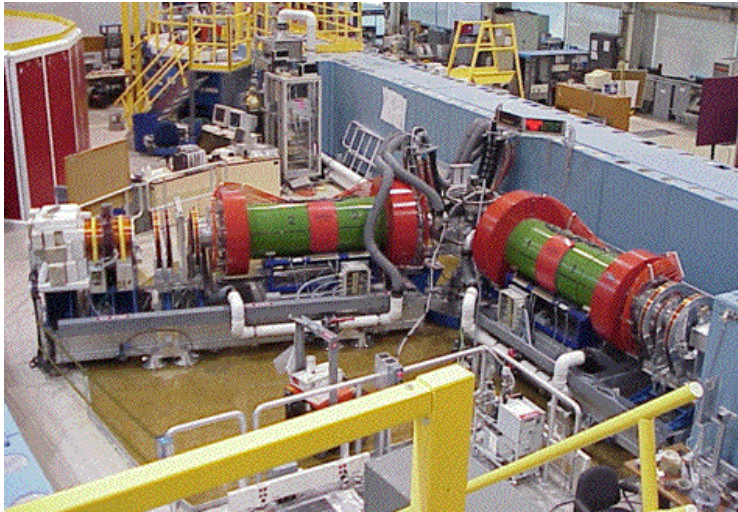


Neutron spin echo spectroscopy and membrane dynamics in solution

Michihiro Nagao

NIST Center for Neutron Research and Indiana University

Dynamics measurement – neutron spin echo



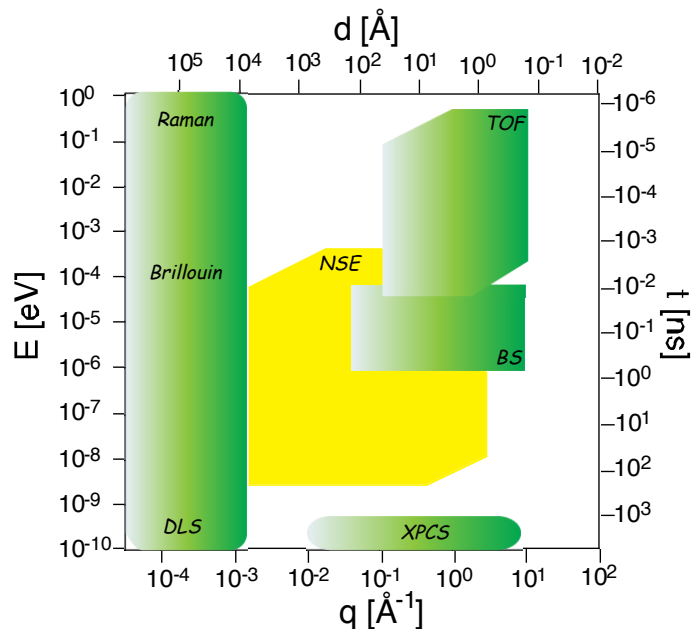
NG5-NSE @ NIST

One of the two NSE spectrometers operational in the North America

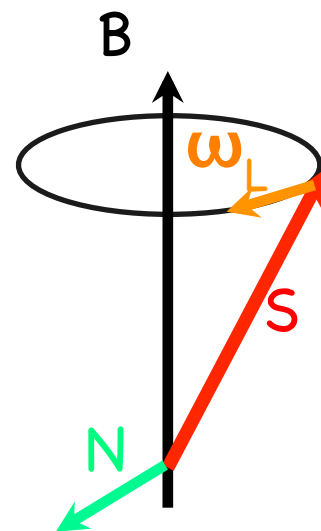
NSE has the highest energy resolution among inelastic neutron spectrometers

NSE directly measures intermediate scattering function $I(q,t)$; working in the time domain

nm length scale and ns time scale dynamics are the subjects to the NSE measurement (energy resolution down to the order of neV or 100s' ns)



neutron Larmor precession



The neutron spin (S) experiences a torque (N) from a magnetic field (B) perpendicular to its spin direction

$$N = S \times B$$

$$\omega_L = \gamma B$$

$$\gamma = 1.83 \times 10^8 \text{ s}^{-1} \text{ T}^{-1}$$



What neutron spin echo measures

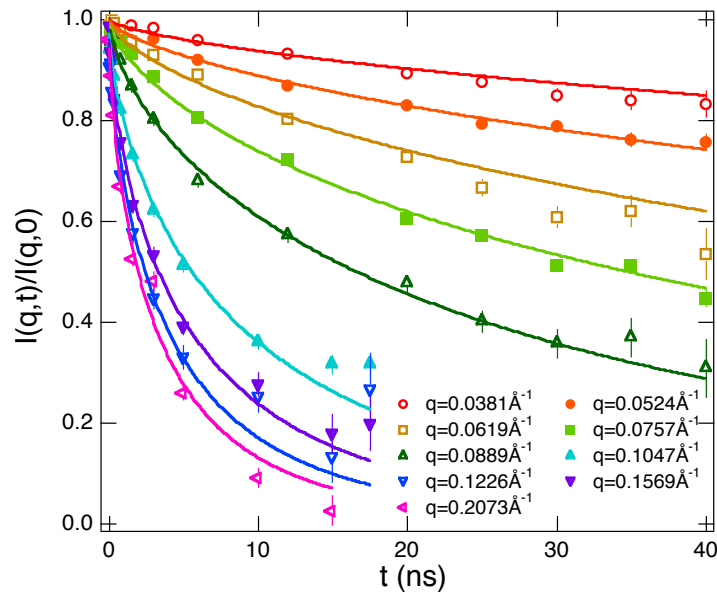
$I(q,t)$; intermediate scattering function = FT of space-time correlation function between an object existing at time t and that existed at $t=0$.

$$I(q,t) = \int G(r,t) \exp(iqr) dr$$

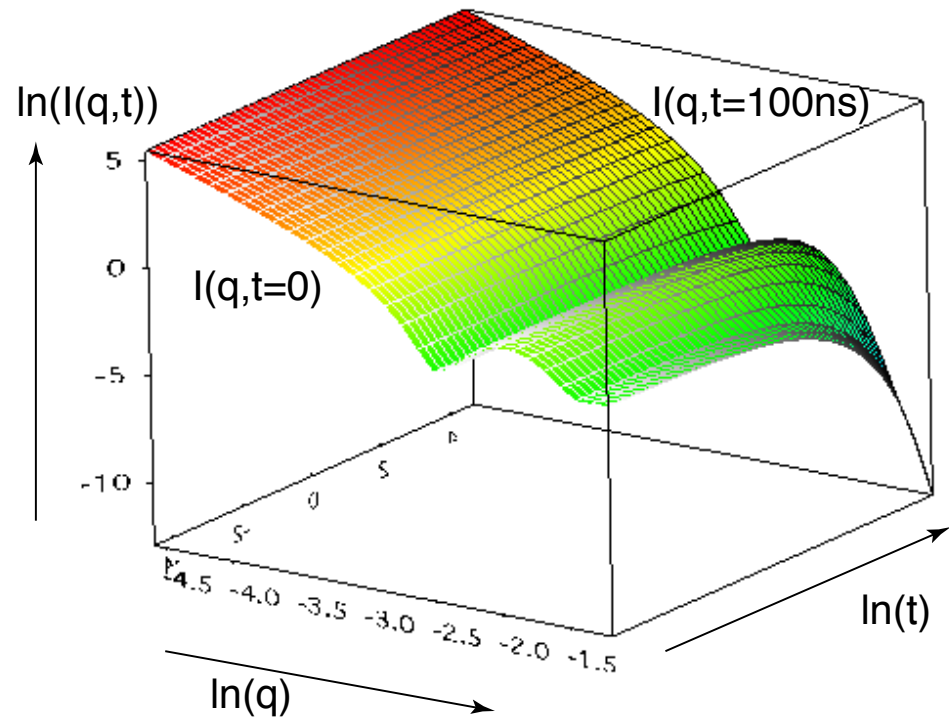
$$= \int S(q,\omega) \exp(i\omega t) d\omega$$

