Neutron scattering from biomembranes

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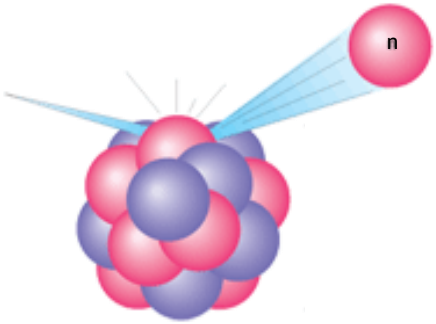

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Why radiation scattering?

How can we determine the relative positions and motions of atoms in a bulk sample of solid or liquid?

We need to 'see' inside the material with a suitable tool

?

By **EYE** we can see only through transparent materials
but
not the atoms

limit for visible light: 10^{-6}m
Interatomic distance in a solid ~ 10^{-10}m

→ Radiation scattering

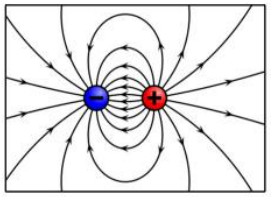
What is a neutron?

Quantum mechanics tells us that,
whilst it is certainly **particulate**,
the neutron also has a **wave** nature
and as such can display the gamut of wave behaviors
including reflection, refraction and diffraction.

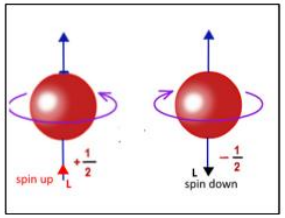
What is a neutron?

Neutron: a subatomic particle, symbol *n* (discovered by James Chadwick in 1932).

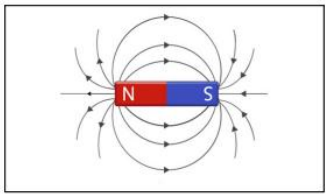
- No electric charge: $q_n=0$
- No electric dipole: $d_n=(0.0\pm1.1\pm0.2)\cdot10^{-26}$ e·cm



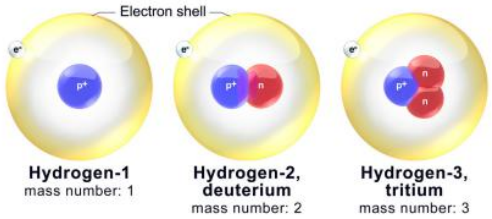
- Spin *(i.e.,* polarization): $s_n=1/2$.



- Large magnetic moment: $\mu_n=-1.913043\ \mu_N = -9.662364\cdot10^{-27}\ JT^{-1}$.



- Mass slightly greater than that of a proton *(p):* $m_n=1.674927\cdot10^{-27}$ kg ($m_p=1.672622\cdot10^{-27}$ kg).
- Neutrons and protons form atomic nuclei: neutrons are in all nuclei except in ^1H. Number of neutrons, *N: N = A - Z (i.e.,* atomic mass number minus proton number).



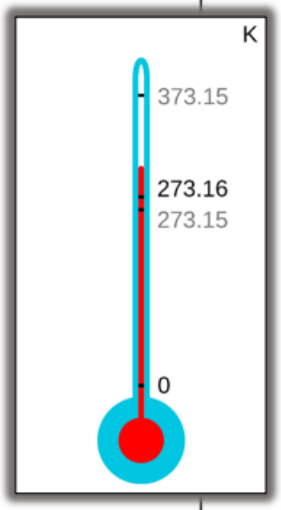
- Lifetime: virtually infinite inside **stable** atomic nuclei. "Half-life", $t_{1/2}=611$ s, when free.

What is a neutron?

First disclaimer: in this lesson we will not study **bound neutrons** (in either stable or instable nuclei), leaving them to nuclear physics.

We will focus on **free neutrons** only, which are usually divided according to their "temperature" T or, more properly, by their (kinetic) energy $E = k_B T$ as:

- **Ultracold:** $E<0.1$ meV $\rightarrow$ $T<1.2$ K
- **Very cold:** 0.1 meV$<E<$0.5 meV $\rightarrow$ 1.2 K$<T<$5.8 K
- **Cold:** 0.5 meV$<E<$ 5.0 meV $\rightarrow$ 5.8 K$<T<$58 K
- **Thermal:** 5 meV$<E<$100 meV $\rightarrow$ 58 K$<T<$1,200 K
- **Epithermal:** 0.1 eV$<E<$ 1 eV $\rightarrow$ 1,200 K$<T<$12,000 K
- **Resonant:** 1 eV$<E<$100 eV
- ….



What is a neutron?

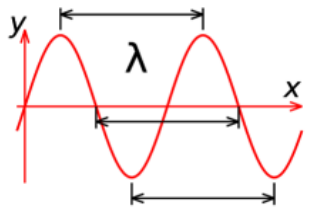
Second Disclaimer: in this lesson we will deal only with the so-called "**slow neutrons**", *i.e.*, those with $E<1$ keV. Among the other things (see later), slow neutrons are totally **non-relativistic objects** since $m_n c^2 = 939.6$ MeV$>>$ 1 keV, and so one can easily work out their speed from $E = 1/2\, m_n v^2$.

- **Ultracold:** $E<0.1$ meV $\rightarrow$ $v<138$ m/s
- **Very cold:** 0.1 meV$<E<$0.5 meV $\rightarrow$ 138 m/s$<v<$309 m/s
- **Cold:** 0.5 meV$<E<$5.0 meV $\rightarrow$ 309 m/s$<v<$978 m/s
- **Thermal:** 5 meV$<E<$100 meV $\rightarrow$ 978 m/s$<v<$4,374 m/s
- **Epithermal:** 0.1 eV$<E<$1 eV $\rightarrow$ 4,374 m/s$<v<$13,830 m/s
- **Resonant:** 1 eV$<E<$100 eV $\rightarrow$ 1.383×10^4 m/s$<v<1.383\times10^5$ m/s
- ....

What is a neutron?

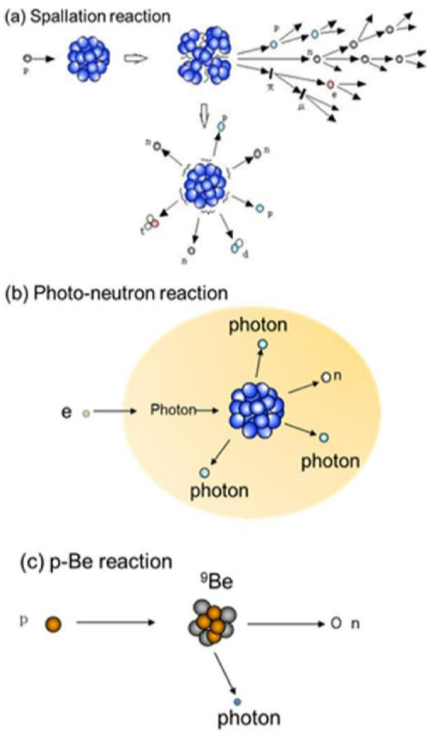
Neutrons are microscopic objects, so they are better described by **quantum mechanics (QM)** rather than by classical Newtonian mechanics. **QM** implies the famous **particle-wave dualism:** every particle with mass m and speed v is associated to a wave, showing a wavelength λ which is expressed by the famous **de Broglie equation,** where h is the **Planck constant.** In the neutron case: $\lambda = h/(m_n v) = h/(2 m_n E)^{0.5}$:

- **Ultracold:** $E<0.1$ meV $\rightarrow$ $\lambda>28.6$ Å [1 Å = 10^{-10} m]
- **Very cold:** 0.1 meV$<E<$0.5 meV $\rightarrow$ 12.8 Å$<\lambda<$26.8 Å
- **Cold:** 0.5 meV$<E<$ 5.0 meV $\rightarrow$ 4.04 Å$<\lambda<$12.8 Å
- **Thermal:** 5 meV$<E<$100 meV $\rightarrow$ 0.904 Å$<\lambda<$4.04 Å
- **Epithermal:** 0.1 eV$<E<$ 1 eV $\rightarrow$ 0.286 Å$<\lambda<$0.904 Å
- **Resonant:** 1 eV$<E<$100 eV $\rightarrow$ 0.029 Å$<\lambda<$0.286 Å
- …



All this is very interesting for **chemico-physical applications** since the typical atomic/molecular size is actually ~1 Å !

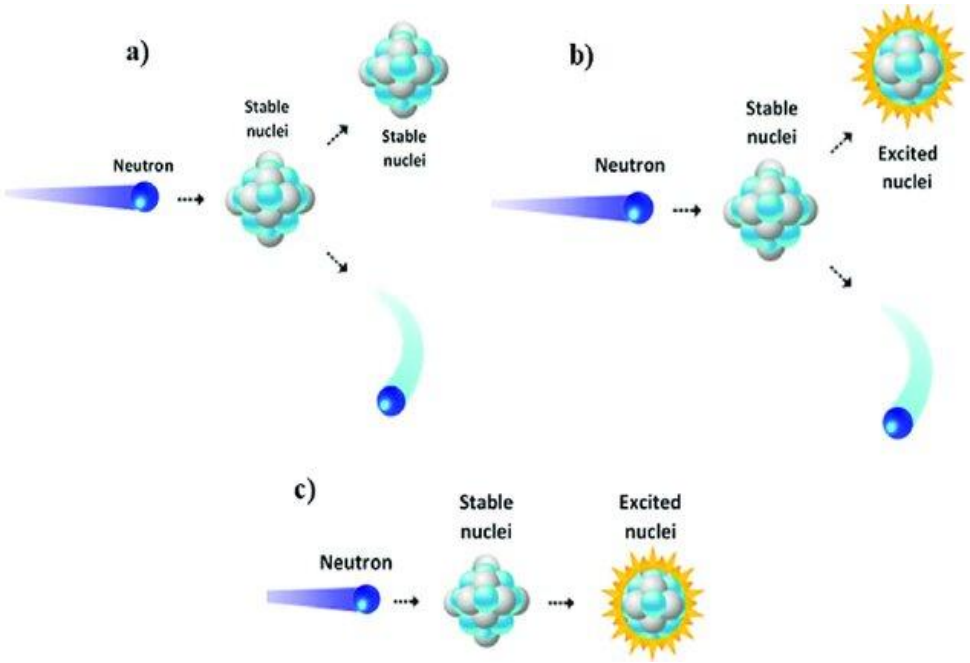
How and where?



Kiyanagi, Y. AAPPS Bull. (2021)

What does ‘scattering’ mean?

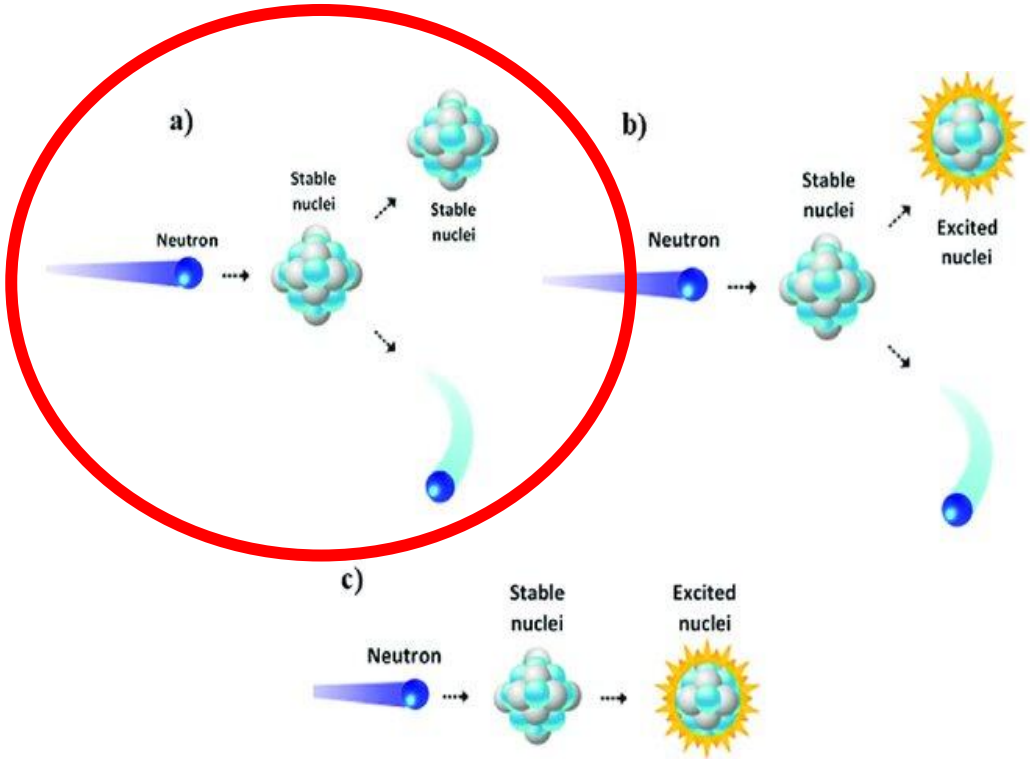
In physics, **scattering** is a process where moving particles or waves, such as light or sound, are forced to deviate from a straight trajectory by interacting with localized non-uniformities in a medium or with other particles.
This deviation can result in a change in direction, energy, frequency, or phase.



10.1016/B978-0-12-820549-5.00010-3

Neutron scattering

We will focus on what happens to the neutrons neither transmitted nor adsorbed by nuclei, but **just scattered** by them



10.1016/B978-0-12-820549-5.00010-3

Double-differential cross section

Since the neutron-nucleus interaction is probabilistic, we need a way to quantify **WHAT PRECISELY IS MEASURED IN A NEUTRON SCATTERING EXPERIMENT**

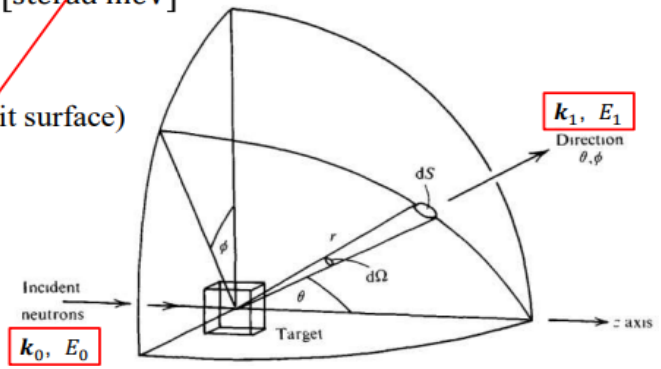
$$\left(\frac{d^2\sigma}{d\Omega dE_1}\right) \doteq \frac{\text{No. of neutrons scattered per second in } d\Omega(\theta,\phi) \text{ and in } [E_1, E_1 + dE_1]}{\Phi d\Omega dE_1} =$$

$$= \frac{1}{\Phi}\frac{d^2N(\theta,\phi,E_1)}{d\Omega dE_1} \qquad \left[\frac{\text{m}^2}{\text{sterad meV}}\right]$$

with

$\Phi \doteq$ flux of incident neutrons
= (neutrons per second through unit surface)

1 barn (bn) $\doteq 10^{-24}$ cm^2



Is a measure of the probability that a specific nuclear interaction will occur between an incident particle and a target nucleus.
It represents the effective target area of a nucleus for a collision with a neutron.
The cross-section **is not the physical size of the nucleus but a quantum mechanical measure of a specific interaction's likelihood**. For example, the scattering cross-section of a nucleus for slow neutrons can be much larger than its actual geometric area.

There are total, absorption, and **scattering cross-sections**.

Double-differential cross section

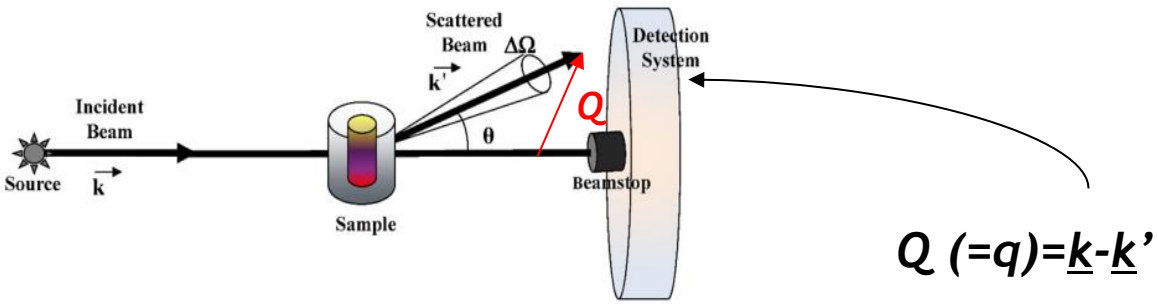
$$\left(\frac{d^2\sigma}{d\Omega dE_1}\right) = \frac{k_1}{k_0}\frac{1}{2\pi\hbar}\sum_{ij} b_i b_j \int_{-\infty}^{\infty} \left\langle e^{-i\boldsymbol{Q}\cdot\boldsymbol{R}_i(0)}\, e^{i\boldsymbol{Q}\cdot\boldsymbol{R}_j(t)} \right\rangle e^{-i\omega t}\, dt$$

Neutron flux and kinematics

Coupling

Sample Properties !
$\boldsymbol{R}_i(0), \boldsymbol{R}_j(t)$
where atoms ARE
what atoms DO

STRUCTURE and DYNAMICS

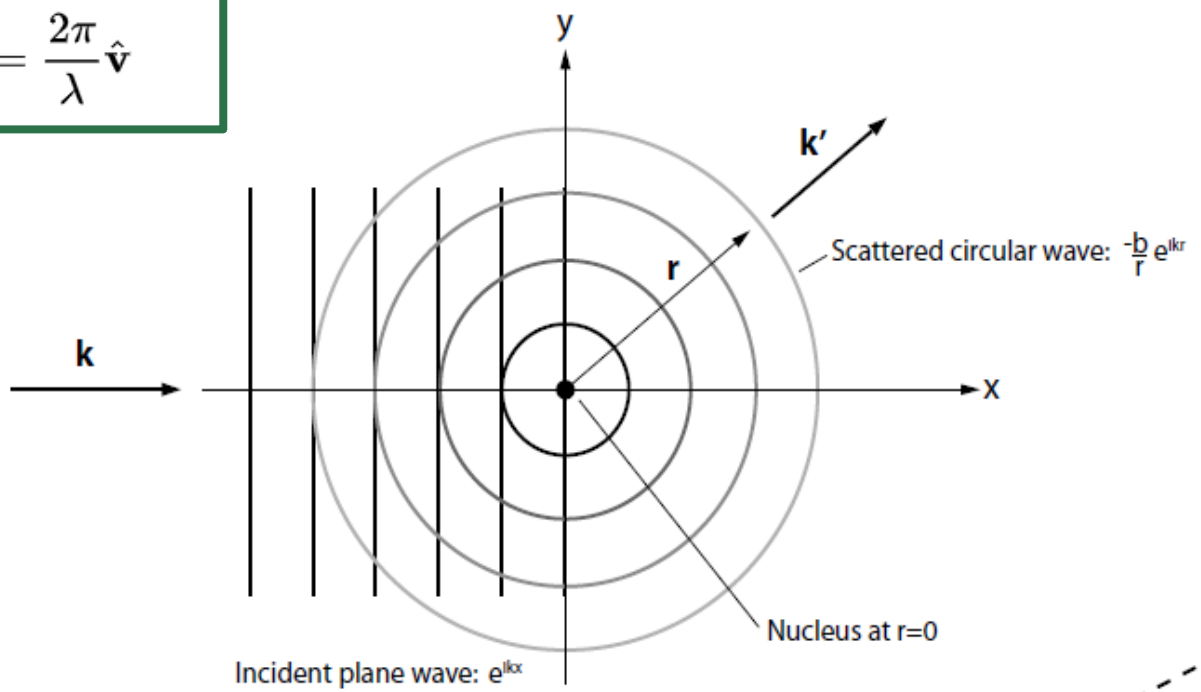


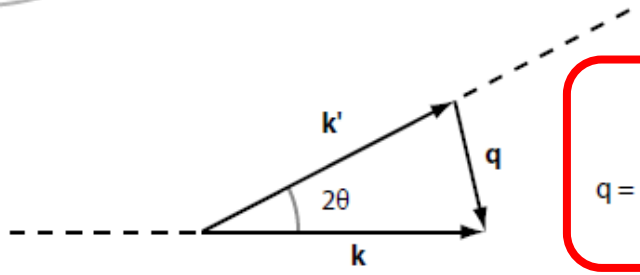
Q (=q)=k-k'

Elastic scattering from a nucleus

Wave vector

$$\vec{\mathbf{k}} = \frac{2\pi}{\lambda}\hat{\mathbf{v}}$$





$$\sin\theta = \frac{|\mathbf{q}|}{2}\frac{1}{|\mathbf{k}|}$$

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

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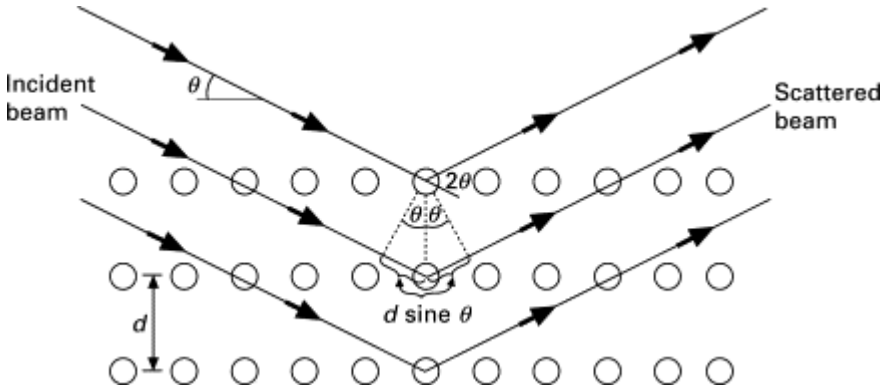
What about an ensemble of nuclei?

Interference of scattered waves



A.R. Bunsell, 2009

What about an ensemble of nuclei?

The first one who applied the properties of the Fourier transform to the experiments of X-ray diffraction in the crystal was W.H. Bragg

IX. Bakerian Lecture.—*X-rays and Crystal Structure.*

By W. H. Bragg, *D.Sc., F.R.S., Cavendish Professor of Physics in the University of Leeds.*

Lecture delivered March 18,—MS. received April 7, 1915.

The method of investigating crystalline structure by the use of X-rays has already been explained in papers read before this Society. It will be convenient nevertheless to re-state its principle very briefly in order to introduce some further considerations which I propose to lay before you.

