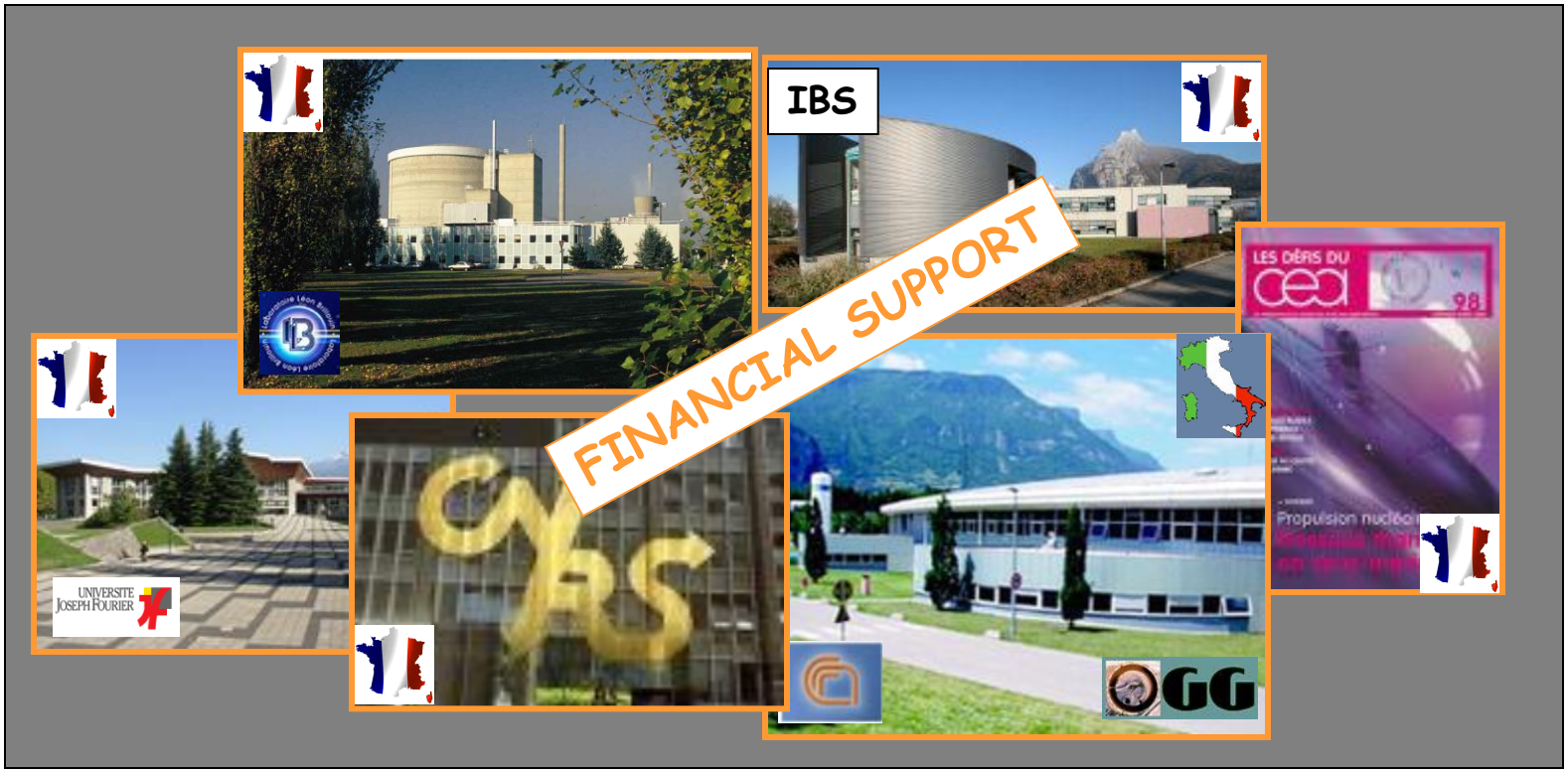
IN13 HIGH ENERGY RESOLUTION BACKSCATTERING SPECTROMETER:

A CRG DEDICATED TO BIOLOGICAL STUDIES

F. NATALI
CNR-IOM & OGG c/o ILL – Grenoble -FRANCE

CONTRACT SITUATION

CRG IN13
CNR
CEA CNRS
IBS LLB UJF
ILL COLLABORATING RESEARCH GROUP



CONTRACT HISTORY

•1st CRG CONTRACT

1st July 1998 – 30th June 2001
3 years
C. Pfister, A. Paciaroni


40 %


60 %

•2nd CRG CONTRACT

1st July 2001 – 30th June 2004
3 years
M. Bèe, F. Natali


60 %


40 %

•3rd CRG CONTRACT

1st January 2006 – 31st December 2010
5 years
F. Natali, J. Peters


55.7 %


44.3 %

BEAM TIME ALLOCATION

CRG of type A

50 %

50 %

22 %

28 %

ORGANISATION AND CONTROLS

Steering Committee (meets once a year)

Composed of:
- the representant of the Presidents of UJF
- one representative of the 3 departments of CNRS ("Vivant", "Chimie" and "MIPPU")
- one representative of the 2 departments of CEA ("Science du Vivant" and "Sciences de la Matière ")
- President of CNR
- 3 representatives of the Italian scientific community chosen by the CNR
- The main Italian responsible & the French co-responsible of the CRG

Its role:
- to evaluate the scientific and financial reports,
- to define the scientific and financial orientations
- to choose the scientific representatives at the SC

Scientific Committee (meets twice a year)

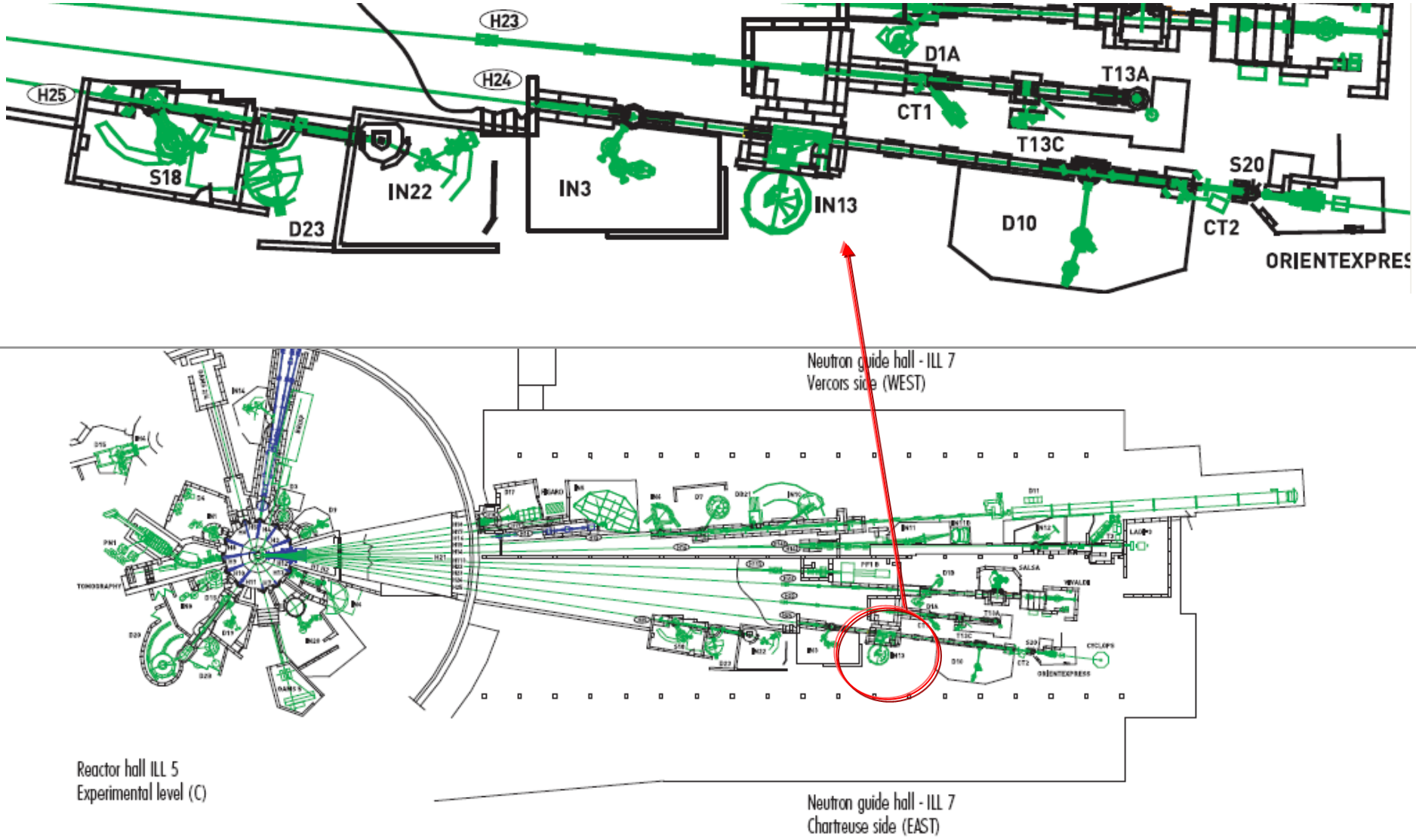
Composed of:
- The two instrument responsibles
- Representatives of scientific groups involved in the CRG (CNR, IBS, LLB, UJF)
- Representatives of the scientific communities

Its role:
To select the proposals submitted within the CRG time

Executive Committee (permanent)

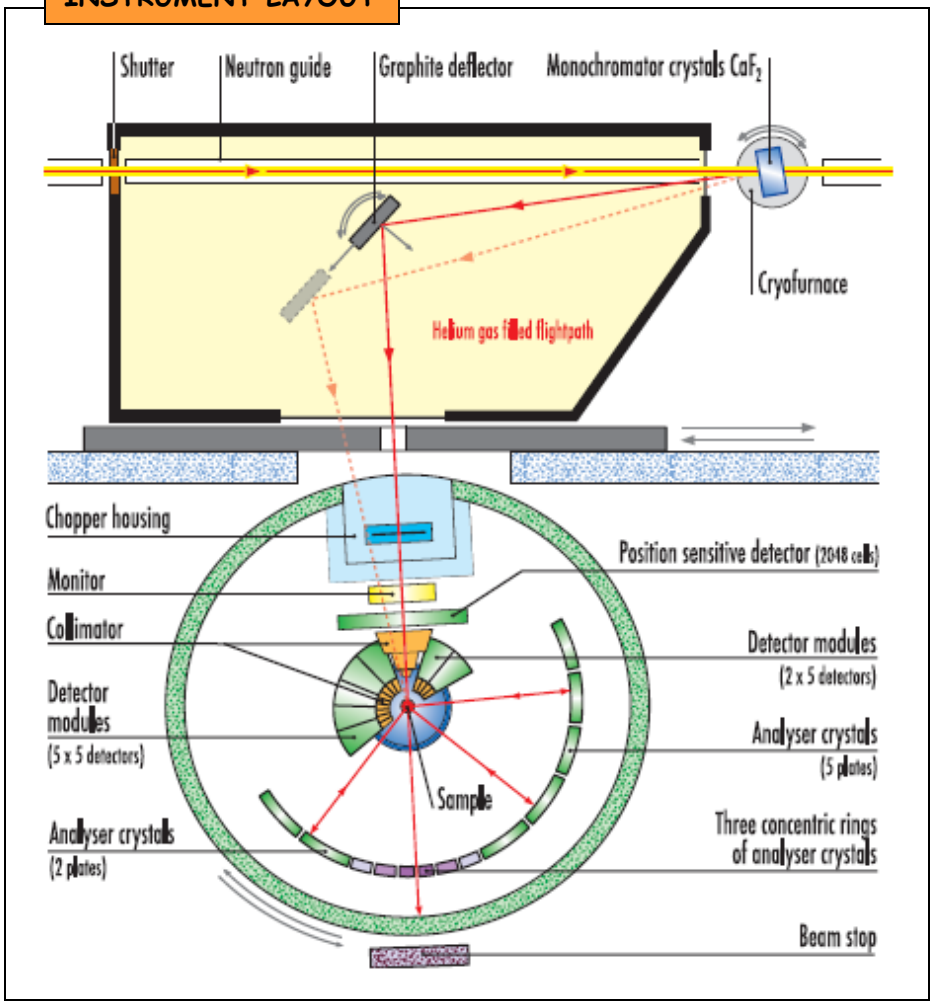
Composed of
- The main project responsible
- The main co-responsible
- CNR responsible of the CRG
- Adjoint responsibles, representatives of French scientific group

Its role:
It is in charge of the global organisation of the CRG (technical needs, staff, budget).


H23
H24
H25
D1A
T13A
CT1
T13C
S20
S18
IN22
IN3
IN13
D23
D10
CT2
ORIENTEXPRES
Neutron guide hall - ILL 7
Vercors side (WEST)
Reactor hall ILL 5
Experimental level (C)
Neutron guide hall - ILL 7
Chartreuse side (EAST)

IN13 IS MAINLY DEVOTED TO LIFE SCIENCES, IN PARTICULAR TO THE STUDY OF THE DYNAMICAL FEATURES OF MACROMOLECULAR COMPOUNDS IN THE MICROEV ENERGY REGION, BUT SCIENTIFIC APPLICATIONS CAN BE ALSO FOUND IN AREAS OF MATERIALS SCIENCE, SOLID-STATE PHYSICS, CULTURAL HERITAGE AND CHEMISTRY.