$$I(q,0) = \int S(q,\omega) \exp(0) d\omega = \int S(q,\omega) d\omega$$

Observed by SANS

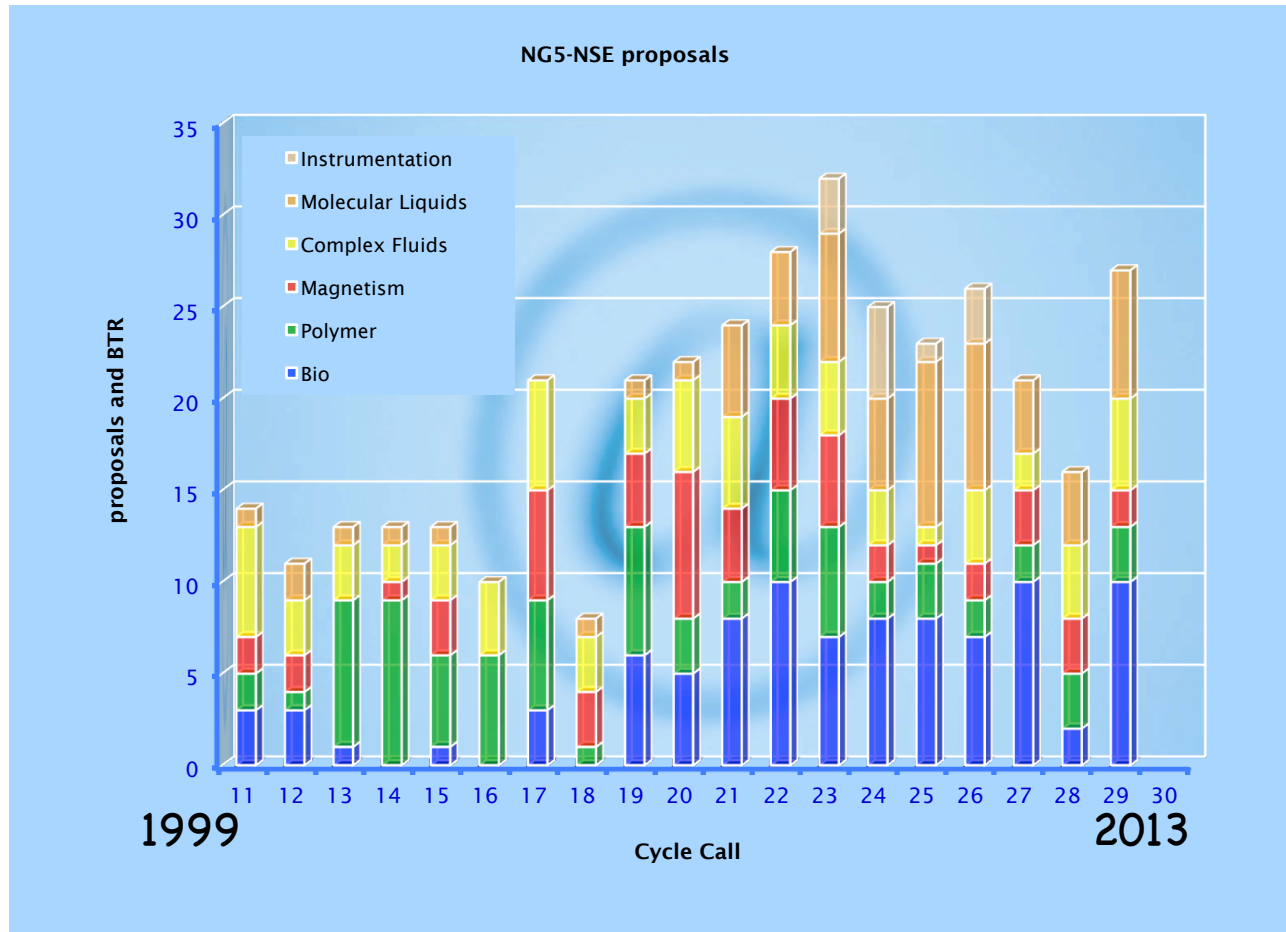


NSE measures intermediate scattering function, $I(q,t)$



NSE: good at measuring relaxation, such as diffusion

Science on NG5-NSE



Bio

model bio-membrane
protein motion

Polymer

segment dynamics
hydro gels
polymer complex
nanocomposite

Magnetism

frustrated magnets
spin glass

Complex Fluids

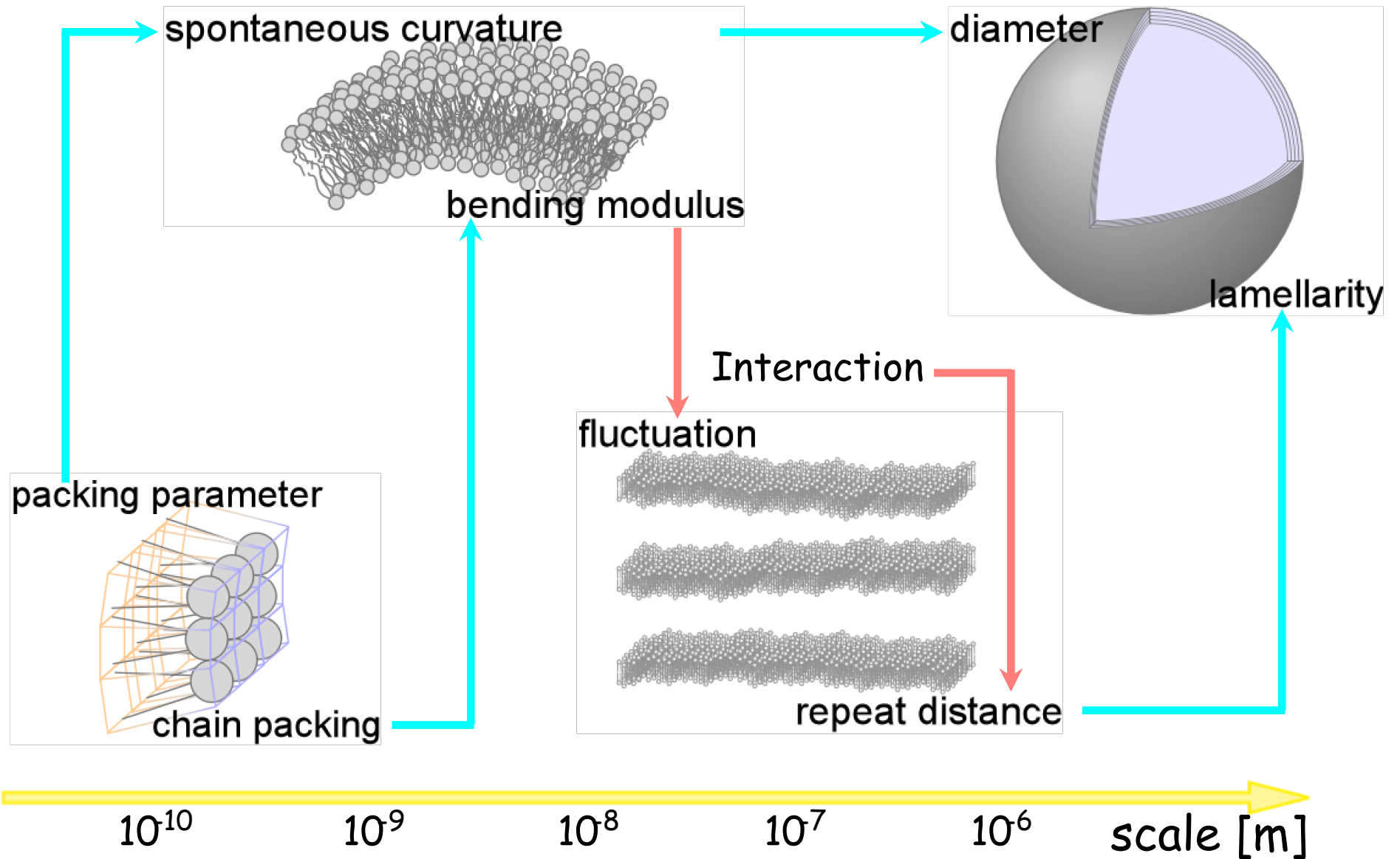
microemulsions
surfactant membranes
cluster dynamics

Molecular Liquids

confined water
ionic liquids

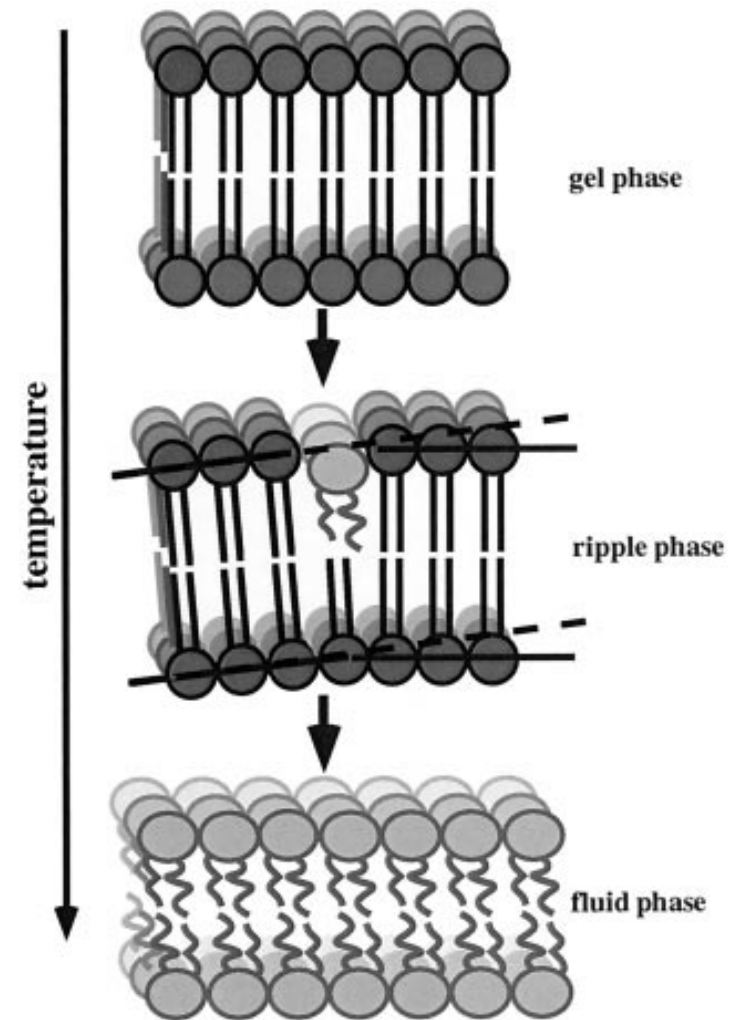
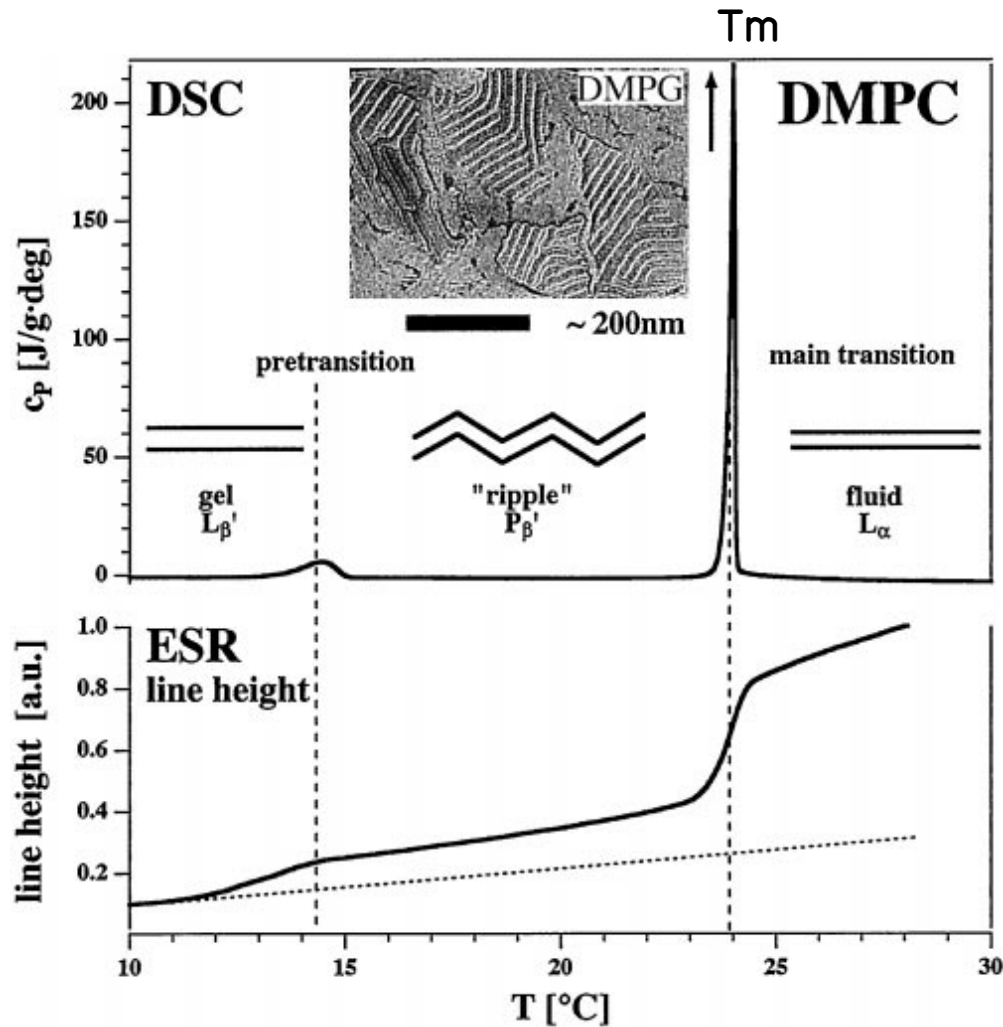
Membrane dynamics measured by Neutron Spin Echo
- focus on thickness fluctuations

Hierarchical structure of lipid vesicles



Phase behavior of lipid bilayers

Lipid molecules undergo fluid-gel phase transition at the main transition temperature T_m

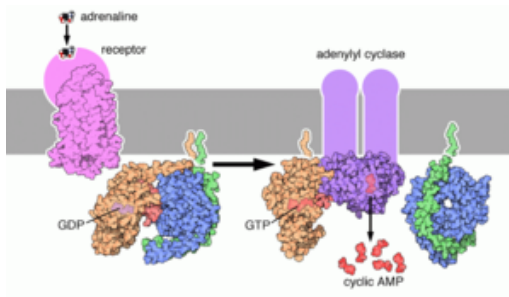


Lipid bilayers are highly dynamic

Lipid membranes are self-assembled highly flexible structures that have the ability to undergo an array of conformational and dynamic transitions which are essential for many biological functions.

