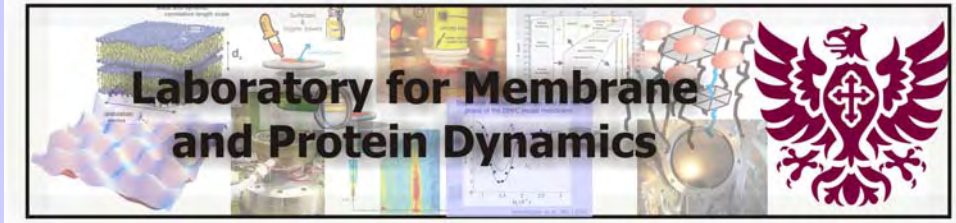


Maikel C. Rheinstädter
Laboratory for Membrane and Protein Dynamics
McMaster University, Hamilton ON
and
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*Pontificia Universidad Catolica de Chile*
*Facultad de Fisica*
*Santiago de Chile, October 2009*

# *Collective Molecular Dynamics in Proteins and Membranes*

**Mini Curso I/III,**
**Red Nacional de Postgrado en Ciencias Físicas:**
"Dynamics in Soft-Matter and Biology Studied by Coherent Scattering Probes"

# Course Outline


Introduction: Membranes and Proteins

Properties of Neutrons and X-rays

Basic Scattering Theory: Elastic and Inelastic Scattering, Coherent and Incoherent Scattering

Scattering Experiments in Biological Materials, Practical Aspects

Molecular Motions in Membranes and Proteins as seen by Neutrons and X-rays

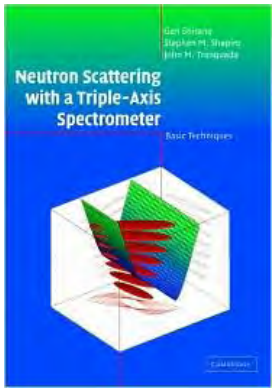
# Course Goals

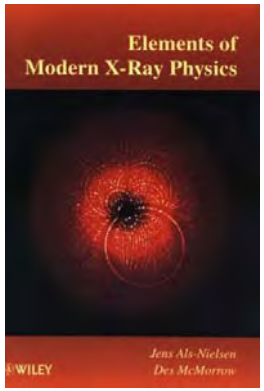


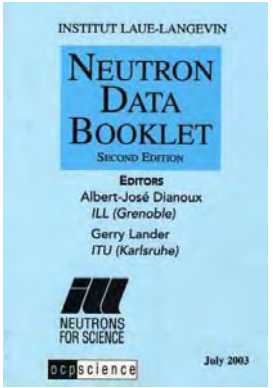


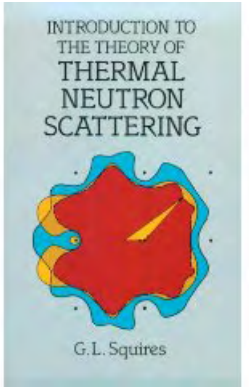
- Scattering jargon, explain concepts (coherent, incoherent, elastic, quasi-elastic, inelastic, selective deuteration)

- Better understand scattering papers if you need them for your work



# Biological Physics is...

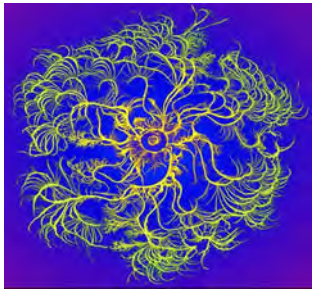

*Aims and Scope, European Biophysical Journal:*

"the study of biological phenomena using physical methods and concepts ... the primary goal ... is to advance the understanding of biological structure and function by application of the principles of physical science"

"... a distinctively biophysical approach at all levels of biological organization will be considered, as will both experimental and theoretical studies"

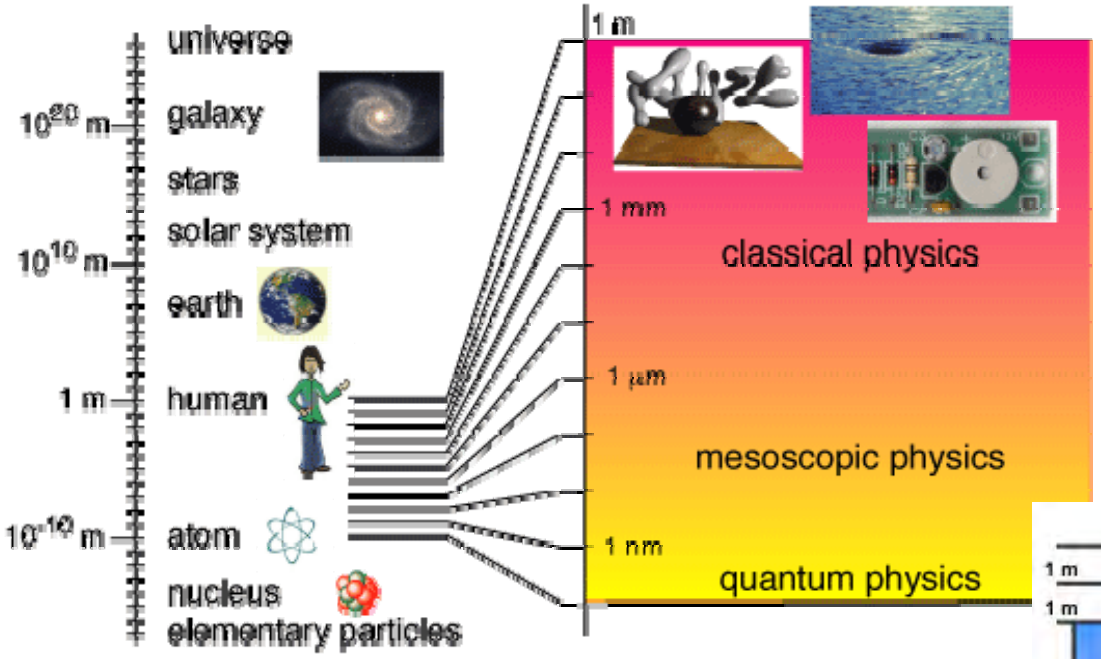
Hierarchy of biological systems:

- Organism
- Organ
- Cell
- Organelle
- Cytoskeleton
- Functional Module
- Biological Macromolecule
- Molecule
- Atom



"Although living systems obey the laws of physics and chemistry, the notion of function or purpose differentiates biology from other natural sciences" (*Hartwell et al.*)

# Length Scales




*NanoBiology*

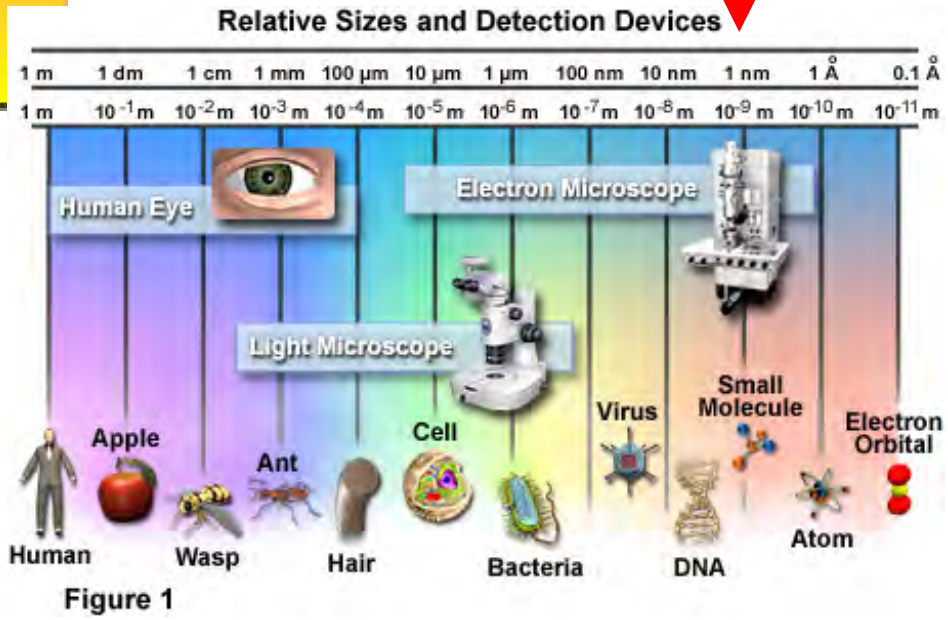
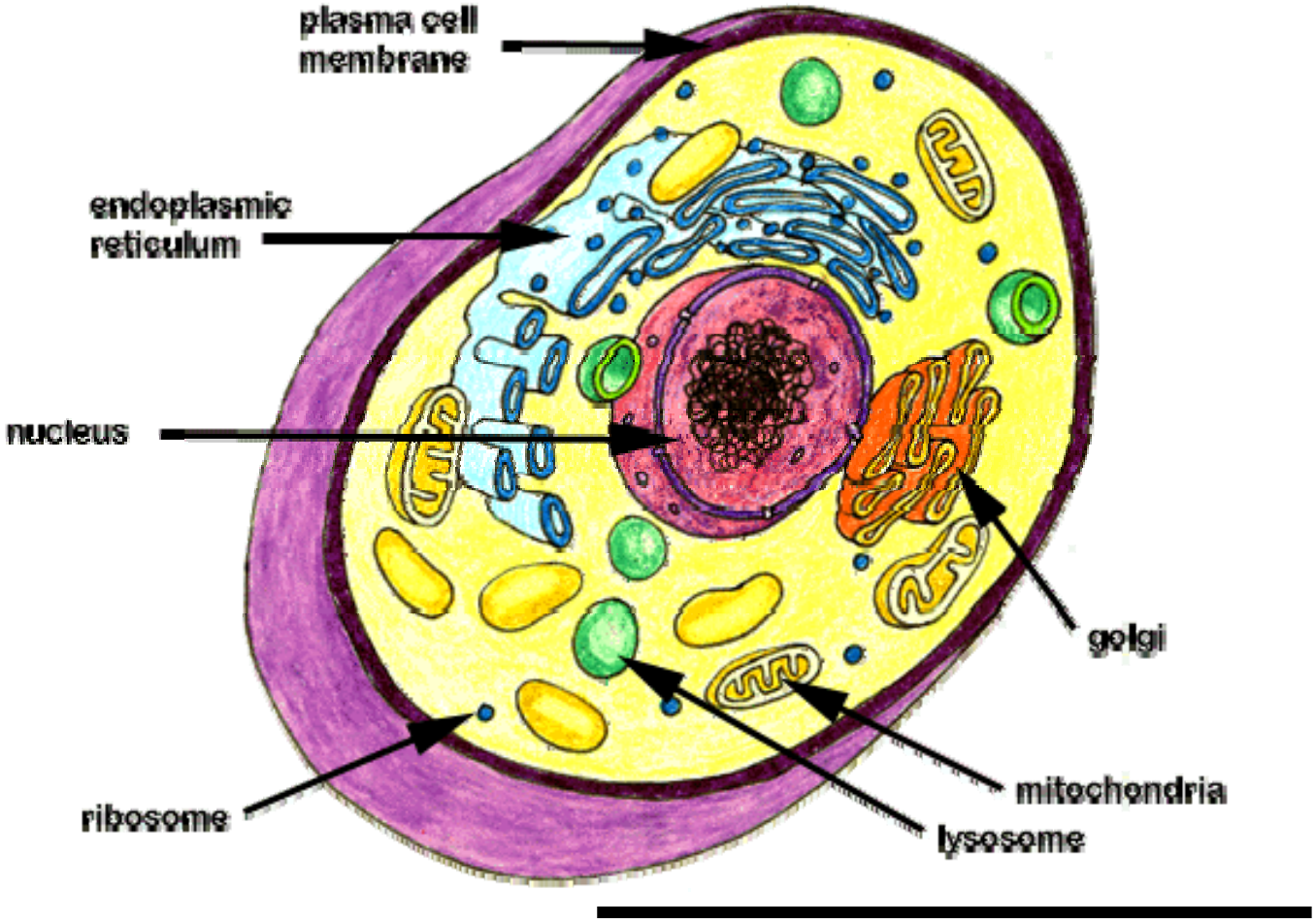



