Study of dynamic properties of reconstituted myelin sheath

MISTRAL
Myelin structure and dynamics in health and disease: a biophysical approach.

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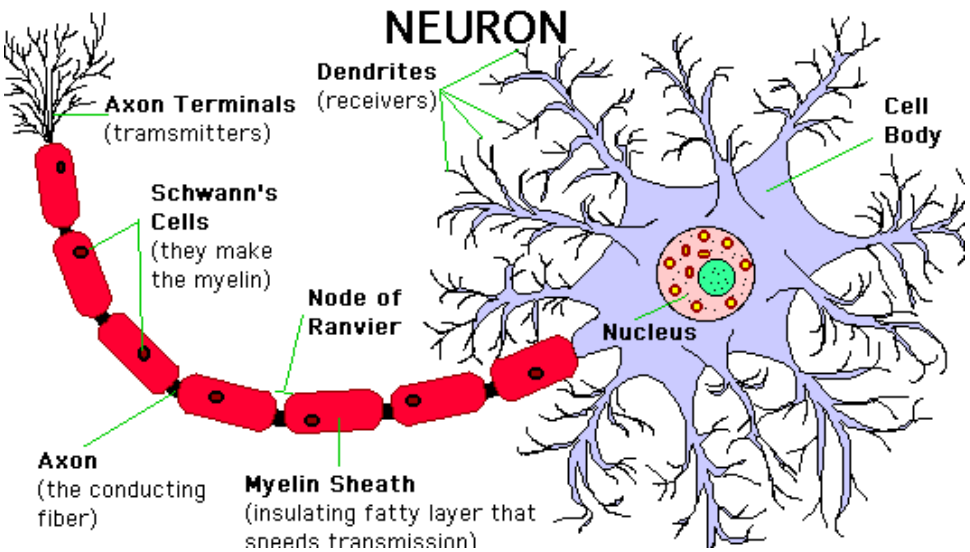
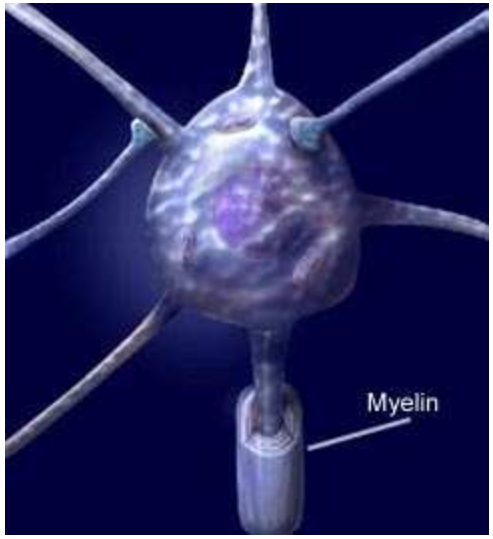
NEUTRONS FOR SCIENCE

UNIVERSITE JOSEPH FOURIER

Istituto Nazionale per la Fisica della Materia

Consiglio Nazionale delle Ricerche


NEURON
Dendrites
(receivers)
Axon Terminals
(tramsmitters)
Cell
Body
Schwann's
Cells
(they make
the myelin)
Node of
Ranvier
Nucleus
Axon
(the conducting
fiber)
Myelin Sheath
(insulating fatty layer that


Myelin

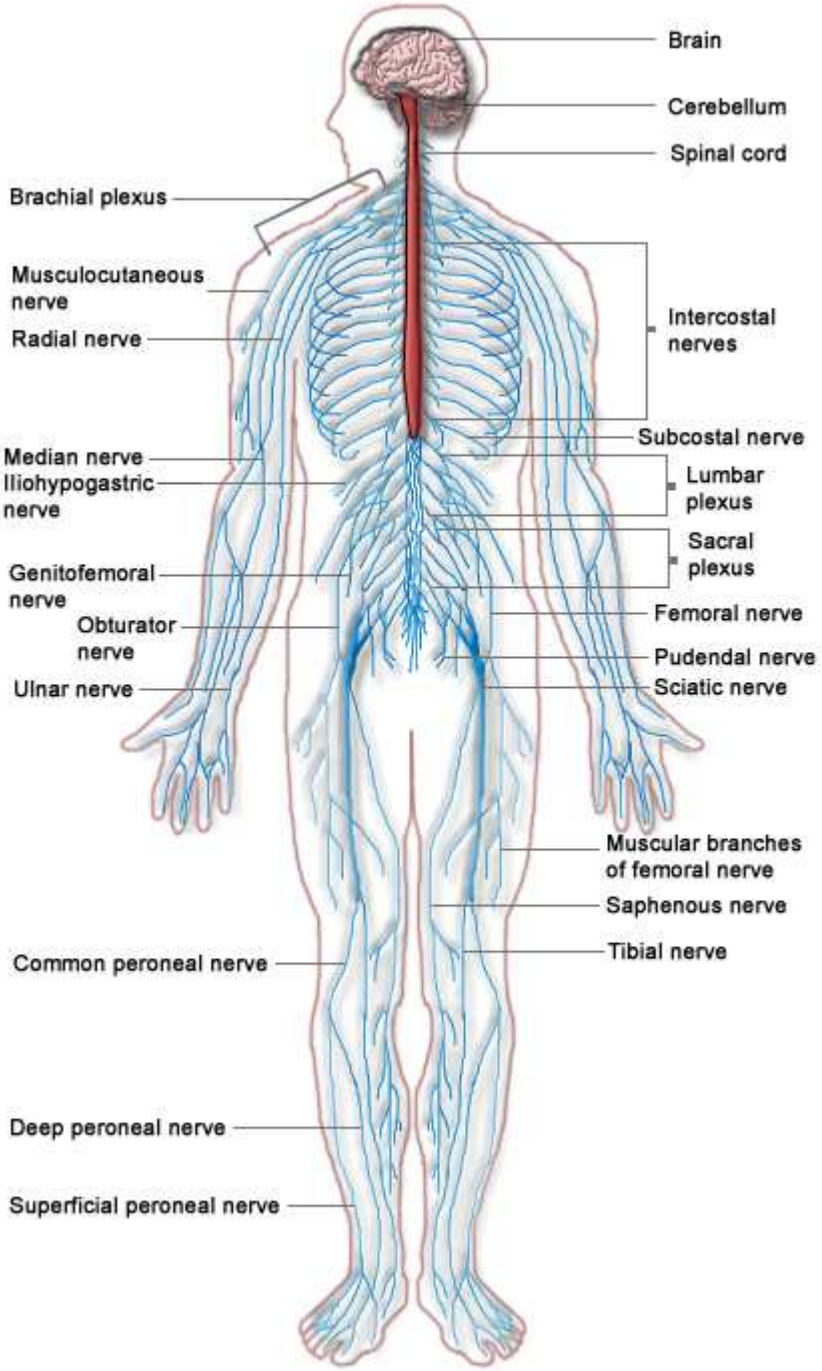
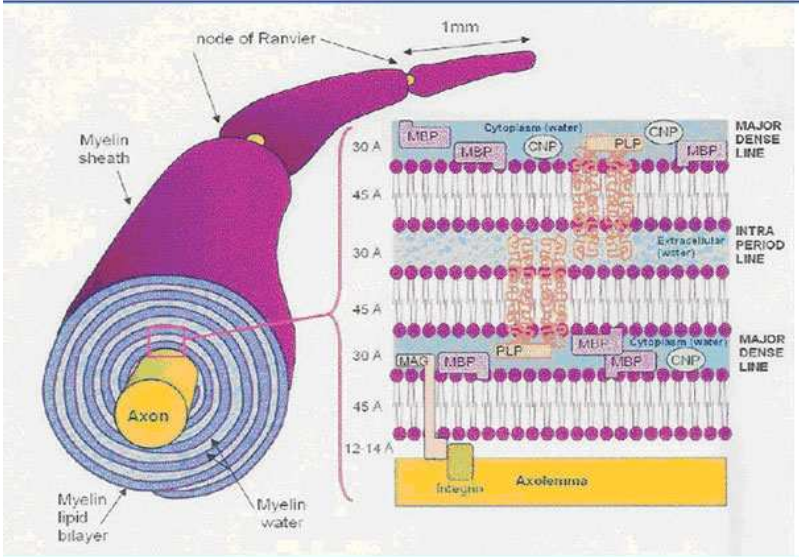

Brain
Cerebellum
Spinal cord
Brachial plexus
Musculocutaneous
nerve
Radial nerve
Intercostal
nerves
Subcostal nerve
Median nerve
Iliohypogastric
nerve
Lumbar
plexus
Sacral
plexus
Genitofemoral
nerve
Obturator
nerve
Femoral nerve
Pudendal nerve
Ulnar nerve
Sciatic nerve
Muscular branches
of femoral nerve
Saphenous nerve
Tibial nerve
Common peroneal nerve
Deep peroneal nerve
Superficial peroneal nerve

Figure 3. Structure of the Myelin Sheath



CNP = cyclic nucleotide phosphodiesterase; CNS = central nervous system; MAG = myelin-associated glycol-protein; MBP = myelin basic protein; PLP = proteolipid protein.