Atomic to molecular length scale:

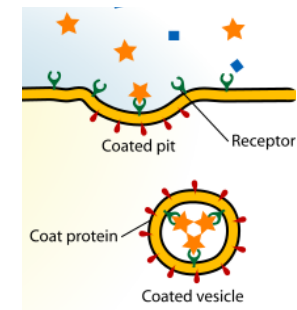
Cell signal transduction is affected by molecular lateral diffusion within the lipid membrane.



Marguet et al., *EMBO J.* **25**, 3446 (2006).

Macroscopic length scale:

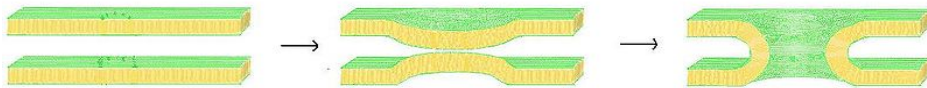
Membrane stiffness and fluidity have been shown to have a large impact on cellular uptake and release.



Weber et al., *Adv. Med. Eng.* **114**, 377 (2006).

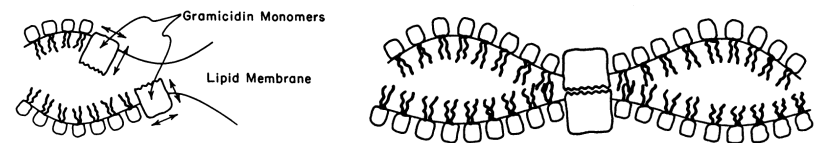
Intermediate length scale :

Membrane thickness fluctuations have been proposed as a mechanism for pore formation.



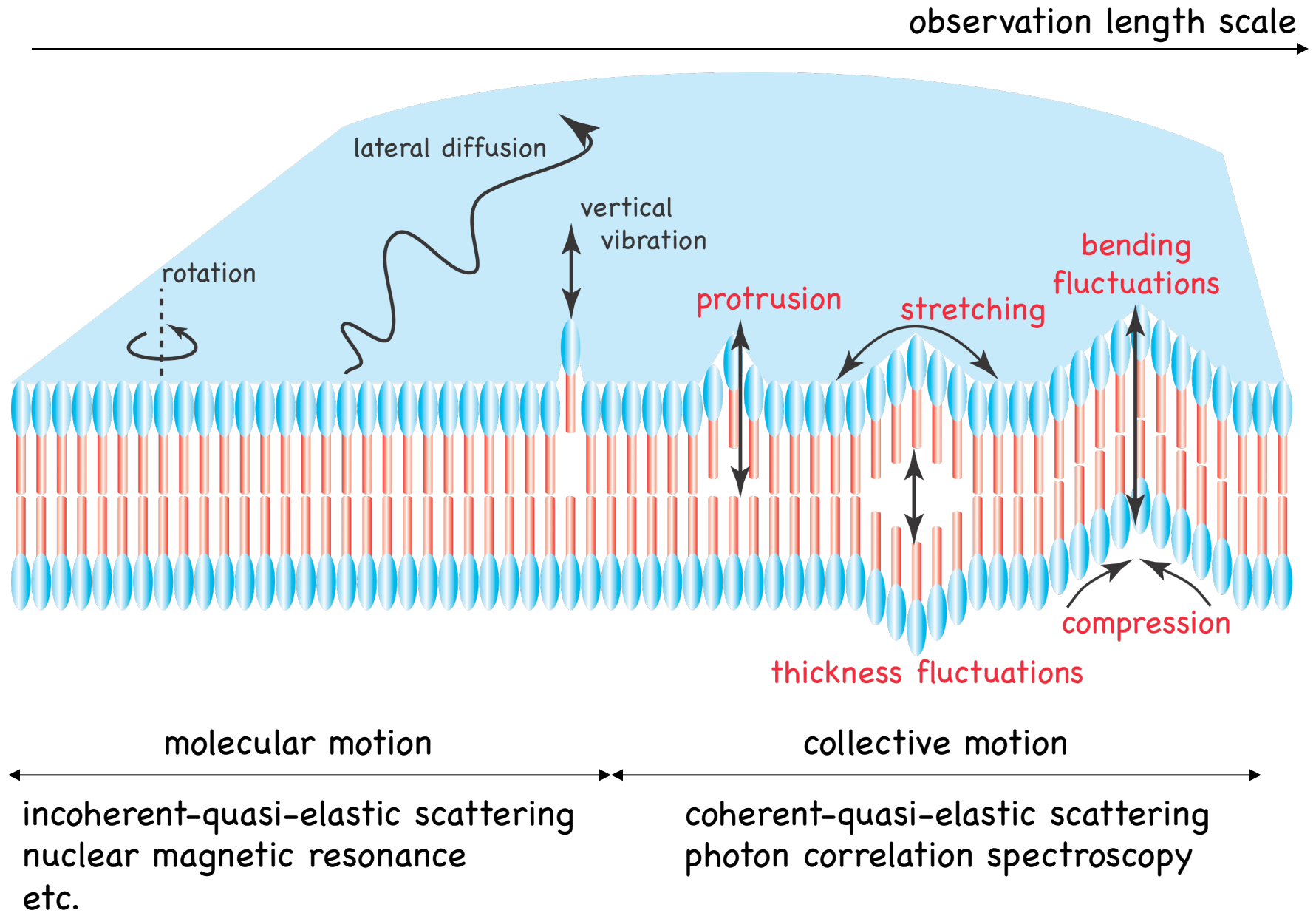
Movilenu et al., *Bull. Math. Biol.* **58**, 1231 (2006).

Also suggested as a mechanism for peptide channel formation.

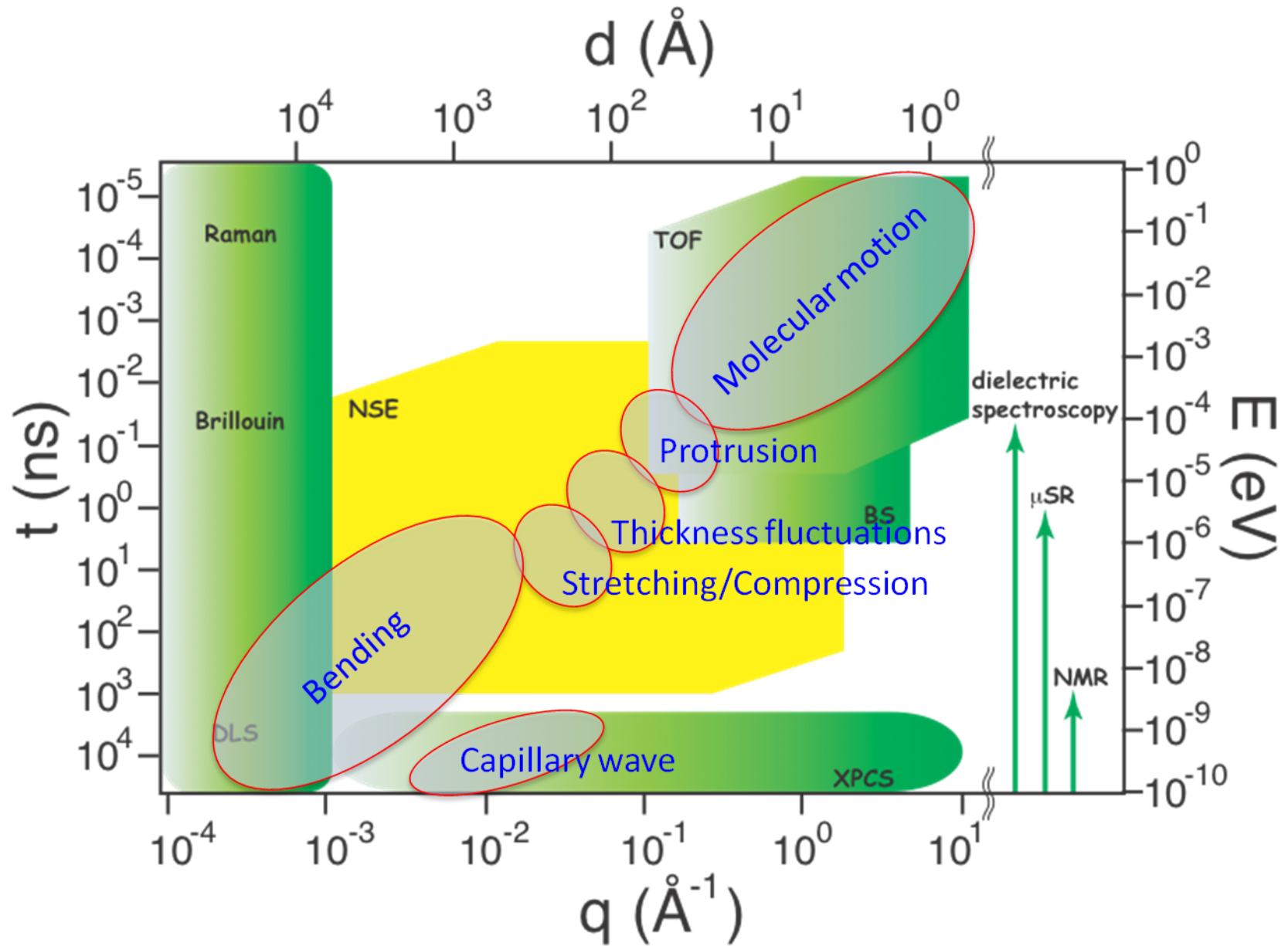


Helfrich and Jakobsson, *Biophys. J.* **57**, 1075 (1990).

Membrane dynamics



Techniques to measure dynamics

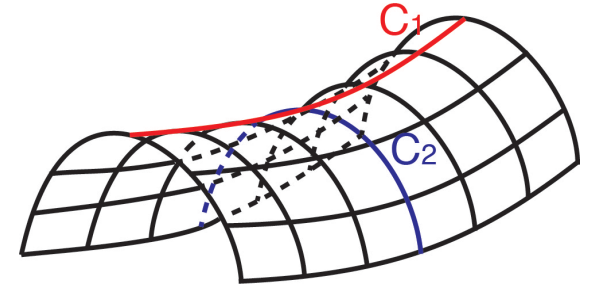


Membrane dynamics – theory for bending –

Helfrich bending energy Helfrich, *Z. Naturforsch.* **28**, 693 (1973).

