Istituto dei Sistemi Complessi
Consiglio Nazionale delle Ricerche

Neutrons
for life sciences

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Life Sciences

Life Sciences involve the scientific study of life – such as microorganisms, plants, and animals including human beings.

Among Life Science disciplines, those that can benefit from neutron techniques are:

- **Biology** – *scientific study of life*
- **Biochemistry** – *study of the chemical reactions required for life to exist and function, usually a focus on the cellular level*
- **Bioinformatics** – *developing of methods or software tools for storing, retrieving, organizing and analyzing biological data to generate useful biological knowledge*
- **Biophysics** – *study of biological processes by applying the theories and methods that have been traditionally used in the physical sciences*
- **Molecular biology** – *the study of biology and biological functions at the molecular level, some cross over with biochemistry, genetics, and microbiology*
- **Structural biology** – *a branch of molecular biology, biochemistry, and biophysics concerned with the molecular structure of biological macro-molecules*

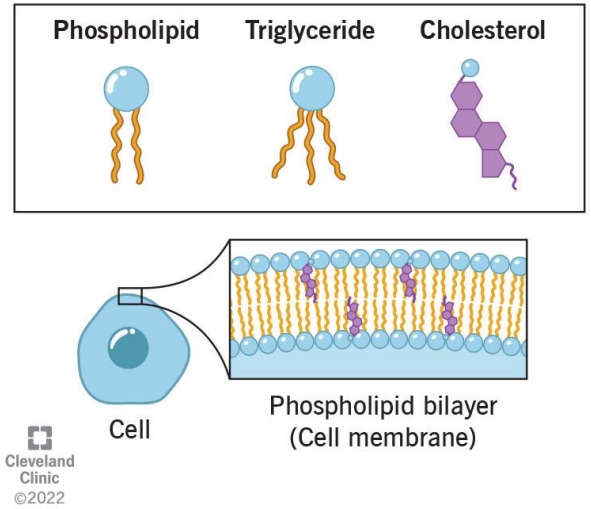
Derived areas

Neutron techniques play a key role for:

- **Biomaterials**
- **Health, Biomedical and Medical Sciences**
- **Bioelectronics**
- **Pharmacology**
- **Proteomics**

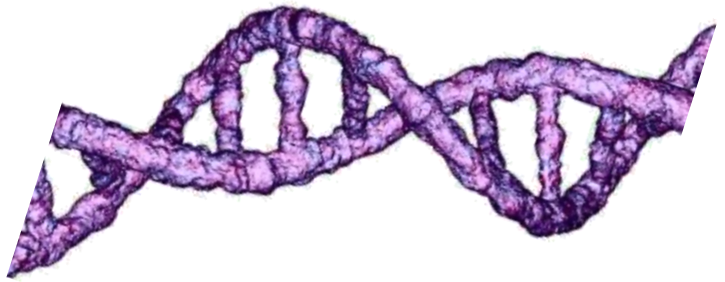
Key systems

Lipids



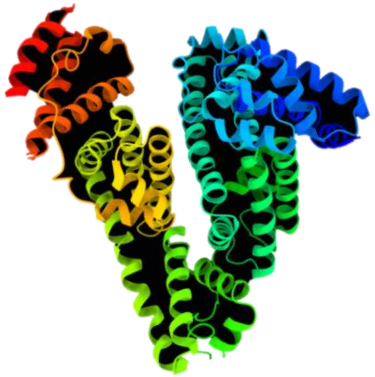
- **Structure**
- **Dynamics**
- **Role of composition** on emerging properties

Biopolymers



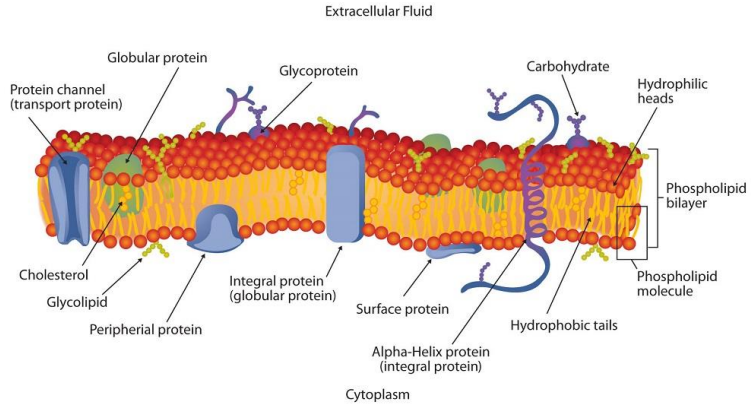
- **Structure**
- **Dynamics**

Proteins & Peptides



- **Structure**
- **Aggregation**
- **Dynamics**
- **Interactions**

Complex membranes



- **Interactions**
- **Structural remodeling**
- **Structure & dynamics VS composition**

Neutron techniques for structural characterisation

Atomic resolution

Neutron Diffraction

1. Crystallography
   - **Main use:** locating **H atoms** in biomacromolecules.
   - **Sample state:** crystalline, to capture structures at atomic resolution.
   - Commonly used for studying enzymes, proteins, and large biological complexes and to **visualize structured solvent networks**.

2. Fiber diffraction
   - **Main use:** understanding the **structure** of **biological fibers.**
   - **Sample state:** well-ordered, periodic fiber form (e.g., crystalline fibers).
   - Commonly used for studying DNA, filamentous bacterial viruses, polysaccharides, amyloids, etc…

3. Small-Angle diffraction
   - **Main use:** understanding the **structure** of **planar oriented** samples.
   - **Sample state:** oriented or ordered, e.g. stacked multilayers, etc…
   - Commonly used for studying lipid and protein multilayers, skin models, myelin sheaths, etc…

Neutron techniques for structural characterisation

From atomic to molecular resolution

Small-Angle Neutron Scattering (SANS)

- **Main use:** characterising **size**, **shape** and **internal structure** of biomacromolecules in **solution**.
- **Sample state:** in solution, typically 1-100 mg/ml.
- Can be combined with *in-situ* Size Exclusion Chromatography (**SEC-SANS**).
- Transversal for the study of many biosystems (lipid vesicles, proteins, their aggregates, etc…).

Neutron Reflectometry (NR)

- **Main use:** characterising **size**, **shape** and **internal structure** of biomacromolecules in **solution**.
- **Sample state:** planar **thin films**, typically **1-500 nm** thick.
- Can be combined with *in-situ* infrared (ATR-IR) and electrical impedance (EIS) spectroscopies.
- Commonly used to study biomembranes, protein films, polymer coatings, etc…

Neutron Imaging

- **Main use:** watch the internal structure (**direct space**) of macroscopic samples.
- **Sample state:** macroscopic, >few 10 mm^3 and in solid state.
- Typically used to study water in biological specimens.

Isotopic substitution for "coherent" techniques

Chemical composition of

- **a generic phopsholipid molecule (DOPC)**: **62% H**, 31% C, 6% O, 0.5% N, 0.5% P
- **a generic protein (HSA)**: **50% H**, 29% C, 10% O, 10% N, 1% P

Techniques with **atomic resolution** exploit **isotopic substitution** to alter the scattering behavior without changing the structural properties of the sample.

$$V(\vec{r}) = \frac{2\pi\hbar^2}{m_n} b^{coh}\delta(\vec{r})$$

$$b^{coh}(^1H) = -3.74\ fm$$

$$b^{coh}(D) = +6.67\ fm$$

Techniques with **lower resolution** exploit **contrast variation** to highlight or mask different parts of a complex system by selectively substituting specific isotopes without changing the structural properties of the sample.

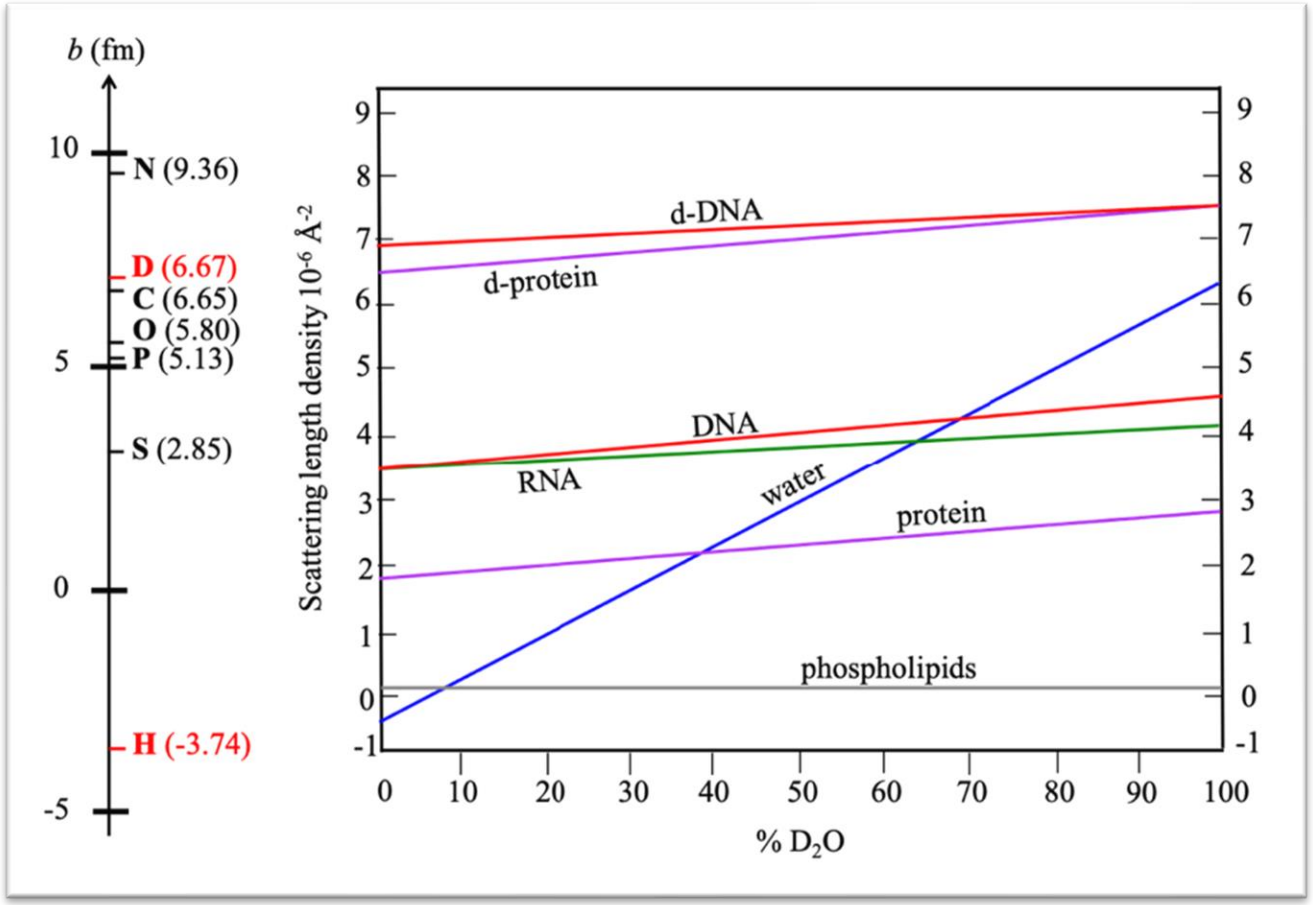
$$\rho = \sum_{j=1}^{N} \frac{b_j^{coh}}{V_m}$$