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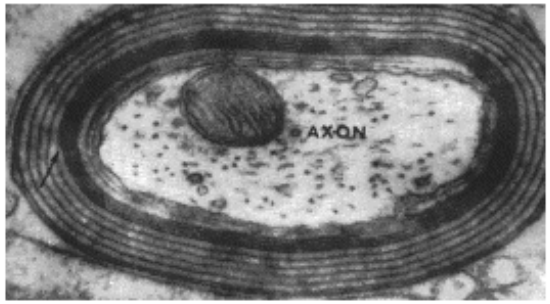
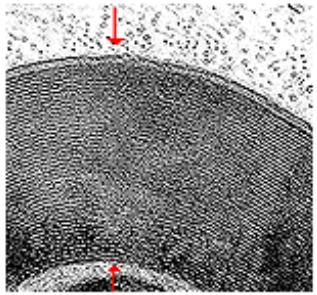
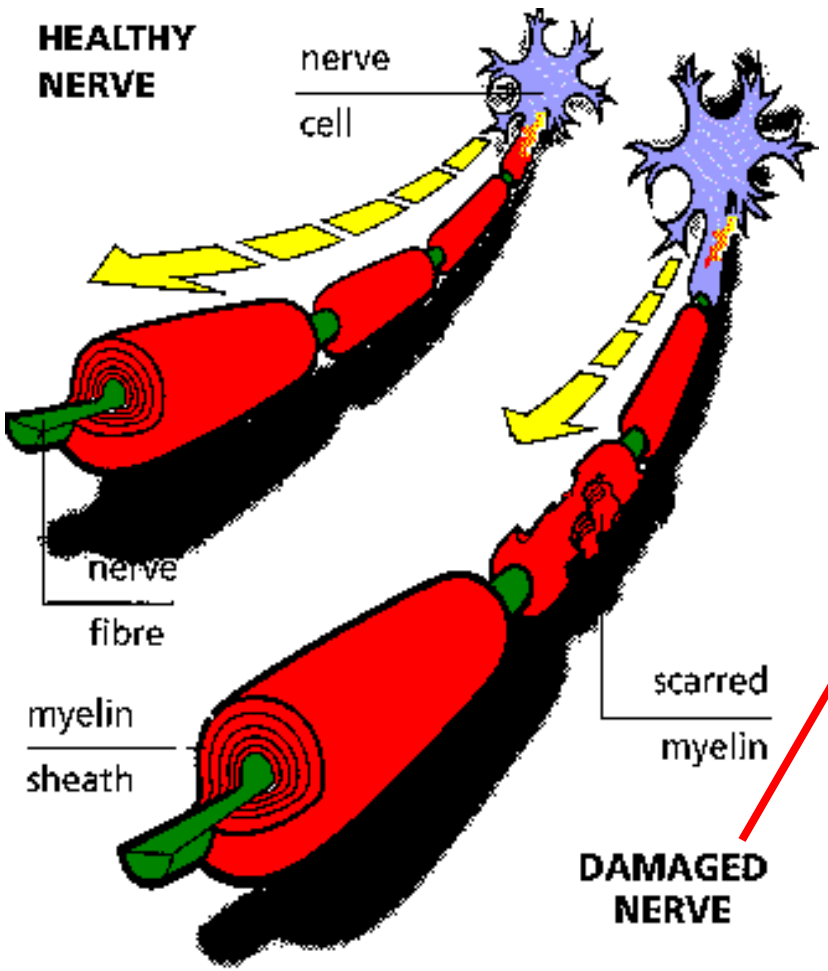
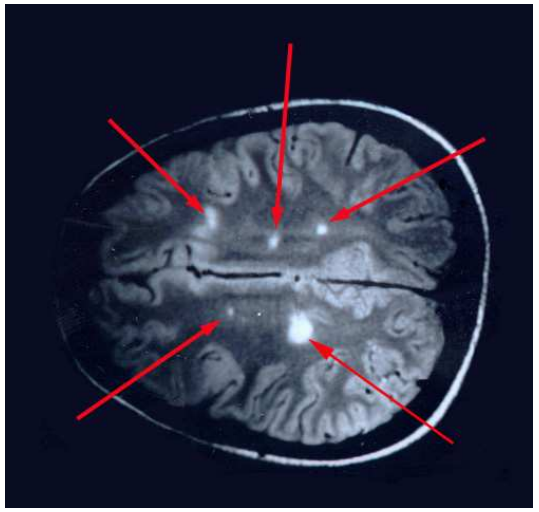




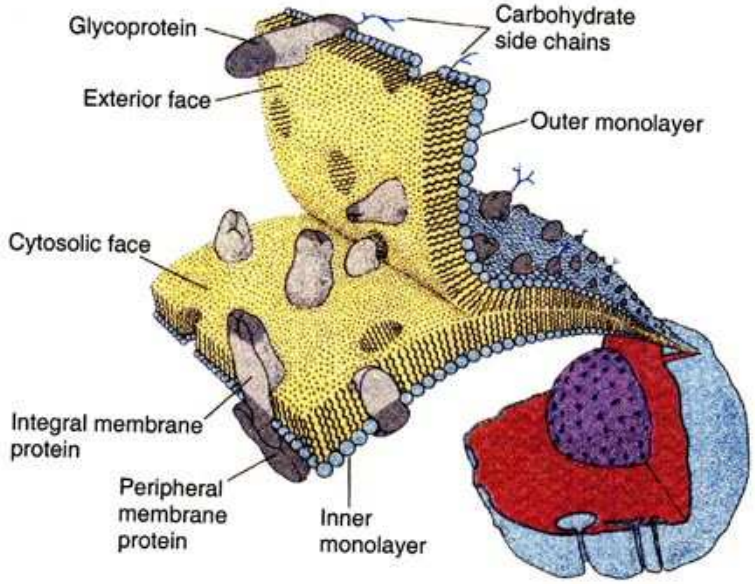


The myelin sheath, lipoprotein complex, protects the neuron´s nerve fiber (the axon) by isolating it and it speeds the transimission of nerve impulses.

Myelin becomes inflammed and the membrane layers break down into smaller components: demyelination process.These components becomes well circumscribed plaques.



Results: obstructing of the nerve conduction leading to neurological dysfunction such as MS in CNS and Guillain-Barré disease in PNS.



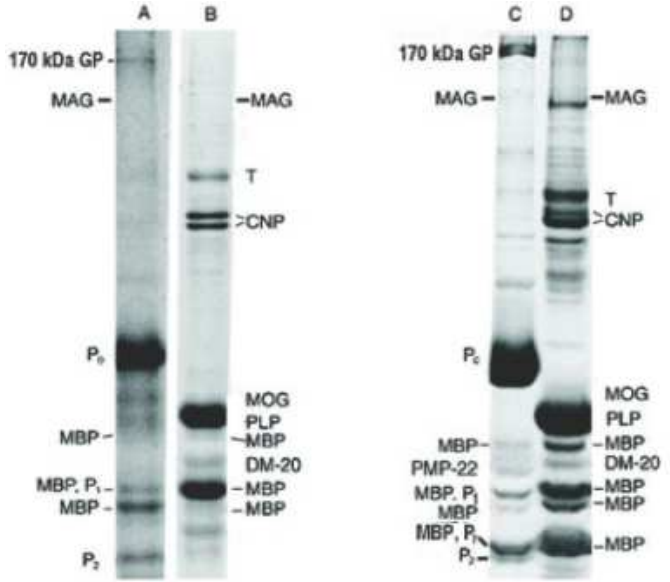
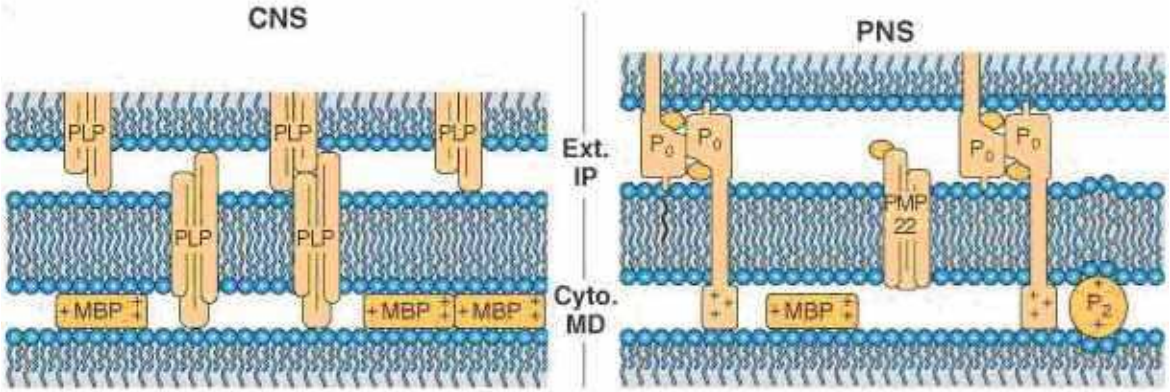


FIGURE 4-12 Polyacrylamide gel electrophoresis of myelin proteins in the presence of sodium dodecyl sulfate (SDS). The proteins of human PNS myelin (**A**), human CNS myelin (**B**), rat PNS myelin (**C**) and rat CNS myelin (**D**) were solubilized with the detergent SDS, electrophoresed and stained with Coomassie brilliant blue. The electrophoretic system separates proteins primarily according to their molecular size with the smallest proteins migrating the farthest toward the bottom of the gel. Abbreviations for the proteins are the same as in the text or