Assuming the membrane is thin enough sheet, which is undulating

$$f = \frac{\kappa}{2} \{ (C_1 + C_2) - C_0 \}^2 + \bar{\kappa} C_1 C_2$$

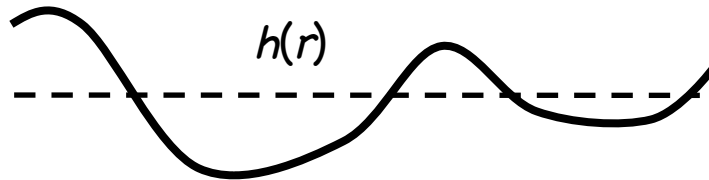


Zilman-Granek theory

Zilman and Granek, *Phys. Rev. Lett.* **77**, 4788 (1996).

Zilman and Granek, *Chem. Phys.* **284**, 195 (2002).

Dynamics of a planar non-interacting Helfrich sheet



$$H = \frac{1}{2} \kappa \int d^2r (\nabla^2 h(\vec{r}))^2 = \frac{1}{2\xi^2} \sum_{\vec{q}} \kappa q^4 h_{\vec{q}} h_{-\vec{q}}$$

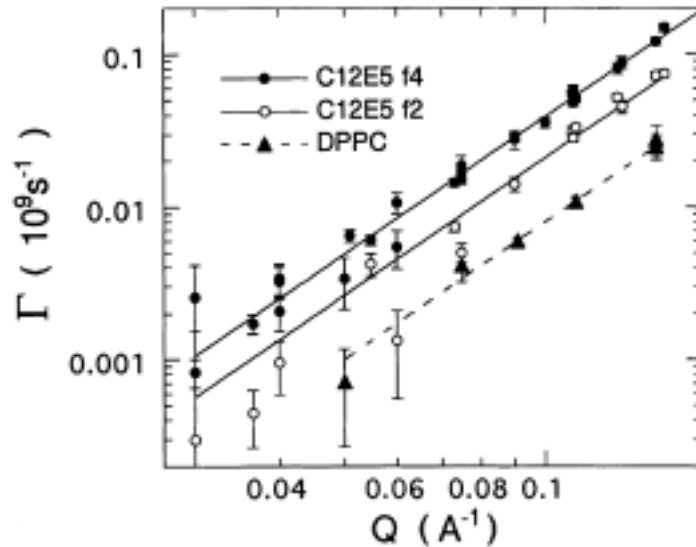
$$\frac{I(q,t)}{I(q,0)} = \exp \left[-(\Gamma t)^{2/3} \right]$$

$$\Gamma = 0.025 \gamma \left(\frac{k_B T}{\kappa} \right)^{1/2} \frac{k_B T}{\eta} q^3$$

κ : bending modulus

η : solvent viscosity

Application of Zilman-Granek theory to lipid bilayers



Takeda et al., *J. Phys. Chem. Sol.* **60**, 1375 (1999).

To get reasonable values of κ ,

$$\Gamma = 0.025\gamma \left(\frac{k_B T}{\kappa} \right)^{1/2} \frac{k_B T}{\eta} q^3$$

$$\eta = (3 \text{ to } 4)\eta_{\text{solv}}$$

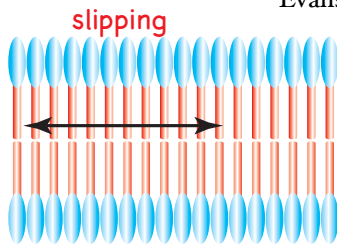
Taking the local dissipation at the membrane into consideration

Watson-Brown theory

Watson and Brown, *Biophys. J.* **98**, L09 (2010).

Extension of ZG theory including slipping of each monolayer

Evans and Yeung, *Chem. Phys. Lipids.* **73**, 39 (1994).; Seifert and Langer, *Europhys. Lett.* **23**, 71 (1993).

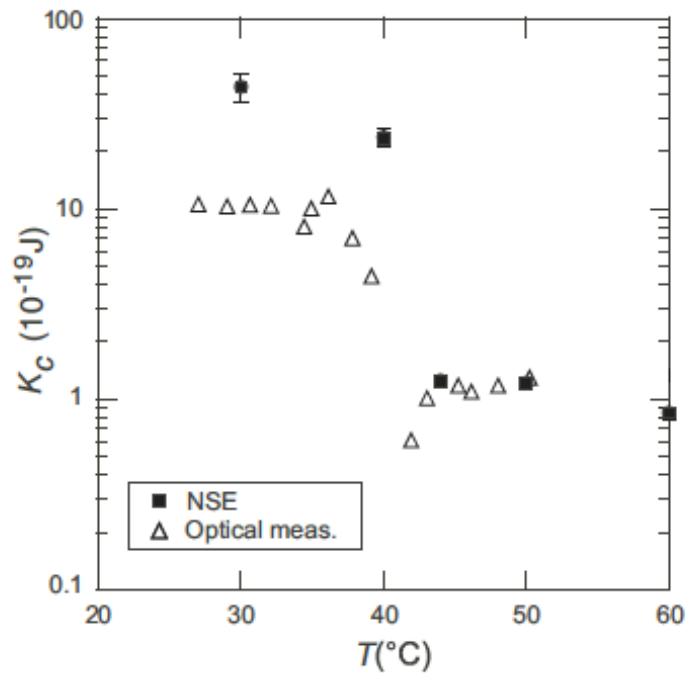


Inter-monolayer friction plays a role, where lateral compressibility k_m of membrane appears in dynamical equation

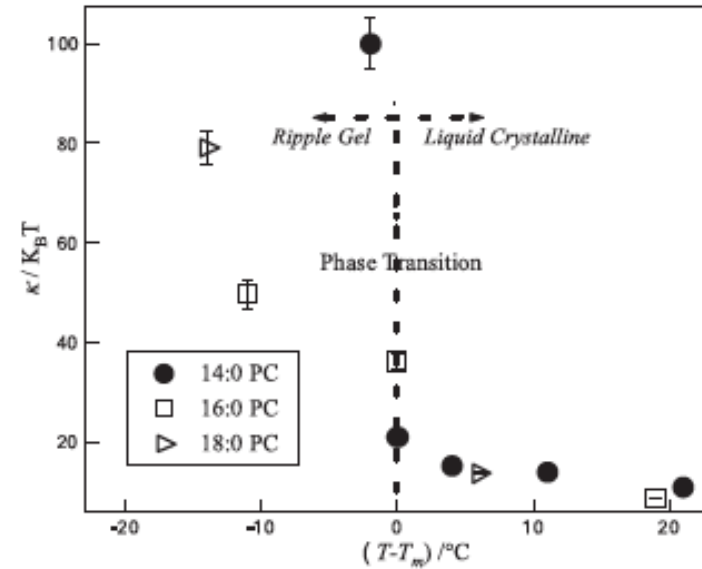
$$\kappa \rightarrow \tilde{\kappa} = \kappa + 2d^2 k_m$$

This correct the effective viscosity problems

Effects of temperature on bending



Seto et al., *Euro. Phys. J. E* **26**, 217 (2008).;
Lee et al., *Phys. Rev. E* **64**, 020901(R) (2001).

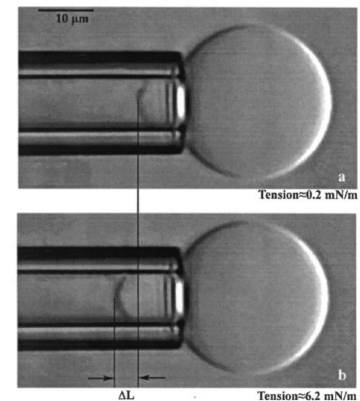


Yi et al., *J. Phys.: Cond. Matter* **21**, 155104 (2009).

Softening of bilayers occur at the main transition, T_m

Bending moduli differ about an order of magnitude below and above the transition

Similar trend is observed using micropipette pressurization



Rawicz et al., *Biophys. J.* **79**, 328 (2000).

Thickness fluctuations in lipid bilayers

Collaboration with

Andrea C. Woodka, NIST – current: US Military Academy

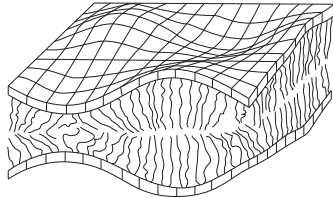
Paul D. Butler, NIST

Lionel Porcar and Bela Farago, ILL

Thickness fluctuations in lipid bilayers

Breathing model of a lipid bilayer by Miller

Miller, *Top. Bioelectrochem. Bioenerg.* **4**, 161 (1981); Bach and Miller, *Biophys. J.* **29**, 183 (1980); Miller, *Biophys. J.* **45**, 643 (1984).



Amplitude of the fluctuations reaches $\approx 15 \text{ \AA}$ or more from the geometrical constraints (volume conservation)

Thickness fluctuations by Hladky and Gruen

Hladky and Gruen, *Biophys. J.* **38**, 251 (1982).

Long wavelength fluctuation amplitude is negligible

Short wavelength fluctuations are severely limited

Intermediate wavelength fluctuation amplitude $< 10 \text{ \AA}$

Deformation free energy of bilayer membranes by Huang

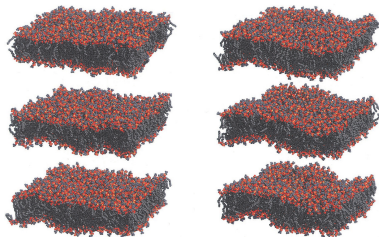
Huang, *Biophys. J.* **50**, 1061 (1986).

$$\sqrt{\langle \text{Amplitude}^2 \rangle} = \frac{k_B T}{\pi \gamma} \left\{ \tan^{-1} \left[\frac{16\pi^2 K_1 + h\gamma}{2h\sqrt{K_1 \bar{B}}} \right] - \tan^{-1} \left[\frac{\gamma}{2\sqrt{K_1 \bar{B}}} \right] \right\} \approx 4.5 \text{ \AA}$$

γ : surface tension, K_1 : splay coefficient, h : membrane thickness, \bar{B} : compressibility coefficient

Computer simulation by Lindahl and Edholm

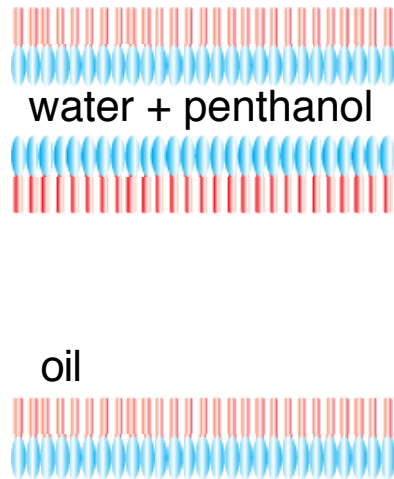
Lindahl and Edholm, *Biophys. J.* **79**, 426 (2000).



