Lipid Bilayers and Membrane Dynamics: Insight into Thickness Fluctuation

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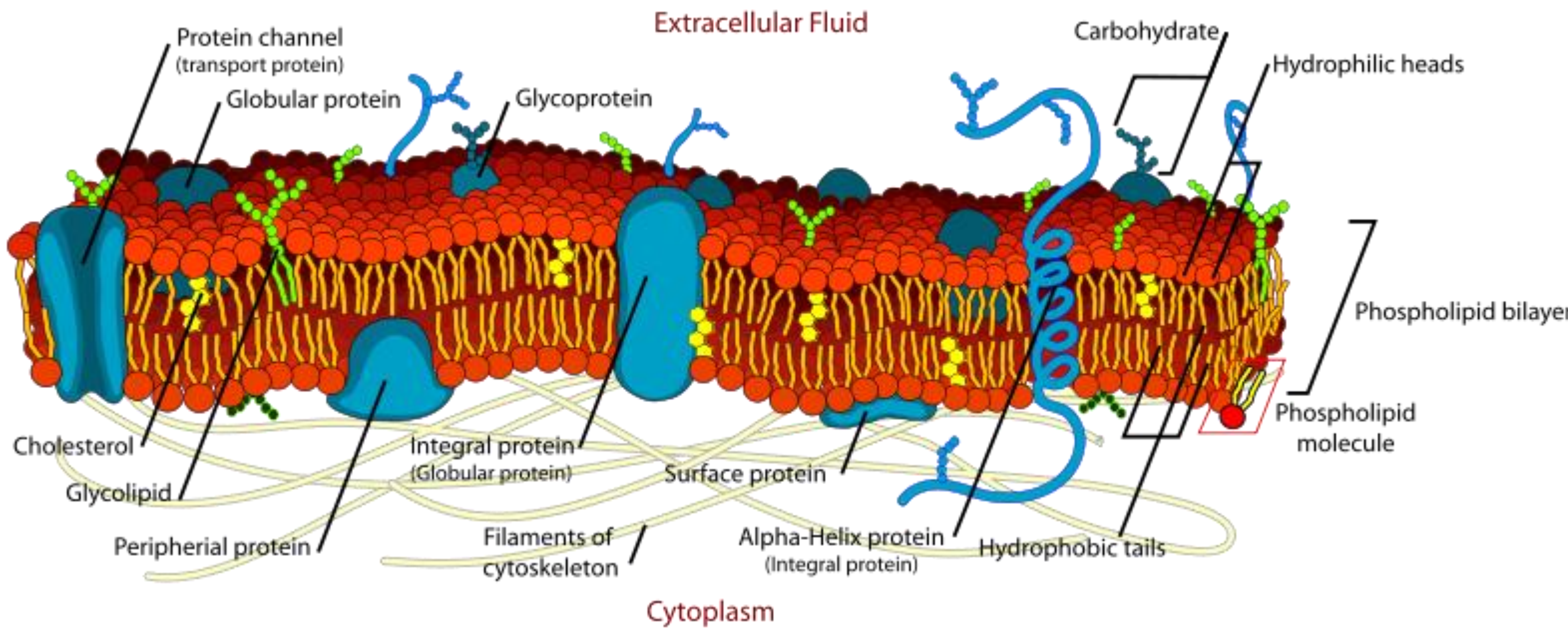
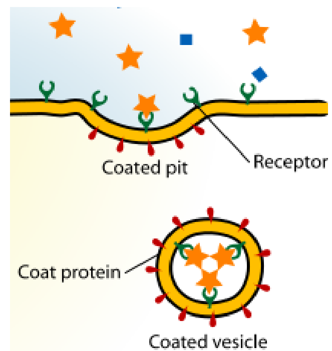


Motivation

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.

