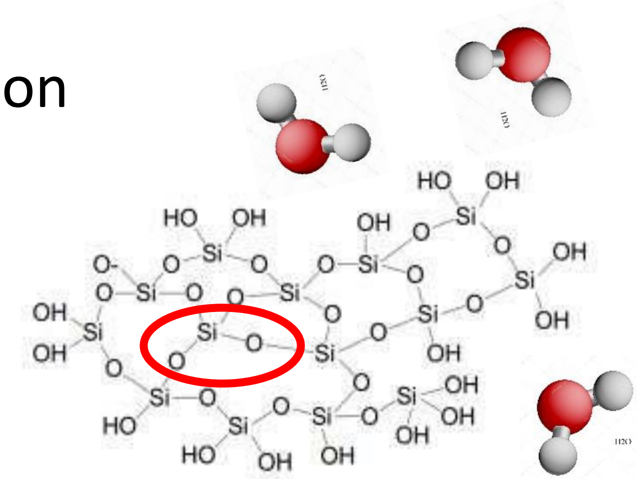






Possibility of solvent contrast variation (*external*)

# Scattering lengths with X-rays

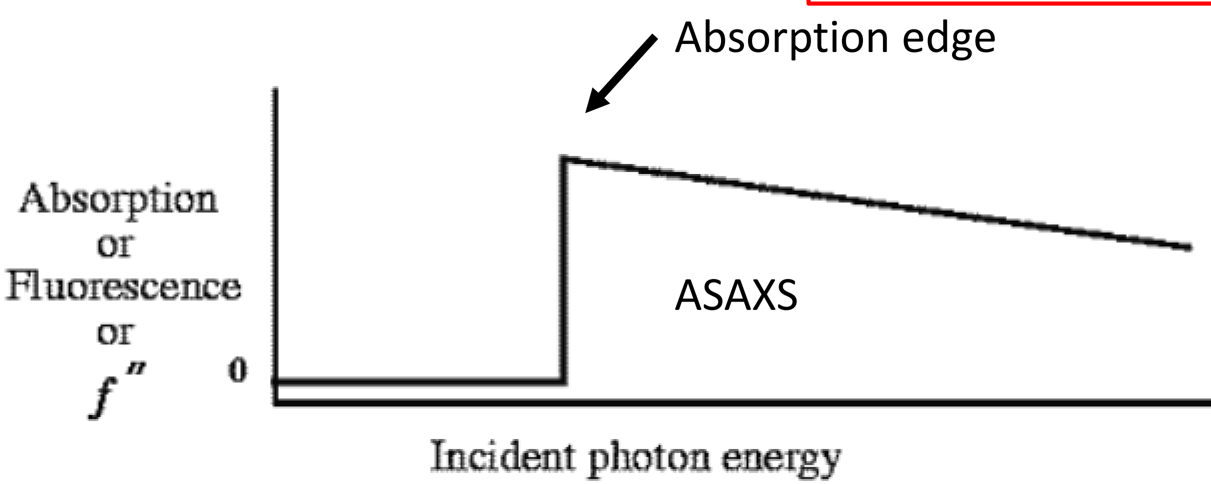
→ Scattering length density Σ b/V depends on radiation



**X-rays:** each electron

$$b_i = \frac{e^2}{4\pi\varepsilon_0 m_e c^2} f_1 = r_e f_1$$

**Take-home message:**
Contrast variation is more delicate.
Combine use of X-rays and neutrons!
See different parts of your sample.



# Take-home message: Varying the SLD of the solvent

## X-rays

- b proportional to number of electrons.
- Heavy elements visible.
- Difficult to contrast match with most solvents.

Add electrons to the solvent: e.g., sugar to water.

Problem: this sometimes messes up phases/conformations/kinetics.

Use ASAXS (but need the right elements).

## Neutrons

- b random but works for H/D !
- Light elements visible.
- Easy to contrast match with most solvents.

Mix $H_2O/D_2O$ or other H/D solvents.

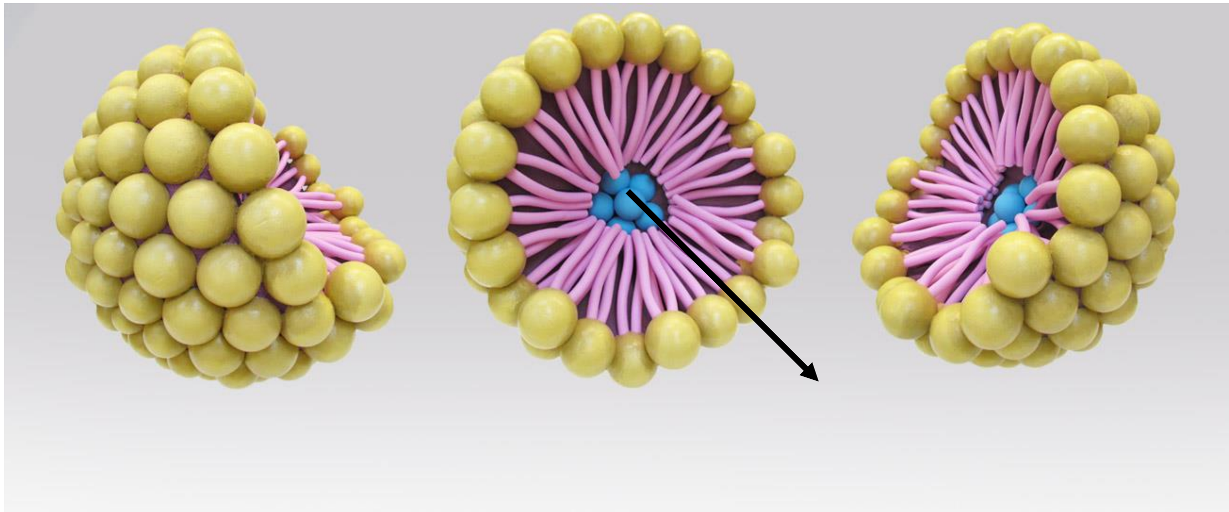
Problem: sometimes messes up phase boundaries.

Chemistry may be expensive!