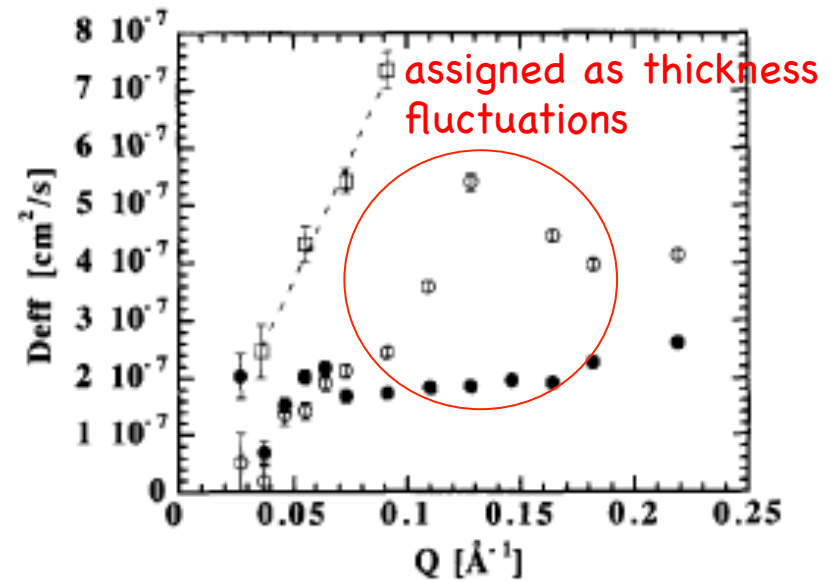
Monolayer RMS amplitude for peristaltic (thickness fluctuation): 2.5 \AA

Thickness fluctuations in surfactant systems

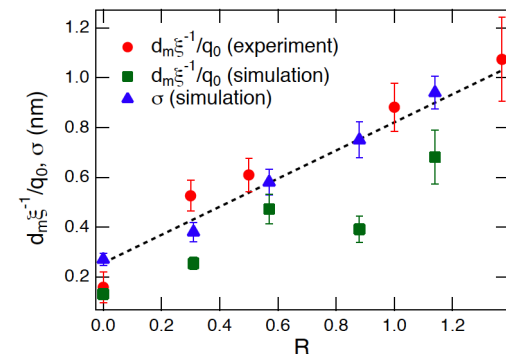
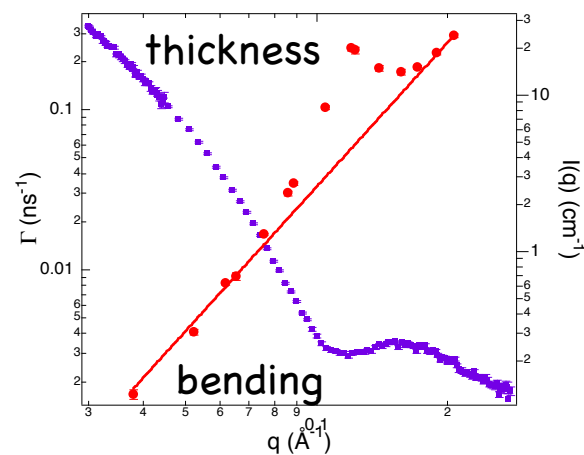
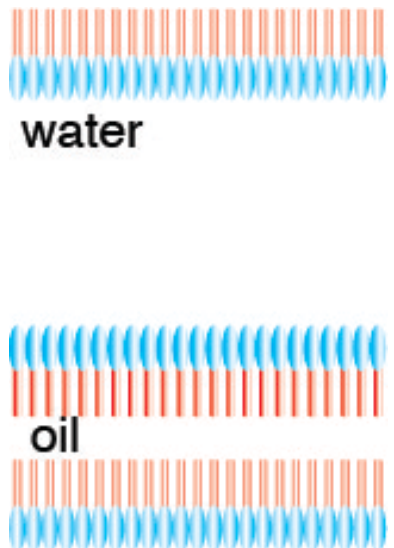
Farago et al., *Physica B* **213&214**, 712 (1995).; Farago, *Physica B* **226**, 51 (1996).



Neutron spin echo (NSE) measured Q dependence of D_{eff} , showing an increase at membrane thickness scale

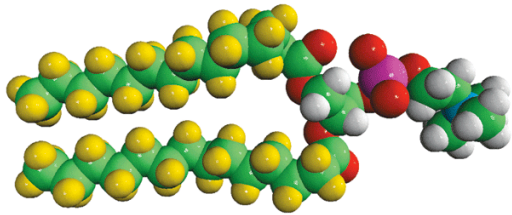


Nagao, *Phys. Rev. E* **80**, 031606 (2009).; Nagao et al., *Soft Matter* **7**, 6598 (2011).; Nagao, *J. Chem. Phys.* **135**, 074704 (2011).

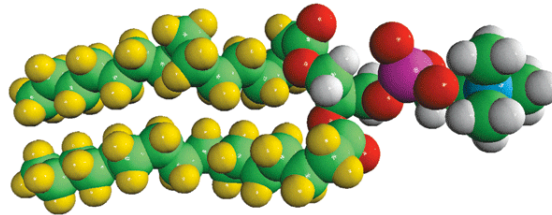


Computer simulation well reproduced the thickness fluctuation amplitude

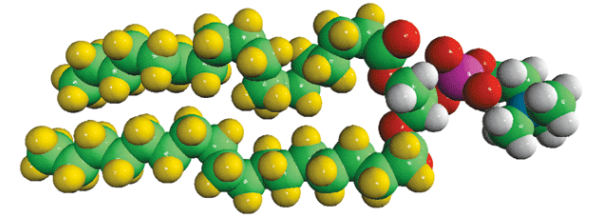
Samples



DMPC (C=14)



DPPC (C=16)

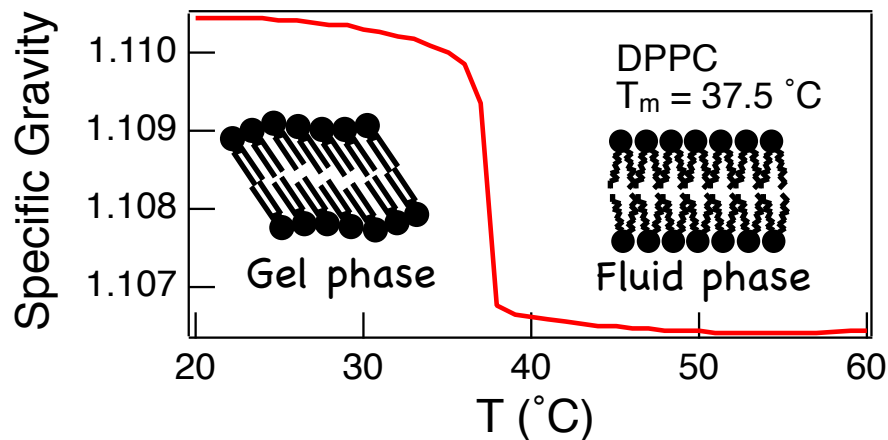


DSPC (C=18)

10 wt% lipids large unilamellar vesicles (LUVs) were prepared extruding through 100 nm polycarbonate filter in D₂O

tail deuterated lipids were used to enhance the signal from thickness fluctuations (scattering contrast matching between the tail region and water medium)

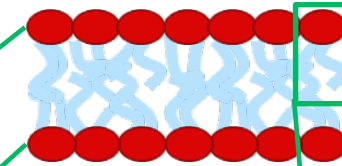
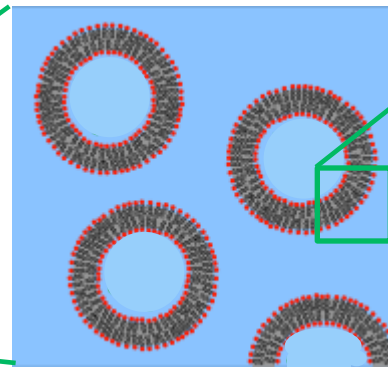
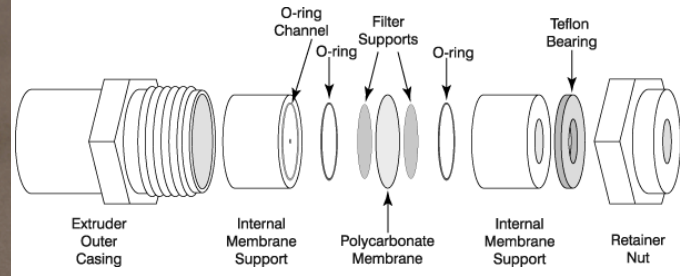
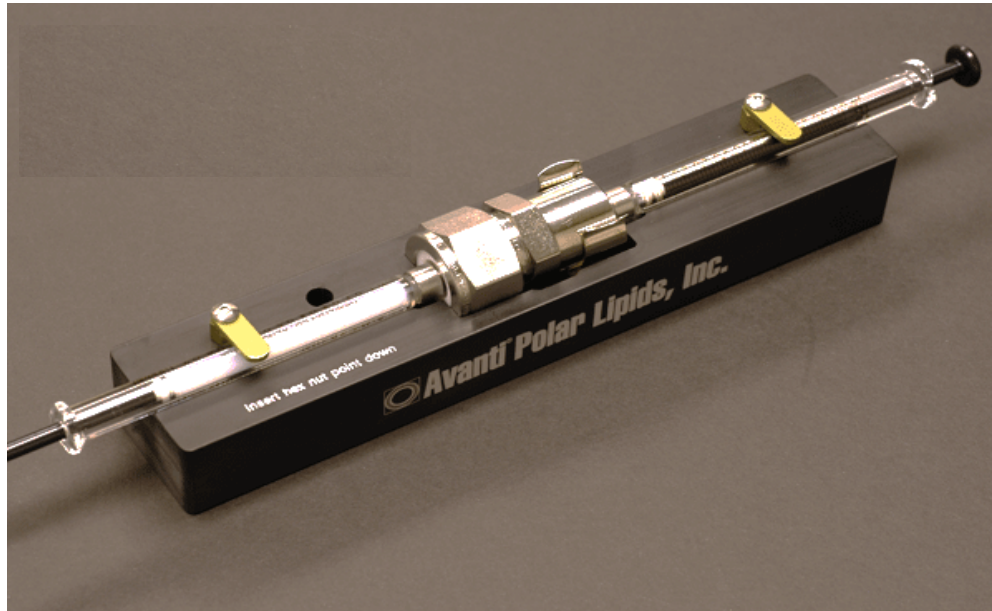
Density measurement to confirm the melting transition temperature, T_m



lipid	DMPC	DPPC	DSPC
T_m (°C) deuterated	20.5	37.5	50.5
T_m (°C) hydrogenated	23#	41#	55#