The statement of the principle may be made in the following way. Let a train of waves of length λ be passing through a medium in which are particles having the power of scattering the radiation. Suppose, further, that the scattering power is not distributed evenly through the medium, but that directions can be found along each of which there is a periodic variation of the scattering power of the material contained in strata perpendicular to the given direction, strata being, of course, taken of equal thickness for comparison. Let the distance of recurrence or spacing be called d. Let θ be the angle between the rays and the strata. Then there will be a "reflection" of the radiation by the medium of $n\lambda = 2d \sin \theta$, where n is any integer.

For instance, the Lippmann process of colour photography produces such a distribution of scattering power in the sensitive film through the agency of stationary waves.* If light is incident on the film it is strongly reflected when $\lambda = 2d \sin \theta$; if the light is white the film selects the appropriate wave for reflection, and this is the origin of the colour manifestation.

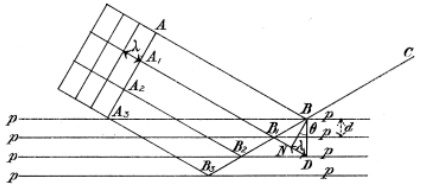


Fig. 1.

The formula is readily explained by aid of the figure, which shows a set of regularly spaced planes p, p, p, ..., each reflecting a minute fraction of the incident

* 'Physical Optics,' Wood, p. 149.

Double differential cross section and scattering length

It is possible to derive that -for elastic scattering in a spherical scattering symmetry- the cross section can be written as:

$$\sigma = 4\pi b^2$$

Where **b** is the (bound) **SCATTERING LENGTH**

b is related to the interaction with each nucleus due to
i) Nuclide composition
ii) Neutron-nucleus combined spin

If we have many nuclei?

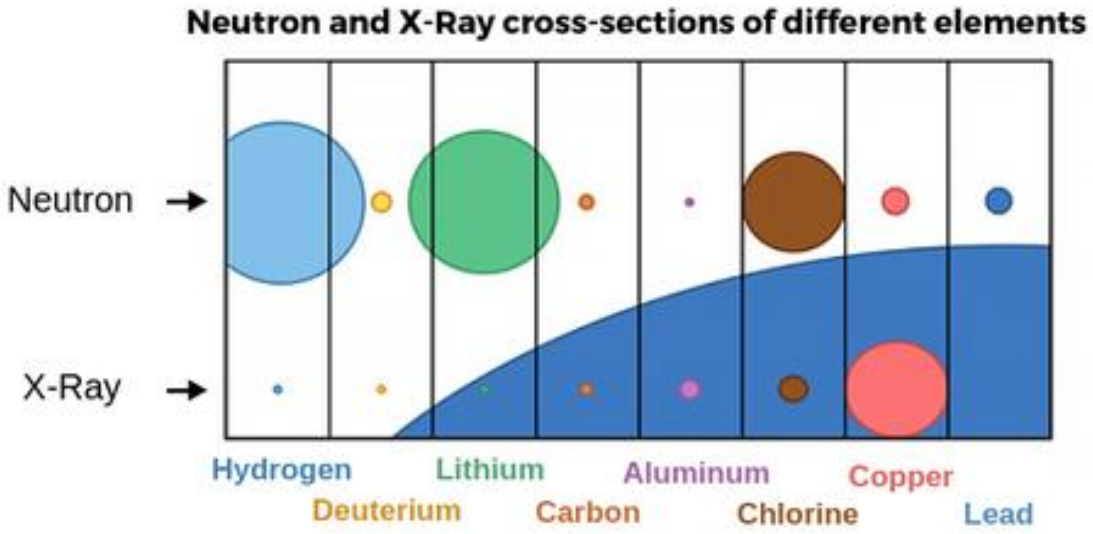
→ We calculate the coherent scattering length of all nuclei in a volume, divided by that volume, also called **SCATTERING LENGTH DENSITY**

$$\mathrm{SLD} = \frac{\sum b_i}{V}$$

Double differential cross section and scattering length

The processes by which neutrons interact with matter are fundamentally different from those of photons.
While photons primarily interact with atomic electrons, neutrons interact mainly with the atomic nucleus.

Describing the interaction of a neutron with a nucleus involves complex interactions among all the nucleons within the nucleus and the incoming neutron. As a result, **there is currently a lack of fundamental theories that can accurately predict the variations in neutron cross-sections.**



To sum up

→ Neutrons interact with nuclei, not the electron cloud

→ We use the concept of cross-section to quantify this probabilistic interaction

→ The specific measurement is the double-differential cross section, which gives detailed information about the direction and energy of scattered neutrons

→ The experimental data is ultimately related to the scattering length density (SLD), key parameter for describing the scattering power of a material at the nanoscale.

To sum up…consequences

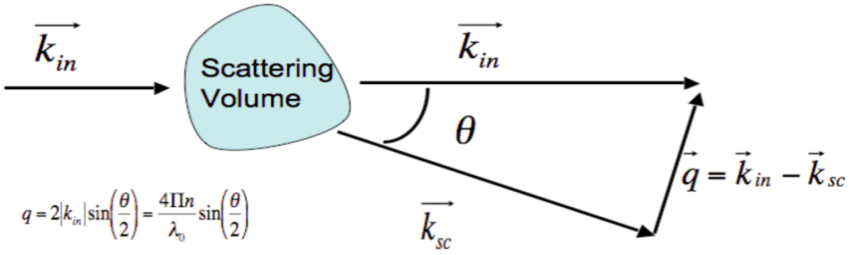
- **Neutrons penetrate bulk samples**

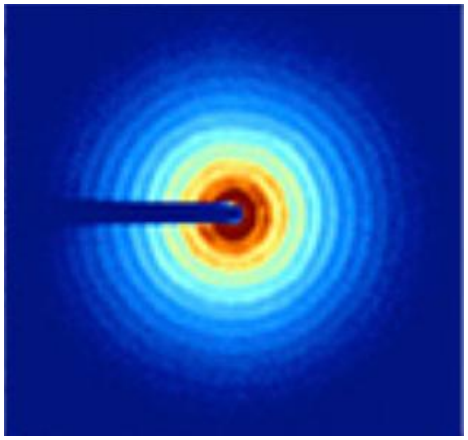
As neutral particles, neutrons are not repelled by the electron clouds of atoms, so they can travel deep into materials and interact directly with atomic nuclei.

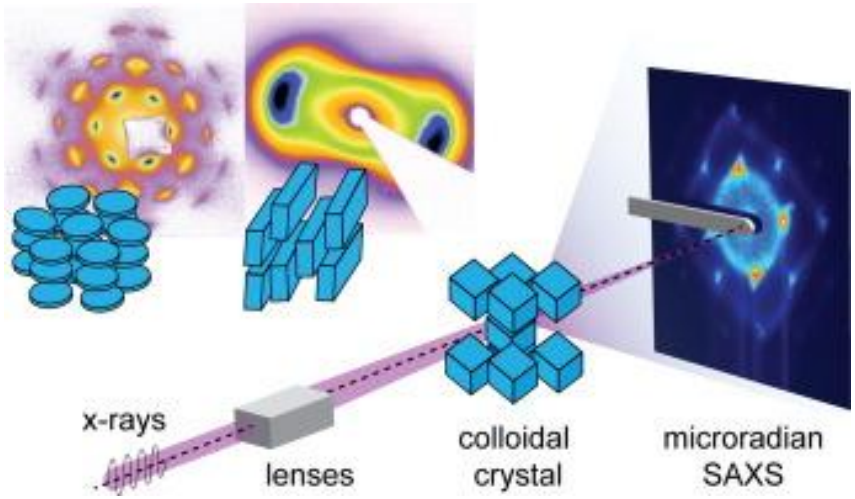
- **Sensitive to isotopes**

Neutron interaction cross-sections vary significantly between different isotopes of the same element, enabling neutron scattering techniques to distinguish between and analyze different isotopes within a material.

What do we see on the detector?

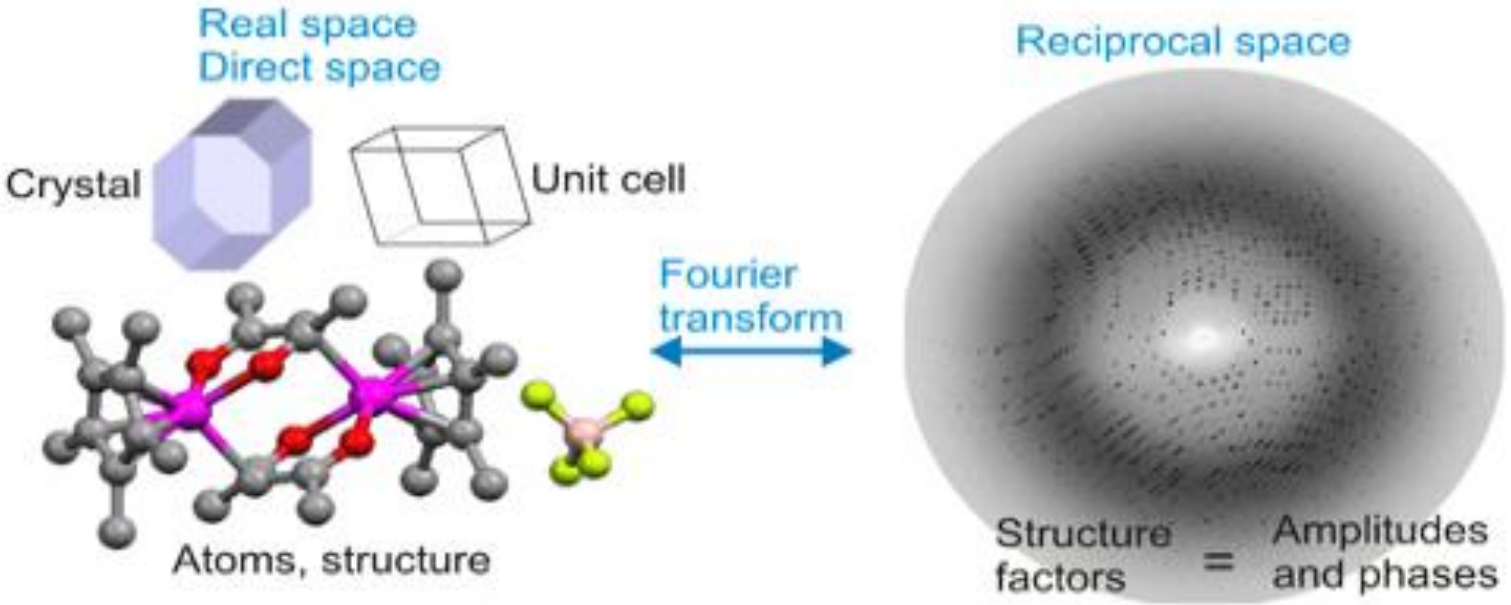






V.PetukhovJanne-MiekeMeijerGert , JanVroege, Current Opinion in Colloid & Interface Science Volume 20, Issue 4, August 2015, Pages 272-281

What space?



$$\rho(xyz) = \frac{1}{V} \sum_{hkl}^{+\infty} |F(hkl)| \,.\, e^{-2\pi i[hx+ky+lz-\phi(hkl)]}$$

Amplitudes

Phases

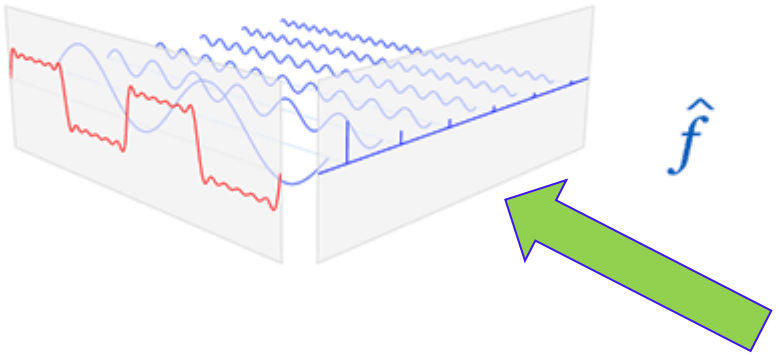
Real space
Direct space

Reciprocal space

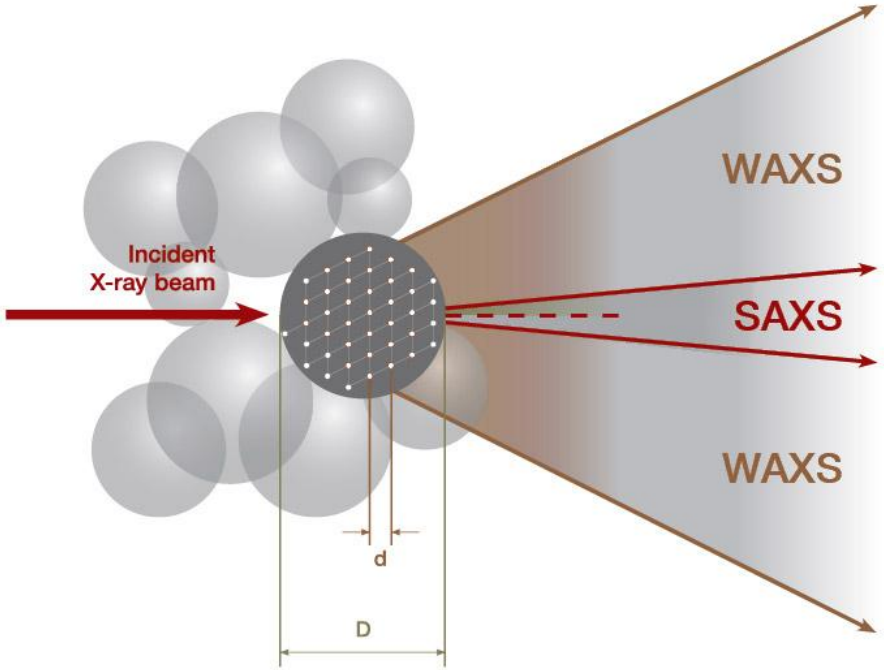
What space?

Fourier transform is a mathematical tool that decomposes any periodic function into a sum of sinusoidal functions (frequency dependent)

$a_n \cos(nx) + b_n \sin(nx)$



What space?



Wide angle scattering

Small angle scattering

https://wiki.anton-paar.com/en/saxs-nanostructure-analysis/

Neutron scattering

Collision can be either ***elastic*** or ***inelastic***

Structure, shape, size

Elastic scattering:

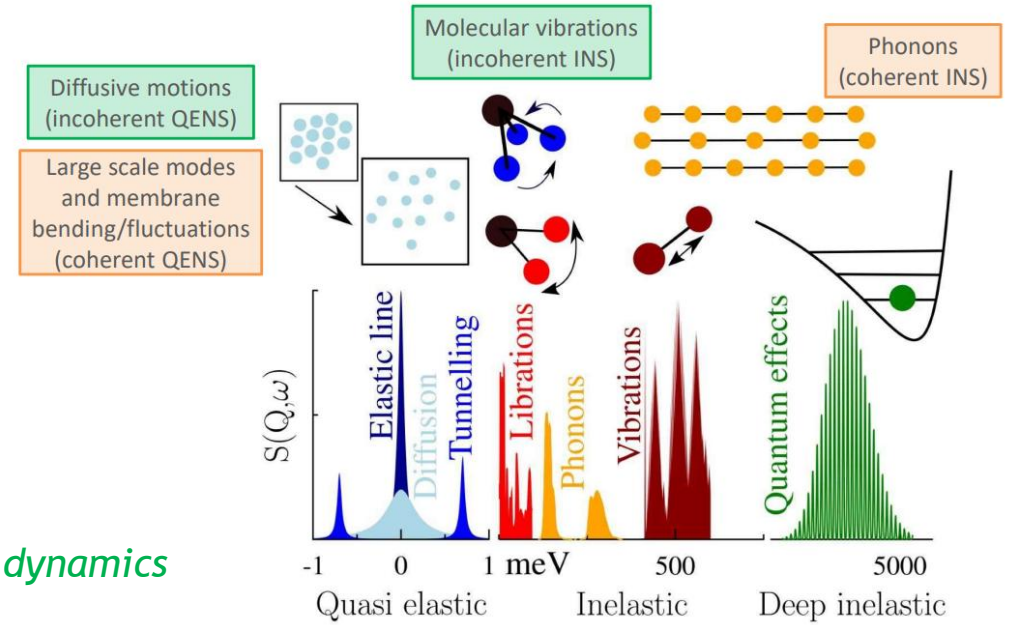
$$|\boldsymbol{k}_i| = |\boldsymbol{k}_f| = \frac{2\pi}{\lambda} \; ; \; Q = 2|\boldsymbol{k}|\sin\theta = \frac{4\pi}{\lambda}\sin\theta$$

$$\Delta E = 0$$

Inelastic scattering:

$$|\boldsymbol{k}_i| \neq |\boldsymbol{k}_f|;$$

$$\Delta \boldsymbol{E} = \hbar\omega = E_i - E_f = \frac{\hbar}{2m}(k_i - k_f)$$



Vibrations, rotations, diffusion, dynamics

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Neutrons vs X-rays

+

✓Interaction with nucleus, not with the electron cloud

✓Sensitivity to isotopes

✓Can measure magnetic structures

✓Can measure lattice vibrations

✓Large penetration depth (depending on isotope)

✓No radiation damage

✓Contrast variation techniques

-

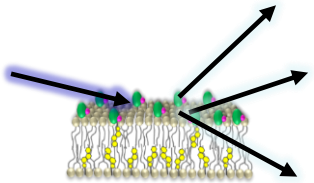
✓Hard to get high neutron flux
- need for big facilities
- need for large samples

✓Samples activation

✓Incoherent background (especially from ^{1}H)

COMPLEMENTARY

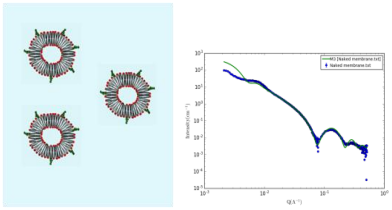
Why elastic neutron scattering on model membranes?



- ✓Probe relevant length (Å to µm) and time (*ps* to *hr*) scales
- ✓Non-destructive
- ✓Possibility of selective deuteration to play with contrast
- ✓Deep material penetration (buried systems)

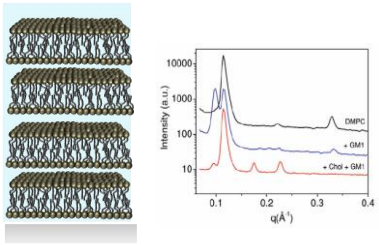
COMPLEMENTARY TECHNIQUES FOR MEMBRANE STRUCTURE

SANS from LUVs



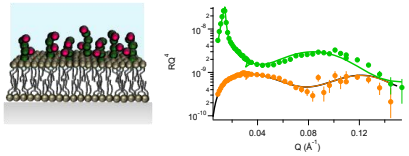
Membrane form factor
Aggregate shape
Structuring in solution

ND from membrane stacks



Membrane thickness/thicknesses (lateral domains)

NR from SLB



Membrane transverse structure

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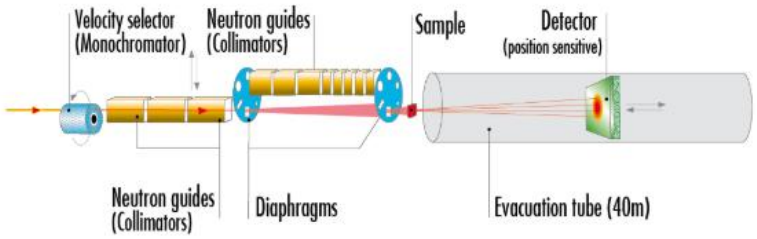
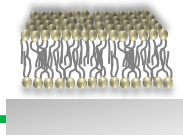
Techniques for structural characterization

In bulk:

Small Angle Neutron Scattering
-SANS-

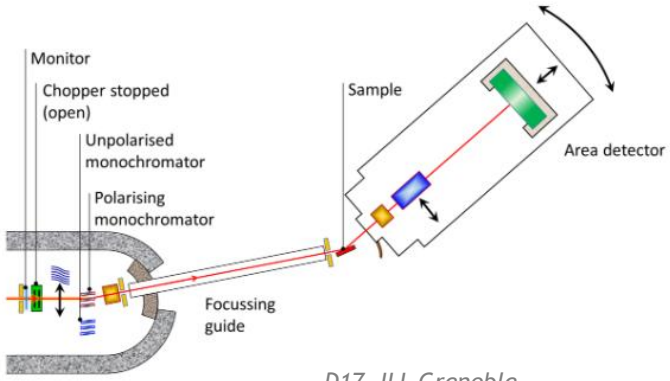


D11, ILL Grenoble



At interfaces:

Neutron Reflectometry
-NR-



D17, ILL Grenoble

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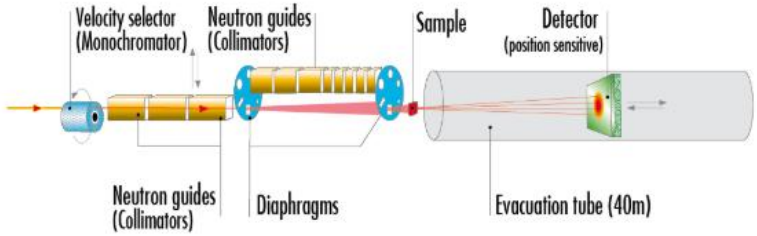
Techniques for structural characterization

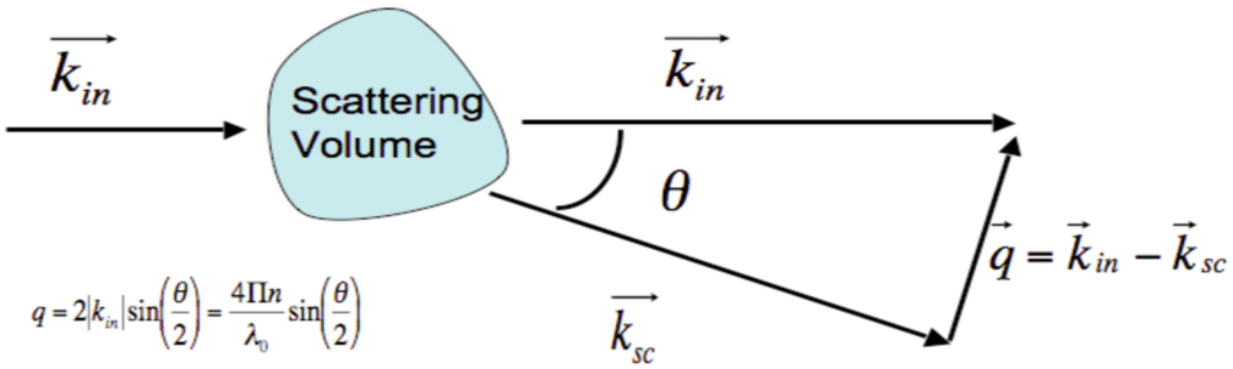
In bulk:

