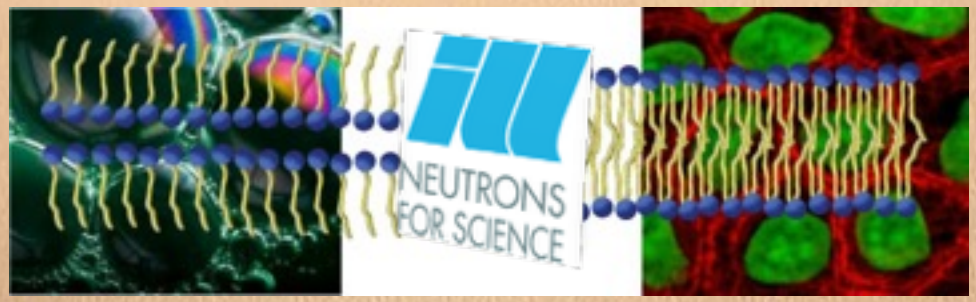
# Neutrons and Soft Matter:

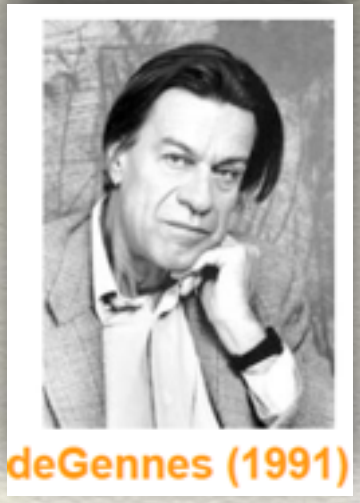
## From Soap Bubbles to Cell Membranes

Giovanna Fragneto



# SOFT MATTER

"Molecular systems giving a strong response to very weak command signal"



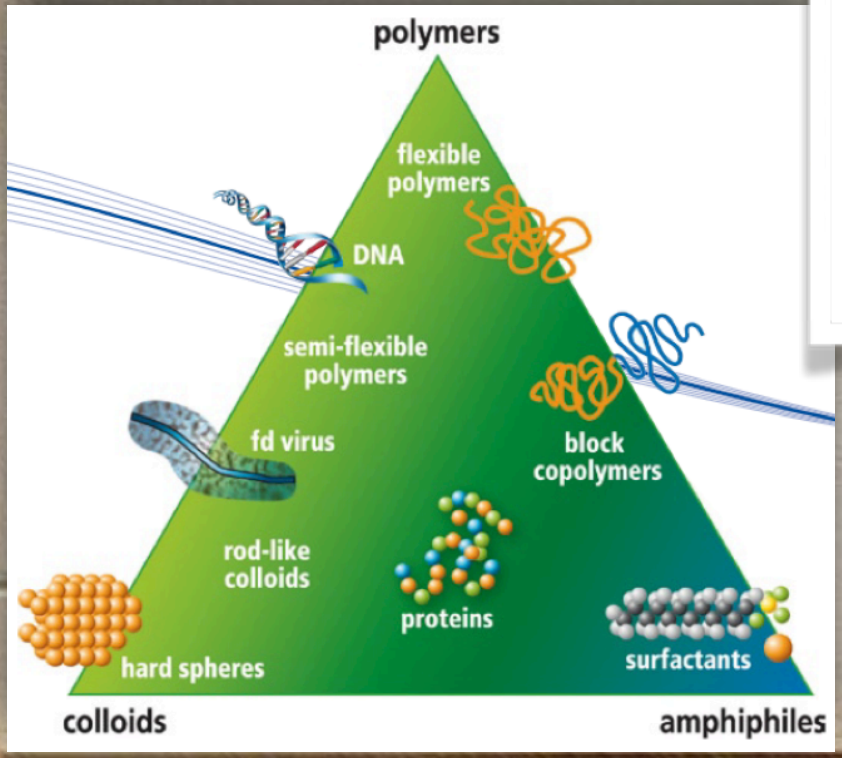

*"founding father of soft matter"*

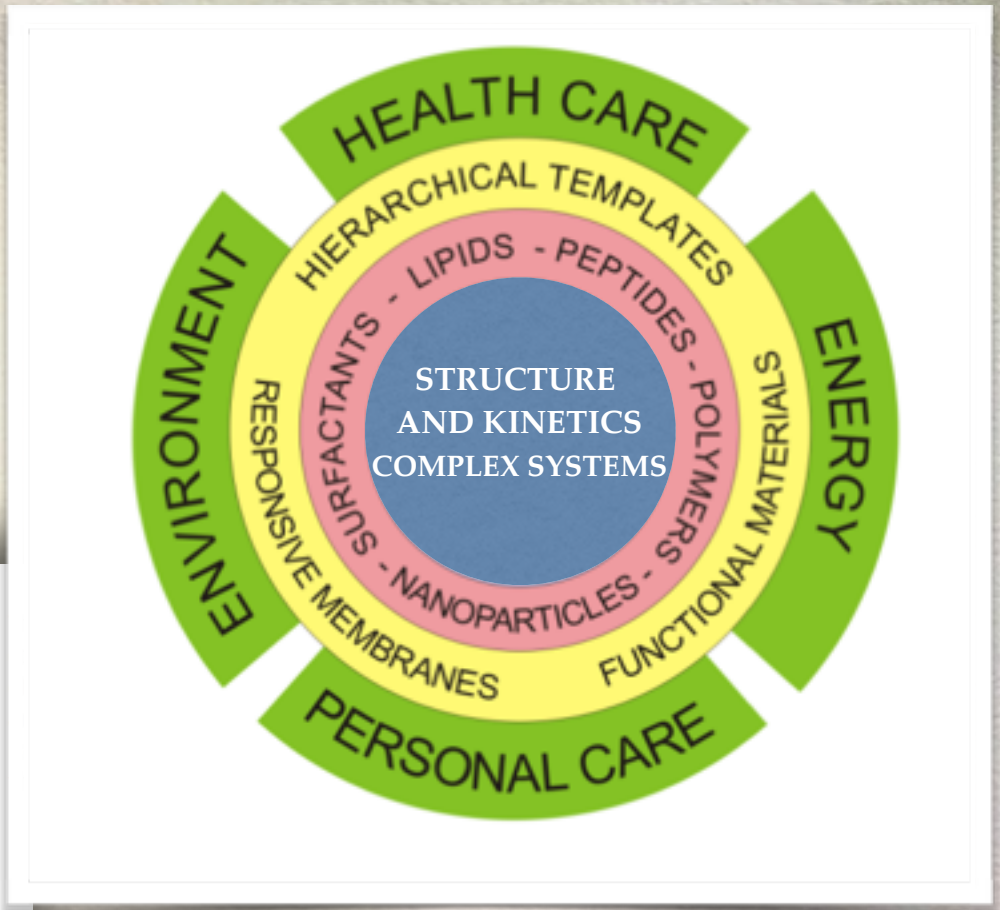
Condensed matter: states are easily deformed by small external fields, including thermal stresses and thermal fluctuations.

Relevant energy scale comparable with room temperature **thermal energy.**

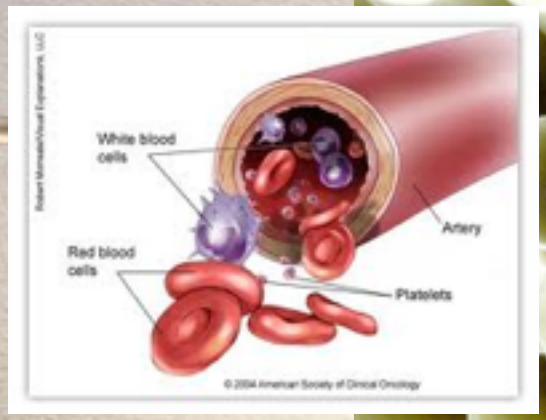
Structures in the size range of **nanometres to a few micrometres.**

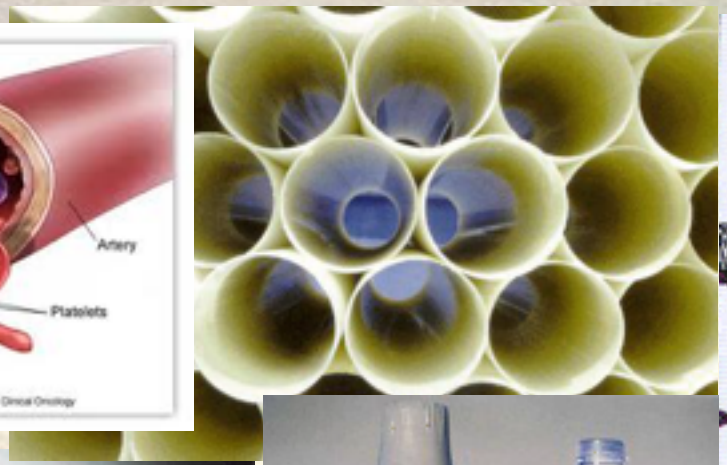
Complex fluids : including colloids, polymers, surfactants, foams, gels, liquid crystals, granular and biological materials.

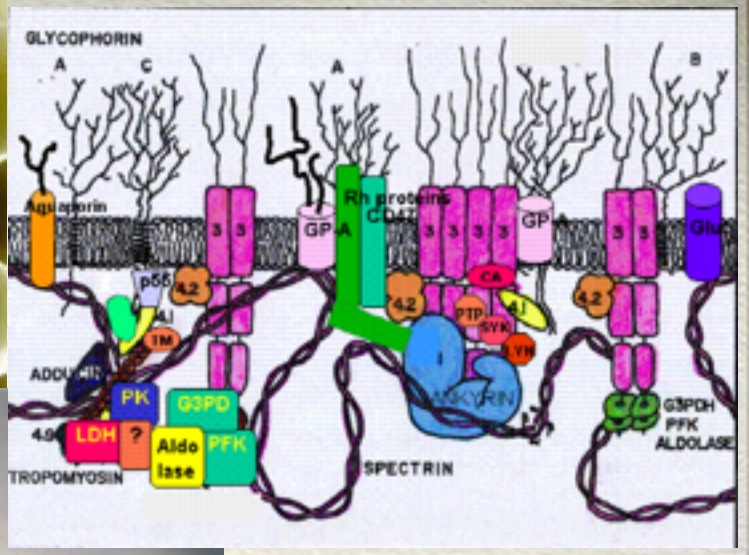



***Soft matter plays an important role in nearly every aspect of our daily life and soft matter research is a driving force in a broad range of innovation fields.***



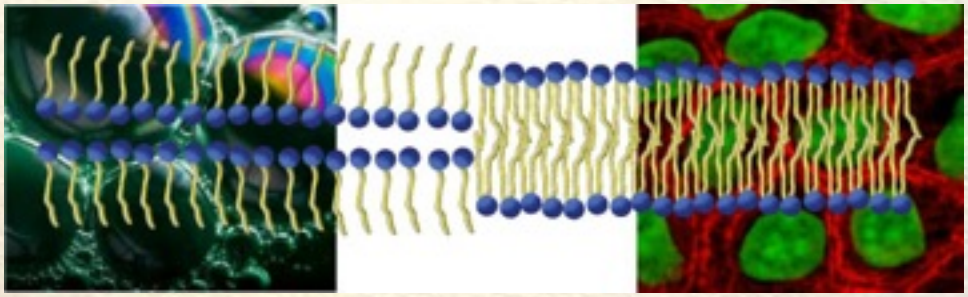








Products where surfactant is a secondary component in the material or the production.

Materials with very different functions sharing common structural features



Soap bubbles and cell membranes are formed by **amphiphilic** molecules able to **self-assemble**, a few **nanometer** thick and which **structure** and **dynamics** can be determined by **scattering** techniques

# Hydrophobic Effect

**Tendency of nonpolar substances to aggregate in aqueous solution and exclude water molecules**

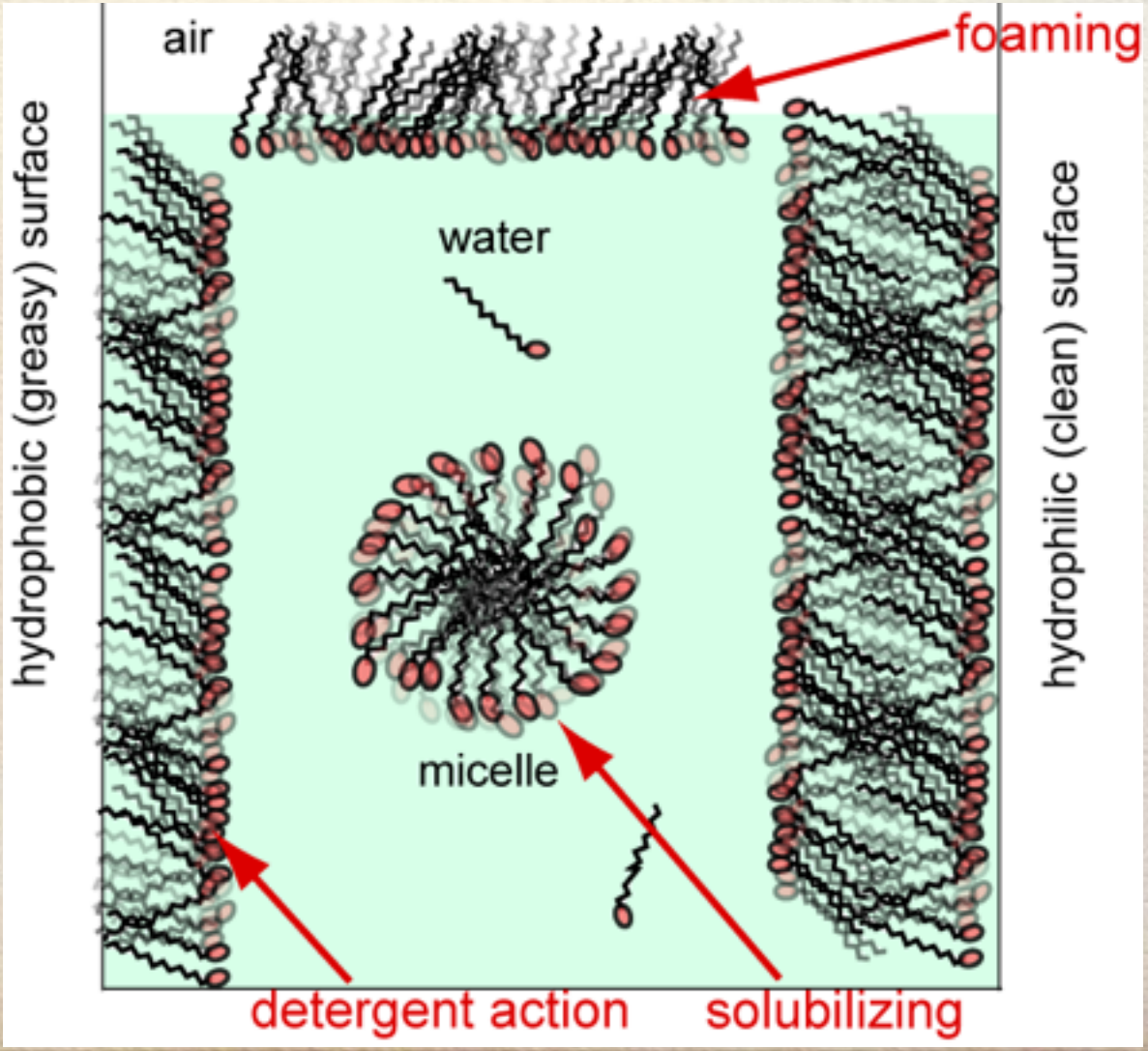
***entropic effect originating from the disruption of highly dynamic hydrogen bonds between molecules of liquid water by the nonpolar solute***

It explains the separation of a mixture of oil and water into its two components, and the beading of water on nonpolar surfaces.

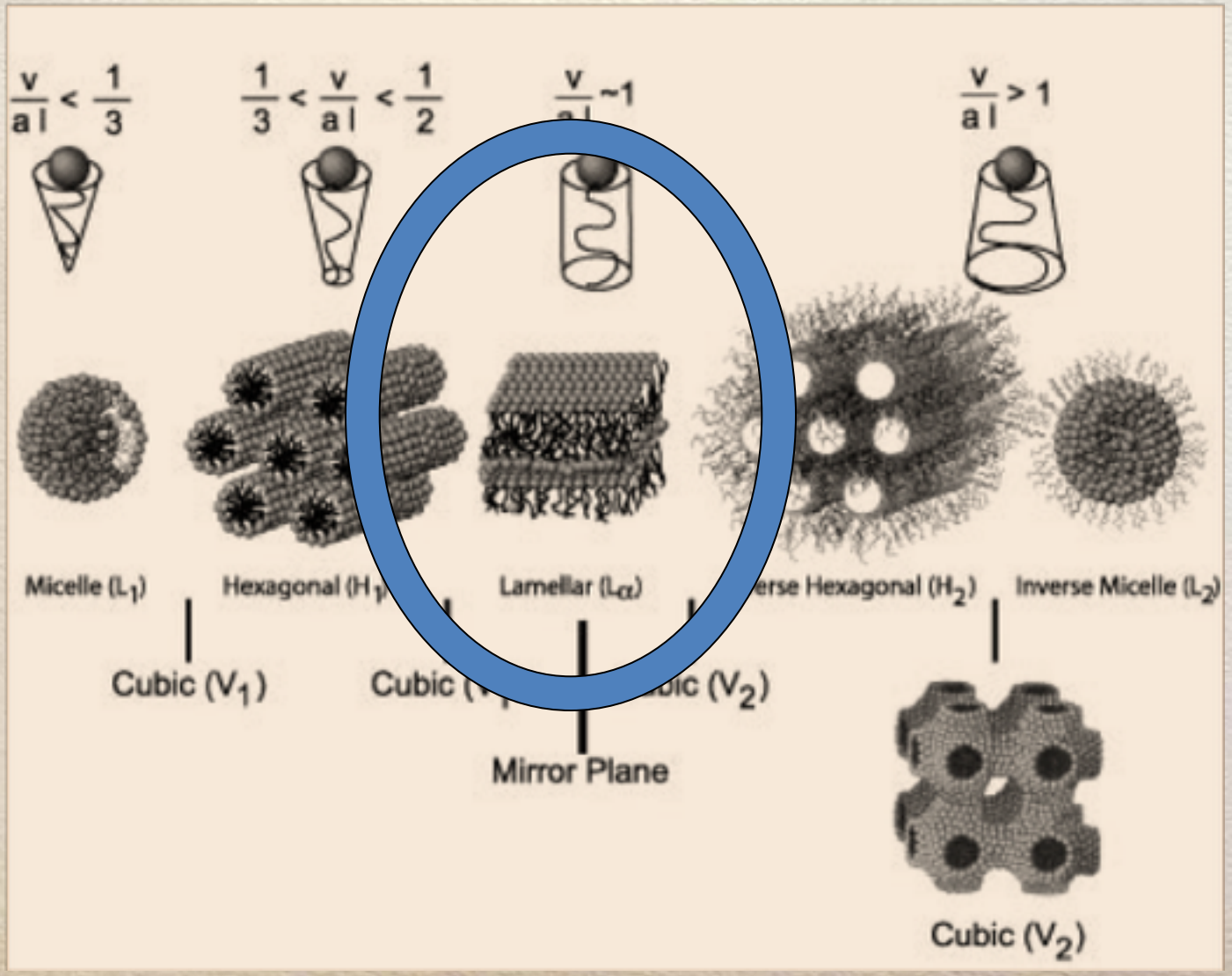
At the molecular level, it is important in driving protein folding, formation of **lipid bilayers and micelles**, insertion of membrane proteins into the nonpolar lipid environment and protein-small molecule interactions.

# Self-assembling amphiphilic systems:

*alcohols, surfactants or soap like molecules and lipids*



# Surfactant Phases

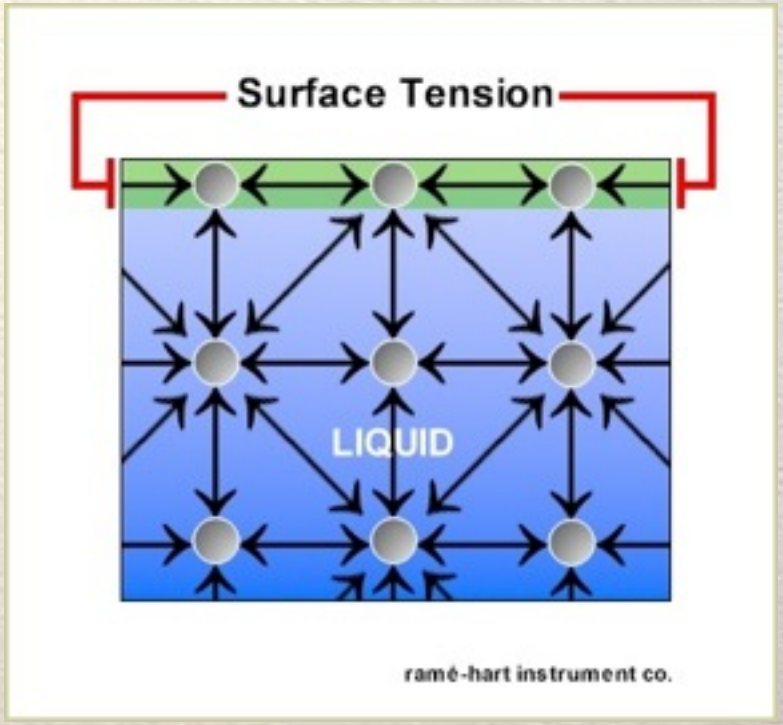


# Surface Tension

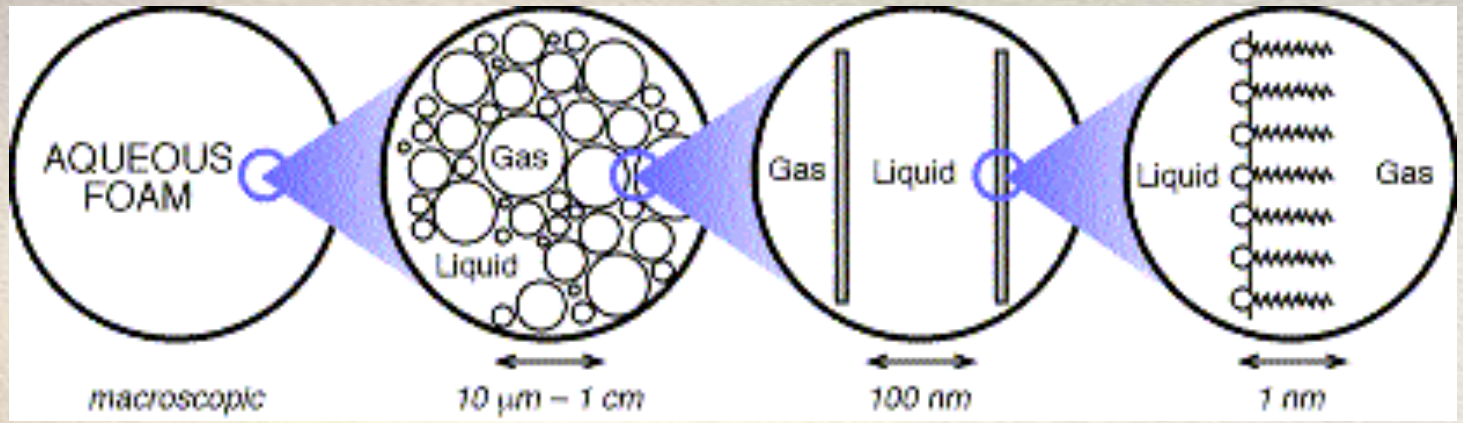
The cohesive forces between molecules down into a liquid are shared with all neighboring atoms.