$$\rho(H_2O) = -0.56 \times 10^{-6}\ \text{Å}^{-2}$$

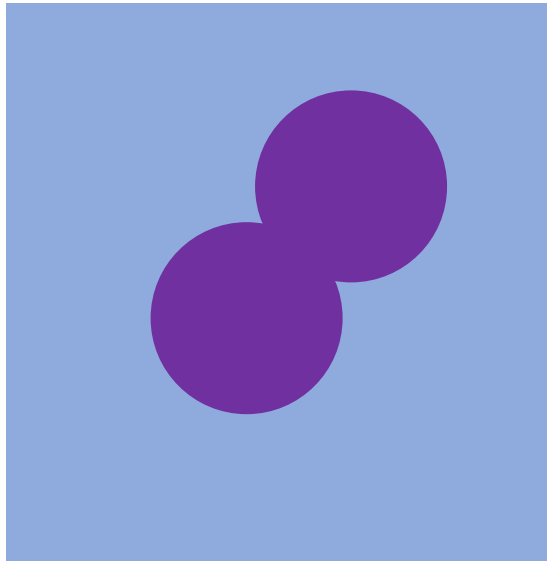
$$\rho(D_2O) = 6.35 \times 10^{-6}\ \text{Å}^{-2}$$

Contrast variation

$$\rho = \sum_{j=1}^{N} \frac{b_{coe}}{V_m}$$



The **contrast** is the **difference** between the **SLD** of the part of the **sample considered** and that of the **surrounding environment**.

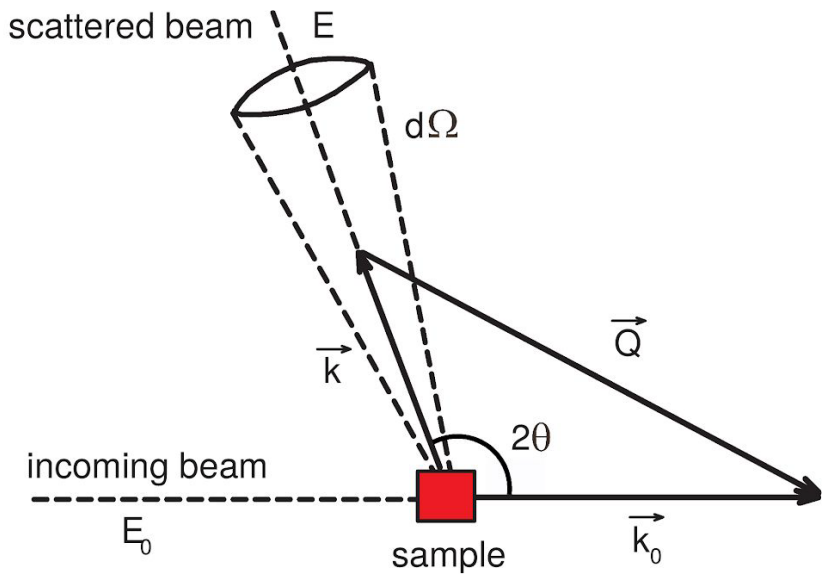


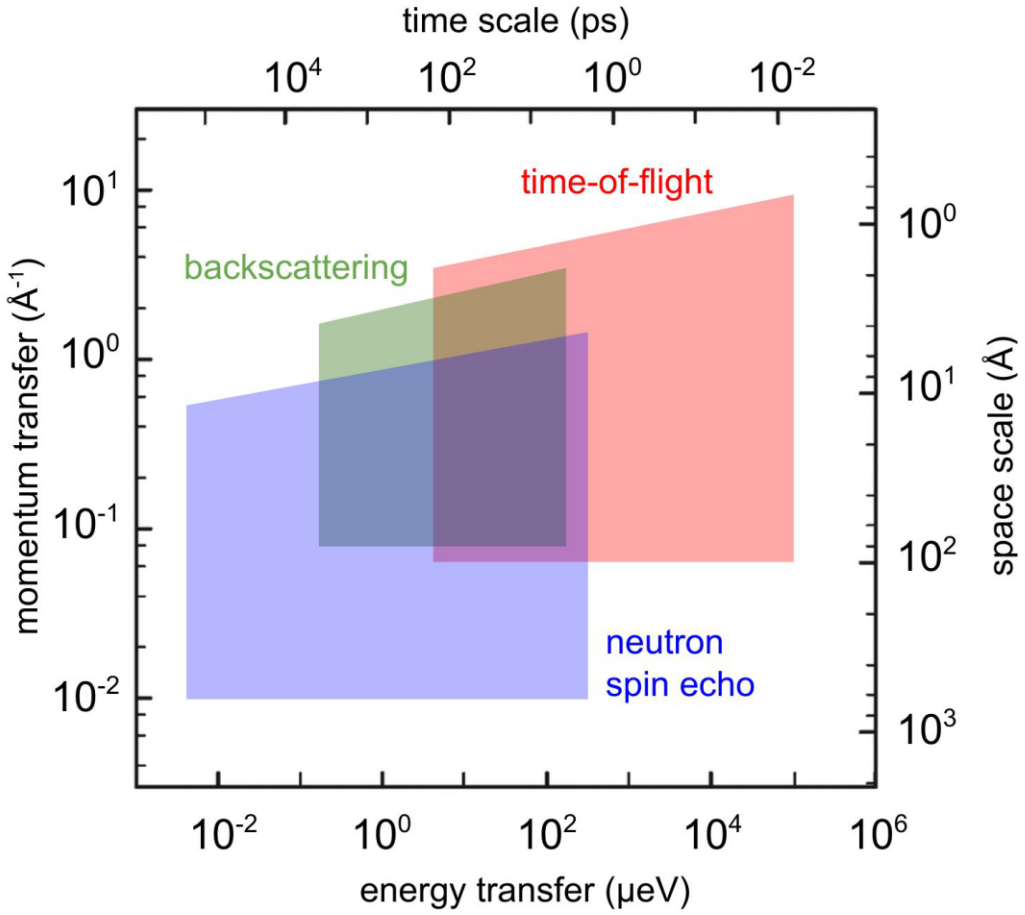
S. Combet *EPJ Web of Conference 2020*, 10.1051/epjconf/202023601001

Neutron Spectroscopy techniques *for life sciences*

$$\hbar\omega = E_i - E = \frac{\hbar^2}{2m_n}\left(k_0^2 - k^2\right)$$

Exchange of **momentum** and **energy**





Neutron techniques for dynamical characterisation

From picoseconds to nanoseconds

Inelastic Neutron Scattering (INS)

- **Main use:** probing the ***collective*** dynamics in biomacromolecules (and larger systems).
- Used to probe collective excitations at large energy transfers (meV regime).

Quasielastic Neutron Scattering (QENS)

- **Main use:** probing ***internal dynamics*** in biomacromolecules, ***diffusive behaviour*** of **water** and **biomacromolecules.**
- Largely used to investigate proteins and lipids in deuterium-rich environments (ps-ns, μeV regime).

Elastic Incoherent Neutron Scattering (EINS)

- **Main use:** probing the **average dynamics** of protiated macromolecules.
- Commonly used to determine the mean-square displacement of H atoms (< μeV regime).

Neutron Spin-Echo (NSE)

- **Main use:** probing slow correlated motions on the nano- to picosecond timescale.
- Commonly used to determine diffusion constants and collective motions.

For **INS**, **QENS** and **EINS samples** are hydrated powder or films and very dense solutions (> 50 mg of "sample" in the beam). For **NSE** concentrated solutions are typically used.

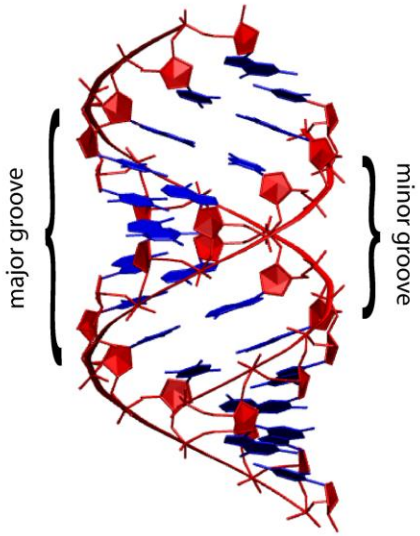
Selected examples

Fiber diffraction

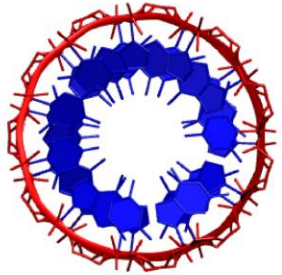
Example taken from

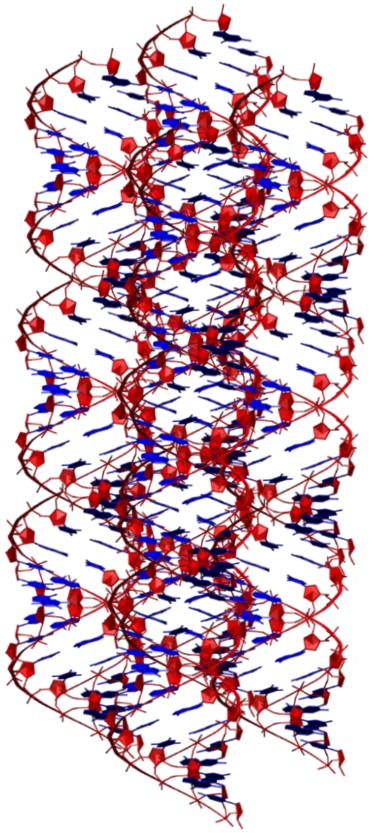
M. Haertlein *et al.* Methods in Enzymology **2016** 10.1016/bs.mie.2015.11.001