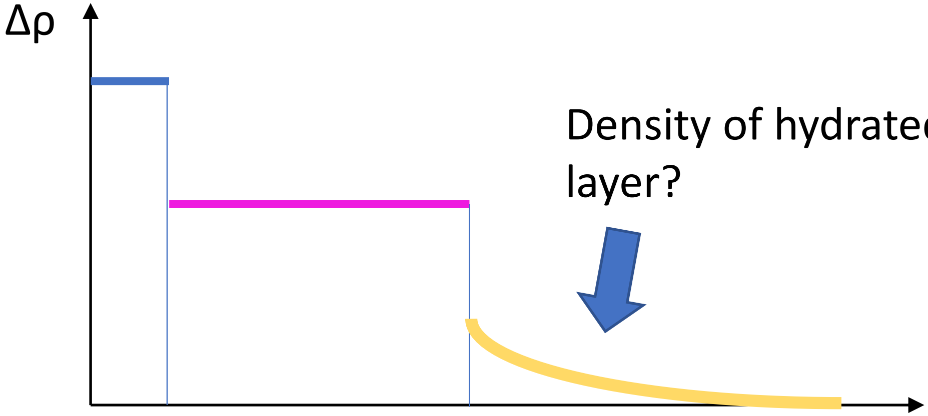
Combine SAXS and SANS !

# Contrast describes structure in space

## Radial contrast functions (spherical symmetry)



Micelle with core filled (e.g. cleaning microemulsion)

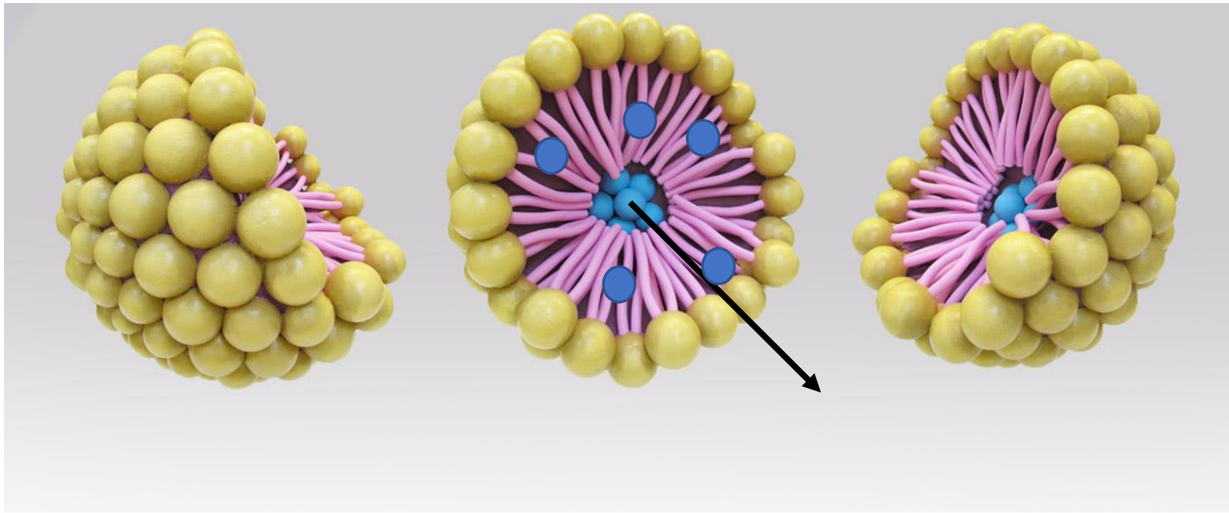



Density of hydrated outer layer?

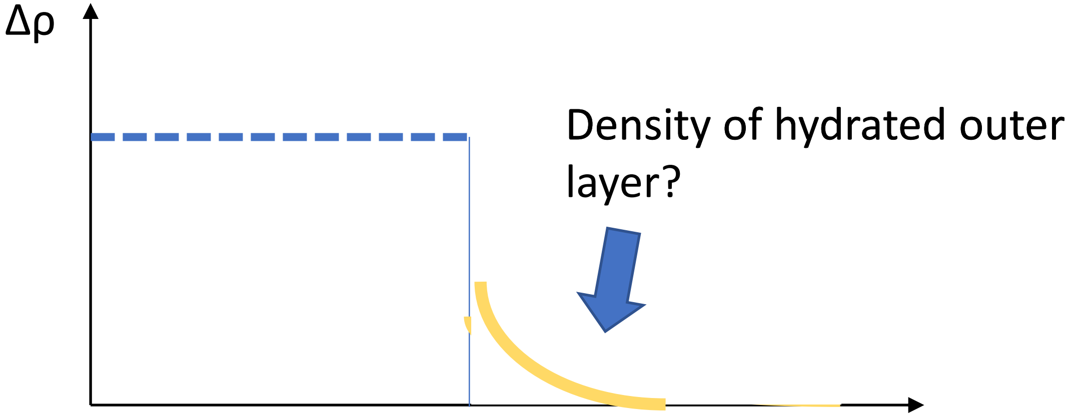
$\Rightarrow$ **How many parameters in this model?**