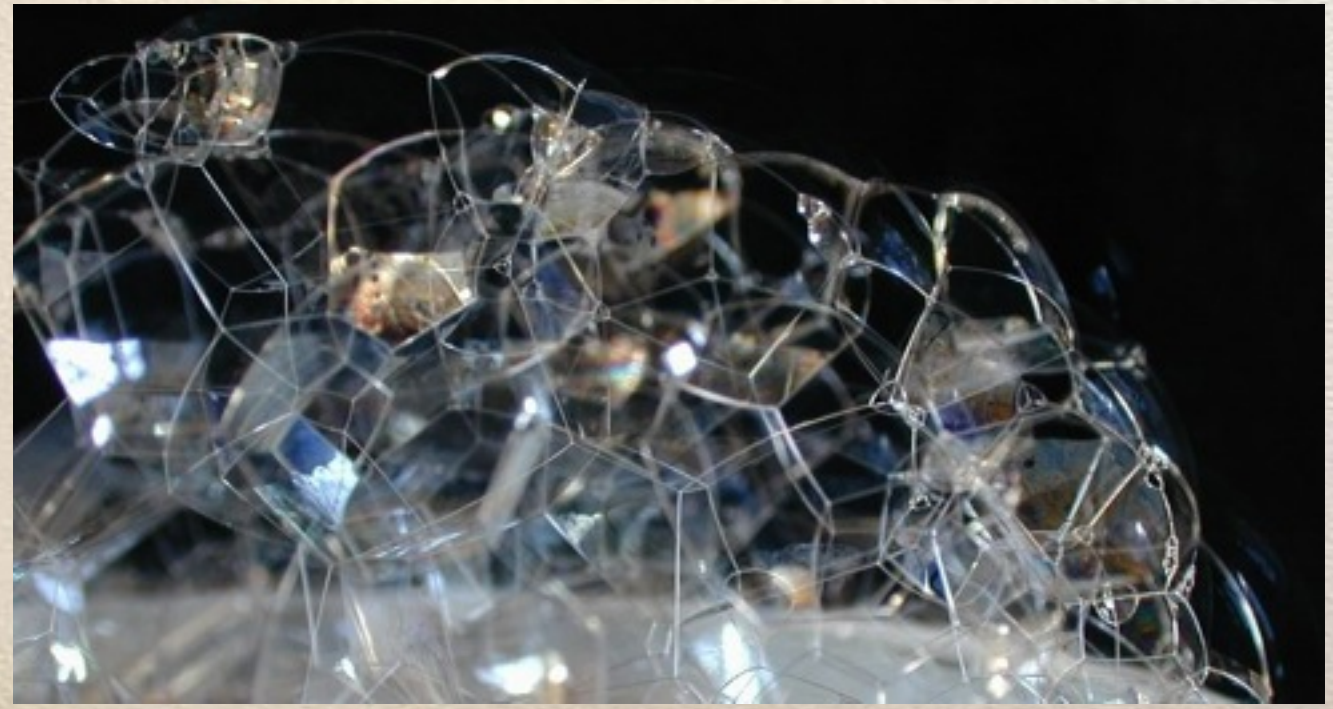
Those on the surface have no neighboring atoms above, and exhibit stronger attractive forces upon their nearest neighbors on the surface.

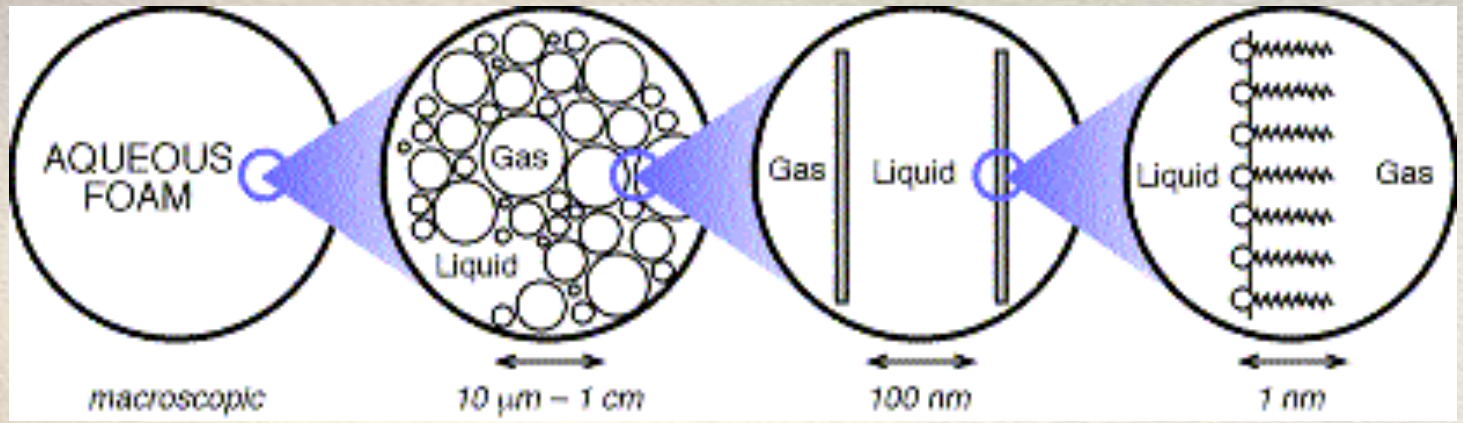
This enhancement of the intermolecular attractive forces at the surface is called ***surface tension.***




***Pure water has a surface tension of ~ 72 mN/m, but a monolayer can cause this to drop nearly to zero.***


AQUEOUS FOAM
Gas
Liquid
Gas
Liquid
Liquid
Gas
macroscopic
10 μm – 1 cm
100 nm
1 nm

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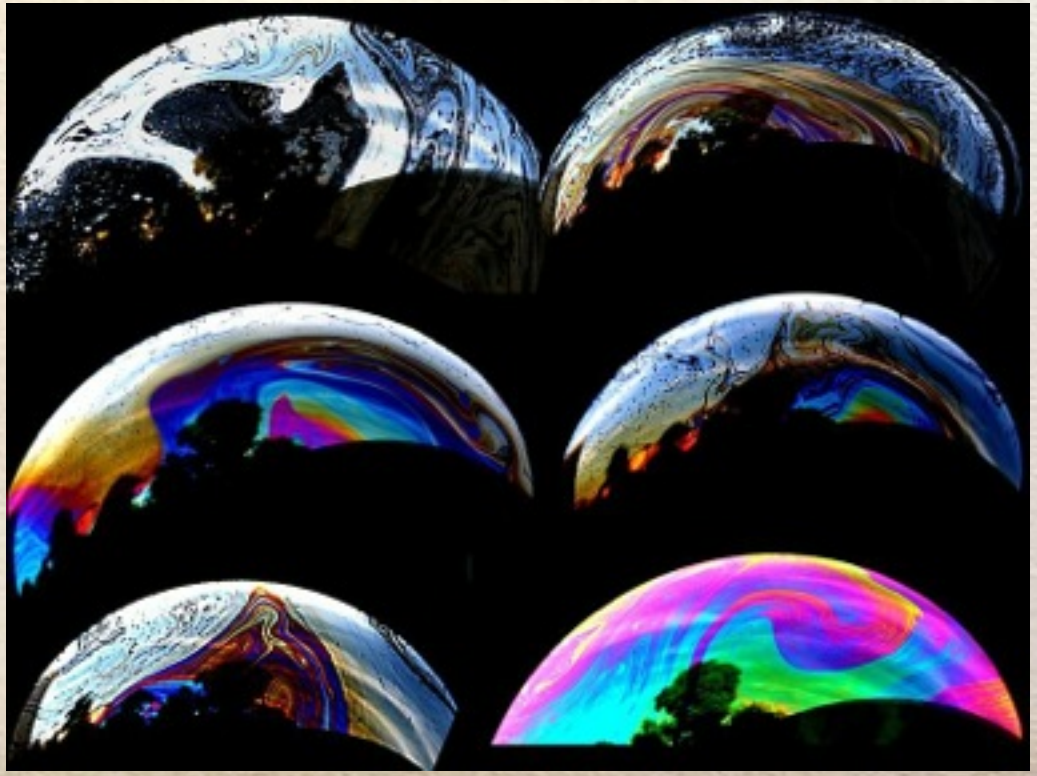
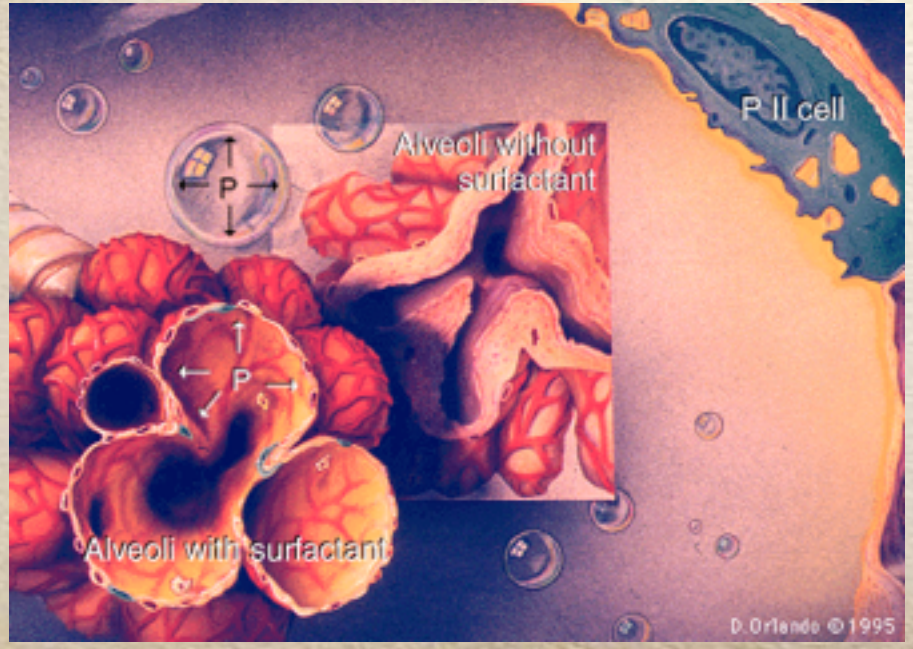


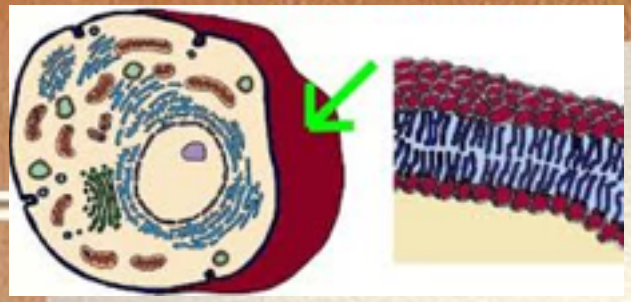
# Lung Surfactants:

The normal surface tension in the lungs is 25 mN/m; at the end of the expiration, compressed surfactant phospholipid molecules decrease the surface tension to near-zero levels.

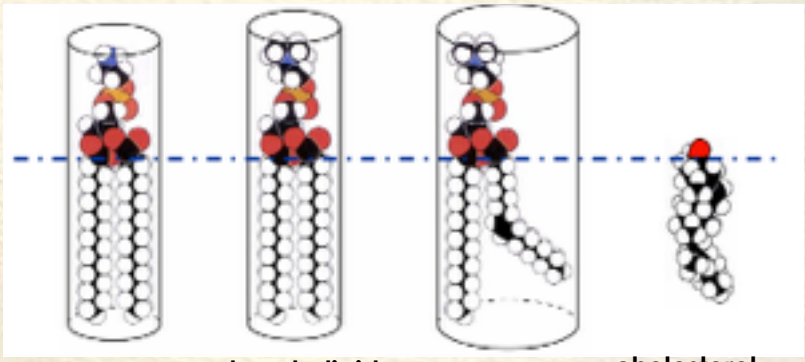
Pulmonary surfactant allow the lung to inflate much more easily, thereby eliminating the work of breathing. It reduces the pressure difference needed to allow the lung to inflate

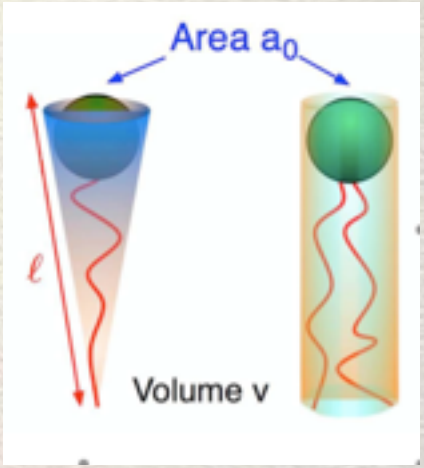



**Premature infants lacking of these surfactants suffer from infant respiratory distress syndrome**



# From soap to lipid bilayers




Lipids are amphiphiles:
in aqueous environment self-assemble
(reduced specific volume ~1 *i.e.* bilayers are formed)

- Highly hydrophobic core forms a barrier : protects content of cell
- Lipid bilayer participates to exchanges between extracellular fluid and cytoplasm

**The structure and organization of the lipid bilayer component of membranes hold the key to understanding the functioning of membranes**

# Why important?

*Total surface of membranes covers an area of ~100 $m^2$ in our body*

**Function of membrane proteins :** dependent on membrane composition, lipid-protein interaction, lipid mediated protein-protein interaction

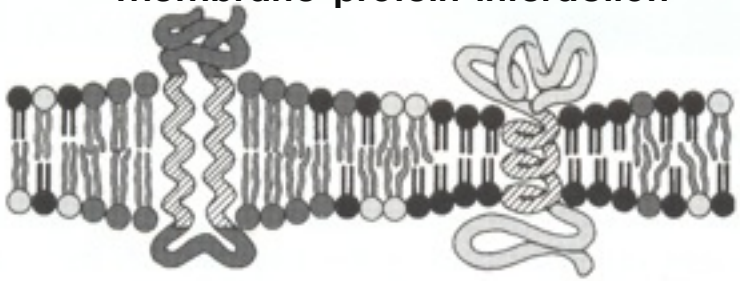
**Pharmacological interest :** Drug transport through membranes (dependent on physico-chemical membrane properties), anti-microbial peptides

Membranes may play a *direct* role in **signal transduction**

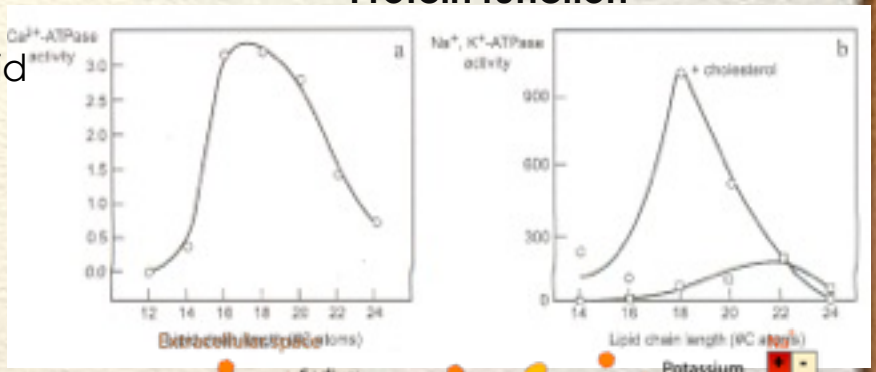
**Diseases** associated with changes in lipid composition (heart disease, obesity, diabetes, cancer and neurodegenerative disorders like schizophrenia, Tay-Sachs syndrome, Alzheimer, Parkinson)

**Cell adhesion**

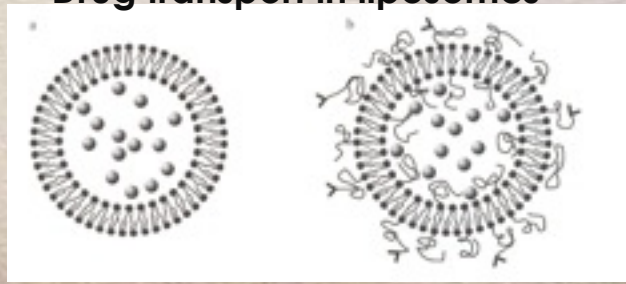
**Membrane-protein-interaction**



**Protein function**

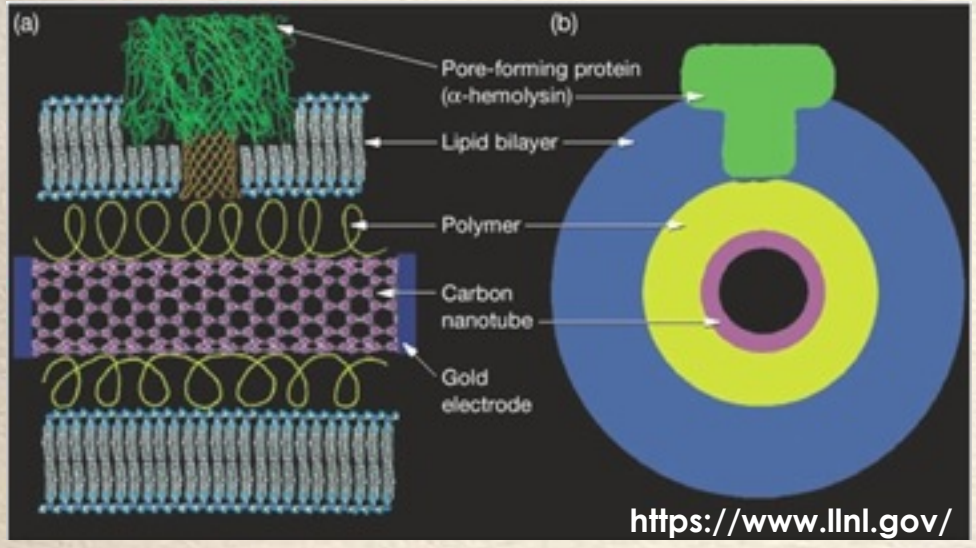



**Drug transport in liposomes**



*O. Mouritsen Life – as a Matter of Fat* Springer (2005)

# Nanobiotechnology Applications




*A new type of biosensor is based on a lipid-coated nanotube*

**Catalytic biosensors** (for example glucose biosensors)
**Affinity biosensors** (antibodies, DNA, peptides and lectins)

***Current problems include: non-specific binding, reproducibility***

**Biofunctional Coatings:**
Artificial Organs and Implanted Medical Devices

# Why Neutron Scattering?

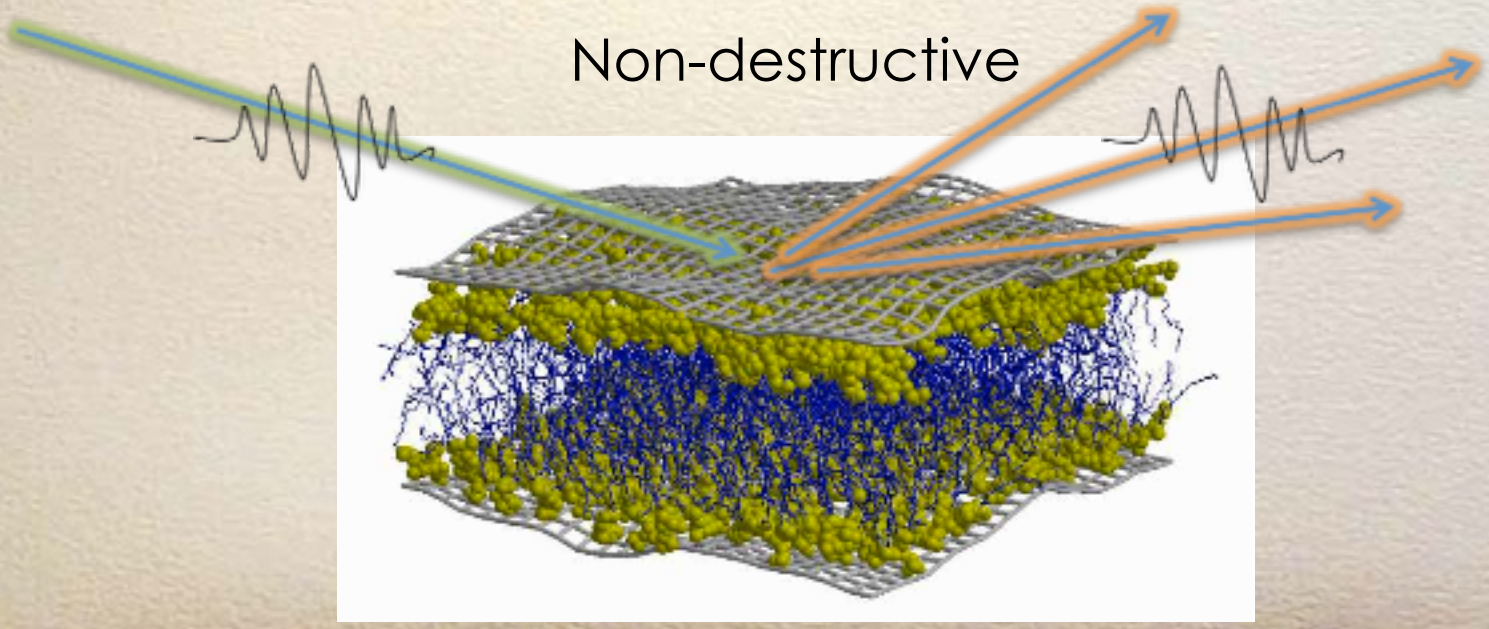
Probe relevant length (**Å to μm**) and time-scales (***ps* to *hr***)

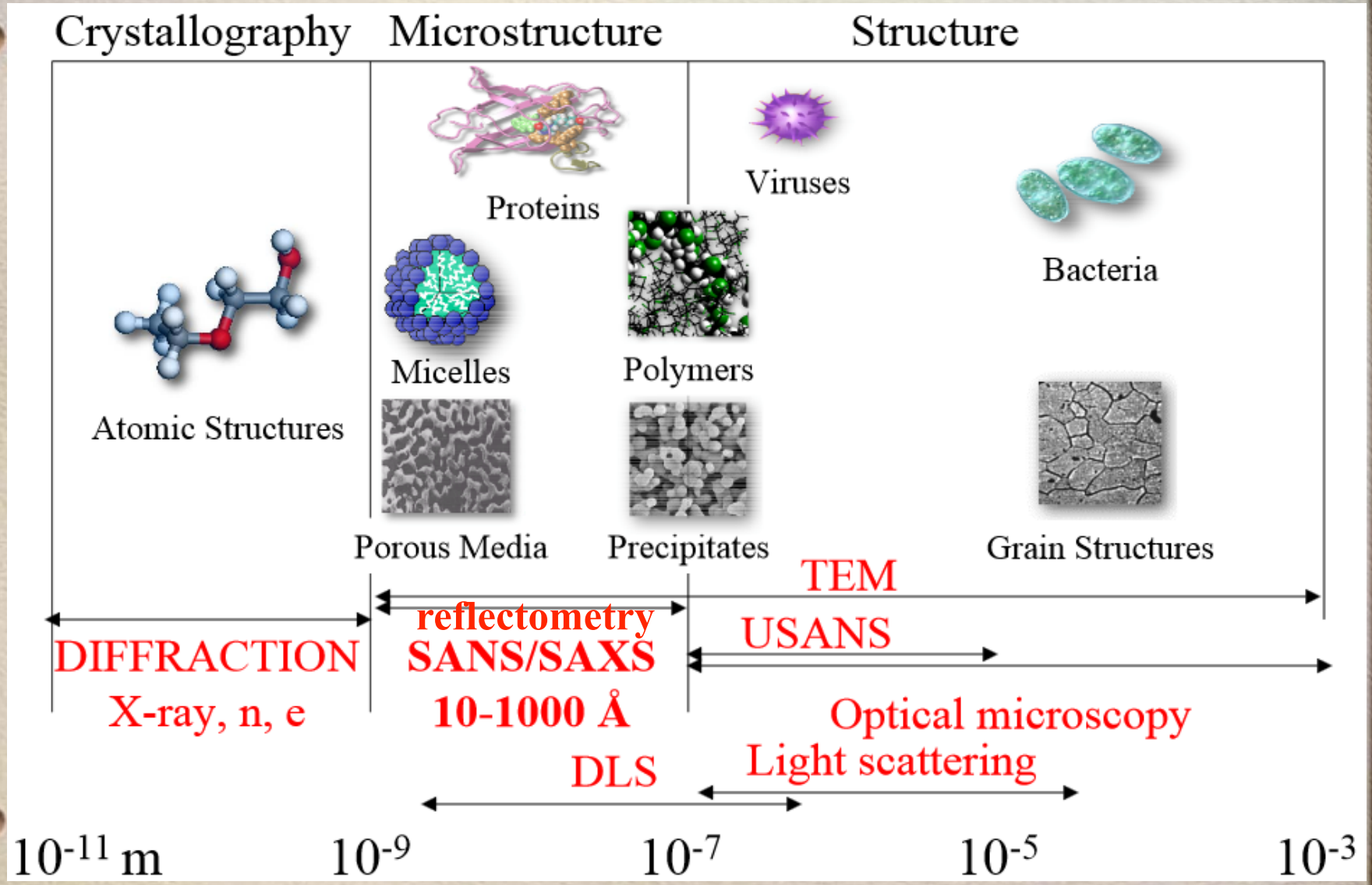
Sensitive to light elements

Buried systems and complex sample environment

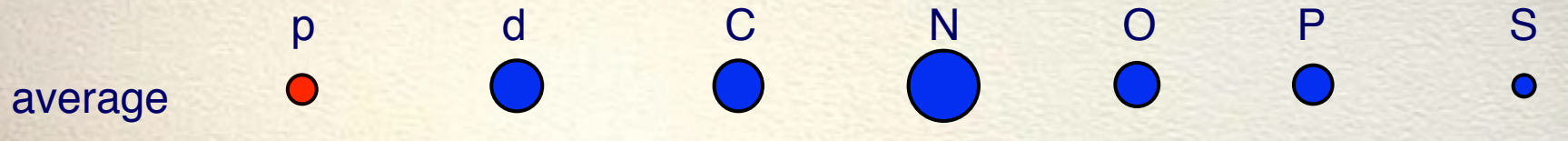
Possibility of **isotopic labelling**

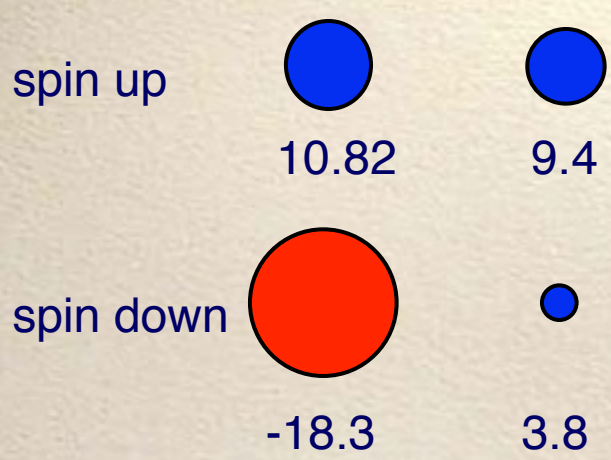
Non-destructive




Crystallography
Microstructure
Structure
Proteins
Viruses
Bacteria
Micelles
Polymers
Atomic Structures
Porous Media
Precipitates
Grain Structures
TEM
reflectometry
USANS
DIFFRACTION
SANS/SAXS
X-ray, n, e
10-1000 Å
Optical microscopy
DLS
Light scattering
10-11 m
10-9
10-7
10-5
10-3