INSTRUMENT LAYOUT



INSTRUMENT DATA

Guide hall n° 1, thermal guide H24

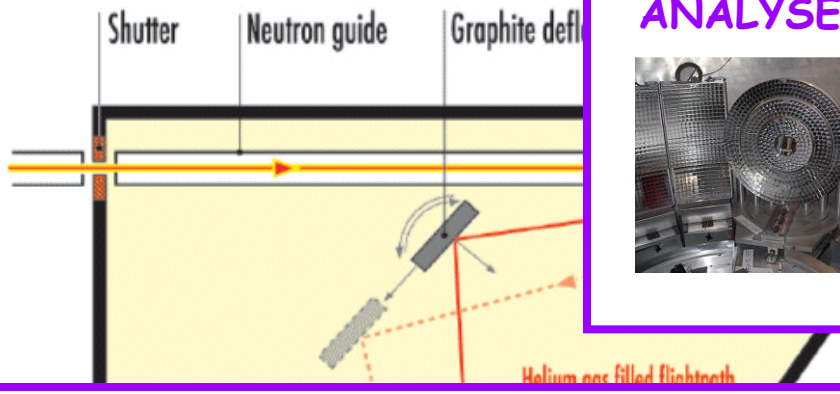
monochromator	
$CaF_2(422)$	
temperature range	$-196 < T_M/°C < 450$
energy range	$-125 < \Delta E/\mu eV < 300$
angular range	$81° < \theta_M < 89°$
incident energy (T_M 25°C)	16.45 meV
incident wavelength (T_M 25°C)	2.23 Å
energy resolution	8 μeV

deflector

sample	
sample size	3.5 x 3.5 cm^2

analyser	
$CaF_2(422)$	
Q-range	$0.2 < Q/Å^{-1} < 4.9$
Q-resolution	$\Delta Q/Å^{-1} < 0.1$

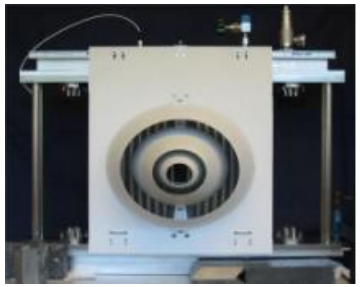
detectors	
2 monitors	
35 ^{3}He detectors	
1 PSD detector for small angles	$0.2 < Q/Å^{-1} < 0.8$



ANALYSERS

CaF_2 crystals.
To select the energy of neutrons scattered by the sample.
Perfect backscattering.

DETECTORS



1 – Monitors
2- Gas counter detectors (He^3 a 10 bar)
3–Position sensitive gas counter detectors (He3 - 6 bar, PSD)

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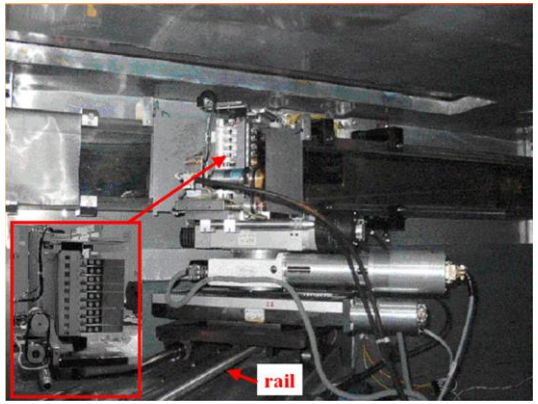
CHOPPE



9 pyrolitic graphite crystals.
Vertically focused.

ings
stals

stop

STAFF OPERATING DURING 2009 ON THE CRG-IN13

RESPONSIBLE

F. NATALI (CNR-IOM)
SCIENTIST - Full time, Permanent position

J. PETERS (Univ. J. Fourier, Grenoble)
PROFESSOR - Full time, Permanent position

PhD STUDENTS

M. TRAPP
(U.J.F., Grenoble)
Since November 2007

W. KNOLL
(U.J.F., Grenoble)
Since June 2009

G. NAGY
(Academy of Science - Budapest)
Since April 2008

TECHNICIAN

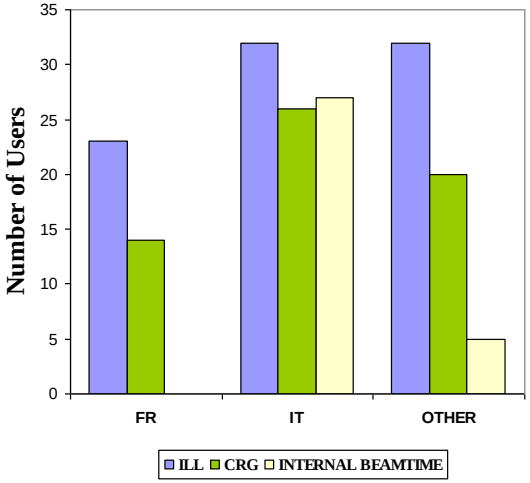
M. LEME (CNRS, Grenoble)
From February 2010

BEAM-TIME STATISTICS of 2006-2009

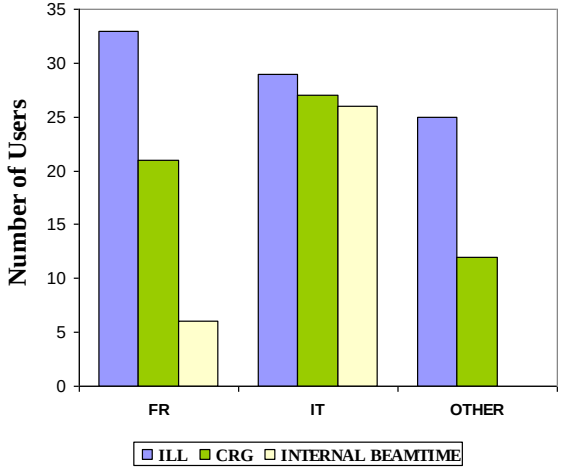
Beamtime Avail. 750 days (including maintenance and commissioning)	BT req.	BT all.	BT req / BT all	# Exp	#public.	# users
ILL	534	315	1.7	48	71	87
CRG	693	327	2.1	50		60
INTERNAL TIME	-----	44	-----	10		32

USER STATISTICS of 2006-2009

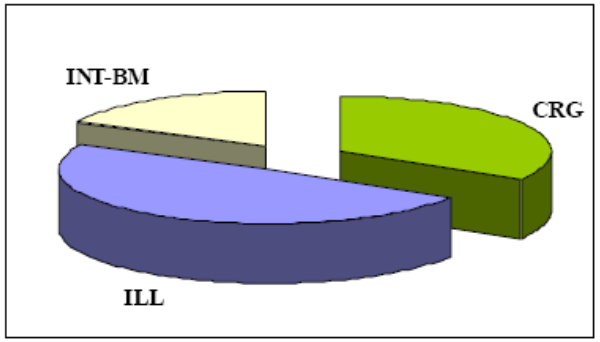
USER NATIONALITY



INSTITUTION

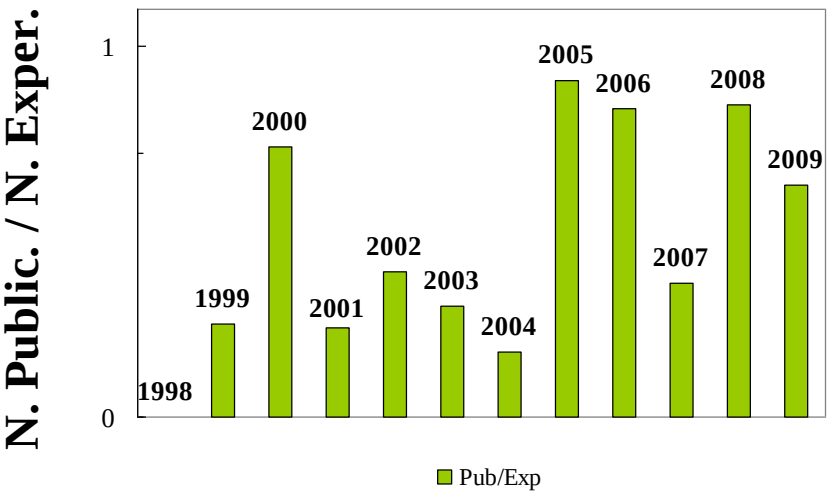


Repartition of the user following their nationality (left panel) and belonging institutions (right panel), performing experiments on IN13 during 2006-2009.

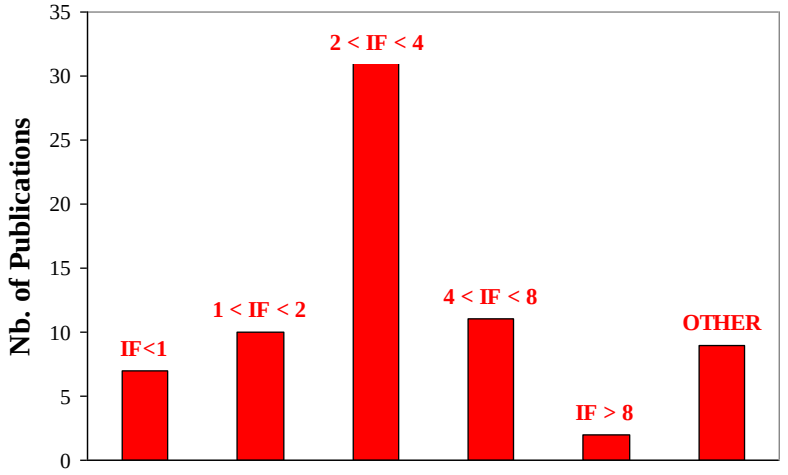


Repartition of the scientific visits registered at ILL during the period 2006-2009 in the ILL/CRG visits.

SCIENTIFIC PUBLICATIONS



Classification of scientific publications of 2006-2009 following the impact factors of the journal

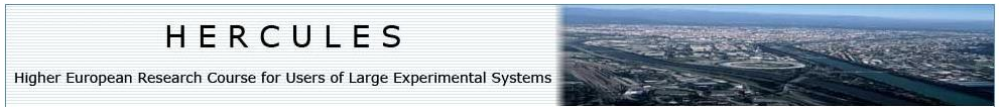


Scientific publications in relation with IN13 from **1998 to 2009** normalised with respect to the number of experiments performed during the same year.

TRAINING PROGRAMME of 2006-2009

The IN13 team is strongly investigating in student's formation. This involves:

- Several 1-day experimental stages of UJF students (Master 1, Physics)
- Several long term stages
- Practical for the Hercules school
- Experimental session of the Italian Neutron School of SISN
- Several PhD students



BUT OF COURSE……PROBLEMS ARE ALWAYS BEHIND THE CORNER!

USE O BEAMTIME

	AVA. DAYS	EXP. DAYS	N. EXP	AVARAGE TIME PER EXP (DAYS)	INTERNAL BT DAYS	INSTR. AND MAINT.	BUFFER DAYS (AT REACTOR RESTARTS)
ILL+CRG	750	642	98	6.5	44	53	11

Tab. 4: Partition of the total available beam time (2006-2009) between experimental days, number of experiments performed, internal dedicated beam-time, maintenance - upgrade and buffer days.

Only 15 days over 600 available days lost

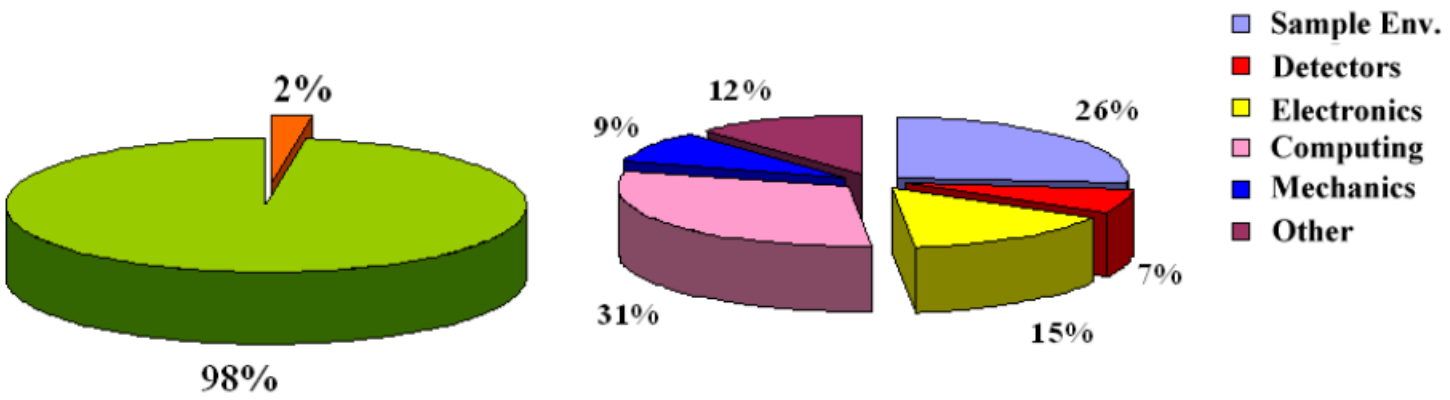


Fig.4: Left panel: lost days with respect to the total available days during 2007-2009.
Right panel: Repartition of the technical problems occurred during 2009 over the different ILL services.

UPGRADES, MAINTENANCE, …

Component		Cost in K€ (TTC)	Gain	Status
Monitor2 automatism		3.5	Precision and reproducibility.	Operational since 2008.
Adjustable slits		8.8	Flexible adaptation of the beam size at the sample position.	Operational since 2006
Encoders for the deflector (6)		3	Reliability of deflector movements.	Operational since 2008
CCD scintillator cameras		4.7	Faster and precise alignment of the sample	Operational since 2008
Renewal of the global detectors electronics (including: time of flight unit & racks)		35	Exploitation of new research area of investigation.	Operational since 2007-2008
Displex (including heating insert, new electronics rotating and 2 standard sample sticks)		50	Cheaper in consumption and easy in use.	Operational since 2008
High pressure cell		14	Exploitation of new research area of investigation.	Operational since 2007-2008
Minor upgrades	- Motors and encoders cables change and reorganization - Endless screw - Nitrogen level sensor + monitor - Helium tide deflector housing	4.1 0.9 1.1 0.5		

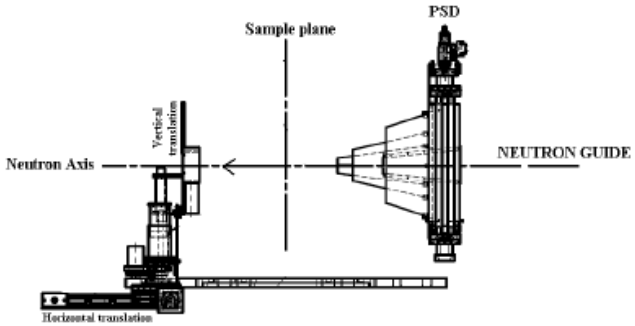


Fig. 16: scheme of the two-axis motorization of monitor2 for transmission measurements.

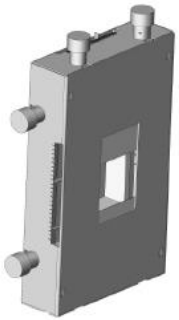


Fig. 17: scheme (left panel) and picture (right panel) of the adjustable slits.