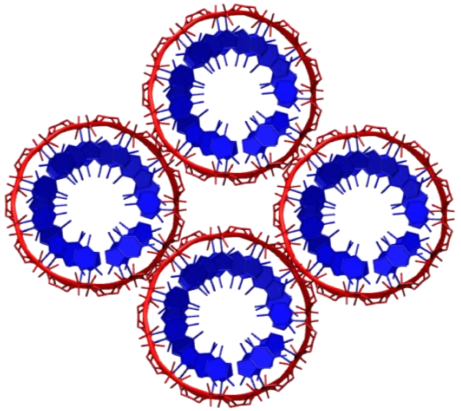
DNA fibers



A-DNA

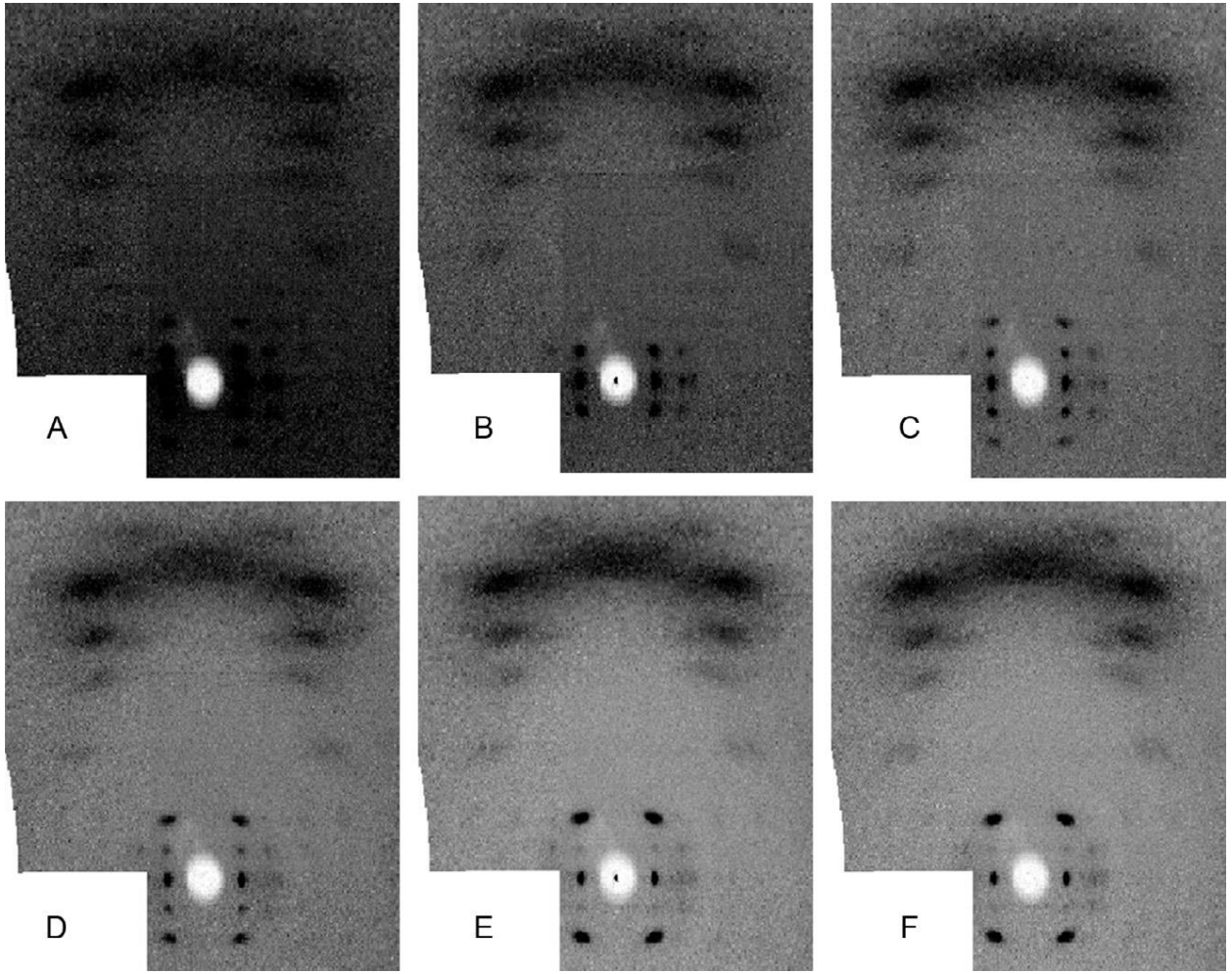






Isotopic substitution

Solvent structure and hydrogen bonding around DNA molecules



Data collected **hydrating** the fibres with water containing from **0% (A)** to **100% (F)** deuterium.

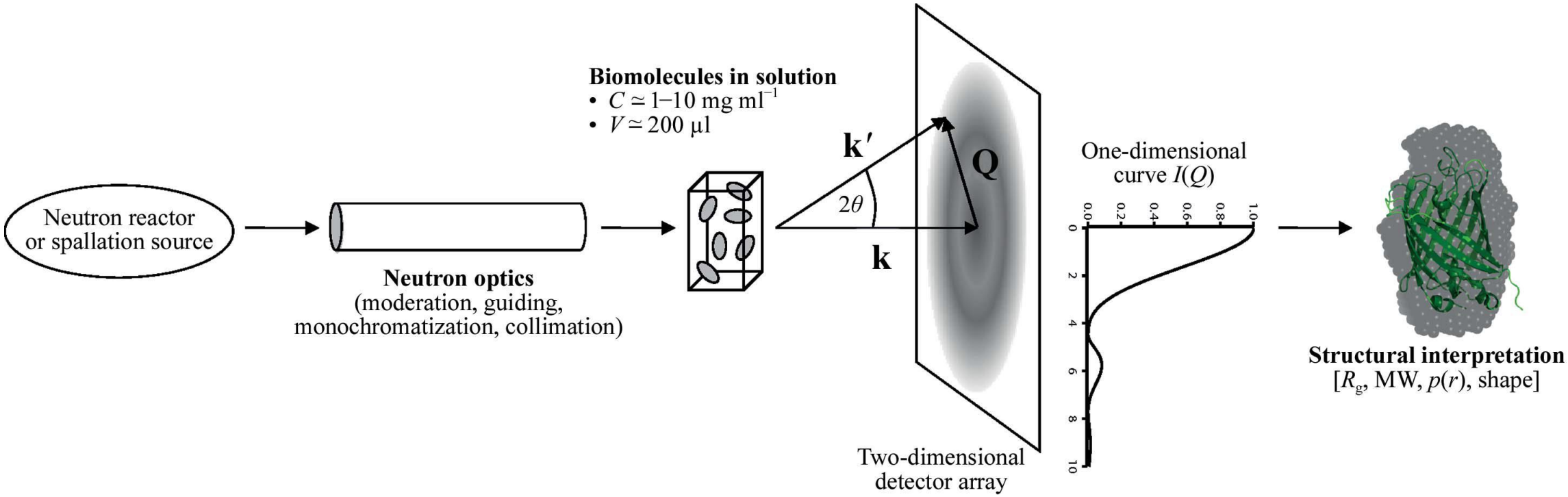
What ca we observe?

1. **Background** is **reduced**: less H in the sample, less the incoherent scattering.
2. **Relative intensity** of **some** diffraction peaks **changes**: labile H atoms of DNA (N-H, O-H groups) are exchanged by D atoms (formation of N-D, O-D groups).
3. Peaks disappearing are due to labile H atoms and by H atoms of water molecules.