# Coherent neutron scattering lengths [fm]




Spin-dependent scattering lengths

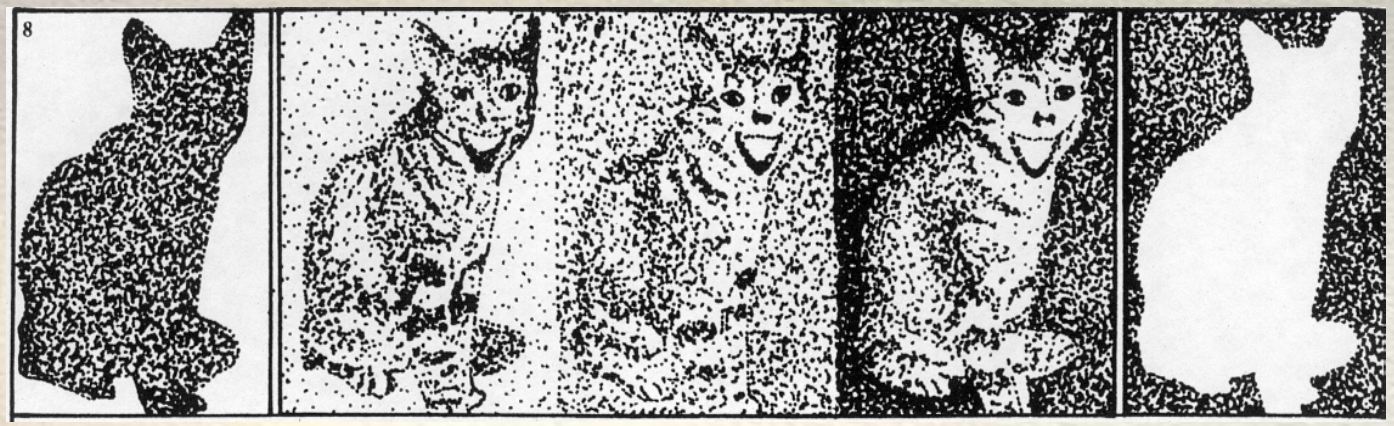
$$\rho_{H_2O} = \frac{2b_H + b_D}{V_{H_2O}} = \frac{(6.67 - 3.74 \cdot 2)\,fm}{30\,\text{Å}^3}$$

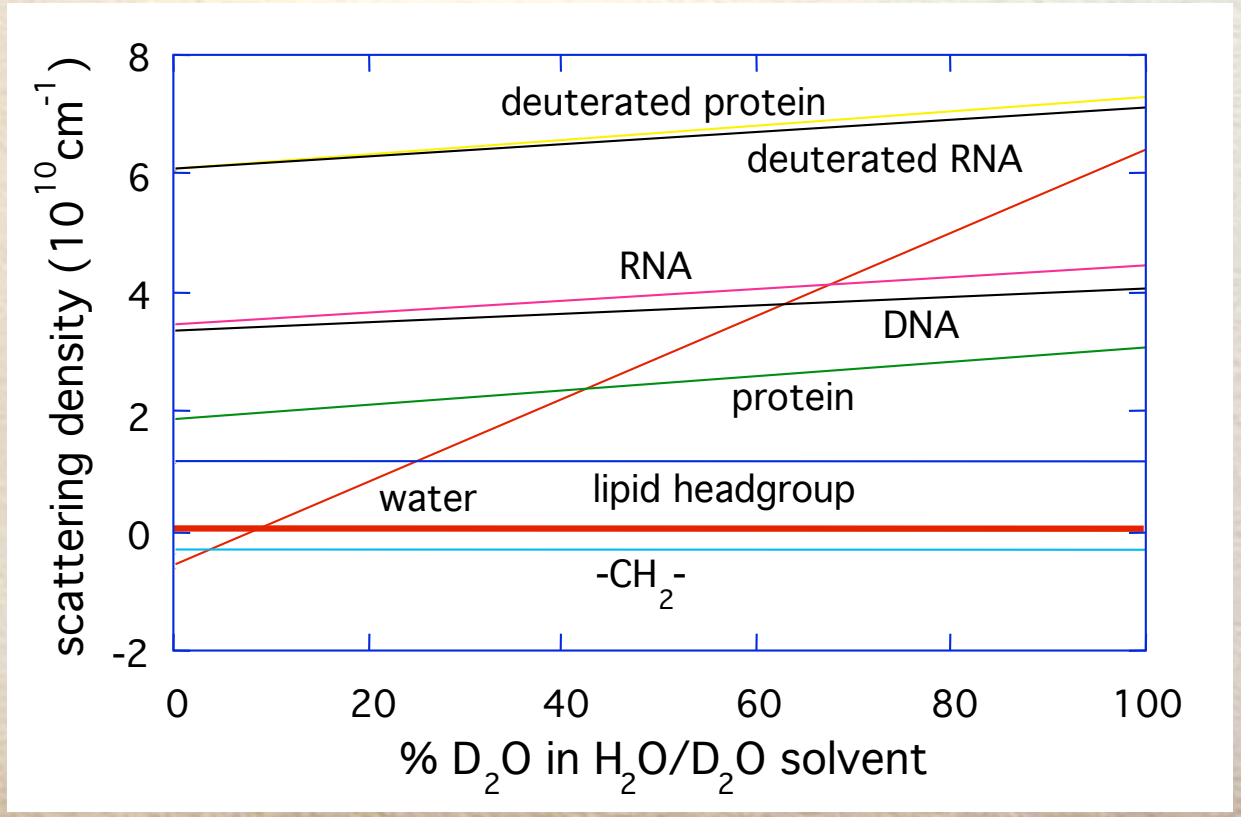
$$\rho_{H_2O} = -0.56 \cdot 10^{10}\,cm^{-2}$$

$$V_{H_2O} = \frac{M_{H_2O}\bar{v}_{H_2O}}{N_A}$$

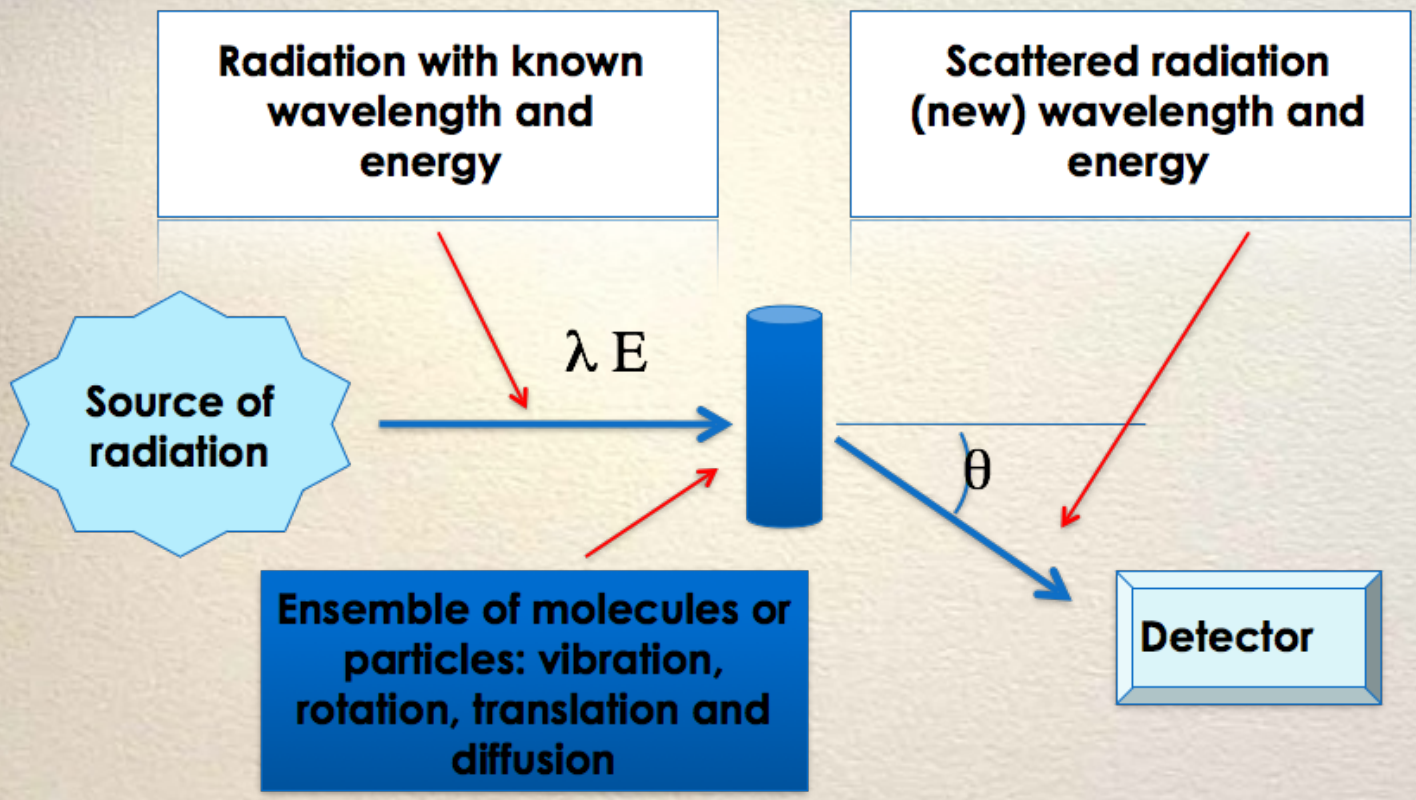
Calculation of the scattering length density

*neutrons deflected from hydrogen are 180° out of phase relative to those deflected by the other elements*


8
100 % $H_2O$
100 % $D_2O$


scattering density ($10^{10}$ $cm^{-1}$)
8
6
4
2
0
-2
deuterated protein
deuterated RNA
RNA
DNA
protein
water
lipid headgroup
-$CH_2$-
0
20
40
60
80
100
% $D_2O$ in $H_2O$/$D_2O$ solvent

# SCATTERING




**elastic or static**

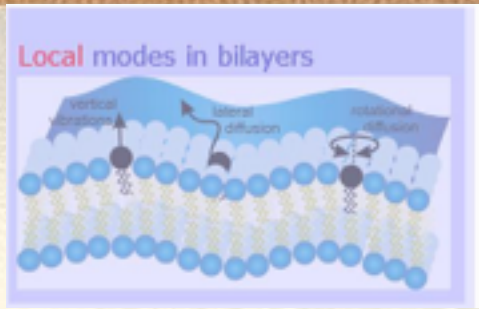
Conformation, structure, size

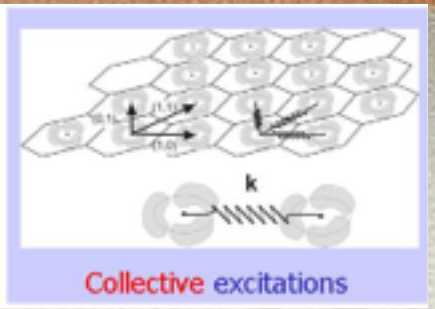
**quasielastic, inelastic or dynamic**

Local and global dynamics, diffusion, vibrations, rotation

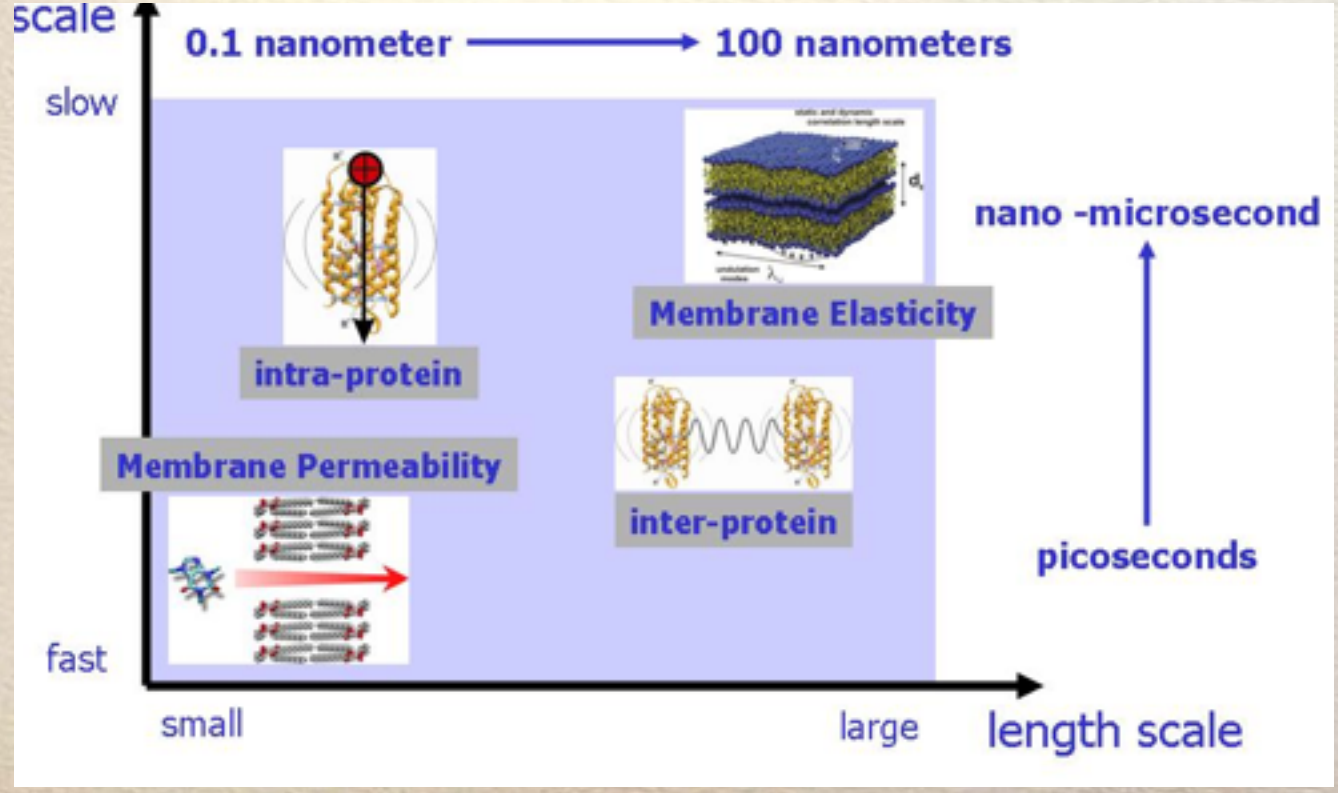
http://www.rheinstaedter.de/maikel/

*time scales from about*
*0.1 ps to almost 1 μs*



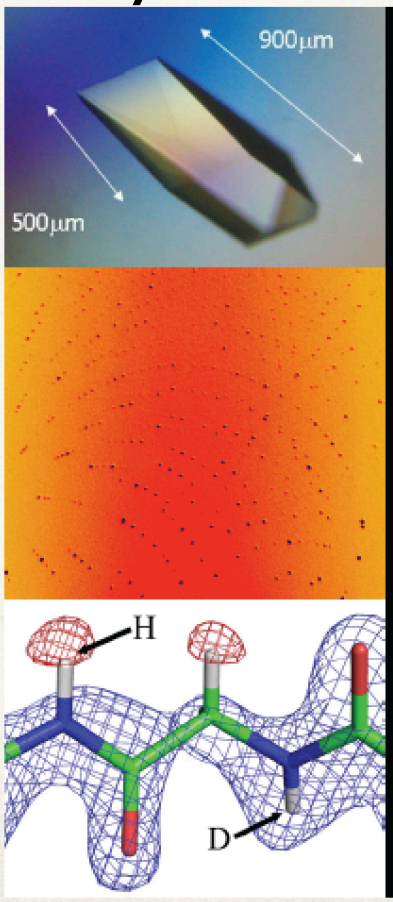

# Inelastic, Backscattering, Spin-Echo



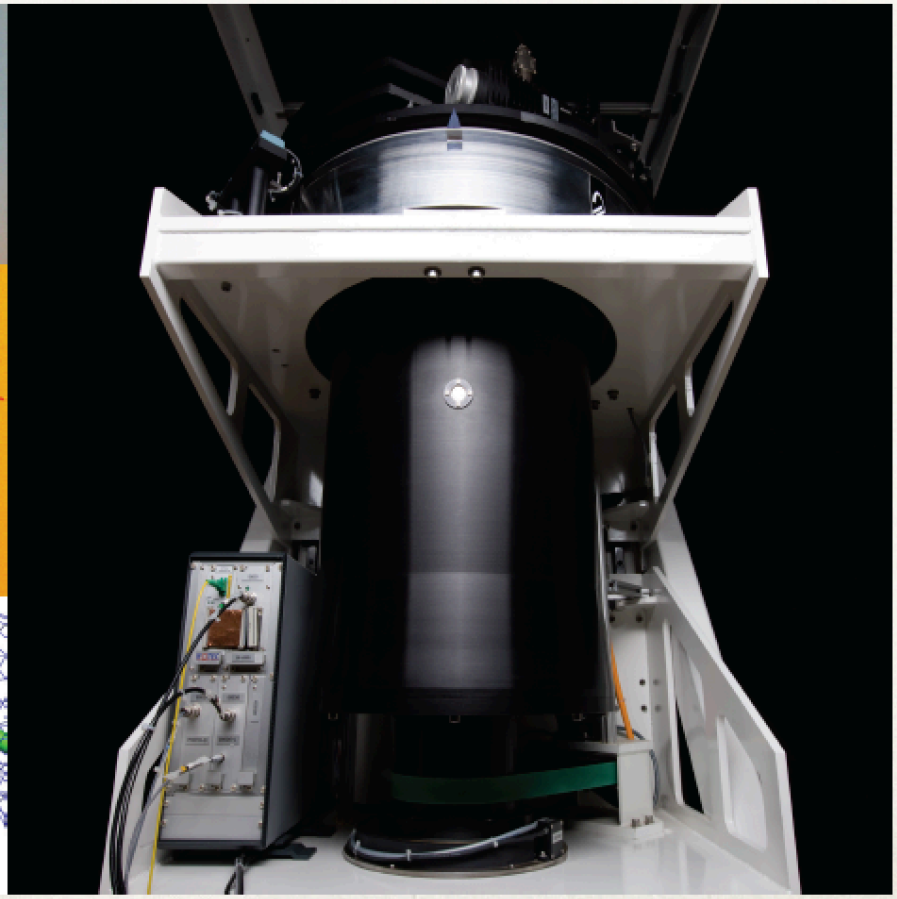
# Protein Crystallography - neutrons see hydrogens

***crystal deuteriation allowes smaller samples and higher resolution data***

**down to 2.0 Å resolution**
**times ~ days**

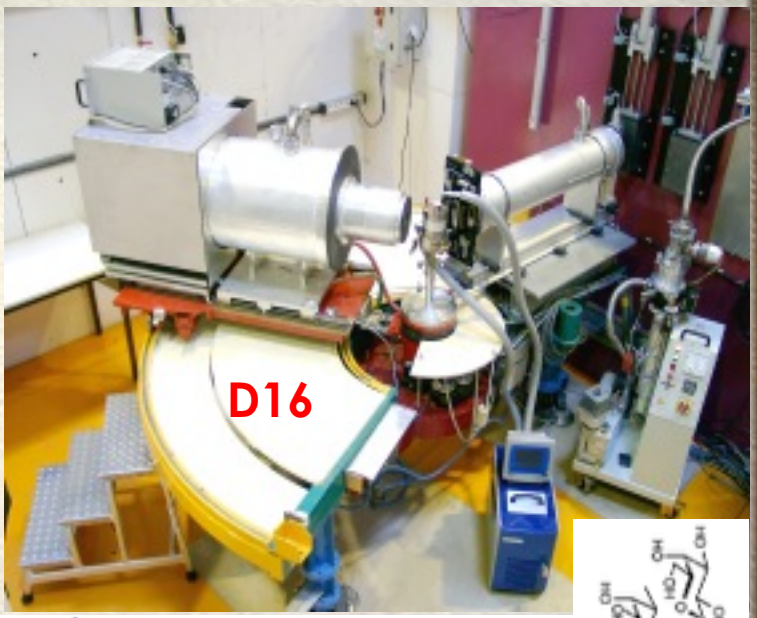


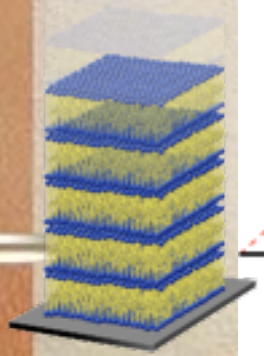

LADI-III

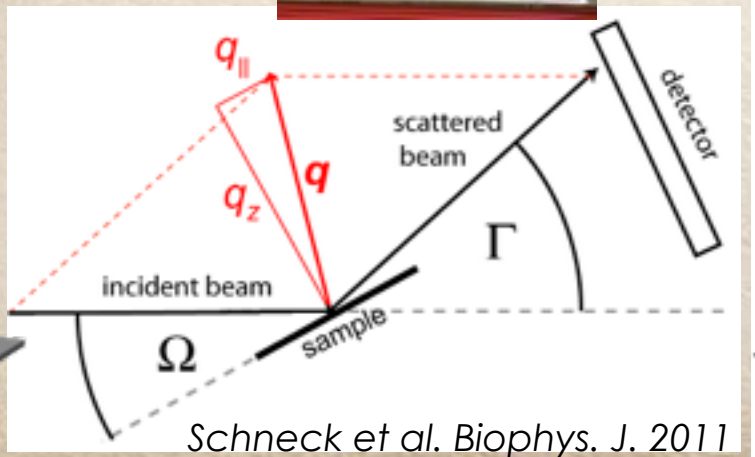


# Diffraction

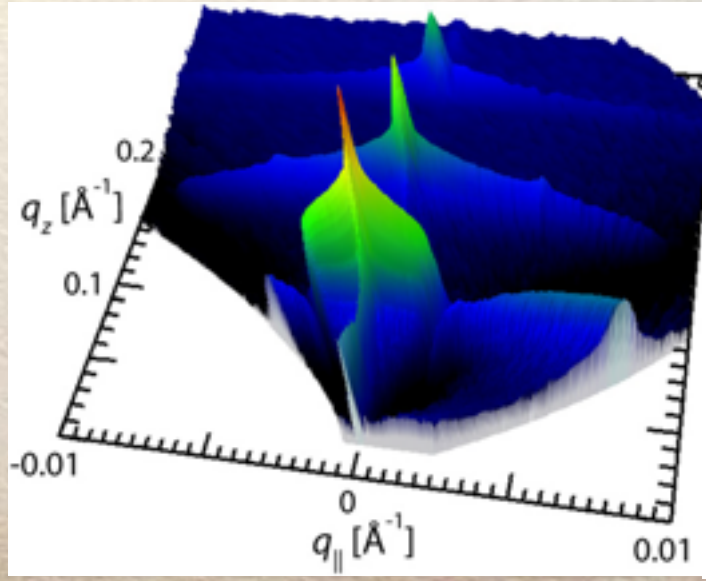
**2-D info ~Å to ~10nm**
**times ~ min**