# Contrast describes structure in space

## Radial contrast functions (spherical symmetry)

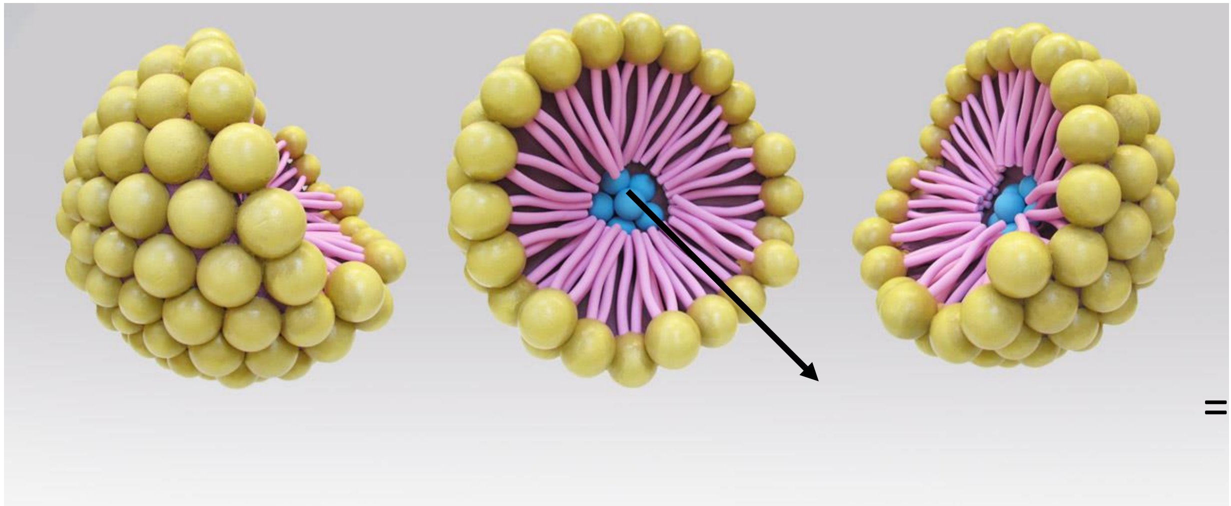


Micelle with core filled (e.g. cleaning microemulsion)




⇒ **Do not start with a prg!**
⇒ **First**: Think your chemistry!
= identify vol's, b's and ρ's.
⇒ **Then** model your *chemistry*
*+ structure.*

# Contrast variation (CV) of simple sphere

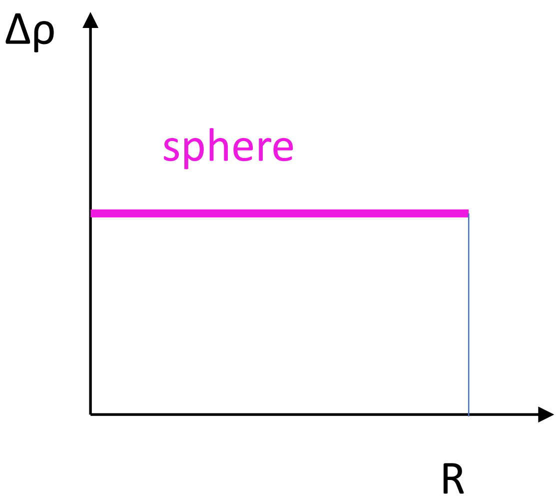


Micelle with core filled (e.g. cleaning microemulsion)

$I_0 = I(q \to 0)$

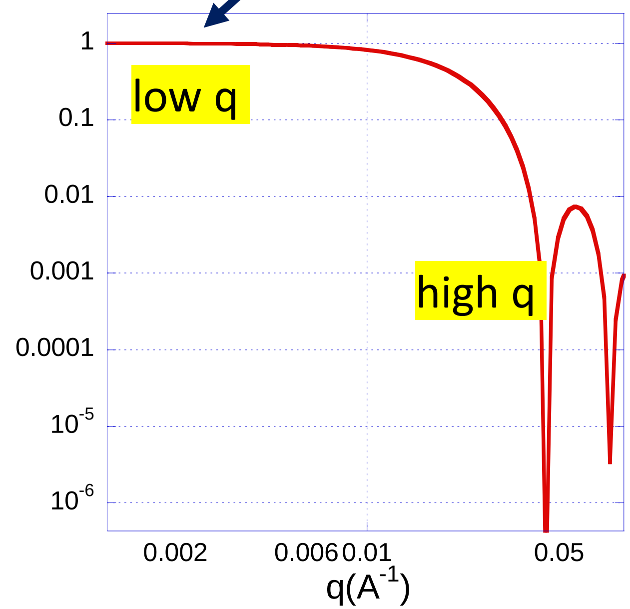
$= \text{number volume}^2 \text{ visibility}^2$