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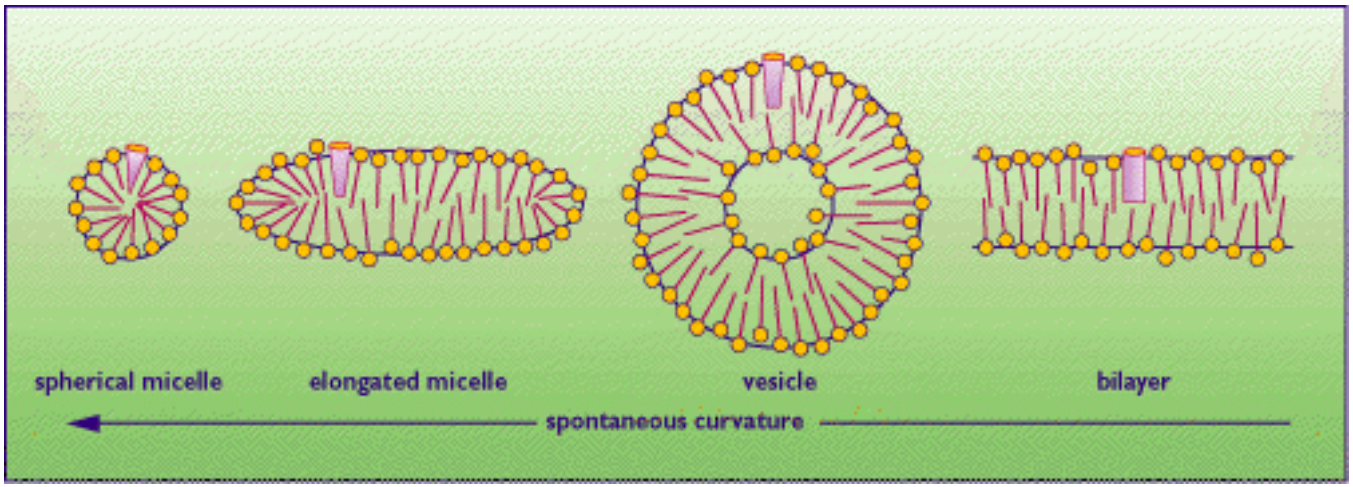
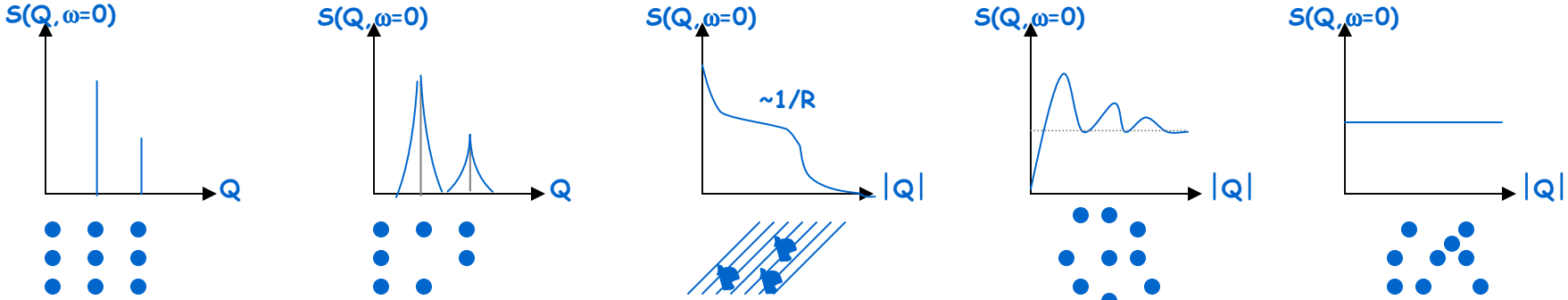
Myelin proteins: primarily for biogenesis and structural stability.Exists in both PNS and CNS.

Structure

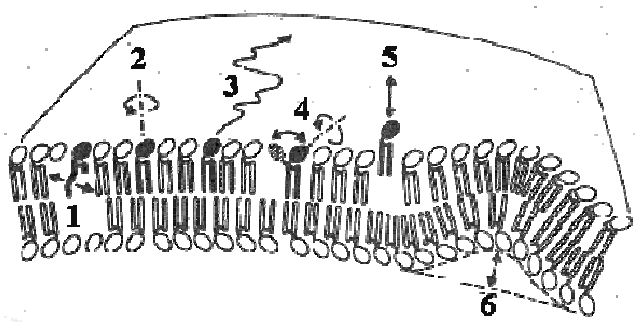


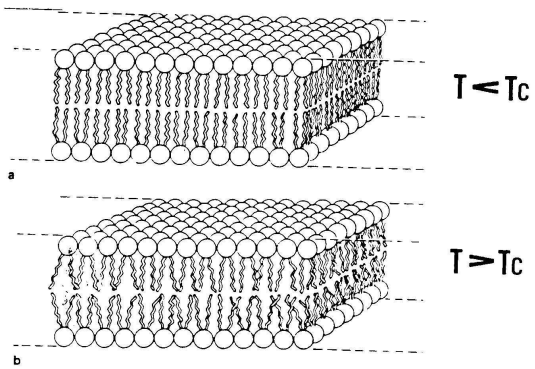


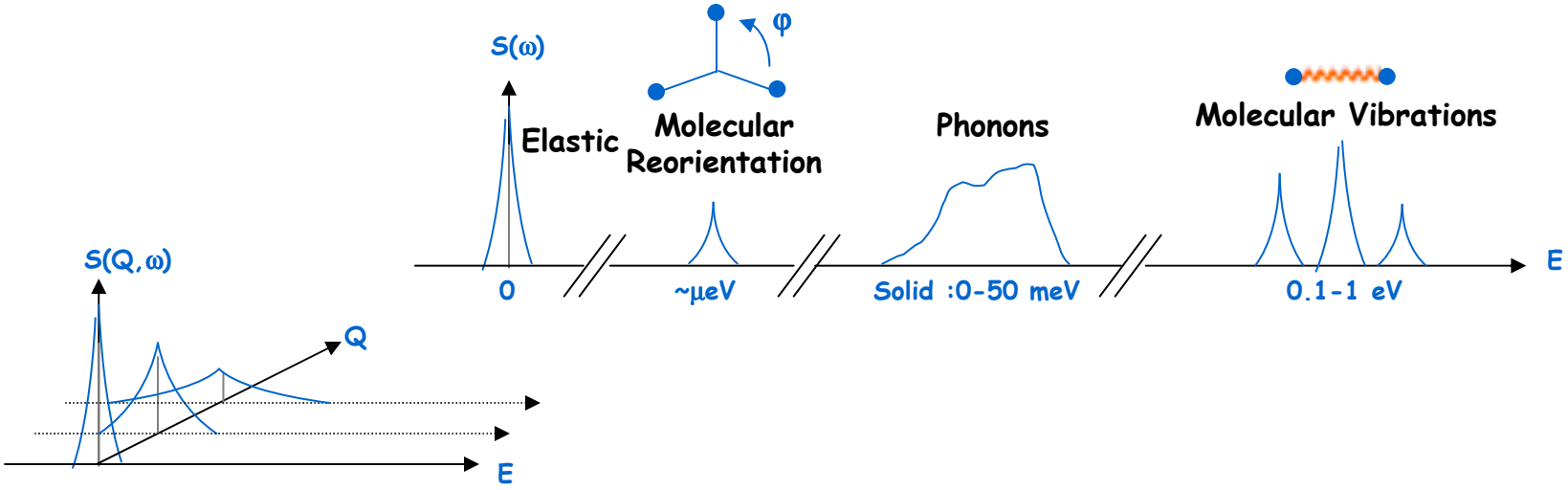
- Optical Microscopy ---> overview
- AFM ---> forces and heights
- SANS (Neutron & X-ray) ---> shapes (low Q, I.e. long d)
- Diffraction (Neutron & X-ray) ---> distances and periodicities
- XAS: EXAFS & XANES (!) ---> distances and angles between atoms
- TDXAS ---> XAS vs temperature

Complementarity !

Dynamics







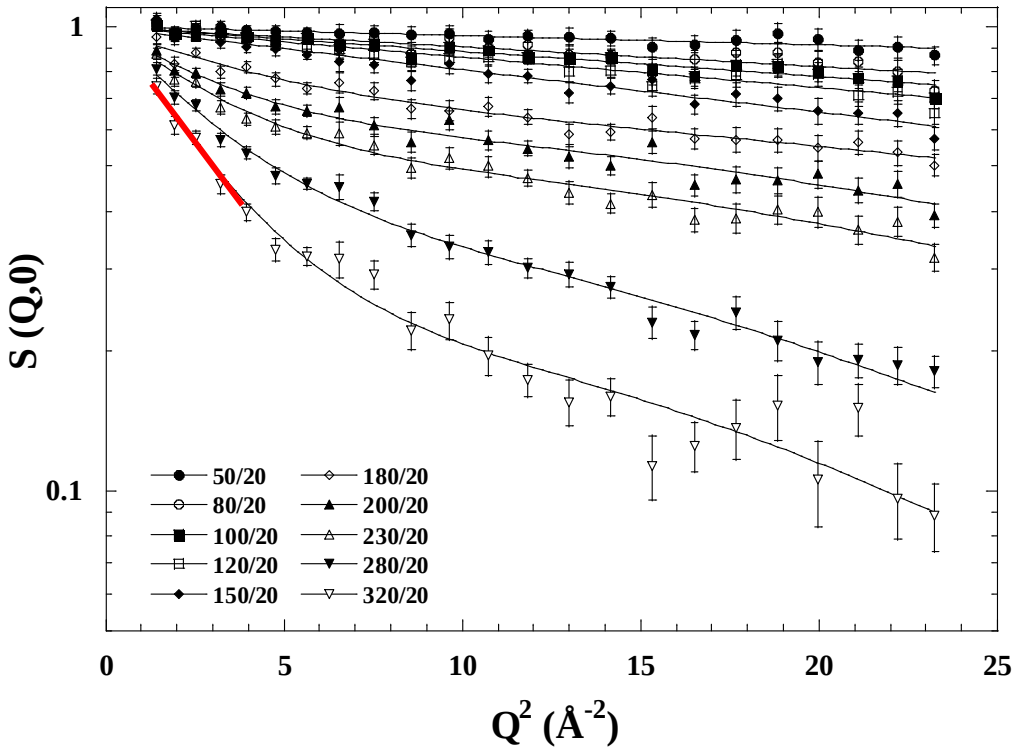
- Rayleigh & Brillouin Spectroscopies ---> Energy shifts due to thermal or pressure fluctuation
- Raman Spectroscopy ---> Molecular vibration
- Quasi-Elastic Neutron Scattering ---> Global Motions
- TXAS ---> Time resolved XAS (Local scale)

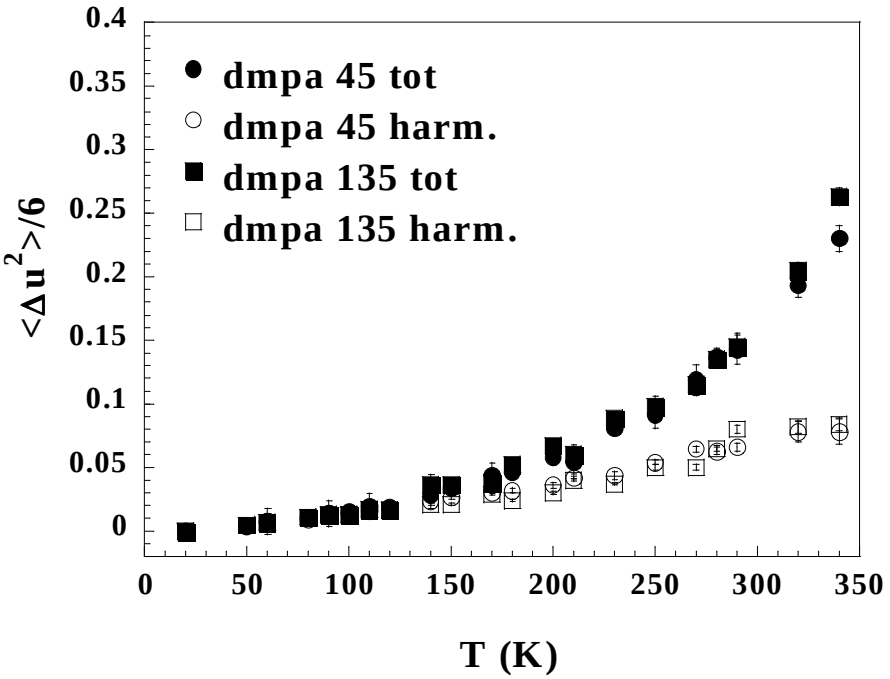
Complementarity in space and time !