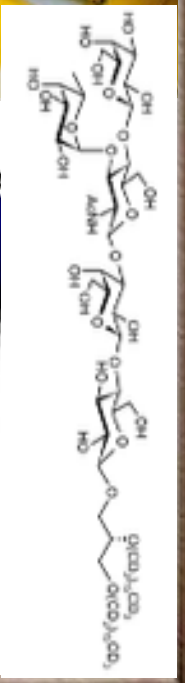




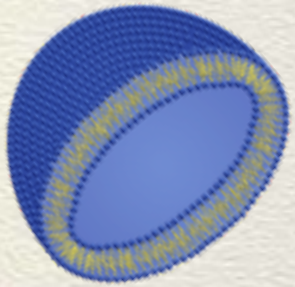

Schneck et al. Biophys. J. 2011

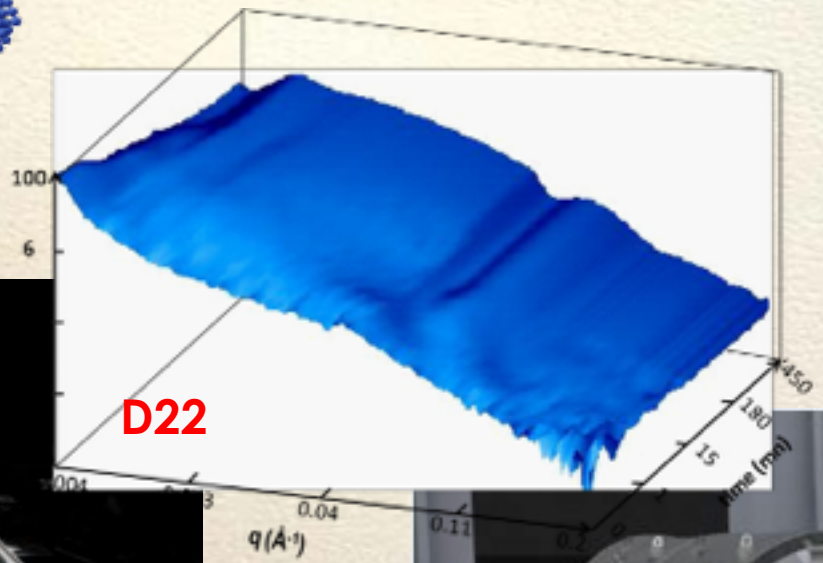


# Small Angle Neutron Scattering

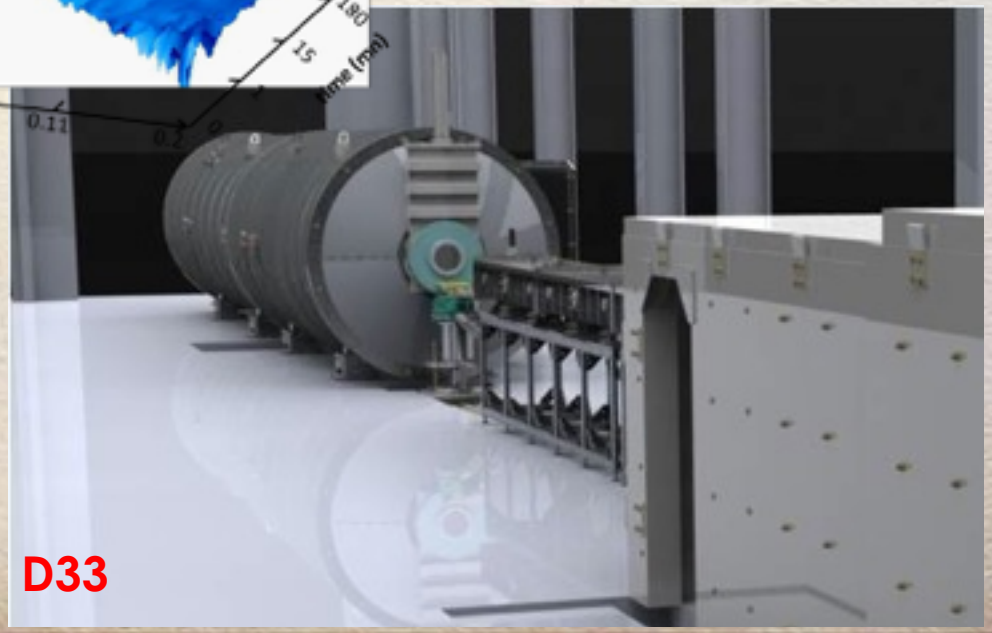
**3-D info ~nm to μm**
**times < min**





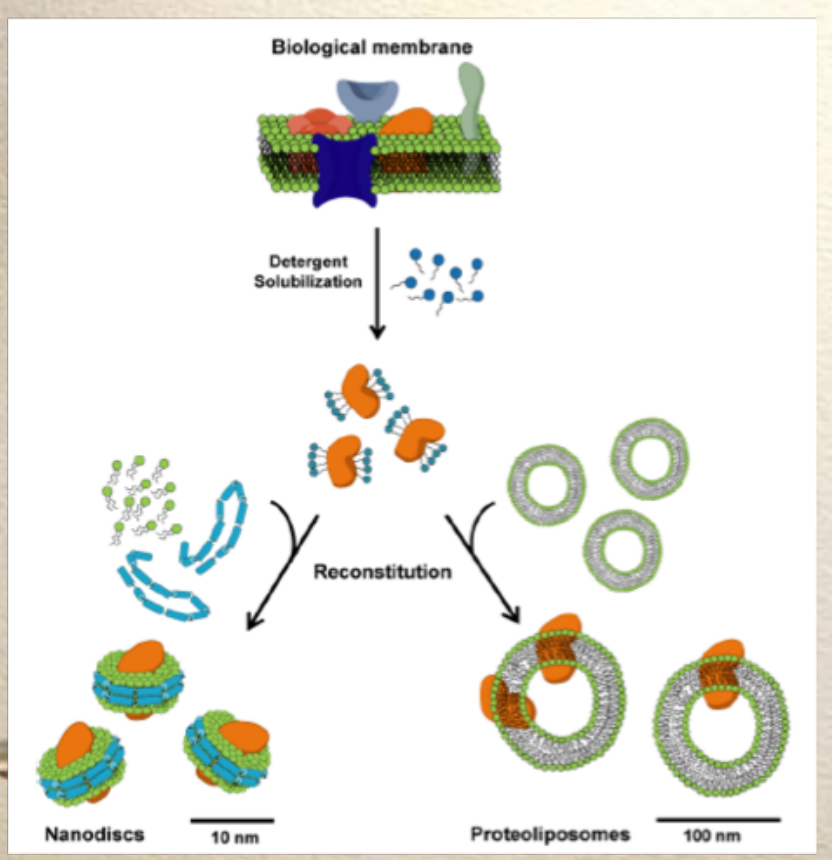

D22

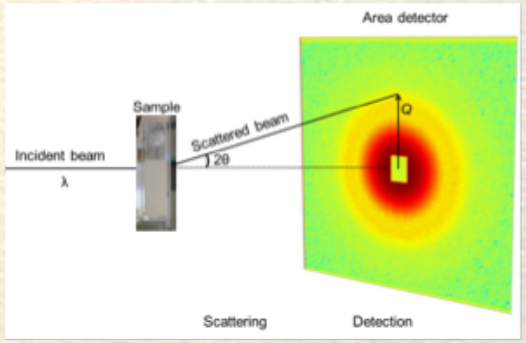
D11



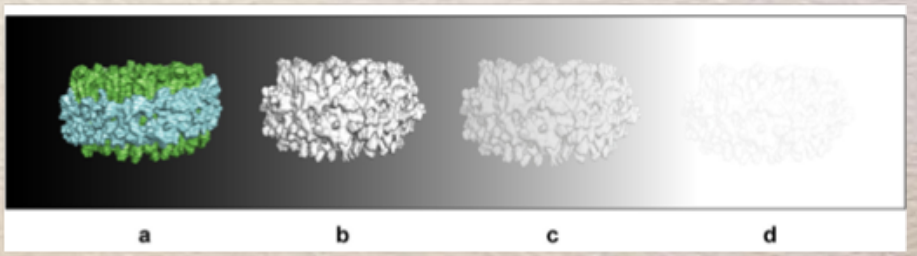
D33

# Membrane proteins structural characterisation by using nanodiscs



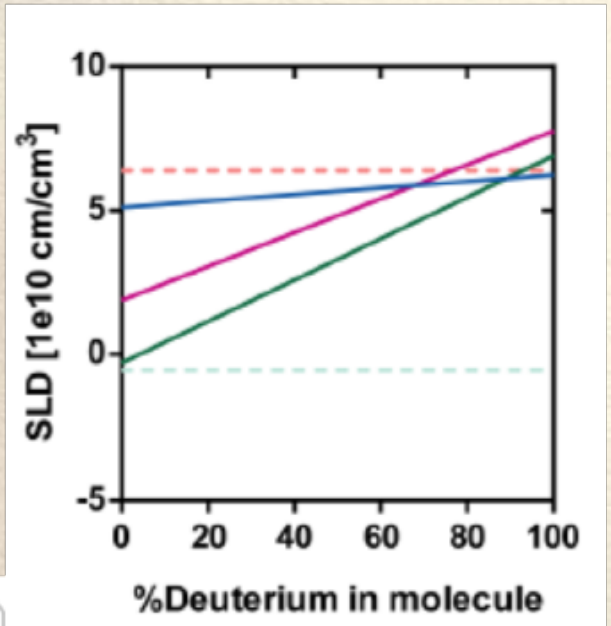



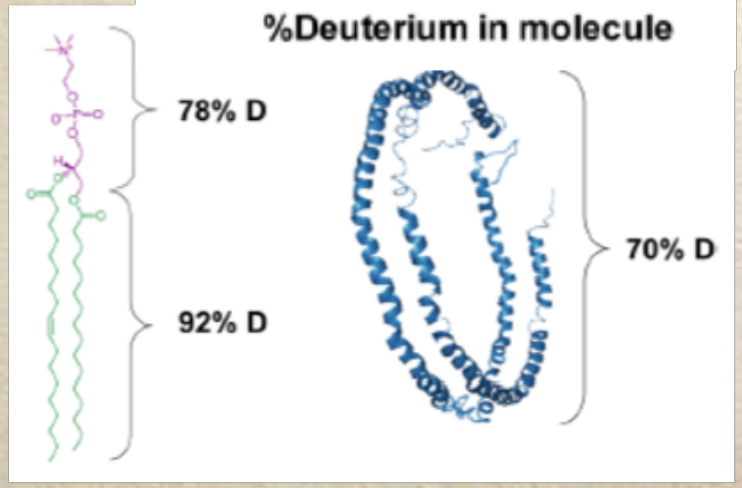

with **contrast variation** in SANS it is possible to highlight the interesting parts of the system

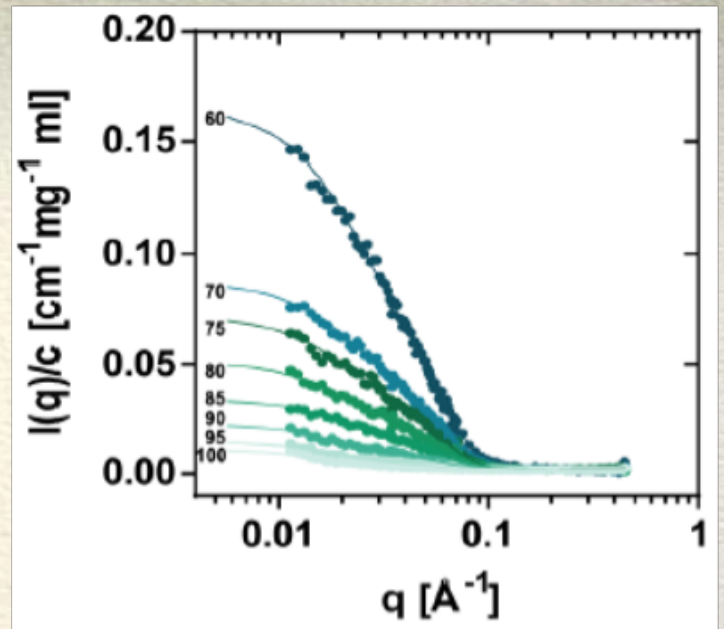


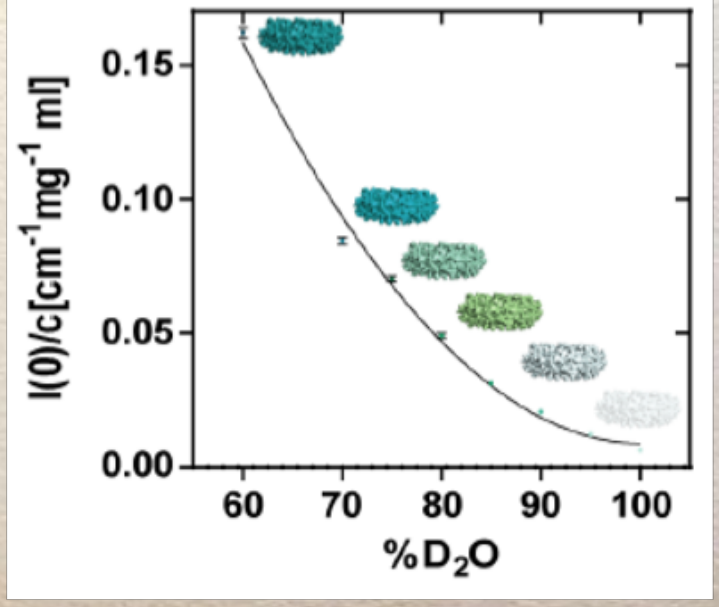

*Maric et al. Acta Cryst. 2013*

# Contrast Variation



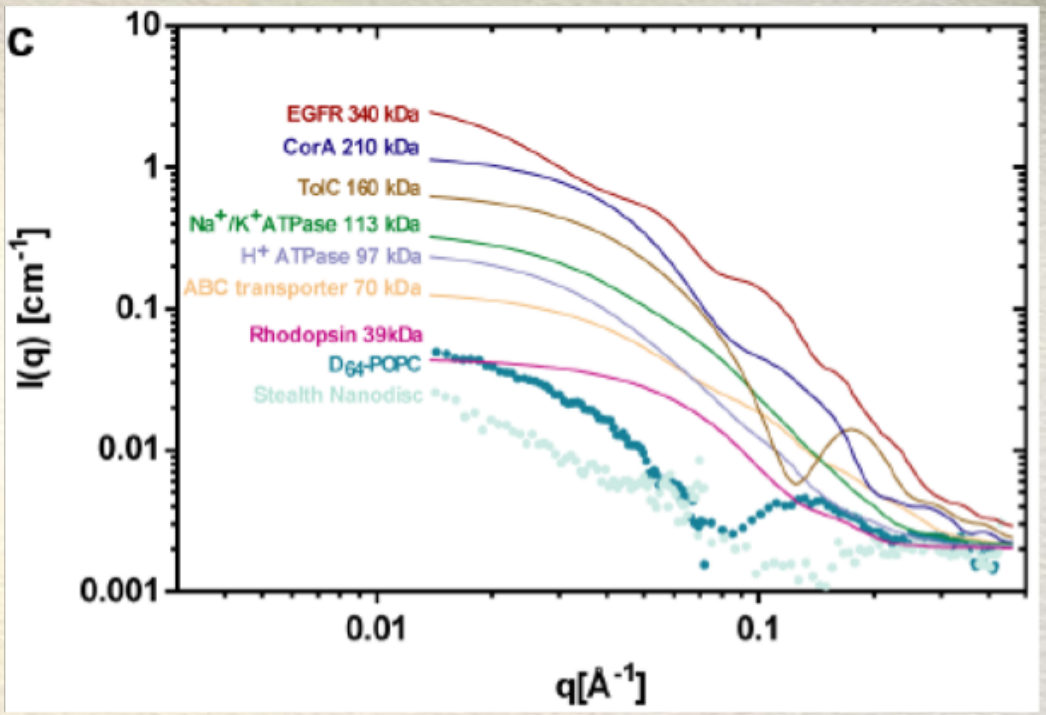



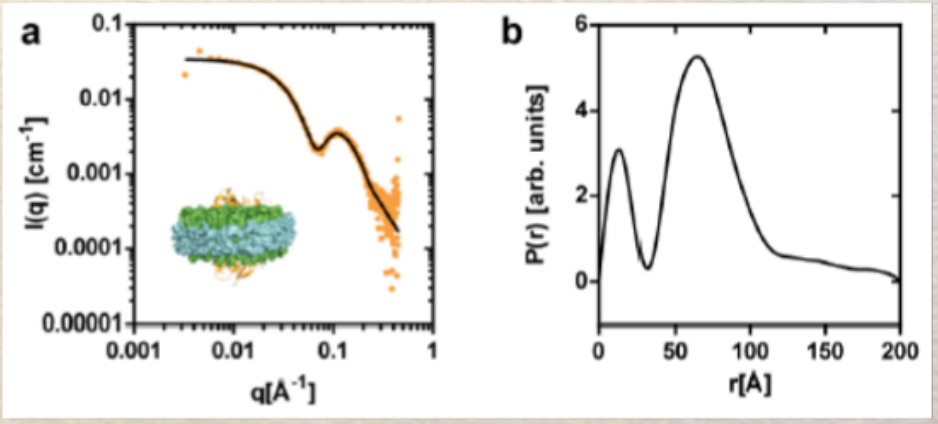




*Courtesy Selma Maric*

# SANS signal expected from membrane proteins

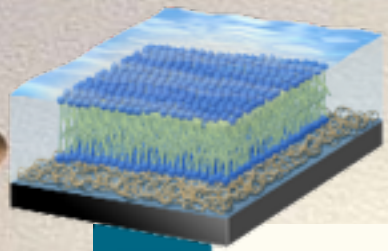
- SAXS from Sensoryrhodopsin-II

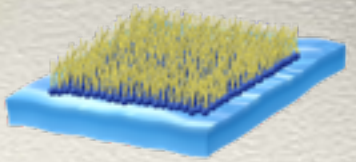


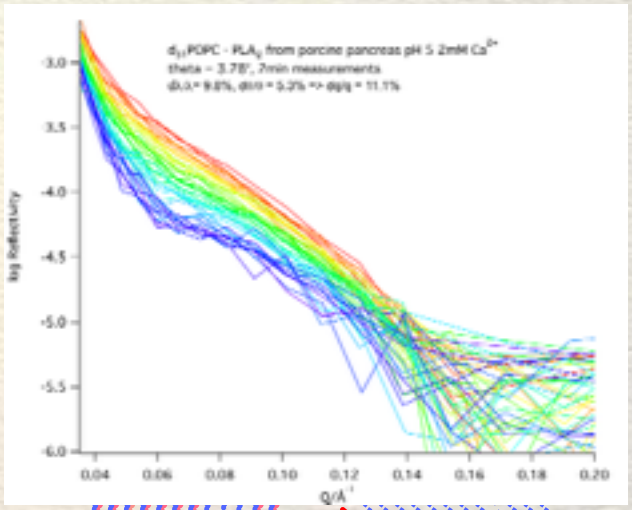

*Courtesy Selma Maric*

# Reflectometry

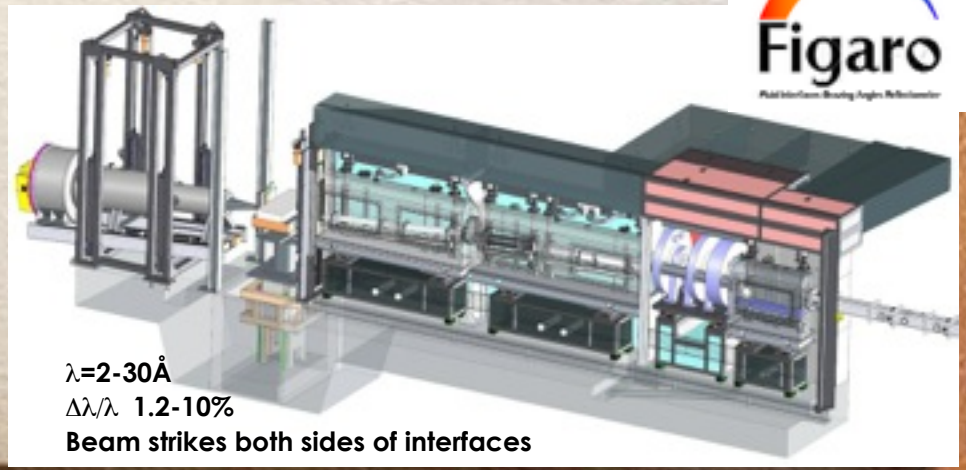




**1-D info ~Å to ~100nm**
**times < min**



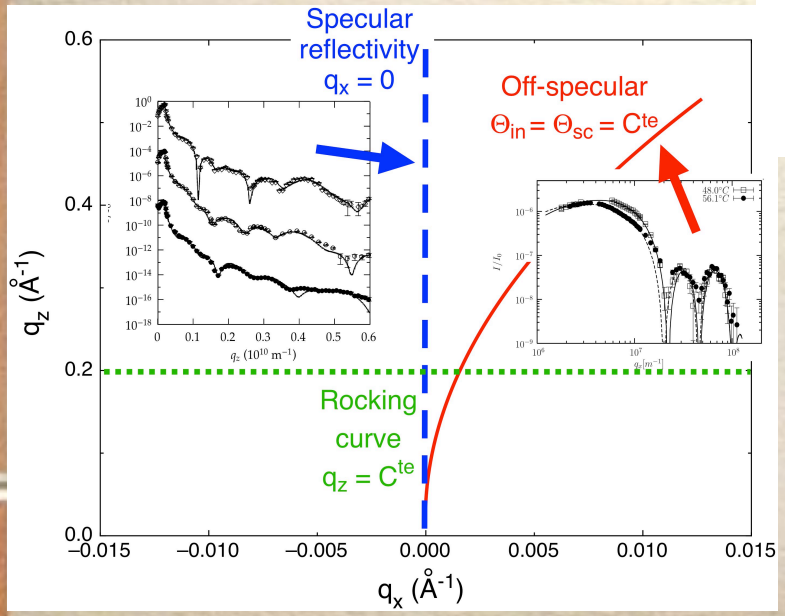


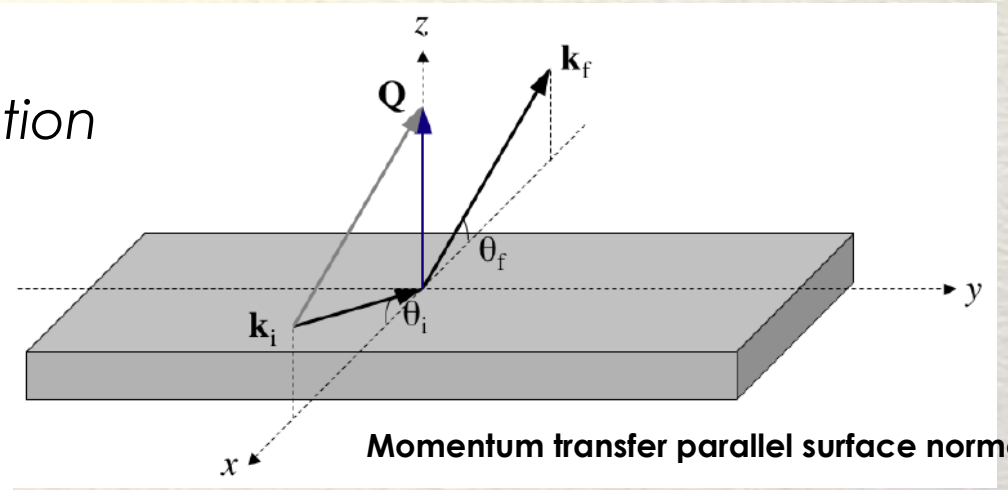

λ=2-30Å
Δλ/λ 1.2-10%
**Beam strikes both sides of interfaces**

# Reflectivity measurements:

## *Specular $\theta_i=\theta_f$*

- *Thickness of layers at interfaces*
- *Roughness/interdiffusion*
- *Composition in the direction normal to the interface*