$= \Phi \, V_{object} \, \Delta\rho^2$




Interferences = Fourier

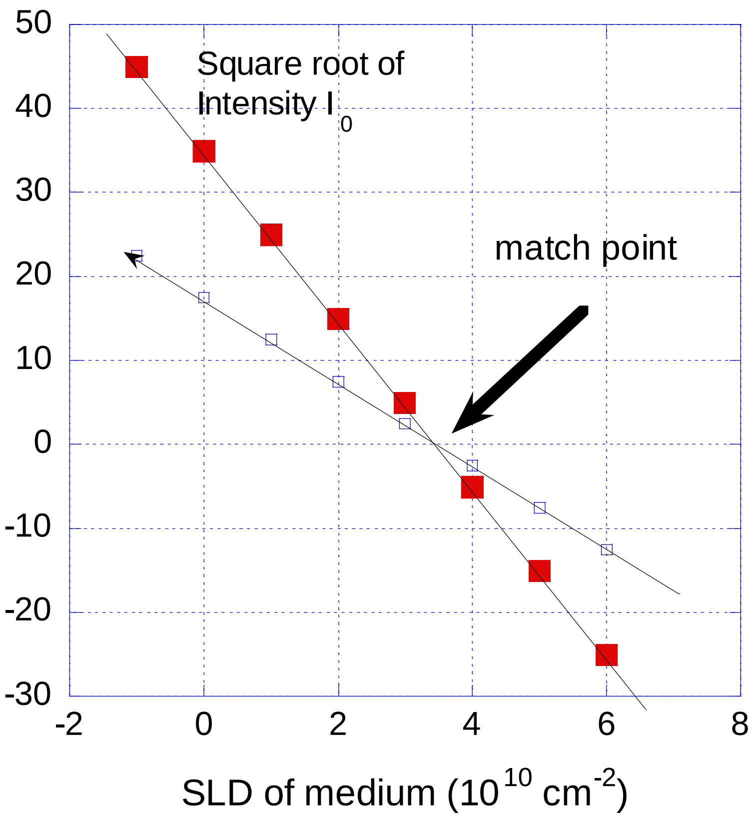
See Martin's talk



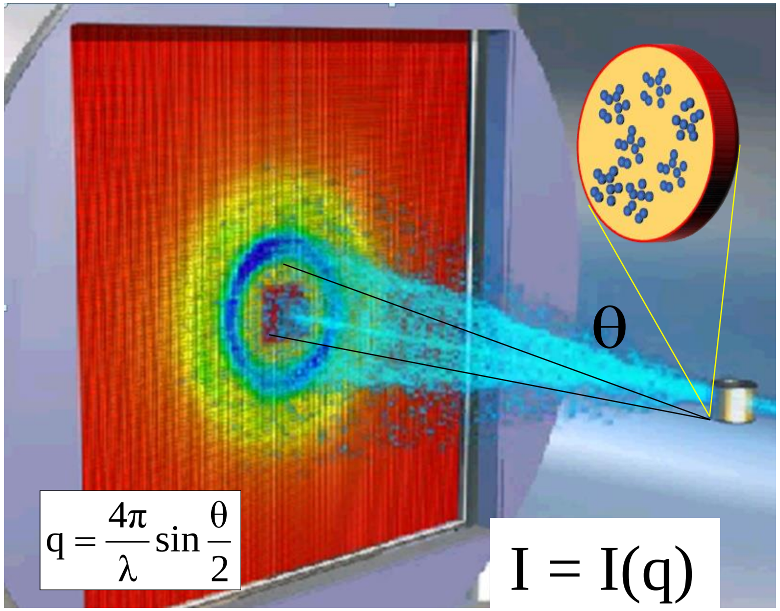
# CV: Zero-angle intensity (form factors)

$$I_0 = I(q \rightarrow 0) = V_{object}\, \Phi\, \Delta\rho^2$$

$$\Delta\rho = \rho_{object} - \rho_{medium}$$




$H_2O$ $D_2O$




**Make use of the match point:**

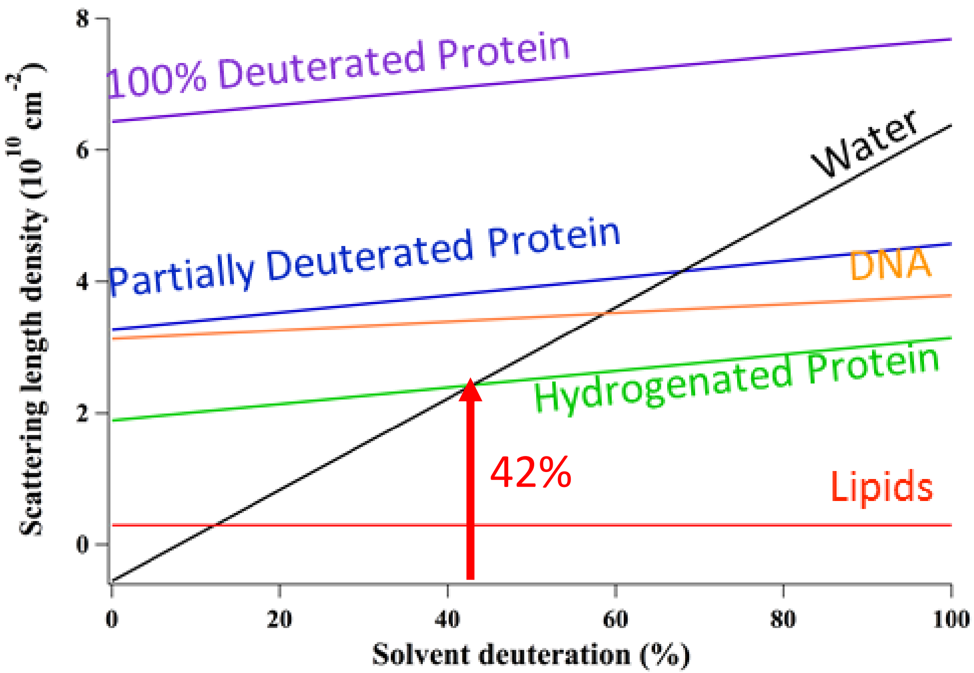
- $\rho_{object}$ is a density (on molecular scale) !
- You can use it to estimate composition: is silica SiO, $SiO_2$, $SiO_3$, or $SiO_4$?
- If you know the composition, you can determine the density in g/cm³.

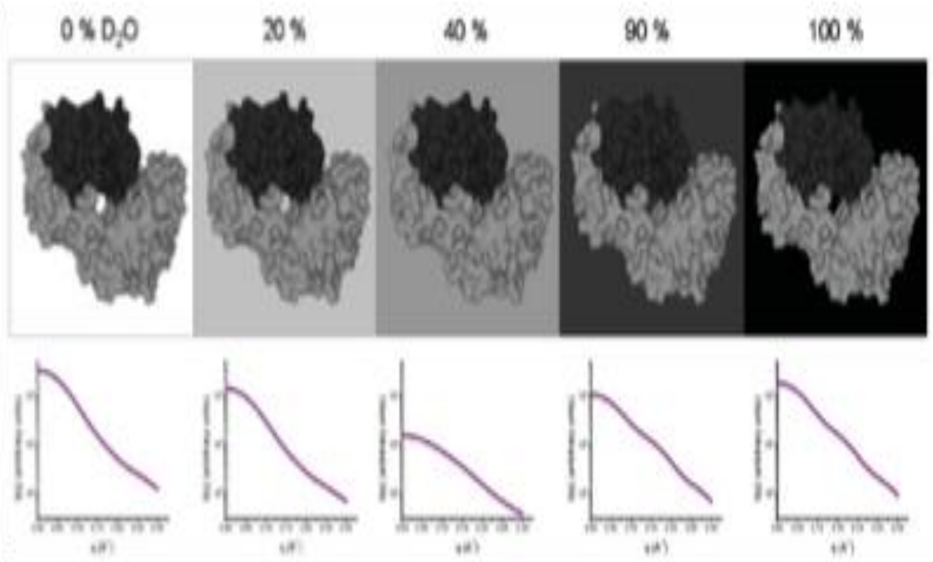
External contrast variation

# PROTEINS

## isotopic contrast and contrast matching with neutrons

50% of atoms of protein are protons: some are fixed (C-H), other are labile (S-H, COOH, NH)