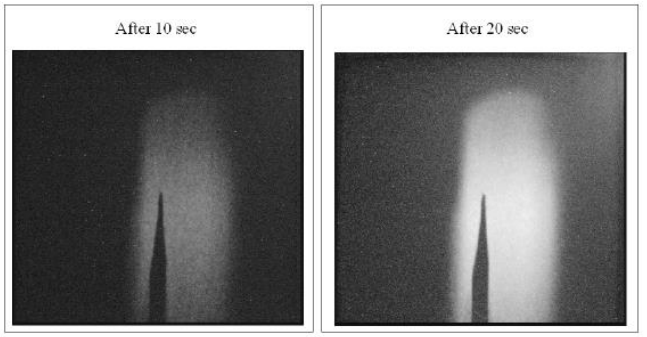
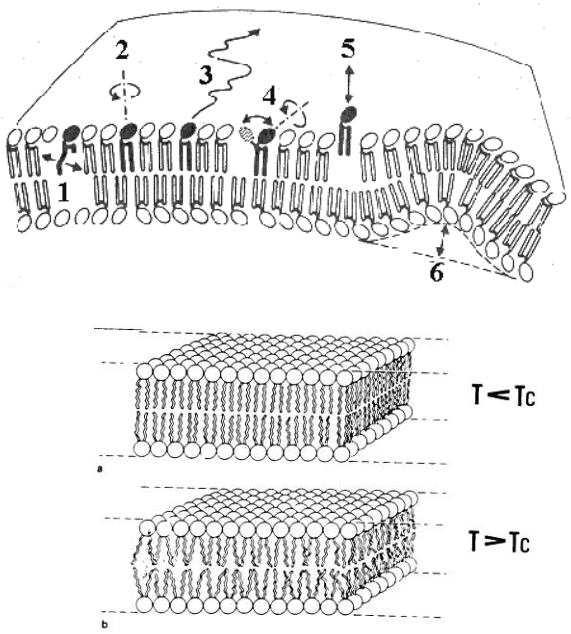
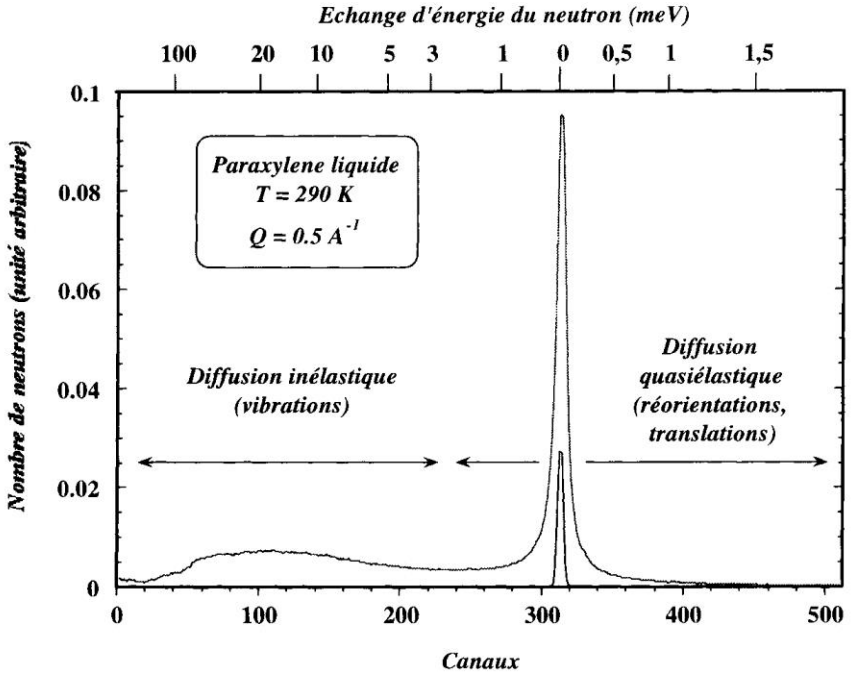
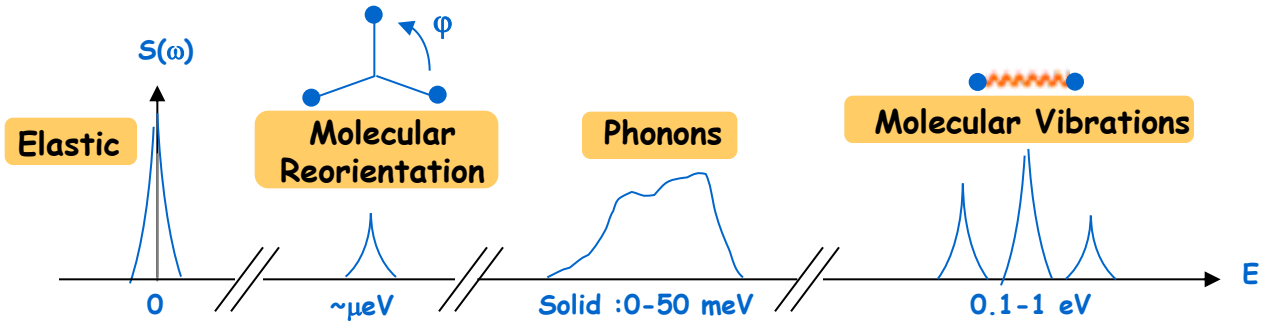


Fig. 21: Aa couple of pictures taken on IN13

THE DYNAMICS



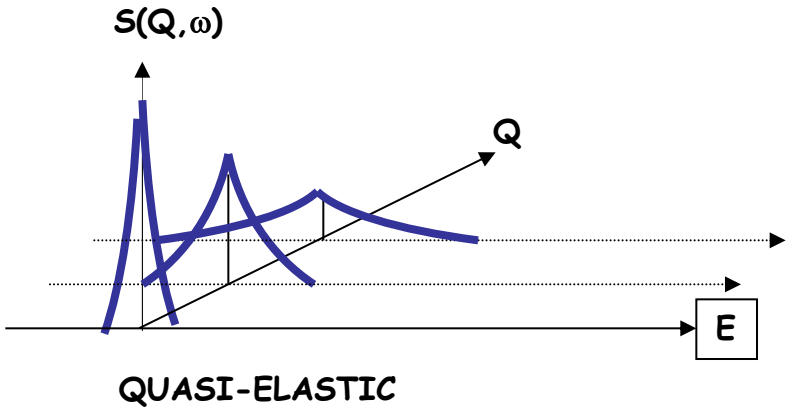




Quasi-elastic neutron scattering

$|k_i|$ = variable, $|k_f|$ = fix

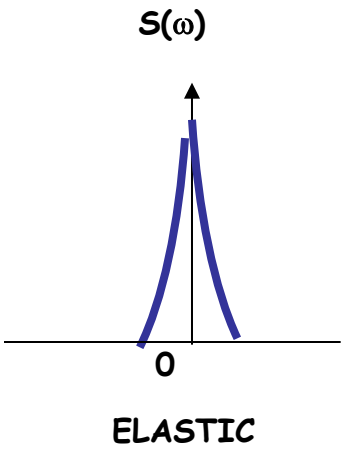
$\hbar\omega = E_f - E_i$



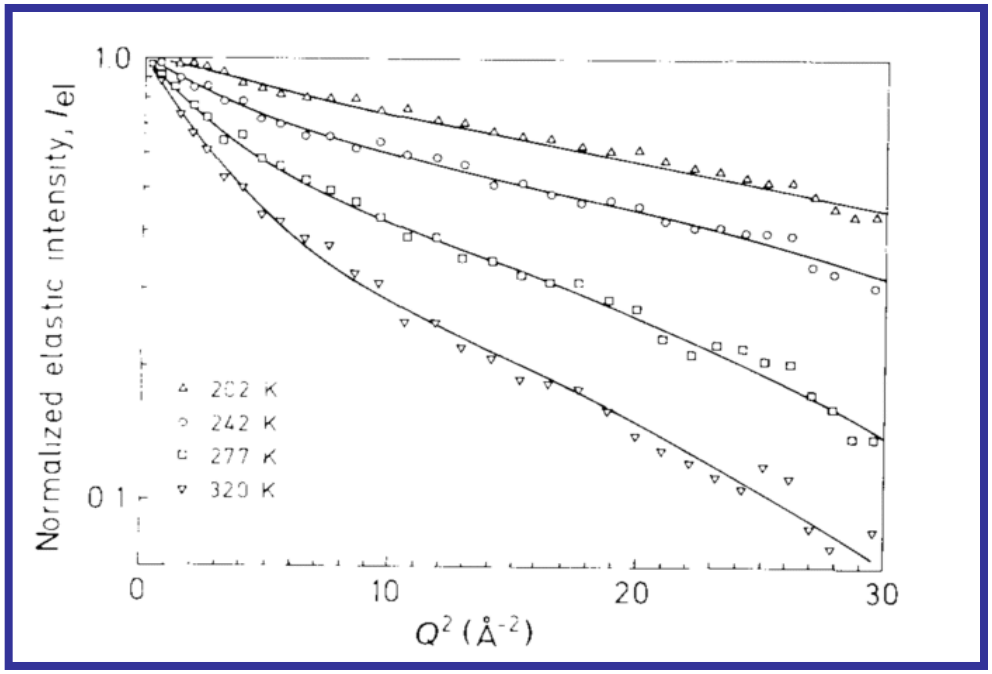
Elastic neutron scattering

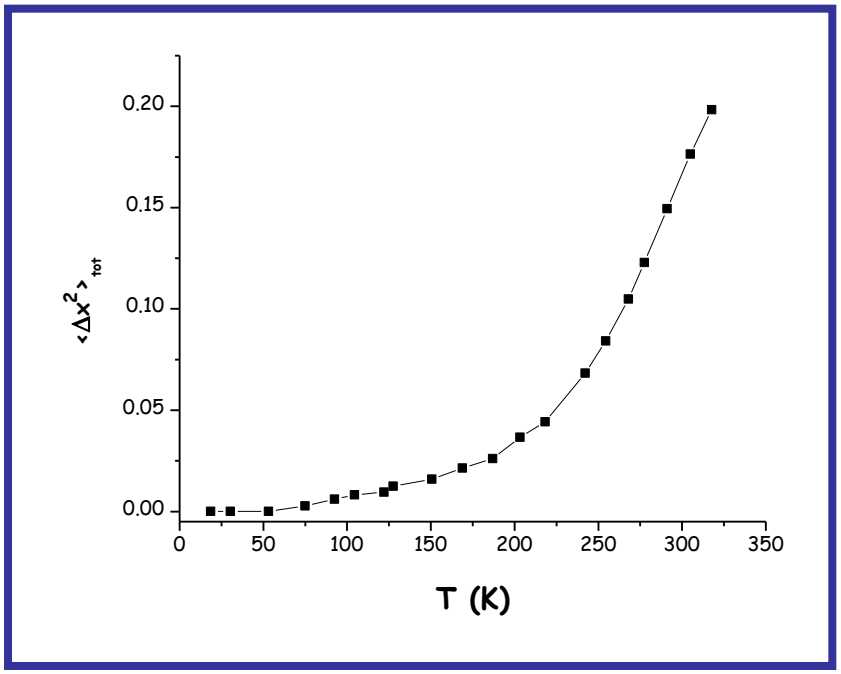
$|\vec{k_i}| = |\vec{k_f}|$ = fix

$\hbar\omega = E_f - E_i = 0$



ELASTIC NEUTRON SCATTERING DATA TREATMENT





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

Gaussian approximation

DOUBLE WELL MODEL

NATURE VOL. 337 23 FEBRUARY 1989

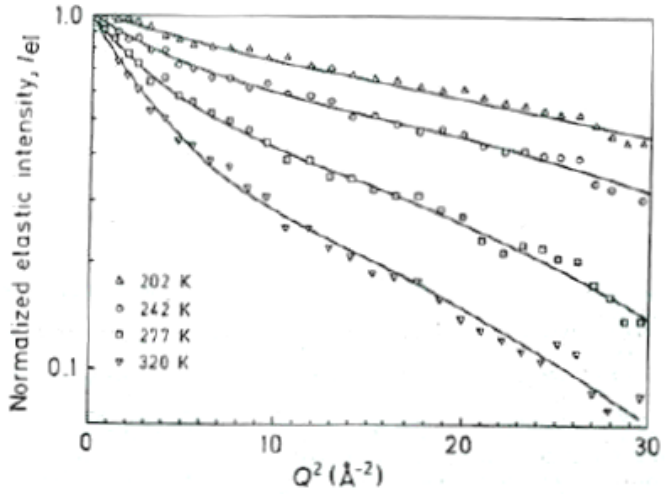
Dynamical transition of myoglobin revealed by inelastic neutron scattering

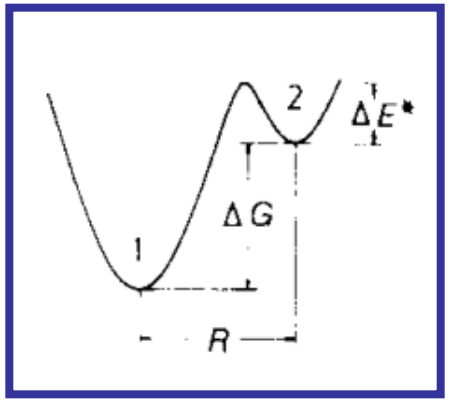
Wolfgang Doster*, Stephen Cusack† & Winfried Petry‡

* Physik Department E13, Technischen Universität München, D-8046 Garching, FRG
† EMBL Grenoble Outstation, Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France
‡ Institut Laue-Langevin, 156X, 38042 Grenoble Cedex, France

Structural fluctuations in proteins on the picosecond timescale have been studied in considerable detail by theoretical methods such as molecular dynamics simulation[1,2], but there exist very few experimental data with which to test the conclusions. We have used the technique of inelastic neutron scattering to investigate atomic motion in hydrated myoglobin over the temperature range 4–350 K and on the molecular dynamics timescale 0.1–100 ps. At globular proteins) suggests a coupling of fast local motions to slower collective motions, which is a characteristic feature of other dense glass-forming systems.