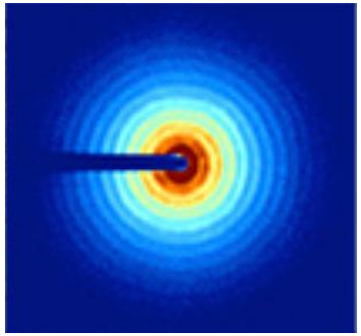
Small Angle Neutron Scattering
-SANS-



D11, ILL Grenoble

Scattering from powders





mass

structure

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

concentration

form

'visibility'

P(q), the FORM FACTOR

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

Interference of X-rays/neutrons scattered from different parts of the same object

For a sphere of radius r

$$P(q) = \left[\frac{3(\sin(qr) - qr\cos(qr))}{(qr)^3}\right]^2$$

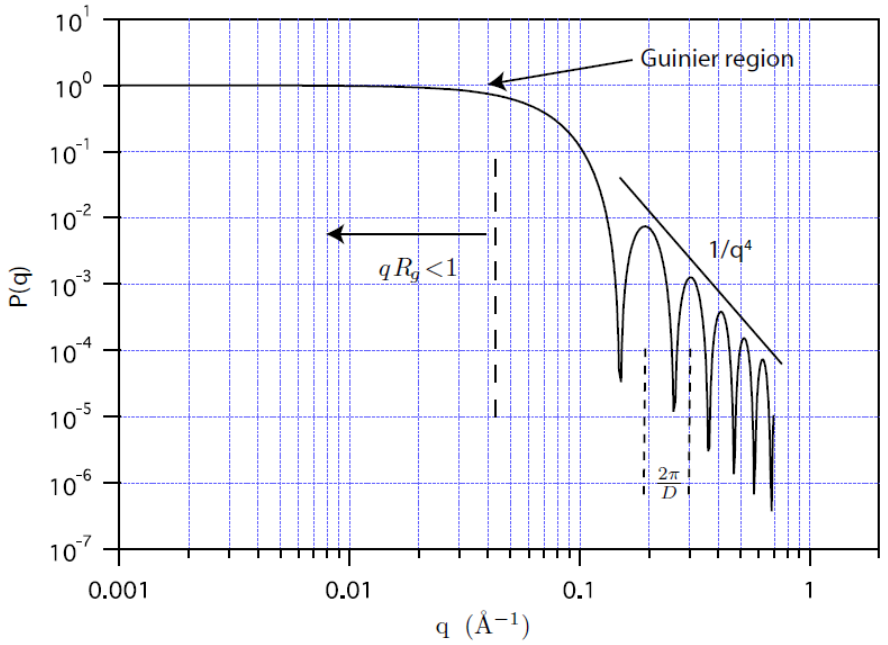
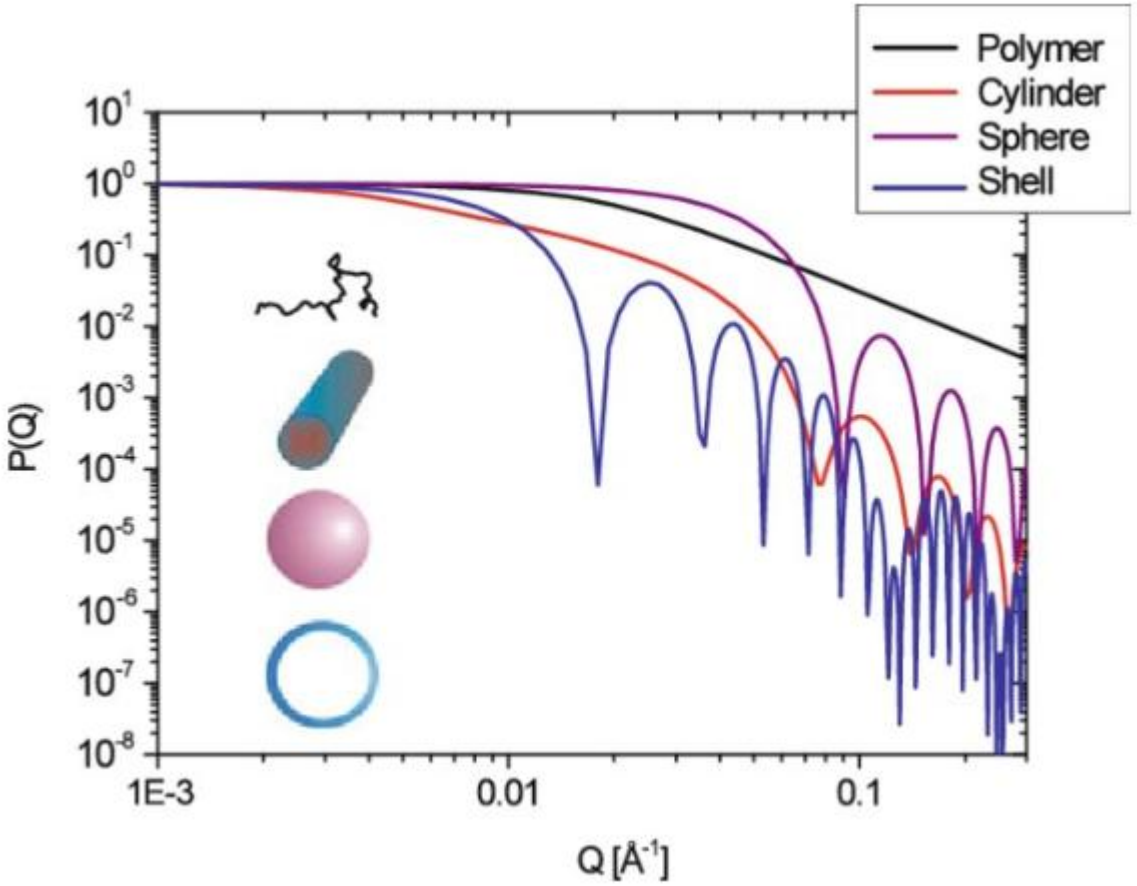


Figure 7: Form Factor spheres of radius 3Å. $R_g = 23$Å

P(q), the FORM FACTOR

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$



R. Lund et al., Advances in Polymer Science 259:51-158 (2013)

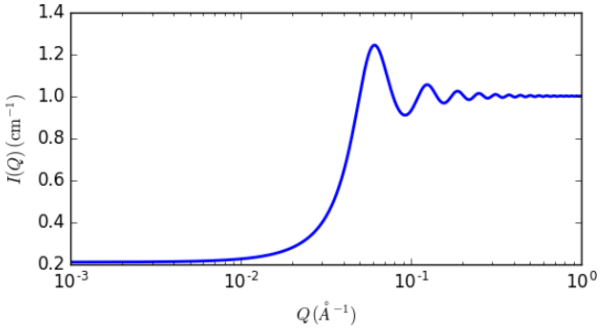
S(q), the STRUCTURE FACTOR (in liquids)

Interference of X-rays/neutrons scattered from different objects in the sample (interparticle correlation)

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

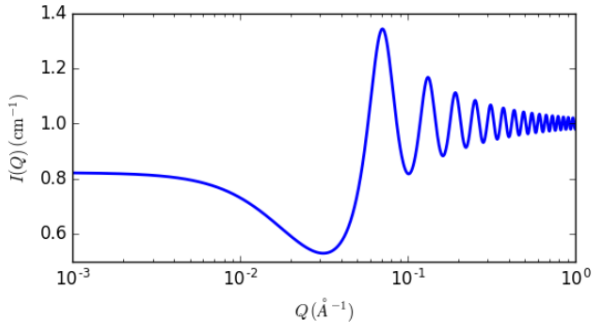
No long range order as crystals

Diluite solutions: interparticle correlation = 0
→ S(q)=1



Hard spheres, radius 50 Å and volume fraction 0.2

J K Percus, J Yevick, *J. Phys. Rev.*, 110, (1958) 1



Sticky spheres, radius 50 Å, volume fraction 0.2, stickiness 0.2

S V G Menon et al., *Chem. Phys.*, 95(12) (1991) 9186-9190

http://www.sasview.org/

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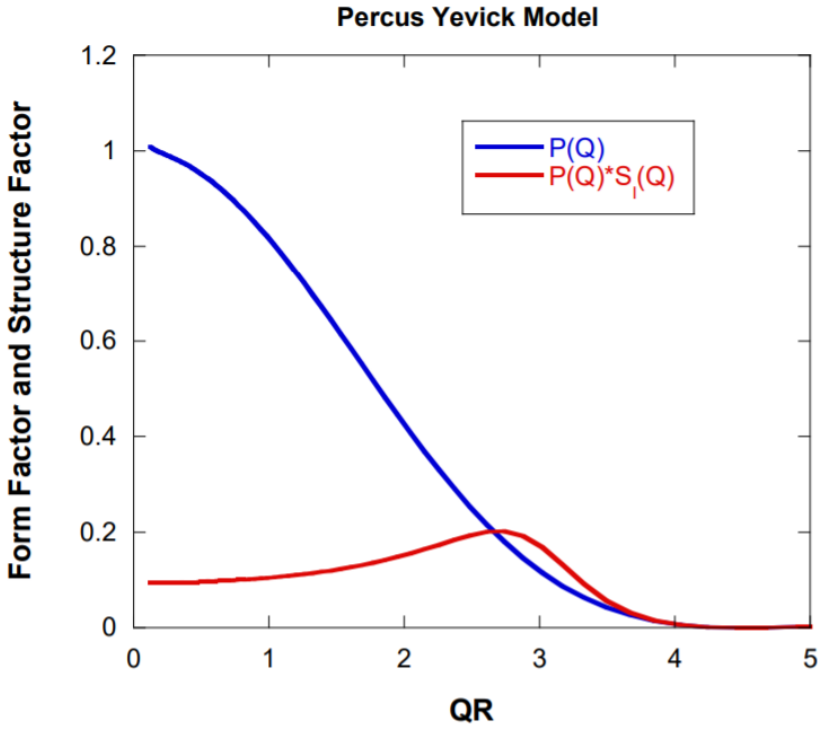
I (q) ÷ c M P(q) S(q) contrast^2



Form factor P(Q) for isolated spheres (infinite dilution limit), and product P(Q)S(Q) for a solution of spheres with a volume fraction of φ = 0.30.

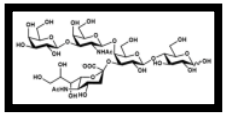
https://www.ncnr.nist.gov/staff/hammouda/distance_learning/chapter_32.pdf

Small Angle Scattering

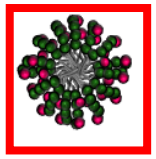
$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

Form factor

Structure factor

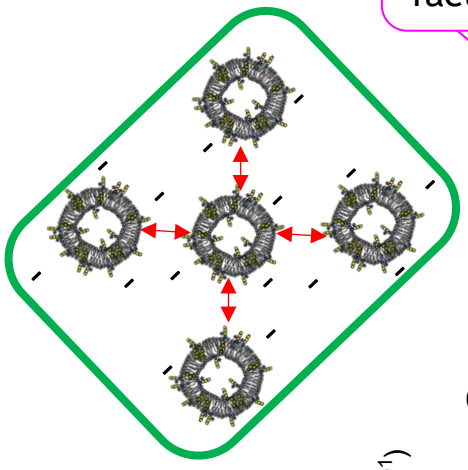


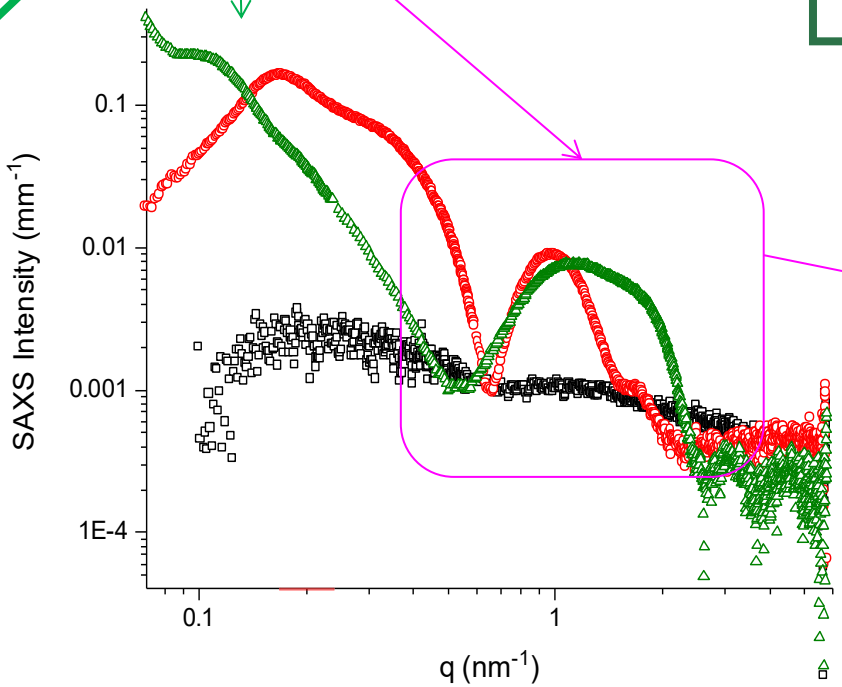
Oligo GM1

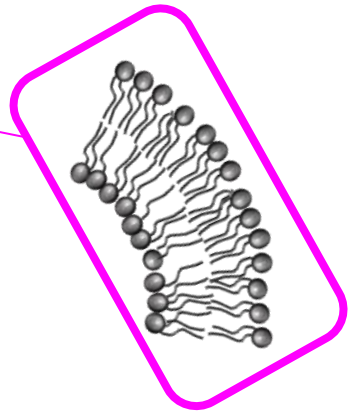


GM1 micelle

Phospholipid+GM1 LUV







The scattering length density (sld)

$$\rho = \frac{\sum_i^n b_i}{V}$$

ρ scattering length density (SLD)
b_i scattering length of the relevant atom
V volume occupied by the *n* atoms

X-rays: electron density ρ_{e-}

Probability of interaction

Contrast: describes the differences in SLDs from different phases of the sample

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

$$\frac{d\Sigma}{d\Omega}(q) = \frac{N}{V}(\rho_1 - \rho_2)^2 V_p^2 P(q) S(q)$$

What counts is the contrast DIFFERENCE

Contrast variation

Isotope	conc	Coh b	Inc b
1H	99.985	-3.7406	25.274
2H	0.015	6.671	4.04

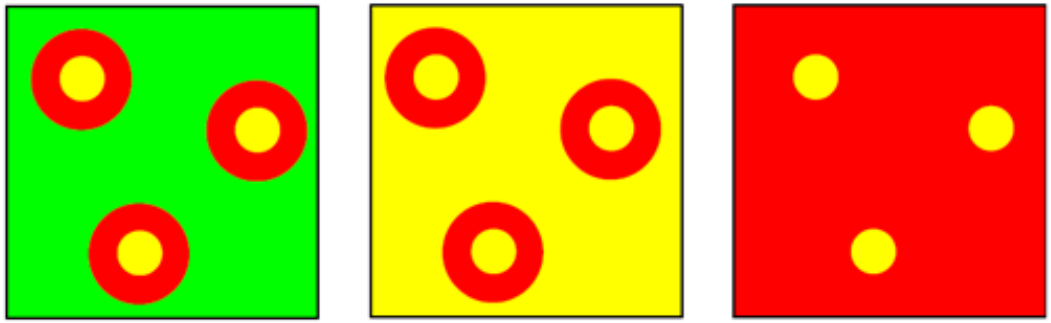
https://ncnr.nist.gov/resources/n-lengths/elements/h.html

$$\rho = \frac{\sum_i^n b_i}{V}$$

$$\frac{d\Sigma}{d\Omega}(q) = \frac{N}{V}(\rho_1 - \rho_2)^2 V_p^2 P(q) S(q)$$

H-D selective substitution

Core-shell particles



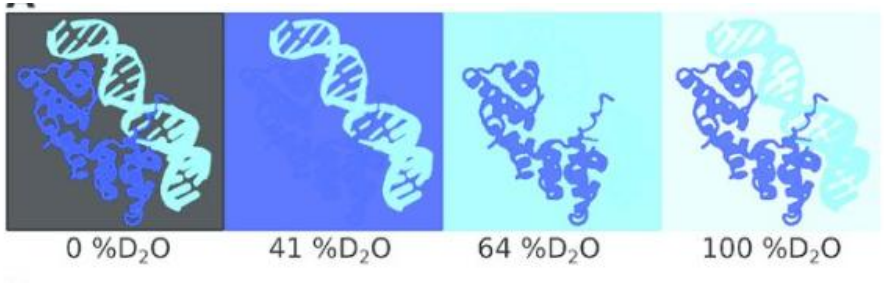
A.J. Jackson, NIST Center for Neutron Research (2008)

We can perform SANS from the different solutions and fit simultaneously to the same model varying only the SLDs

Contrast variation

Z. Bu et al., Adv Protein Chem Struct Biol. 2011;83:163-221

Protein-DNA complex



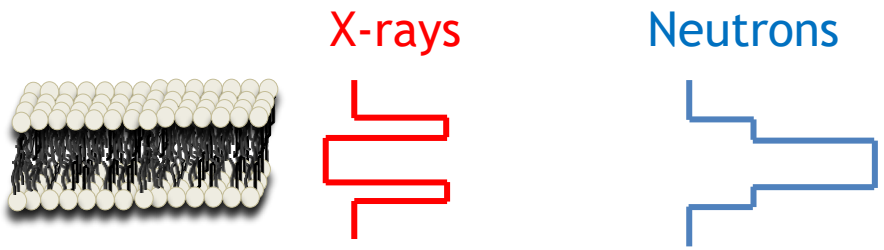
M.M. Castellanos et al., Computational and Structural Biotechnology Journal (2016)

Why different radiations?

$$I(q) \div c\ M\ P(q)\ S(q)\ \text{contrast}^2$$

X-ray

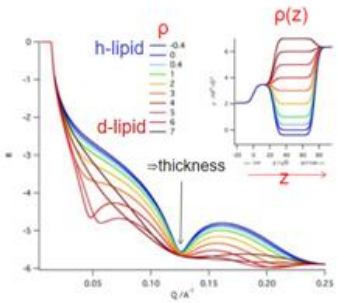
Good contrast for SUGARS (high electron density)



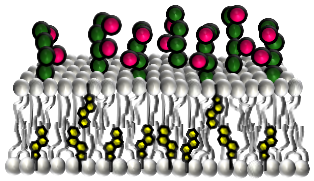
Neutron

Hydrogen coherent scattering length : −3.74 fm
Deuterium coherent scattering length : +6.67 fm

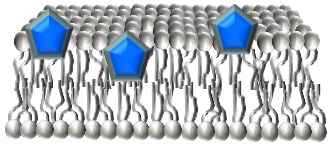
Playing with selective deuteration **protiated molecules can be evidenced in the deuterated phospholipid matrix**



H. Wacklin, ESS



Membrane components distribution



External interacting molecules distribution

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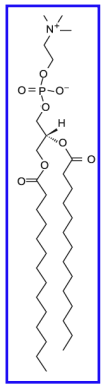
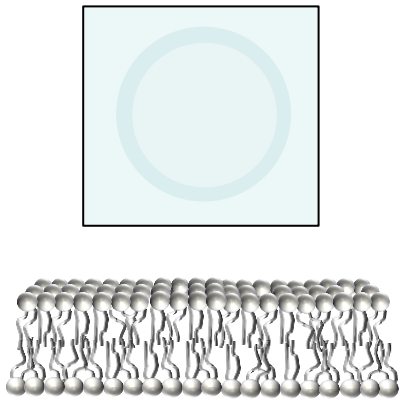
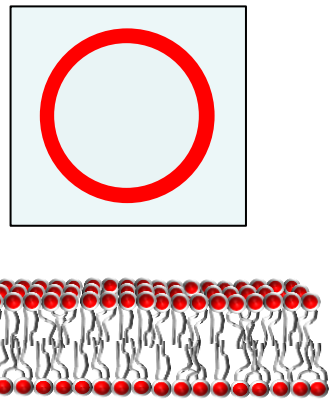
Small Angle Neutron Scattering SANS

DPPC vesicles

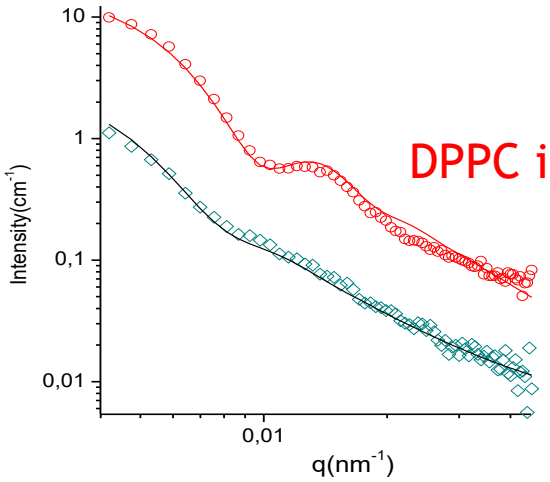




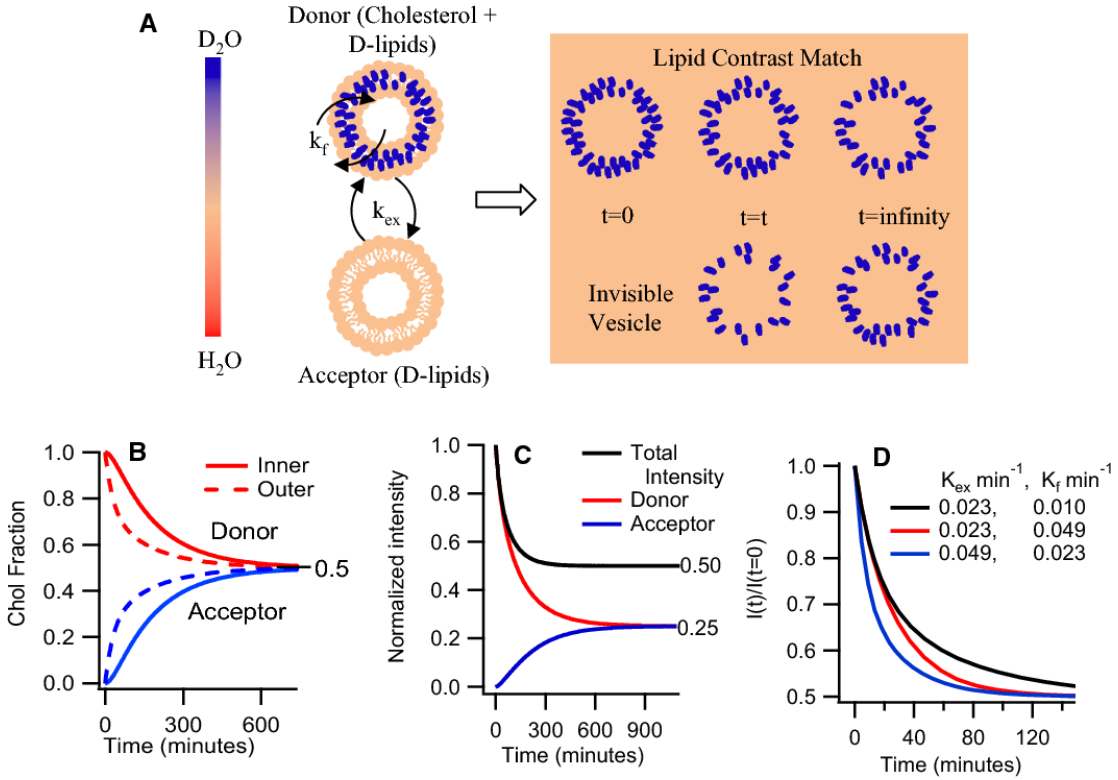


Play with selective deuteration

Play with contrast, visibility!