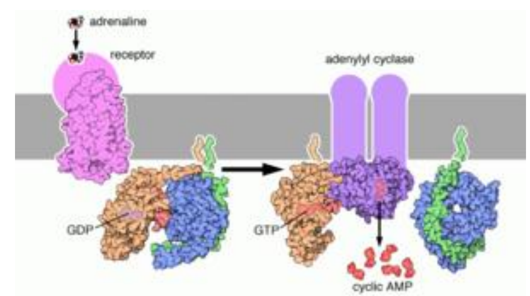
Microscopic length scale:

Membrane stiffness and fluidity have been shown to have a large impact on cellular uptake and release.[2]



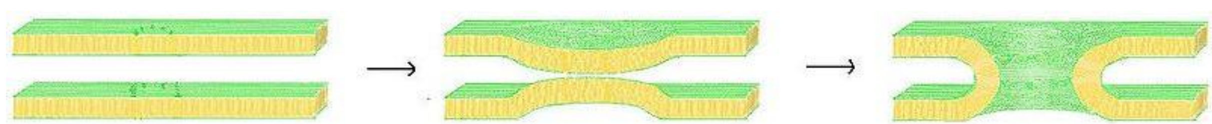
Spectroscopic length scale:

Cell signal transduction is affected by molecular lateral diffusion within the lipid membrane.[1]



Intermediate length scale :

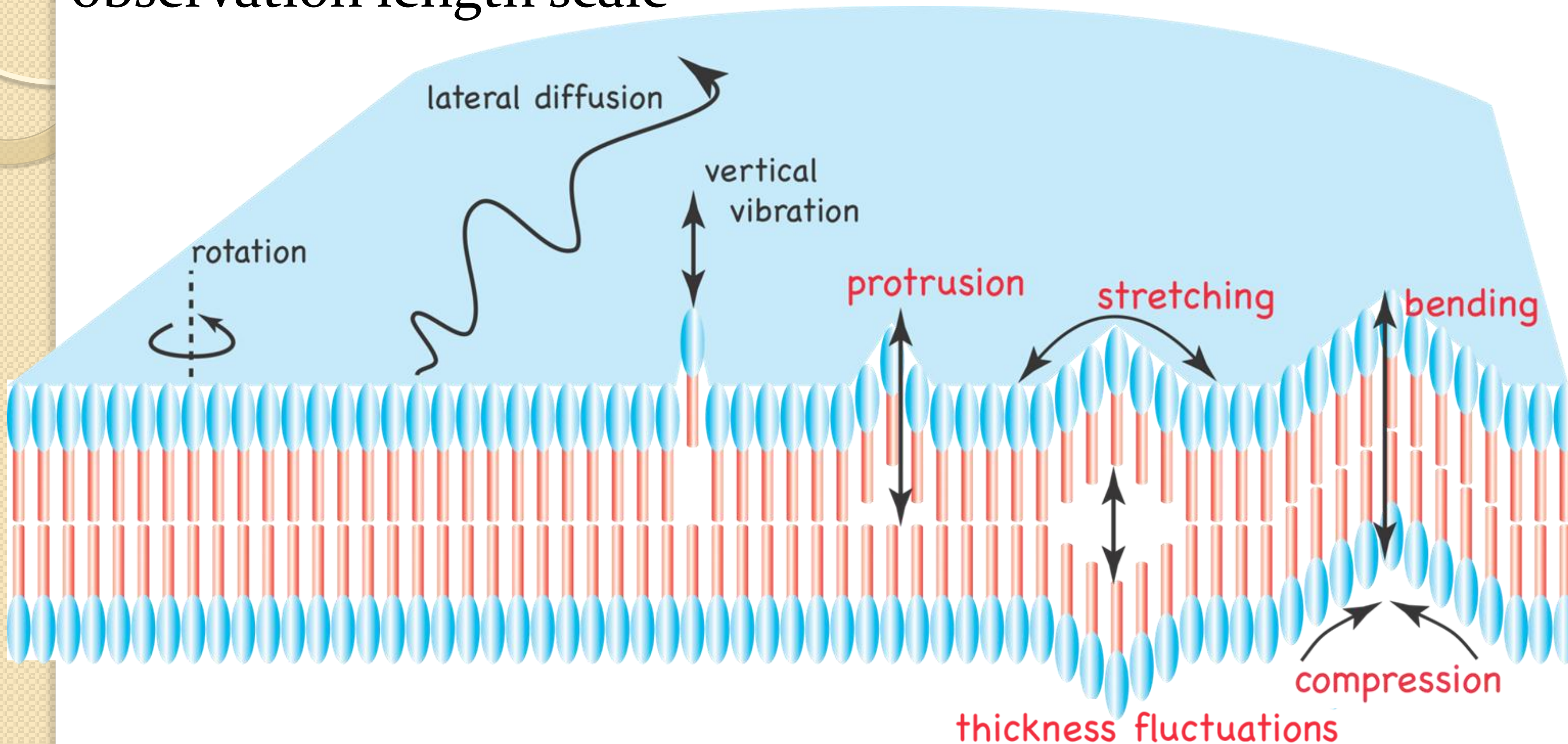
Membrane thickness fluctuations have been proposed as a mechanism for pore formation.[3]