Neutrons vs X-rays

Neutrons, interacting with the nuclei and not with electrons, do not promote radiation damaging !
Neutrons Scattering is not scaling with Z ---> light atoms can be observed !
The Incoherent Neutron Scattering cross section of H atoms is particularly high: optimal probe for biological molecules !
Neutrons allow to choose the 'target' to monitor using selective deuteration !
BUT: Neutrons require BIG samples !

Incoherent Elastic Neutron Scattering

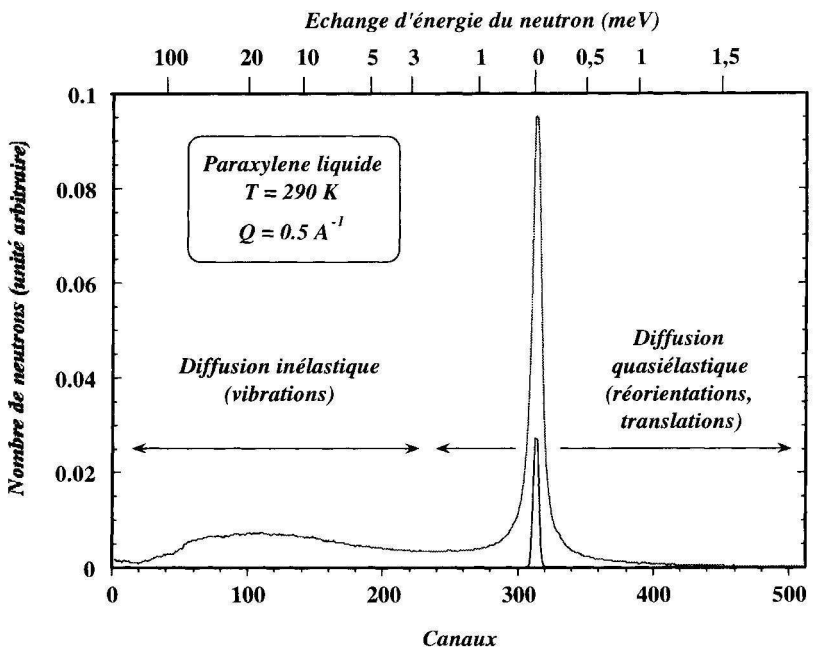


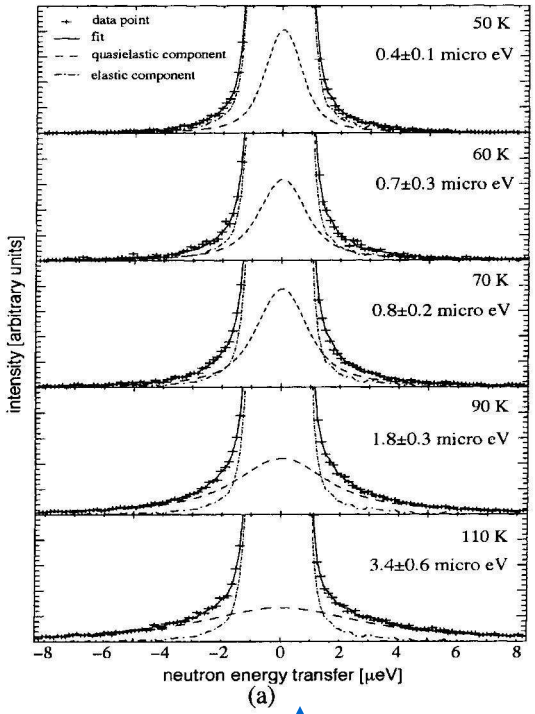


$$\lim_{Q\to 0} S(Q,\omega=0) = I_0 e^{-\frac{<\Delta u^2>}{6}*Q^2} \Leftrightarrow \frac{<\Delta u^2>}{6} = \frac{dS(Q_{\to 0},\omega=0)}{dQ^2}$$

Gaussian approximation

Quasi-Elastic Neutron Scattering





$$S_{inc}(Q,\omega) = A_0(Q)\times\delta(Q)\times R(\omega) + [1 - A_0(Q)]L(Q,\omega)\otimes R(\omega)$$

Elastic Incoherent Structure Factor (EISF=$A_{0(Q)}$)

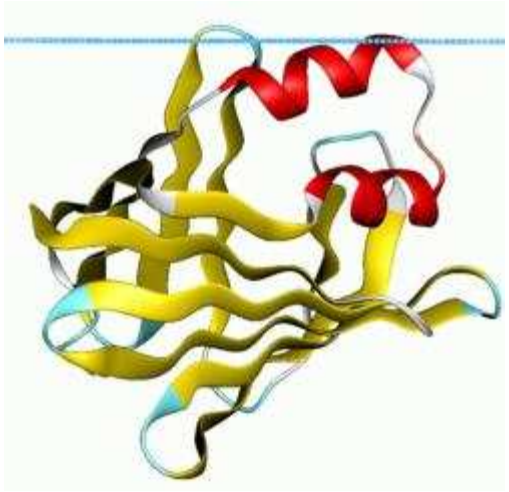
Diffusion Function

Long Range Diffusion

$$A_0(Q) = \frac{I_{el}}{I_{el} + I_{Qens}}$$

$$\frac{1}{\pi}\times\frac{\tau}{1+\omega^2\tau^2} \rightarrow \frac{1}{\pi}\times\frac{DQ^2}{\omega^2 + \left(DQ^2\right)^2}$$

The Myelin Basic Protein (MBP)



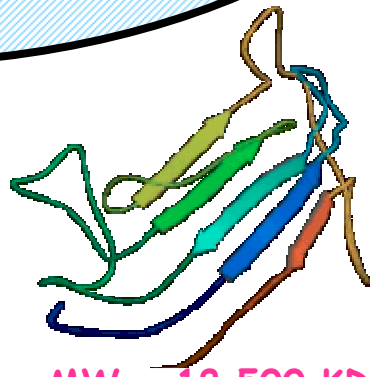
Insulation of nerve axon --> aid in the conductivity of the nerve impulse.

Myelin proteins : primarily for biogenesis and structural stabilityExists in both PNS and CNS.

Crucial in human demyelination diseases: hypersensitivity of MS subjects to MBP.

Open, Highly extended, Double-chain

MBP highly basic character: electrostatic interaction with negatively charged lipids

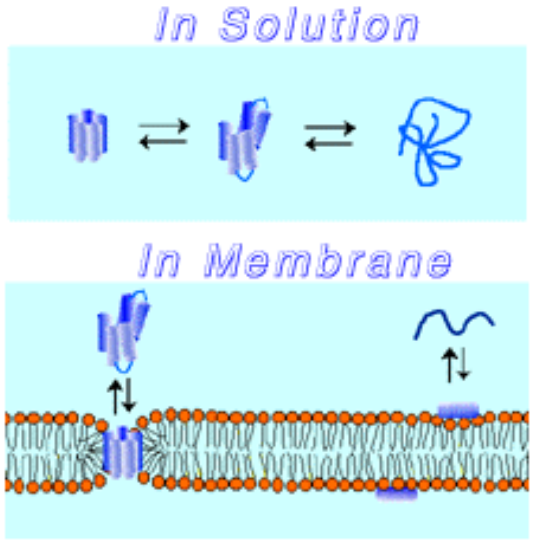


MW ~ 18.500 KDa

The MBP protein, when combined with acidic lipids, forms a double leaftlet having remarkable similarities to myelin

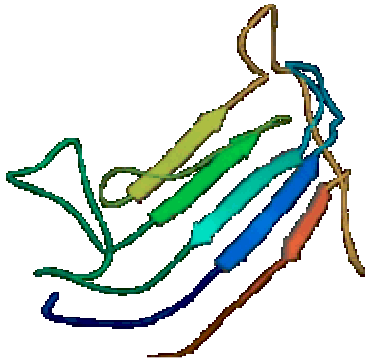
The Myelin Basic Protein (MBP)

Highly basic character: electrostatic interaction with negatively charged lipids



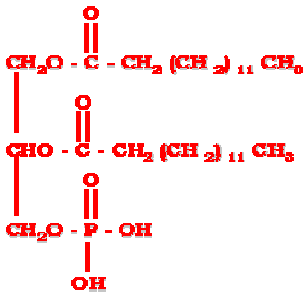
Open,
Highly extended,
Double-chain

More compact

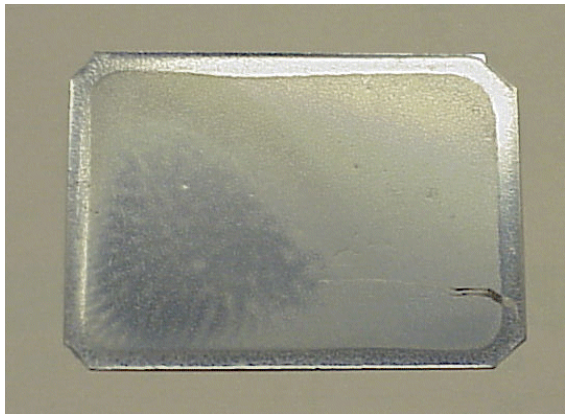


MW ~ 18.500 KDa

+



DMPA
L - α - Phosphatidic Acid
Dimyristoyl ($C_{31}H_{60}O_8P$)