SANS reveals cholesterol transport in lipid membranes



S. Garg *et al.*, Noninvasive neutron scattering measurements reveal slower cholesterol transport in model lipid membranes, Biophysical Journal (2011)

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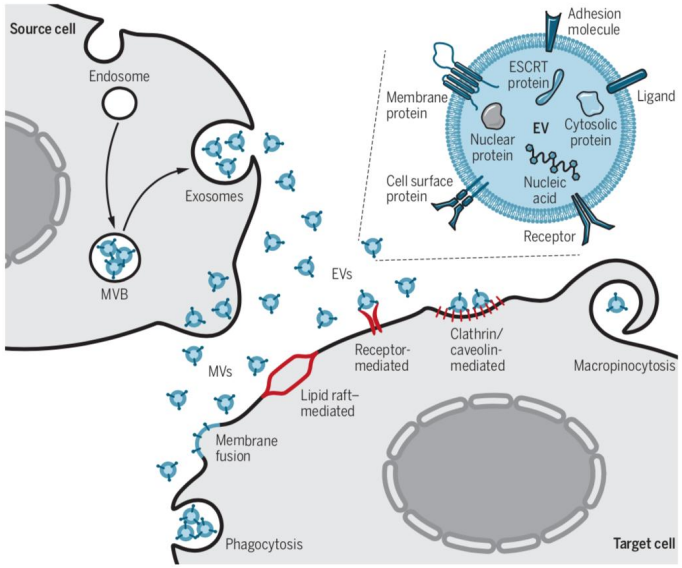
Extracellular vesicles - EVs



Wiklander et al., Science Trans. Medicine (2019)

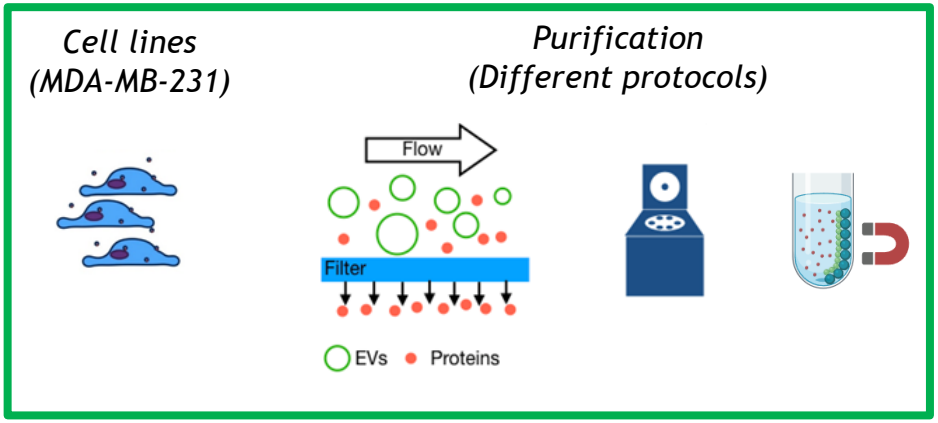
Definition

« Particles that are released from the cells, delimited by a lipid bilayer and cannot replicate on their own »

Specific signatures from originating cells

→ Influence on the fate of recipient cells

→ Biomarkers



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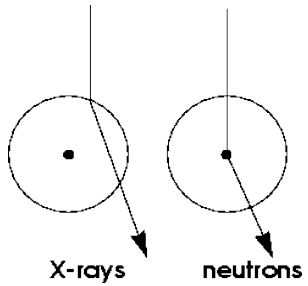
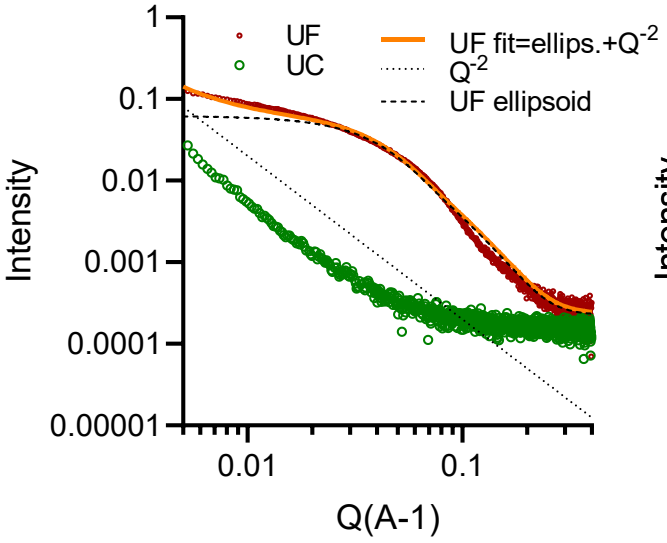
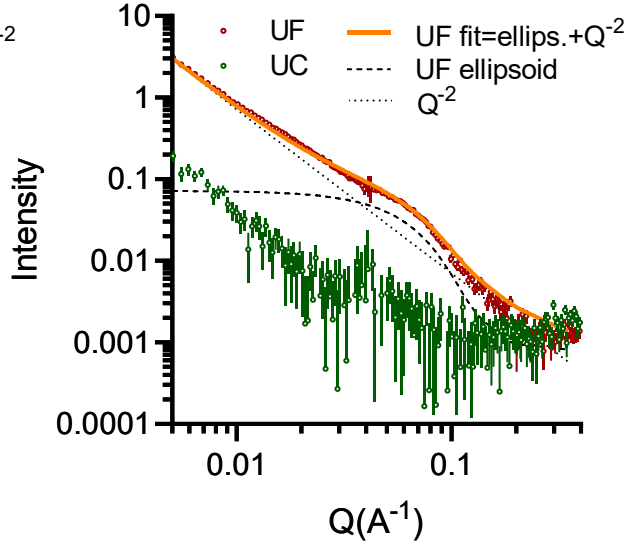
EVs characterization



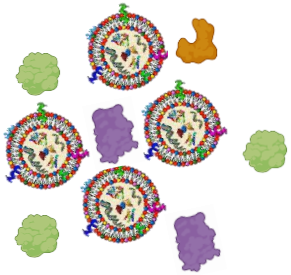
X-ray scattering



Neutron scattering

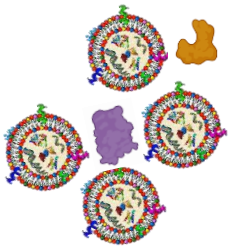


Complementary!



Ultrafiltered: 2D objects + ellipsoidal objects (proteins)

Ultracentrifuged: mainly 2D objects (membranes)



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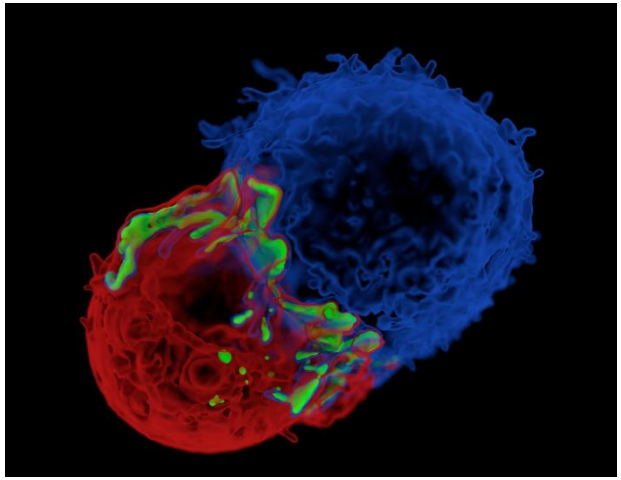
Cell (plasma membrane) interaction with approaching bodies

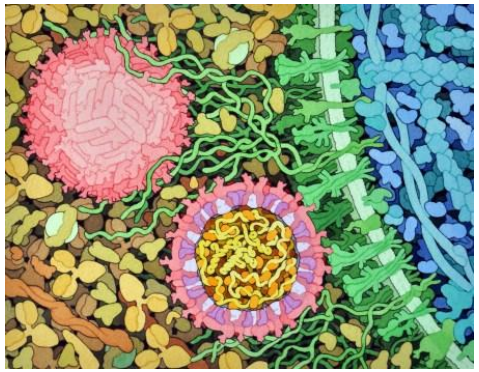
Biology question is far...

what can we face?



→ ***simplified*** systems

keeping the ***main 'bio'-features***



ARTISTIC RENDERING OF THE ZIKA VIRUS PREPARING TO ENTER A CELL (BLUE) BY BINDING TO ITS PROTEIN RECEPTORS (GREEN)

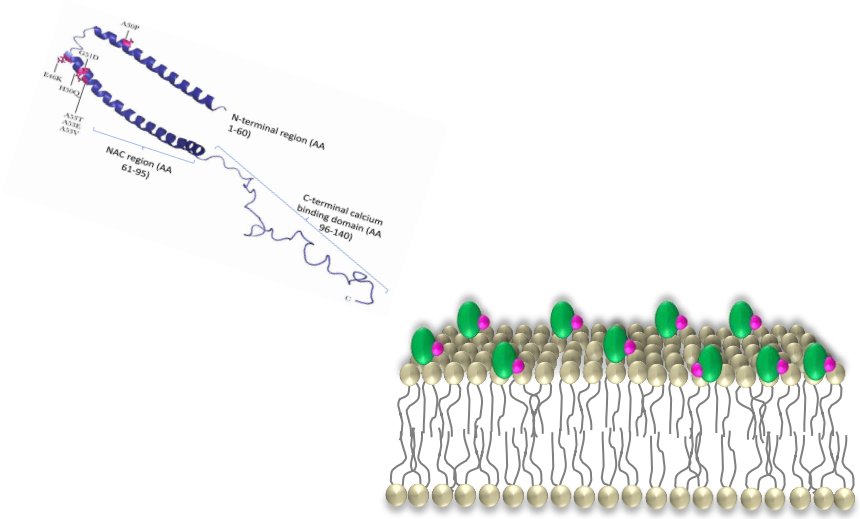
David Goodsell, The Scripps Research Institute

Challenging aspects

→ *The model*

→ *The accessibility (the techniques)*

Protein-membrane interaction

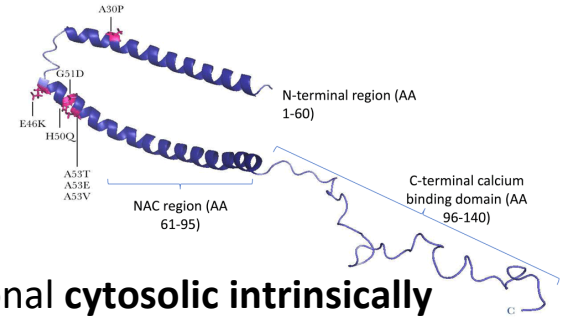


Interaction of Alpha synuclein,

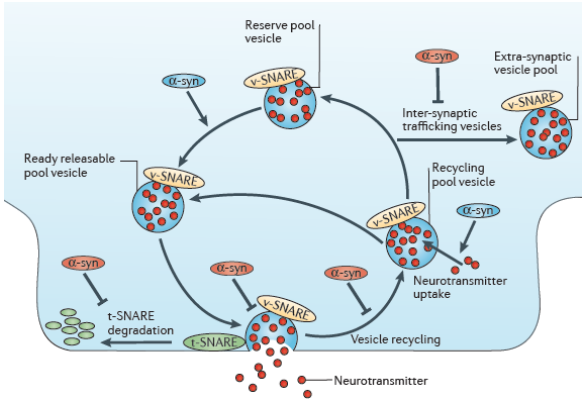
the main protein involved in Parkinson's disease,

with artificial lipid bilayers

The protein: Alpha synuclein

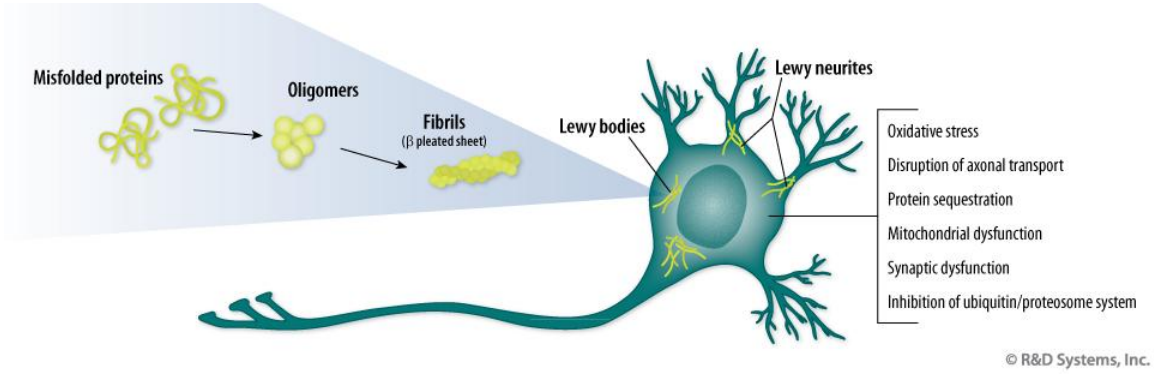


- 140 aa neuronal **cytosolic intrinsically disordered protein**
- **Presynaptic vesicle homeostasis** (*in-vivo* lipid raft binding)



Lashuel et al. Nat. Rev. Neurosci. (2012). 14, 38-48.

In Parkinson's disease: misfolding and aberrant aggregation



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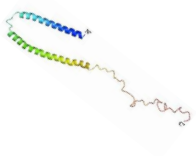
Aim

Investigate the interacion of Alpha synuclein and its aggregates with different model membranes

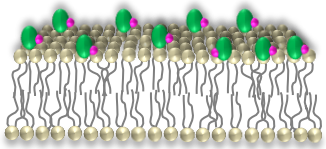
Why?

Type and extent of interaction may depend on membrane composition (chemistry and lipid phase)

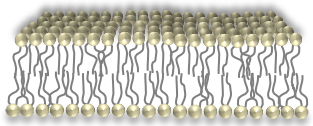
Monomer



Oligomer
(Fe mediated formation)



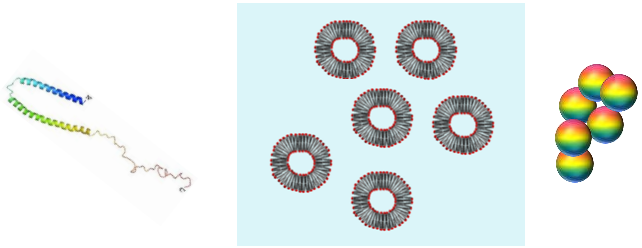
Raft-like
(Ceramide-lipid containing)



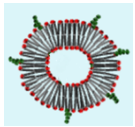
Non raft
(phospholipid only)

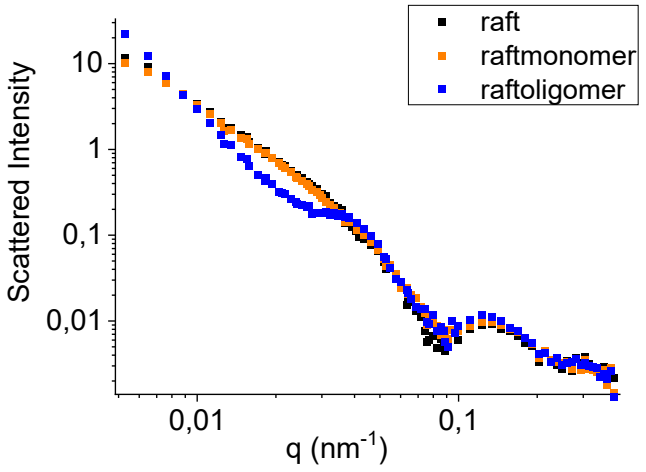
SANS for structural investigation

The system: vesicles in solution

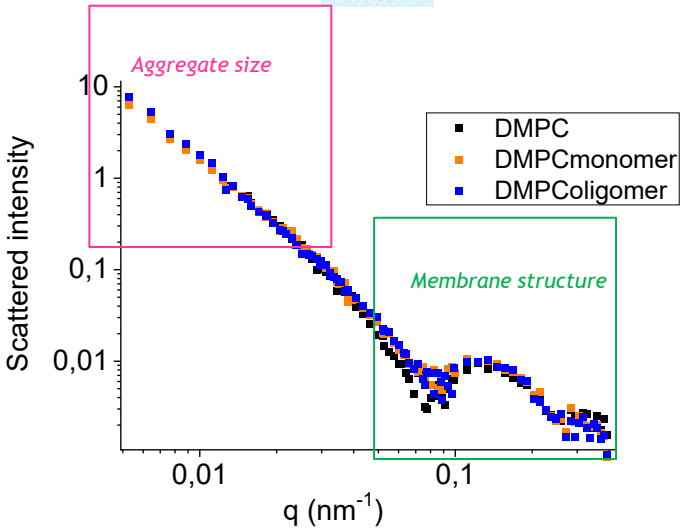


DMPC-GM1 ganglioside (Raft like)



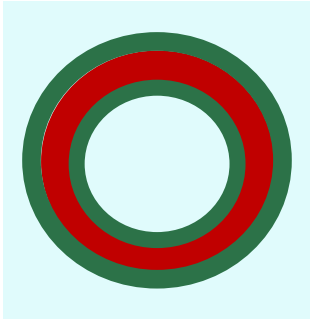


DMPC (non raft)



SANS for structural investigation

Core Multi-Shell Model

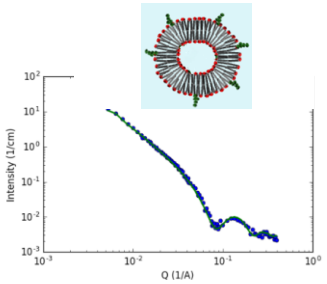


$$P(q) = \frac{\text{scale}}{V} F^2(q) + \text{background}$$

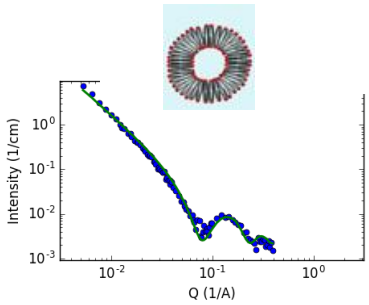
$$F(q) = \frac{3}{V_s}\left[V_c(\rho_c - \rho_s)\frac{\sin(qr_c) - qr_c\cos(qr_c)}{(qr_c)^3} + V_s(\rho_s - \rho_{\text{solv}})\frac{\sin(qr_s) - qr_s\cos(qr_s)}{(qr_s)^3}\right]$$

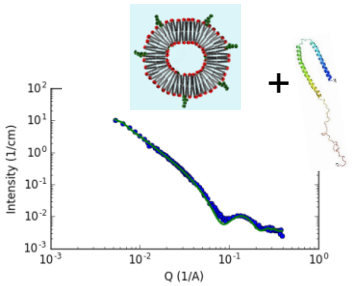
(Guinier, 1955)

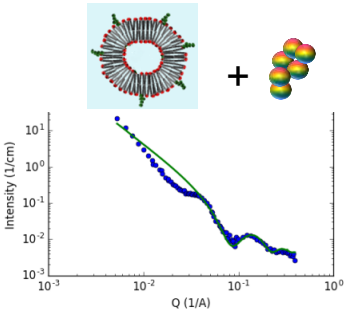
Raft like

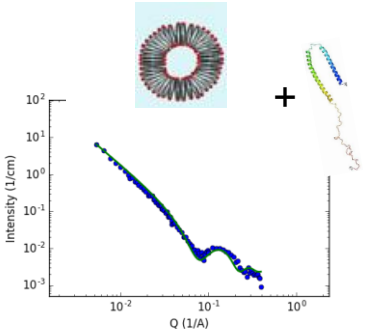


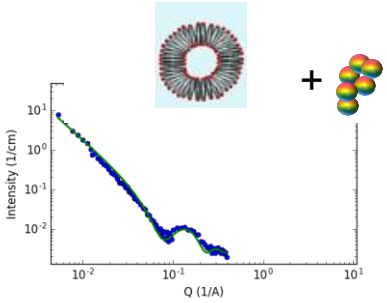
Non raft











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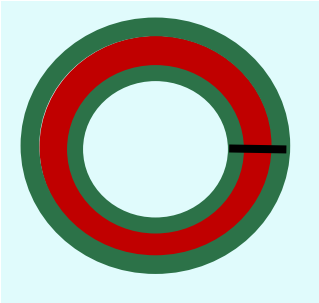
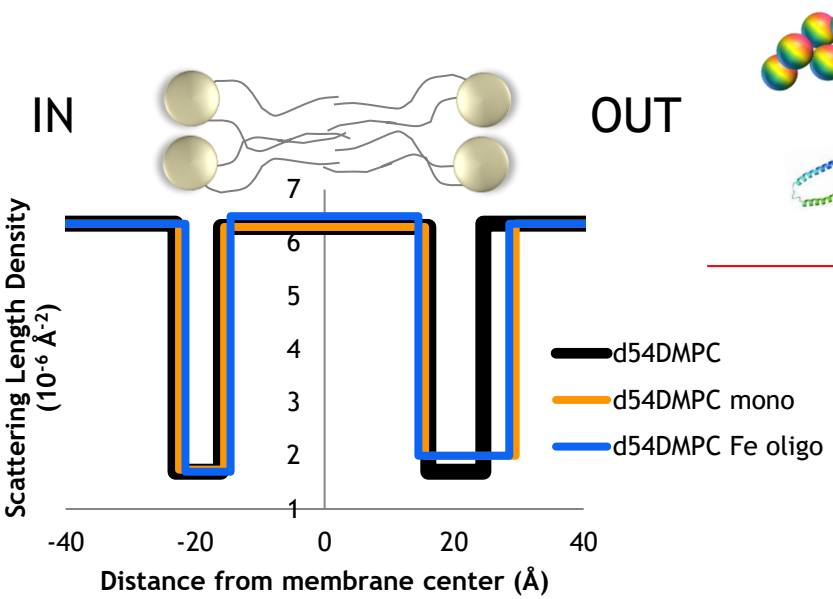
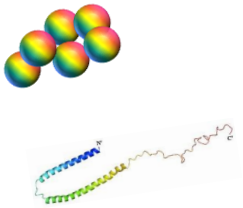
SANS for structural investigation
Bare phospholipid target membrane



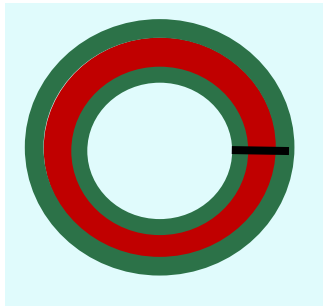


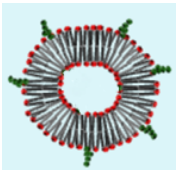


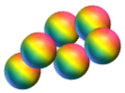
Increased heads thickness

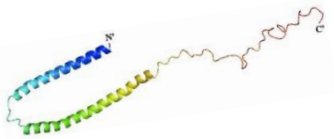
Something on the surface?