Figure 1

1 nanometer = $10^{-9}$ m = 0.000000001 m

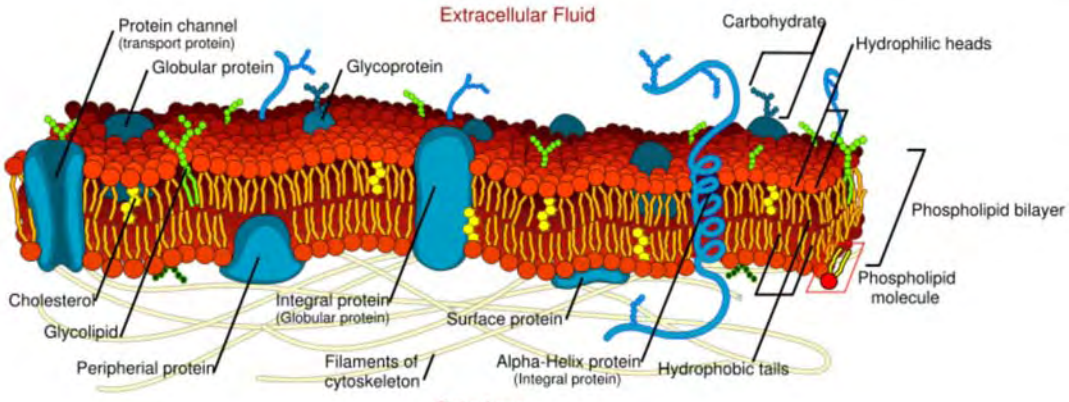
# The Cell




plasma cell membrane
endoplasmic reticulum
nucleus
golgi
ribosome
mitochondria
lysosome
10 µm

# The Cell Membrane

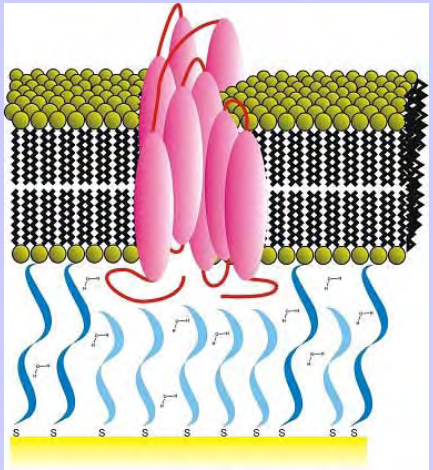
Laboratory for Membrane and Protein Dynamics



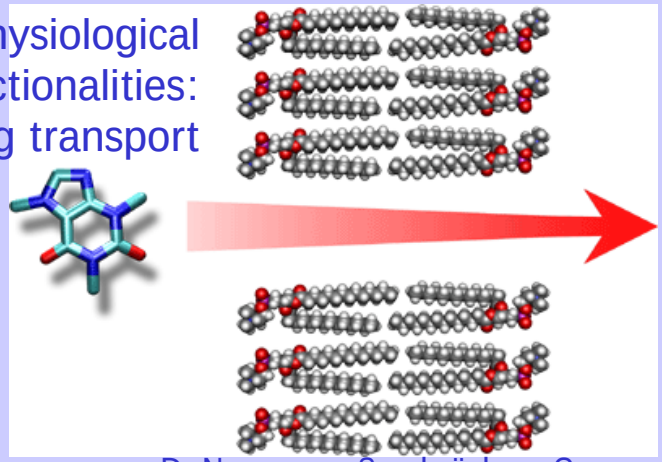

Mariana RuizVillarreal (http://commons.wikimedia.org/)

## **Applications**:

Bioengineering:
tailor membranes with
specific properties



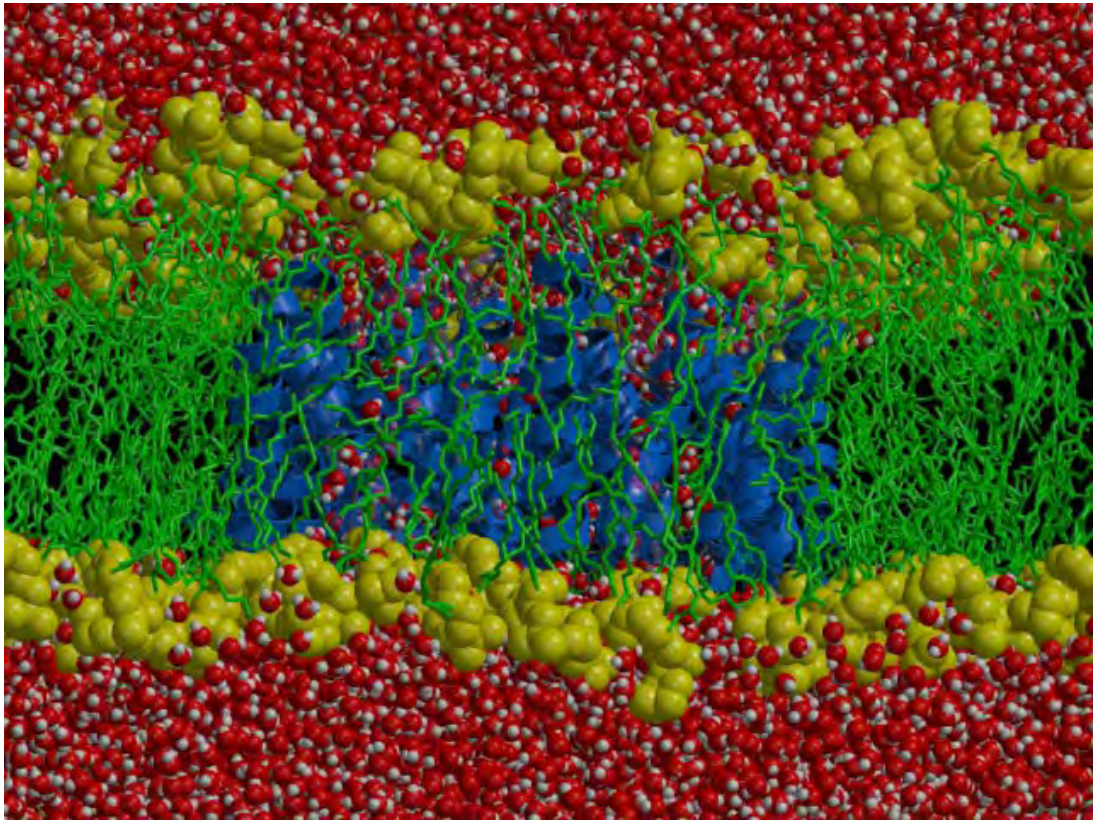
**Understanding** of physiological
and biological functionalities:
Drug transport



D. Neumann, Saarbrücken, Germany

Membrane is the primary site of (inter)action

# Membrane Dynamics



Bert L. de Groot, Rainer A. Böckmann, and Helmut Gruber

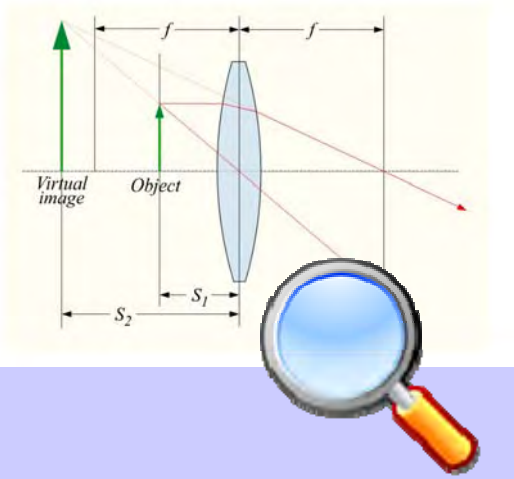
**'multi-scale'**: relevant dynamics in a large range of length and time scales

How can we study dynamics in membranes and proteins?

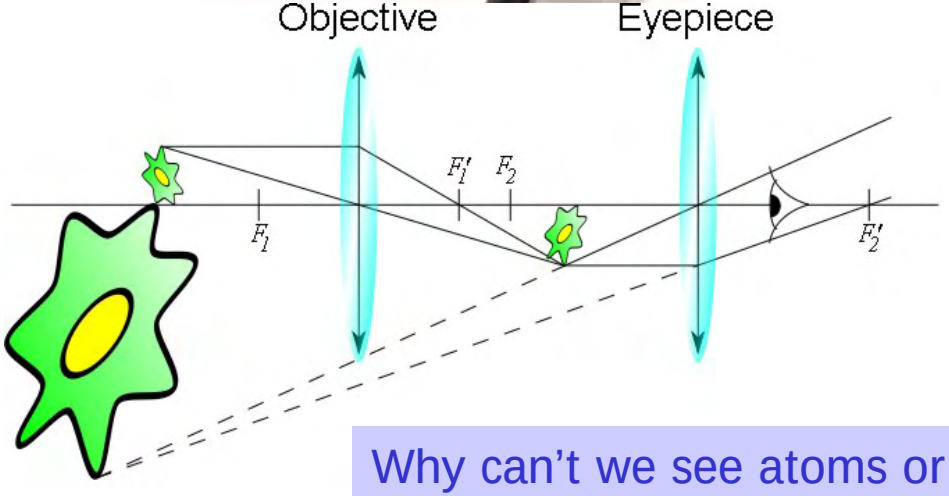
# Optical Techniques



## Magnifying Glass

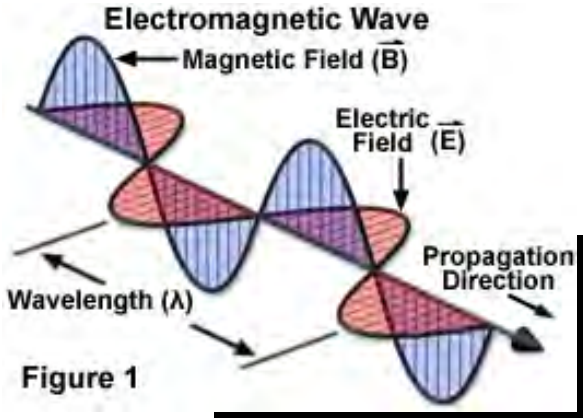


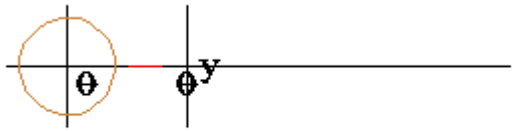

## Optical Microscope



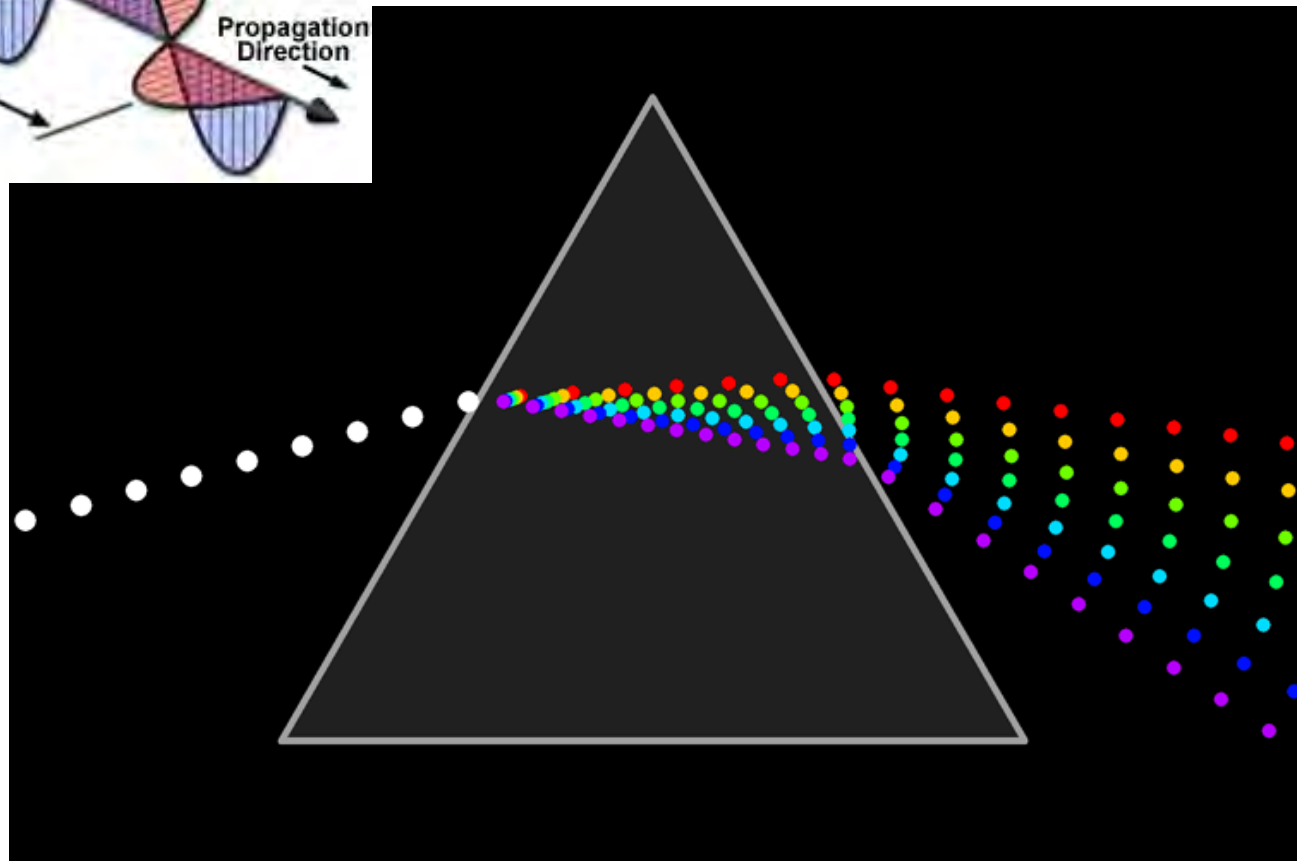

Why can't we see atoms or molecules?