#from Avanti Polar Lipid web site

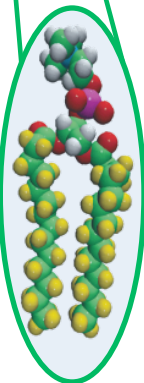
Unilamellar lipid vesicle preparation



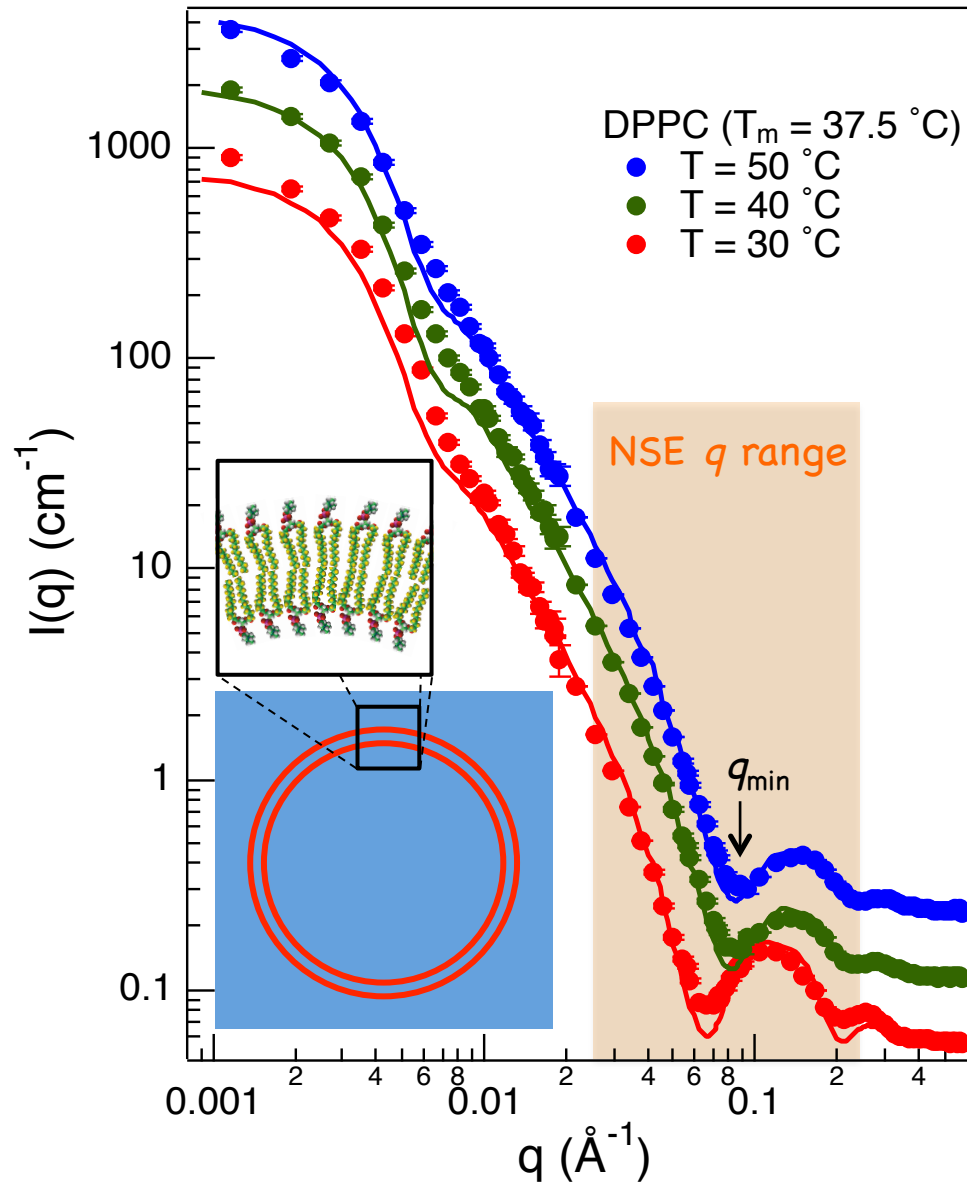
(D54)DMPC = C14

(D62)DPPC = C16

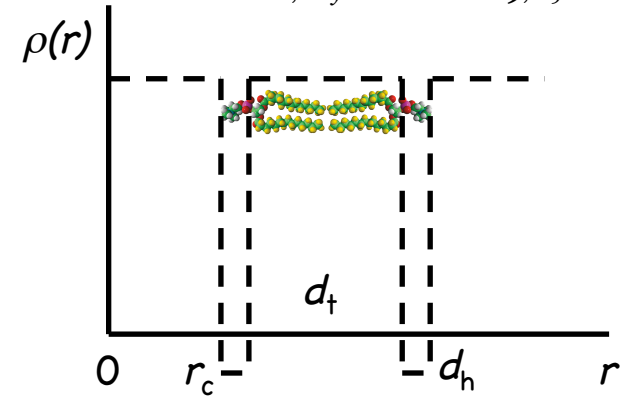
(D70)DSPC = C18



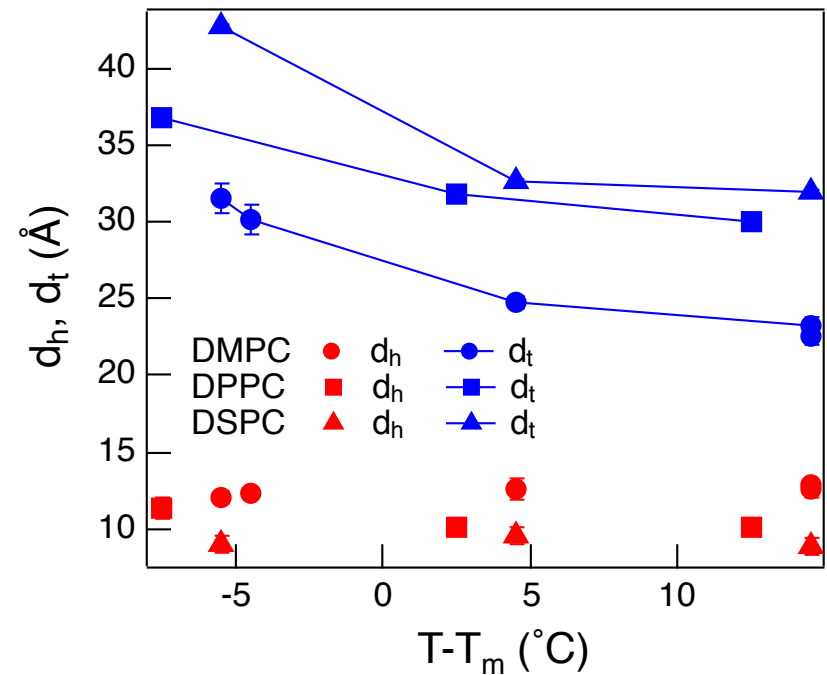
Small-angle neutron scattering (SANS) results



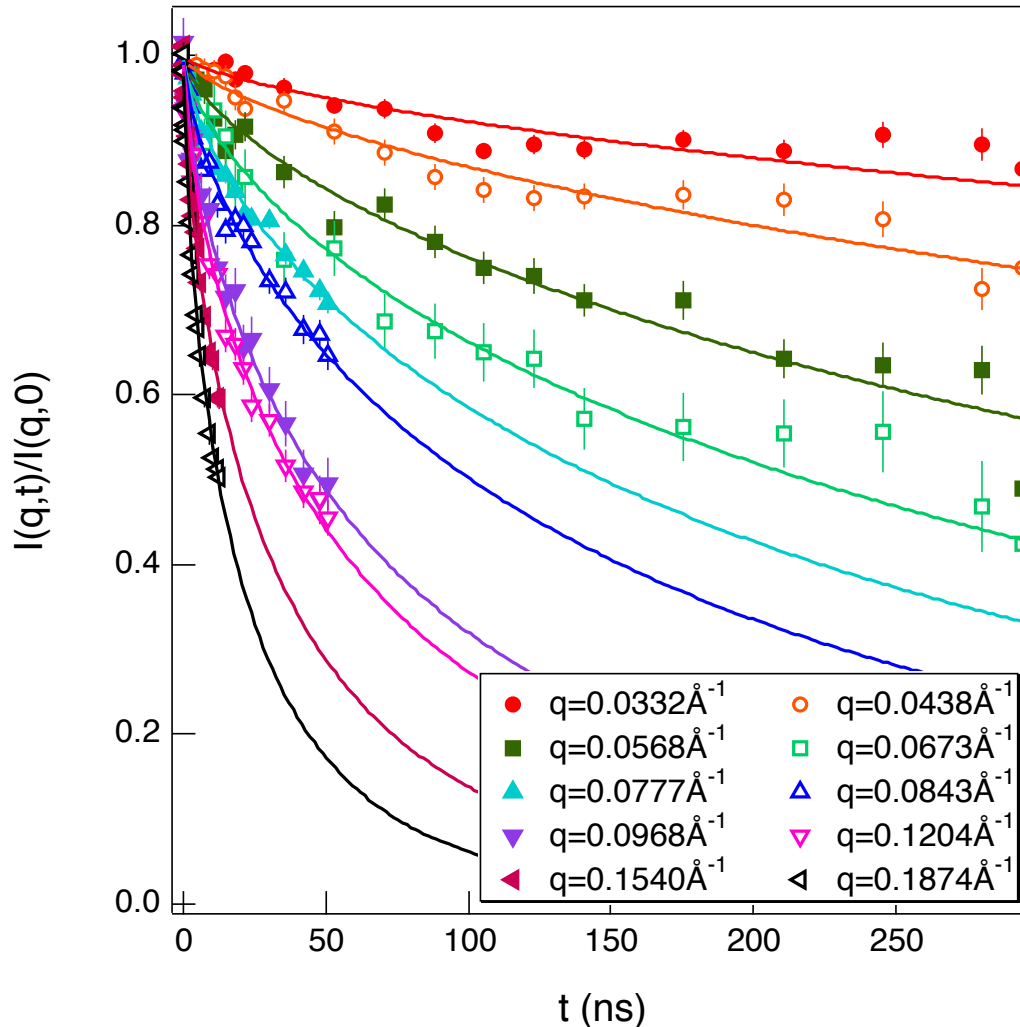
Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



Structure model for the fit



Intermediate scattering functions from DMPC bilayers



$$k_m = \frac{24\kappa}{d_t} \quad d_t: \text{thickness of tail}$$

Rawicz et al., *Biophys. J.* **79**, 328 (2000).

Bending motion is explained as a single membrane dynamics model

Zilman and Granek, *Phys. Rev. Lett.* **77**, 4788 (1996);
Zilman and Granek, *Chem. Phys.* **184**, 195 (2002).

$$\frac{I(q, t)}{I(q, 0)} = \exp \left[- (\Gamma t)^\beta \right]$$

Γ : decay rate, $\beta=2/3$

$$\frac{\Gamma_{\text{Bend}}}{q^3} = 0.025\alpha \sqrt{\frac{k_B T}{\tilde{\kappa}} \frac{k_B T}{\eta_{\text{D}_2\text{O}}}}$$

$\tilde{\kappa}$: effective bending modulus,
 η : solvent viscosity, $\alpha \approx 1$

Considering slipping friction

$$\tilde{\kappa} = \kappa + 2d^2 k_m$$

Watson and Brown, *Biophys. J.* **98**, L9 (2010).