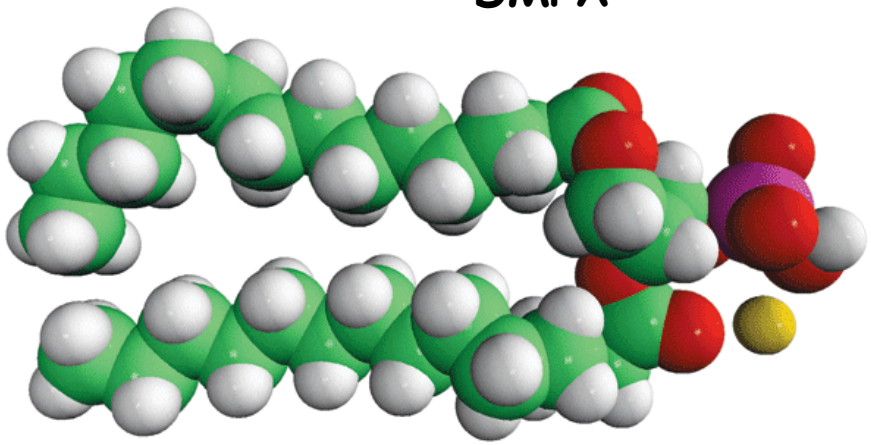


LIPOSOME PREPARATION

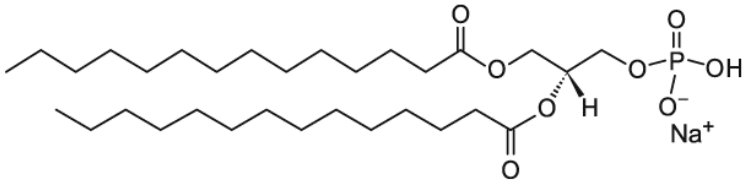
- Lipid powders dissolved in chloroform by vortexing;
-Film deposition by drying;
-Dissolving in D_2O at (50 mg/mL) lipid concentration, heating at T ~ 60 C and vortexing
-Thawing/heating procedure to make the membranes more fragile
-Liposomes extrusion (100 nm pore size)
-Eventually adding protein solution (in D_2O) at a given concentration, vortexing

INFLUENCE OF THE MYELIN PROTEINS ON THE DYNAMICS OF DMPA LIPOSOMES

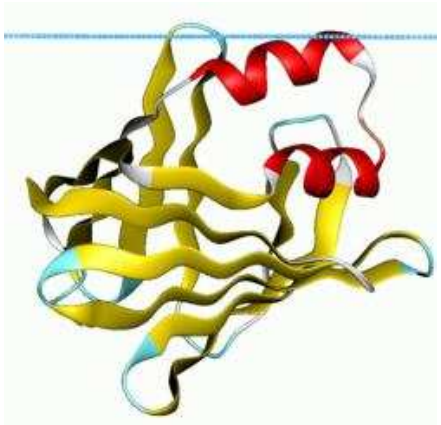
DMPA



MW= 614.767, T_r = 50°C, Anionic



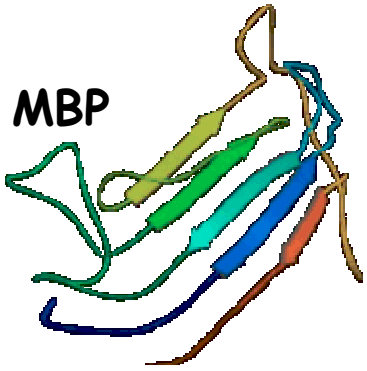
1,2-dimyristoyl-*sn*-glycero-3-phosphate (sodium salt), $C_{31}H_{60}O_8PNa$