SANS for life sciences

A generic SANS experiment

on a protein solution

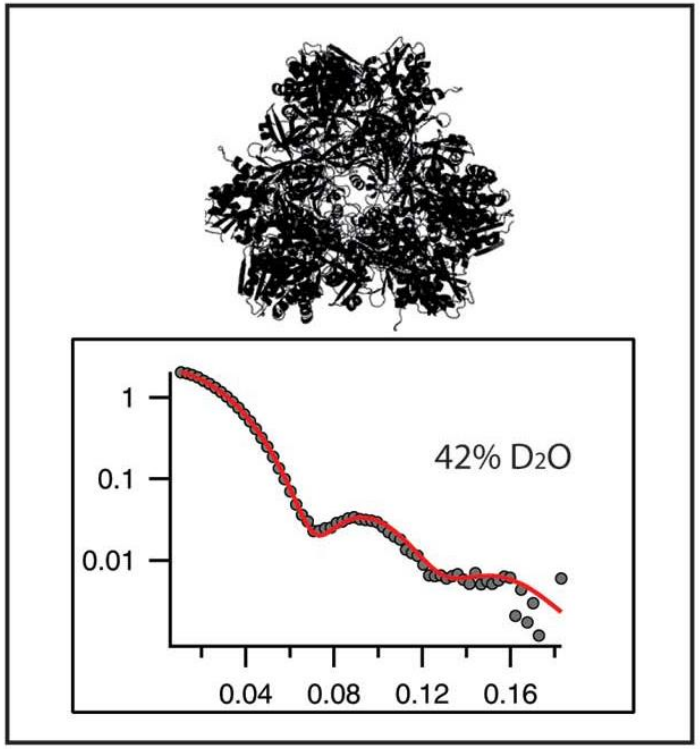


Mahieu & Gabel *Acta Cryst.* 2018, 10.1107/S2059798318005016

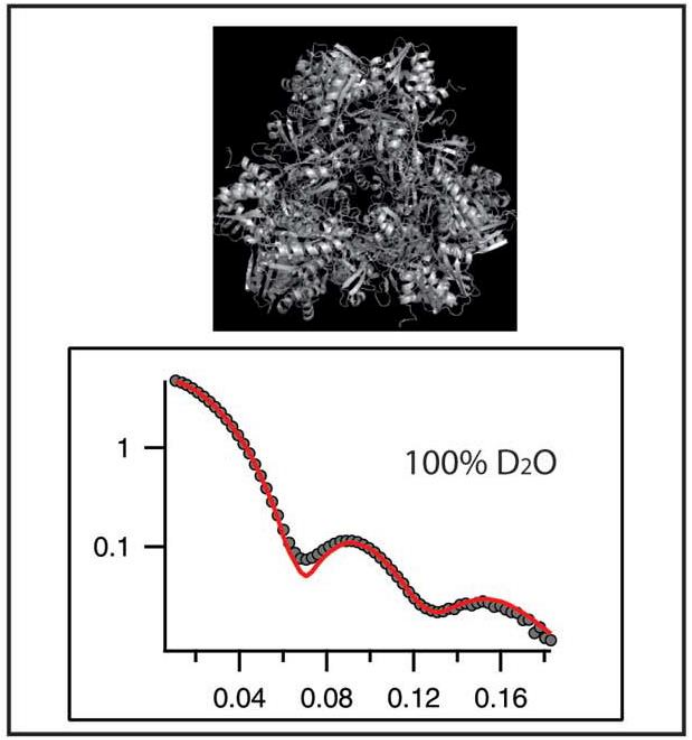
The structure of a protein complex

Pyrococcus horikoshii TET2–TET3 aminopeptidase complexes

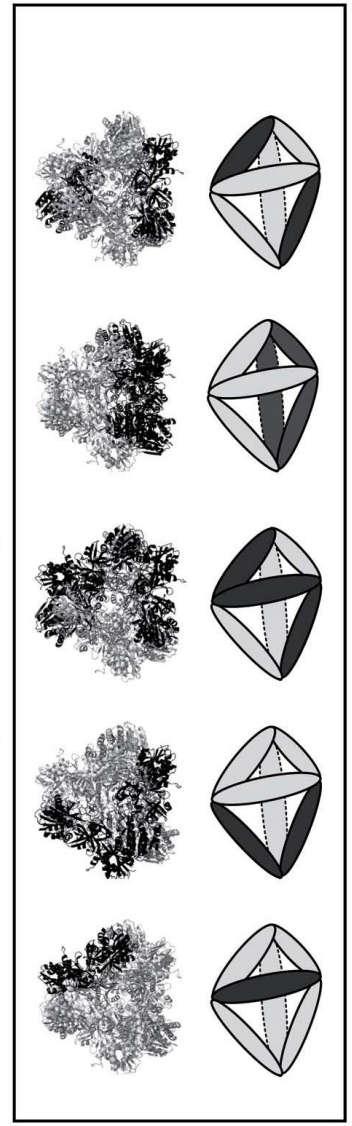
deuterated TET2 in 42% D2O



hydrogenated TET3 in 100% D2O



hetero-oligomers formed upon re-oligomerization of de-oligomerized TET2 and TET3



Mahieu & Gabel *Acta Cryst.* 2018, 10.1107/S2059798318005016
Appolaire *et al. Acta Cryst.* 2014, 10.1107/S1399004714018446

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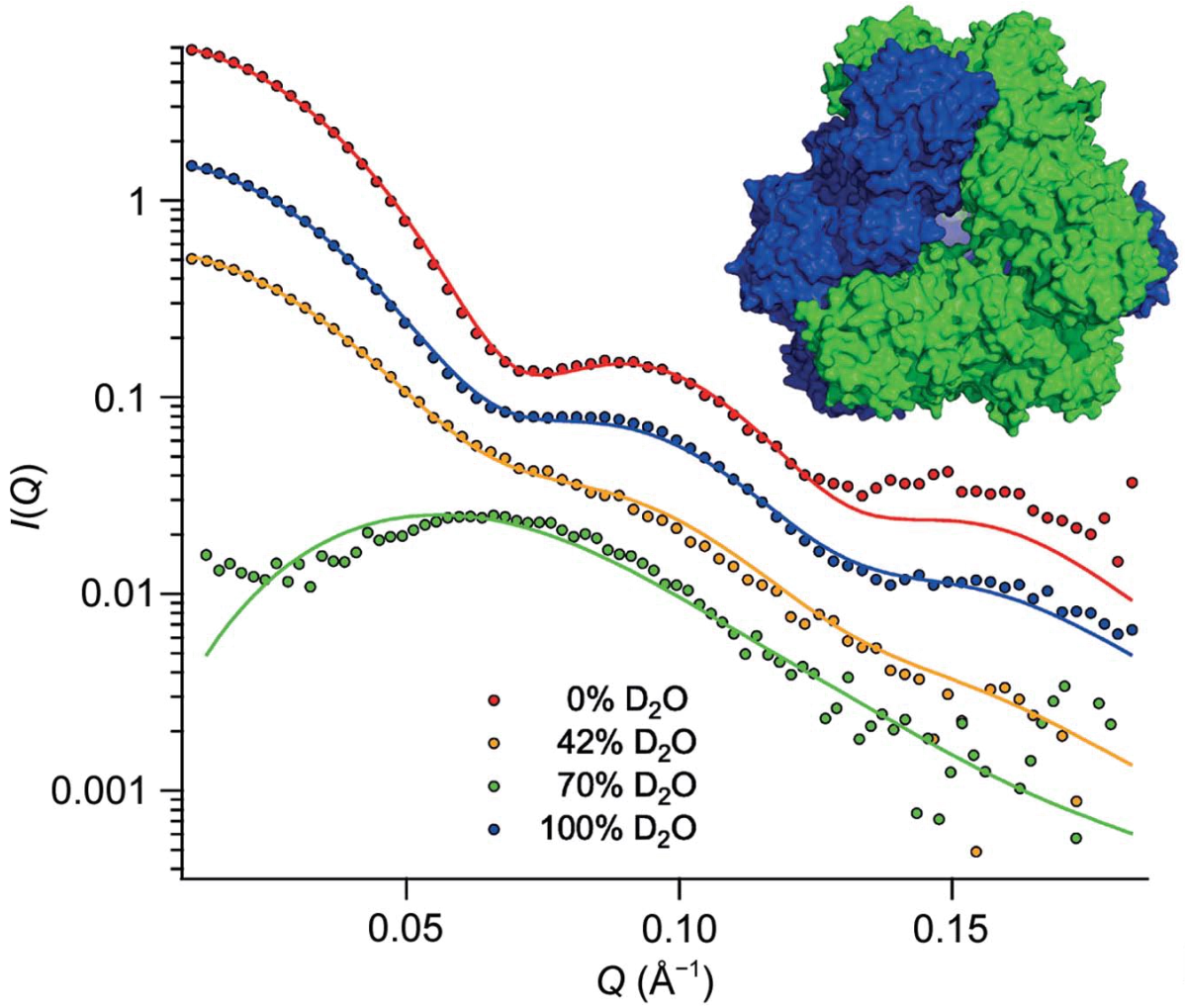
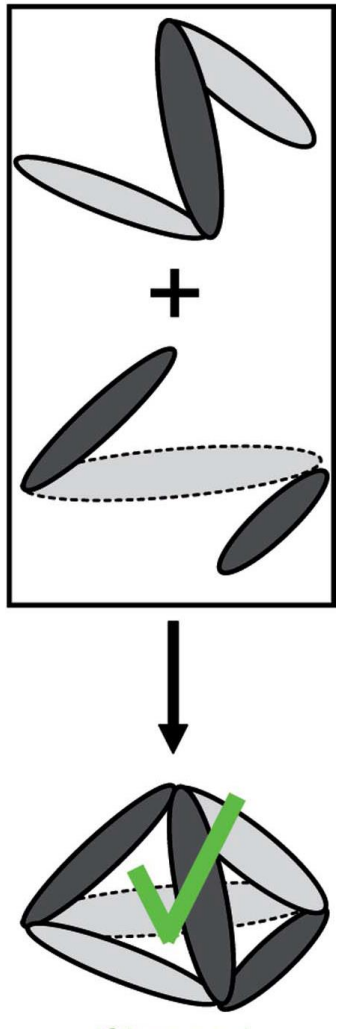


Final structural refinement benchmarked against all possible configurations



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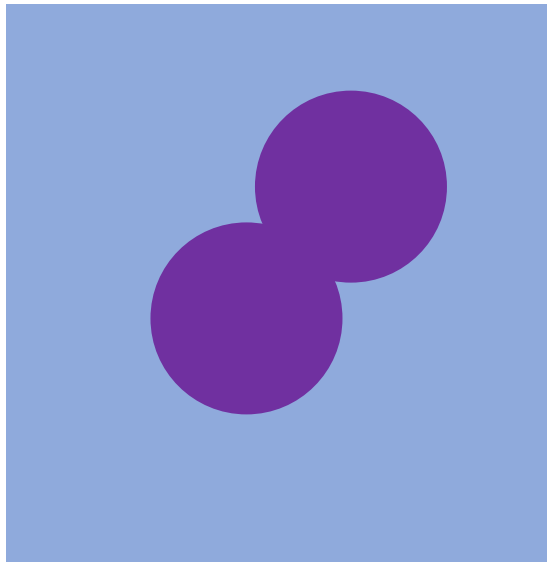
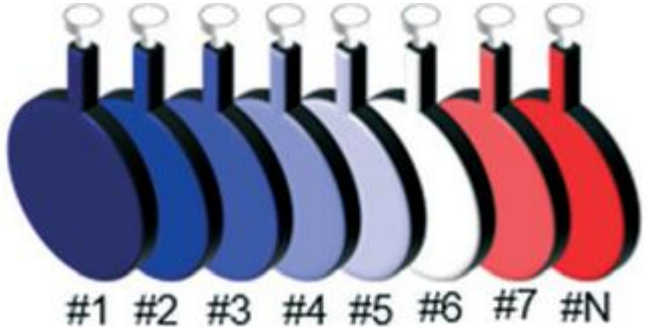
A model free approach

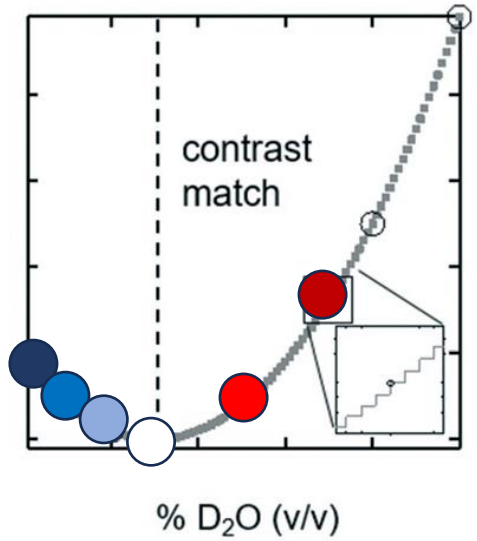
Composition from contrast variation series

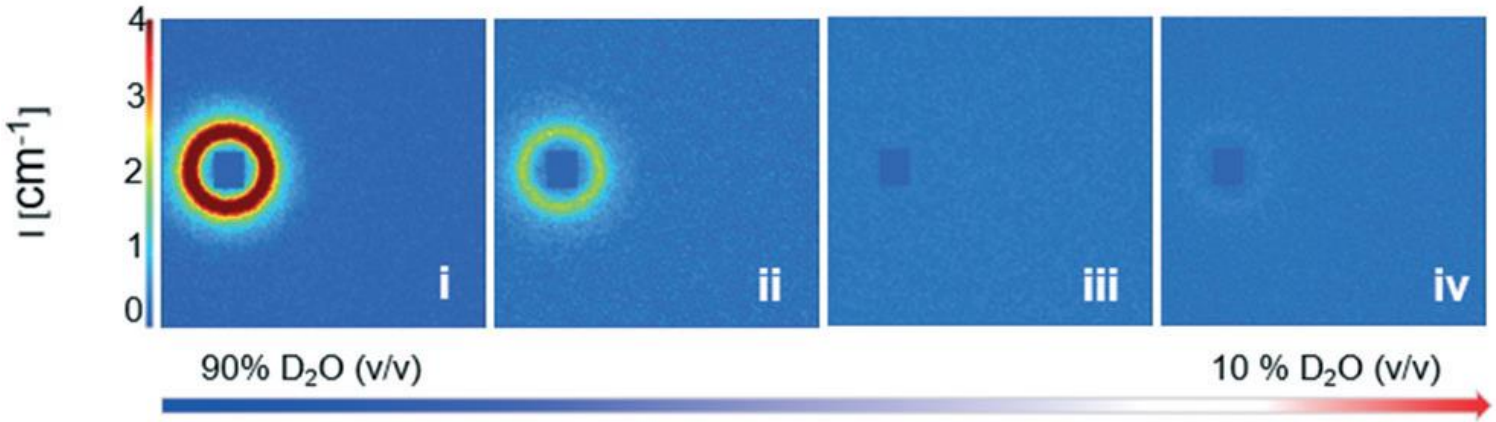


constant concentration, different D2O content



measure and integrate I(q)