# Light is waves




$$y = \sin \theta$$



Visible Light Wavelength
700 nm ($10^{-9}$m)

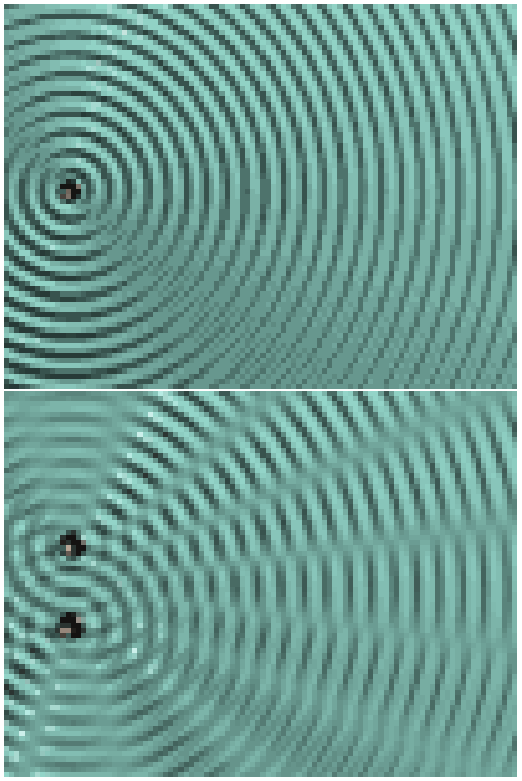
550 nm

450 nm

# Waves Interfere

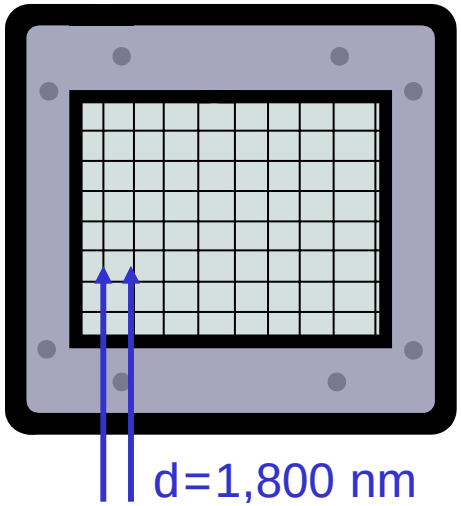
Laboratory for Membrane and Protein Dynamics

## Ripple Tank



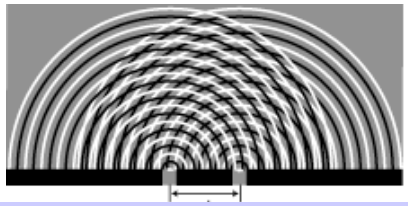
Wave character becomes important when things get ‘small’

## Diffraction grating




Grating: 13,500 lines/inch

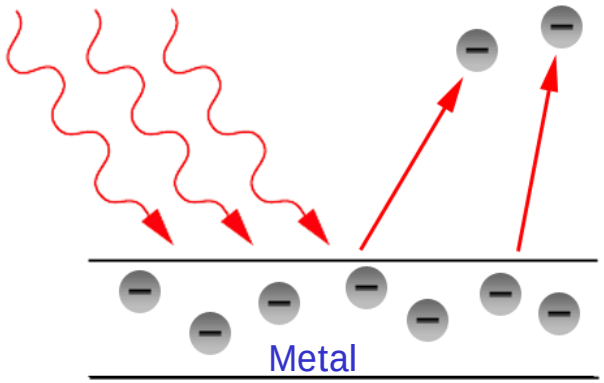
Green Laser: 532 nm



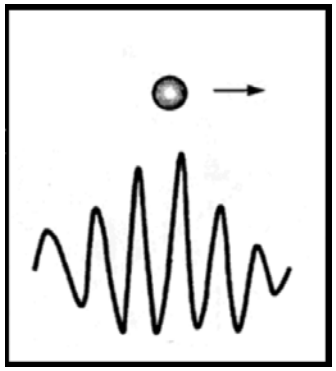
‘Diffraction limit’ when wavelength meets object size: Limit for optical techniques

# Particles can be waves

Nobel Prize Albert Einstein 1921
'Photoelectric Effect'



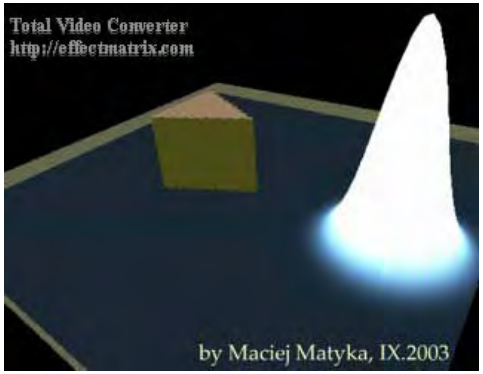

Nobel Prize Louis de Broglie, 1929
'Wave nature of electrons'



Wavelength=quantum constant/(Mass x velocity)

## Wave-Particle Duality

Use particle waves to come to smaller wave lengths

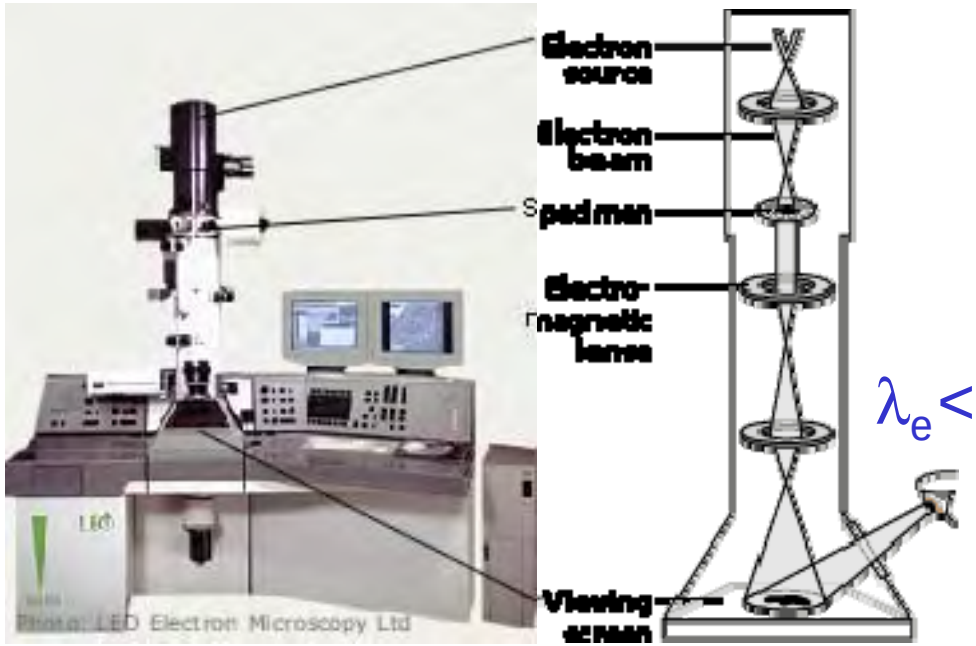



Quantum Particle collides with obstacle

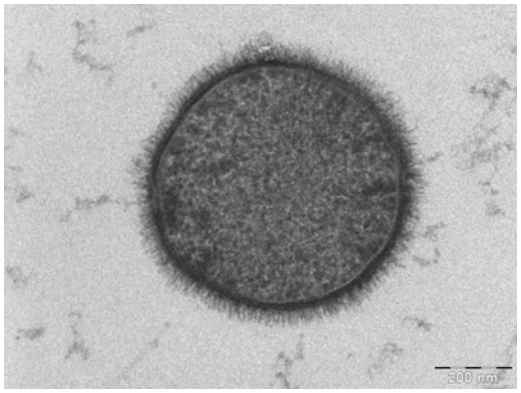
# Electron Microscopy

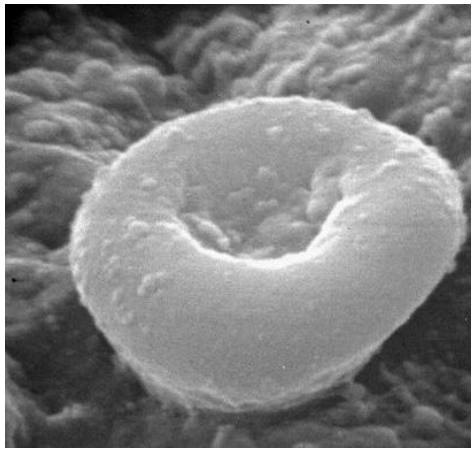
Laboratory for Membrane and Protein Dynamics

Nobel Prize Ernst Ruska, 1986
'Design of the first electron microscope'




$\lambda_e$<0.1 nm



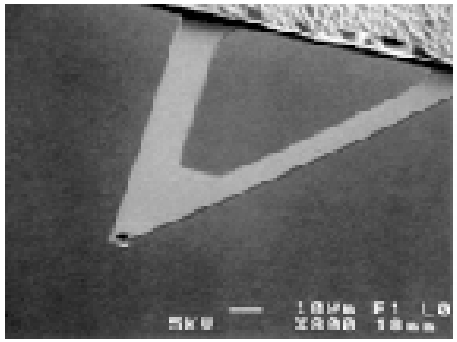



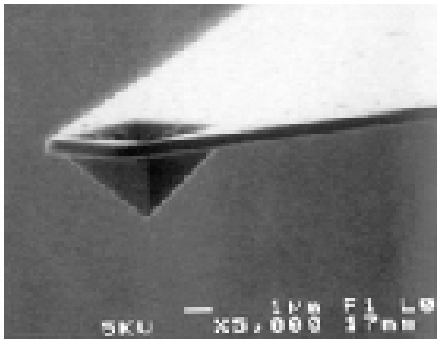
Red blood cell (8000 nm)

- Sample must be in vacuum
- Sample damage
- Complex sample preparation

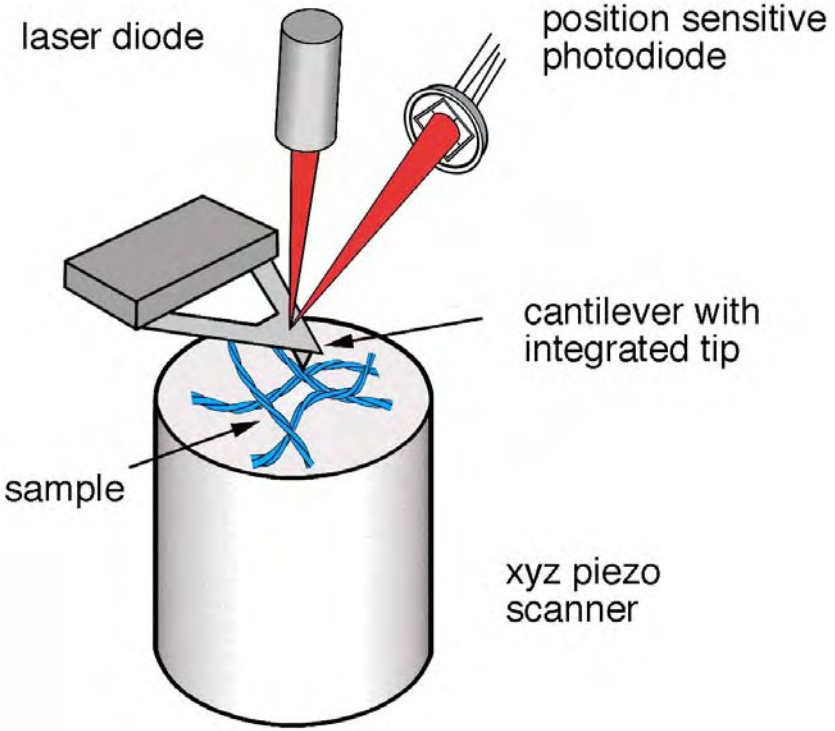
# A different Approach: Atomic Force Microscopy

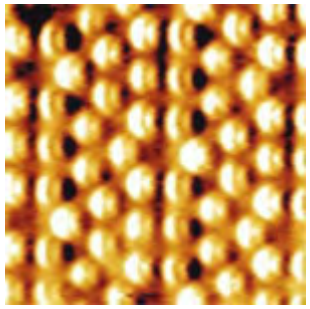
Nobel Prize Gerd Binnig, Heinrich Rohrer, 1986
'for their design of the scanning tunneling microscope '