Inelastic neutron scattering is a spectroscopic technique which can be used to study protein motions on exactly the same timescale (0.1–100 ps) as is now widely accessible by computer simulation[11]. Because of the anomalously large incoherent neutron cross-section of the ^1H nucleus, the method specifically probes the motion of hydrogen atoms. As hydrogens are abundant and are uniformly distributed in proteins, the method gives a global view of protein dynamics. The quantity measured experimentally is the incoherent dynamic structure factor $S(\mathbf{q}, \omega)$, where $\hbar\mathbf{q}$ and $\hbar\omega$ are respectively the momentum and energy transfers between system and incident neutron. $S(\mathbf{q}, \omega)$ is the Fourier transform of the time-correlation function of the density fluctuations in a system and can be directly calculated from the results of a molecular dynamics simulation. For samples of myoglobin hydrated with D_2O, we have measured as a function of temperature both the elastic intensity $S(q, \omega \approx 0)$, which gives information on the geometry of motions, and $S(q, \omega > 0)$, which gives the timescale (or spectrum) of the corresponding diffusive and vibrational motion[12,13].



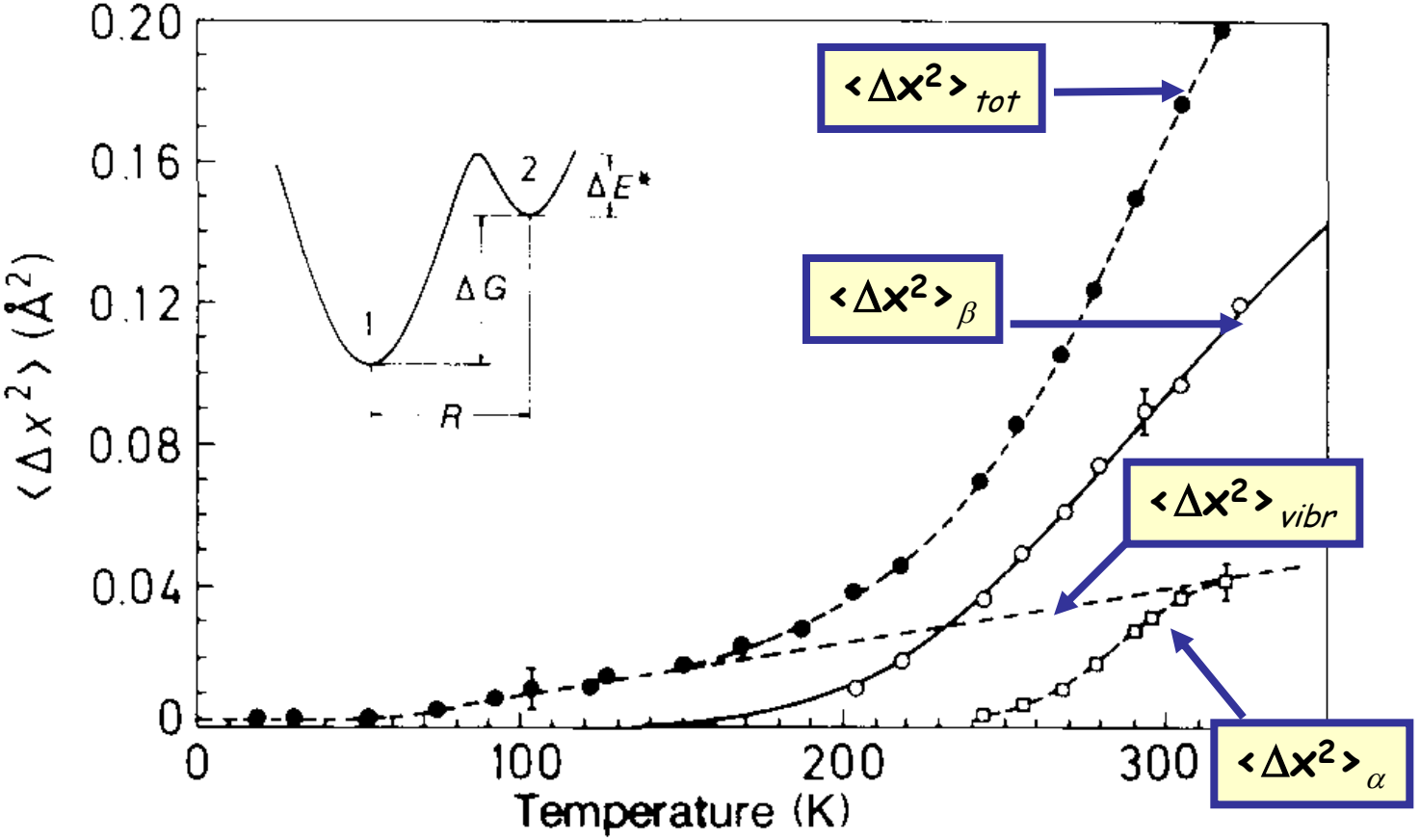


$$S(q,0) = e^{-q^2\langle\Delta x^2\rangle_G}\left\{1 - 2p_1p_2\left(1 - \frac{\sin(qd)}{qd}\right)\right\}$$

$$\langle\Delta x^2\rangle = -\left(\frac{d\ \ln\{S(q,0)\}}{d(q^2)}\right)_{q=0}$$

$$= \langle\Delta x^2\rangle_G + \frac{p_1p_2d^2}{3}$$

Hydrated Myoglobin powder:



$$\langle \Delta x^2 \rangle_{tot} = \langle \Delta x^2 \rangle_{vibr} + \langle \Delta x^2 \rangle_{\alpha} + \langle \Delta x^2 \rangle_{\beta}$$

$\langle \Delta x^2 \rangle_{vibr} + \langle \Delta x^2 \rangle_{\alpha} = \langle \Delta x^2 \rangle_{G}$

$\langle \Delta x^2 \rangle_{\beta} = p_1 p_2 d^2/3$

$\Delta H = (12 \pm 2)$ *Kj/mol*
$\Delta S/R = 3.0 \pm 0.1$

BICOUT et ZACCAI MODEL

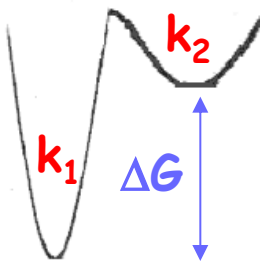
Biophysical Journal Volume 80 March 2001 1115–1123

Protein Flexibility from the Dynamical Transition: A Force Constant Analysis

D. J. Bicout*† and G. Zaccai†‡

*INFM-Operative Group Grenoble CRG IN13 and †Institut Laue-Langevin, 38042 Grenoble Cedex 9, France; and ‡Laboratoire de Biophysique Moléculaire, Institut de Biologie Structurale, F-38027 Grenoble Cedex 1, France

For the sake of simplicity we introduce the model by assuming that all particles are dynamically equivalent, i.e., $x_\alpha = 1$. In the spirit of mode coupling theory (Götze and Sjögren, 1992), the model assumes that two classes of conformational fluctuations essentially control atomic motions in a protein in the native state. First are fluctuations of the local environment in which the particle undergoes movements about its equilibrium position. Second are interaction-mediated fluctuations of the protein molecule that allow larger excursions of the particle within a cage formed by its neighboring molecules. For a protein atom this can be regarded as two, i.e., a large and a small, conformational cages fitting together within which it is compelled to move in a potential $V(\mathbf{r})$. Many conformational substates for the protein in the native state may correspond to the s position $\mathbf{r}$ of the particle at time t.



$$\langle R^2(T)\rangle \simeq \begin{cases} k_B T/k_1; & \theta < T < T_0, \\ k_B T/k_3 - a^2; & T_0 < T \sim T_m, \end{cases}$$

$$\langle R^2(T)\rangle = [1-\phi(T)]\frac{k_B T}{k_1} + \phi(T)\frac{k_B T}{k_2};$$

where

$$\phi = \frac{1}{1+e^{\beta\Delta G}};$$

$$\frac{1}{k_3} = \frac{[1-\phi(T_t)]}{k_1}\left\{1 - \frac{\Delta H}{k_B T_t}\phi(T_t)\right\} + \frac{\phi(T_t)}{k_2}\left\{1 + \frac{\Delta H}{k_B T_t}[1-\phi(T_t)]\right\},$$

$$a^2 = \Delta H\left(\frac{1}{k_2} - \frac{1}{k_1}\right)\phi(T_t)[1-\phi(T_t)].$$

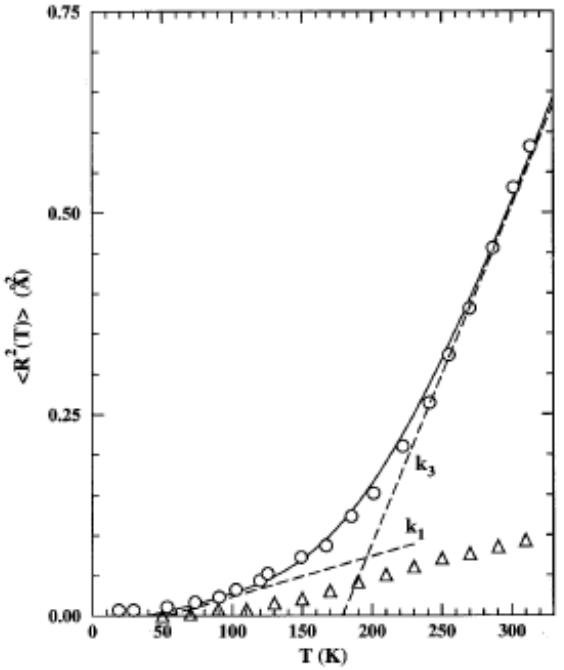


FIGURE 1 Hydrogen mean-square displacement $\langle R^2(T)\rangle = 3\langle x^2(T)\rangle$ versus temperature for hydrated myoglobin (*circles*) (from Doster et al., 1989). The solid line is the best fit to the data (*circles*) using the expression in Eq. 12b with parameters listed in Table 1. The quoted dashed curves correspond to linear approximations in Eq. 15 with the force constants k_1 and k_3 (see Table 1). The triangles represent elastic scans for the trehalose-coated CO-myoglobin (from Cordone et al., 1999). Note the absence of the dynamical transition for trehalose-coated CO-myoglobin.

DISPLACEMENTS DISTRIBUTION MODEL

Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochimica et Biophysica Acta 1749 (2005) 173 – 186
BIOCHIMICA ET BIOPHYSICA ACTA BBA
http://www.elsevier.com/locate/bba

Invited Review

Protein–water displacement distributions

Wolfgang Doster*, Marcus Settles

Technische Universität München, Physik Department E 13 and Institut für Röntgendiagnostik, D-85747 Garching, Germany

Received 3 February 2005; received in revised form 18 March 2005; accepted 18 March 2005
Available online 9 April 2005

Abstract

The statist
one probes e
independent
the displacem
along the pro
vitrified prote
of displacem

Keywords: Pro

$$I(q,T,t_{\mathrm{res}}) = A_1 \cdot \exp\left(-q^2\langle \Delta x_1^2\rangle/2\right) + A_2 \cdot \exp\left(-q^2\langle \Delta x_2^2\rangle/2\right)$$

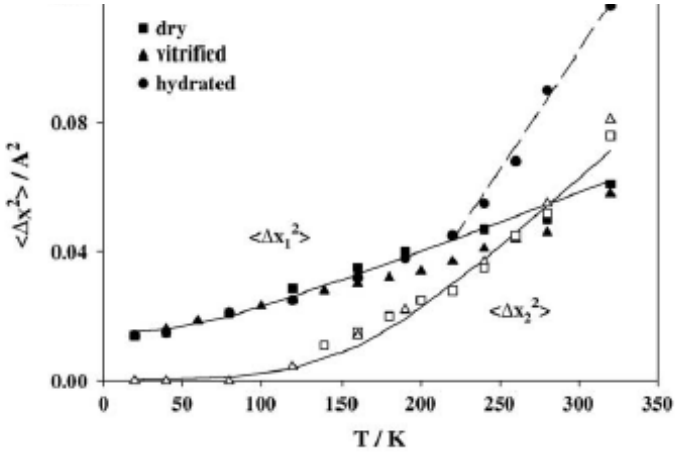


Fig. 7. Second moment of the displacement distribution including the zero-point vibration (myoglobin, dry, D-glucose-vitrified and hydrated (0.35 g/g) at a fixed time of 50 ps, derived from data in Fig. 6. Closed symbols: component 1(vibration + water-induced), open symbols: component 2 (torsion), lines: from fits in Fig. 6, dashed: dynamical transition. Instrument: IN13, ILL.

PRL **95,** 038101 (2005) PHYSICAL REVIEW LETTERS

Onsets of Anharmonicity in Protein Dynamics

J. H. Roh,[1] V. N. Novikov,[1,4] R. B. Gregory,[2] J. E. Curtis,[3] Z. Chowdhuri,[3] and A. P. Sokolov[1]
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[2]*Department of Chemistry, Kent State University, Kent, Ohio 44242, USA*
[3]*National Institute of Standards and Technology, Gaithersburg, Maryland 20899, USA*
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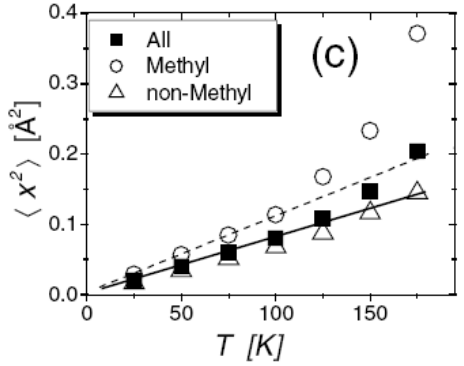
(Received 5 August 2004; published 12 July 2005)

Two onsets of anharmonicity are observed in the dynamics of the protein lysozyme. One at $T \sim 100$ appears in all samples regardless of hydration level and is consistent with methyl group rotation. T second, the well-known dynamical transition at $T \sim 200$–230 K, is only observed at a hydration level greater than ~0.2 and is ascribed to the activation of an additional relaxation process. Its variation wi hydration correlates well with variations of catalytic activity suggesting that the relaxation process directly related to the activation of modes required for protein function.

DOI: 10.1103/PhysRevLett.95.038101 PACS numbers: 87.14.Ee, 87.15.He, 87.15.