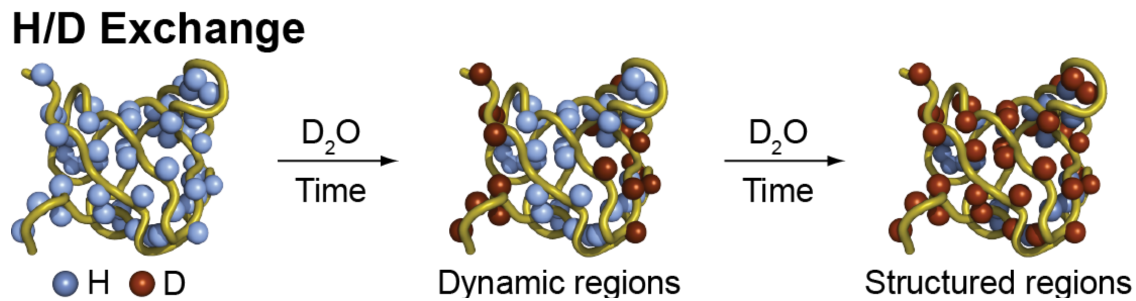

Jacques, D. A. & Trewhella, J. (2010). Prot. Sci. 19:642-657

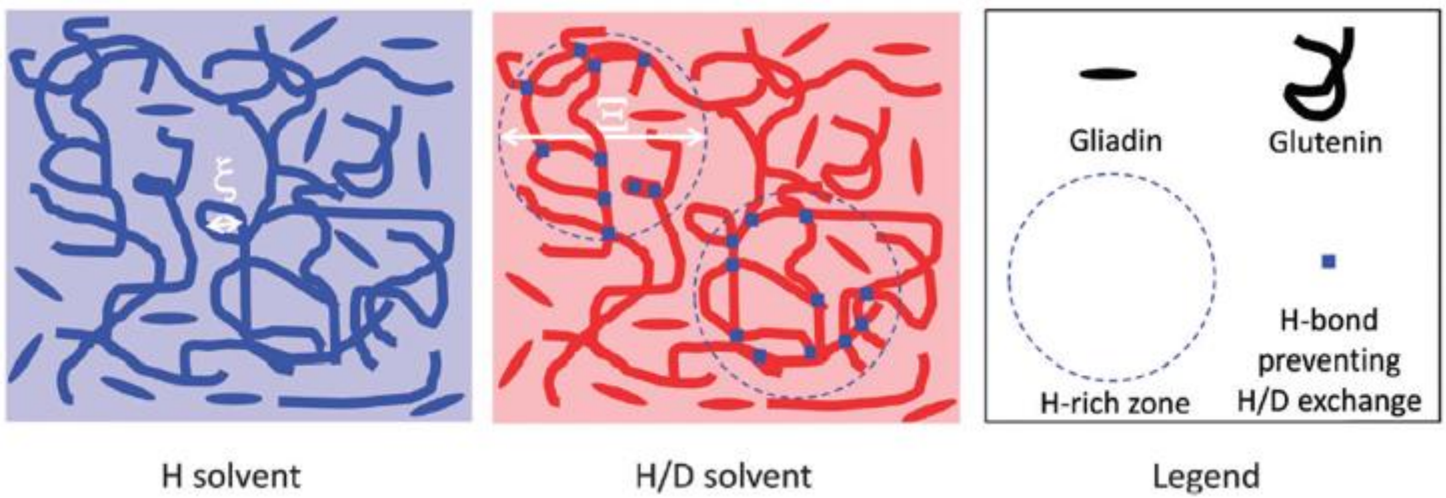
- Variation of the $H_2O$:$D_2O$ ratio highlights different components of biological complexes.
- For protein-protein complexes, isotopic labelling with deuterium is needed for contrast studies.

# PROTEINS

## isotopic contrast and contrast matching with neutrons

Possible H-D exchange:



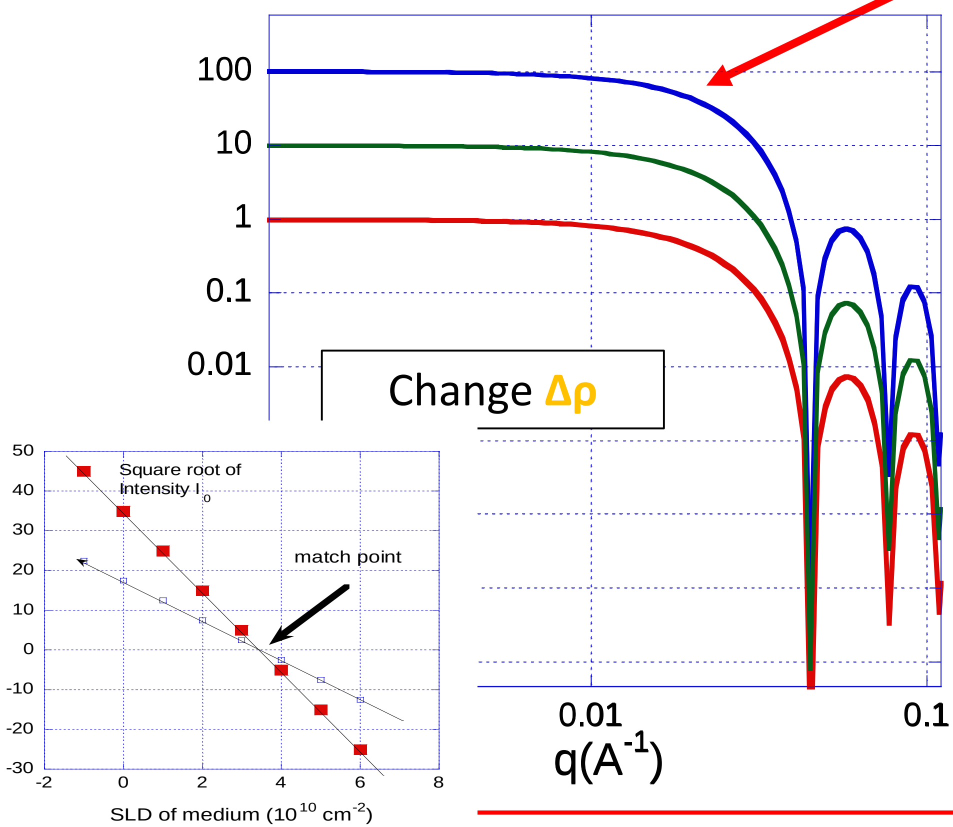

**Caveats:**
- $H_2O$ and $D_2O$ are *not* the same solvents (anomaly of water, solubility).
- Careful close to solubility limit.
- Unfolding: solvent may access and modify new areas = composite behaviour.