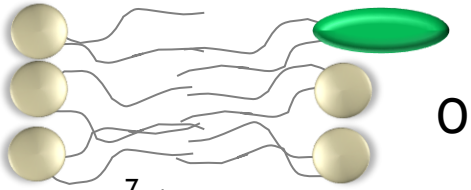
SANS for structural investigation
RAFT target membrane

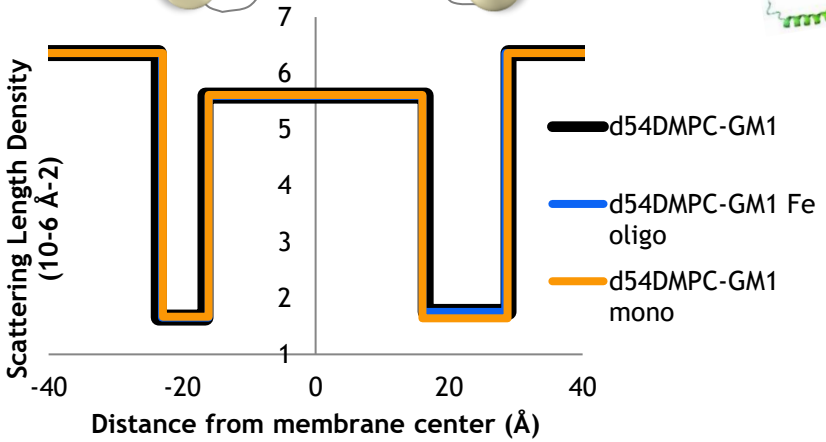






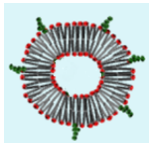


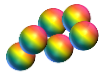


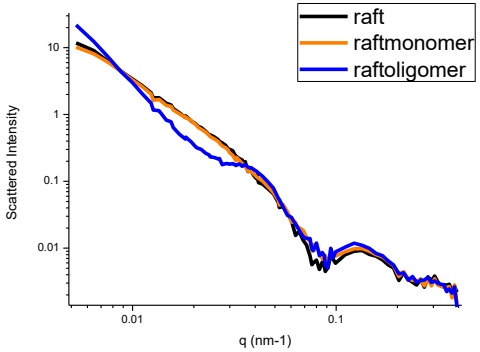


SANS for structural investigation
RAFT target membrane

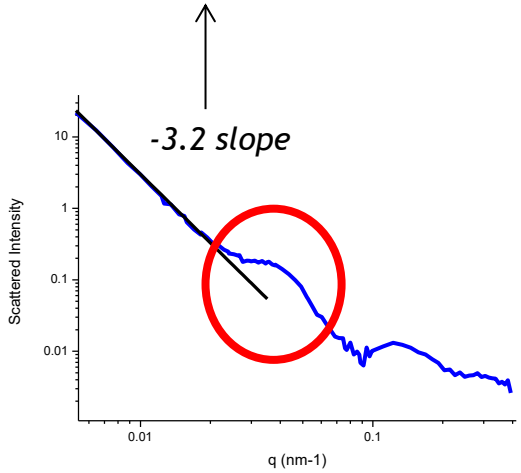
Raft membrane + oligomers





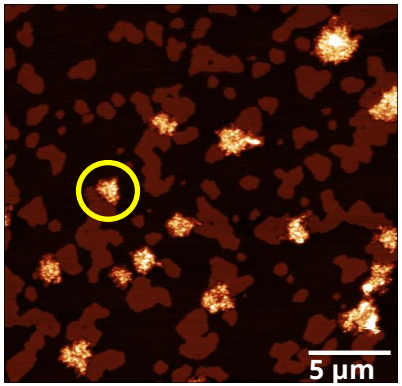


Surface fractal large objects *(Porod regime)*



20 nm sized structures

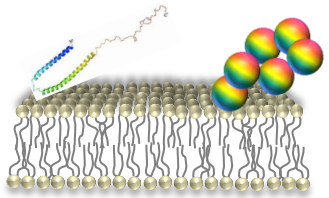
Oligomer cluster on membrane surface?



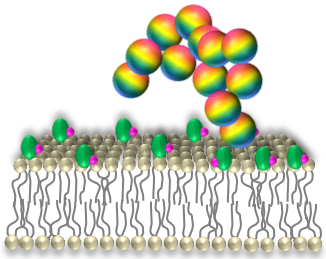
AFM

SANS for structural investigation

- ✓ Both monomers and oligomers interactions is limited to the external membrane leaflet in non-raft domains and results in membrane thicking → role in non raft membrane destabilization?



- ✓ Oligomers accumulate on raft-like domains → strengthen the hypothesis of a critical role of lipid rafts not only for the biological function of the protein, but even for the development and progression of the disease

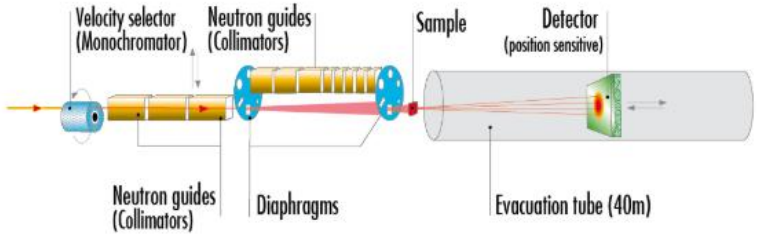


- ✓ MULTITECHNIQUE APPROACH

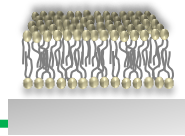
Techniques for structural characterization

In bulk:

Small Angle Neutron Scattering
-SANS-

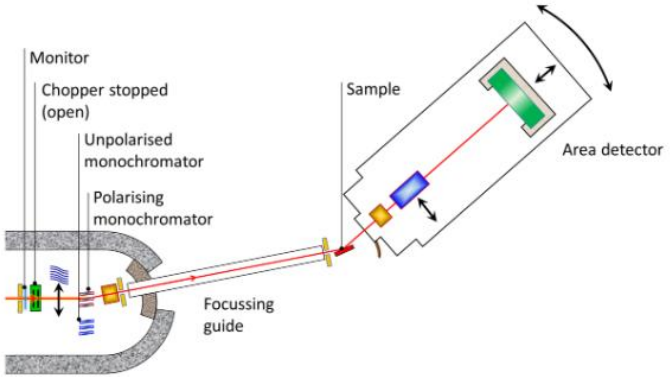


D11, ILL Grenoble



At interfaces:

Neutron Reflectometry
-NR-



D17, ILL Grenoble

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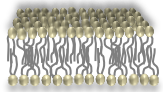
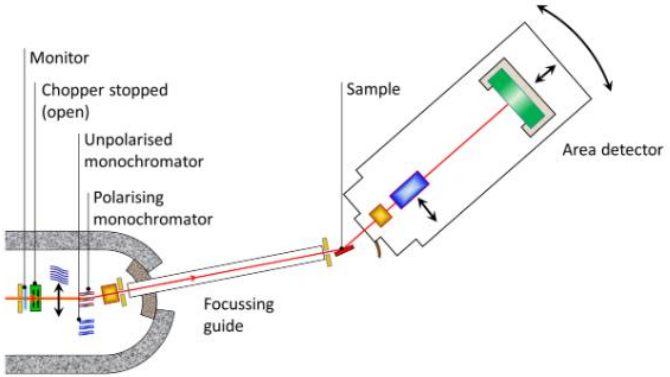
Techniques for structural characterization



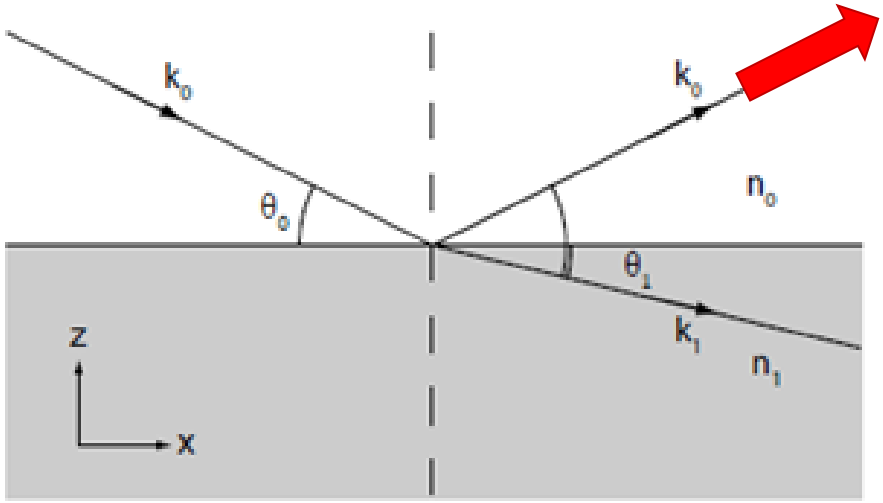
At interfaces:

Neutron Reflectometry
-NR-



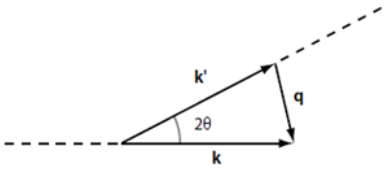
D17, ILL Grenoble

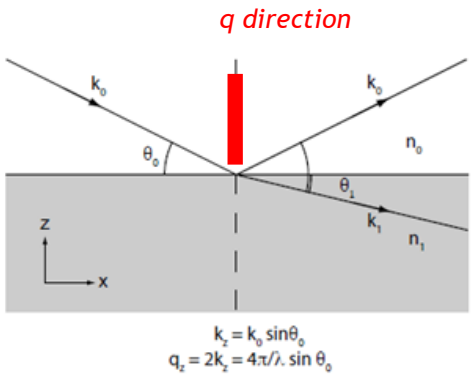
Reflectivity



$$k_z = k_0 \sin\theta_0$$
$$q_z = 2k_z = 4\pi/\lambda . \sin\theta_0$$

Reflectivity





As for small angle scattering, the differential cross section is given by the Fourier transform of the scattering length density distribution over the whole sample. Taking into account the specular geometry such that only $\mathbf{q_z}$ varies we can obtain an expression for the reflectivity

$$R(\mathbf{q_z}) = \frac{16\pi^2}{q_z^2}|\hat{\rho}(q_z)|^2$$

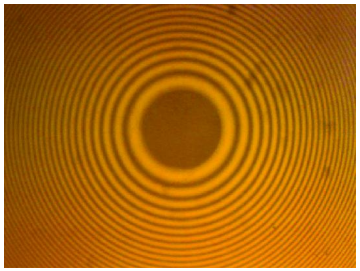
where $\hat{\rho}(q_z)$ is the *one-dimensional* Fourier transform of the scattering length density profile normal to the interface.

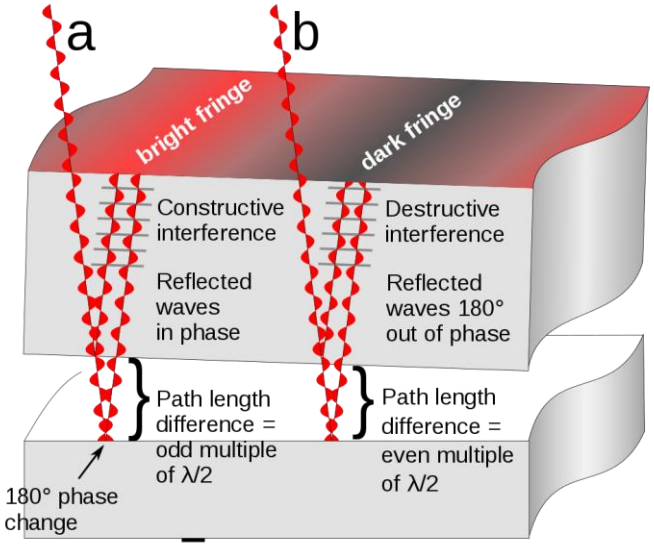
$$R(q) = \frac{16\pi^2}{q^4}\left|N_b^{'}(q)\right|^2$$

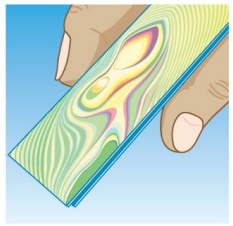
$$N_b = \frac{\sum_i n_i b_i}{V}$$

Interference of reflected beams

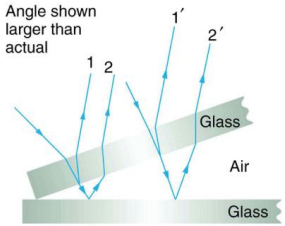
NEWTON'S RINGS
monocromatic beam







(a)

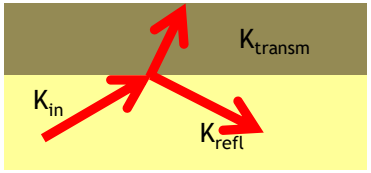


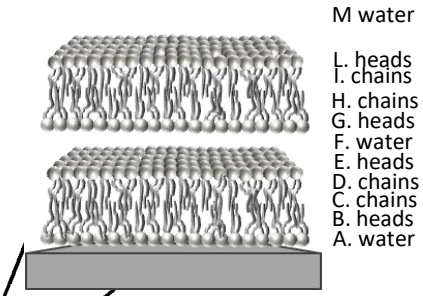
(b)

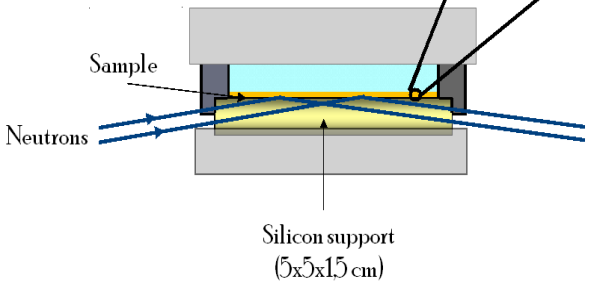
Light striking a thin film is partially reflected (ray 1) and partially refracted at the top surface. The refracted ray is partially reflected at the bottom surface and emerges as ray 2. These rays will interfere in a way that depends on the thickness of the film and the indices of refraction of the various media.

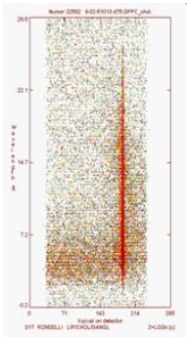
When we have more than one interface: A Neutron Reflectivity experiment

For the incident beam (Neutrons, X-Rays) is as if the sample is divided in layers, depending on their composition (water, polar heads, chains)

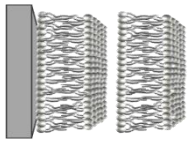


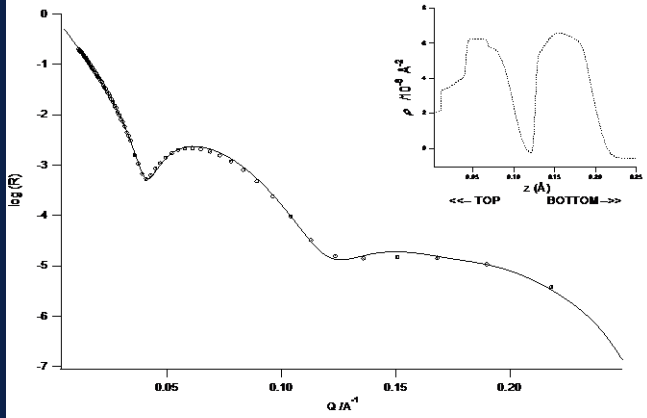






The Reflectivity spectrum obtained is given by the **interference** of the waves **reflected** from the top and bottom of each layer





SAMPLE CONTRAST PROFILE

Information about the transverse structure of the sample, layer by layer:

thickness, composition, compactness, roughness

LIPID «RAFTS» - GEMs

functional and structural domains

recognition

cell adhesion

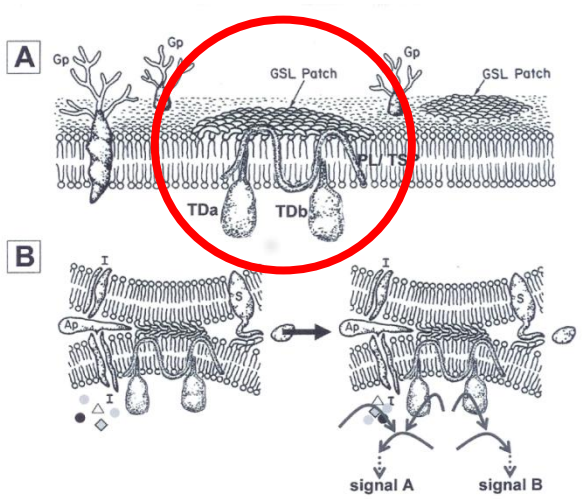
transfer of information

cell growth

proliferation

apoptosis

…



Hakomori (1998)

•lipid driven

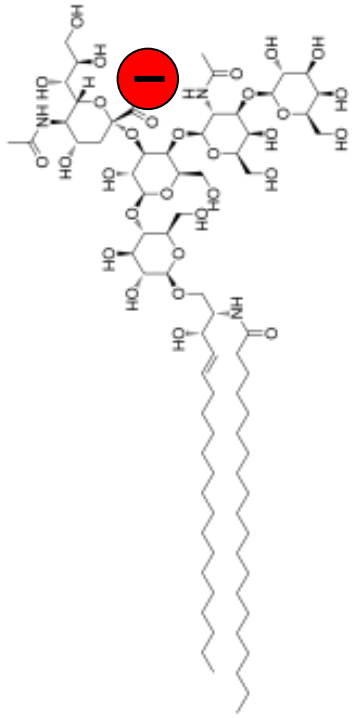
•glycolipids only on the outer side of the membrane, cholesterol mainly in the inner

Valid model for lipid rafts - GEMs

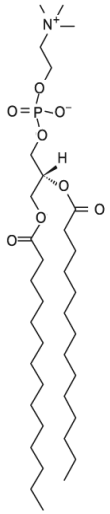
→lipid composition

→structural asymmetry

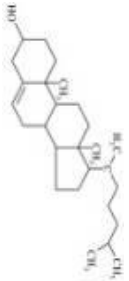
Glycosphingolipid



Glycerophospholipid

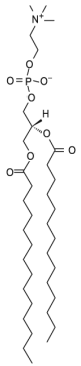


Cholesterol

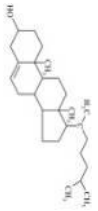


The contrast

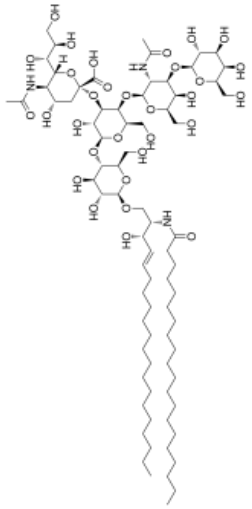
DPPC

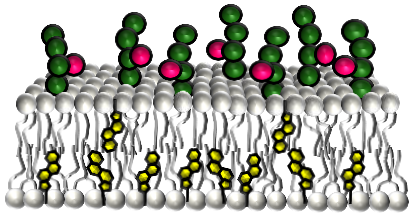


Cholesterol



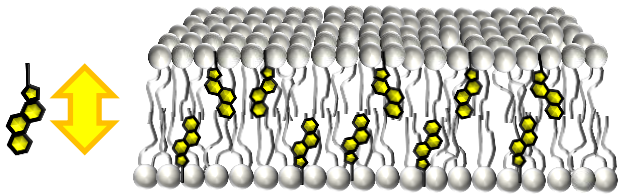
GM1





Material	SLD (10^{-6}Å^{-2})[a]
H_2O	-0.56
D_2O	6.36
Cholesterol	0.22
GM1 chains (gel phase)	-0.41
GM1 heads	1.88
Lipid D-heads	5.70
Lipid D-chains (gel phase)	7.66
Lipid D-chains (fluid phase)	6.13

Is asymmetry possible? ...conditions to be kept?



V. Rondelli et al., J. Phys. (2012)

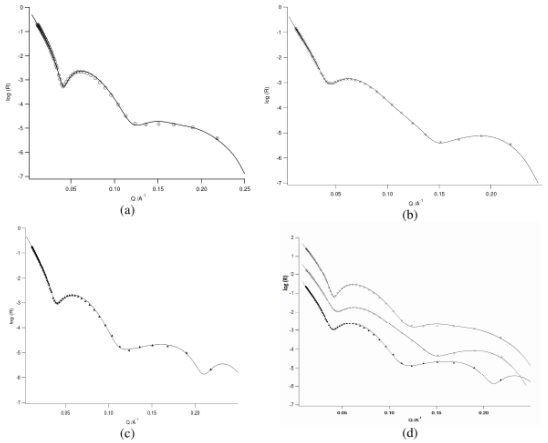


Figure 3. Reflectivity spectra of the cholesterol-containing sample in H_2O (a) at 22°C before annealing, (b) at 51°C, (c) at 22°C after annealing.
In insert (d) the curves have been arbitrarily shifted by 5 (51°C) and 10 (22 °C) for better clarity in comparing them: upper curve: 22°C before annealing. Middle curve: 51°C. Lower curve: 22°C after annealing.