P2

MW= 14.5 KDa, Folded

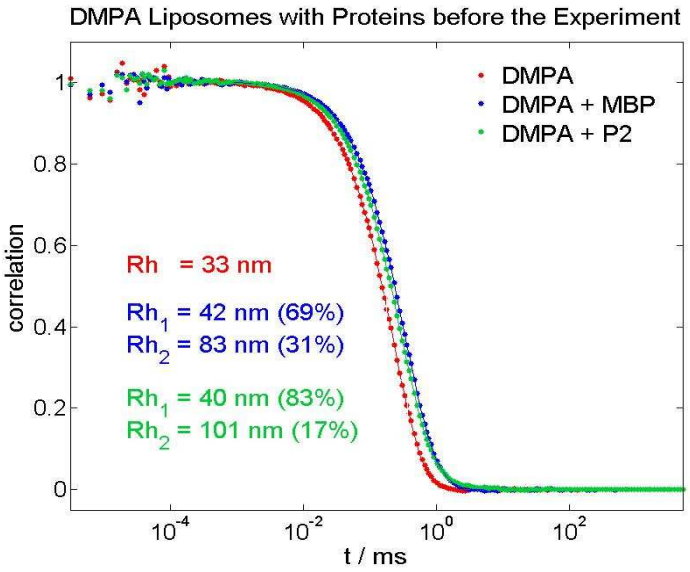
MBP

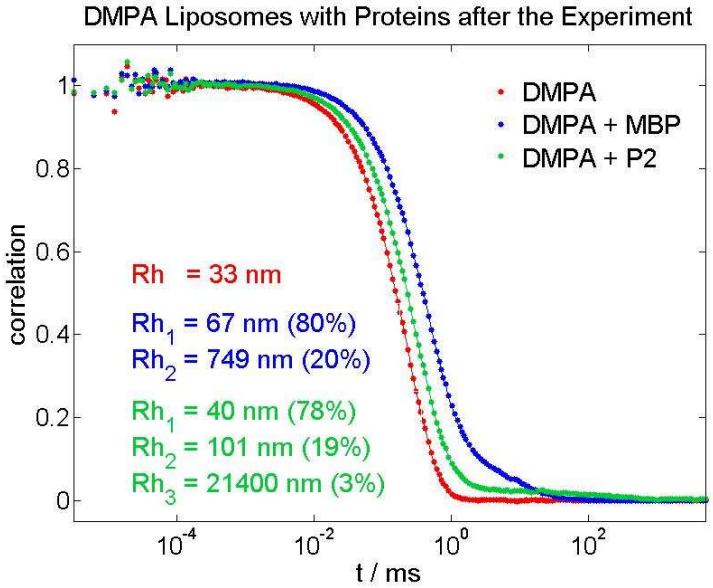


MW= 18.5 KDa, Unfolded

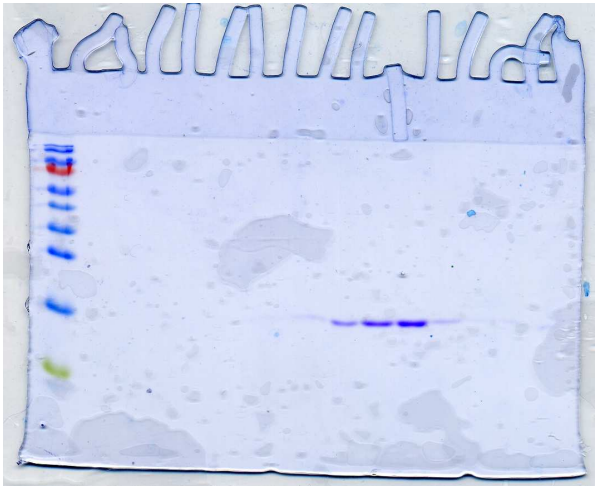
SAMPLE CHARACTERIZATION

DYNAMIC LIGHT SCATTERING

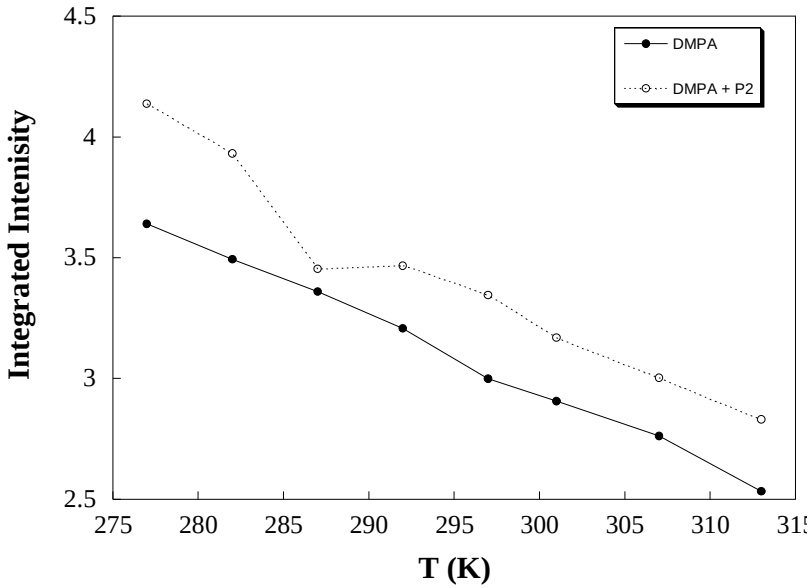


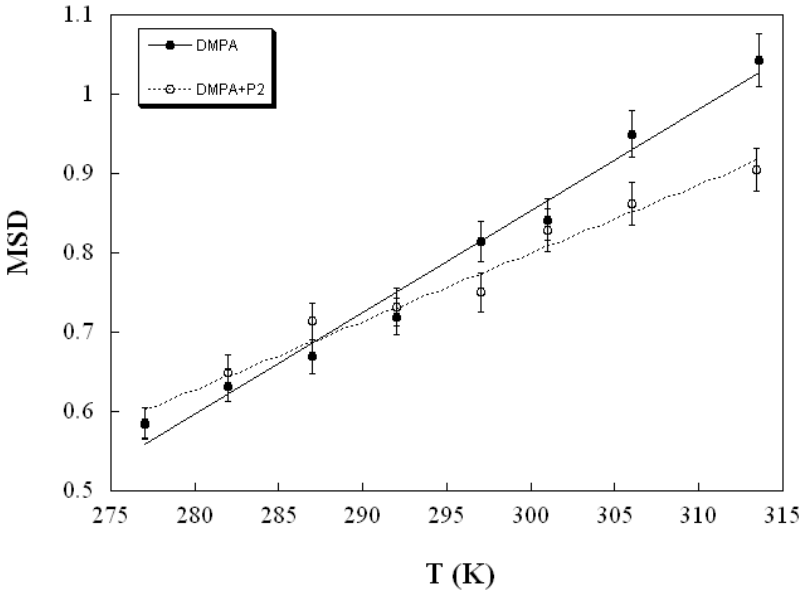


SUCROSE GRADIENT



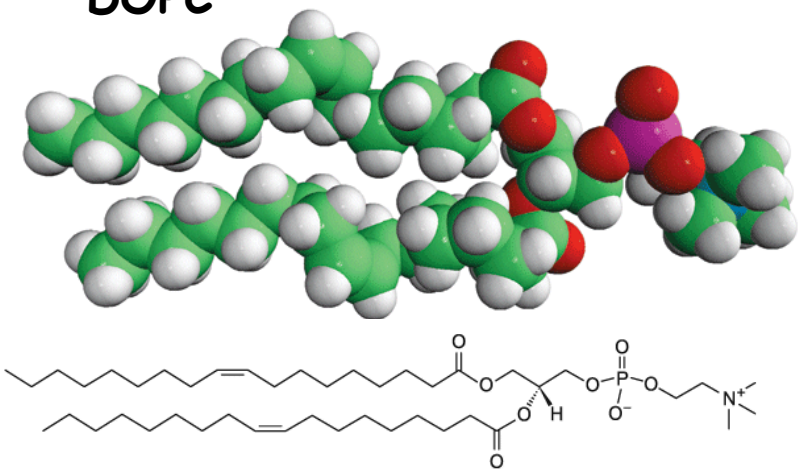
MEMBRANE DYNAMICS





INFLUENCE OF THE MYELIN PROTEINS ON THE DYNAMICS OF DOPC-DOPS MIXED LIPOSOMES

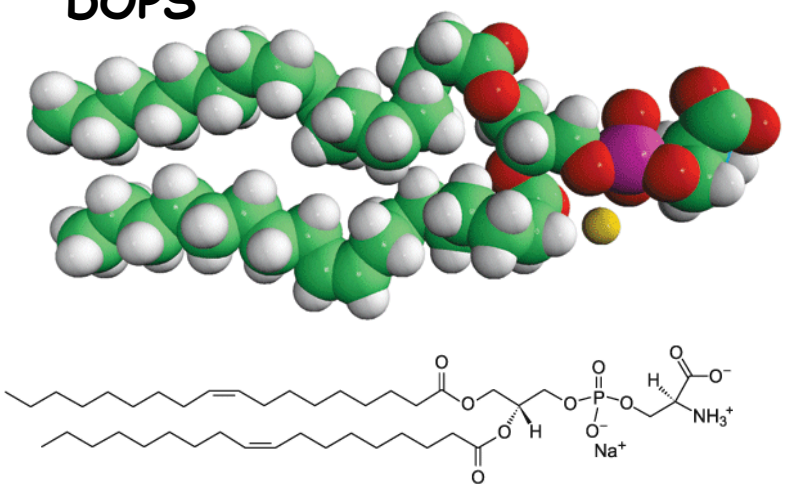
DOPC



1,2-dioleoyl-*sn*-glycero-3-phosphocholine, C44H84NO8P

M.W.: 786.113
T_r = -20°C
Neutral

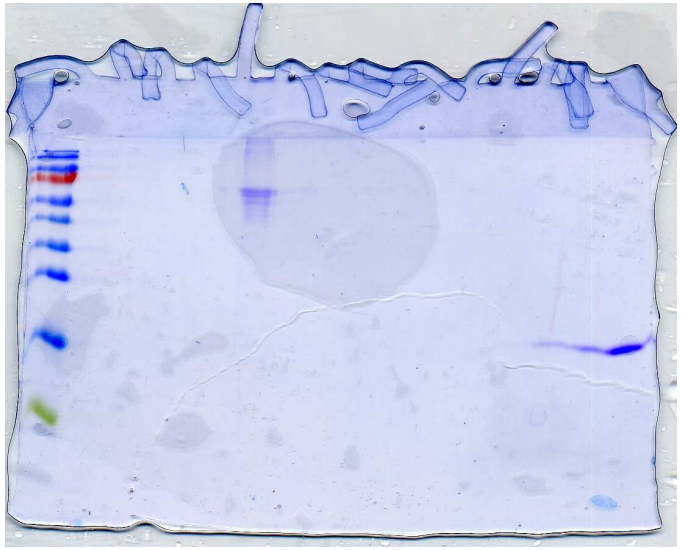
DOPS



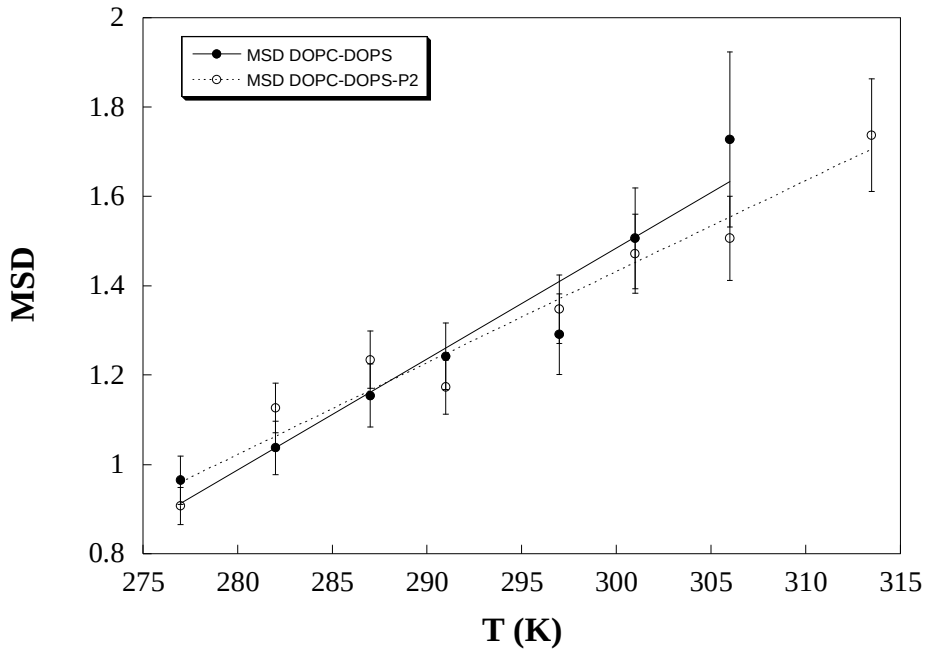
1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine (sodium salt), C42H77NO10PNa