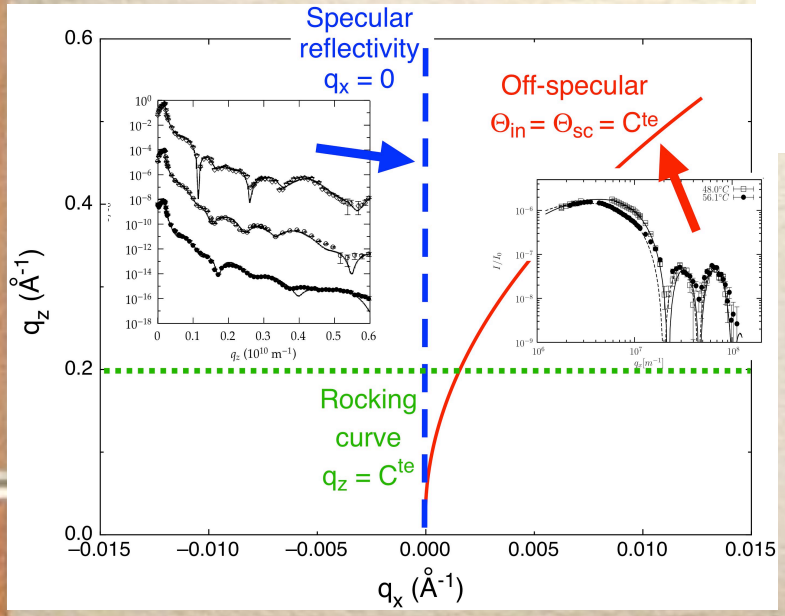



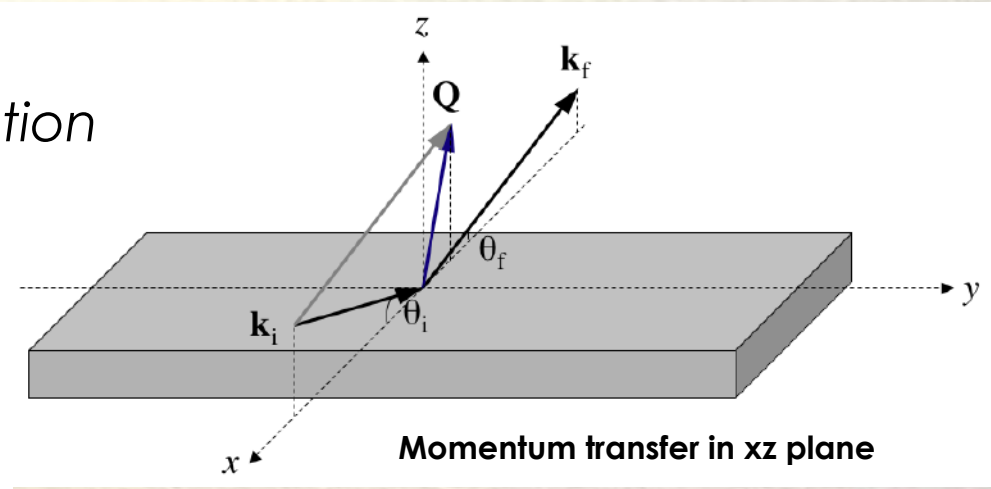

**Momentum transfer parallel surface normal**

# Reflectivity measurements:

## *Specular $\theta_i=\theta_f$*

- *Thickness of layers at interfaces*
- *Roughness/interdiffusion*
- *Composition in the direction normal to the interface*

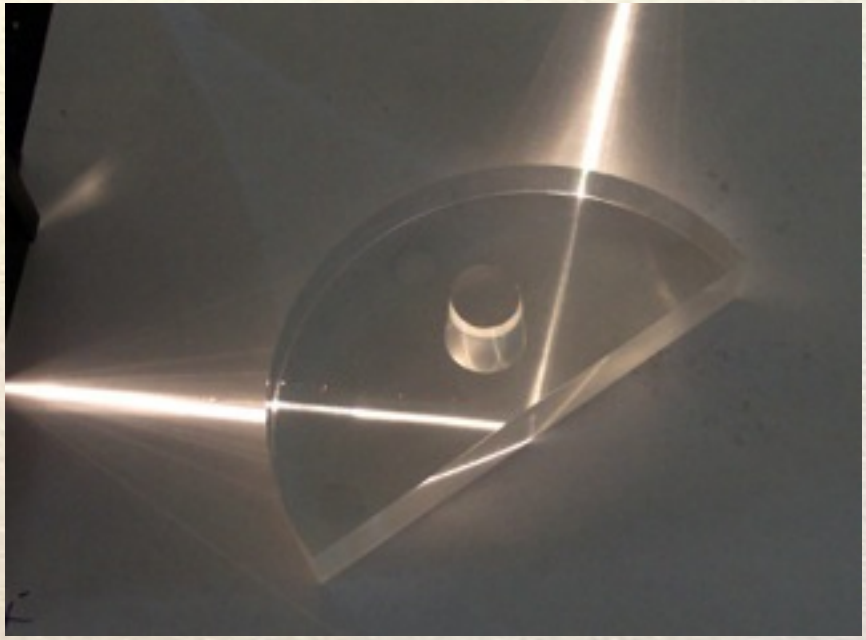



**Momentum transfer in xz plane**

*In-plane features (height fluctuations, domains, holes ...) can be probed by off-specular measurements: for thin films synchrotron radiation is more suitable*

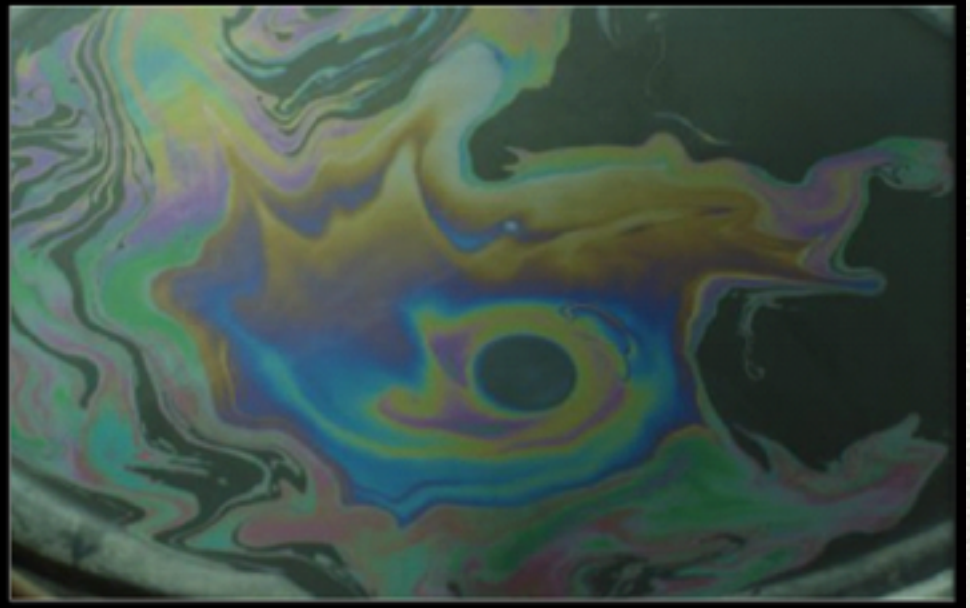
**For both kinds of radiation the refractive index is a function of the scattering length density and wavelength.**

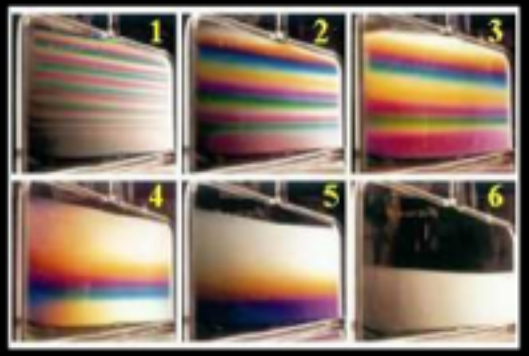


**As with light, total reflection may occur when neutrons pass from a medium of higher refractive index to one of lower refractive index.**

**Two well known examples of light interference from thin films**

Oxidized oil film on water





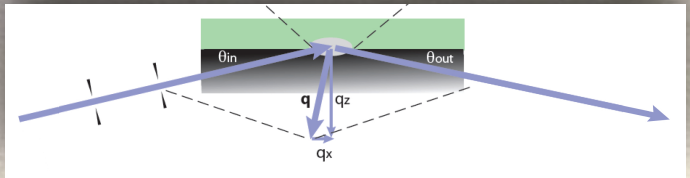

Drainage of soap film. Note the absence of reflection in the top half of the film in 6.

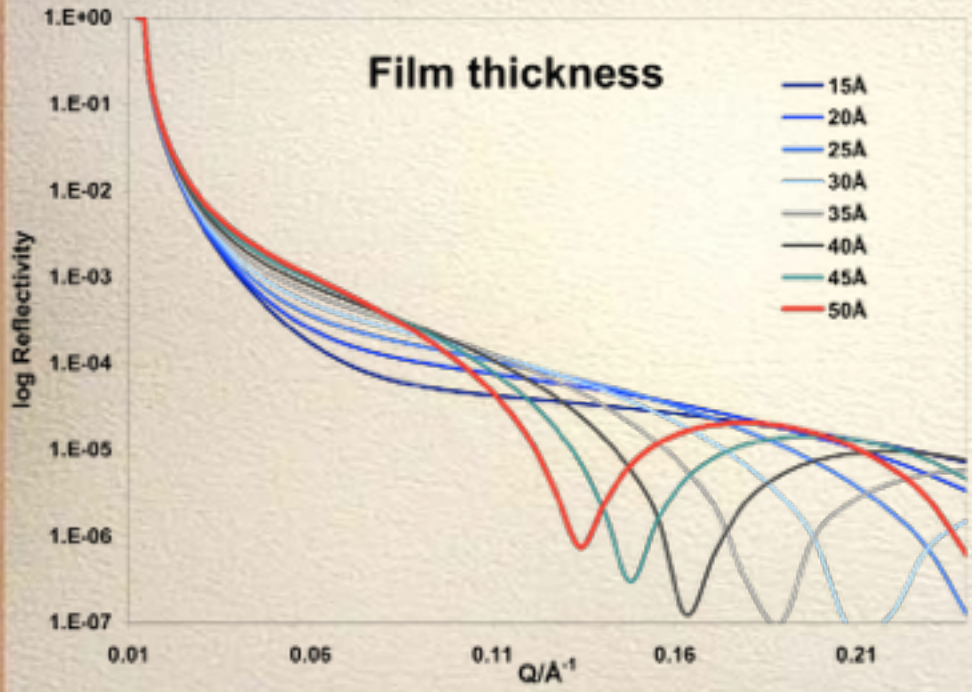
# Optical Demonstrations

Reflection from a thin film

Newton's Rings

Courtesy of R. K. Thomas
http://rkt.chem.ox.ac.uk/techniques/nrmain.html

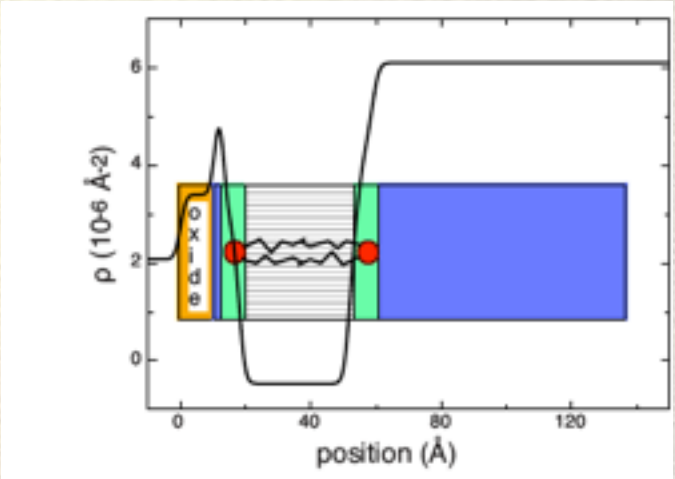



$$R(\vartheta,\lambda) = \frac{I_{out}(\vartheta,\lambda)}{I_{in}(\lambda)}$$

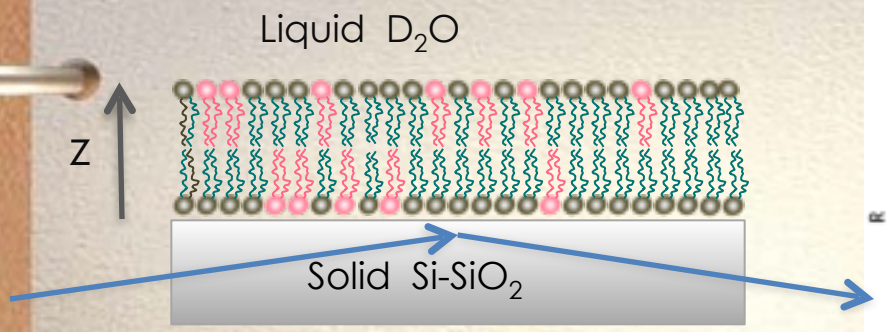
$$q = \frac{4\pi}{\lambda}\sin\theta$$

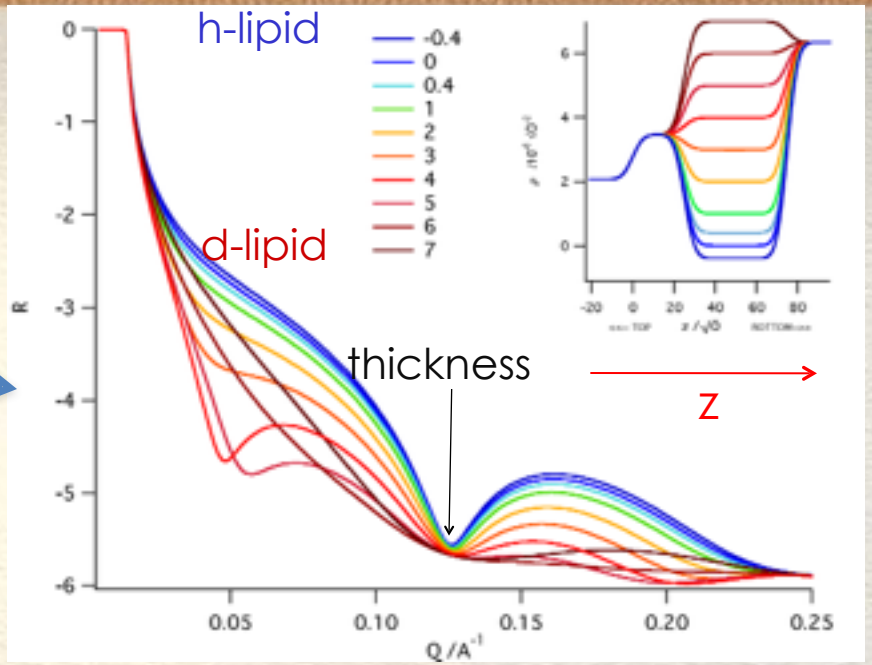
## Scattering length density profile extracted from data analysis




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

Scattering length density – related to layer composition




**Reflected intensity depends on structure and composition at the interface**

**Born Approximation**

**$q >> q_c$**

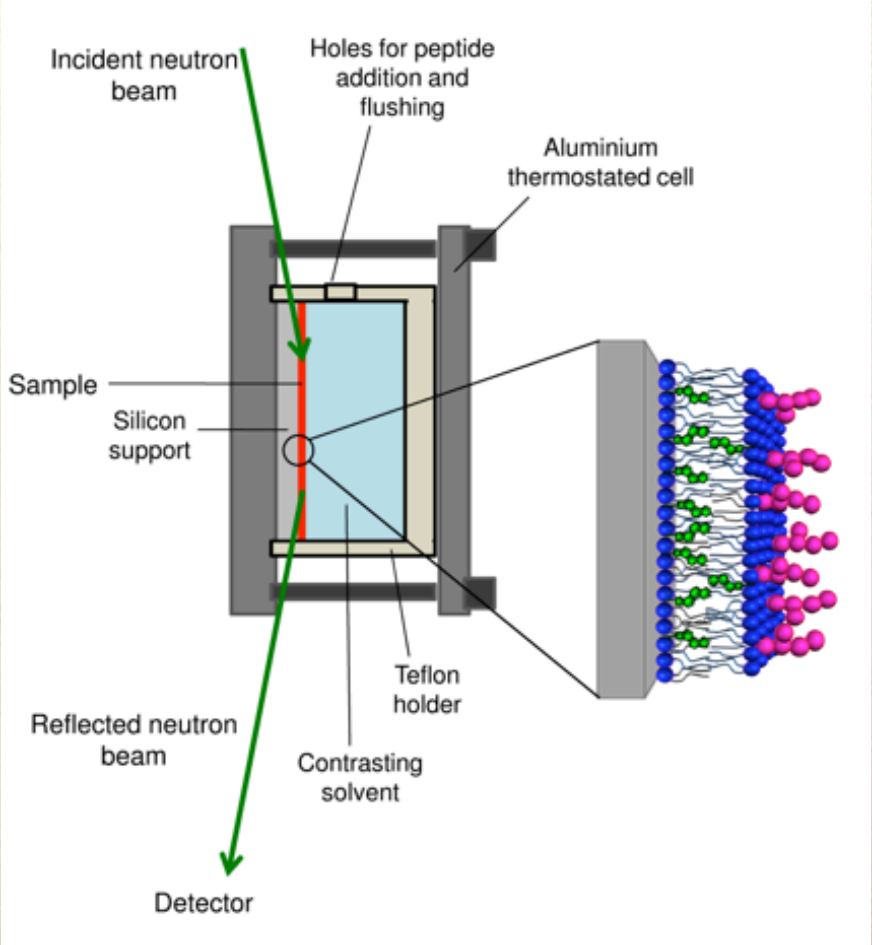
$$q_c = \sqrt{16\pi N_b}$$

*Ignored double scattering processes because these are usually very weak*

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

$$N_b'(q) = \int_{-\infty}^{+\infty} \exp(iqz)\frac{dN_b}{dz}dz$$

# Schema of sample holder



# Data modelling

Thin Film – Neutron Reflection

Contrast

Courtesy of R. K. Thomas
http://rkt.chem.ox.ac.uk/techniques/nrmain.html

SANS

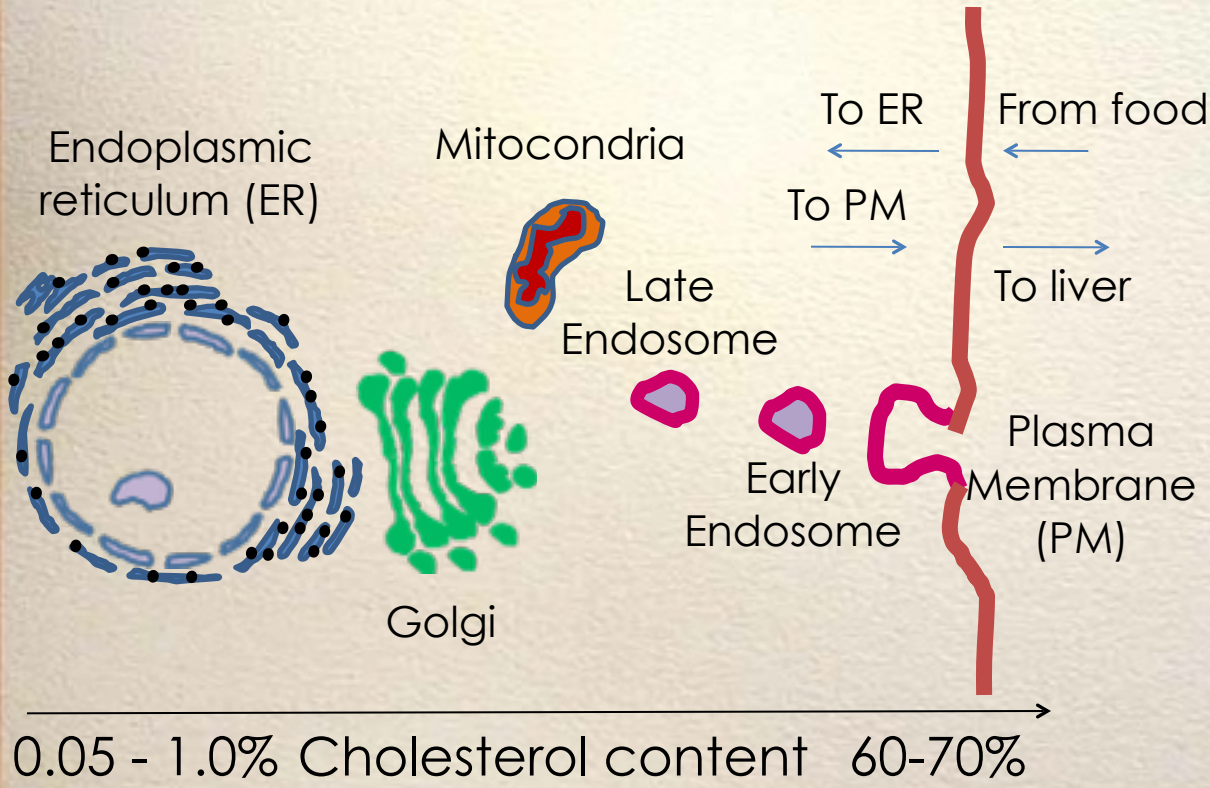
# Understanding Intra-cellular Cholesterol Transport

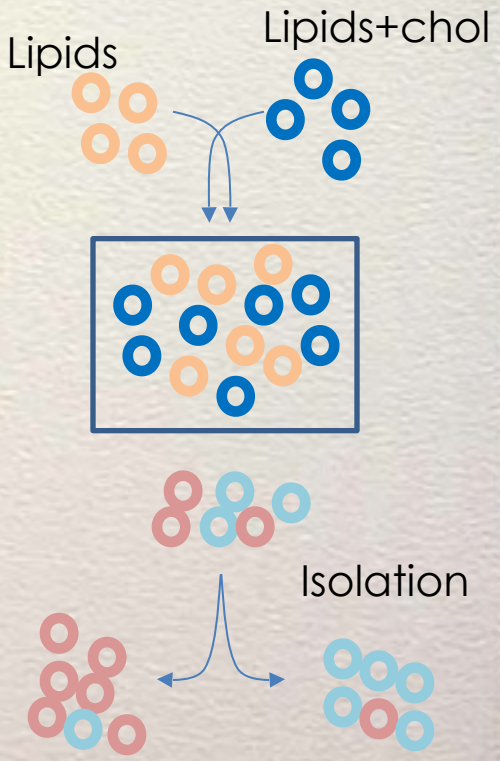
*Garg et al. 2011*

Courtesy Lionel Porcar

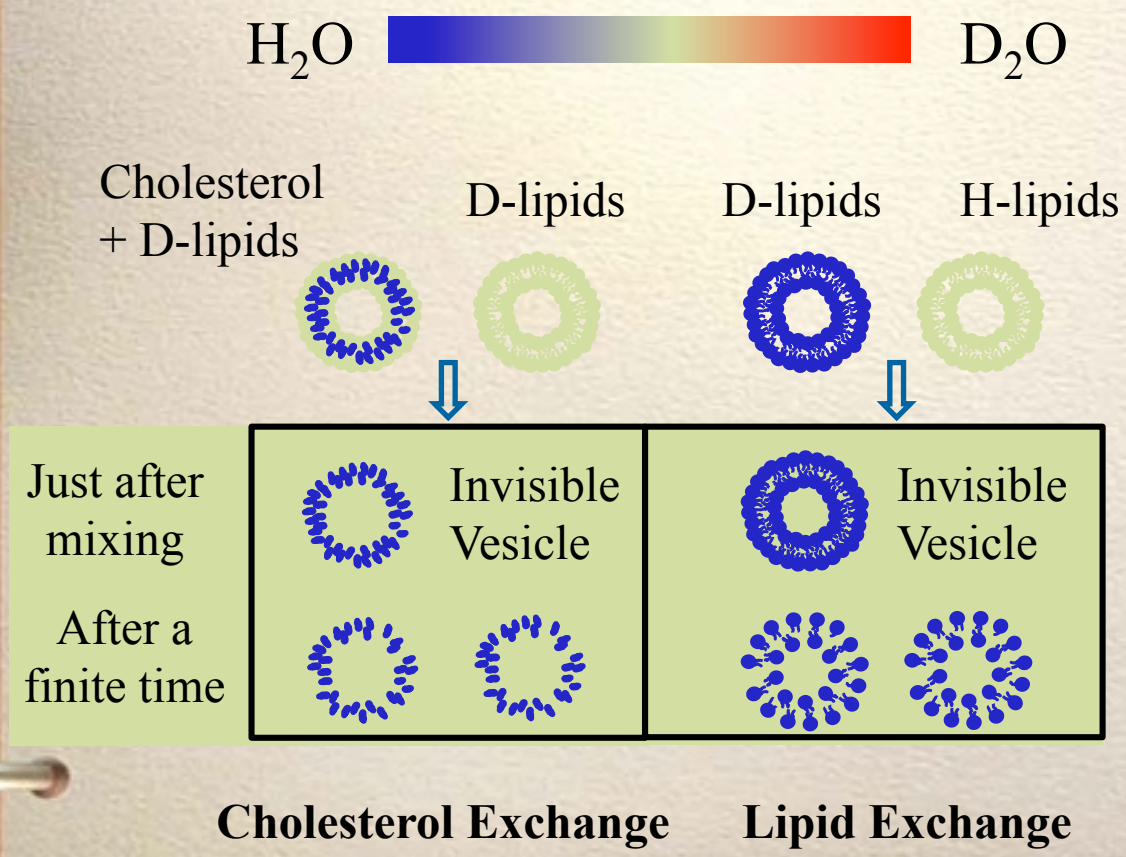
# In-vivo

# In-vitro



# Aim: validate results from in-situ Transfer by Time-Resolved SANS

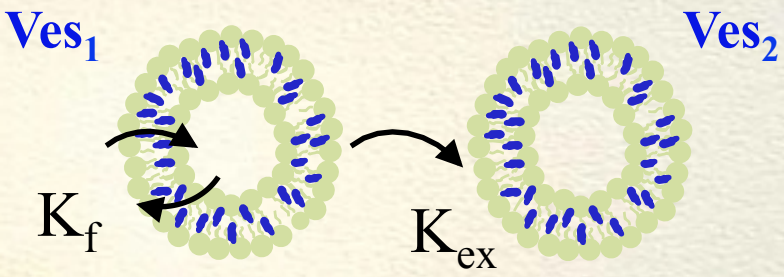



1) Does not require vesicles isolation (in situ technique)
2) No need of fluorescent or tag cholesterol
3) Access early stage of transfer
4) Accurate control on lipid membrane composition and structure
5) Can be applied to anything (just require deuterated materials)