M. Adamo *et al. Lab on a Chip* 2017, [10.1039/c7lc00179g](10.1039/c7lc00179g)

A model free approach

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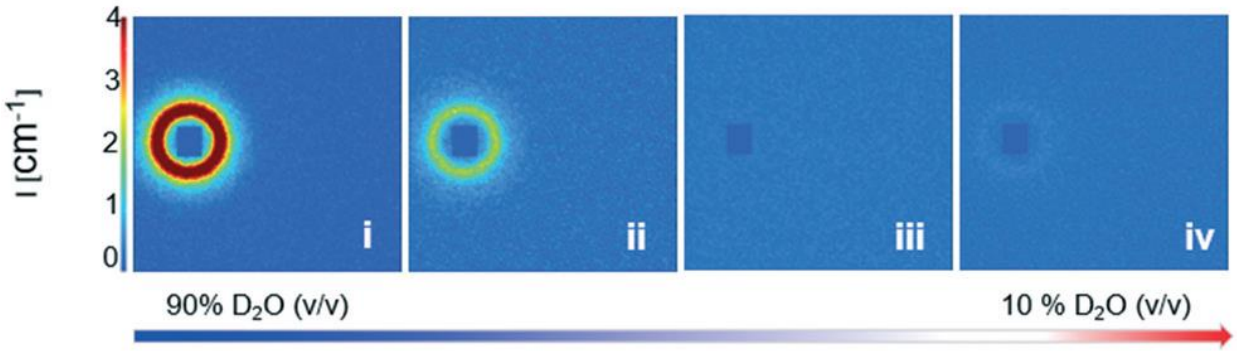
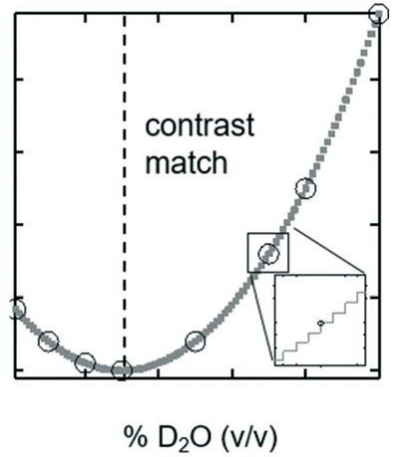


$$\frac{d\Sigma_c(q)}{d\Omega} = \left(\frac{\mathrm{N}}{\mathrm{V}}\right) \mathrm{V}_\mathrm{p}^2 \Delta\rho^2 P(q) S(q) + bkg$$

$\Delta\rho = 0$ defines **the match point** of the sample.

If $\Delta\rho = 0$, $\int dq I(q)$ is minimum.



For multi component complexes/aggregates: $I(q) \approx \sum_j \left(f_j \Delta\rho_j v_j\right)^2$

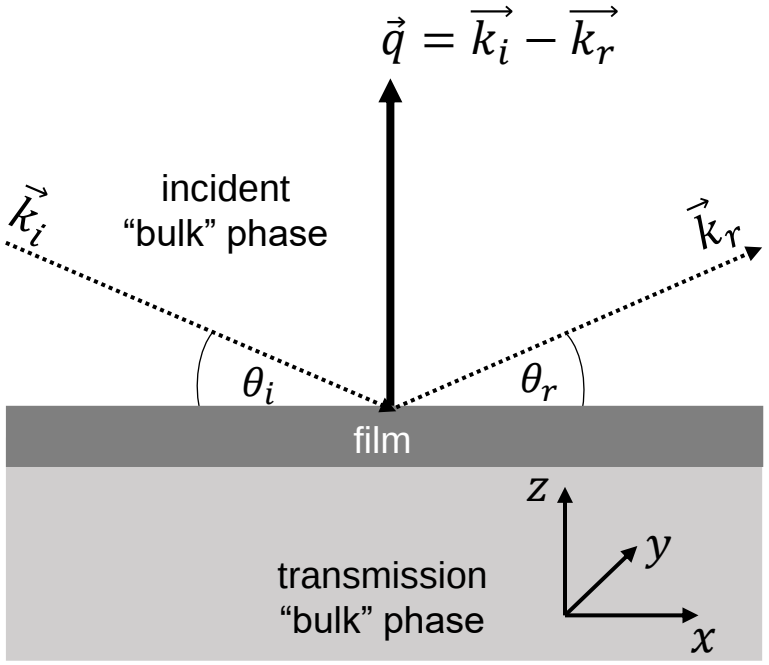
$$\rho_{MP} = 0 = \sum_j f_j \rho_j$$

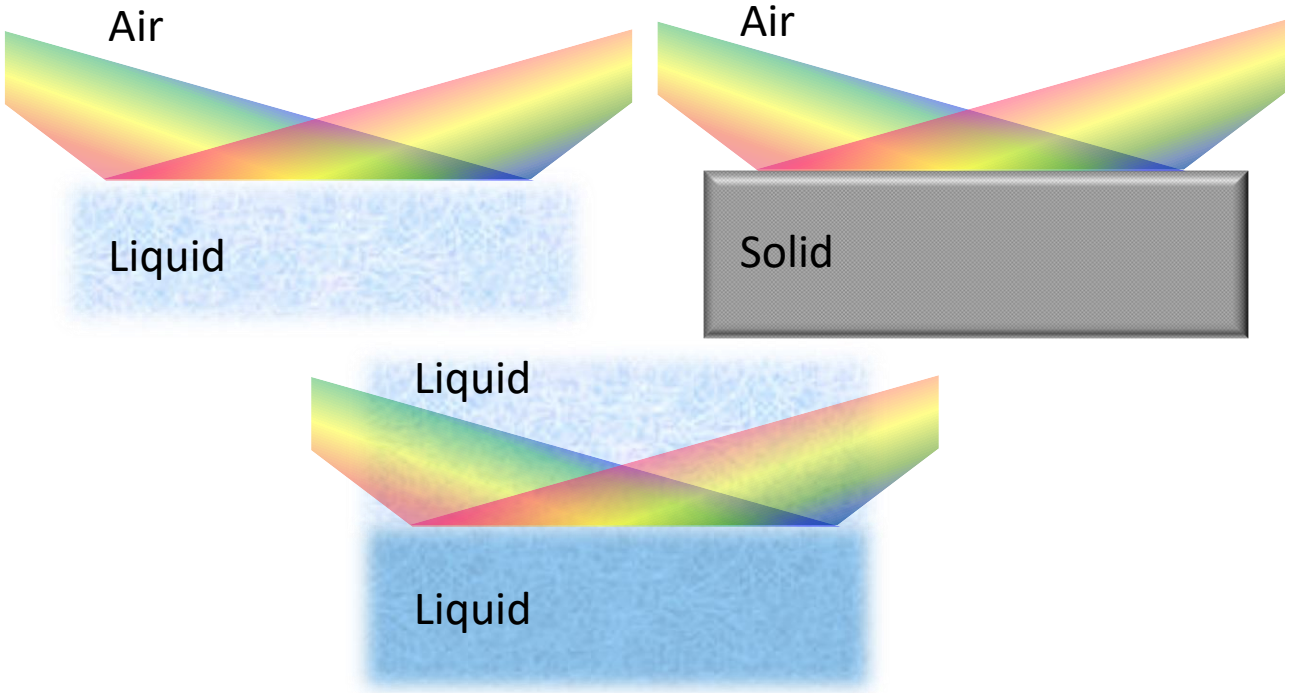
M. Adamo *et al. Lab on a Chip* 2017, [10.1039/c7lc00179g](10.1039/c7lc00179g)

Specular NR for life sciences

General experiment layout

specular NR





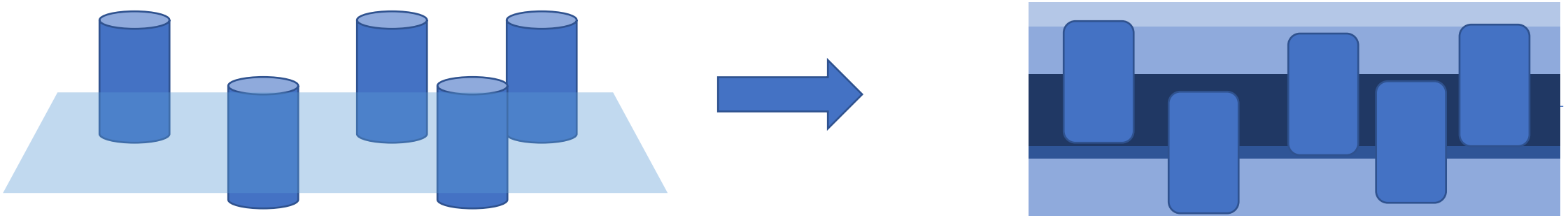
Reflectivity (R) is the quantity measured in a reflectometry experiment