NON-INVASIVE INSIGHT IN THE INTERNAL STRUCTURAL MECHANISMS

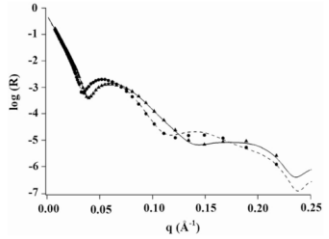


Fig. 3. Modification of the neutron reflectivity spectrum induced by the incubation of GM1 (*before*: triangles, *after*: circles) in a d_{75}-DPPC+cholesterol bilayer. Straight and dashed lines represent the curves obtained from the parameters used to fit the data (see Tables 2 and 3). The solvent is H_2O and T = 49.5 °C. Error bars are smaller than graphical symbols.

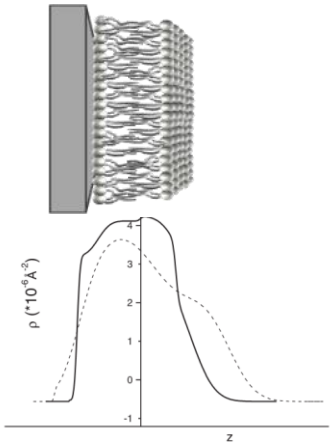
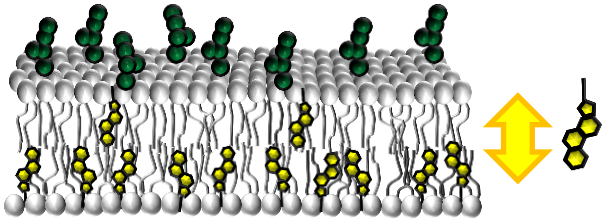


Fig. 4. Contrast profiles, ρ(z), of the floating bilayer of Sample A at 49.5 °C in H_2O. Full line: before GM1 incubation. Dashed line: after GM1 incubation. In the figure the vertical axis is placed roughly at the center of the floating bilayer, to guide the eye. The contrast profile account for lipid components, included solvent and roughness.



V. Rondelli et al., BBA – Biomembranes (2012)

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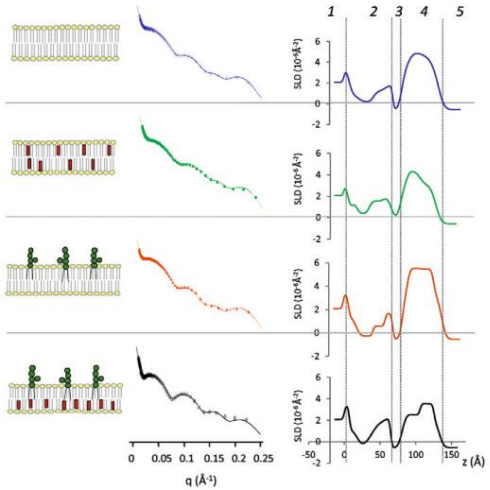
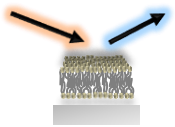
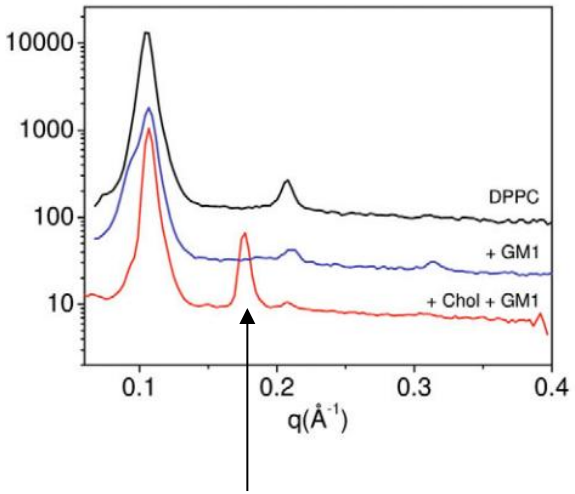
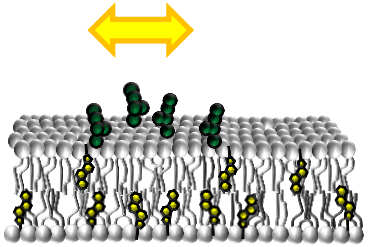
What about lateral distribution?

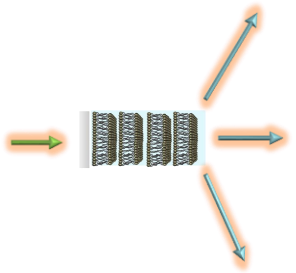




NR





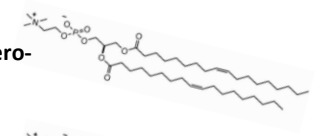


Neutron Diffraction

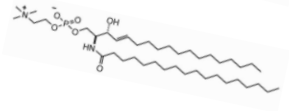
V. Rondelli et al., Eur. Phys. J. E (2013)

Sugar-free raft model

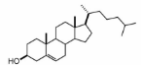
1,2-dimiristoyl-sn-glycero-3-phosphocholine (DMPC)



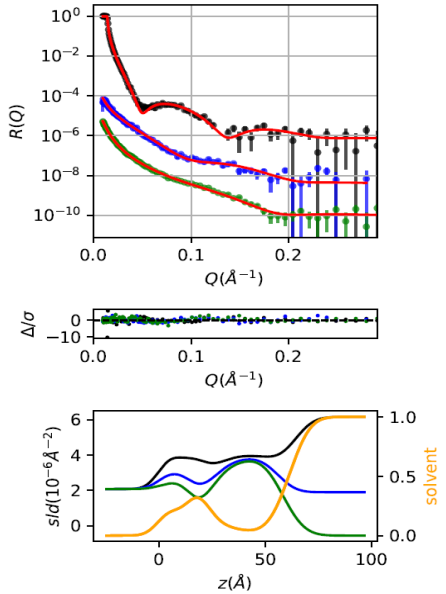
Sphingomyelin (SM)

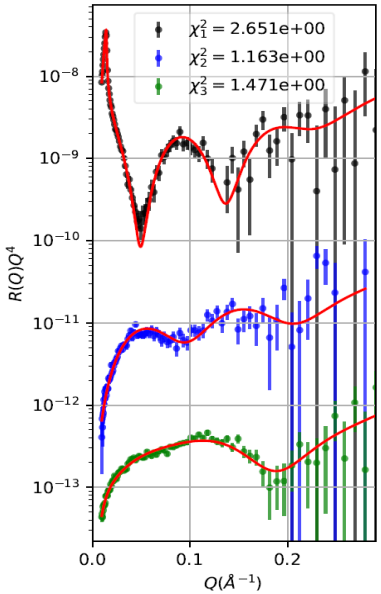


Cholesterol (Chol)

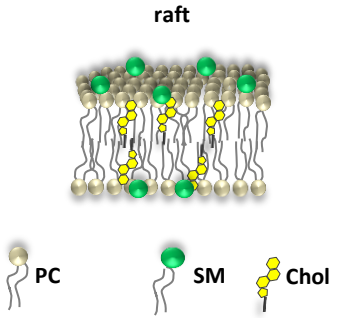


SM and Chol co-localize **symmetrically**





PC:SM:Chol 2:1:0.15 mol



S. Abdalla et al., JCIS (2025)

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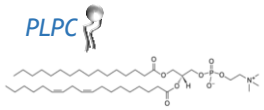
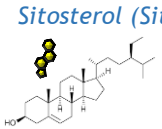
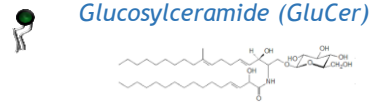
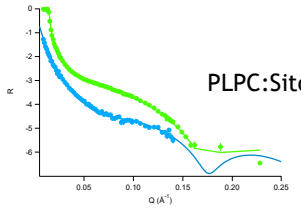
What about plant membranes?

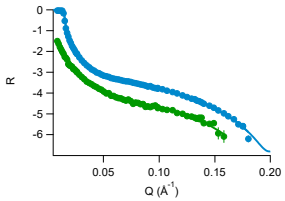




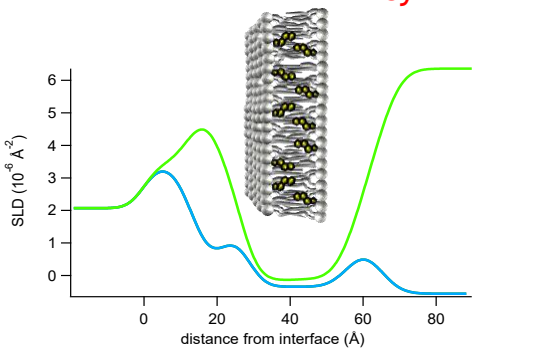


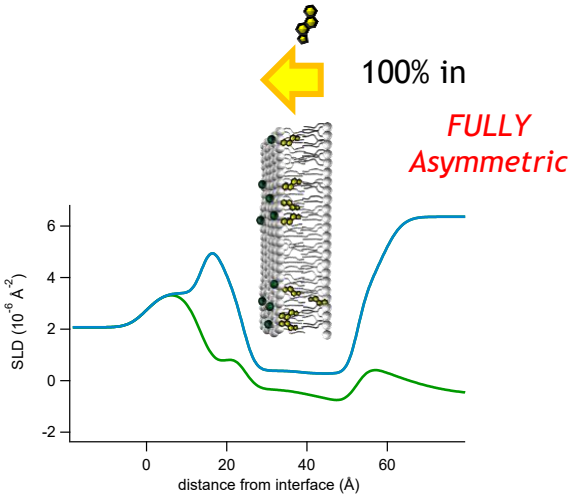


PLPC:Sito



PLPC:Sito:GluCer



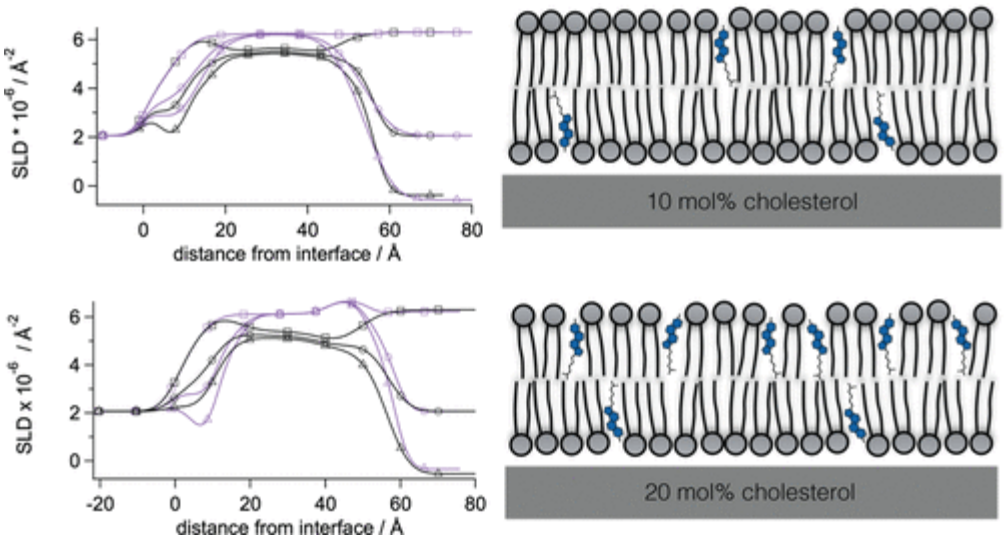


→ *Uneven sitosterol distribution in leaflets ONLY in presence of Glucosylceramide*

V. Rondelli et al., Sci. Rep. (2021)

Deuterated lipid mixtures from Escherichia Coli

	SLD * 10^{-6} / Å^{-2}	Volume / Å^3
Si	2.07	
SiO_2	3.47	
dPC tail	6.1	905[1]
dPC head	6.1	322[2]
h-cholesterol	0.2	622[3]
d-cholesterol	7.2	622[3]

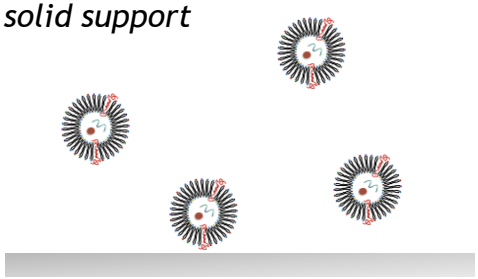


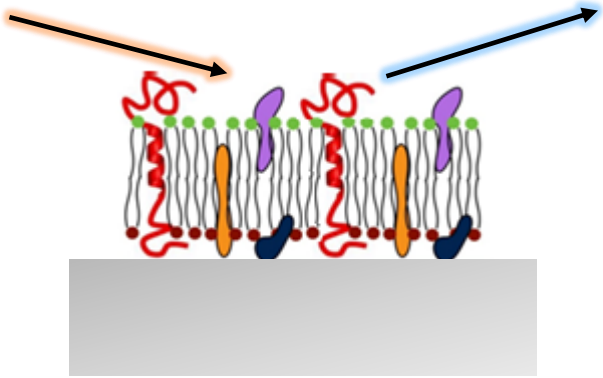
Cholesterol is located closer to the lipid head group–tail interface in natural PC extract rather than in the center of the core of the bilayer as seen for very thin or polyunsaturated membranes.

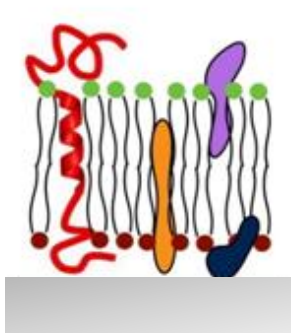
S. Waldie et al., Langmuir (2018) 34, 472–479

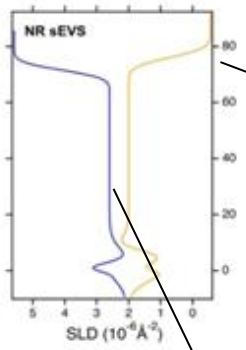
Neutron reflectometry on EVs-derived supported bilayers

Extracellular Vesicles fusion on solid support









Thickness:

6.9 ± 0.2 nm

single bilayer containing molecules other than lipids, as **large proteins**

SLD 2 ± 0.2 x10^{-6} Å^{-2}

lipid : protein
22 : 78
(by volume)

$$R \approx \left(\frac{16\pi^2}{q^4} N_b^2 \right) e^{-q_z^2 \sigma^2}$$

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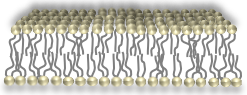
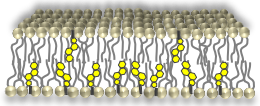
Membrane models

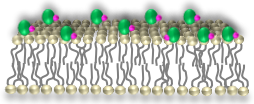
phospholipids



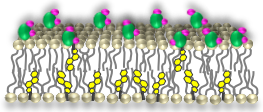
\+
cholesterol



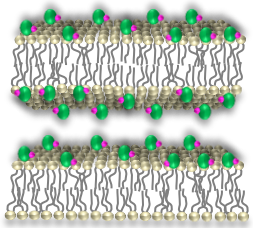
\+
glycolipids



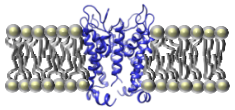
raft models



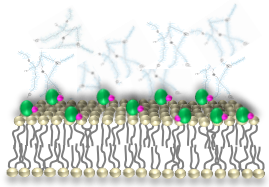
glycolipid-containing
*facing **double**-membranes*



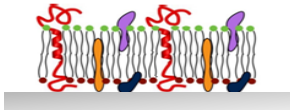
\+
transmembrane protein (K^+ channel)



\+
gel forming proteins (mucin)



from native extracts



IT COULD WORK

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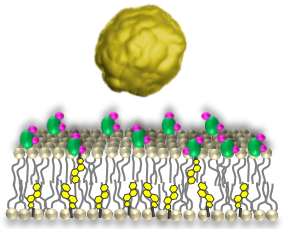
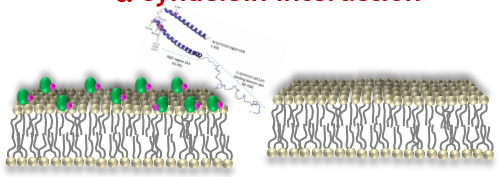
We can model interactions at membrane surface

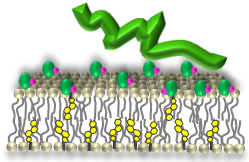
Enzymatic digestion on membrane surface



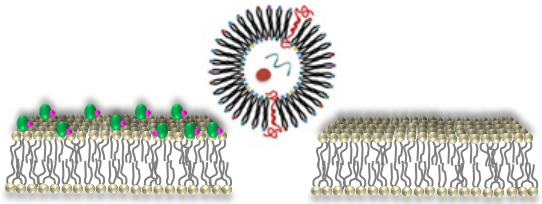
α-synuclein interaction



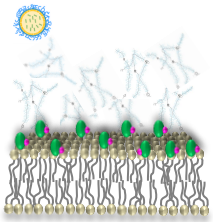
Aβ Peptide interaction



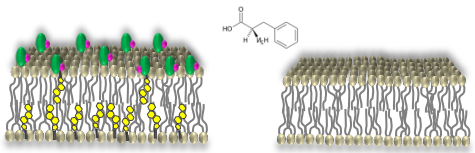
Extracellular vesicles interaction



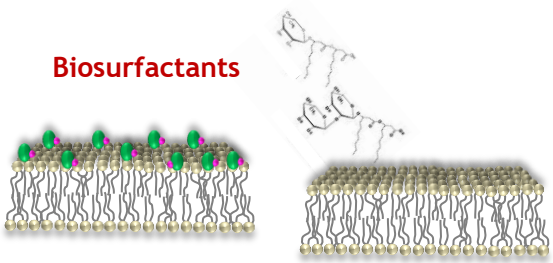
Transmucosal delivery



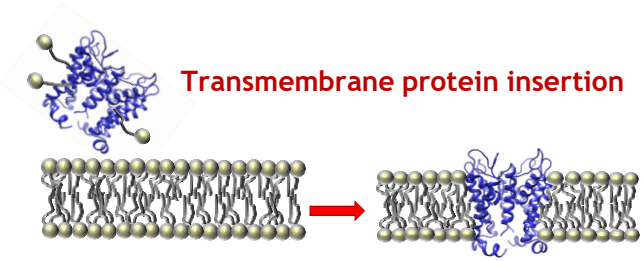
Amino acid interaction



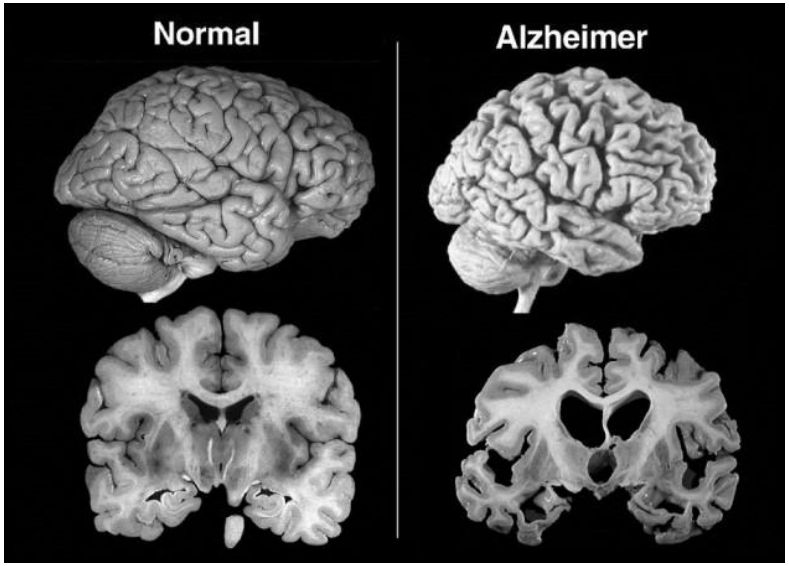
Biosurfactants

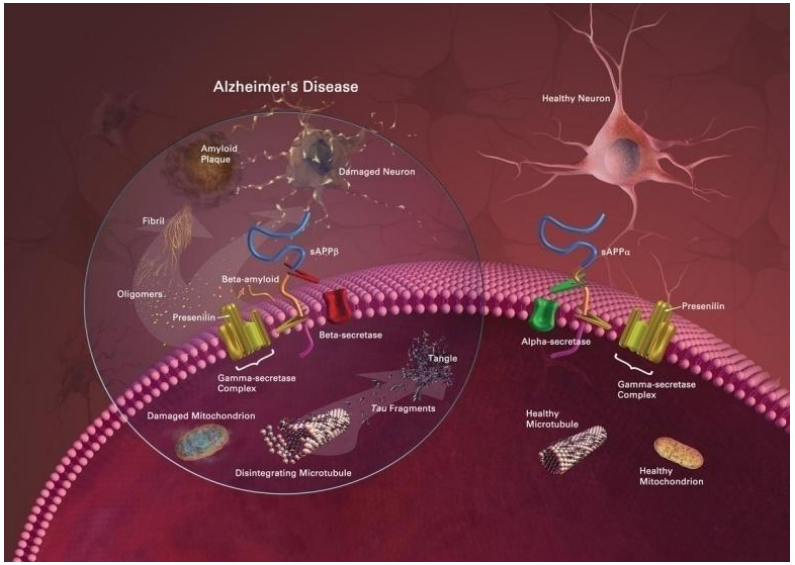


Transmembrane protein insertion



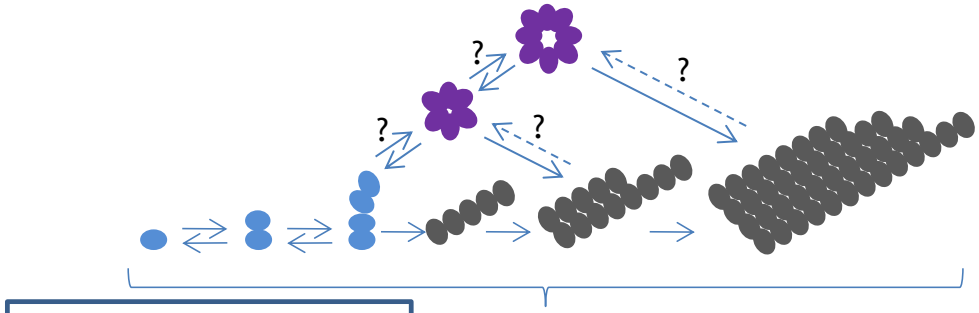
Aβ evidence and hypothesis





Complex landscape

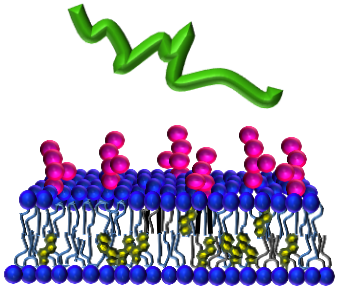
Simplified experimental models



Disruption of cellular ionic homeostasis

Loss of synaptic efficacy, neuronal dysfunction and degeneration

Membrane-active species



Structural modifications induced by membrane-peptide interaction

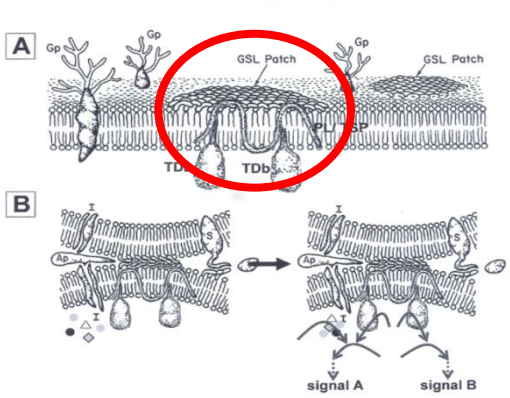
integrated approach with complementary techniques