*Courtesy Lionel Porcar, P. Butler, U. Perez-Salas*

# Time-Resolved SANS approach

(Garg et al., NIST, Biophys. J., 2011)




## Kinetics

$$\frac{dC_{in1}}{dt} = -K_f C_{in1} + K_f C_{out1}$$

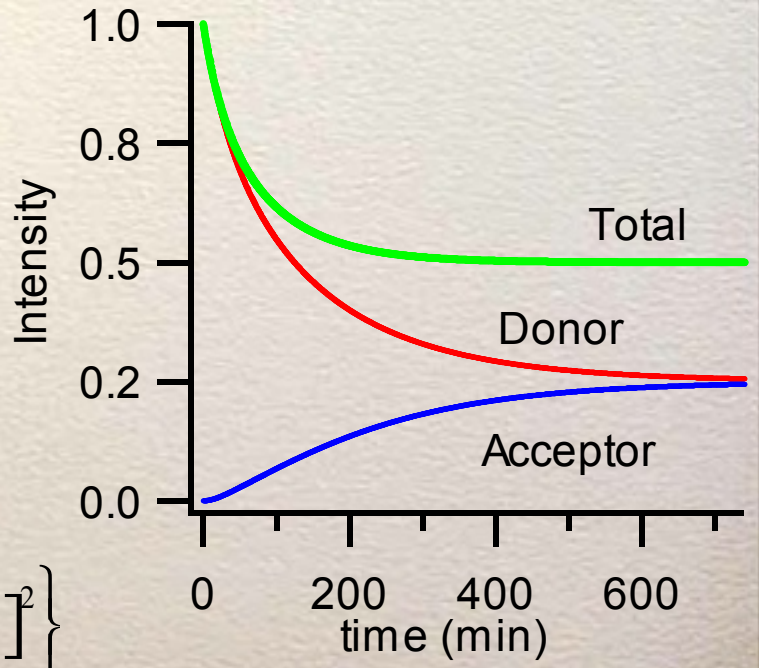
$$\frac{dC_{out1}}{dt} = K_f C_{in1} - K_f C_{out1} - K_{ex} C_{out1} + K_{ex} C_{out2}$$

## Scattering

$$I \sim \phi_{Ves1} V_1 \left(SLD_{ves1} - SLD_{solvent}\right)^2 FF$$

$$+\phi_{Ves2} V_2 \left(SLD_{ves2} - SLD_{solvent}\right)^2 FF$$

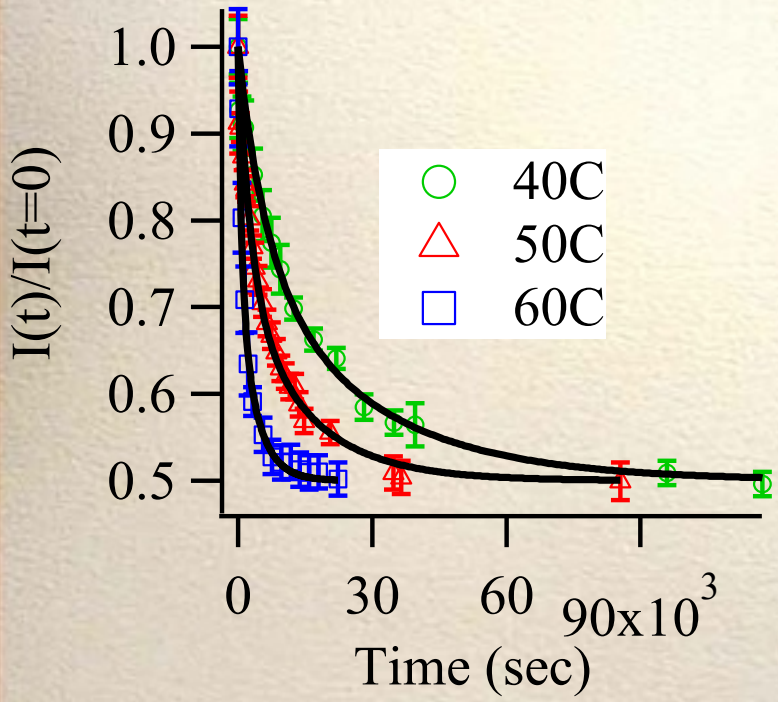
$$I \sim \frac{\phi_{Ves}}{2}\left\{\left(\left(\phi_{chol1}^{vol}\right)^2 + \left(\phi_{chol2}^{vol}\right)^2\right)\left[SLD_{Chol} - SLD_{solvent}\right]^2\right\}$$

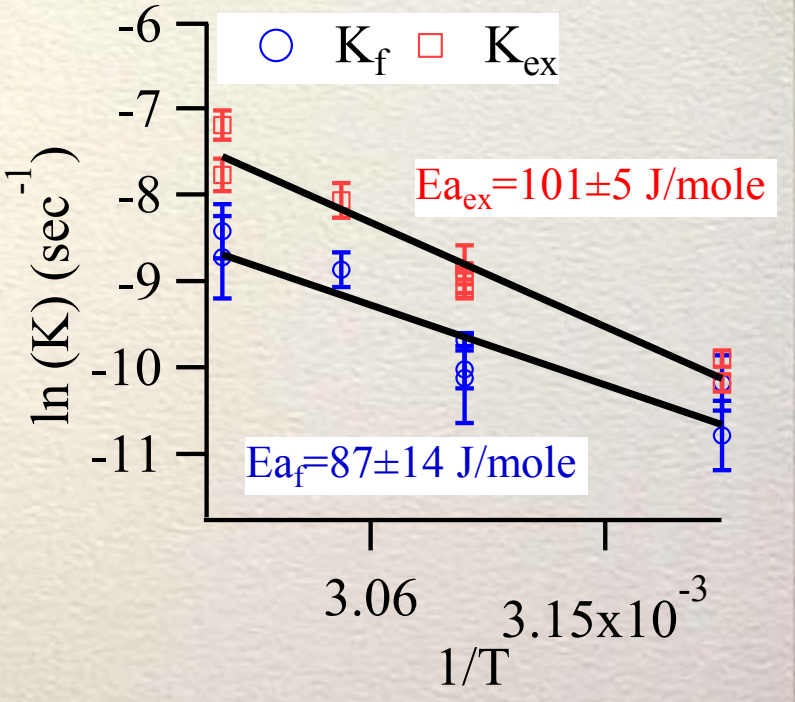



*Courtesy Lionel Porcar, P. Butler, U. Perez-Salas*

*Courtesy Lionel Porcar*

# Cholesterol's transfer in POPC vesicles



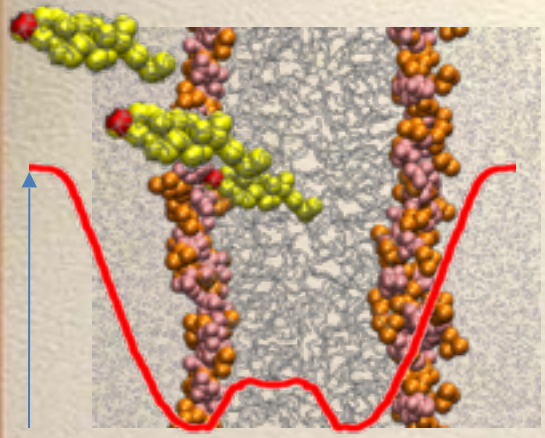

**Total cholesterol exchange!!**

**Half life for exchange: ~100min**
**Half life for flipping: ~250min (surprisingly slow)**

*Courtesy Lionel Porcar*

# Comparison with MD simulations and literature

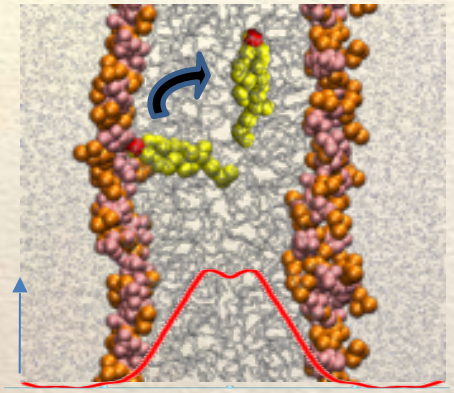
**Exchange**



distance

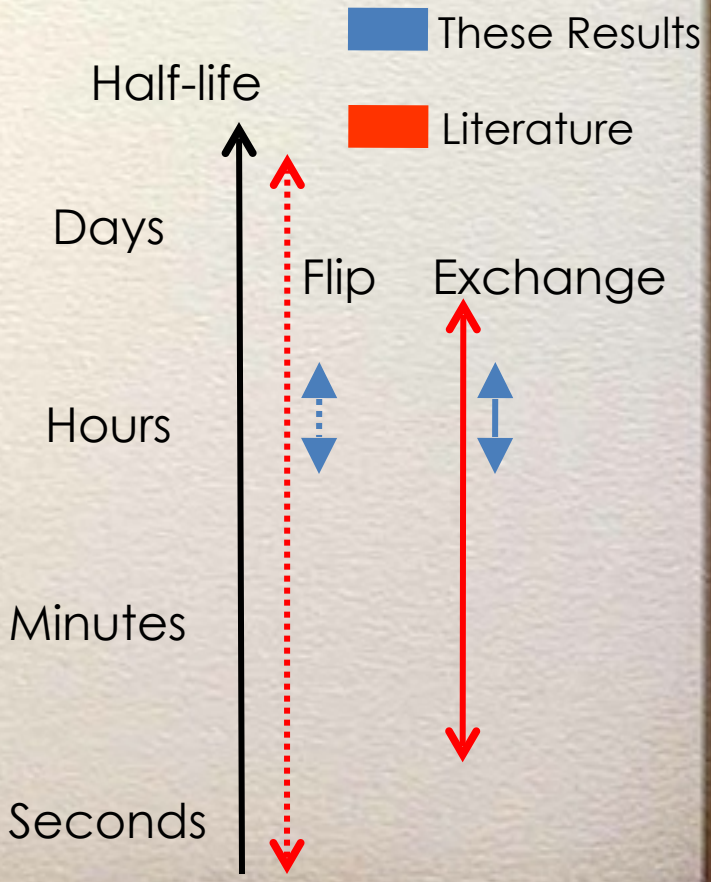
$\Delta G^*_{exch} \approx 80\ KJ\ mol^{-1}$

**Flipping**



angle

$\Delta G^*_{flip} \approx 25\ KJ\ mol^{-1}$

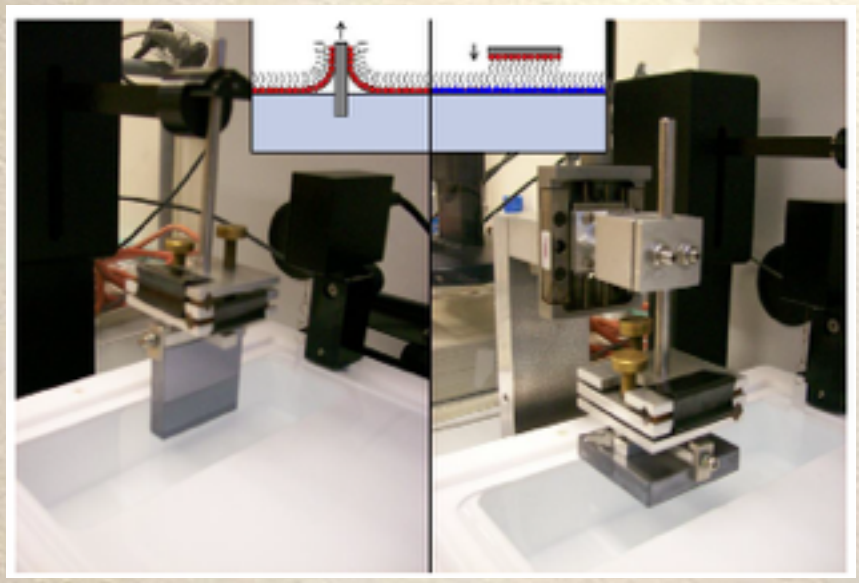


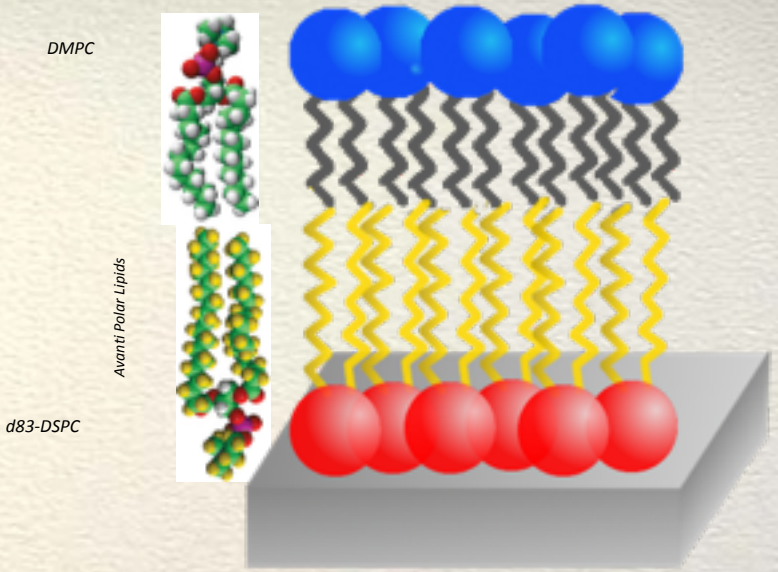
**Asymmetric deposition**

**12°C**

**40 mN/m lateral pressure**

- **d83-DSPC by LB (inner)**
- **DMPC by LS (outer)**



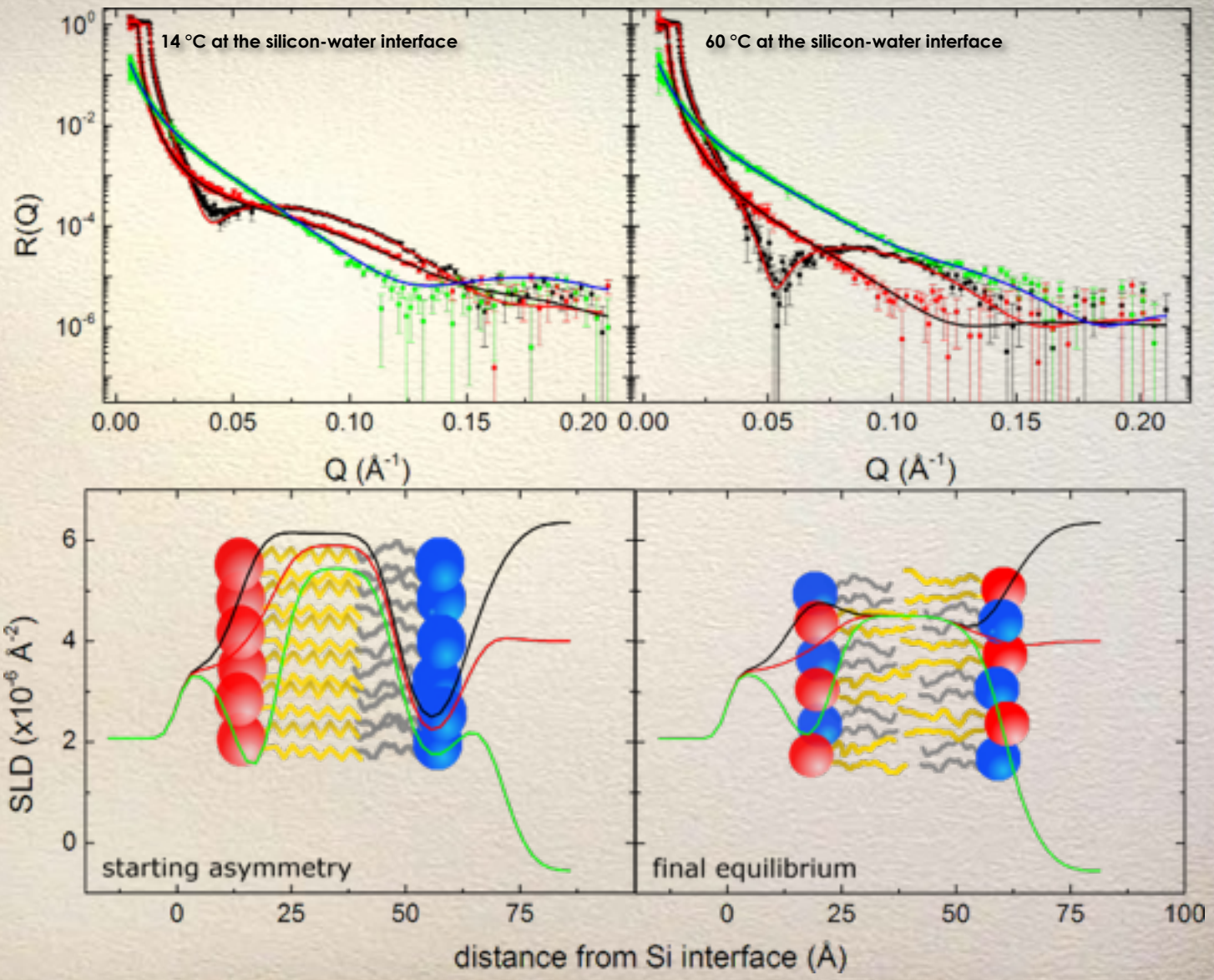



Thermal cycle for NR measurements

14°C →30°C→60°C→30°C → 14°C

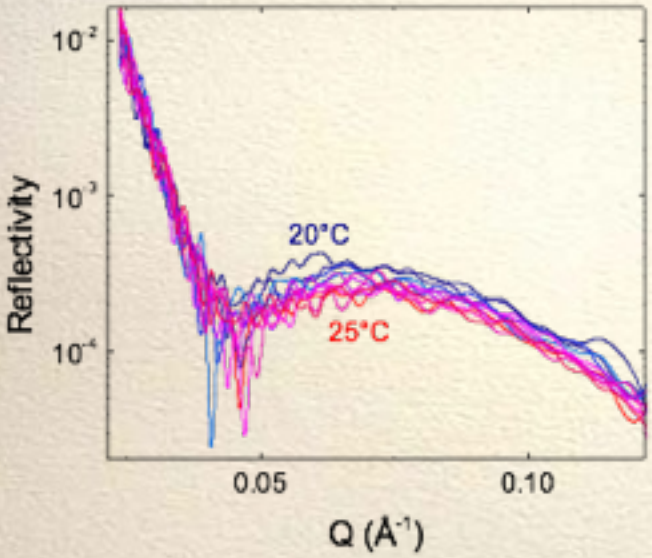
| | | |
|---|---|---|
| $T_m$ | d83-DSPC | 50.5°C |
| $T_m$ | DMPC | 23.5°C |

State kept up to 12 hours without any observed modification !!!