$$R = \frac{num.reflect.neutrons}{num.incident\ neutrons}$$

Key considerations

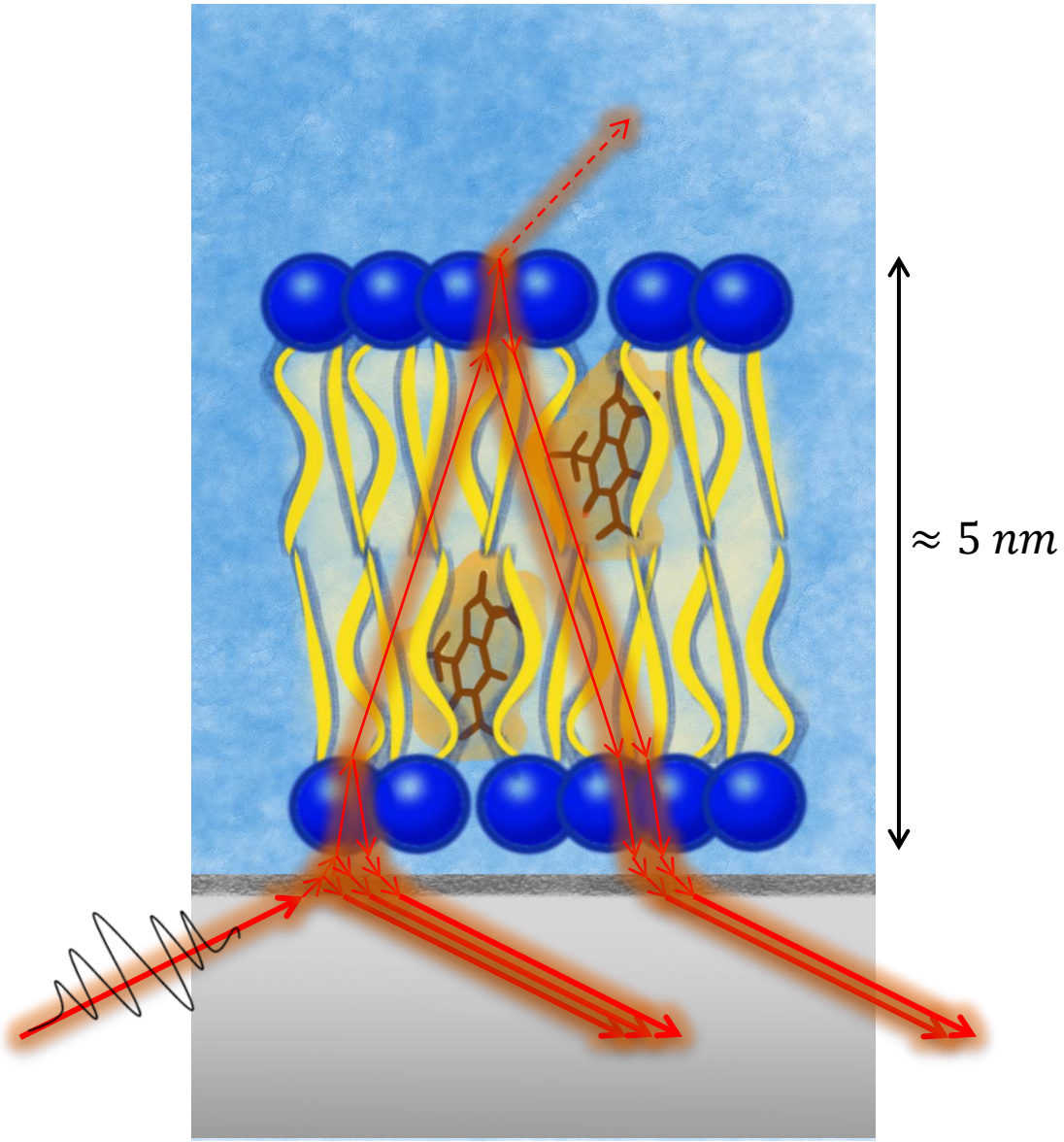
"Reflectometry is a technique used to determine the thickness and the internal structure of thin films at interfaces"

- **Thickness**: depends on sample and instrumentation, but from few (2-3) Å up to 10^3-10^4 Å, as rule of thumb.
- **Visible** to the probe i.e. with a ***good contrast*** with respect to the environment.
- Sensitivity on **internal structure**: down to few Å.



Always look to the sample from the side and imagine a layered structure!

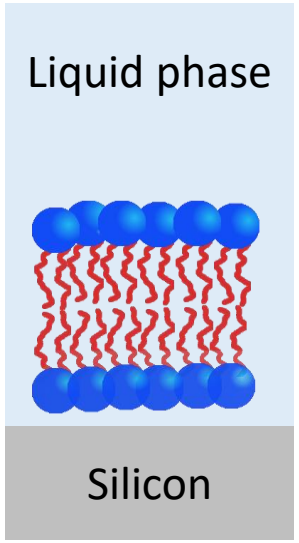
A visual example with a lipid bilayer

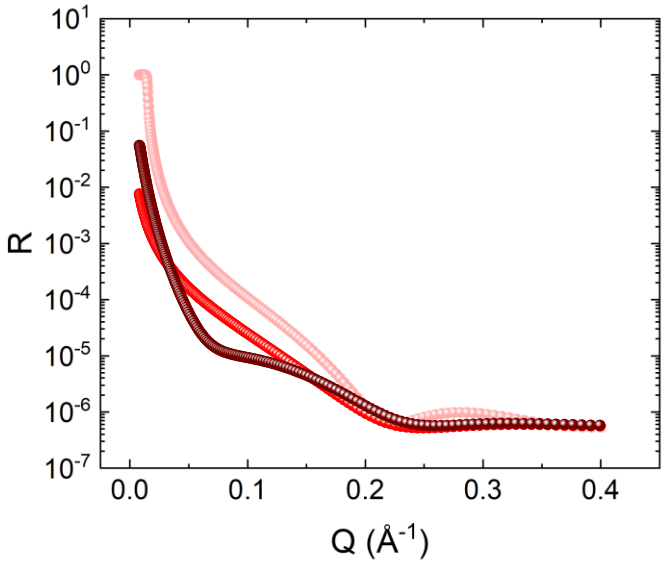


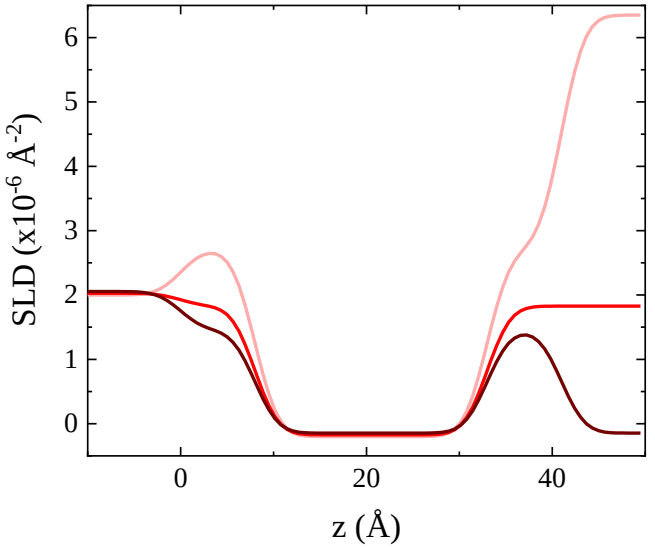
The result of a NR experiment

$$R(q) = \frac{16\pi^2}{q^4}\left|\int \frac{\partial \rho(z)}{\partial z} e^{i\vec{q}\cdot\vec{z}} d\vec{z}\right|^2$$

Master formula (valid at large q)





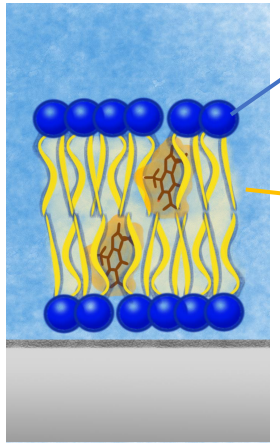


Contrast variation and multi-component materials

SLD for multi-component materials

$$\rho_{total} = \sum_i \phi_i \rho_i$$

$$\phi_i \equiv \frac{V_i}{\sum_j V_j} \equiv \frac{V_i}{V_{total}}$$



headgroups + water

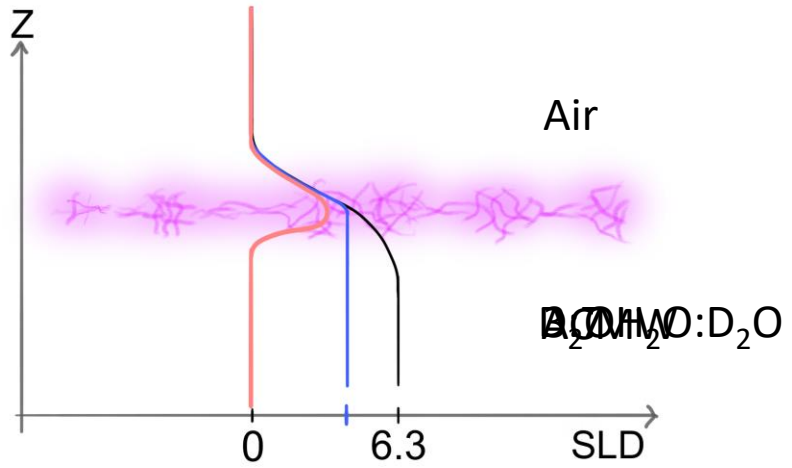
$\boldsymbol{\rho_B} = \phi_{HG} \cdot \rho_{HG} + \phi_w \cdot \rho_w = (1 - \phi_w) \cdot \rho_{HG} + \phi_w \cdot \rho_w$

tails + caffeine

$\boldsymbol{\rho_Y} = \phi_T \cdot \rho_T + \phi_C \cdot \rho_C = (1 - \phi_C) \cdot \rho_T + \phi_C \cdot \rho_C$