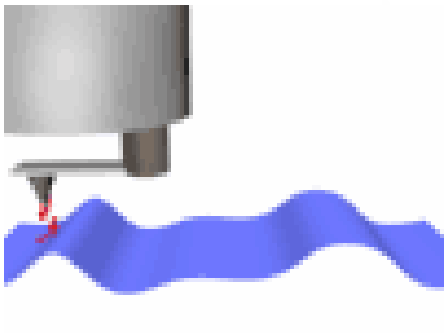


Size of the tip: ~40 nm

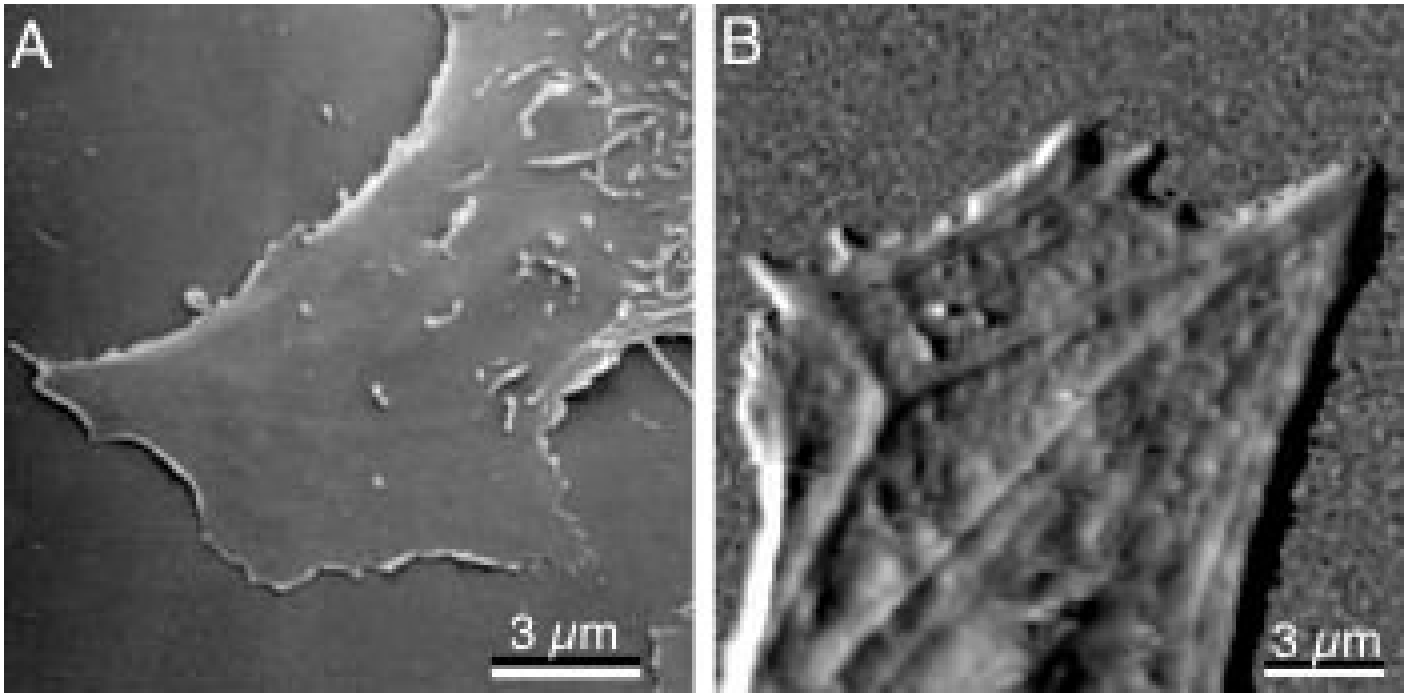


Si(111)-(7x7) surface



# A different Approach: Atomic Force Microscopy

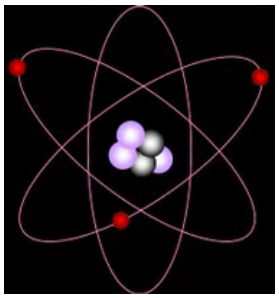



- Surface sensitive
- No dynamics

# Neutron Scattering

Laboratory for Membrane and Protein Dynamics

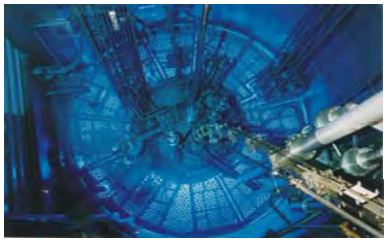
## Neutron part of the nucleus



$\lambda_n$<1 nm

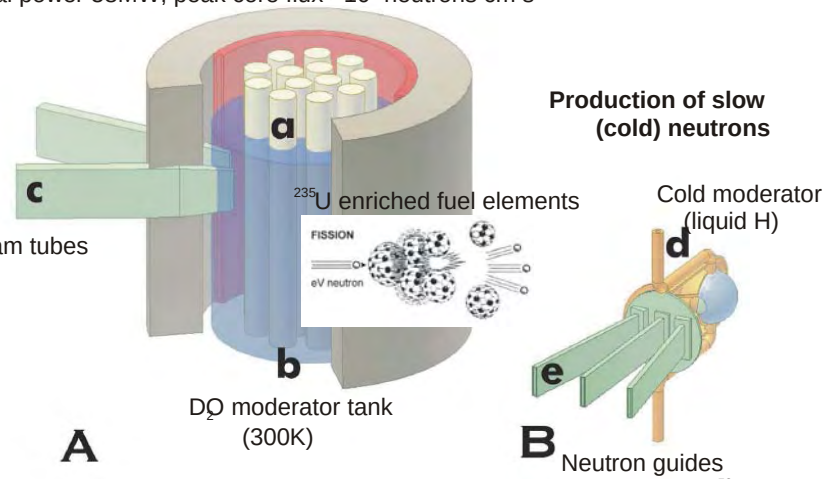
### Production

#### Reactor Sources



The Institut Laue-Langevin (ILL) in Grenoble, France, operates the world's most powerful neutron reactor

Thermal power 58MW, peak core flux >10 $^{15}$ neutrons cm$^{-2}$ s$^{-1}$




Beam tubes

$^{235}$U enriched fuel elements

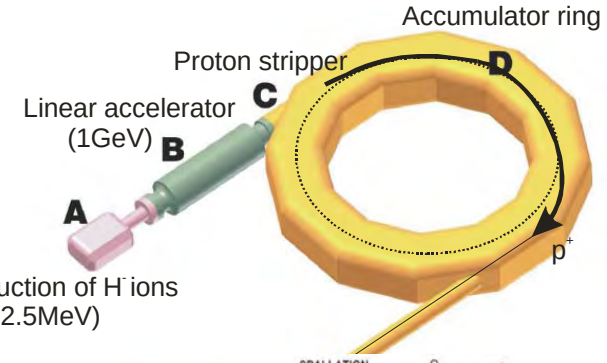
**Production of slow (cold) neutrons**

Cold moderator (liquid H)

$D_2O$ moderator tank (300K)

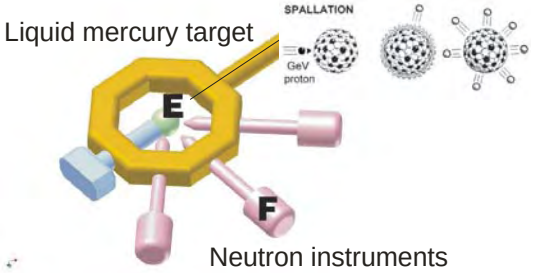
Neutron guides (total reflection by Ni $^{58}$ coating)

#### Spallation Sources



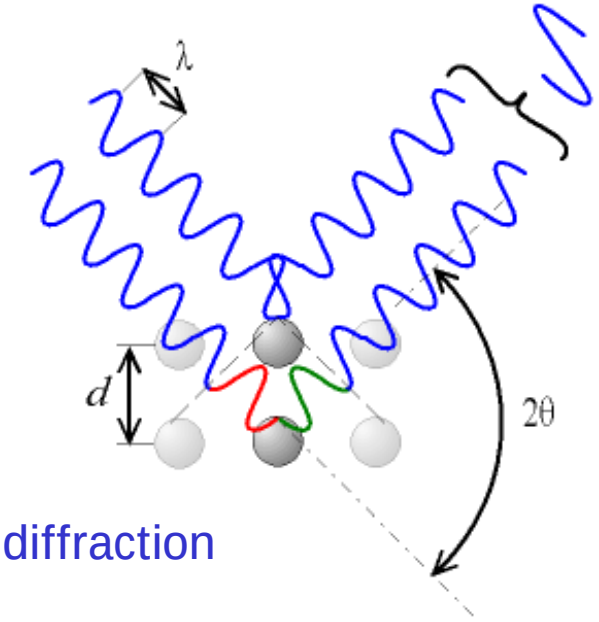


2 MW spallation neutron source




The Spallation Neutron Source (SNS) in Oakridge, USA, is the world's most powerful neutron spallati on source

# Scattering-Reciprocal Space




diffraction

"...where the atoms are and how they move."

Scattering vector

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

**Nobel Prize Bertram Brockhouse, Clifford Shull, 1994**

'for pioneering contributions to the development of neutron scattering techniques for studies of condensed matter '