14°C →30°C

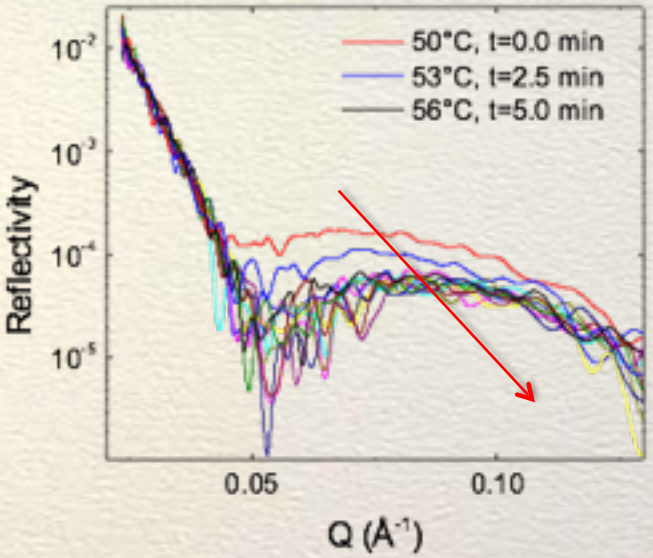
DMPC melts but not DSPC



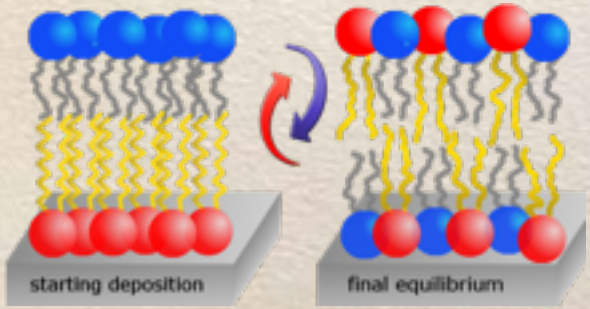

**Slight decrease of the total thickness**

30°C→60°C

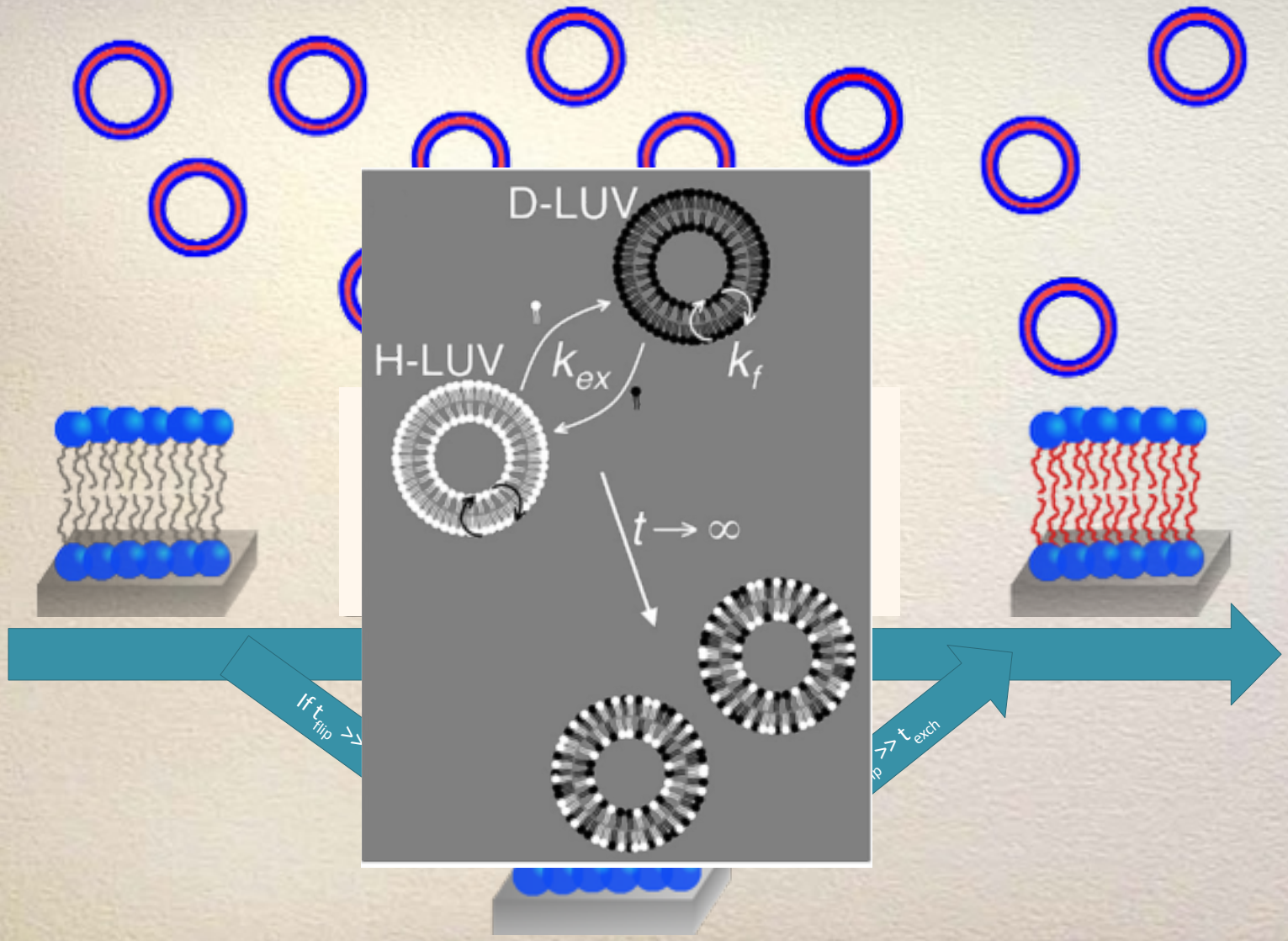
… also DSPC becomes fluid




**Decrease of internal contrast**

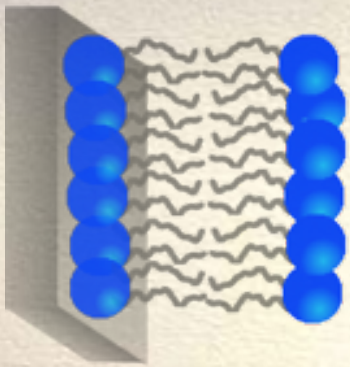


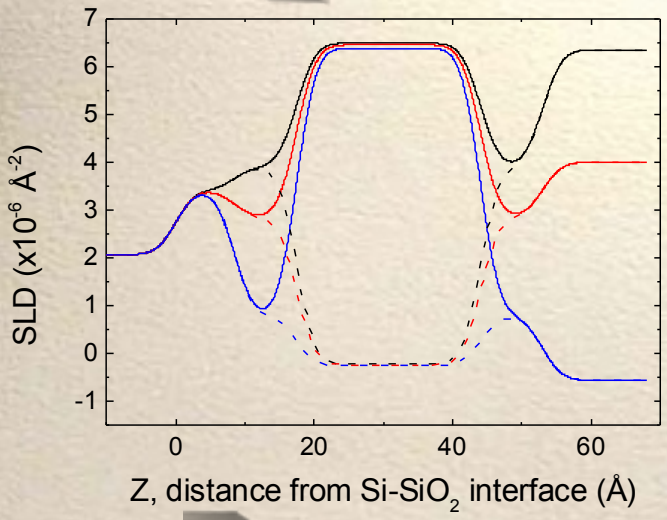

Gerelli Y., *et al.*, **Langmuir** 2012(28), 15922

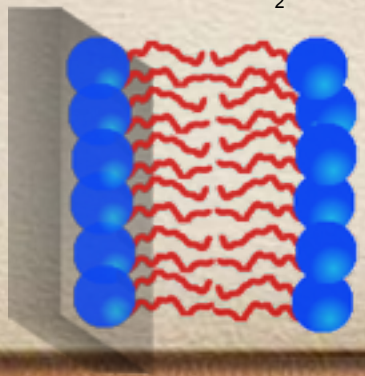



*from Nakano, M. et al. Phys. Rev. Lett. 2007, 98, 238101*

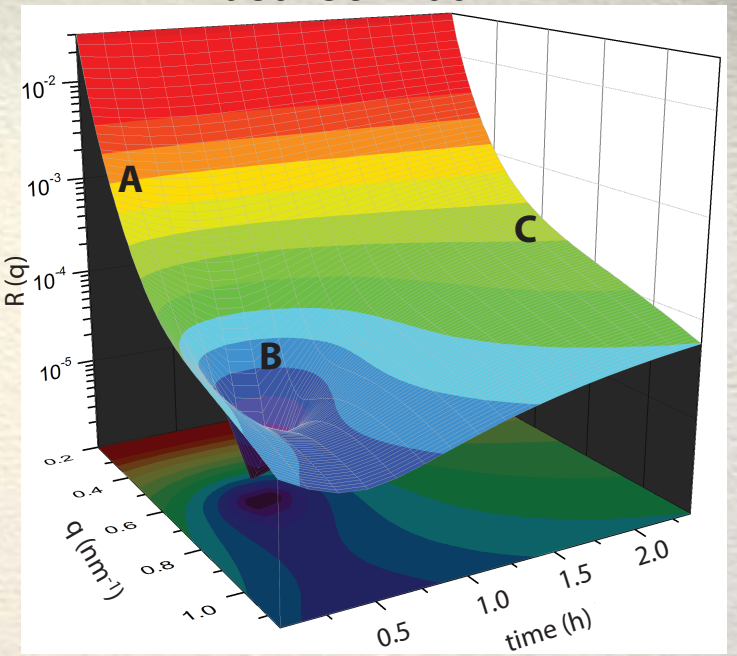
*starting and final points characterised in three water contrasts*

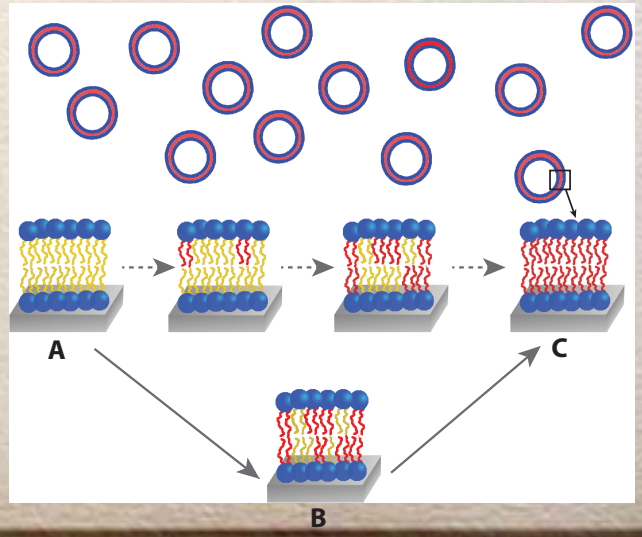




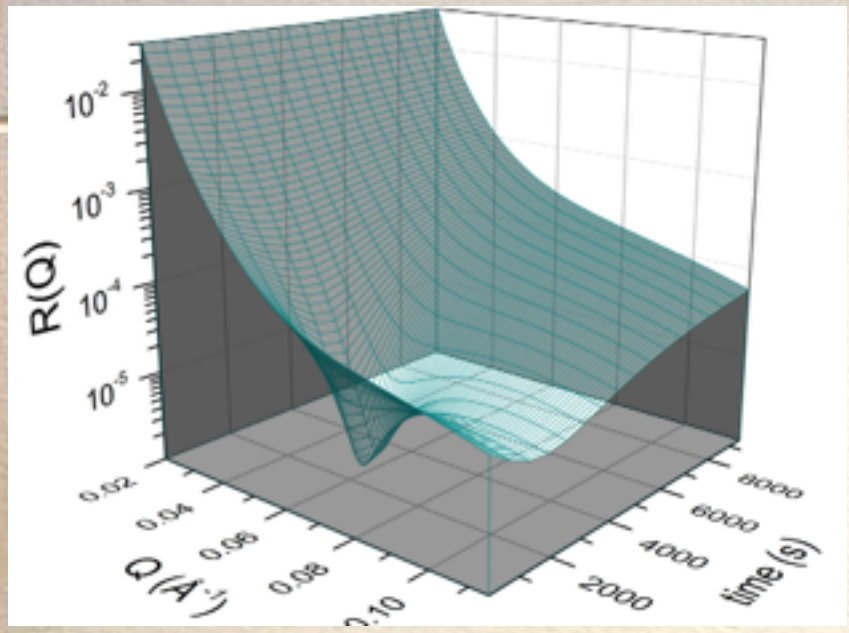



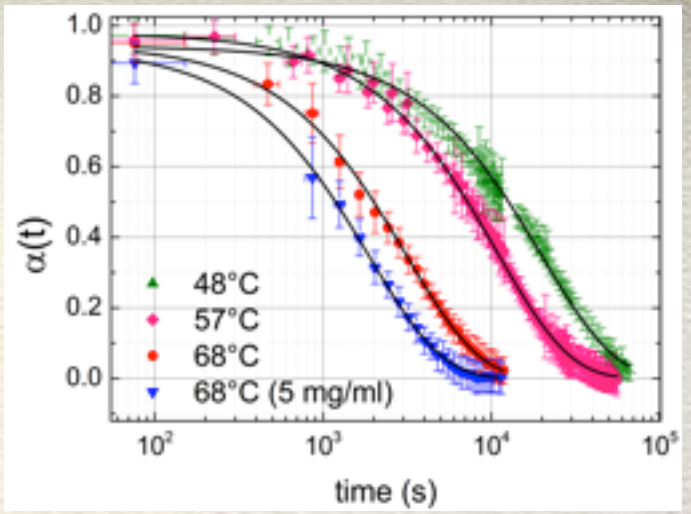
*intermediate points characterised in best contrast*




Gerelli Y., *et al.*, Langmuir 2013(29), 12762

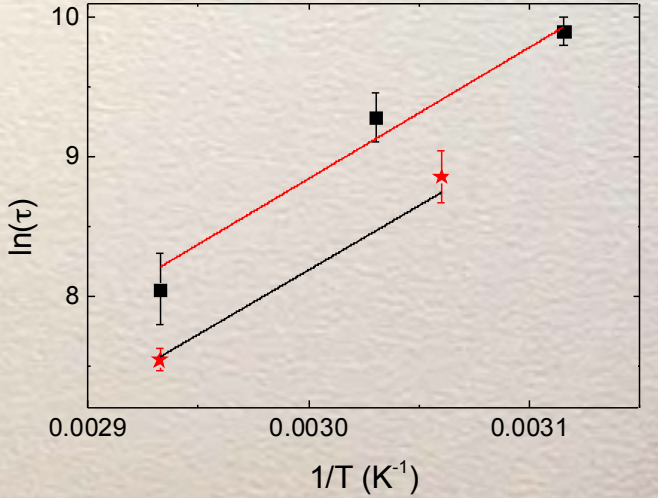



$$\alpha(t, T) = \alpha(0)\, e^{-t/\tau(T)}$$

$$E_a^{ex} \equiv R\left[\frac{\partial \ln \tau(T)}{\partial(1/T)}\right]$$

| Technique | $E_a$ |
|---|---|
| *1:1 LUV–LUV Exchange* | *85 ± 2* |
| *LUV–Bilayer Exchange* | *81 ± 7* |




[a] *Nakano, M. et al. Phys. Rev. Lett. 2007, 98, 238101*

Gerelli Y., *et al.*, **Langmuir** 2013(29), 12762

*For pure DMPC :*

Preliminary indications of a disagreement with SANS data, *flip-flop seems to be faster than the exchange and therefore NOT visible.*

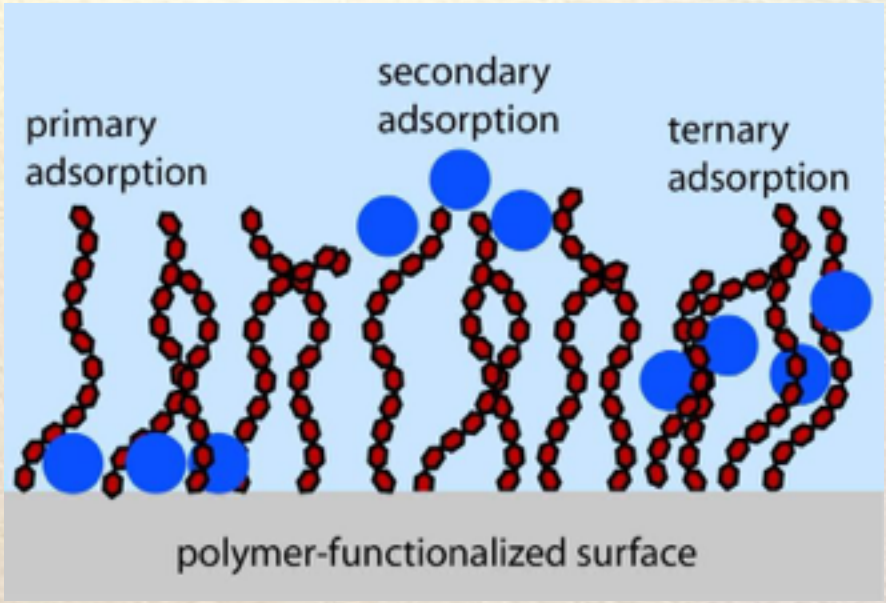
*Is this due to planar geometry and coupling to the substrate in the NR case?*

**or**

*Is this due to the different experimental conditions (h/d-lipids relative concentrations)?*

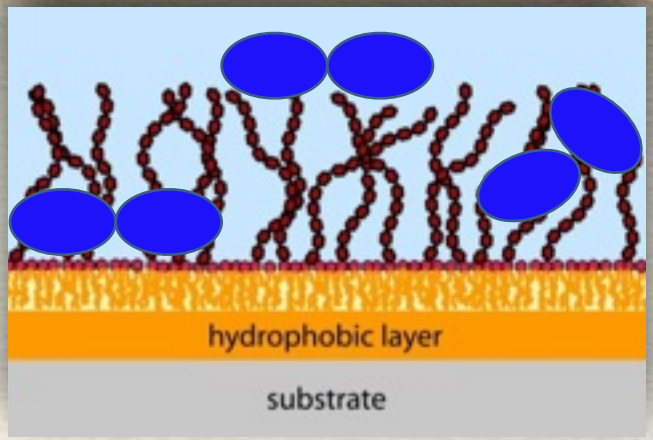
***Essential to select well the sample conditions***

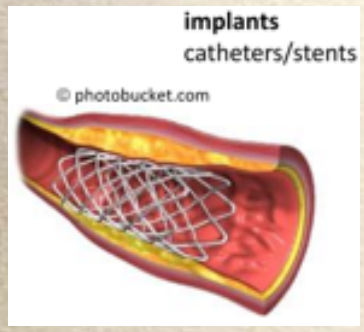
# Neutron reflectometry and deuteriation to probe density profiles of proteins adsorbed onto polymer brushes




*Emanuel Schneck, Audrey Schollier, Avi Halperin, Michele Sferrazza, Michael Haertlein, Martine Moulin*

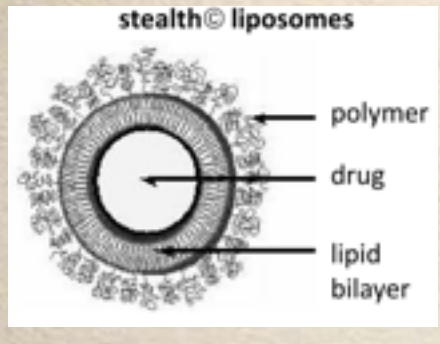
# Density Profiles of Proteins in Polymer brushes



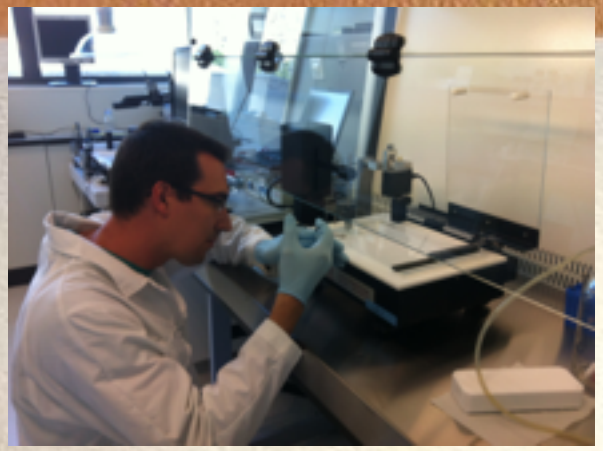

**biocompatible surface functionalization**

**“brush failure” via protein adsorption**




**modes of protein adsorption: primary, secondary, ternary**

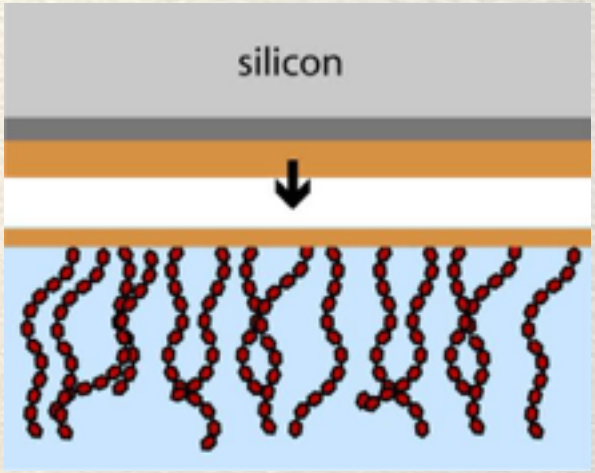
**structural characterization for “rational design” of protein resistant functionalization (role of grafting density and polymer length)**



# Sample Preparation

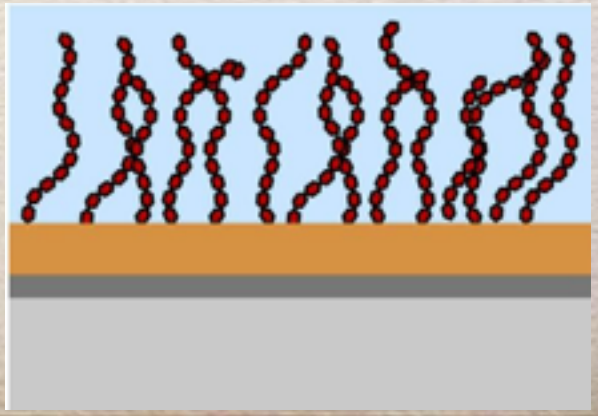
**Preparation steps**

- Planar silicon substrates
- Hydrophobic functionalization
- Brushes at air/water interface (Langmuir trough) of PS-PEG diblock copolymers or PE-PEG lipid anchored polymers

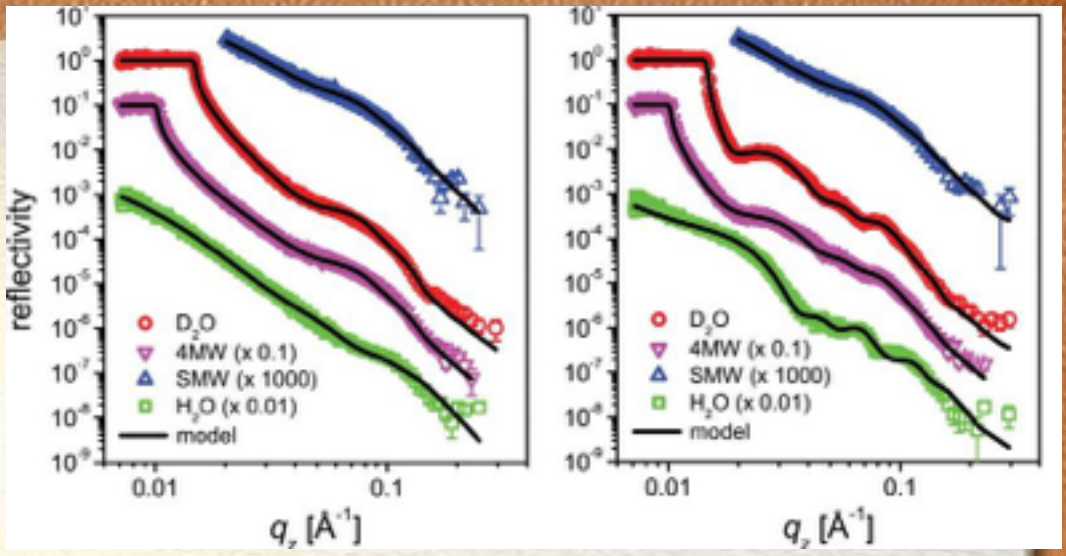



**Resulting brush**

- defined grafting density, $\sigma$
- defined polymer length, ***N***
- hydrophilic/hydrophobic grafting surface



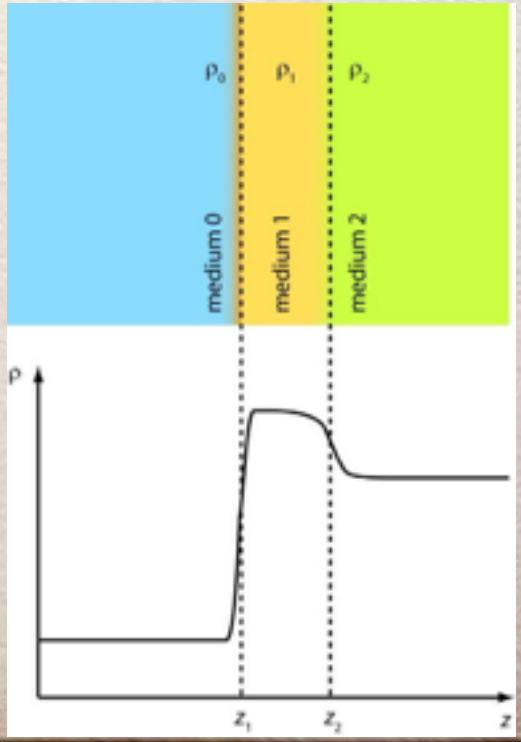
# Scattering length density profile




**SLD profile ϱ(z) gives the profile of the organic compounds;**

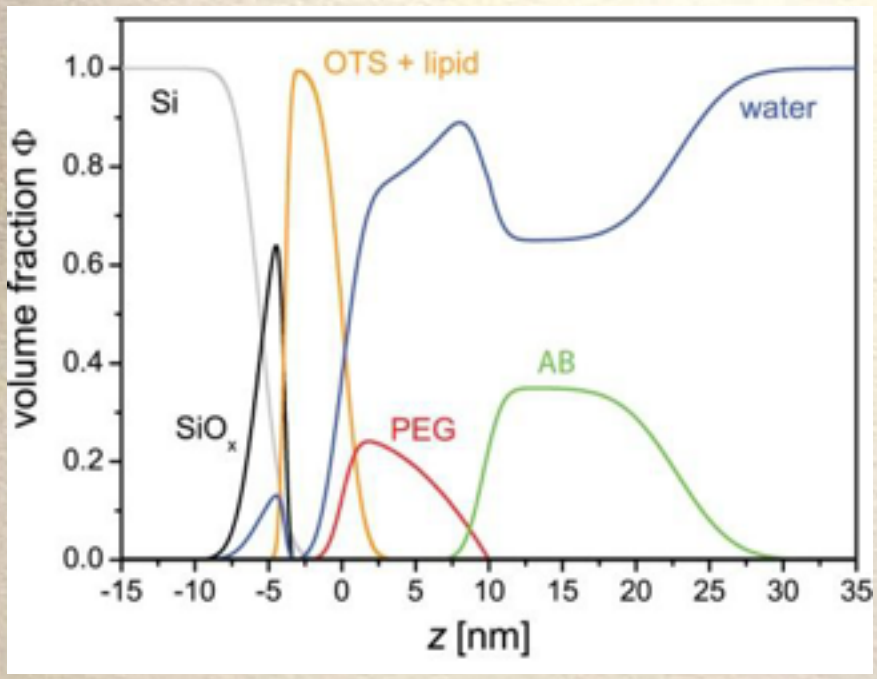
**NOT UNIQUE:**

- Unambiguous result can be obtained by step-wise build-up of sample architecture; use of contrast variation
- SIMULTANEOUS ANALYSIS of 8 reflectivity curves/ sample (4 water contrasts before and after protein adsorption)