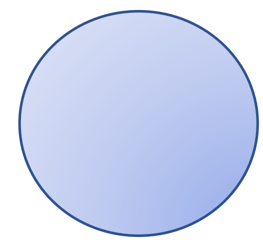
# Non-zero-angle intensity (form factors)

Guinier expression:

$$I = I_0 \exp(-q^2 R_g^2/3)$$

$$I_0 = I(q \rightarrow 0) = V_{object}\, \Phi\, \Delta\rho^2$$



Homogeneous sphere
R = 10 nm

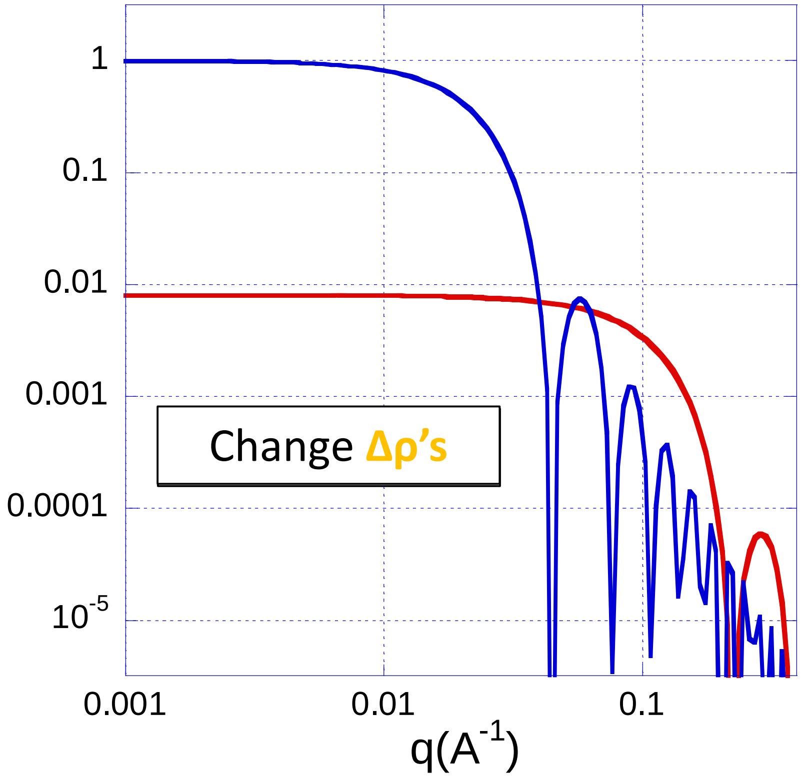
I = prefactor(ρ) * Form factor(q)

**Change ρ or q?**

- If homogeneous, $\Delta\rho^2$ factors out, otherwise same function.
- Change contrast = prefactor = same shape !
- Same shape = same radius (of gyration).

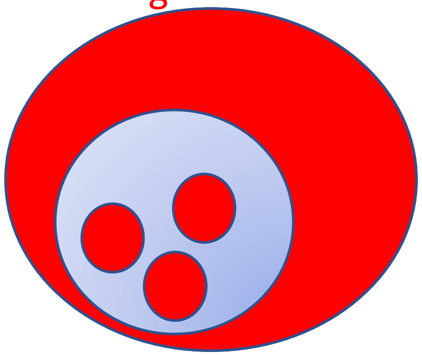
**=> Change q = better stats.**

# Non-zero-angle intensity + heterogeneity (form factors)




Guinier expression:

$$I = I_0 \exp(-q^2 R_g^2/3)$$



Heterogeneous sphere

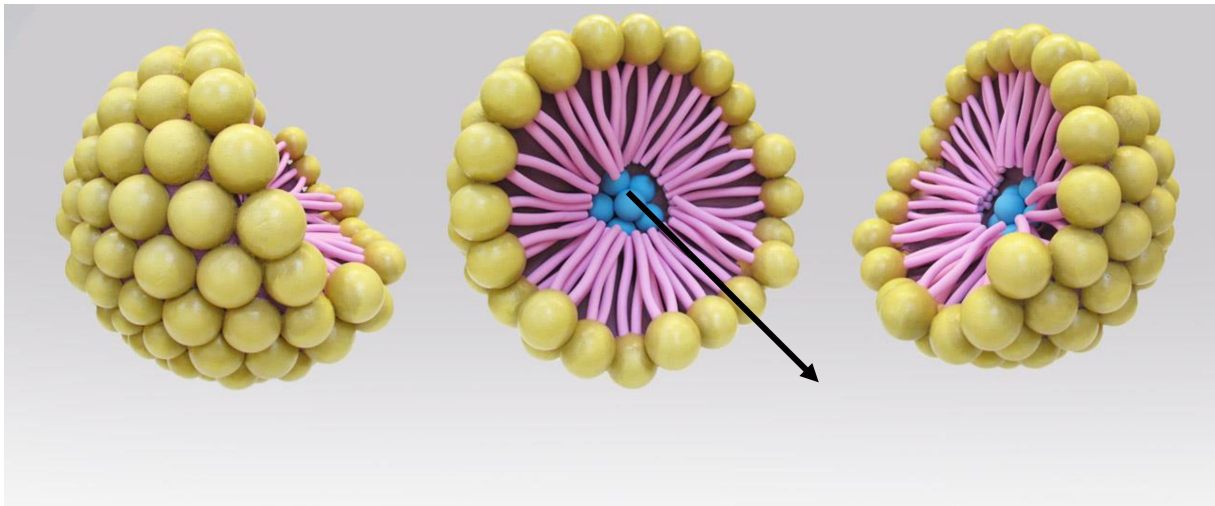
$R_1$ = 10 nm, $R_2$ = 2 nm

$$I(q) = [\Delta\rho_1 A_1 + \Delta\rho_2 A_2]^2$$

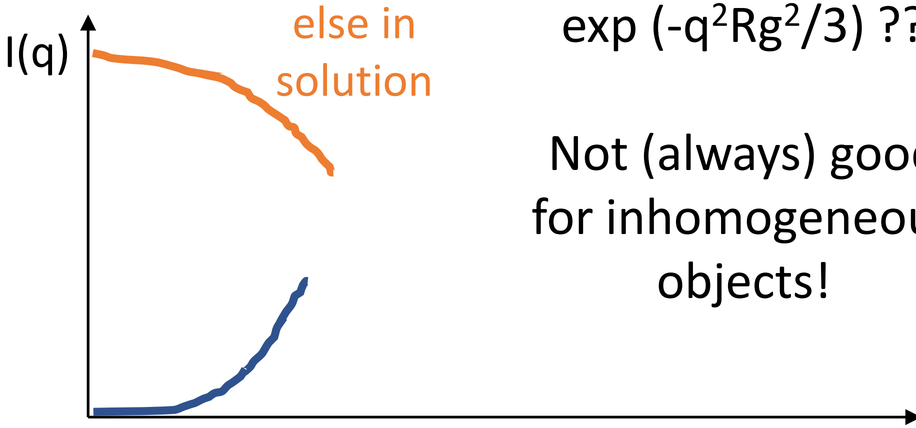
**Now q matters:**

- Low-q: average particle composition.
- Non-zero q: the radius of gyration depends on contrast.
- In particular: zero average contrast: $I_0 = 0$, followed by increase, imaginary $R_g$ !

# Radial contrast functions: special case



Micelle with core filled (e.g. cleaning microemulsion)




Guinier
$\exp(-q^2 Rg^2/3)$ ??

Not (always) good for inhomogeneous objects!

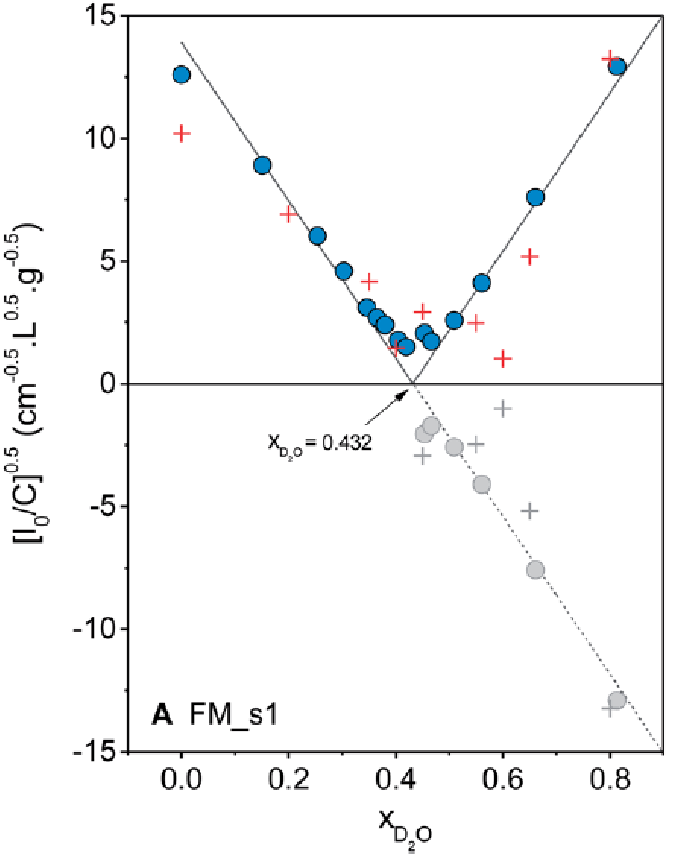
**If contrast is negative/positive**

Total $\mathbf{\Sigma\, b_i}$:

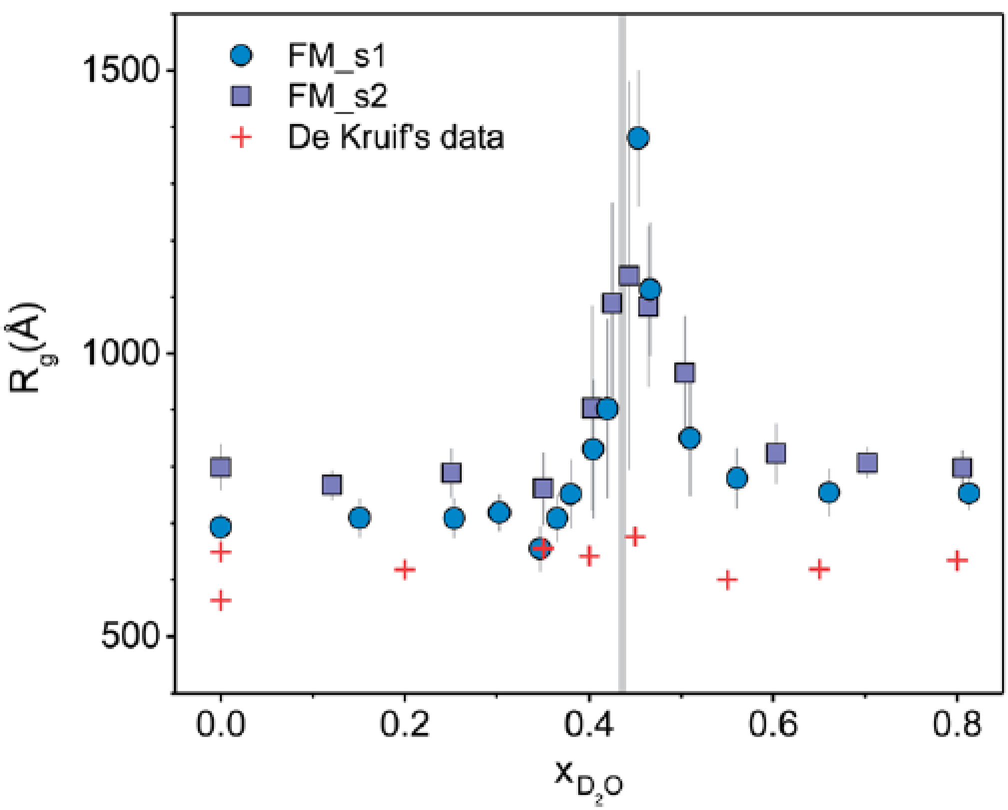
$\int \Delta\rho\, dV = V_1\, \Delta\rho_1 + V_2\, \Delta\rho_2 + V_3\, \Delta\rho_3$

May be zero....