Fourier Transformation

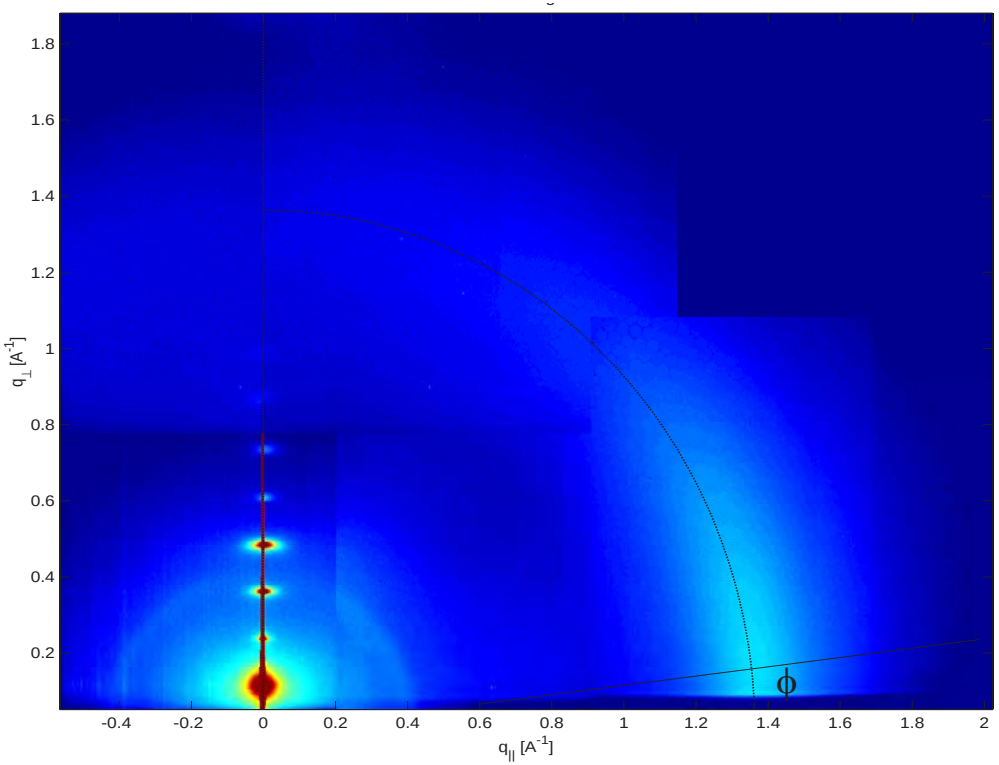
*'real space'* ⟷ *'reciprocal space'*

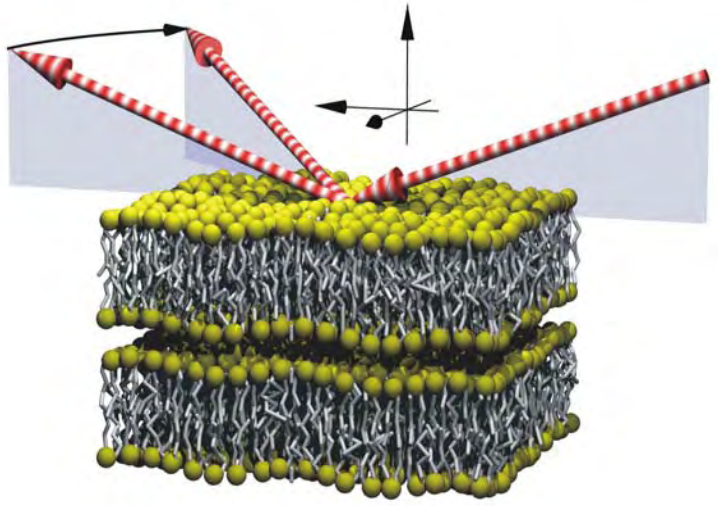
## Scattering laws

momentum $\vec{q} = \frac{m}{\hbar}(\vec{v}_1 - \vec{v}_2)$

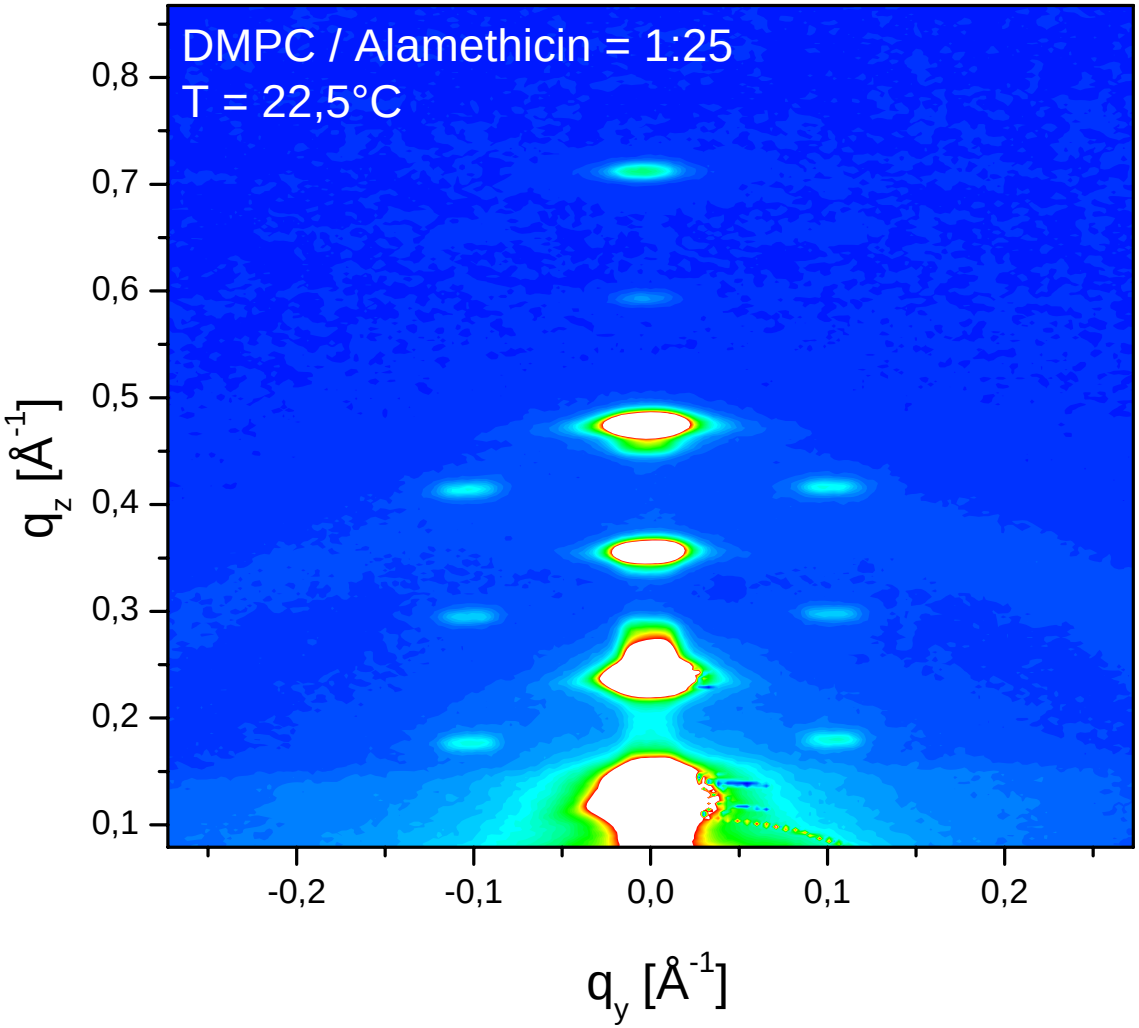
energy $\hbar\omega = \frac{1}{2}m\left(v_2^2 - v_1^2\right)$

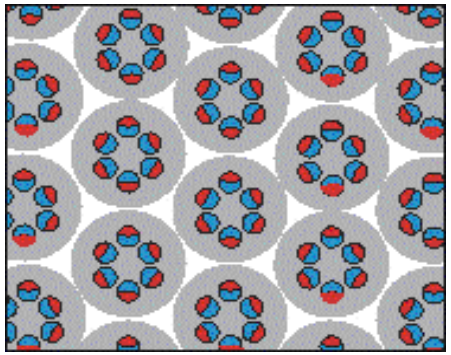
# Reciprocal Space of a Membrane

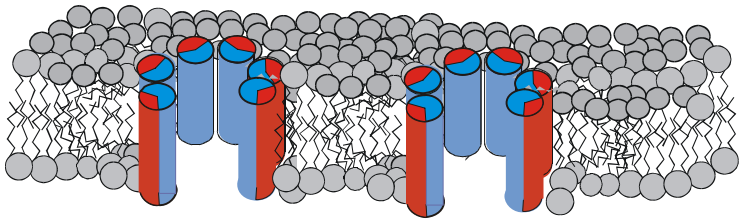


# Complex Membrane





# Biological Physics and Techniques

Hierarchy of biological systems:

Organism

Organ

Cell

Organelle

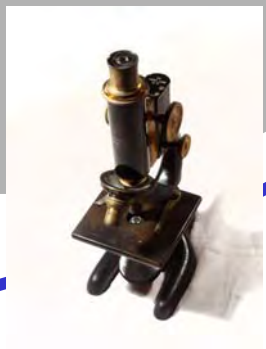
Cytoskeleton

Functional Module

Biological Macromolecule

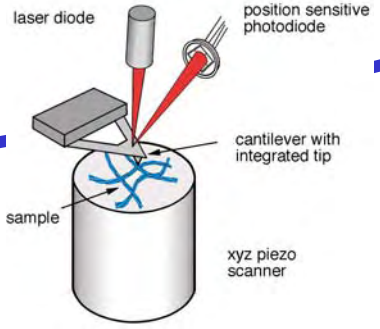
Molecule

Atom

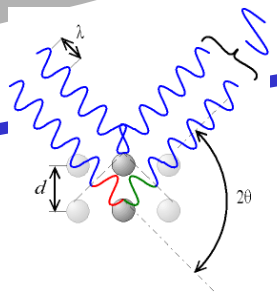


Optical Techniques

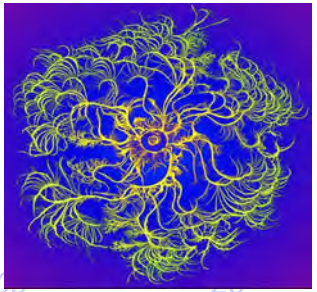
Electron Microscope




Atomic Force Microscope

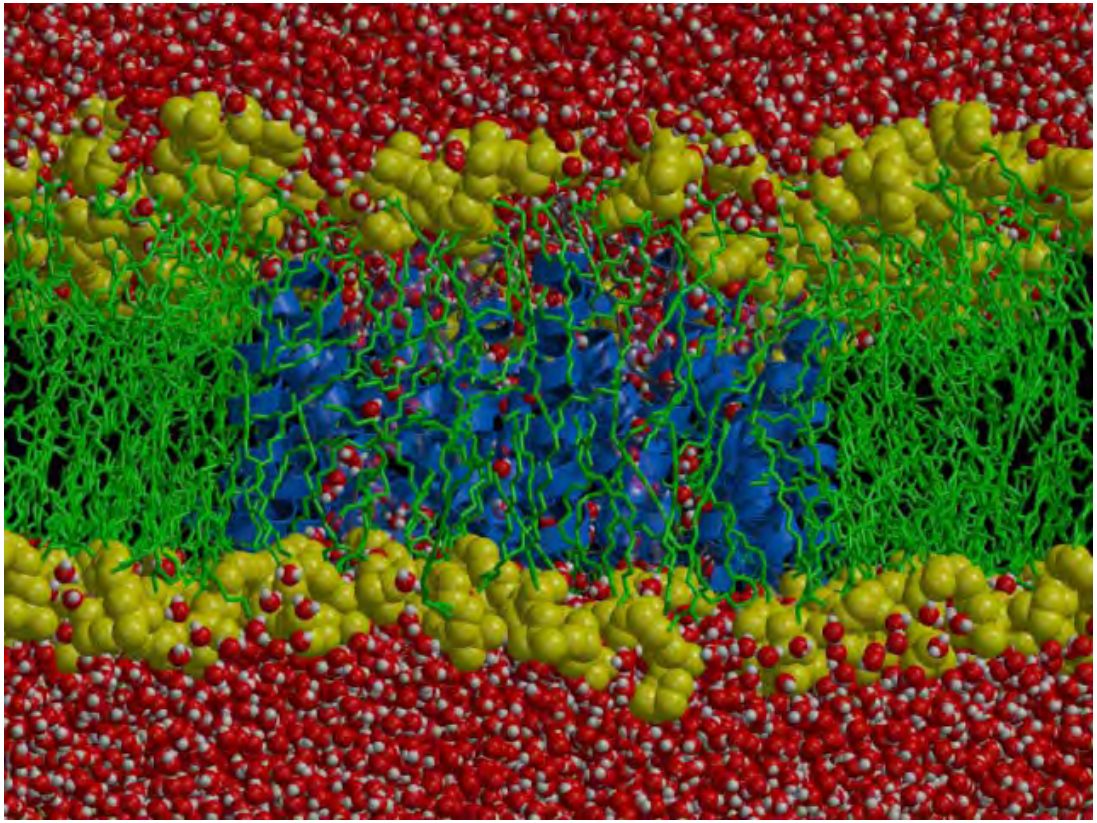



Neutron, X-ray Scattering



# Part II

# Membrane Dynamics



Bert L. de Groot, Rainer A. Böckmann, and Helmut Gruber

Membrane is the primary site of (inter)action

**'multi-scale'**: relevant dynamics in a large range of length and time scales

missing or not well developed periodic **structure** (BZ concept)

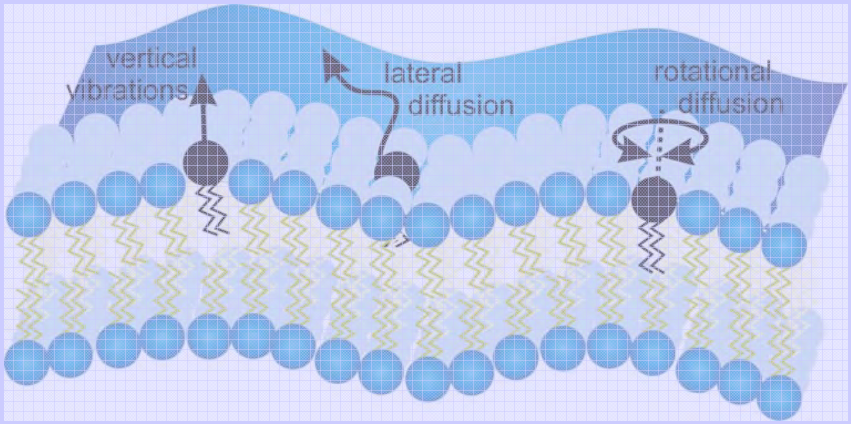
**high 'intrinsic' background**

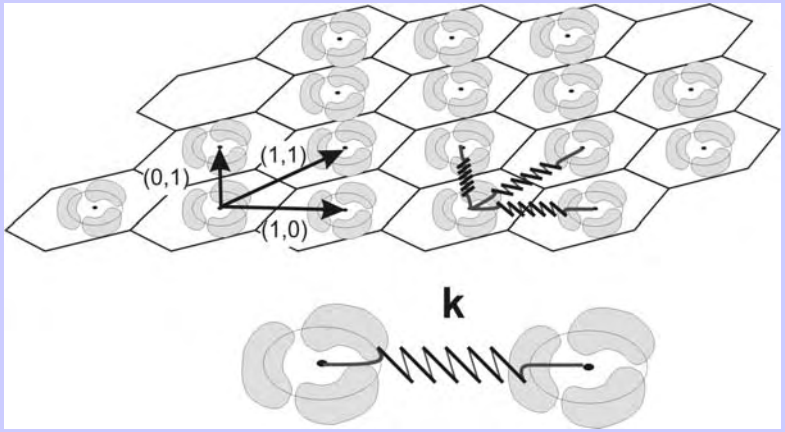
- different molecular components
- single and collective molecular motions