κ : bending modulus,

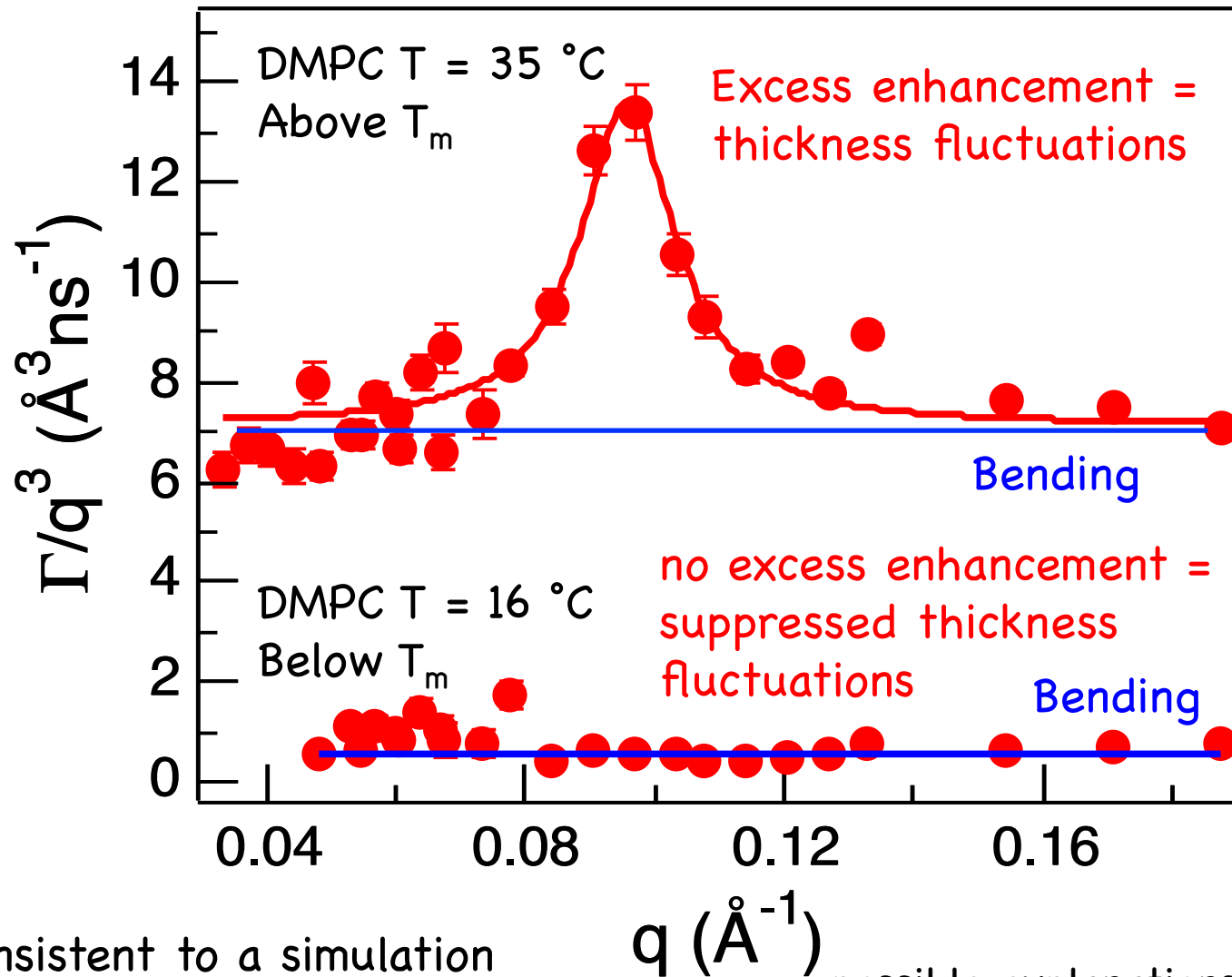
k_m : monolayer compressibility modulus

$$\frac{\kappa}{k_B T} = \left(172.4 \frac{\eta_{\text{D}_2\text{O}}}{k_B T} \frac{q^3}{\Gamma_{\text{Bend}}} \right)^2$$

Lee et al., *Phys. Rev. Lett.* **105**, 038101 (2010).

q-dependence of the decay rate

Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



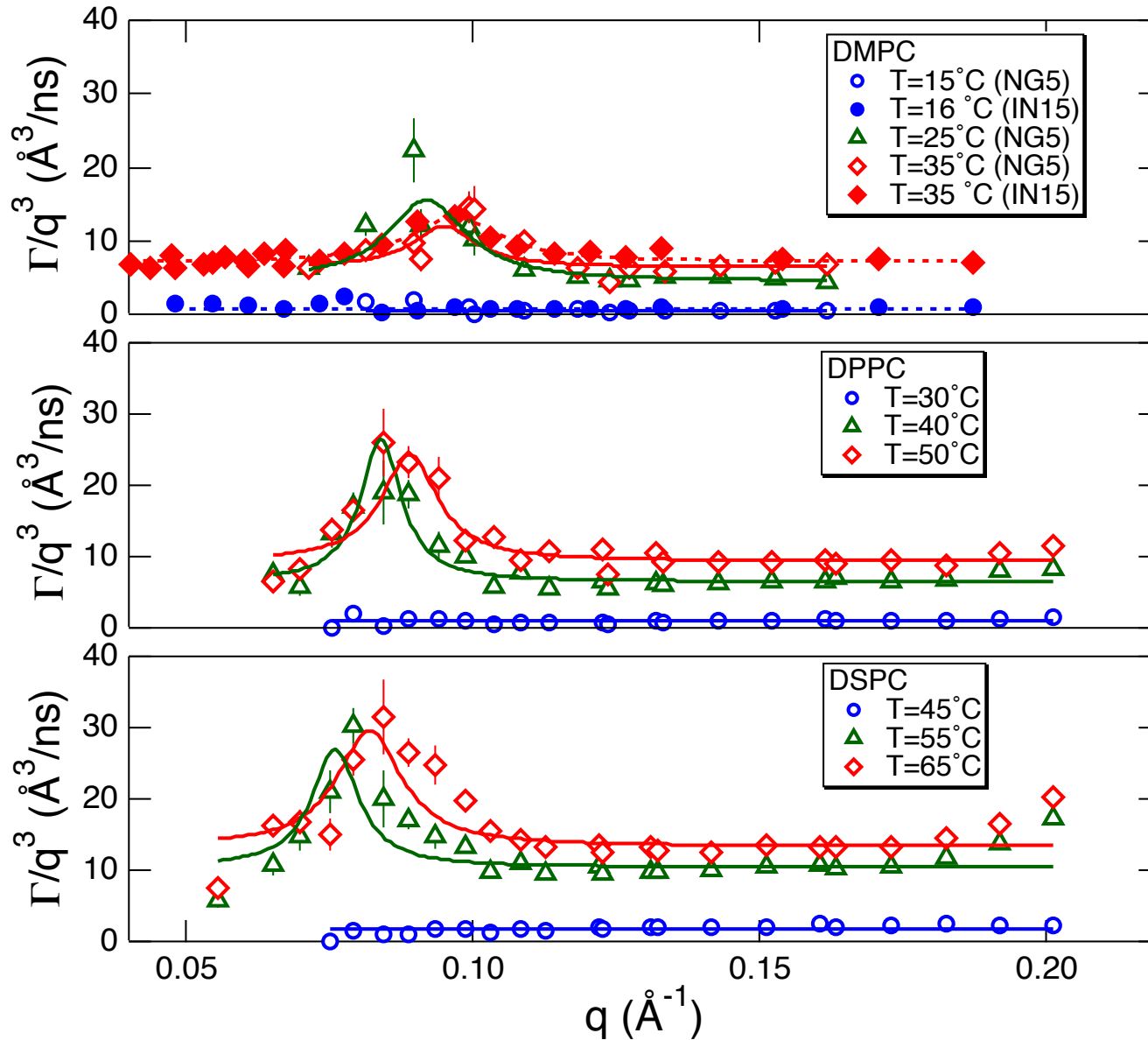
Consistent to a simulation

West and Schmid, *Soft Matter* **6**, 1275 (2010).

possible explanations:
complete suppression
slow down to outside the window

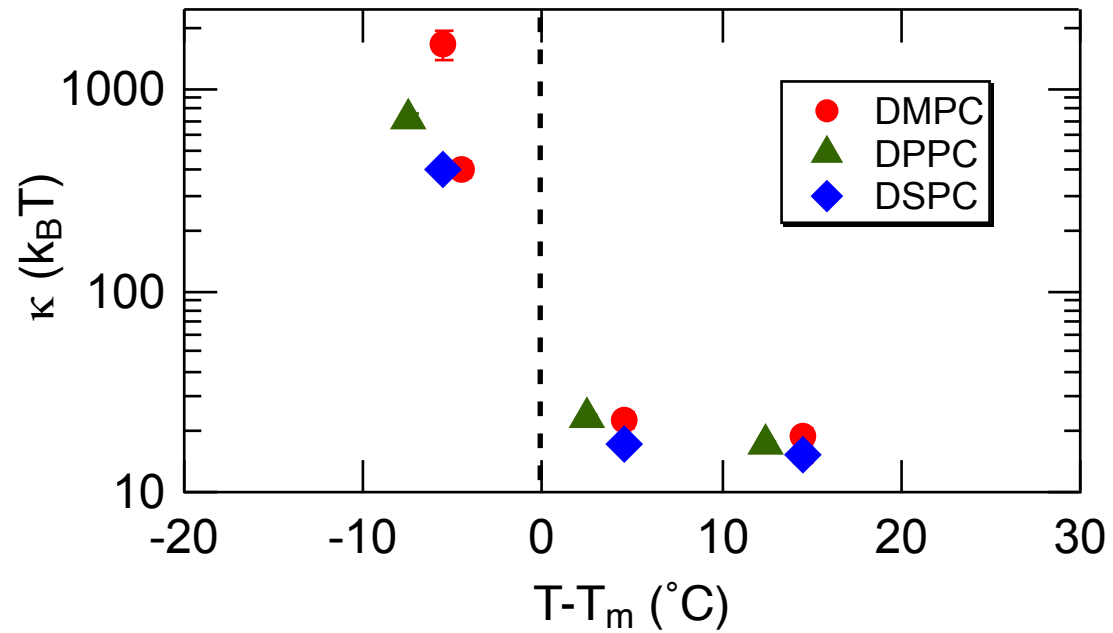
q-dependence of the decay rate for various lipids

Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



Estimated bending modulus

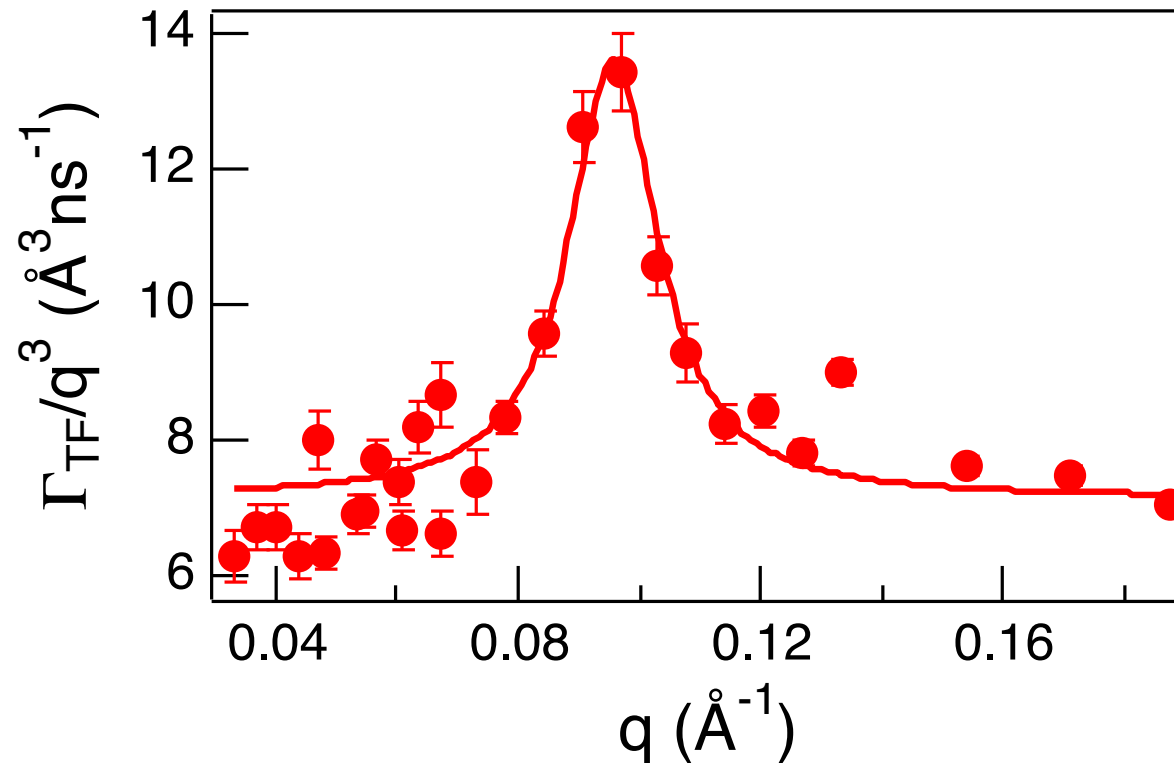
Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



Reasonable trends in temperature dependence

Observation of thickness fluctuations

Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



Empirical equation used for surfactant systems

Bending modulus

TF relaxation time

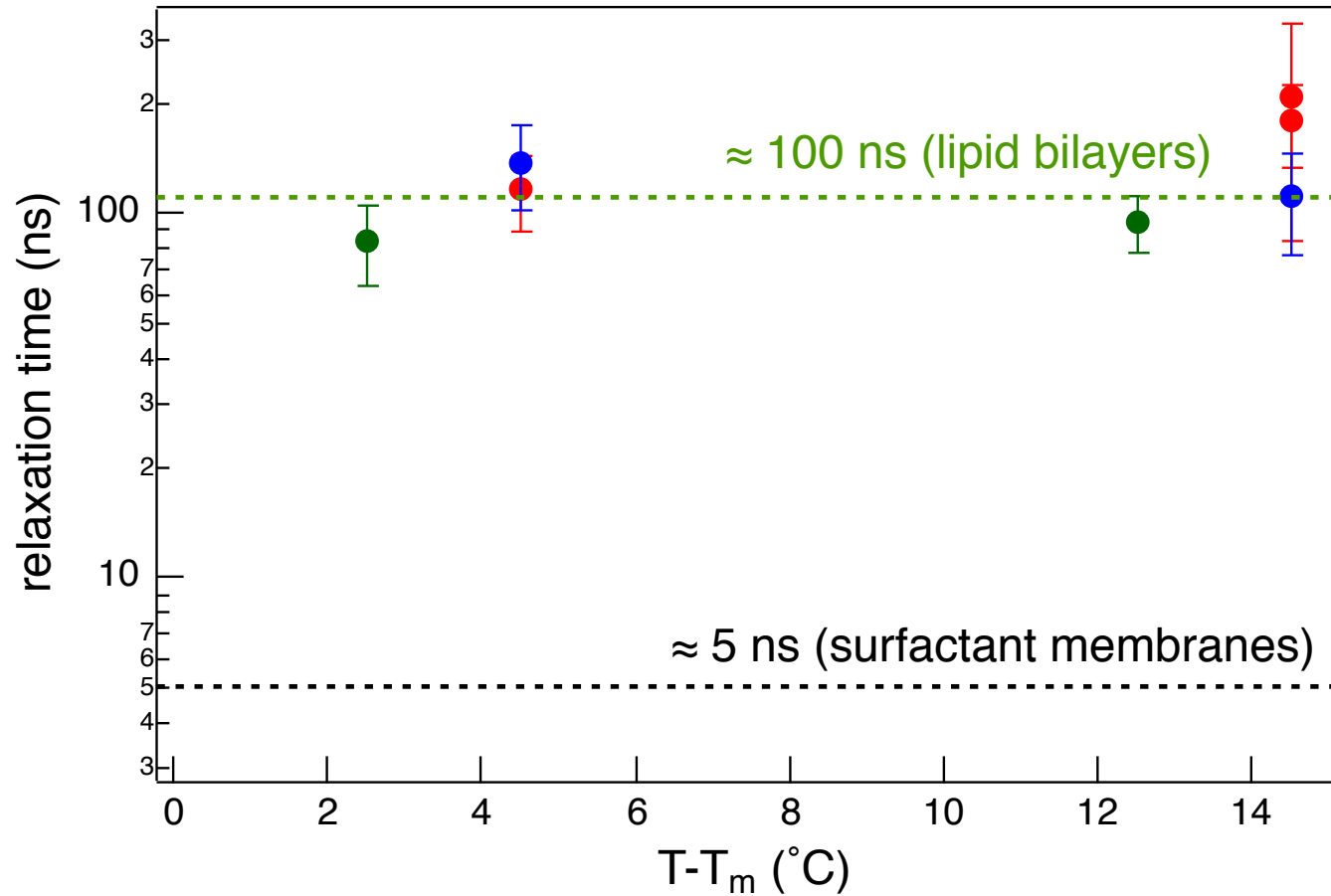
$$\frac{\Gamma}{q^3} = \frac{\Gamma_{\text{Bend}}}{q^3} + \frac{(\tau_{\text{TF}} q_0^3)^{-1}}{1 + (q - q_0)^2 \xi^{-2}}$$

TF amplitude

Nagao, *Phys. Rev. E* **80**, 031606 (2009).; Nagao et al., *Soft Matter* **7**, 6598 (2011).; Nagao, *J. Chem. Phys.* **135**, 074704 (2011).

Relaxation time of thickness fluctuations

Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).



Above T_m : independent of either temperature or tail chain length

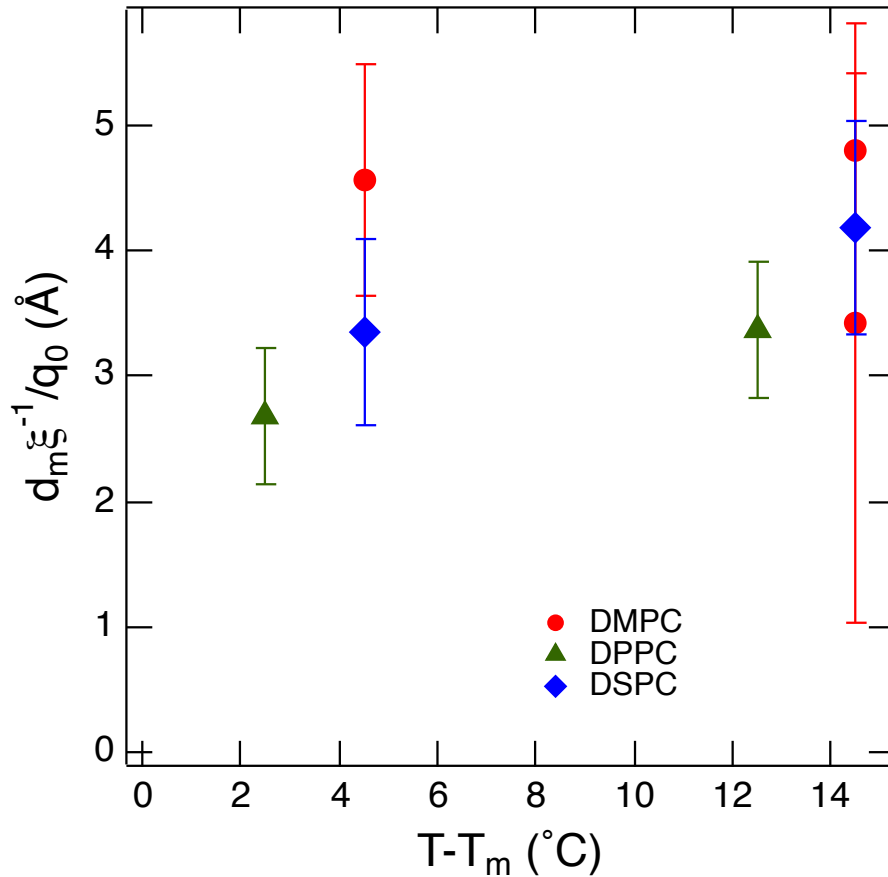
approximately an order slower than surfactant membranes

Thickness fluctuation amplitude

Woodka et al., *Phys. Rev. Lett.* **109**, 058102 (2012).

Width of Lorentz peak relates to the fluctuation amplitude

Nagao et al., *Soft Matter* **7**, 6598 (2011).



Mean amplitude = $3.7 \text{ \AA} \pm 0.7 \text{ \AA}$

experiment

Huang's mean amplitude $\approx 4.5 \text{ \AA}$

Huang, *Biophys. J.* **50**, 1061 (1986).

theory

Lindahl & Edholm's amplitude $\approx 5 \text{ \AA}$

Lindahl and Edholm, *Biophys. J.* **79**, 426 (2000).

simulation

$\approx 8 \%$ of the membrane thickness;
close to the value seen in
surfactant membranes ($\approx 12 \%$)

Suggesting TF amplitude is defined by
geometrical constraints, like volume conservation

Summary

NSE is useful & powerful technique to measure ns scale dynamics.

Good to measure dynamics in small scattering angle (large scale objects)

Good to measure slower dynamics in wide-angle scattering region, slower motion than that accessible by other inelastic neutron spectrometers.

Thickness fluctuations was captured in lipid bilayer vesicles.

Mode relaxation time scale: order 100 ns

Mode amplitude: < 10 % of the thickness $\sim 4 \text{ \AA}$