For hydration or solvent penetration in any layer

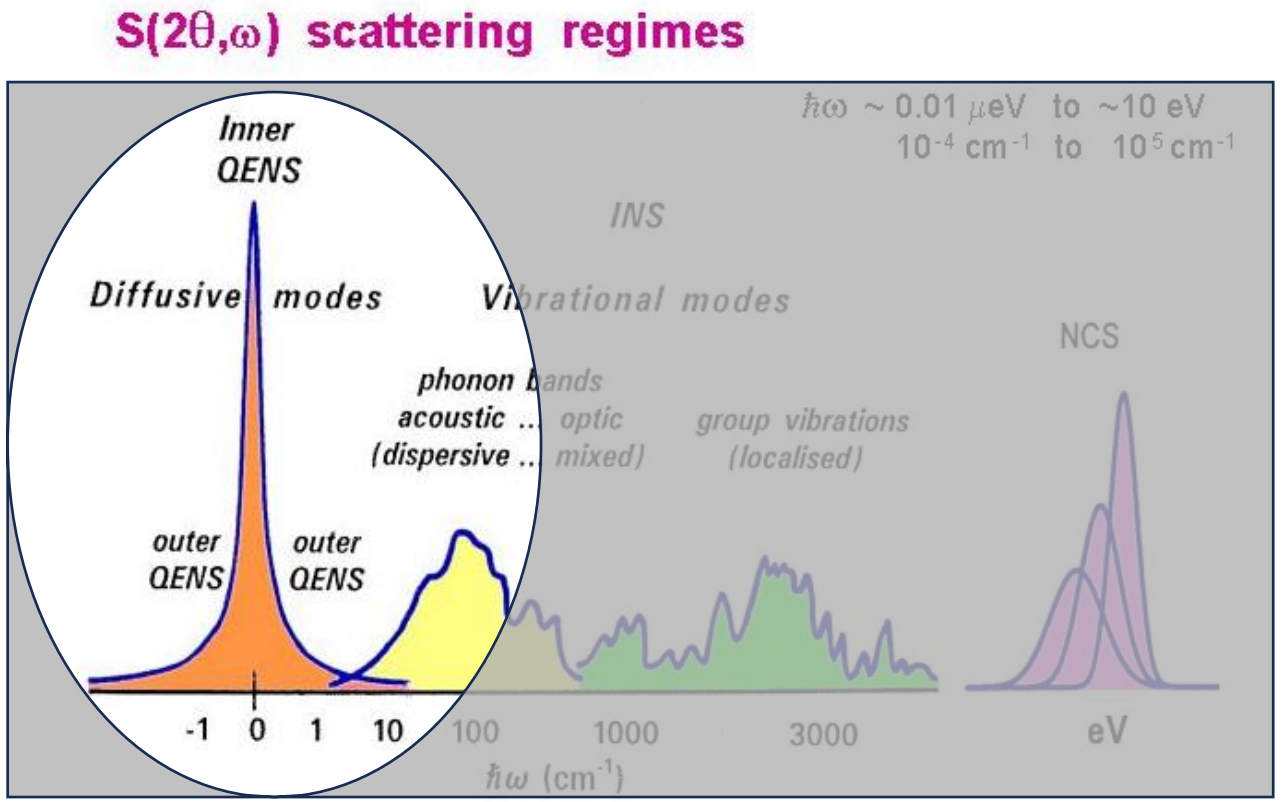
$$\rho_l = \phi^{dry} \rho^{dry} + \phi_w \rho_w = (1 - \phi_w)\rho^{dry} + \phi_w \rho_w, \quad \phi_w + \phi^{dry} = 1$$

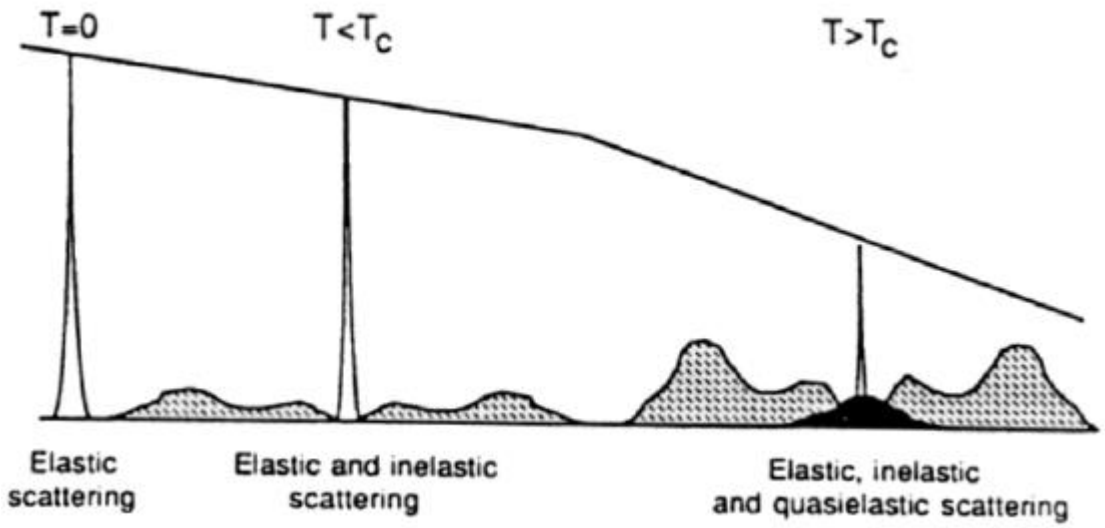


ACMW = **a**ir **c**ontrast-**m**atched **w**ater,
SLD = 0 i.e. **92:8** H_2O:D_2O by volume.

QENS for life sciences

Energy landscape





Incoherent Structure factor

Fourier transform of the self-correlation function, G_s

$$S_{incoh}(\vec{q},\omega) = \frac{1}{2\pi\hbar}\int G_s(\vec{r},t)e^{i(\vec{q}\cdot\vec{r}-\omega t)}d\vec{r}dt$$

For biological systems, a QENS spectrum consists of an elastic contribution and, at least, of a quasi-elastic broadening.

The **q-dependence** of the **elastic peak intensity** gives information on the

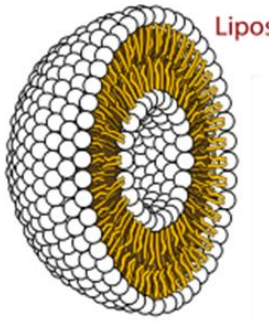
1. ***fraction*** of ***immobile***** H-atoms.
2. ***Geometry*** of ***confined*** motions, if any.

The **q-dependence** of the **QE broadening and intensity** gives information on the

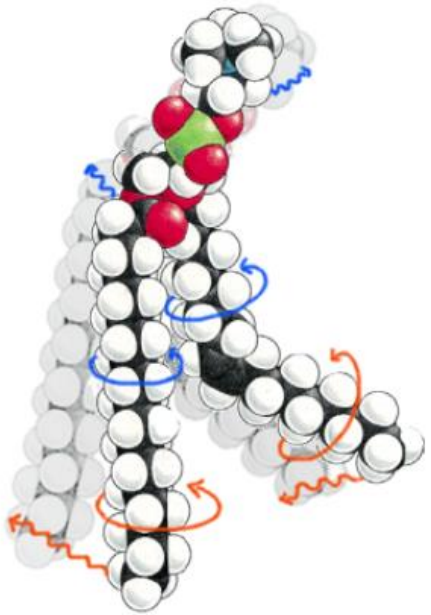
1. ***timescale*** and ***diffusion constants*** of the motions.
2. ***number*** of motions detected.

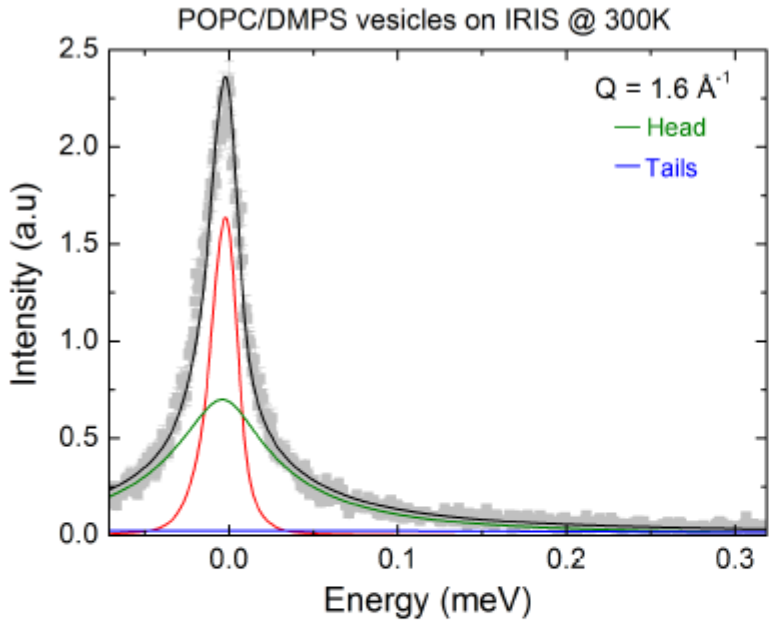
An example of phospholipid vesicles

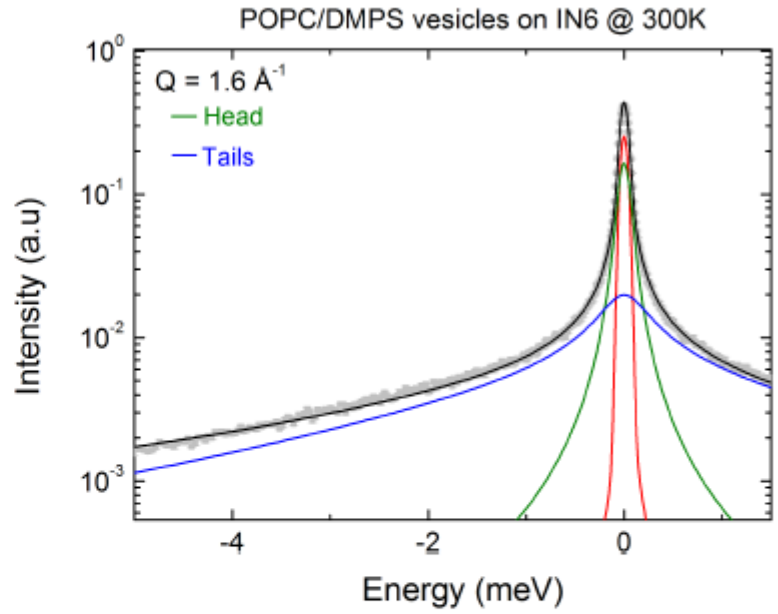
measured at different resolutions



Freeze-dried and re-hydrated in D_2O







IRIS @ *high resolution*

- The presence of a narrow quasi-elastic component is clear.
- The **broader** component could be **misinterpreted** as a flat background.

IN6 @ *low resolution*

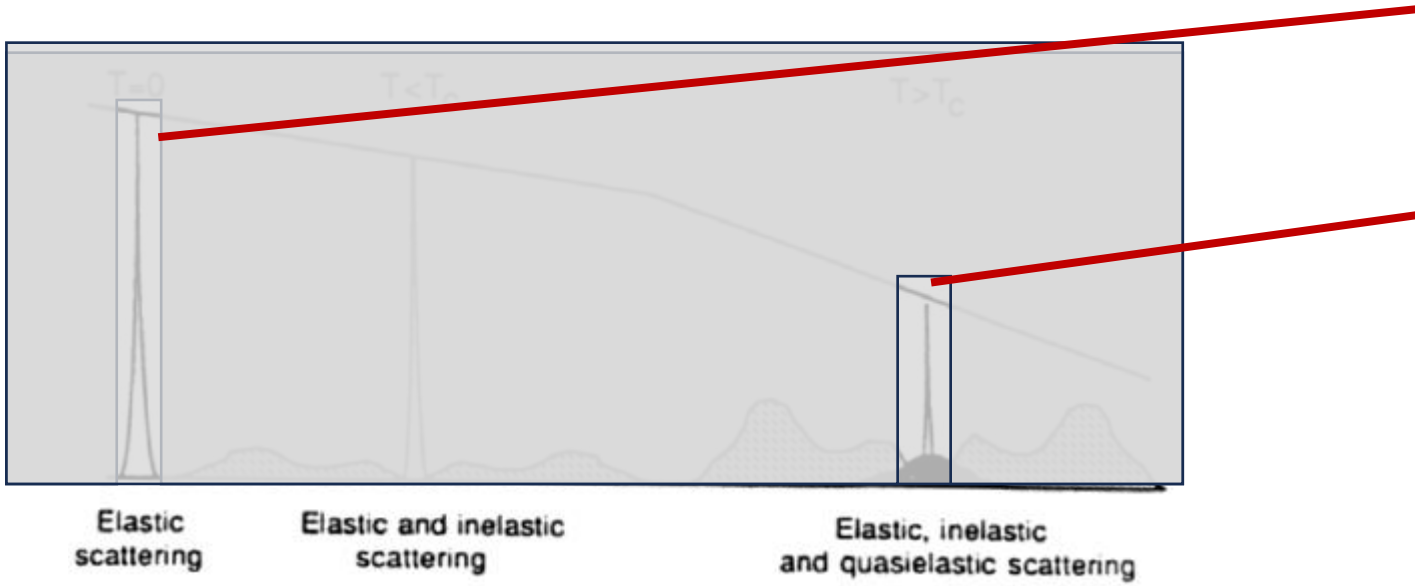
- The presence of the second sub-spectrum has been clarified.
- Its contribution to the total signal is the 25-30% of the total one.