# Membrane Dynamics

Laboratory for Membrane and Protein Dynamics

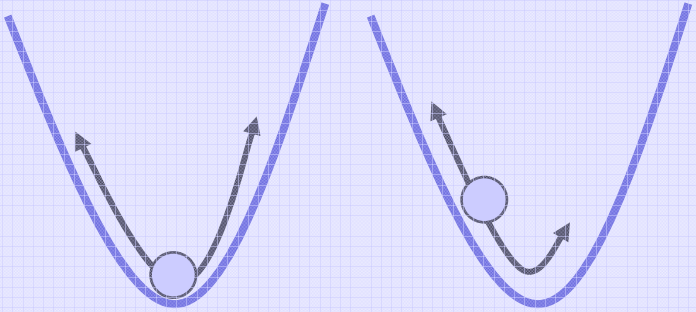
## Local modes in bilayers



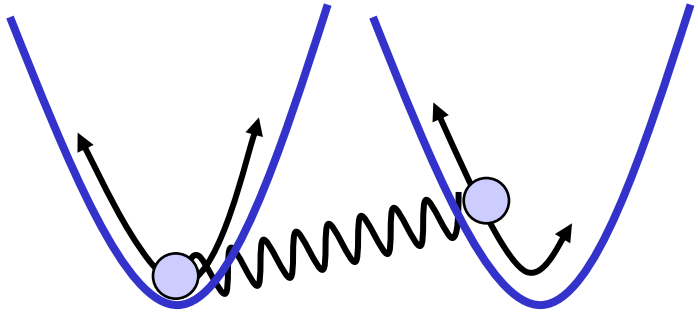




Collective excitations

## Incoherent, single molecule

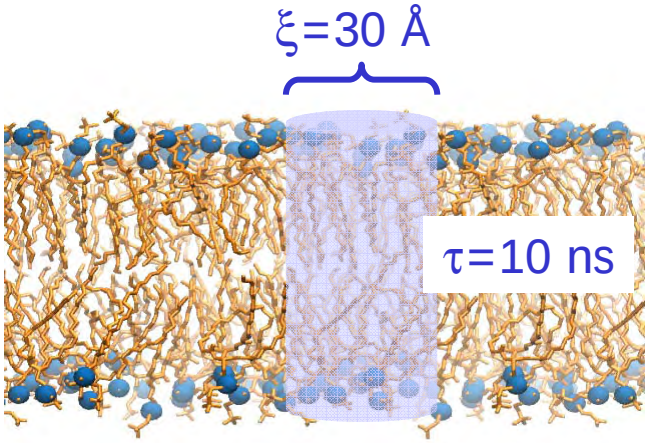


## Coherent, interactions

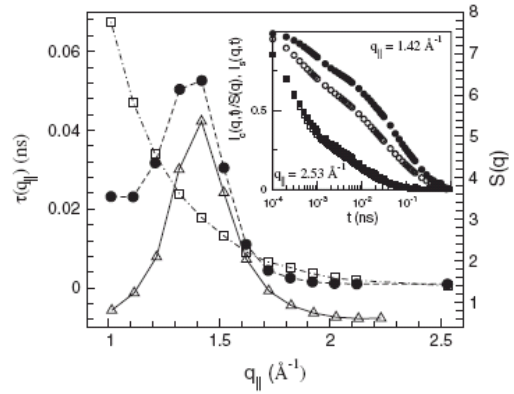


**Correlated molecular motions drive "functionalities" of membranes and proteins and structural changes**

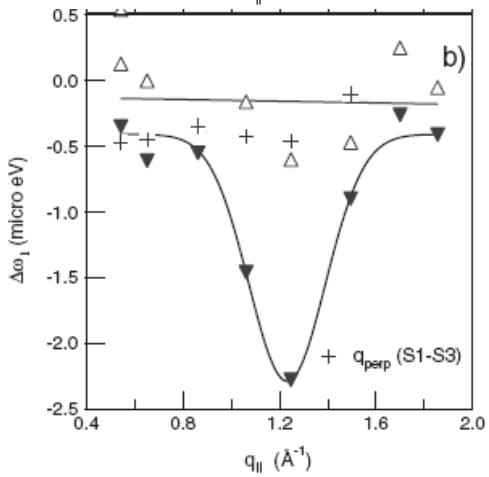
# Coherence in Biology




Fluid lipid bilayer at T=30°C




Simulation




Experiment

Coherent structural relaxation
"Motional Coherence"

## Access to coherent properties

| Bilayer | Hydration |
|---|---|
| partially (chain) deuterated | $D_2O$ |
| partially (chain) deuterated | $H_2O$ |
| protonated | $D_2O$ |
| protonated | $H_2O$ |

**Coherence is a fundamental property of biological materials**

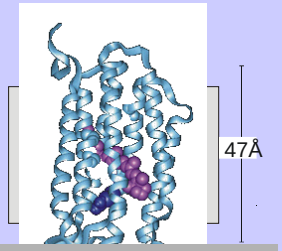
PRL 101, 248106 (2008)

# Dynamics - Function

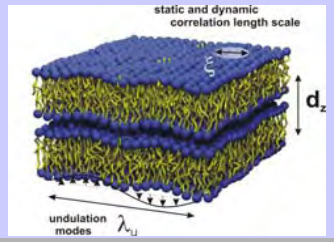

time scale

slow

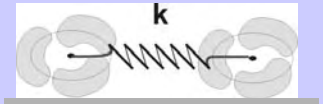
**0.1 nanometer** → **100 nanometers**




**intra-protein**

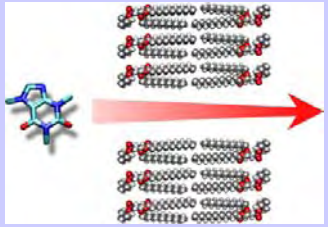



**Membrane Elasticity**




**inter-protein**

**Membrane Permeability**



**nano -microsecond**

**picoseconds**

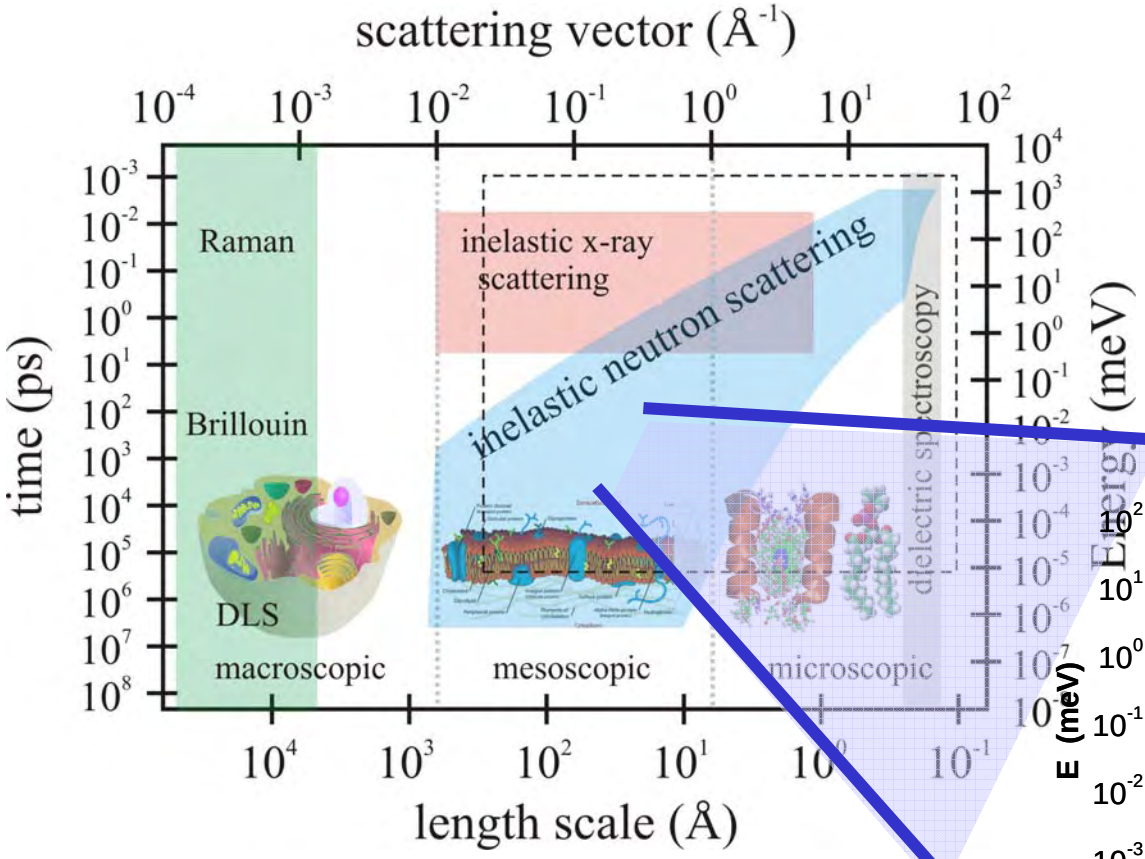
fast

small

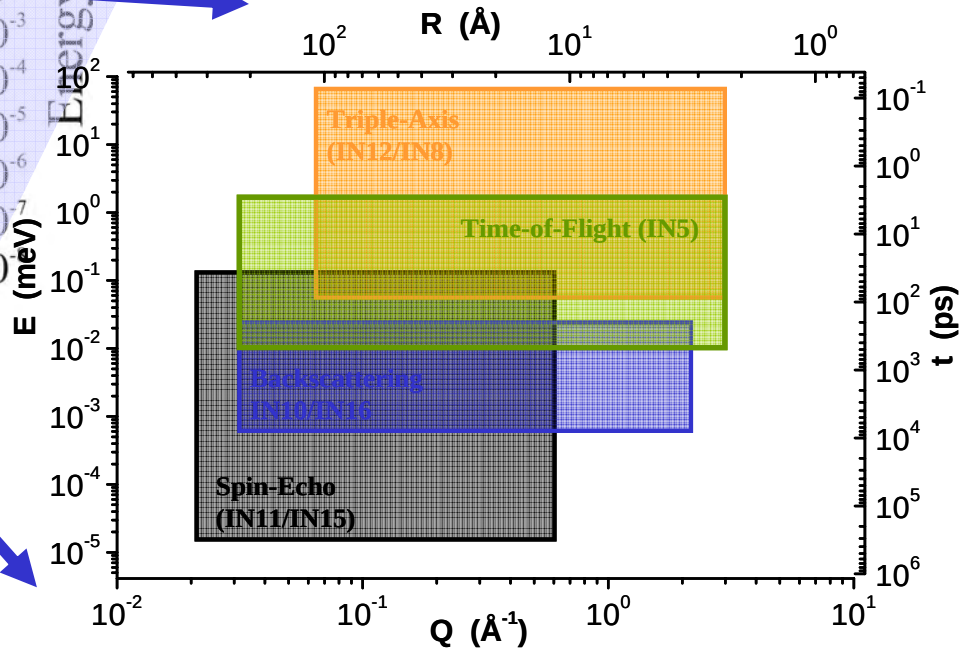
large

length scale

# Membrane and Protein Spectroscopy


Inelastic neutron scattering gives wave vector resolved access to dynamics




excitations
relaxations
⟷ specific motions

# Road Map

Laboratory for Membrane and Protein Dynamics

Inelastic Scattering Experiments

Cholesterol

**Membrane Properties**

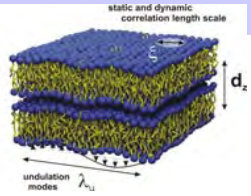
Elasticity

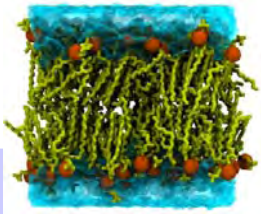
Permeability

Ethanol

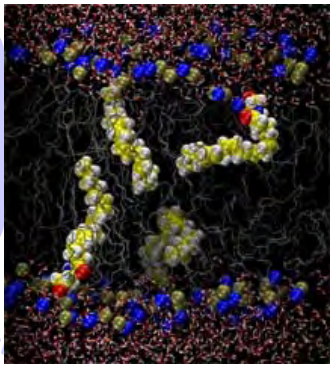
Inter and Intra Protein Dynamics

Solvent interaction



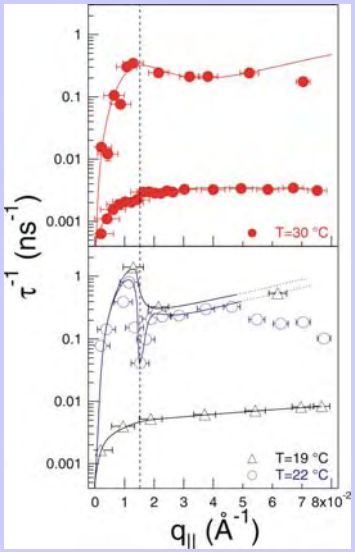
How does membrane composition and properties determine protein dynamics and function?