(c) Simulated $\langle x^2 \rangle$ for all nonexchangeable protons, methyl protons, and nonmethyl protons in dry lysozyme. Harmonic behavior is shown as a linear extrapolation from low-temperature data for all protons (solid line) and for methyl protons (dashed

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**

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→ **MAGNETIC EXCITATIONS**

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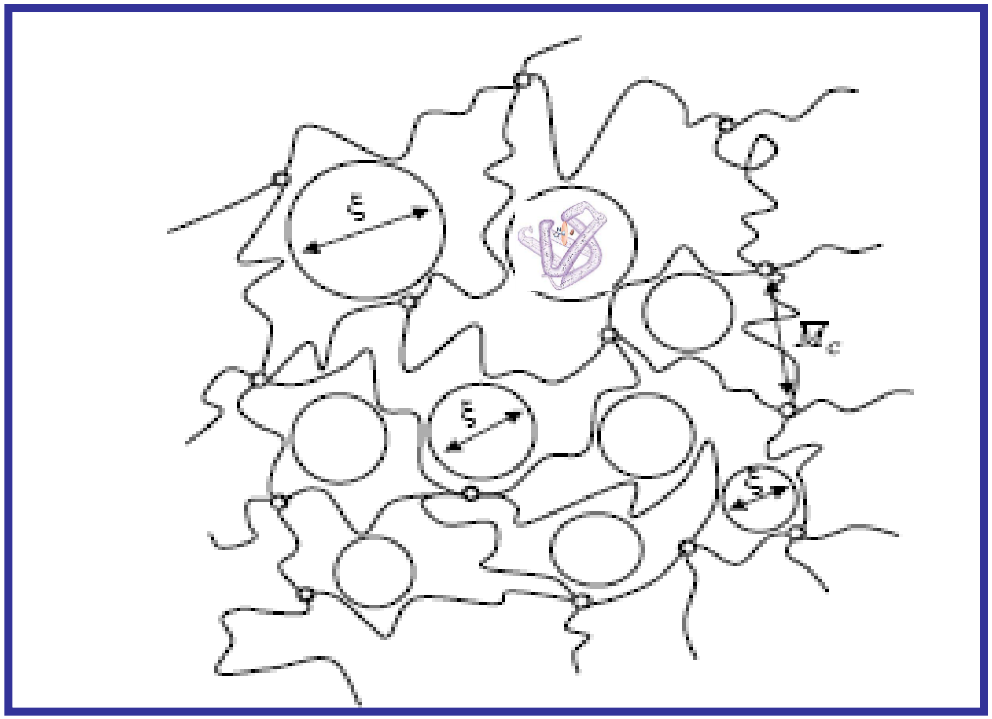
→ **AMORPHOUS AND GLASSES**

Dynamics of myoglobin in confinement:

an elastic and quasi-elastic neutron scattering study

Giorgio Schirò[a], Michele Sclafani[a], Francesca Natali[b], M. Plazanet[c5], and Antonio Cupane[a]



EMBEDDED PROTEINS IN SILICA HYDROGELS MAINTAIN ALMOST UNCHANGED THEIR FUNCTIONAL PROPERTIES, AND SHOWN INCREASED CONFORMATIONAL STABILITY AND RESISTANCE AGAINST DENATURATION.

1- INDUSTRIAL APPLICATIONS: BIOSENSOR

DEVICE THAT COMBINE THE RECOGNITION OF BIOLOGICAL MOLECULES WITH ADVANCED TRANSDUCERS WHICH CONVERT THE BIOLOGICAL SIGNAL INTO A MEASURABLE OUTPUT.

FERRIC HEMOGLOBIN I (HbI) from Lucina pectinata:

PHYSIOLOGICAL RECEPTOR FOR HYDROGEN SULFIDE – A GAS WHICH MAY PROVOKE LOSS OF CONSCIOUSNESS AND DEATH.

MYOGLOBIN:

RECOGNITION ELEMENT FOR THE DETECTION OF NITRIC OXIDE (NO), CYANIDE (CN), CARBON MONOXIDE (CO).

2- BASIC RESEARCH

HOMOGENEOUS AND OPTICAL TRANSPARENCY DOWN TO 5 K.

OPTICAL ABSORPTION MEASUREMENTS ALLOWED AT CRYOGENIC TEMPERATURES.

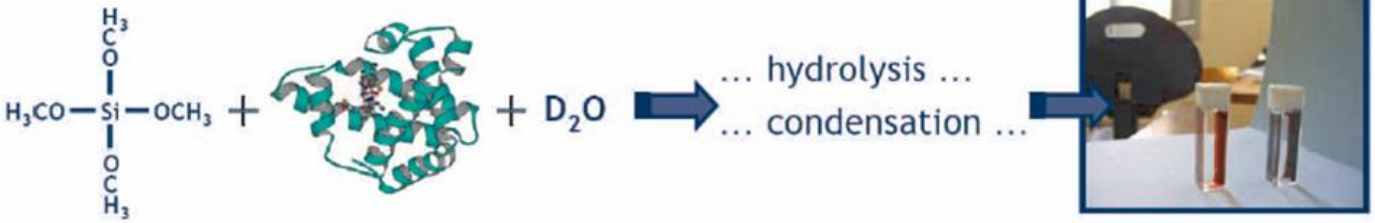
THE PROTEIN IS TRAPPED IN CAGES. ROTATIONS AND GLOBAL CONFORMATIONAL CHANGES ARE RESTRICTED, BUT LOCAL MOTIONS REQUIRED FOR LIGAND BINDING ARE STILL PERMITTED.

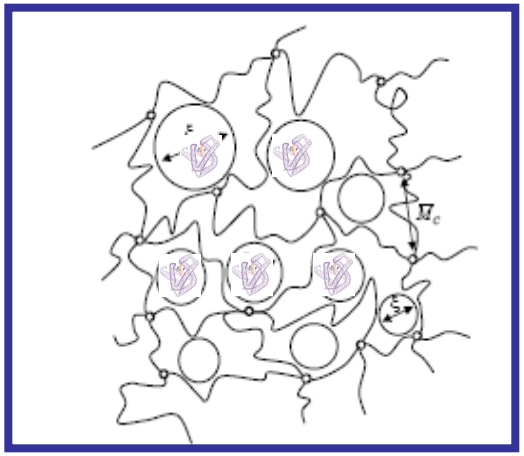
THE PROTEIN CAN BE TRAPPED IN A GIVEN QUATERNARY STATE (T, R) IN THE GEL, AND THE MATRIX SUPPRESS THE R-T INTERCONVERSION.

THE EQUILIBRIUM AND THE KINETICS OF LIGAND BINDING TO THE PROTEIN CAN BE STUDIED IN A GIVEN QUATERNARY CONFORMATION.

UNFOLDING & REFOLDING OF ENCAPSULATED PROTEIN MAY BE FOLLOWED.

Hydrolysis and polycondensation of an alkoxide precursor lead to the formation of a porous and disordered SiO_2 matrix in which water and proteins can be trapped

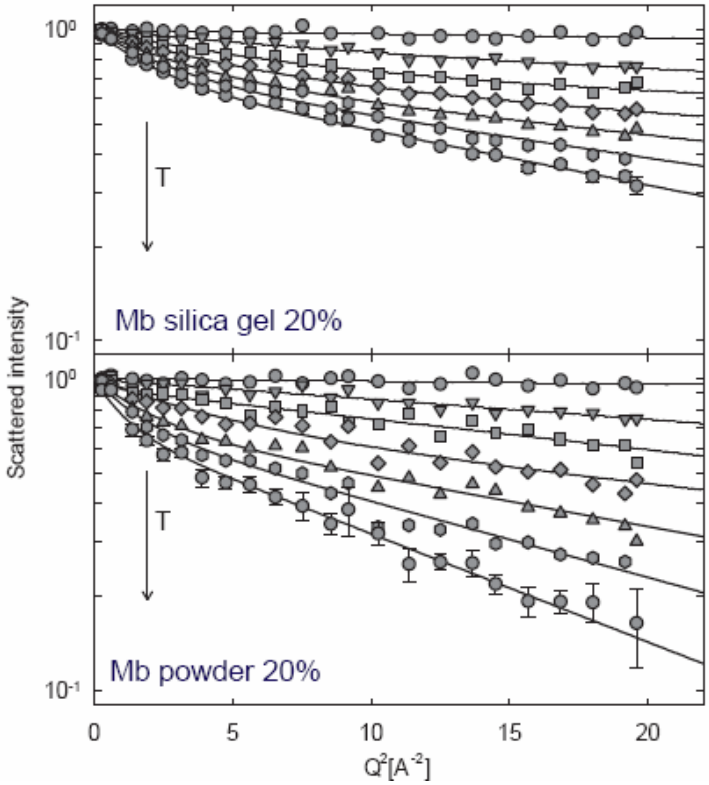


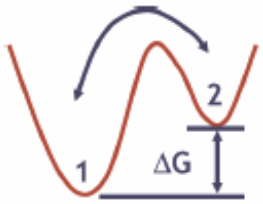


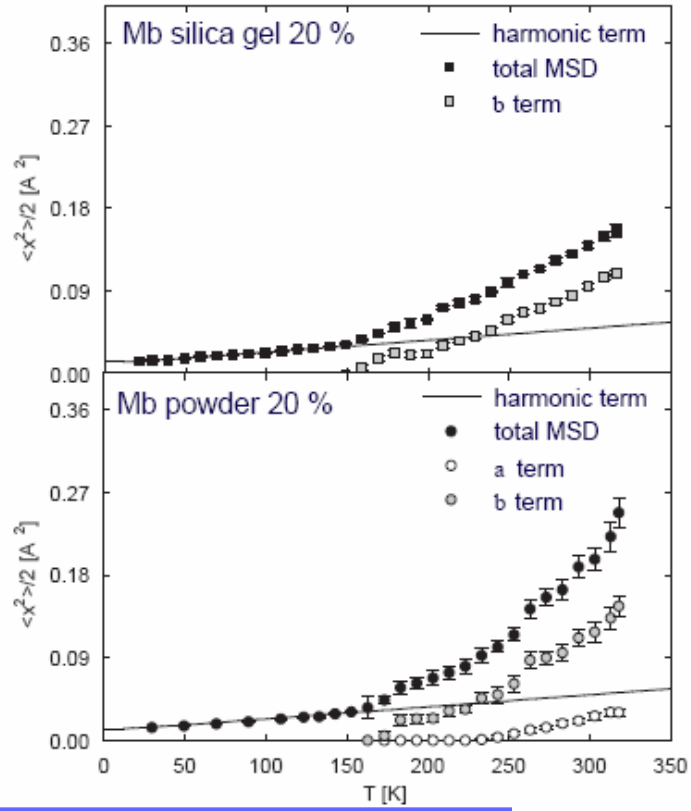
TRIDIMENSIONAL SiO_2 MATRIX: DISTRIBUTION PORES SIZES CENTERED AT 50 ANGS

MYOGLOBIN GIRATION RADIUS: ~ 30 ANGS.

ONE OR NONE MYOGLOBIN MOLECULE TRAPPED IN EACH CAGE + SURROUNDING WATER MOLECULES.







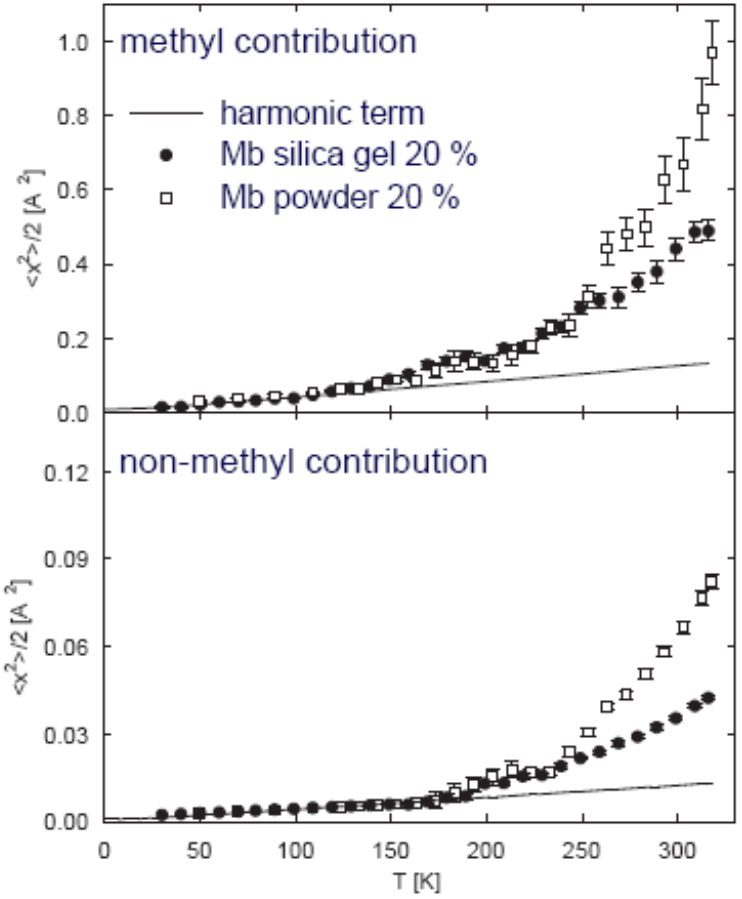
Double well model: $S(Q,0) = I_0 e^{-x_G^2 Q^2}(1 - 2p_1p_2(\frac{sin(Qd)}{Qd}))$

Within the frame of double well model, the decrease of encapsulated protein dynamics at high temperature is due to:

- a quenching of α-relaxations (slow motions?)
- a reduction of β-relaxations

Bimodal distribution:

$$I(q,T,t_{\mathrm{res}}) = A_1 \cdot \exp\left(-q^2\langle \Delta x_1^2\rangle/2\right) + A_2 \cdot \exp\left(-q^2\langle \Delta x_2^2\rangle/2\right)$$

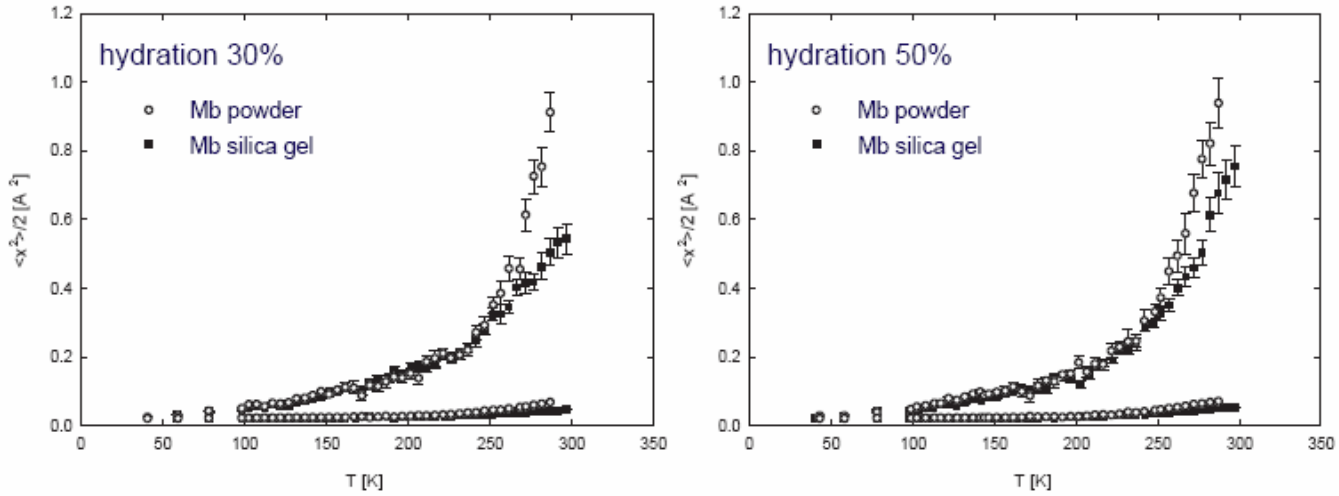


Two onsets of anharmonicity:

- 100-150 K, methyl group rotations (structural assignment supported by observations in polymer systems)
- 200-250K, dynamical transition coupled with solvent dynamics (activation of biological activity?)