(1) D Marguet, et al. EMBO J, 25, 3446 (2006)
(2) P. Weber, et. al. Adv Med Eng, 114, 377(2006)
(3) L. Movilenu, et al. Bull Math Biol, 58, 1231 (2006)

Membrane Dynamics

observation length scale



molecular motion

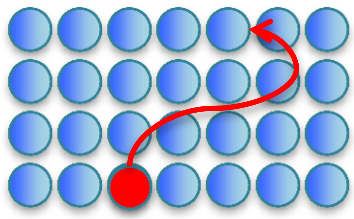
collective motion

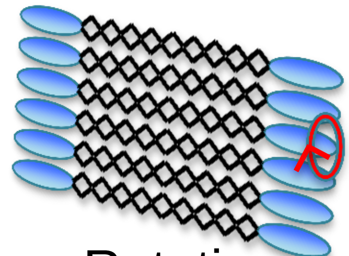
incoherent-quasi-elastic scattering
nuclear magnetic resonance etc.

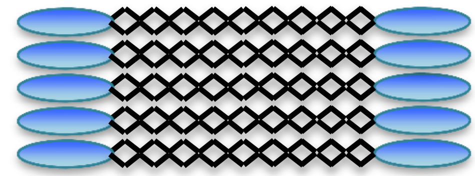
coherent-quasi-elastic scattering
photon correlation spectroscopy

The Dynamics in Lipid Bilayers

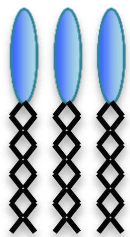
Molecular movements (incoherent movements of molecules)

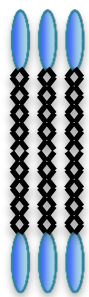

Lateral diffusion


Rotation

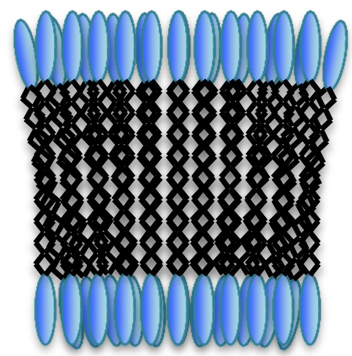

Vertical vibration

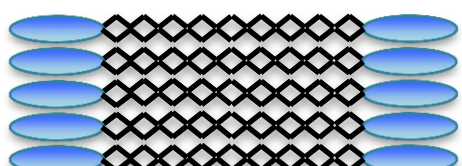
Coherent movements of molecular assemblies