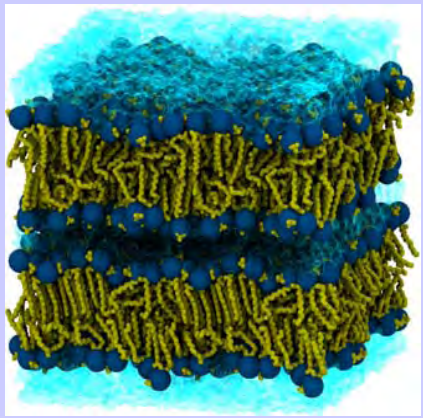
# Interdisciplinary Approach

Laboratory for Membrane and Protein Dynamics

## Experiments

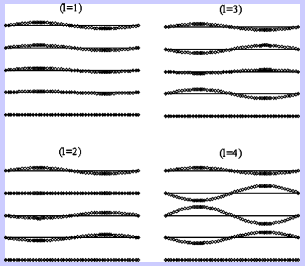



## Simulations

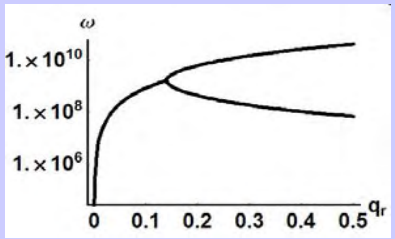


**System S2: 48,880 atoms**
**(256 lipids + 6224 waters)**

## Analytical Theory

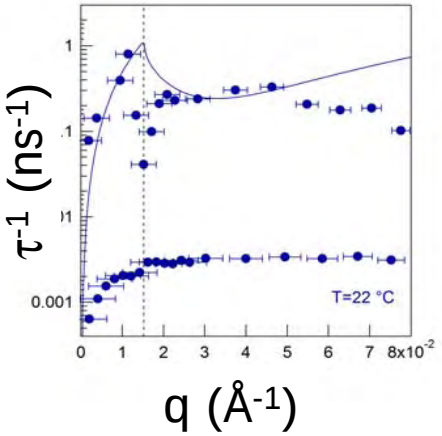



$$\tau^{-1}(q_{||}) = \frac{\kappa / d}{\eta_3} q_{||}^2 \frac{q_{||}^4 + (\pi/(\Lambda D))^2}{q_{||}^4 + \frac{1}{\mu\eta_3}(\pi D)^2}$$



# Mesoscopic Membrane Fluctuations

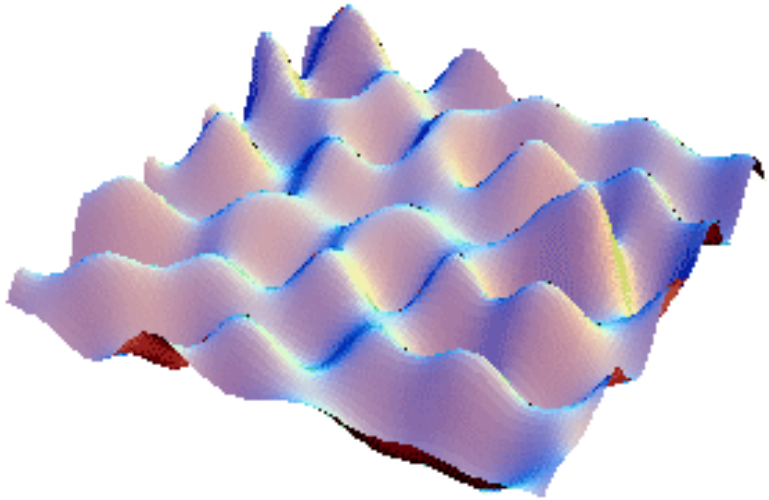
## Dispersion relation

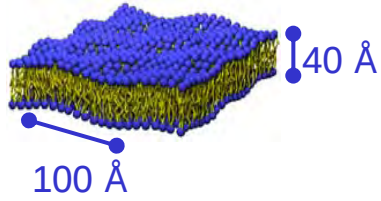



Contains 'dynamic' information

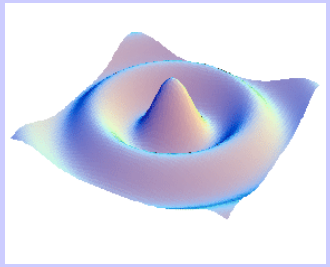
q-dependence of excitation frequencies and relaxation rates

Thermal membrane fluctuations

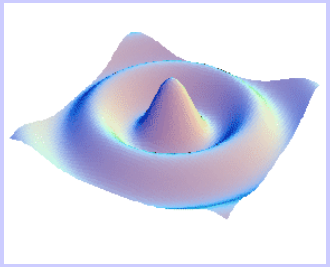




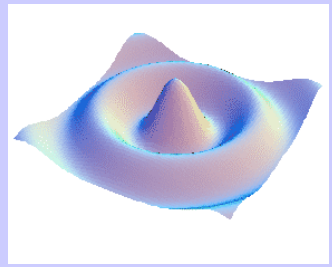

Elementary excitations


Propagating

+


Oscillating

+

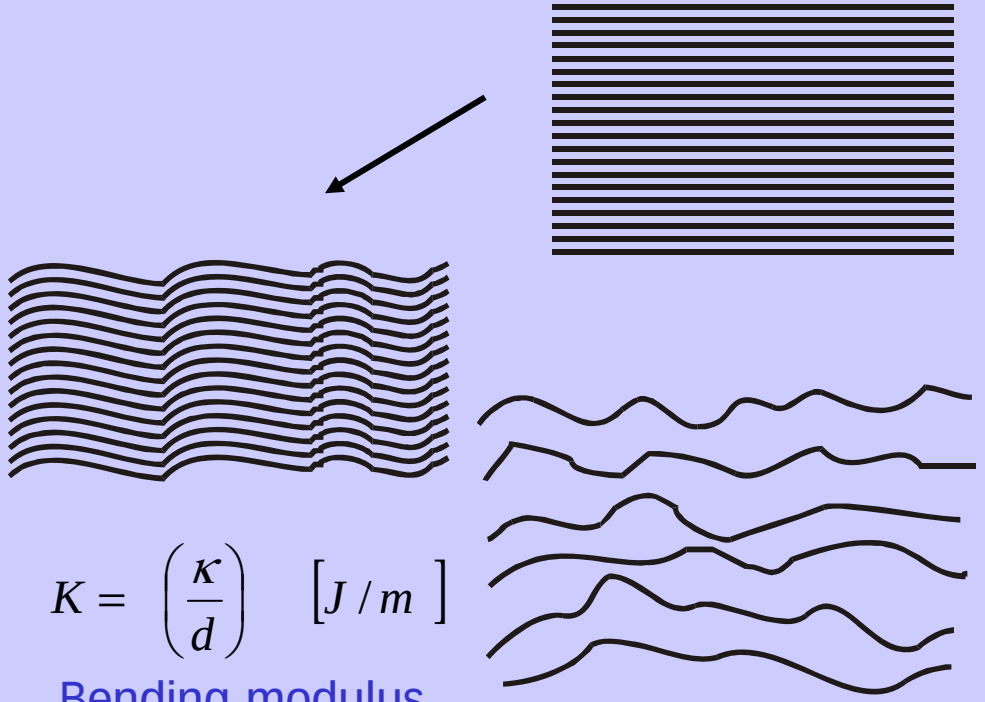

Relaxating

Mode

# Membrane Elasticity

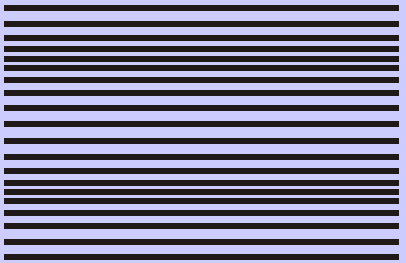
Laboratory for Membrane and Protein Dynamics

## Mesoscopic membrane fluctuations



$$K = \left(\frac{\kappa}{d}\right) \quad [J/m]$$

Bending modulus

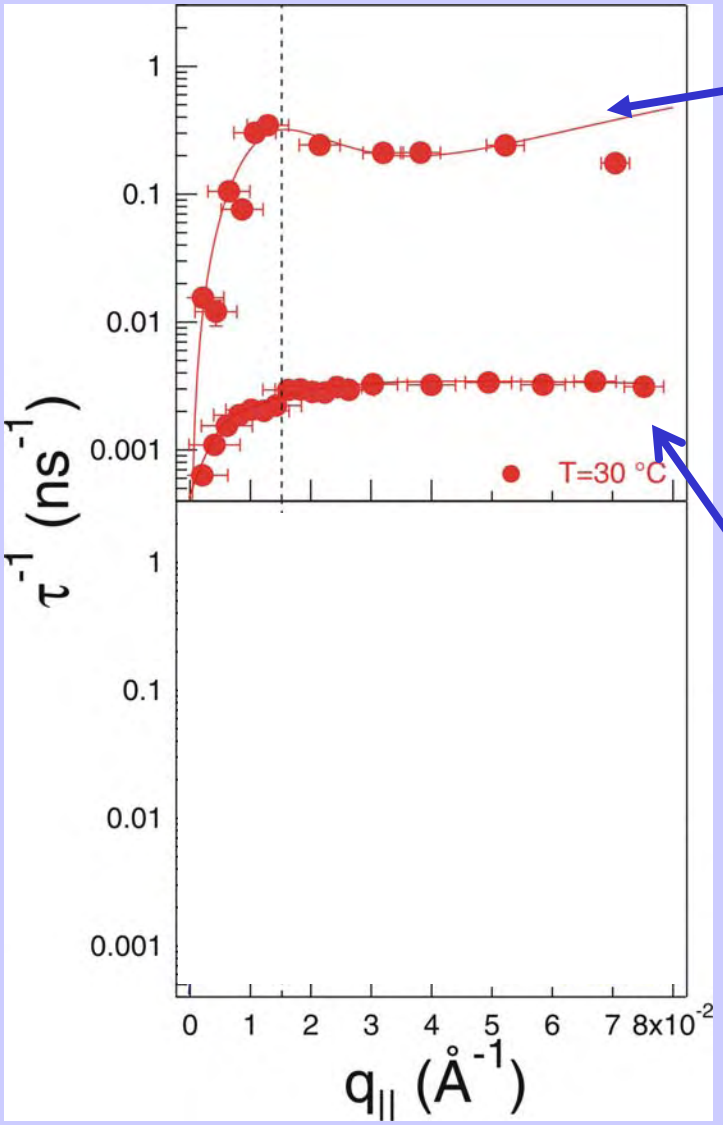


$$B = -d\left(\frac{\partial \Pi}{\partial d}\right) \quad [J/m^3]$$

Compressional modulus

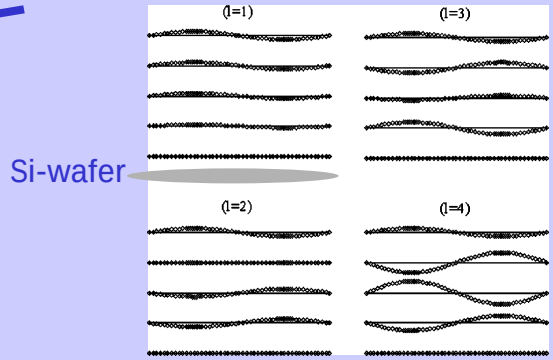
$$H = \int dV \left\{ B\left[\frac{\partial u}{\partial z}\right]^2 + K[\nabla^2 u]^2 \right\}$$

# Mesoscopic Membrane Dynamics

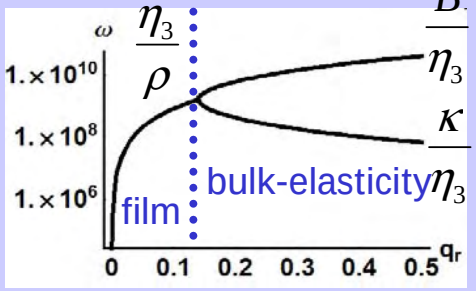



PRL 97, 048103 (2006)

## Undulations

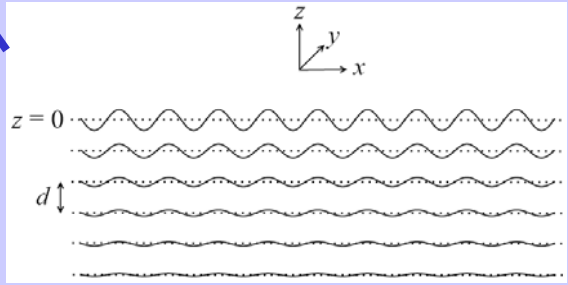



## Relaxation




Romanov and Ul'yanov, PRE 66, 061701 (2002)

## Surface mode




Bary-Soroker and Diamant,
Europhys. Lett., 73, 871 (2006), March 15, 2006

Fit to smectic hydrodynamic theory
Ribotta, PRL 32, 6, (1974).