⟶ complementary information on different properties

GEMs are claimed to be involved in

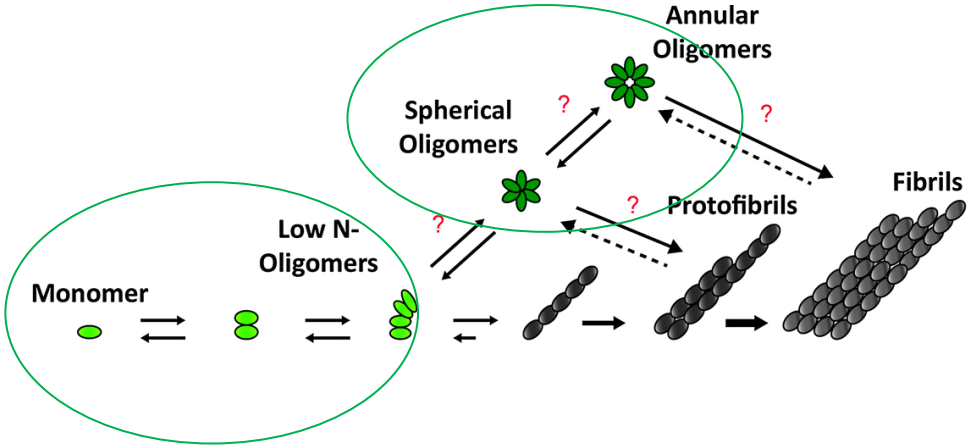
“membrane-mediated” amyloidogenesis

(K. Matsuzaki)

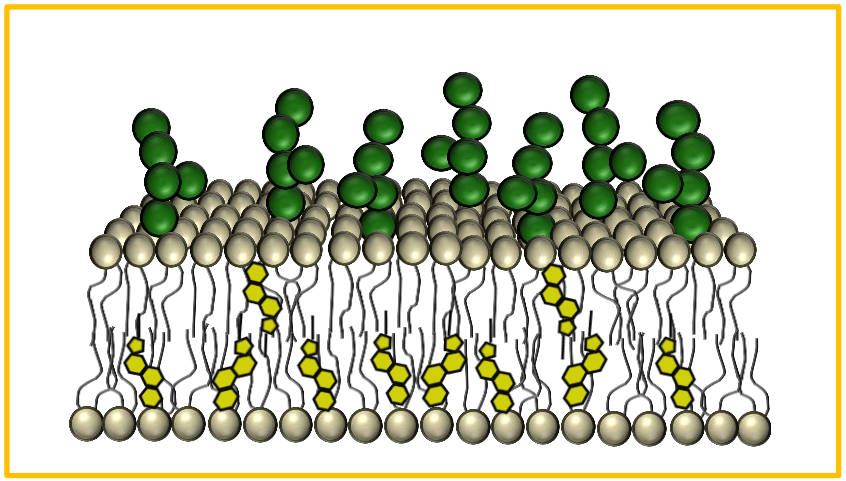


Hakomori (1998)

$A\beta_{1-42}$ interaction with a single raft mime



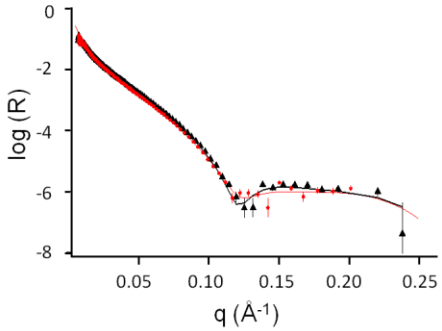
dDSPC:chol:GM1
10:1.25:0.5 mol

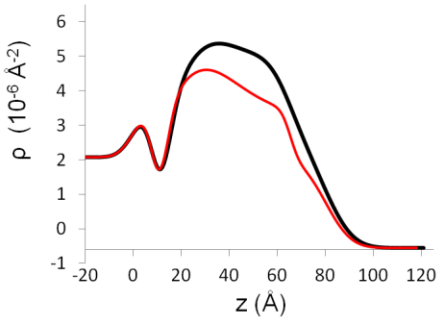


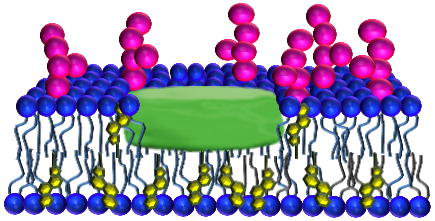
Protiated peptide in deuterated membrane

RAFT + structured oligomers

50 mM
Phosphate buffer
22° C
5h incubation

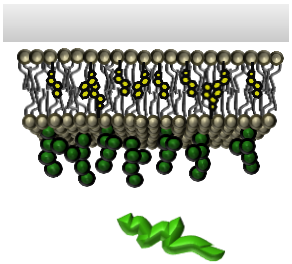






Embedded as such in the external leaflet of the membrane

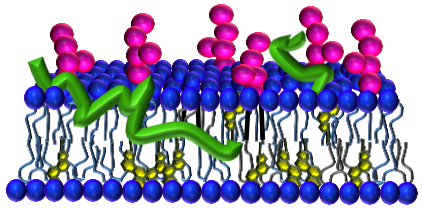
...seed?

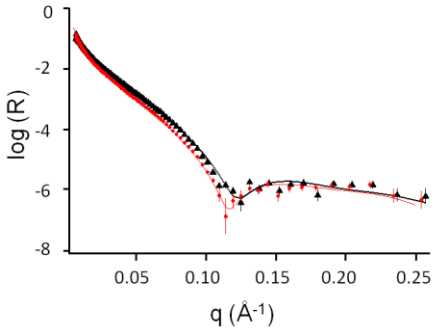


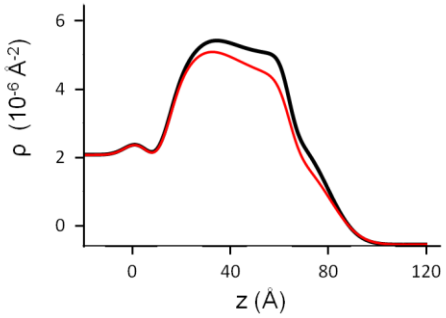
	Naked Membrane A				Membrane A after interaction with Aβ1-42 structured oligomers						
							Aβ1-42 penetration hypothesis			lipid loss hypothesis		
	T	r	$\rho_{lip}(z)$	W	T	r	$\rho_{lip}(z)$	W	Aβ penetration (%vol)	$\rho_{lip}(z)$	W	H_2O penetration (%vol)
hydrophilic in *(3)*	6	5	4.87	30	6	5	4.87	30		4.87	30	
hydrophobic in *(4)*	21	8	7.01	18	21	8	6.99	18		7.01	18	
hydrophobic out *(5)*	23	9	6.89	25	23	***15***	***5.9***	25	***19%***	6.89	***39***	***14%***
hydrophilic out *(6)*	16	3	4.21	31	17	4	***3.2***	31	***37%***	4.21	***52***	***21%***

RAFT + early oligomers

DEPSIPEPTIDE
1 μM

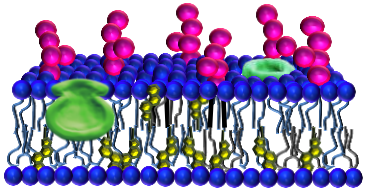


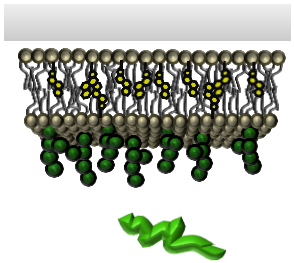




Aggregation templated by the membrane

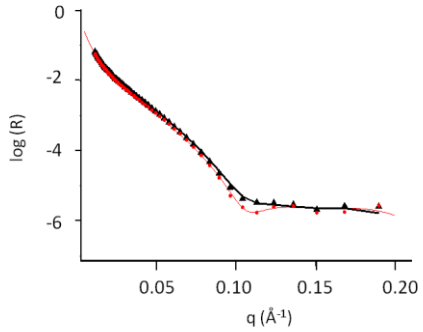
…pore formation?

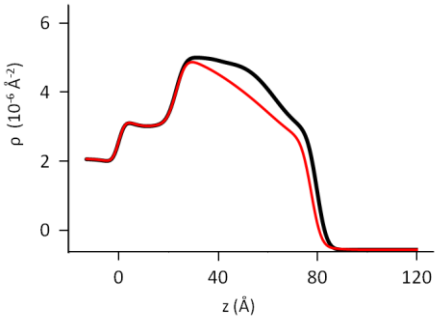


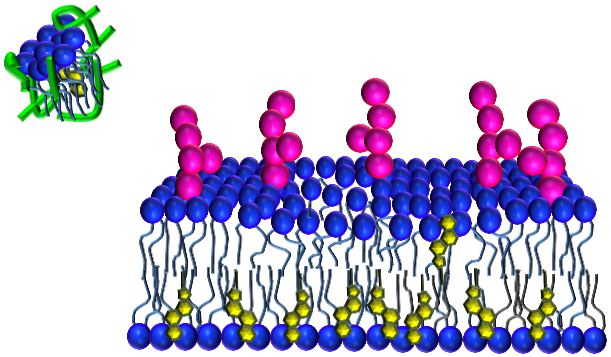


	Naked Membrane B				Membrane B after interaction with Aβ1-42 early oligomers							
							Aβ1-42 penetration hypothesis			lipid loss hypothesis		
	T	r	$\rho_{lip}(z)$	W	T	r	$\rho_{lip}(z)$	W	Aβ penetration (%vol)	$\rho_{lip}(z)$	W	H_2O penetration (%vol)
hydrophilic in *(3)*	6	4	4.87	30	6	4	4.87	30		4.87	30	
hydrophobic in *(4)*	21	9	7.01	18	21	***12***	***6.7***	18	***6%***	7.01	***22***	***4%***
hydrophobic out *(5)*	23	9	6.89	25	25	***15***	***4.6***	25	***42%***	6.89	***55***	***30%***
hydrophilic out *(6)*	16	7	4.21	31	14	***3***	***3.3***	31	***32%***	4.21	***49***	***18%***

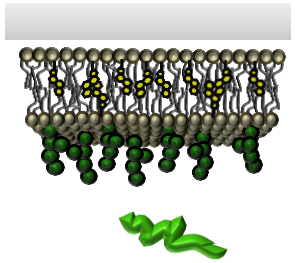
RAFT + N-terminus





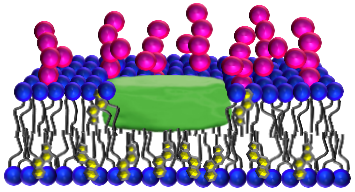


Role in membrane destabilization?

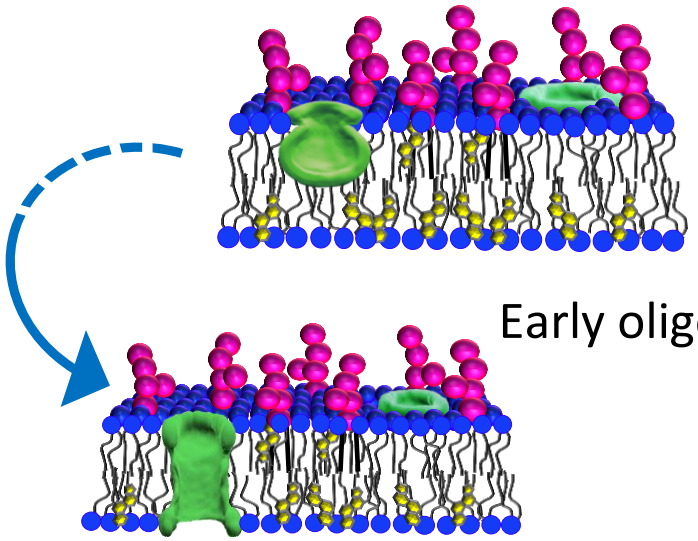


	Naked Membrane C				Membrane C after interaction with Aβ1-6							
							Aβ1-6 penetration hypothesis			lipid loss hypothesis		
	T	r	$\rho_{lip}(z)$	W	T	r	$\rho_{lip}(z)$	W	Aβ penetration (%vol)	$\rho_{lip}(z)$	W	H_2O penetration (%vol)
hydrophilic in *(3)*	9	8	4.87	34	9	8	4.87	34		4.87	34	
hydrophobic in *(4)*	17	3	7.01	27	17	3	6.99	27		6.99	27	
hydrophobic out *(5)*	23	3	6.89	27	**22**	***10***	***5.9***	27	***20%***	6.89	***40***	***13%***
hydrophilic out *(6)*	17	9	4.21	30	**15**	***12***	***3.6***	30	***26%***	4.21	***43***	***13%***

Model raft + $A\beta_{1-42}$



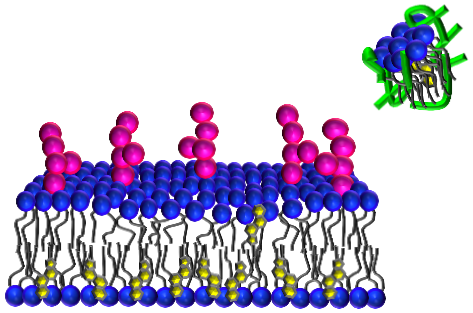
Structured oligomers



Early oligomers

Therapeutic hint?

Model raft + $A\beta_{1-6}$

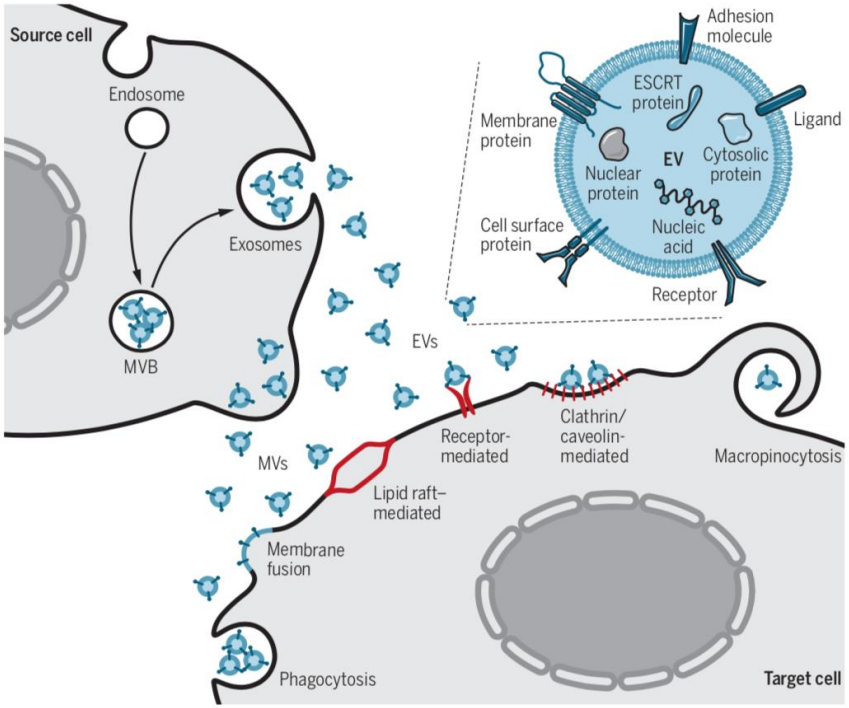


V Rondelli, et al., *Scientific Reports* (2016)

Back to extracellular vesicles - EVs

Definition

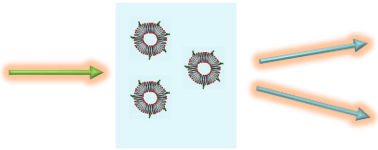
« Particles that are released from the cells, delimited by a lipid bilayer and cannot replicate on their own »



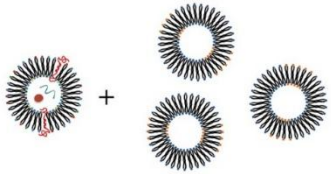
Wiklander et al., Science Trans. Medicine (2019)

Back to extracellular vesicles - EVs

SANS



sEVs : dDMPC Vs
1: 15000
1:3000
1:2700

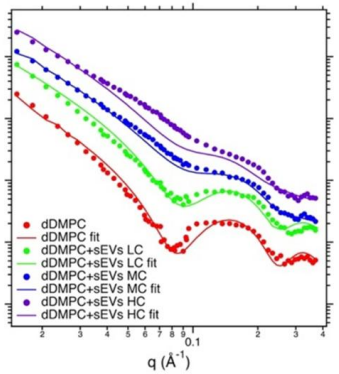


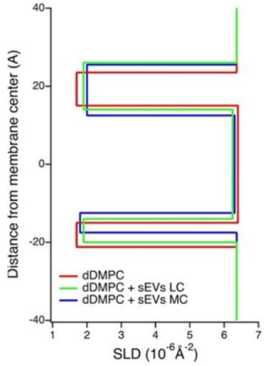
FITTING MODEL
Multilayered spherical form factor

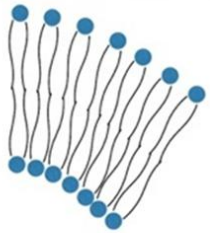
$$P(q) = \frac{\text{scale}}{V} F^2(q) + \text{background}$$

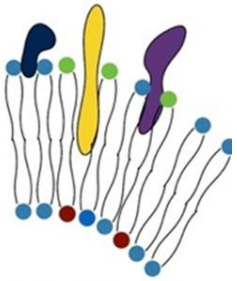
$$F(q) = \frac{3}{V_s}\left[V_c(\rho_c - \rho_s)\frac{\sin(qr_c) - qr_c\cos(qr_c)}{(qr_c)^3} + V_s(\rho_s - \rho_{\text{solv}})\frac{\sin(qr_s) - qr_s\cos(qr_s)}{(qr_s)^3}\right]$$

(Guinier, 1955)

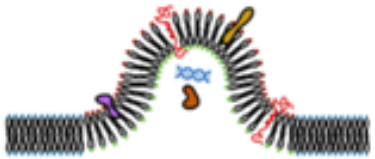




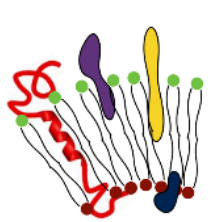


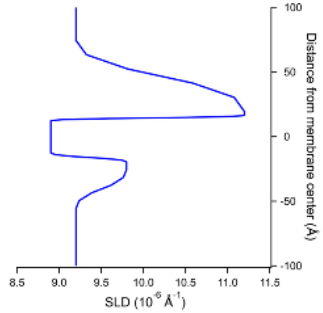


- Change in contrast spans whole membrane thickness
- Asymmetric



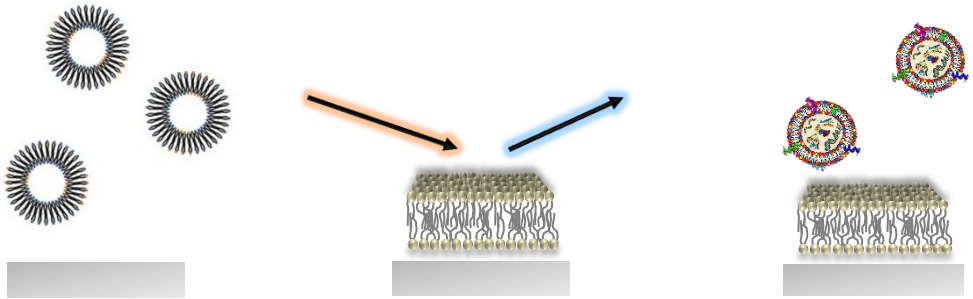
EVs SLD profile



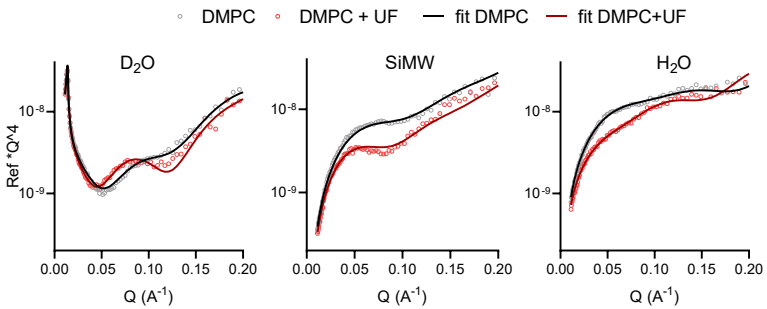


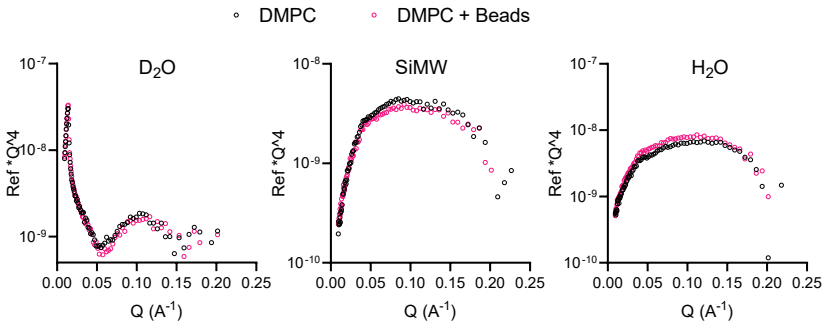
EVs uptake mechanisms - Neutron Reflectometry

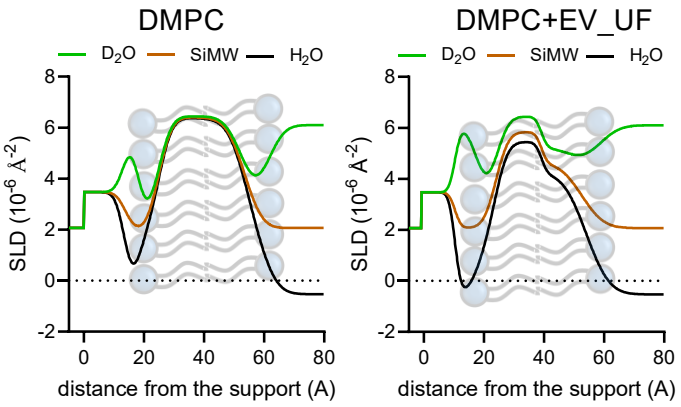
DMPC vesicles fusion → *DMPC characterization* → *EVs injection from bulk water* → *mixed system characterization*

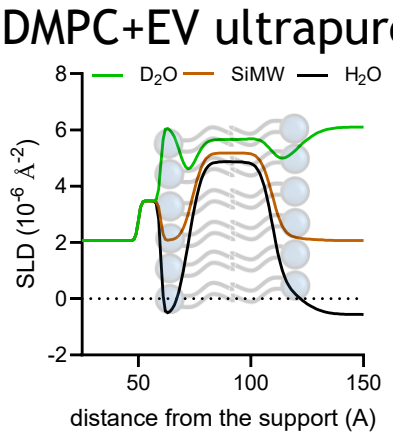


Material	SLD (10^{-6}Å^{-2})
H_2O	-0.56
D_2O	6.36
Lipid chains	-0.33
Protein	2 - 2.5









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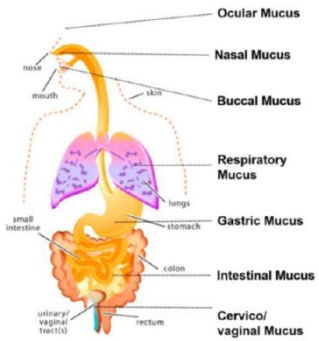
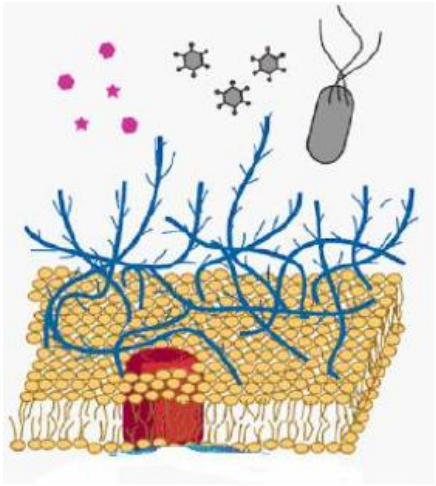
The mucus selective barrier

MUCUS: highly viscoelastic secretion, covering the epithelia surfaces of the gastrointestinal, pulmonary, oral, nasal and genital tracts.