T$\geq$250K, MSD of met-Mb powder are systematically larger than those of encapsulated met-Mb

Let's increase protein hydration...



Also at higher hydration levels, encapsulation reduces solvent-coupled motions (probably with a weaker effect), and does not influence low temperature dynamics / structure (activation of methyl group rotations).

Elastic data:

- at all the investigated hydration levels, the internal dynamics of methyl groups is unaffected by encapsulation while the solvent-coupled dynamical activation above 250K is clearly reduced in silica gel
- the analysis in terms of an anharmonic potential suggests that this high temperature effect is due to the hindering of α relaxations in encapsulated proteins

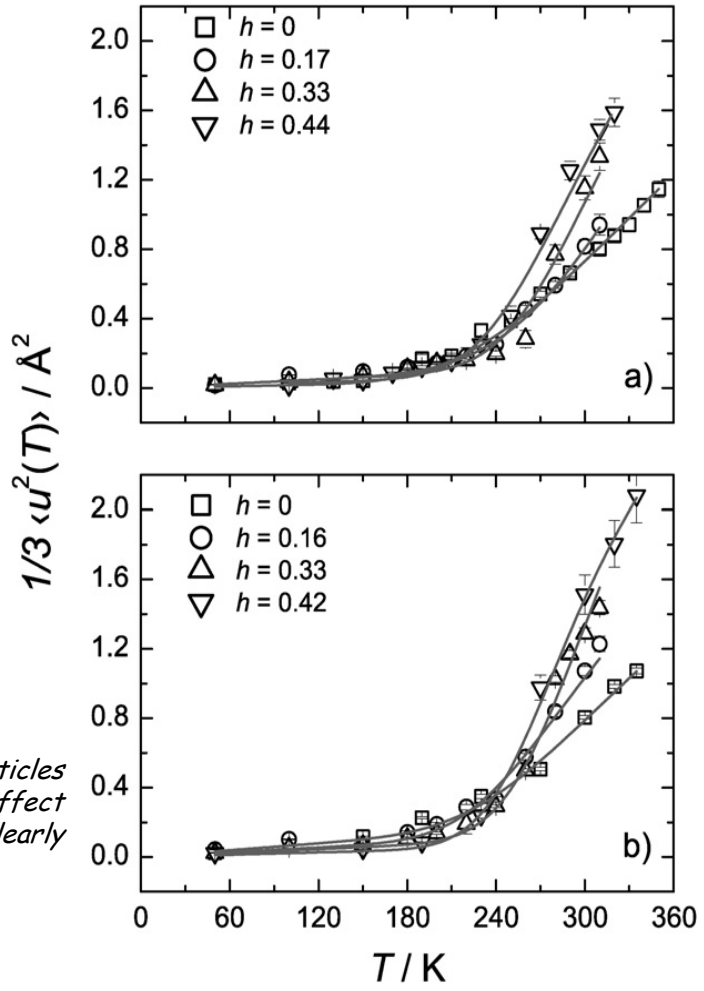
Our results suggest that encapsulation modifies protein dynamics mainly *via* a modification of solvent dynamics

Flexibility and drug release features of lipid/saccharide nanoparticles

Yuri Gerelli,*[a] Maria Teresa Di Bari,[a] Stefano Barbieri,[b] Fabio Sonvico,[b] Paolo Colombo,[b] Francesca Natali[c] and Antonio Deriu[a]

Received 6th August 2009, Accepted 17th November 2009
First published as an Advance Article on the web 21st December 2009
DOI: 10.1039/b916139b

The effect of lipophilic additives (excipients and drugs) on the behavior of lipid/saccharide nanoparticles has been investigated by incoherent elastic neutron scattering. Temperature scans from 20 K to 350 K have been performed on lecithin/chitosan particles loaded with isopropyl myristate and cetyl-stearyl alcohol, two lipophilic molecules with different melting temperatures which are commonly added to improve drug loading efficiency. In a similar way the effect of tamoxifen citrate, a lipophilic drug frequently used in breast cancer therapy, has also been studied. The different melting points of the two excipients affect mostly the low-temperature behavior of the nanoparticles. At physiological temperatures they both improve the particle flexibility. On the other hand addition of tamoxifen leads to stiffer structures and to lower amounts of released drug. The macroscopic features of the drug release appear to be correlated to the microscopic flexibility determined by neutron scattering. The data confirm also the role of chitosan as a stiffening and stabilizing agent of the lipid particles.



Temperature dependence of the MSD for nanoparticles (panel a) and for lecithin vesicles (panel b). The effect of the stiffer structure of the nanoparticle is clearly visible at high hydration h.

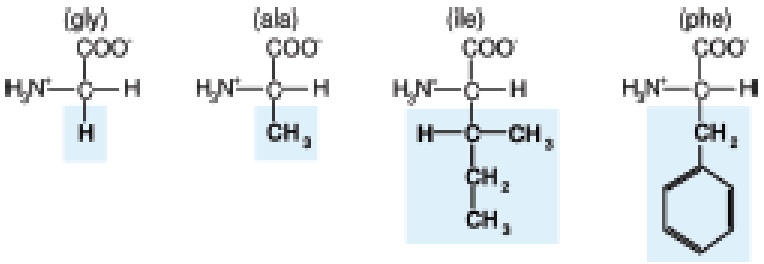
Direct Evidence of the Amino Acid Side Chain and Backbone Contributions to Protein Anharmonicity

Giorgio Schiró,[†] Chiara Caronna,[‡,||] Francesca Natali,[§] and Antonio Cupane*,[†]

Department of Physical and Astronomical Sciences, University of Palermo, via Archirafi 36, I-90123 Palermo, Italy, European Synchrotron Radiation Facility, B.P. 220, F-38043 Grenoble, France, and INFM-CNR OGG and CRS-SOFT, c/o ILL, 6 Rue Jules Horowitz, BP 156-38042 Grenoble, France

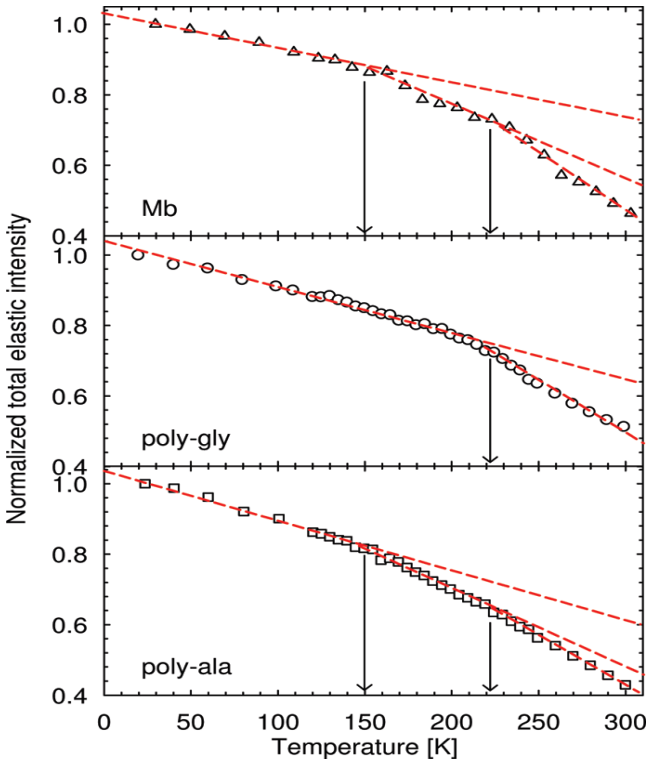
Received October 9, 2009; E-mail: cupane@fisica.unipa.it

Abstract: Elastic incoherent neutron scattering has been used to study the temperature dependence of the mean-square displacements of nonexchangeable hydrogen atoms in powders of a series of homomeric polypeptides (polyglycine, polyalanine, polyphenylalanine and polyisoleucine) in comparison with myoglobin at the same hydration level ($h = 0.2$). The aim of the work was to measure the dynamic behavior of different amino acid residues separately and assess the contribution of each type of side chain to the anharmonic dynamics of proteins. The results provide direct experimental evidence that the first anharmonic activation, at ~150 K, is largely due to methyl group rotations entering the time window of the spectrometer used; however, contributions on the order of 10−20% from the motions of other groups (e.g., the phenolic ring and the methylene groups) are present. Our data also indicate that the dynamical transition occurring at ~230 K can be attributed, at least at the hydration level investigated, mainly to motions involving backbone fluctuations.



The results provide direct experimental evidence that the first anharmonic activation, around 150 K, is largely due to the activation of methyl groups rotations, but that contributions on the order of 10-20 % of motions of other groups (e.g. the phenolic ring and the methylene groups) are present. Our data also indicate that the dynamical transition occurring around 230 K can be attributed, at least at the hydration level investigated, mainly to motions involving backbone fluctuations.

J. AM. CHEM. SOC. 2010, *132*, 1371–1376

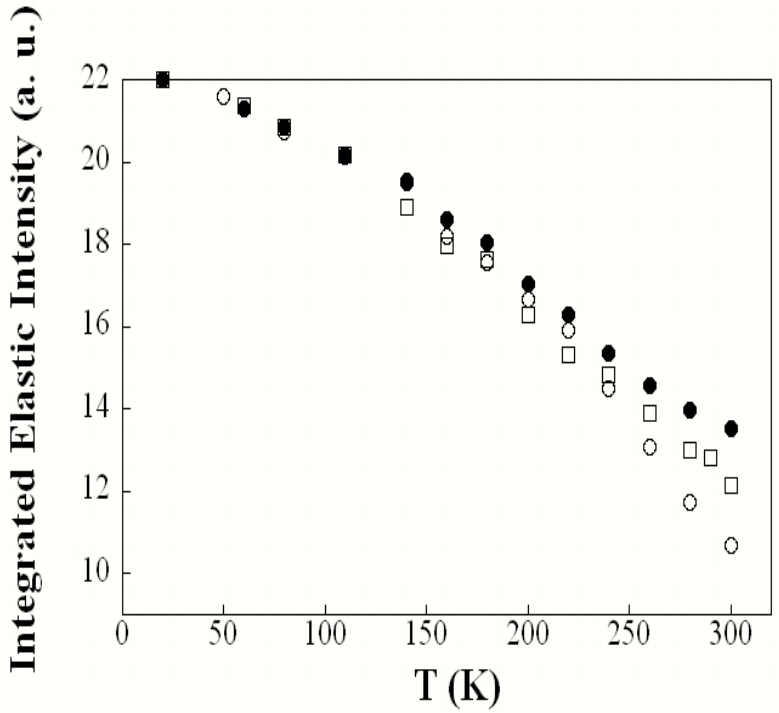


Elastic intensity binned over Q as a function of T for hydrated powders (h=0.2) of myoglobin, polyglycine and polyalanine. The arrows mark the temperatures at which breaks in the temperature dependence are observed.

Study of protein dynamics *vs* amyloid formation

F. Natali, C. Marasini, R. Ferrando, A. Gliozzi
Z. Phys. Chem. 224 (2010) 215-225

Protein fibril formation has been often associated to manifestation of serious and devastating amyloyd diseases, including Alzheimer and BSA. The main mechanism for the formation of amyloid fibrils is the accumulation of protein aggregates in body's organs. In this paper, we try to compare the dynamical behaviour of two amyloidogenic proteins, the Insulin and the Myoglobin. Insulin has been chosen for its pharmacological extensive use in diabetes's therapy, while Myoglobin is used as control, since its dynamics is now largely known. The investigation has been performed through incoherent elastic neutron scattering over a wide temperature range. Our results suggest a stiffer structure for the Ins hexamer with respect to Mb, confirming its enhanced stability, required for pharmacological applications. Explanation in terms of influence of both quaternary structure and water organization around the protein surface have been proposed.