MW= 810.025
T_r = -11°C
Anionic

SUCROSE GRADIENT

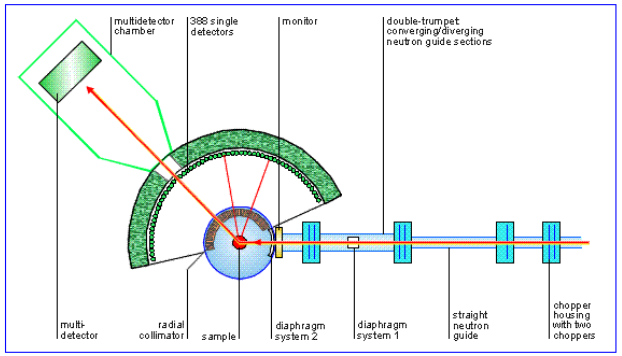


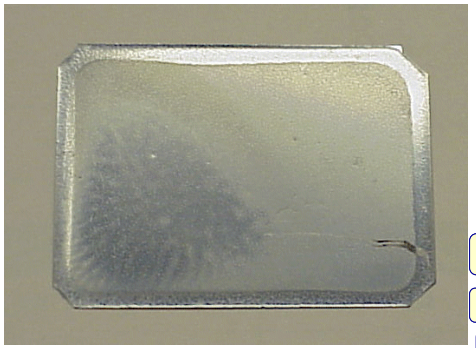
MEMBRANE DYNAMICS



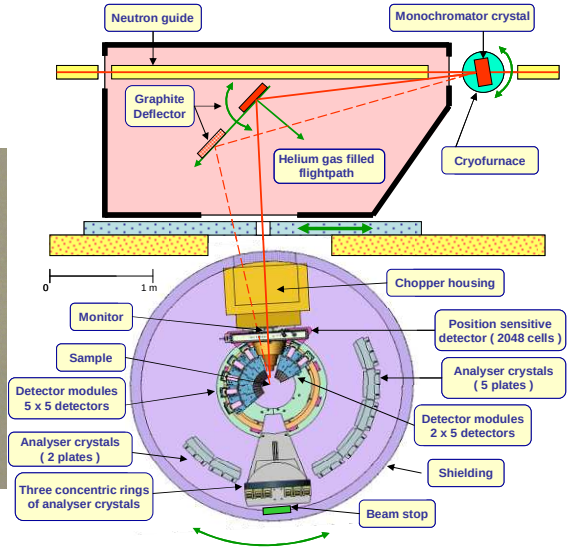
PERSPECTIVES

V3 - Time-of-Flight Spectrometer (NEAT) back



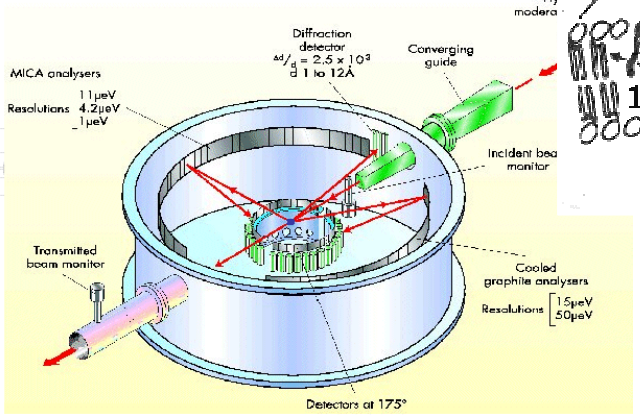


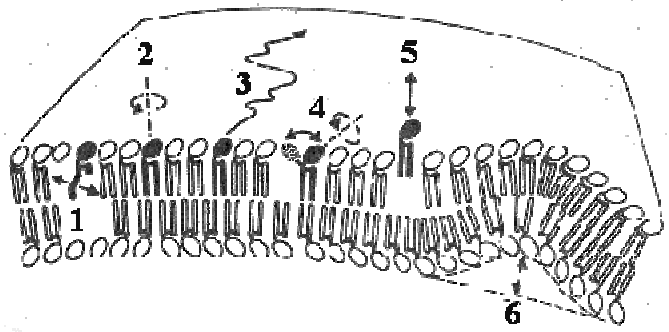
V3

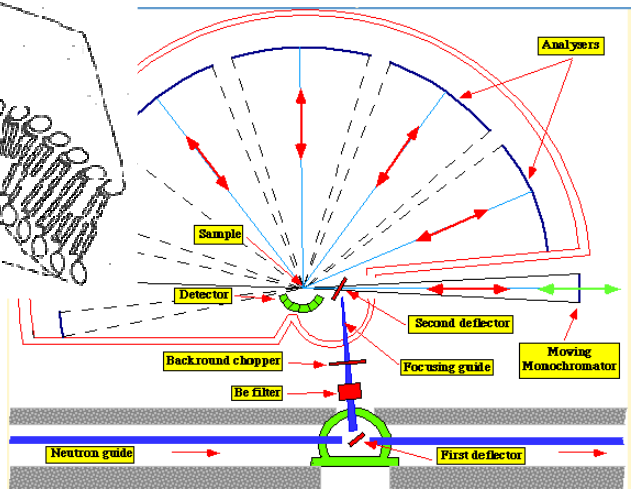


IRIS

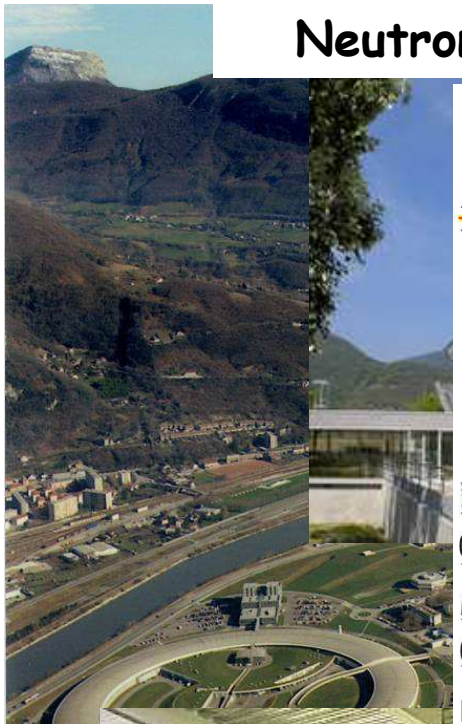
A High Resolution Quasi-Elastic and Inelastic Neutron Scattering Spectrometer with long d-spacing diffraction capal

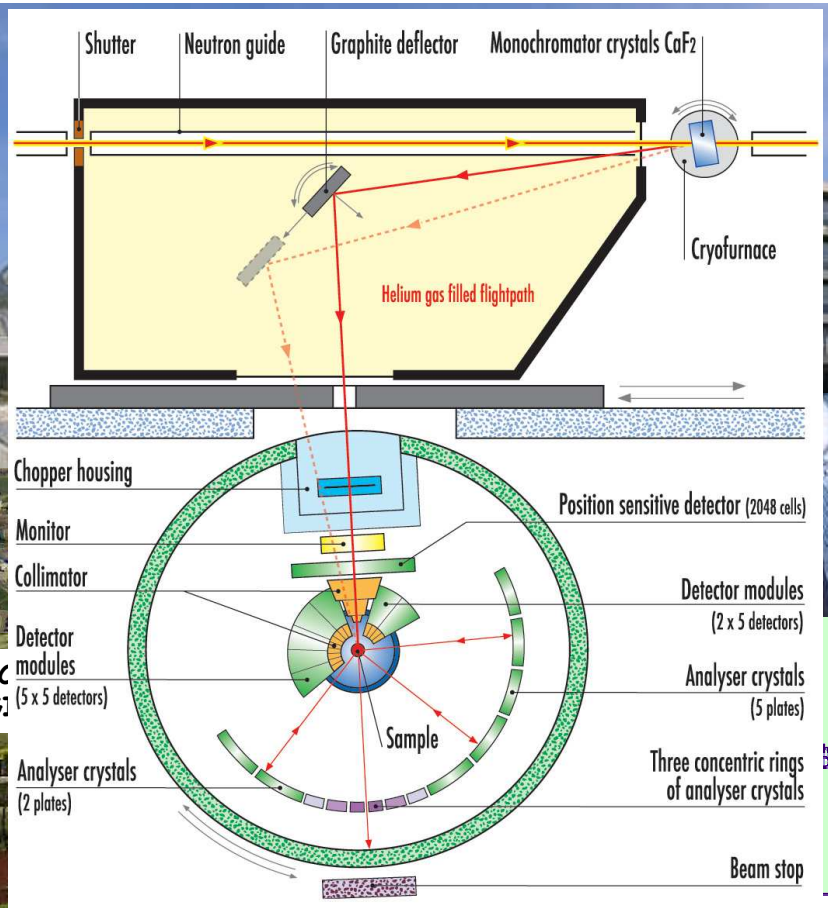




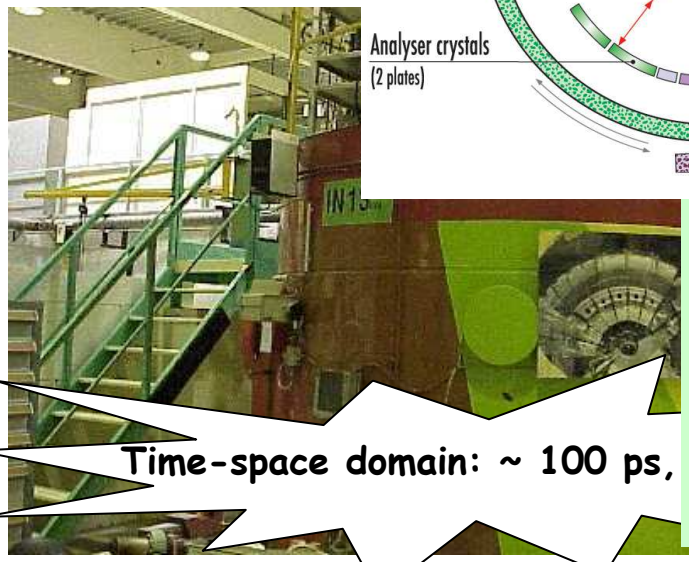


Neutron thermal backscattering spectrometer IN13, ILL

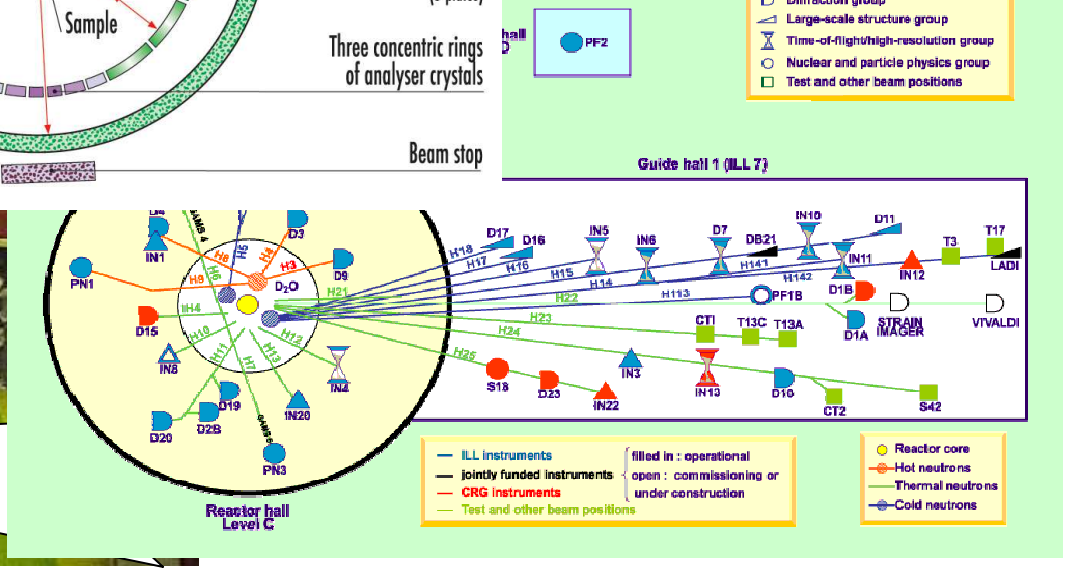




IN13 BACKSCATTERING SPEC
A CRG DEDICATED TO BIOLOGI



Time-space domain: ~ 100 ps,



SCIENTIFIC CRG PROGRAMME

CELLULAR RESPONSE TO EXTERNAL SIGNALS AND EXTREME CONDITIONS
Molecular dynamics of proteins, molecular machines and membranes in living cells

→ **CELLULAR RESPONSE TO EXTERNAL SIGNALS AND EXTREME CONDITIONS**

→ **DYNAMICS OF BIOLOGICAL MEMBRANES AND MODEL SYSTEMS**
- Ganglioside aggregates
- Lipid membranes and lipid-protein interactions
- Purple Membrane

→ **MOLECULAR DYNAMICS OF PROTEINS**
- Dynamics of proteins at high pressure
- Dynamics of folding states of small globular model proteins
- Dynamic properties and structural stability of hemoproteins embedded in silica hydrogels
- Effect of bio-protecting glassy matrices on the biomolecules dynamics