***Schneck, Schollier et al., Langmuir 2013***

# Data Analysis




*Schneck, Schollier et al., Langmuir 2013*

**Layers below grafting surface**

- slabs with adjustable thickness, dry SLD, water content, interface roughness

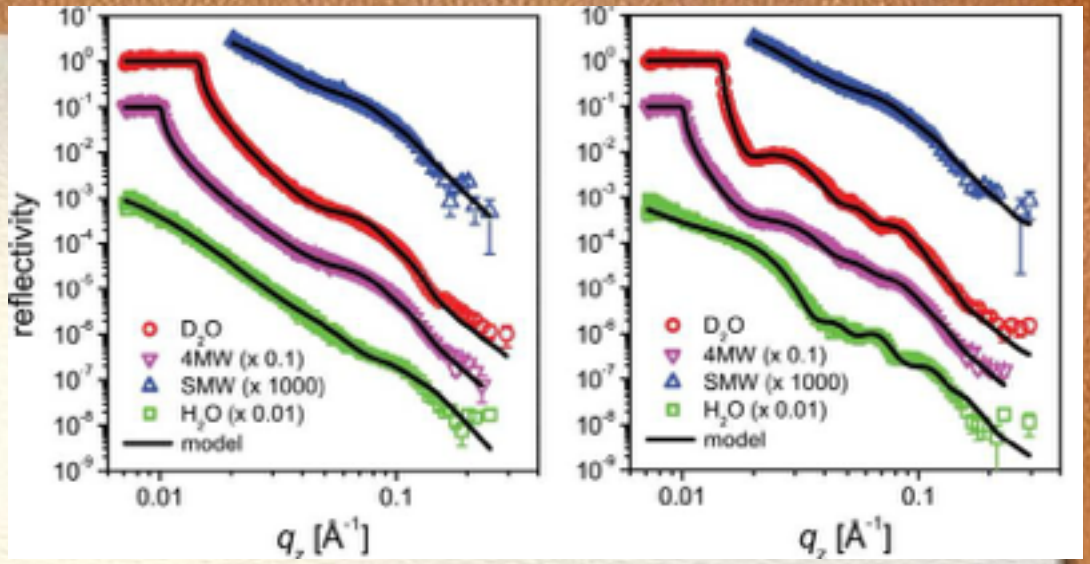
**PEG brush**

- parabola (SCF theory) with adjustable brush length and density

**After protein adsorption**

- protein distribution that allows for primary, secondary, ternary adsorption (rough slabs+Gaussians)
- SLD of PEG and protein fixed
- dependence of protein SLD on water contrast (H/D exchange) taken into account

# Data Analysis



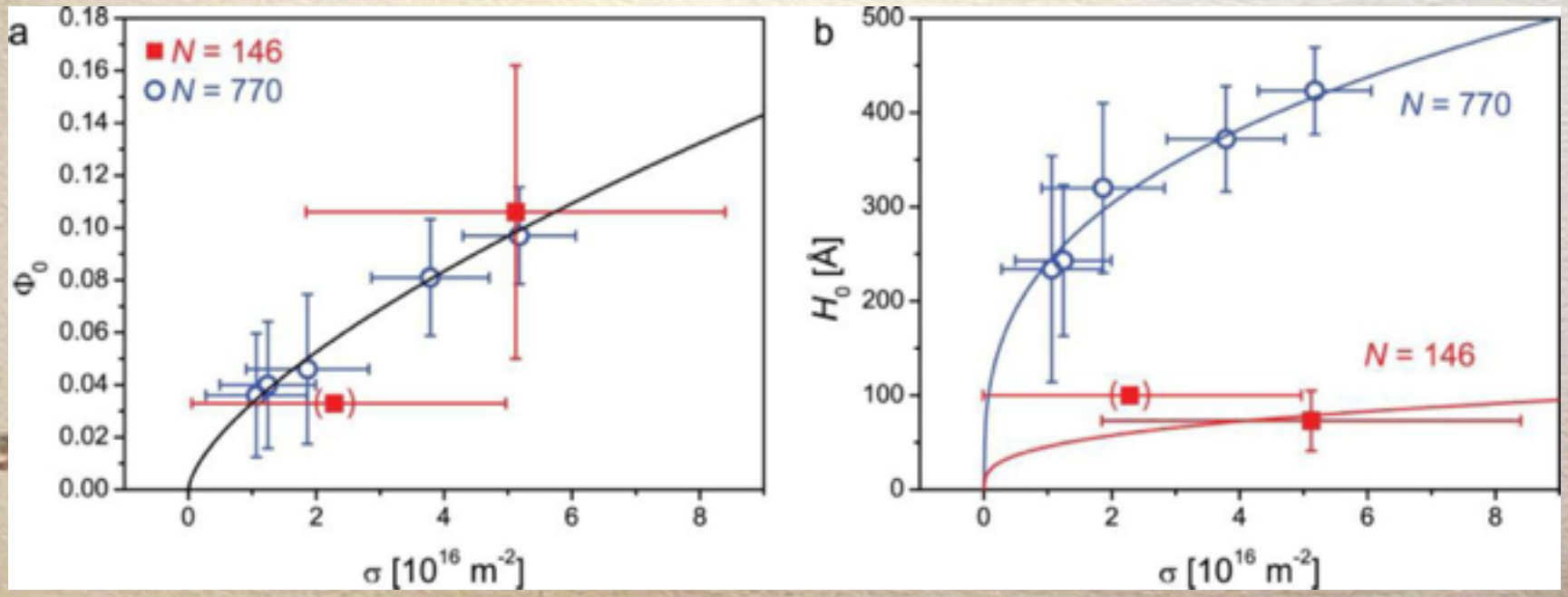

**for each parameter set:**

- compute SLD profiles corresponding to all measurement conditions;
- discretized into 1Å slices;
- compute corresponding reflectivity curves (dynamical treatment: Fresnel reflection coefficients, Parrat formalism)
- **parameters are varied** to achieve best agreement between measured and modelled reflectivity curves

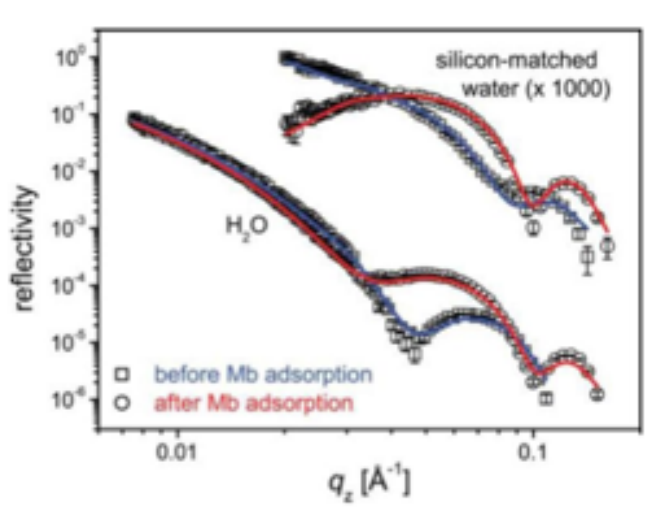
# Bare Brushes
# Results consistent with SCF theory

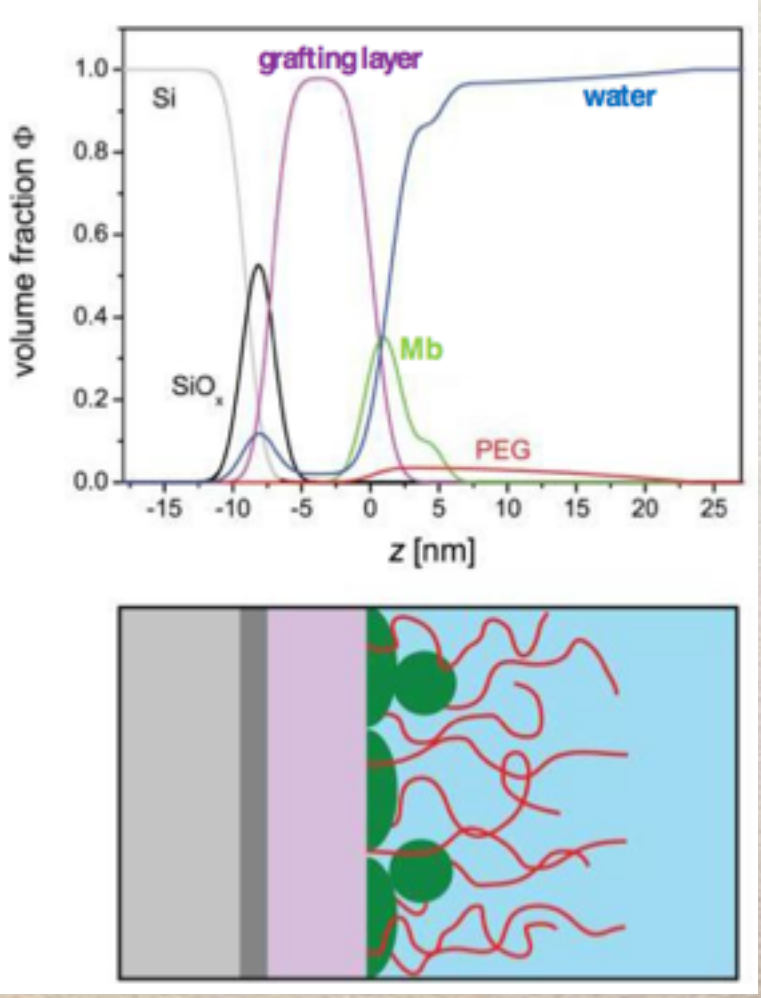
- PEG 114<N<770 up to $\sigma \sim 2\times10^{17} m^{-1}$ (*5nm$^2$ per chain*)

- **parabolic brush model** gives density, $\Phi_0$ and length, $H_0$



# Adsorption of deuterated myoglobin to PEG brushes grafted on hydrophobic polystyrene surfaces

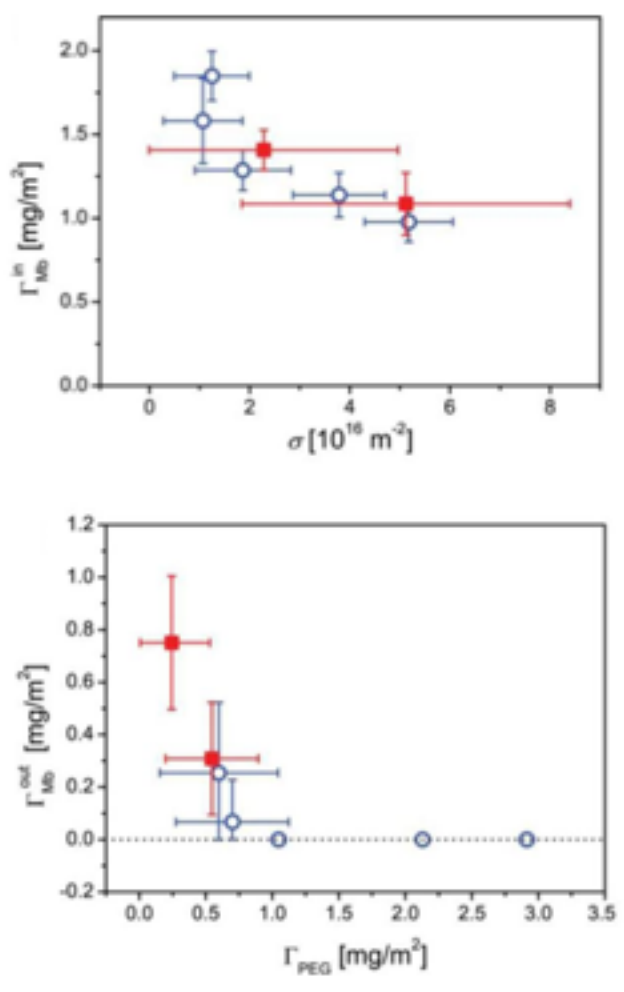



- Significant adsorption for all brush parameters
- only primary adsorption

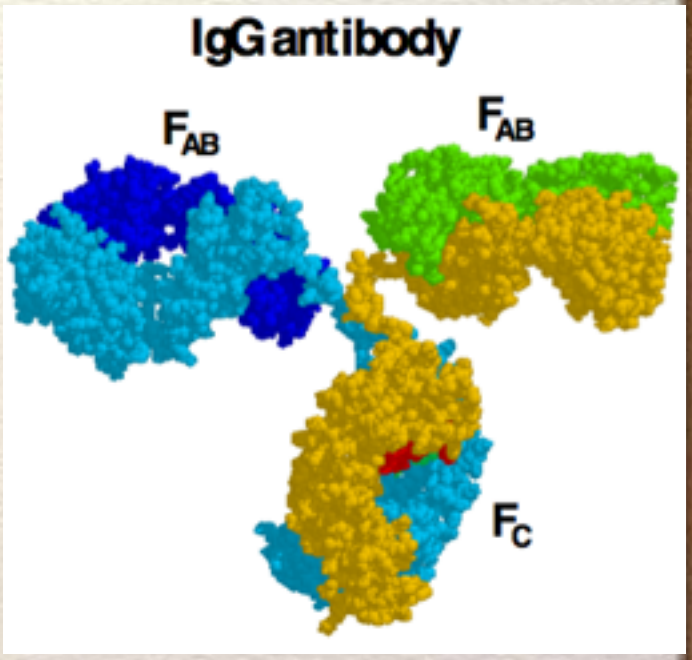
# Adsorption of deuterated myoglobin to PEG brushes grafted on hydrophobic polystyrene surfaces

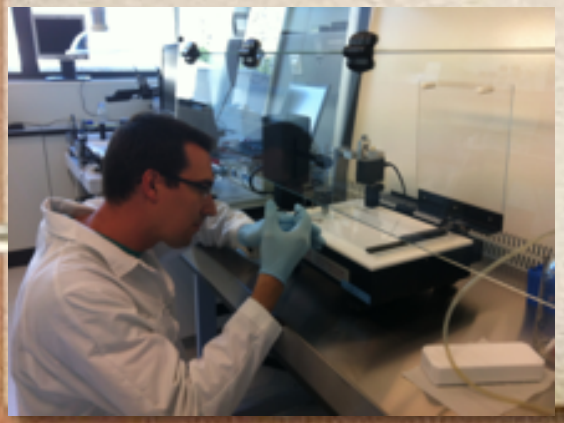
- inner-layer: protein amount decreases with grafting density
- anchoring points obstacles adsorption
- outer protein layer depends on overall PEG amount and protein-protein interactions are altered by the presence of PEG
- **Information only accessible with neutron reflection combined with protein perdeuteration**

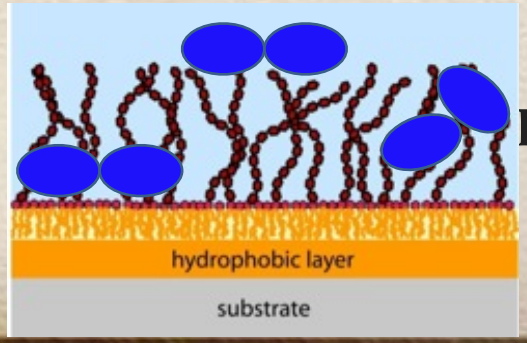


# Specific adsorption: PEG antibodies

- Classically PEG purely repellent, in fact it is antigenic
- PEG antibodies produces in animals (0.1% - 25% in humans)
- Implications on brush functioning - failure?
- IgG AB bind specifically to end segments of PEG



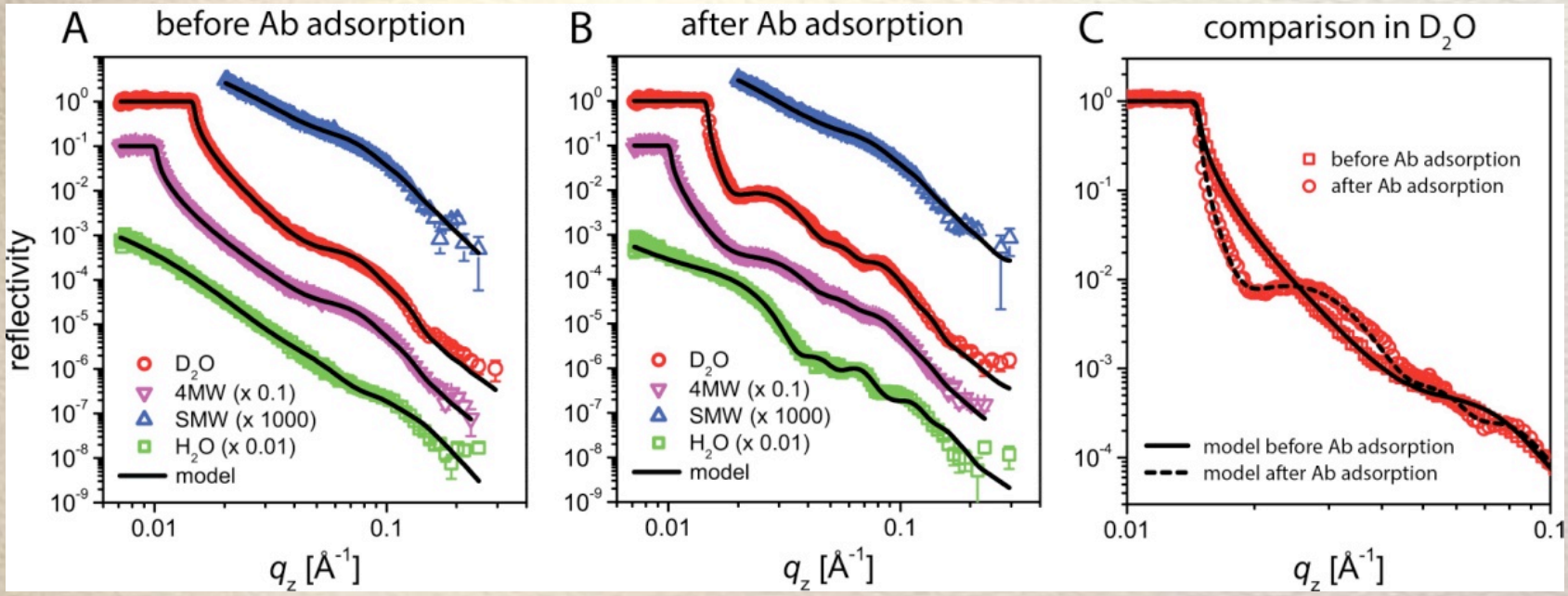






Brushes grafted to hydrophilic phospholipid surface to prevent primary adsorption

# Specific adsorption: PEG antibodies

**Neutron reflectometry measurements**

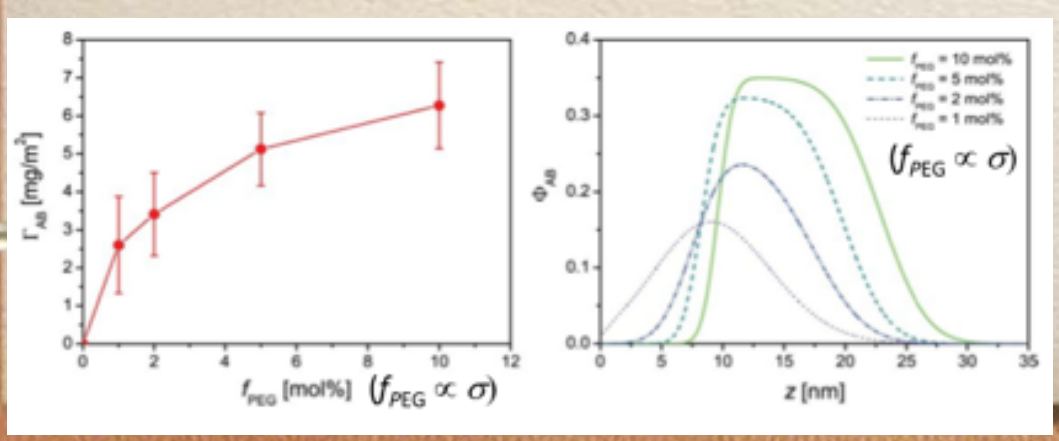


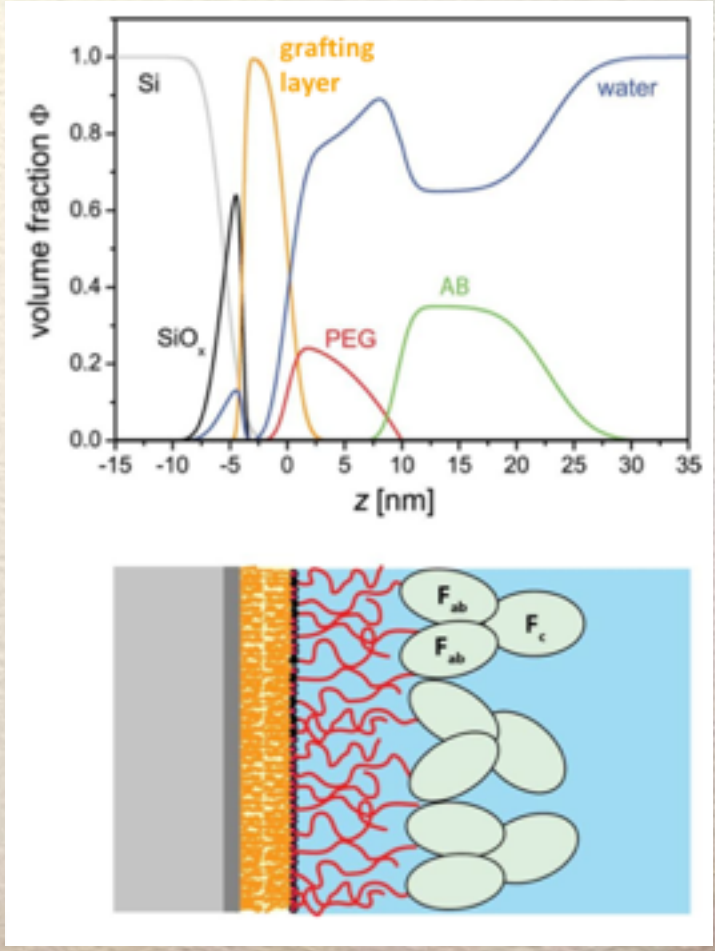

**Brushes grafted to hydrophilic phospholipid surface to prevent primary adsorption**

# Specific adsorption: PEG antibodies

- Antibodies adsorbs at brush periphery
- No primary adsorption
- Amount increases with grafting density
- Saturation - molecular crowding

**Antibodies become the dominant surface:**
**Brush no more functional**
**foreign-body reaction**



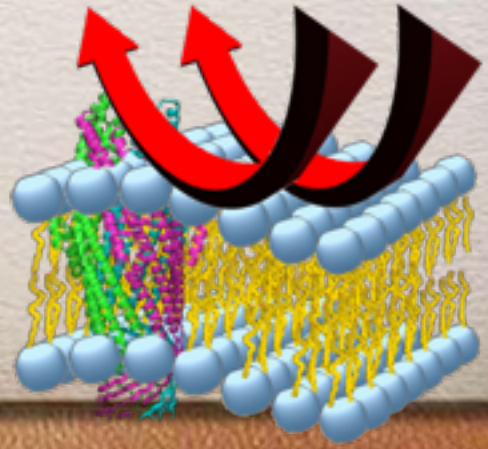




*Schneck et al., in preparation*

- Many open questions regarding protein adsorption to polymer brushes
- Neutron reflectometry (coupled to protein deuteriation) promising approach
- Detailed structural insight
- Unique tool to investigate the structure of biological interfaces and interfaces relevant for biotechnological applications

# Conclusions

- Neutron scattering remains an essential tool for the study of structure at the nanometer level of soft self-assembled systems.
- Complementary to x-ray and synchrotron radiation, advantages include high penetration, sensitivity to light elements (H, C, O, N, …) and isotopic labelling/contrast variation.
- Possibility to work in real (physiological) conditions
- Possibility for in-situ studies of systems under deformation.
- Need optimised sample preparation
- Perspectives in biology are very numerous.



# Thank you for your attention