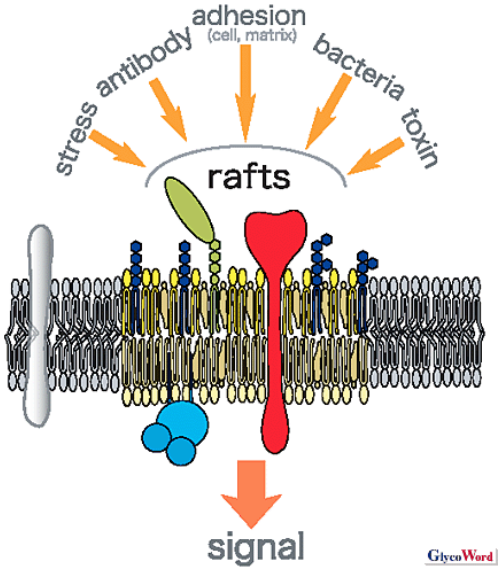


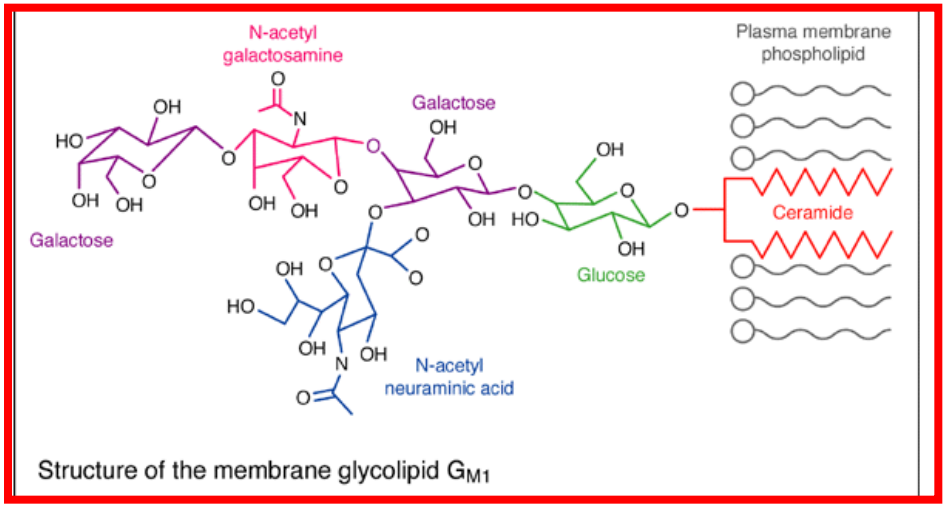
Normalised elastic scattering intensities measured at 110K (filled symbols) and 300K (empty symbols). Circles: dry Mb; squares: hydrated Mb; trian- gles: hydrated Ins.

The dynamics of gangliosides in bilayer domains

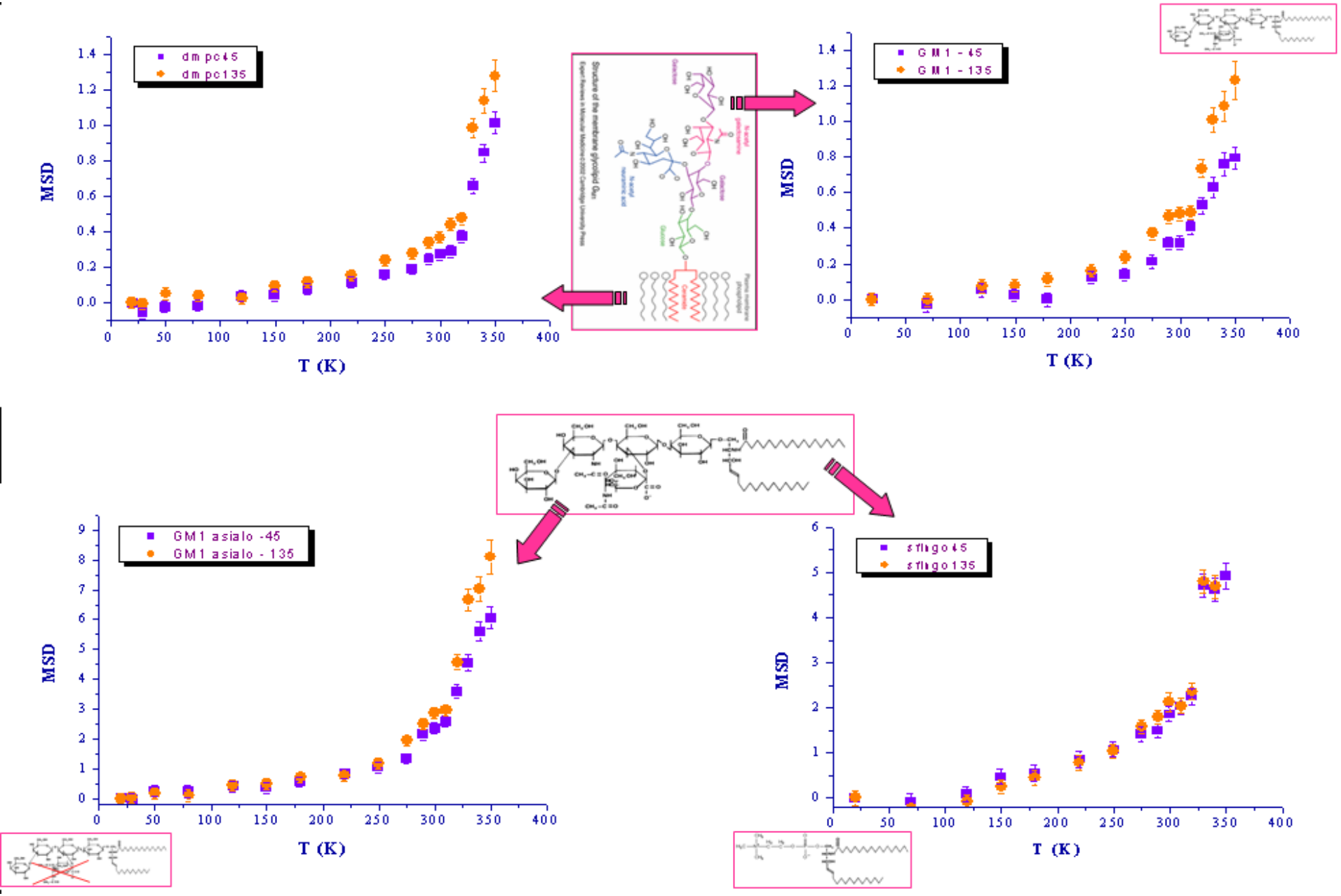
Caronna C.[1], Natali F.[2], Cantu' L.[3], Deriu A.[4], Corti M.[5], Del Favero E.[6], Brocca P.[7]

[1]*ESRF, Grenoble, France and INFM Palermo,* [2]*INFM and CNR-SOFT, OGG Grenoble, France,* [3]*Dept. Medical Chem. Biochem. Biotechn., UNIV. MILANO,* [4]*Physics Dept., UNIV. PARMA and INFM Parma,* [5]*Dept. Medical Chem. Biochem. Biotechn., UNIV. MILANO and INFM Milano,* [6]*Dept. Medical Chem. Biochem. Biotechn., UNIV. MILANO and INFM Milano,* [7]*Dept. Medical Chem. Biochem. Biotechn., UNIV. MILANO and INFM Milano*





Lipid raft are plasma membrane microdomains rich in cholesterol and sphingolipids, which provide a particularly ordered lipid environment. Rafts are involevd in signal transduction and intracellular trafficking. In neurons, lipid rafts act as platforms for the signal transduction. Rafts are also important for neural cell adheion, axon guidance and synaptic transmission.
→ Lipid rafts are structurally unique components of plasma membranes, crucial for neural development and fucntion.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dmpc135
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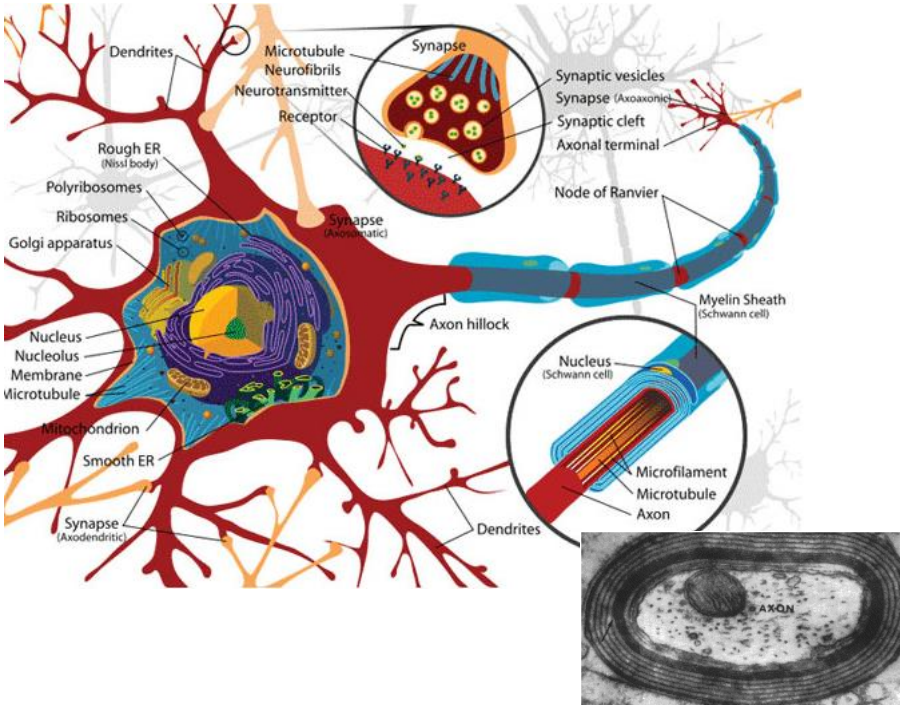
MSD
T (K)
sfingo45
sfingo135
MSD
T (K)

THE ROLE OF MYELIN IN DEGENERATIVE DISEASES

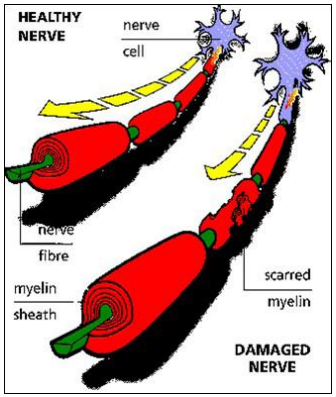
F. NATALI
CNR-IOM & OGG c/o ILL, Grenoble - FRANCE

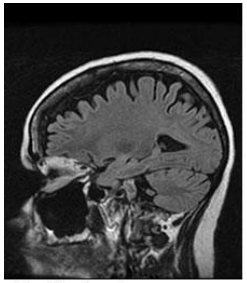
The myelin sheath is a lipid-rich tightly packed multi-layered membrane structure surrounding and insulating the nerve axons in the central nervous system (CNS) and the peripheral nervous system (PNS). It enables the fast transfer of nerve impulses. Myelin is destroyed by autoimmune processes in inflammatory demyelinating diseases such as multiple sclerosis (MS) in the CNS and the Guillan-Barré syndrome in the PNS.

THE MYELIN

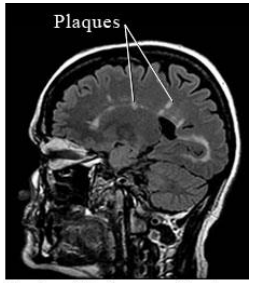


DEGENERATIVE DISEASES





Healthy brain



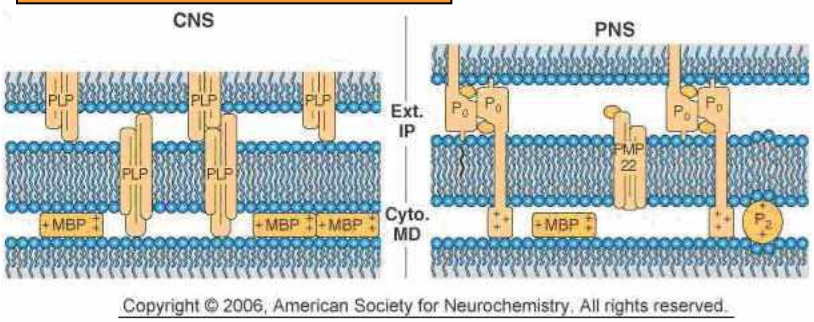
Brain with damage (lesions or plaques) caused by MS

SCIENTIFIC APPROACHES

- **Investigation of dynamics in reconstituted model membranes simulating the myelin fibers**
- **Investigation of more complex scenario: the biological tissues in Central Nervous System**

THE MODEL MEMBRANE in collaboration with the University of Basilicata and Genova, IT

THE MYELIN COMPOSITION



Myelin proteins : primarily for biogenesis and structural stability.
In Central Nervous System (CNS): MBP: up to 30% w of total proteins
In Peripherical Nervous System (PNS): MBP: from 5 to 15%; P2: from 1 to 15%

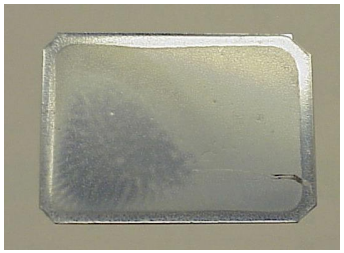
First studies of the influence of the Myelin Basic Protein (MBP) in CNS:
Natali, et al. (2002) Applied Physics A. 74, 1582-1584.
Natali, et al. (2003) Chem. Phys. 292 (2-3), 455-464.
Natali, et al. (2004) Physica B, 350 (1-3), E623-E626.

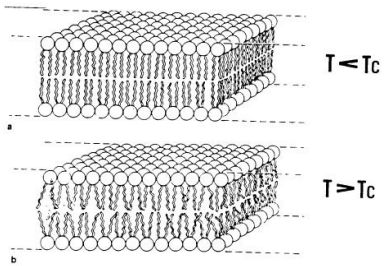
We address now the CNS !