→ **DYNAMICAL PROCESSES IN ORGANISED STRUCTURES OF SACCHARIDE SYSTEMS IN AQUEOUS SOLVENT**

SCIENTIFIC ILL PROGRAMME

→ **BIOLOGICAL STUDIES**

→ **POLYMERS**

→ **INCLUSION COMPOUNDS**

→ **MAGNETIC EXCITATIONS**

→ **PHASE TRANSITIONS**

→ **AMORPHOUS AND GLASSES**

48 ILL news
FOR REACTOR USERS

Workshops & Events

Workshops

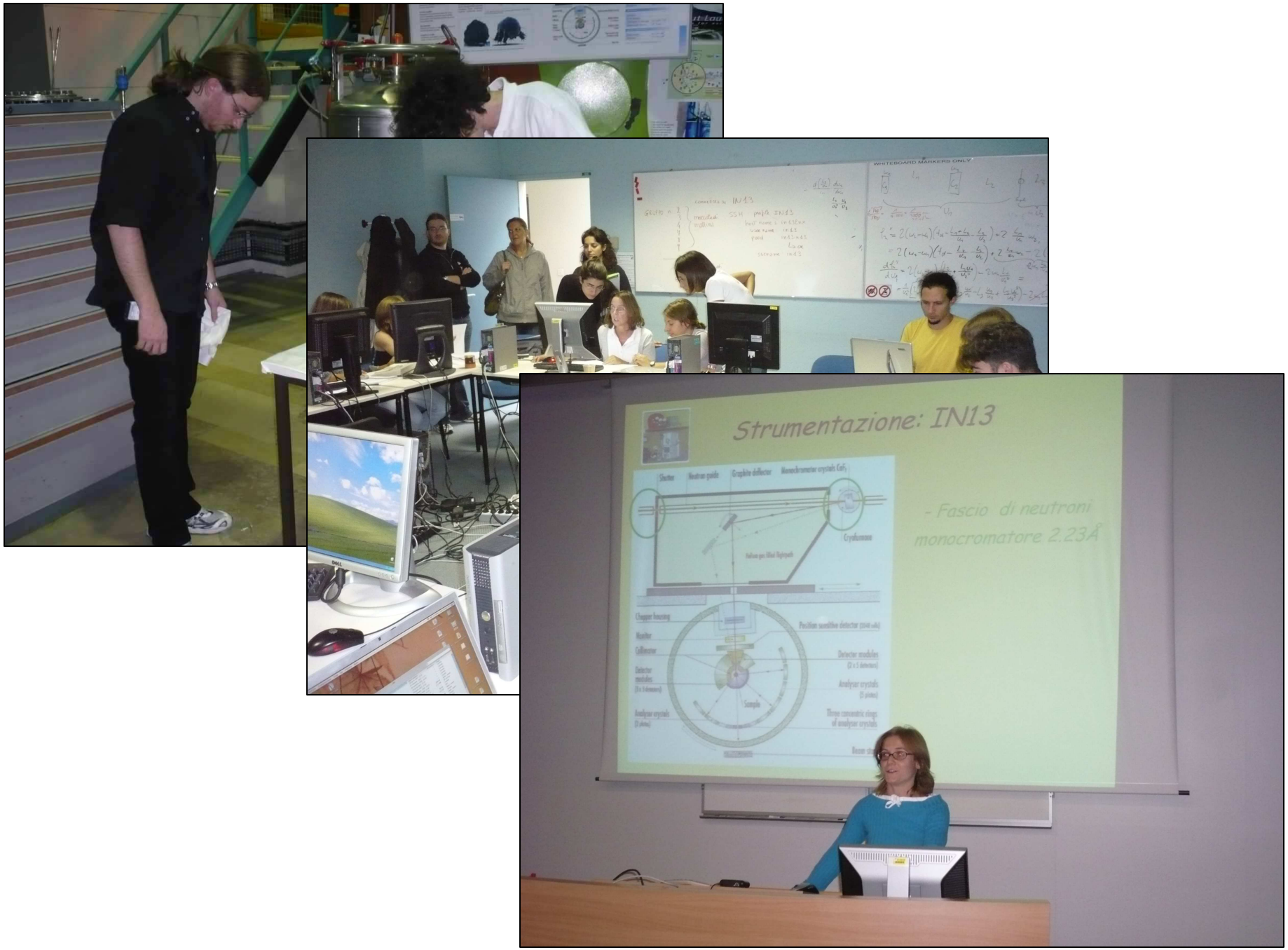
4th Neutron School of the Italian Society of Neutron Spectroscopy

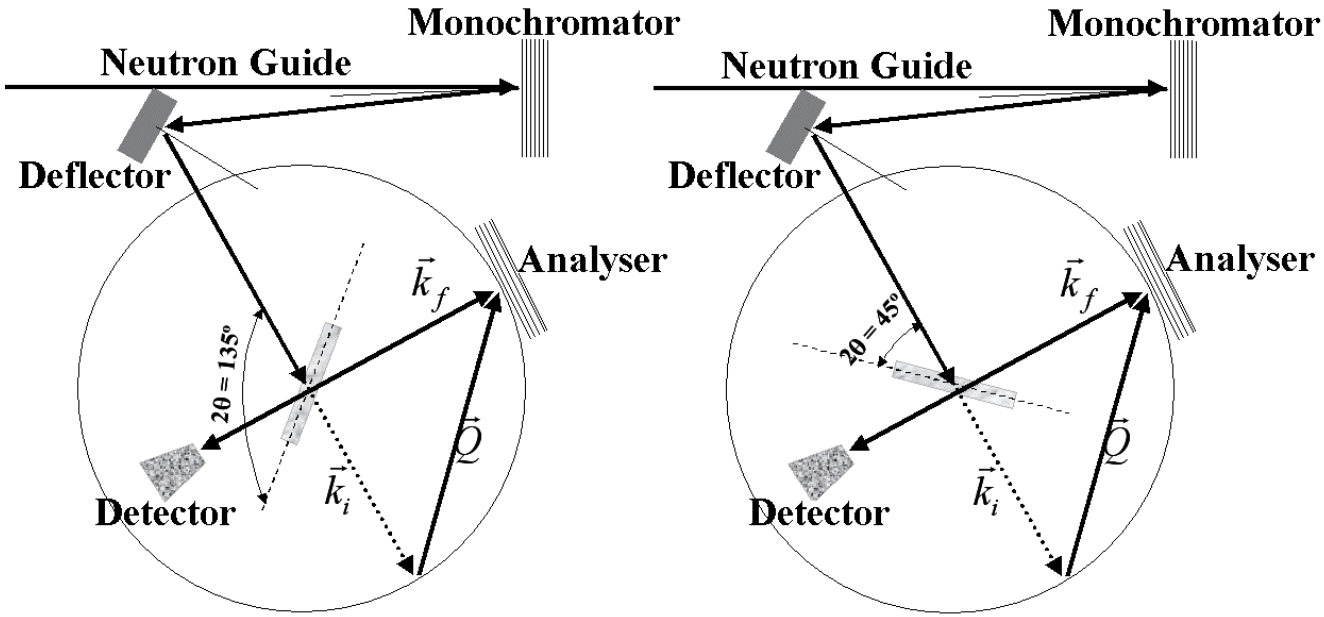
The Italian Neutron School, organised by the Italian Society of Neutron Spectroscopy (SISN) addresses to students, PhDs and young researchers aiming to approach the neutron spectroscopy by learning its performance and power in research application.

The 4th edition of the school – which took place this year - was

Fondazione Mediaterraneo-Convento dell' Annunziata, Sestri Levante (GENOVA)
10-12 settembre 2007
Institut Laue-Langevin, Grenoble (FRANCIA)
15-20 settembre 2007

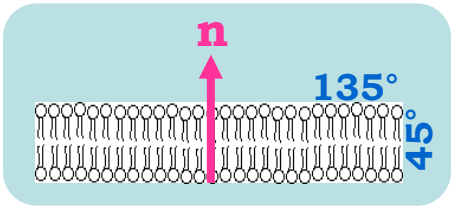
Directors of the School: F. Natali and A. Orecchini


WHITEBOARD MARKERS ONLY
Strumentazione: IN13
- Fascio di neutroni
monocromatore 2.23Å

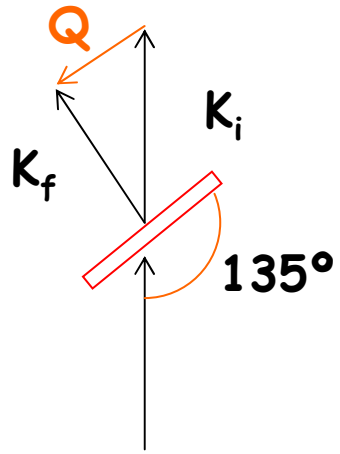


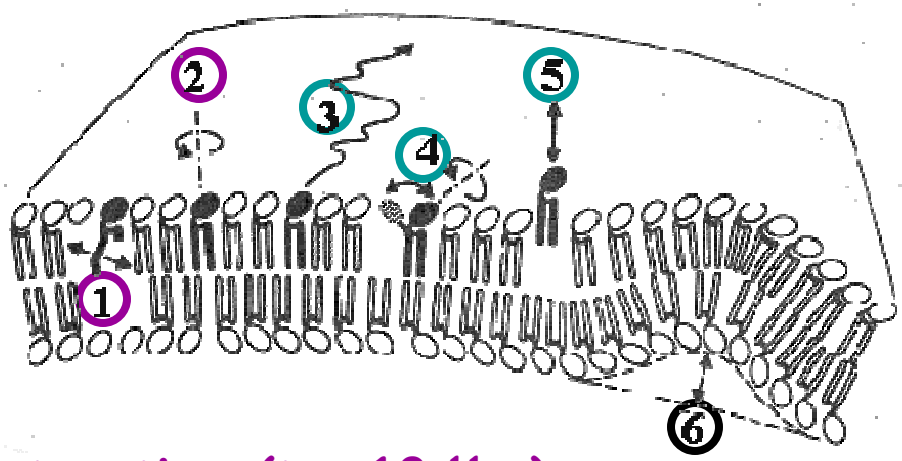
a) In-plane geometry

b) Out-of-plane geometry



Neutron Scattering





1 - Chain defect motions ($t \sim 10^{-11}$ s)

2 - Rotational diffusion about their long molecular axis ($t \sim 2*10^{-11}$ s)

3 - Lateral diffusion in the plane of the bilayers ($t \sim 10^{-9}$ s)

4 - Rotational and flip flop motion of the lipid head groups ($t \sim 3*10^{-9}$ s)

5 - Vertical vibrational motion of the lipid molecules ($t \sim 10^{-9} - 10^{-10}$ s)

6 - **Collective undulations of the bilayer** ($t \sim$ s)

determine the dynamic surface roughness of membranes

Incoherent Elastic Neutron Scattering

IN13: 8 μeV, 1.3-5.5 Å

Fast motion

Incoherent Quasi-Elastic Neutron Scattering

IN16: 0.8 μeV, 0.2-1.9 $Å^{-1}$

Slow motion