Tasks:
-to protect mucosal tissues from dehydration, mechanical stress
-to act as SELECTIVE BARRIER against microorganisms and toxic substances regulating the distribution of drugs, ions and proteins

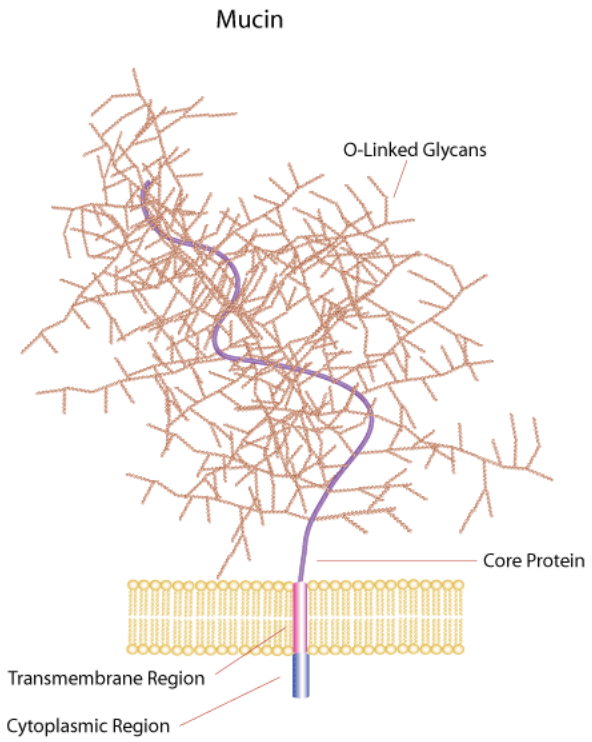


Structure:

-water (up to 98%)
-lipids
-small proteins
-nucleic acids
-high molecular weight glycoproteins: MUCINS

Mucins: a mucus model environment

MUCIN:
amino-acidic backbone
+
regular distribution of sugar brushes

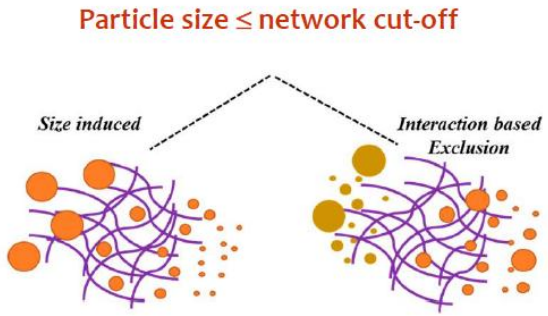


Mechanical and viscoelastic preperties of mucus depend on mucin

Delivery in mucus environments

Why transmucosal delivery?

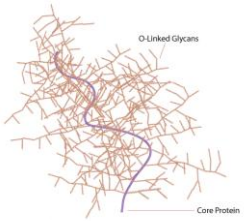
The transmucosal membranes are relatively permeable, have a rich blood flow and hence
allow the rapid uptake of a drug into systemic circulation to avoid first pass metabolism



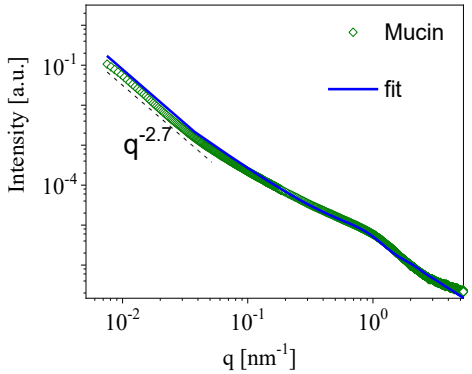
O. Lieleg, K. Ribbeck in *Trends Cell. Biol.* **21**(9), 2011

→penetrating particle
→adjuvants for mucus destabilization

Mucines: a mucus model environment

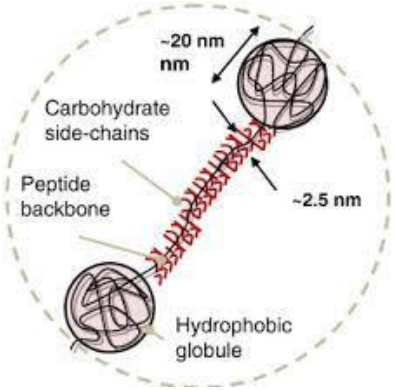


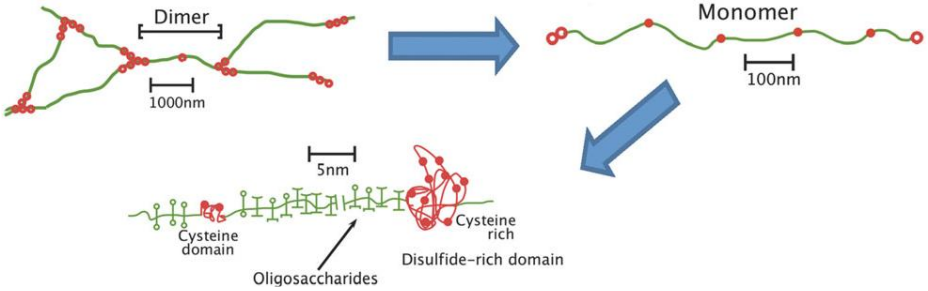
SAXS from bulk systems



Mucin

dumbbell-like shape
form factor





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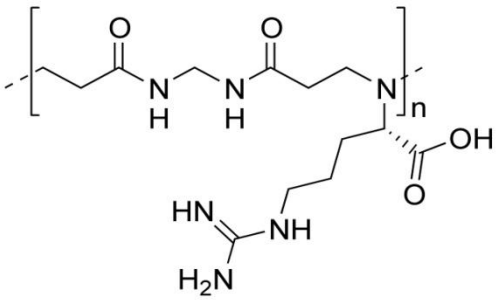
D,L-ARGO7 polymer

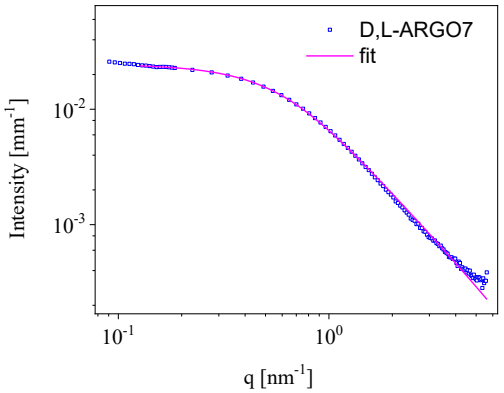


MW= 10 kDa

D,L-ARGO7

- Water soluble poliamidoamyne
- Amphoteric
- good **biocompatibility** and **biodegradability**
- promising for DNA and siRNA delivery.
- strong **protection** actions against virus infection
- **low cytotoxicity**

SAXS from bulk systems



Gaussian polymer model, radius of gyration 2.5 nm

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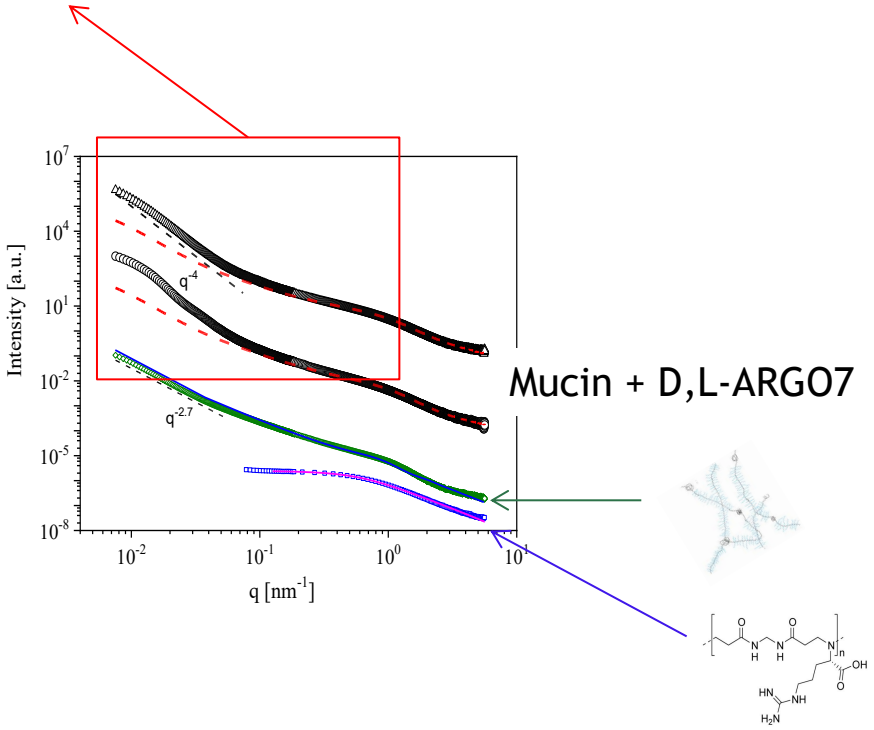
D,L-ARGO7 polymer interaction with mucin

SAXS from bulk systems

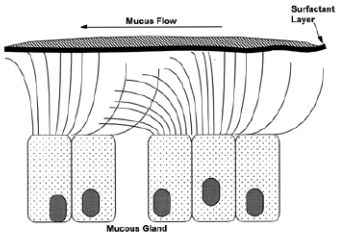
-Large scale objects formation
-Mucin mesh modification



Is it enough?

In bulk we observe the mean of systems behaviour and phases

CLEARENCE

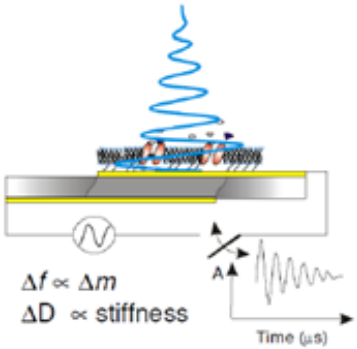


We care about destabilization, phase separation and changes in mobility

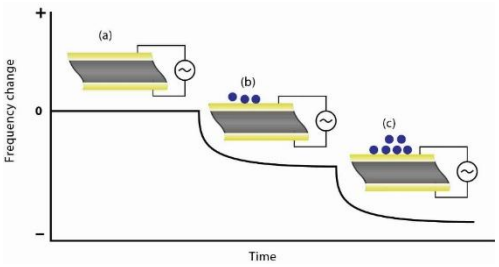
→ Thin films

QCM-D from thin layers

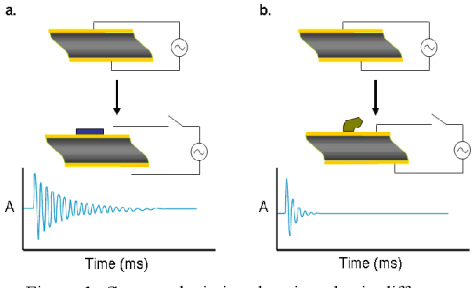
Excitation resonance of a freely oscillating quartz crystal



Resonance frequency:
information about the mass (ng to μg) deposited

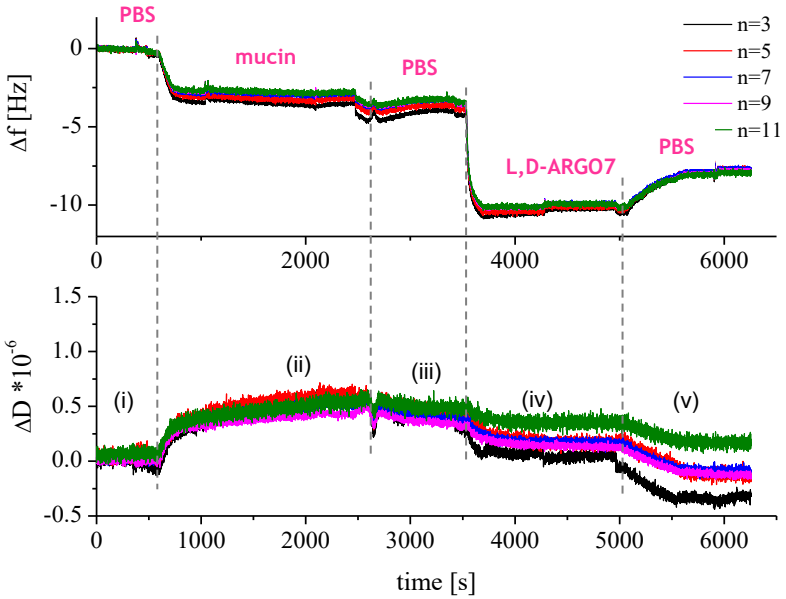


Dissipation:
information about the viscoelastic properties of the deposited sample

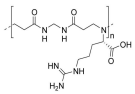


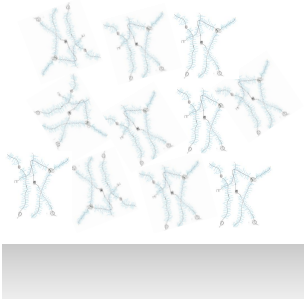
Mucin thin film and its interaction with the polymer

(i) PBS solution injected to get a baseline
(ii) injection of mucin
(iii) injection of PBS to remove the substance not surface confined
(iv) injection of polymer solution
(v) rinsing in PBS



mucin 20 mg/L
D,L-ARGO7 (pH 7.4) 5:1 ratio
SiO_2 support (mucin layer 20 Å thickness/ rigid layer)

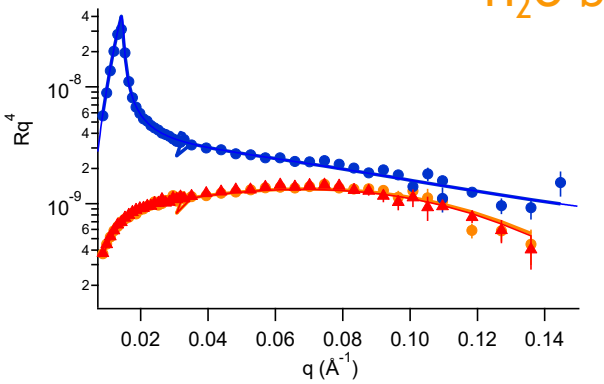


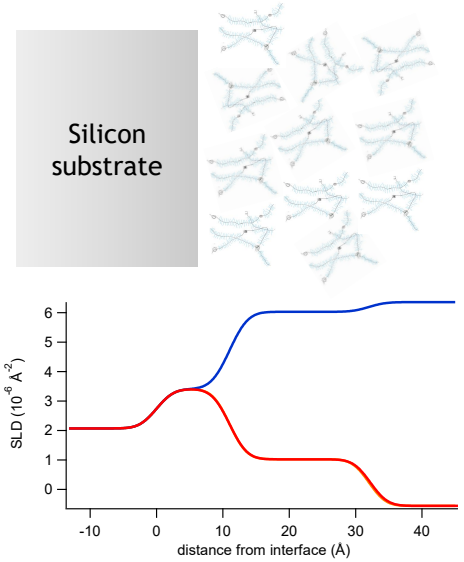


→ What is flushed?

Neutron reflectometry

H_2O
D_2O
H_2O back





20Å mucin layer
Stable under solvent flushing

Table S1. Fit parameters of the mucin layer deposited on the silicon surface. Parameters correspond to a contemporary fit performed on H2O and D2O solutions with 150mM NaCl. Errors have been estimated by changing the parameters up to a variation of two in the $\chi 2$.

	Thickness (±1 Å)	SLD (± 0.05*10⁻⁶Å⁻²)	Solv p (±5%vol)	Roughness (±2Å)
Mucin	21	2.5 (H_2O) 5.6 (D_2O)	40	3

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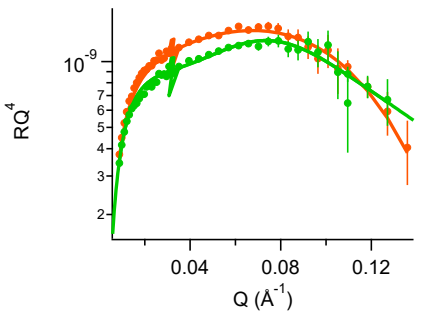
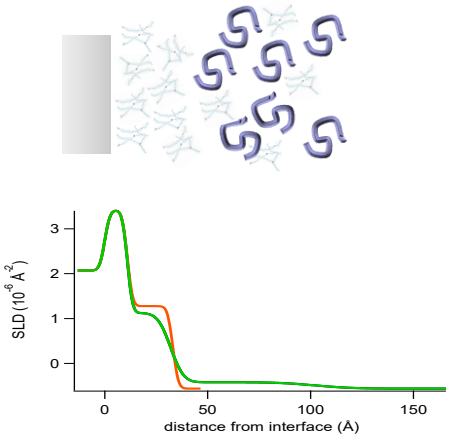
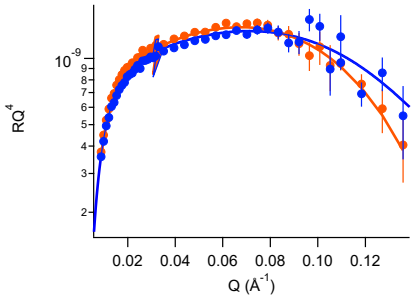
Mucin + D,L-ARGO7: neutron reflectometry

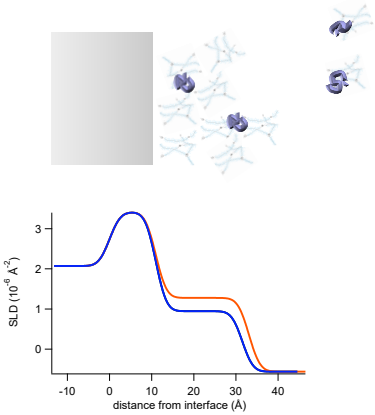


Mucin
Mucin + D,L-ARGO7



Mucin
Mucin + D,L-ARGO7 after flush





Mucin destabilization and partial removal by flushing

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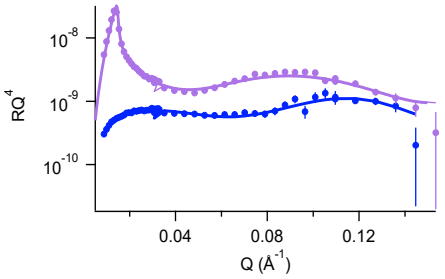
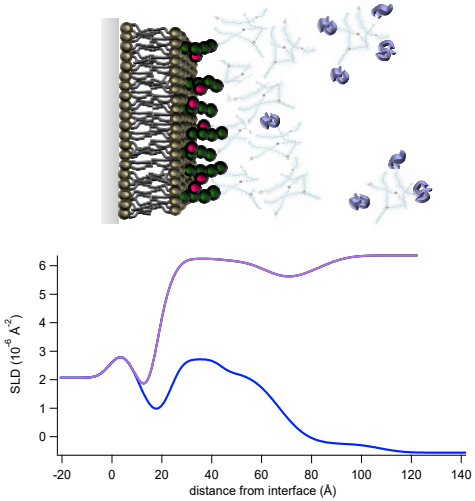
A model for epithelial mucus covered cells

D_2O
H_2O

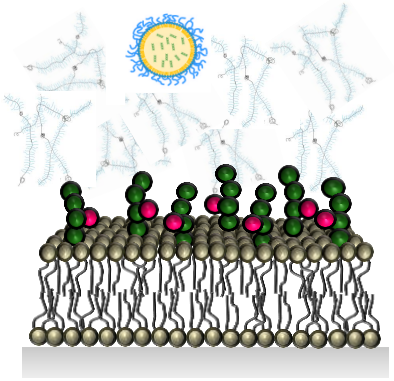




Rondelli V. *et al.*
International Journal of Molecular Sciences (2019)

→ *Mucin destabilization and partial removal by flushing*

→ *Complex system stability*



Access not only to the **fate of particle and mucus** but also to the **cross details of model tissue-biomolecules interaction** with few Å sensitivity

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CONCLUSIONS

Neutron scattering/reflectometry are essential complementary techniques for cross-structural investigations down to the nanoscale of self assembled systems at interfaces

Selective deuteration allows for selective visibility of components

Possibility to work in physiological conditions

Possibility for in-situ interaction studies

Perspectives in biology are numerous

Literature

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