MEMBRANE RECONSTITUTION and SAMPLE CHARACTERIZATION

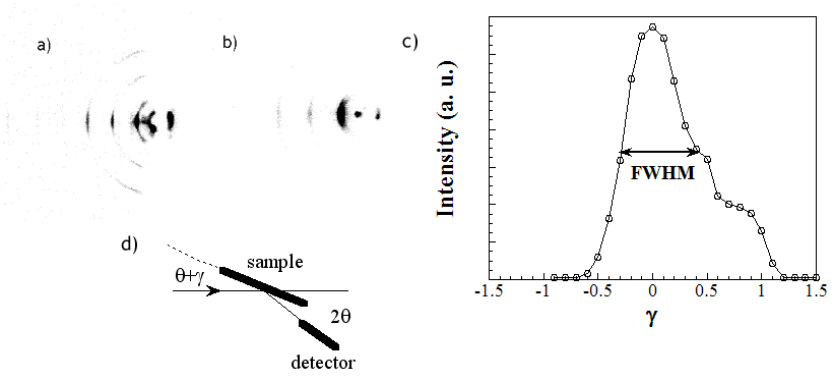
Liposomes made of artificial lipids, DMPA, spread on silicon wafers to promote membrane orientation. MBP is added to study its effect on the membrane dynamics. Gel-to-liquid lipid phase transitions: **T~ 320 K.**

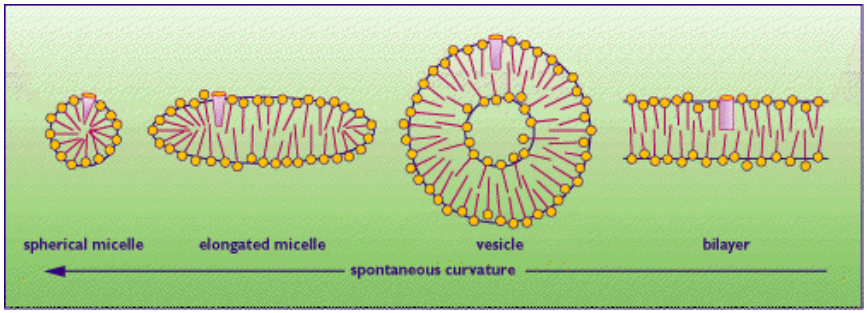
Prior to neutron scattering experiments all samples are firstly characterized using standard lab techniques including Light Scattering, sucrose gradient process to check liposome sizes, polidispersity and protein-liposome binding.



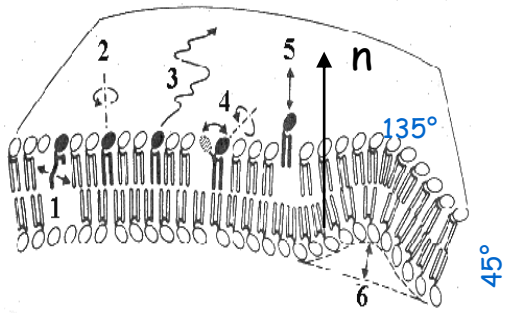


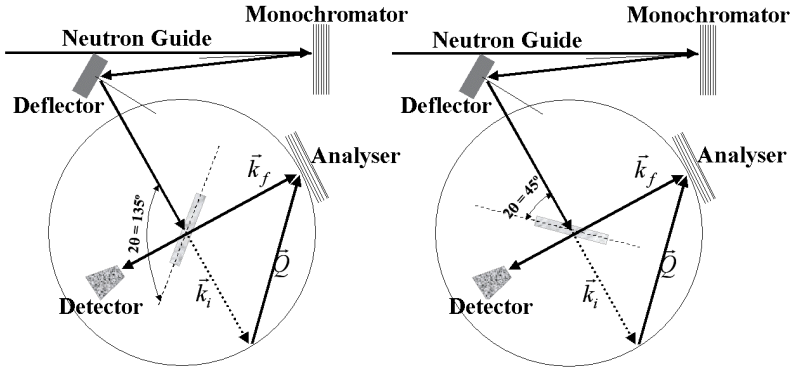
STRUCTURAL PROPERTIES





MEMBRANE RECONSTITUTION and SAMPLE CHARACTERIZATION

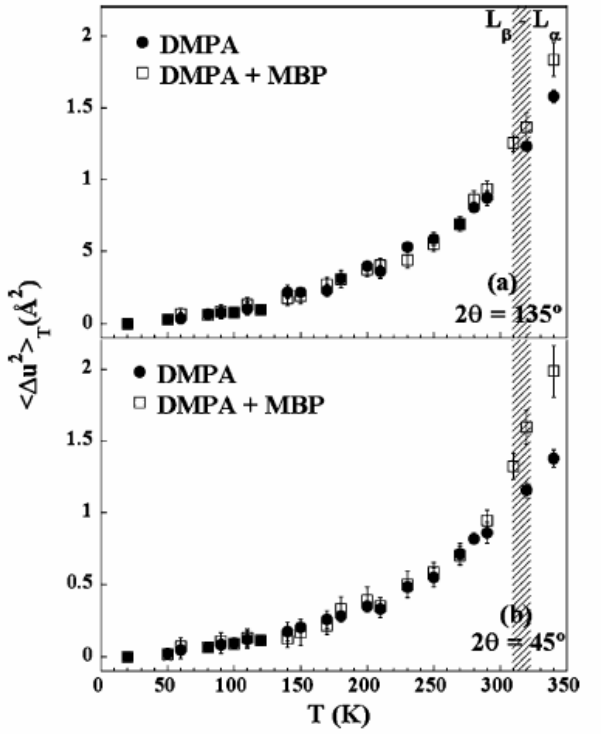


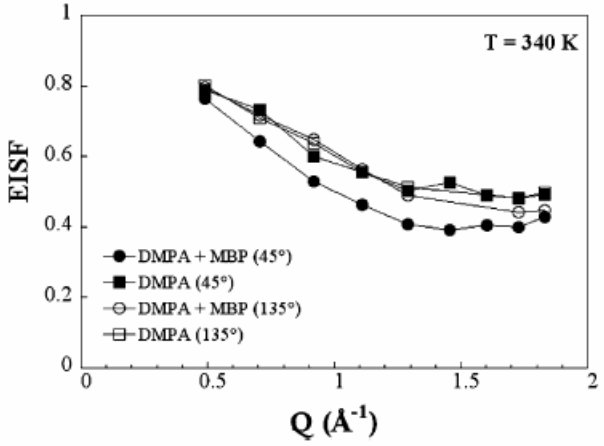


a) In-plane geometry

b) Out-of-plane geometry

DYNAMICAL PROPERTIES – FIRST RESULTS

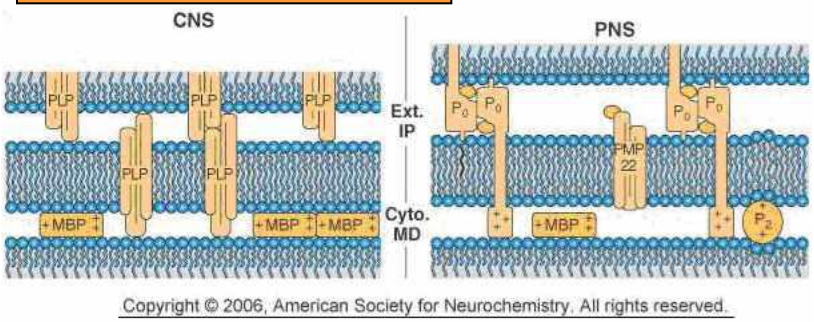




Neutron scattering data acquired on IN13 and IN16 at ILL (FR, 8 μeV FWHM and 0.8 μeV FWHM), respectively.

THE MODEL MEMBRANE in collaboration with the University of Oulu, Oulu, Finland

THE MYELIN COMPOSITION



Myelin proteins : primarily for biogenesis and structural stability.
In Central Nervous System (CNS): MBP: up to 30% w of total proteins
In Peripherical Nervous System (PNS): MBP: from 5 to 15%; P2: from 1 to 15%

First studies of the influence of the Myelin Basic Protein (MBP) in CNS:
Natali, et al. (2002) Applied Physics A. 74, 1582-1584.
Natali, et al. (2003) Chem. Phys. 292 (2-3), 455-464.
Natali, et al. (2004) Physica B, 350 (1-3), E623-E626.

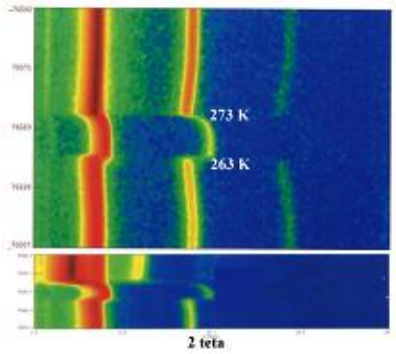
We address now the CNS !

MEMBRANE RECONSTITUTION

Liposomes made of artificial lipids, DOPC and DOPS at 50% w/w.

The major myelin proteins, MBP and P2 are added one by one to study their effect on the membrane dynamics. Gel-to-liquid lipid phase transitions: $\mathbf{T_{DOPC} \sim 253\ K}$ **and** $\mathbf{T_{DOPS} \sim 262K.}$

STRUCTURAL PROPERTIES

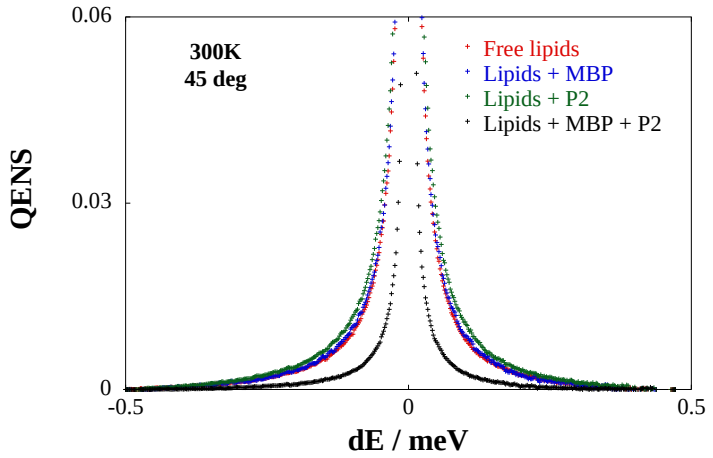


Diffraction images taken on D16 at ILL in function of the temperature for the protein free lipids (upper panel) and MBP-lipid complex (lower panel). The lipid phase transitions are clear visible, suggesting a shift of at least one (or both) of the transition temperature towards higher value.

and SOON …

To investigate the role of the mutant C8 of MBP, detected e.g. in aggressive cases of multiple sclerosis, on the membrane stability.

DYNAMICAL PROPERTIES – FIRST RESULTS



Neutron scattering data acquired at IN5 at ILL (10 μeV FWHM).
Strong out-of-plane membrane stabilization is induced by the mixed MBP+P2 proteins at relative concentration typically found in physiology (MBP: 5% - P2: 2.5%). Effect less pronounced in the in plane dynamics and in the gel phase (230K).

DATA ANALYSIS UNDER PROGRESS.

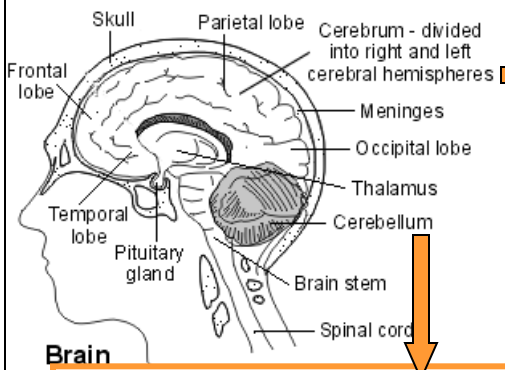
Complementary data acquired on NEAT (DE), OSIRIS (UK) and IN13 (ILL).

VERY RECENTLY….an ambitious project in collaboration with the Depart. of Veterinary Clinical Science at the Univ. of Padova

SCOPE

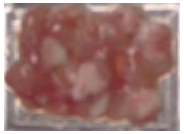
1- To qualitatively compare the dynamics of different sections of the Central Nervous System from bovines, where Myelin is present at different amount.
2- To observe if data are reproducible analyzing tissues from different animals.
3- To compare the effect of different preserving methods: cryogenic towards formalin addition.

THE CENTRAL NERVOUS SYSTEM



Hemisphere Rx from bovine

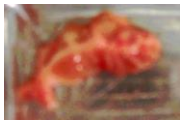
Myelin: external, 23% of total mass
Neurons: internal



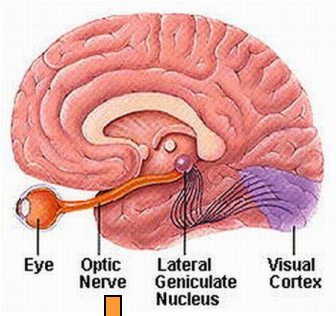
cryopreserved

Formalin makes the tissues dry and more rigid changing also their visual aspect.

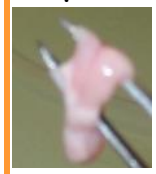
Cerebellum from bovine - cryopreserved



In the Cerebellum the Myelin is internal (white 'filaments' in the figure), while the neurons are external. Less myelin content.

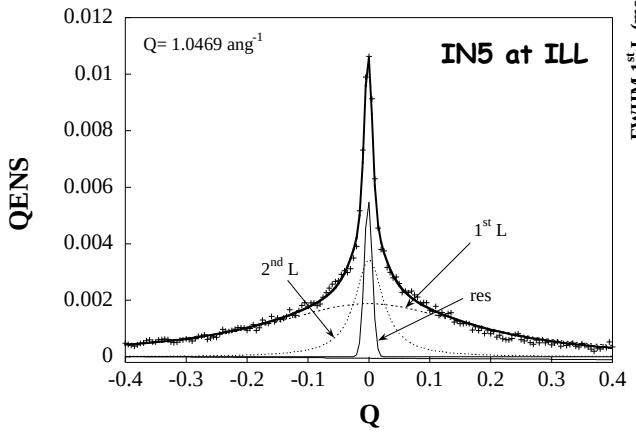


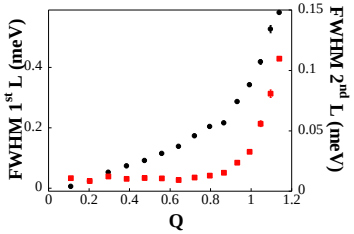
Optic Nerve from bovine - cryopreserved



In the optical nerve the Myelin is external, while the neurons are internal. Myelin is the major constituent of the nerve.

NEUTRON SCATTERING DATA





Two main components, one diffusive and the other confined, have been identified in the global tissue dynamics falling in the energy resolution adopted (10 μeV FWHM).

FIRST RESULTS

- Data are extremely reproducible (tissues from different animals give same scattering response).
- Data from different brain sections show changes in the QENS signal.
- No free water are found in tissues (no bragg reflection at low T) even though water is present in all the investigated tissues at ~70% in weight.
- Formalin and cryopreserving methods affect differently the tissue dynamics. **DATA UNDER ANALYSIS !!!**

and SOON …

- To correlate how all this is linked to the myelin content by isolating the myelin fibers from the different brain sections.
- To compare results with myelin affected by degenerative diseases
- To extend the investigation to healthy and suffering human CNS.

STUDENTS ARE WELCOME!!!

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