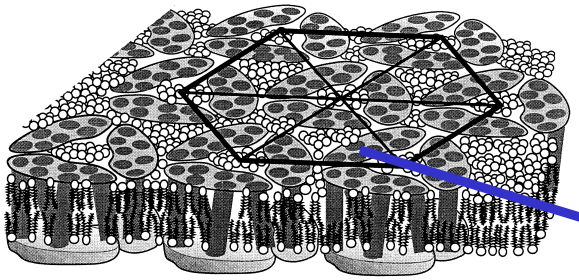
$$\tau^{-1}(q_{||}) = \frac{\kappa / d}{\eta_3} q_{||}^2 \frac{q_{||}^4 + (\pi/(\Lambda D))^2}{q_{||}^4 + \frac{1}{\mu\eta_3}(\pi D)^2}$$

## Quantitative access to membrane properties

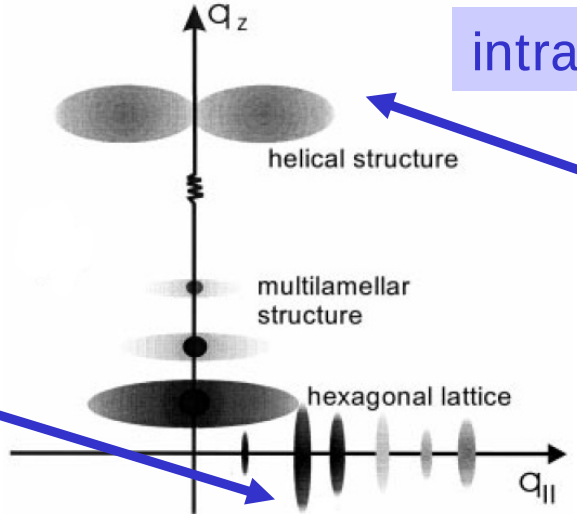
# Cooperative Protein Dynamics

Laboratory for Membrane and Protein Dynamics

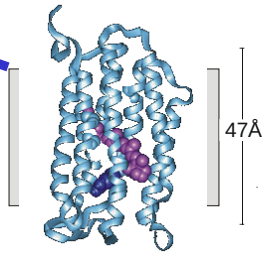
## Bacteriorhodopsin in Purple Membrane



Sample: Dieter Oesterhelt, MPI Munich



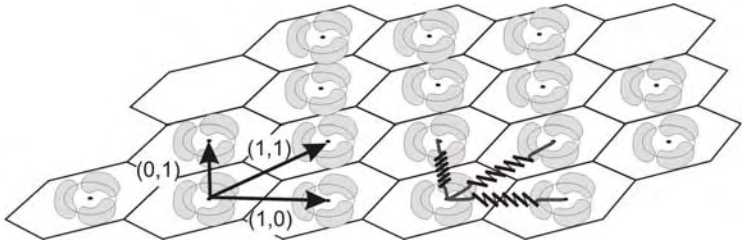

intra protein dynamics

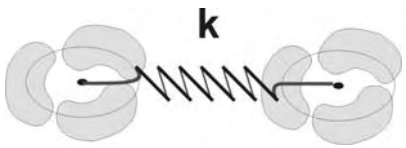


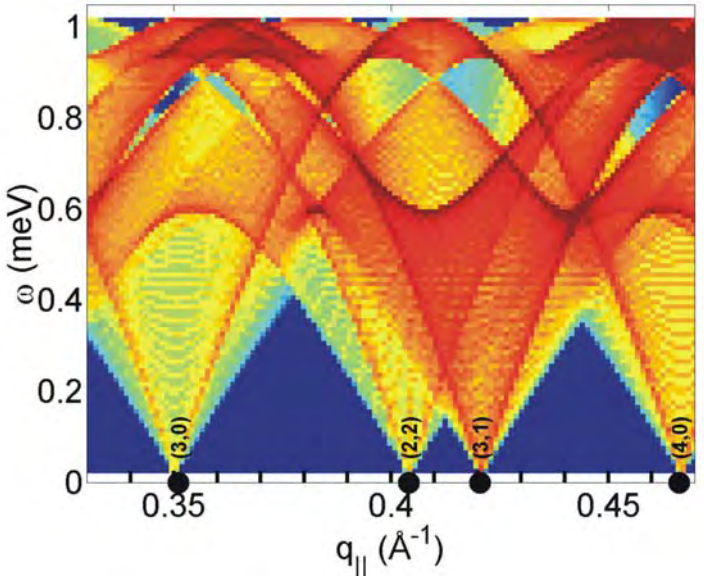

inter protein dynamics

Karin Schmalzl, Dieter Strauch, ILL+U Regensburg

a=62 Å

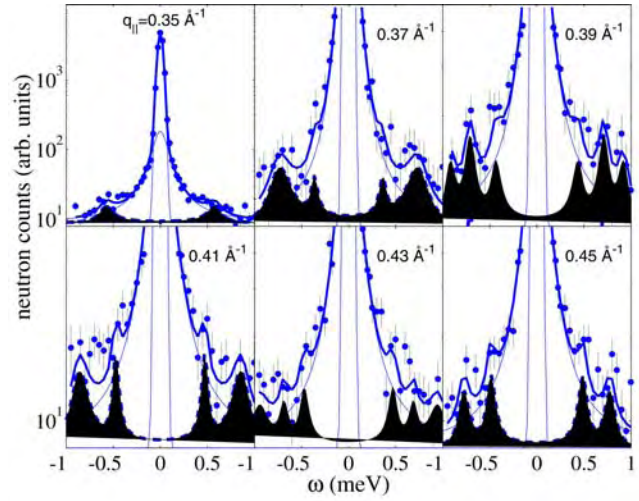


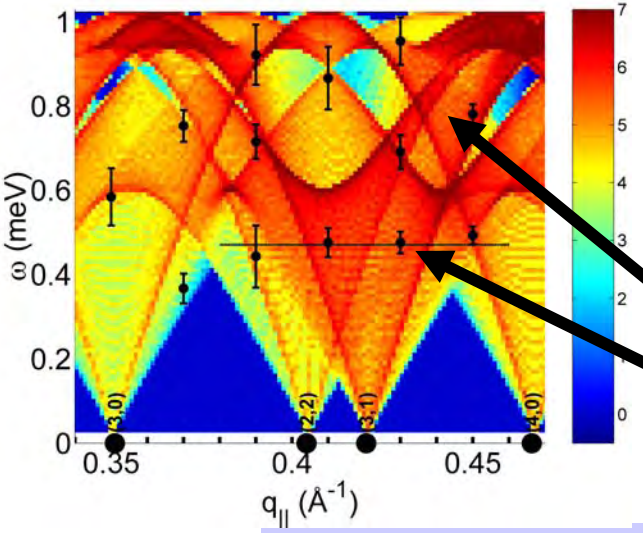




PRL 103, 128104 (2009)

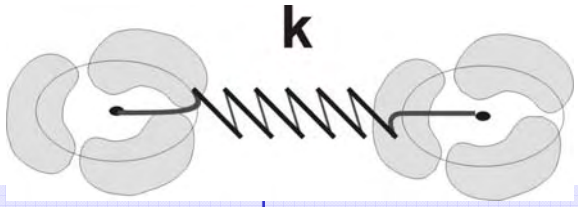
# Cooperative Protein Dynamics




$k = (l + t) \approx l$ (longitudinal)

$M_{BR}\omega^2 = 2(3l + t) \approx 6l$




Equipartition theorem:

$\frac{1}{2}k\langle r^2\rangle = \frac{1}{2}k_BT$

| **k** | 52 N/m |
|---|---|

acoustical

optical

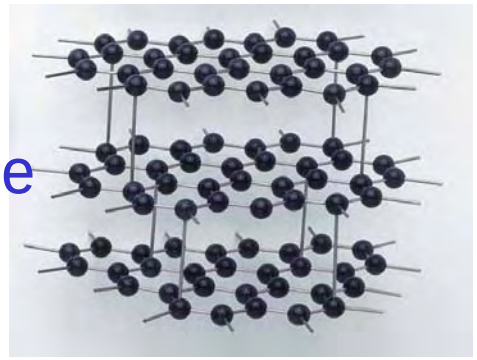
} phonons

Protein-Protein interaction in biological membranes

# Protein “Communication”

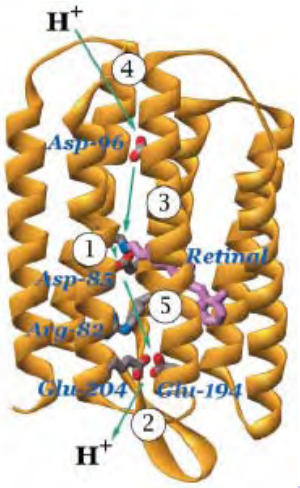

$k_{PM}$~50 N/m

graphite



$k_{inter}$~0.1 N/m

$k_{C\text{-}C}$~10,000 N/m




BR

} 1.7 Å

H. Luecke, Science, 1999

Photocycle

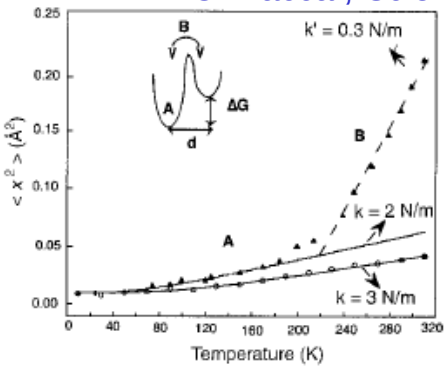
Organized Proteins may work more efficiently

# Diffusive vs. Collective Dynamics

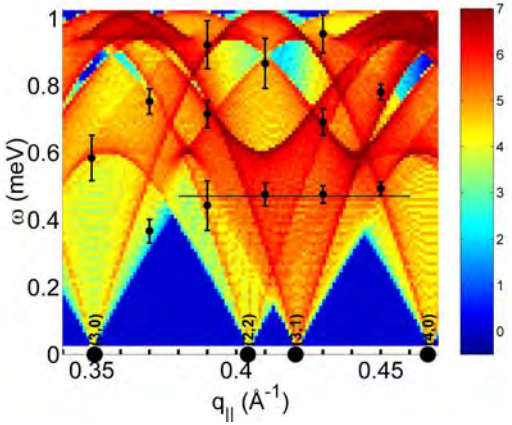
Laboratory for Membrane and Protein Dynamics

## How "soft" is a Protein?

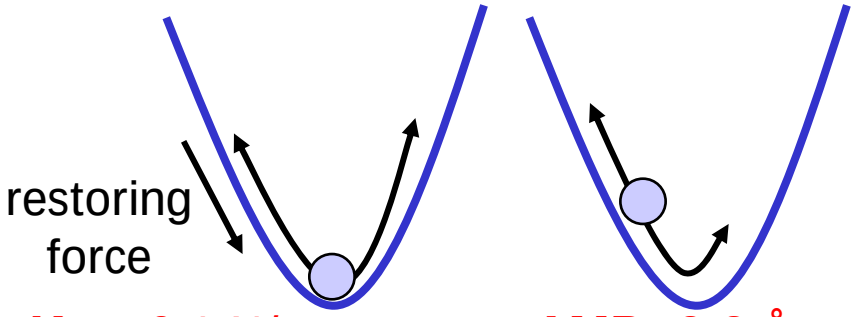
G. Zaccai, Science, 2000




## How interactive is a membrane?



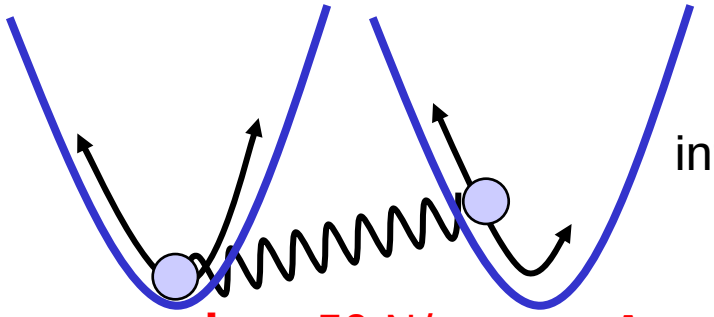

### Diffusive Motion of Proteins in PM




**$K_{PM}$**=0.1 N/m

**AMP**=2-3 Å

### Collective Motion-Protein Interactions




**$k_{PM}$**=50 N/m

**Amp**=0.2 Å

## Thermal Fluctuation Spectrum

## Interactions: 1.7 Å ~ 9 nN