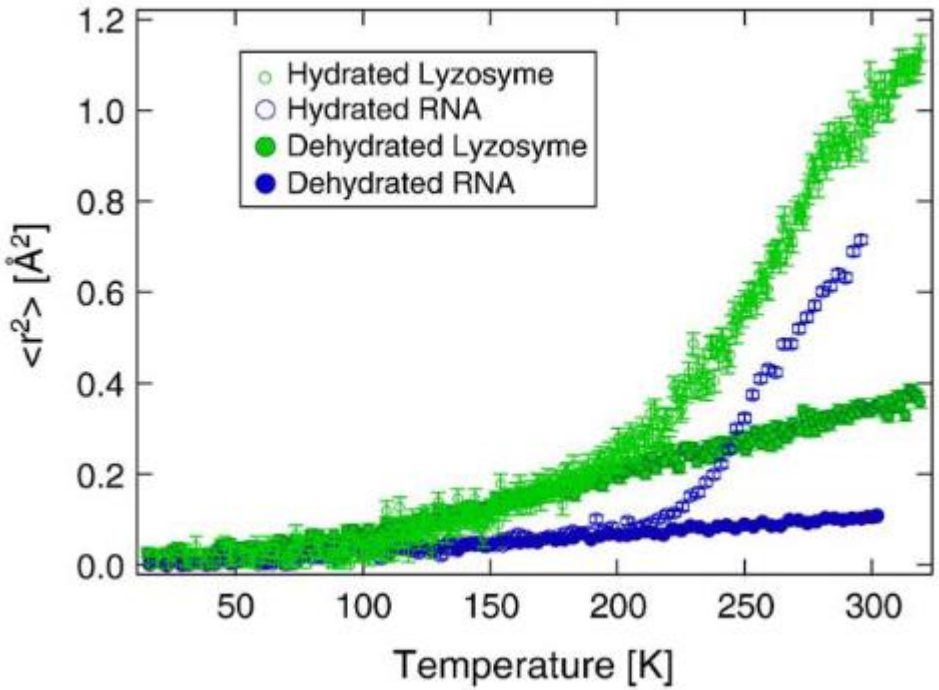
Y. Gerelli *et al. Soft Matter* 2011, 10.1039/CoSM01301C

Fixed elastic window scan

Some assumption:

1. the sample complexity limits the detail of the information that can be derived from this type of measurements.
2. The measurements are informative on the localisation, in temperature or other external parameters, of different dynamical regimes shown by the sample.
3. It is possible** to extract a mean square displacement information that is analogous to the Debye-Waller factor for solids.





Dynamics of hydration water

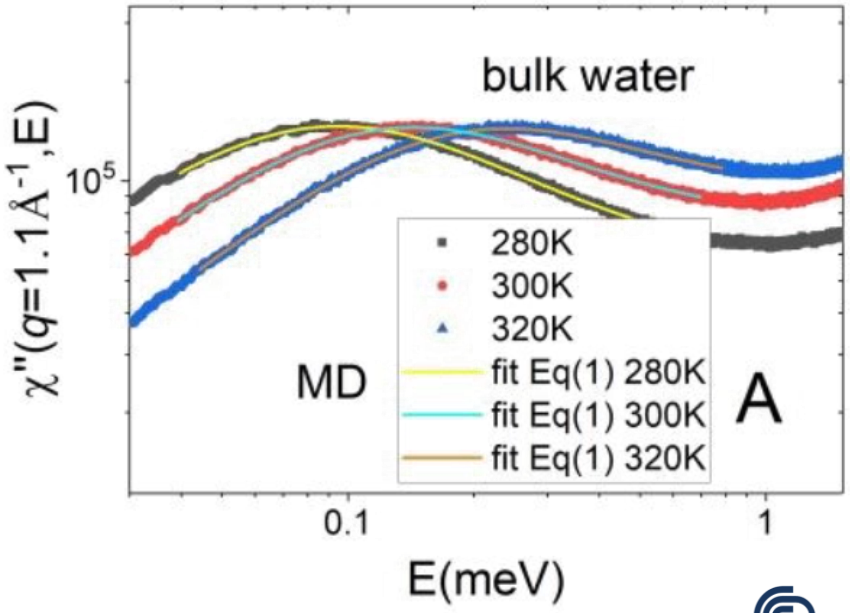
$$\chi'' = \frac{S(q,\omega)}{n_B(\omega)}, \text{neutron suscpeptibility}$$

$$n_B(\omega) = \left[e^{\frac{\hbar\omega}{k_B T}} - 1\right]^{-1}, \text{Bose factor}$$

$$\chi''(q,\omega) \approx A(q) \times \frac{\Gamma_T(q)}{\hbar^2\omega^2 + \Gamma_T^2(q)} + B(q) \times \frac{2\Gamma_r(q) + \Gamma_T(q)}{\hbar^2\omega^2 + \left(2\Gamma_r(q) + \Gamma_T(q)\right)^2}$$

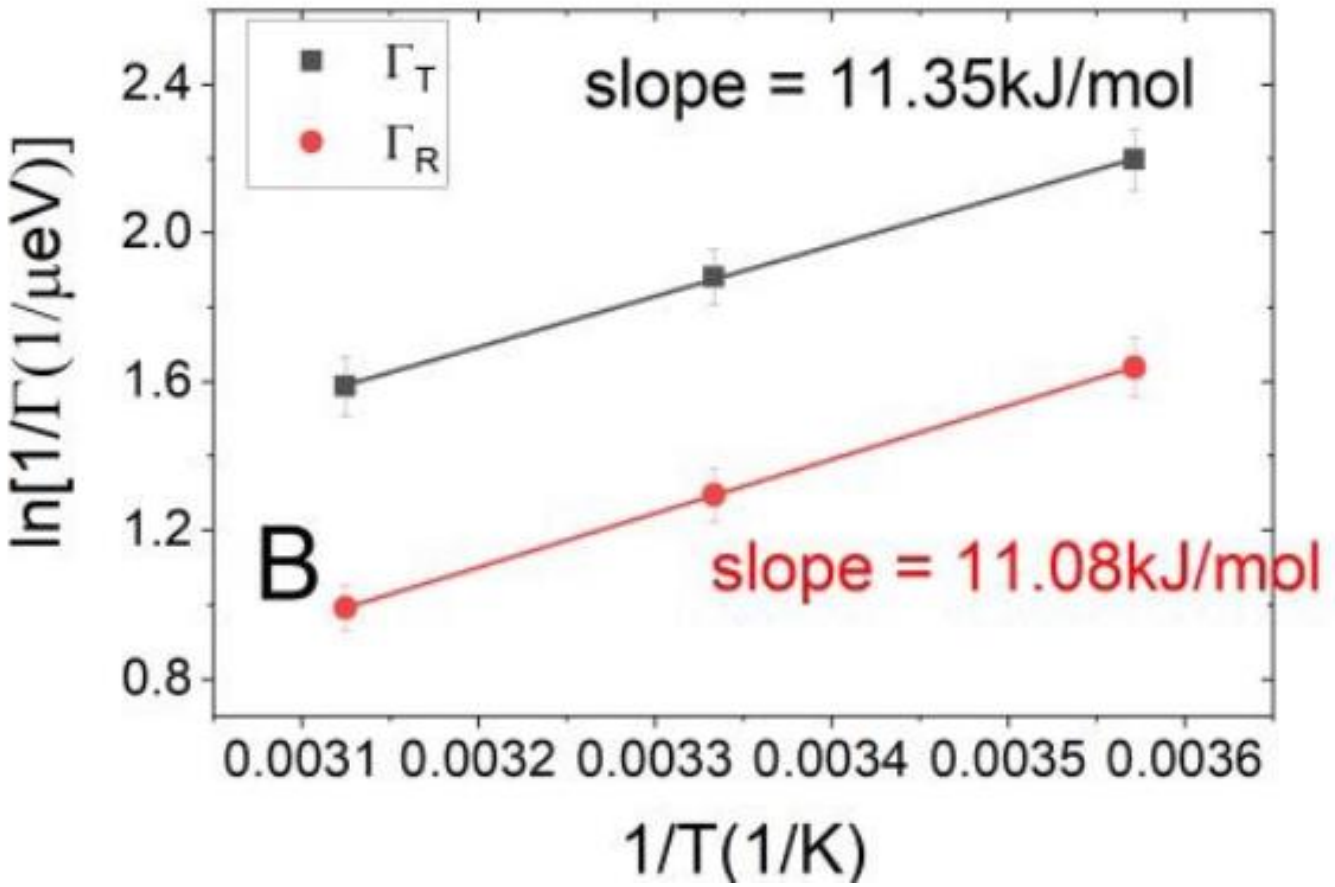
$\Gamma_T(q) \rightarrow$ measures the time for a particle to diffuse a distance 1/q. It increases with q.
$\Gamma_R(q) \rightarrow$ measures frequency of rotational motions. It is q-independent.

Sample:
perdeuterated protein powder
+
hydration water (H_2O).



P. Tan *et al. PCCP* 2020, 10.1039/DoCP02416C

Characterisation of individual motions



P. Tan *et al. PCCP* 2020, 10.1039/DoCP02416C

Final remarks – *for all techniques*

- The complexity of the sample poses challenges in data analysis.
- Detailed modeling, whether numerical or analytical, is necessary for proper data analysis.
- Knowledge from complementary techniques must be employed to build accurate structural and dynamical models.
- Separation of coherent and incoherent signals is not as straightforward as described in textbooks.
- Isotopic substitution might affect biological samples…