# Example of casein micelles




Not zero at matchpoint !




Sudden increase in size?

**Two contributions:** big fat droplets and casein miceles

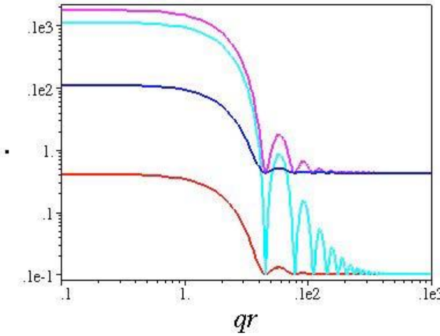
$$I(q) = [\Delta\rho_1 A_1]^2 + [\Delta\rho_2 A_2]^2$$

**Structural heterogeneity of milk casein micelles: a SANS contrast variation study†**


Antoine Bouchoux,‡*[ab] Jorge Ventureira,[ab] Geneviève Gésan-Guiziou,[ab] Fabienne Garnier-Lambrouin,[ab] Peng Qu,[ab] Coralie Pasquier,[ab] Stéphane Pézennec,[ab] Ralf Schweins[c] and Bernard Cabane[d]

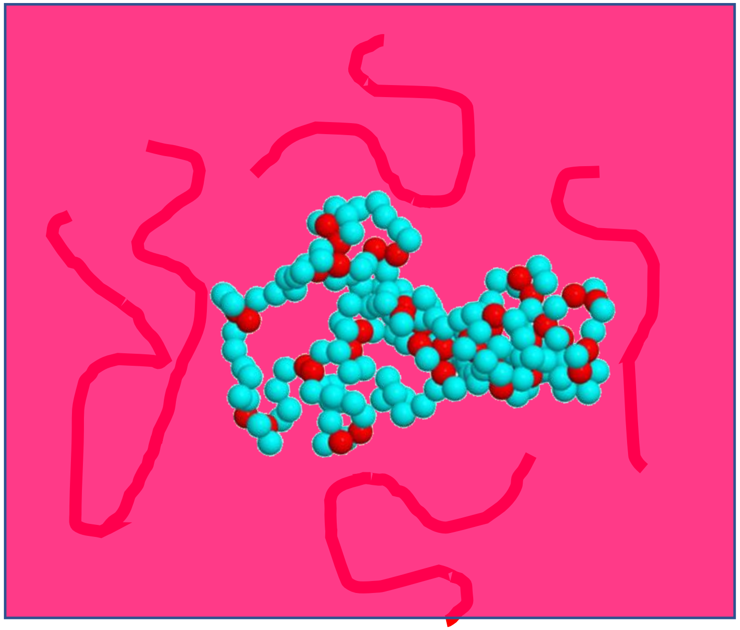
# In practice

**External contrast variation: vary the solvent**




- determine density // **s**cattering **l**ength **d**ensity
- increase **signal** (or decrease to avoid multiple scattering)
- decrease **noise** (incoherent background with neutrons)
- Many deuterated solvents available
- Match part of **two-phase system** (microemulsions, SCNPs …)

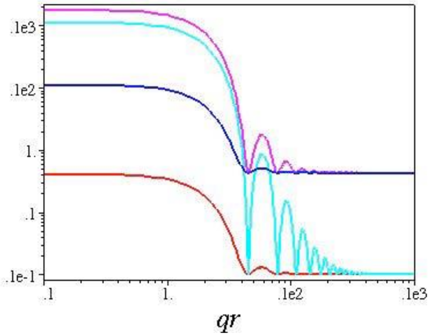
Add solvent dDMF

Arantxa Arbe & Juan Colmenero, Single chain NPs, *Macromolecules* **2019**

# In practice

## External contrast variation: vary the solvent

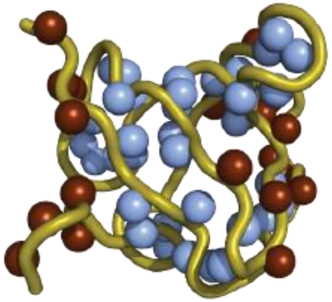



- determine density // scattering length density
- increase signal (or decrease to avoid multiple scattering)
- decrease noise (incoherent background with neutrons)
- Many deuterated solvents available
- Match part of two-phase system (microemulsions, SCNPs …)

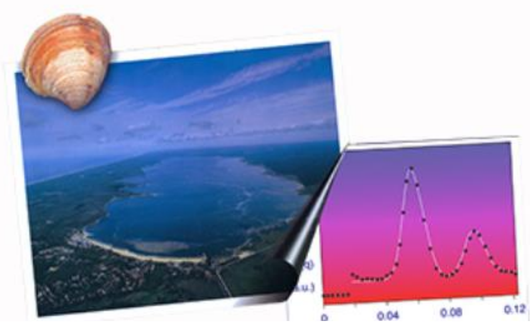
## Internal contrast modification: synthesis

- (per)deuteration: chemistry or biology
- form factor of polymer chains in melt: SANS vs SAXS

## Explore natural differences in contrast (zones):



- proteins
- protein assemblies (eg. casein micelles)
- particles (X-rays) vs polymer chains (neutrons)
- Zera average contrast for composite materials




# Bombannes 2024

**15th European Summer School on "Scattering Methods Applied to Soft Condensed Matter"**

4 – 11 June 2024