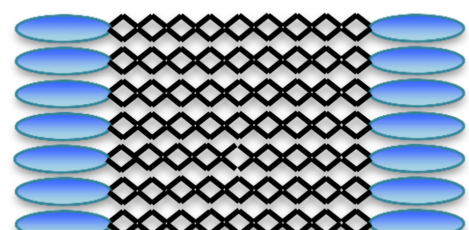

Stretching
Compression


Stretching

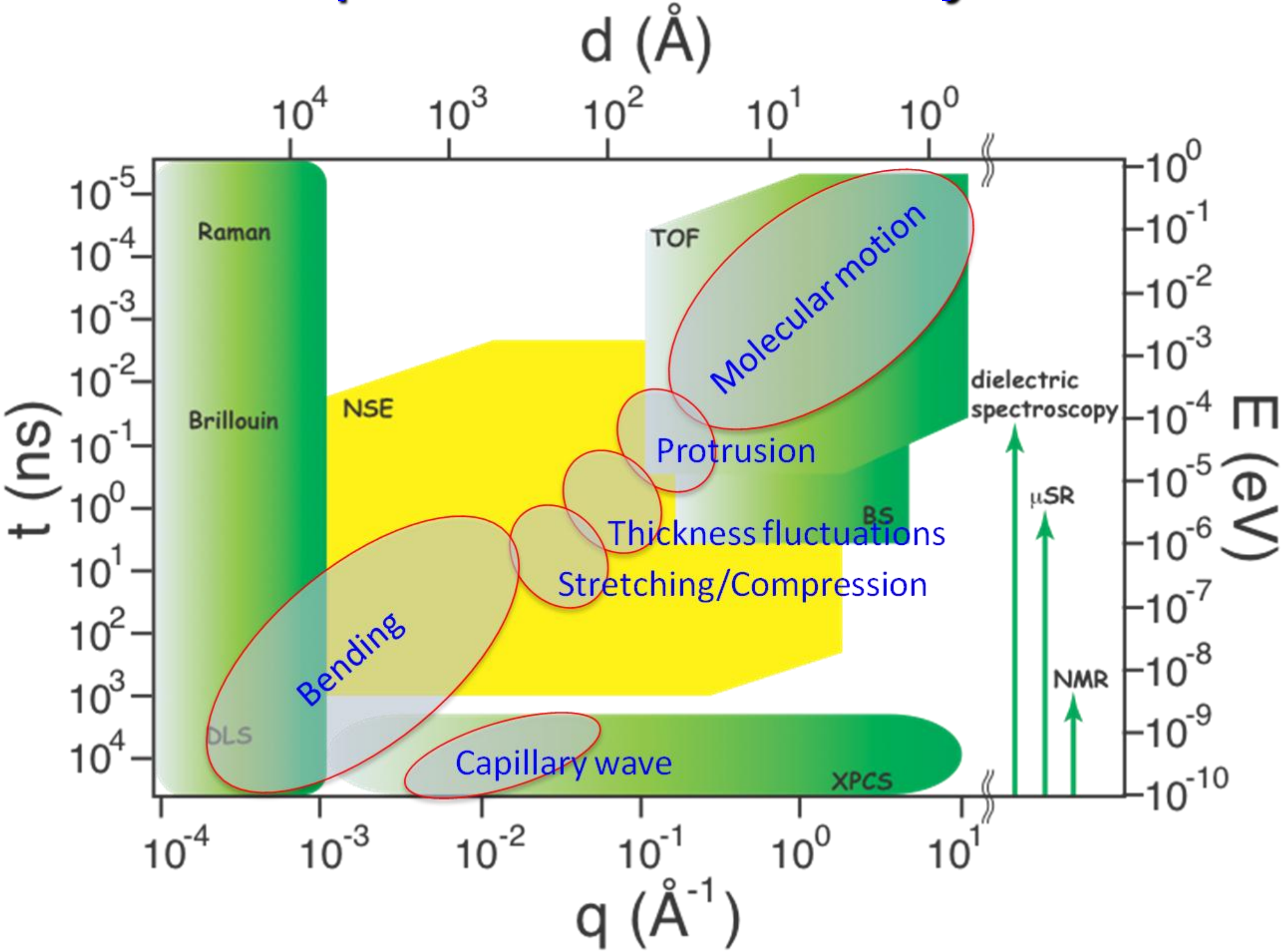
Compression


Bending

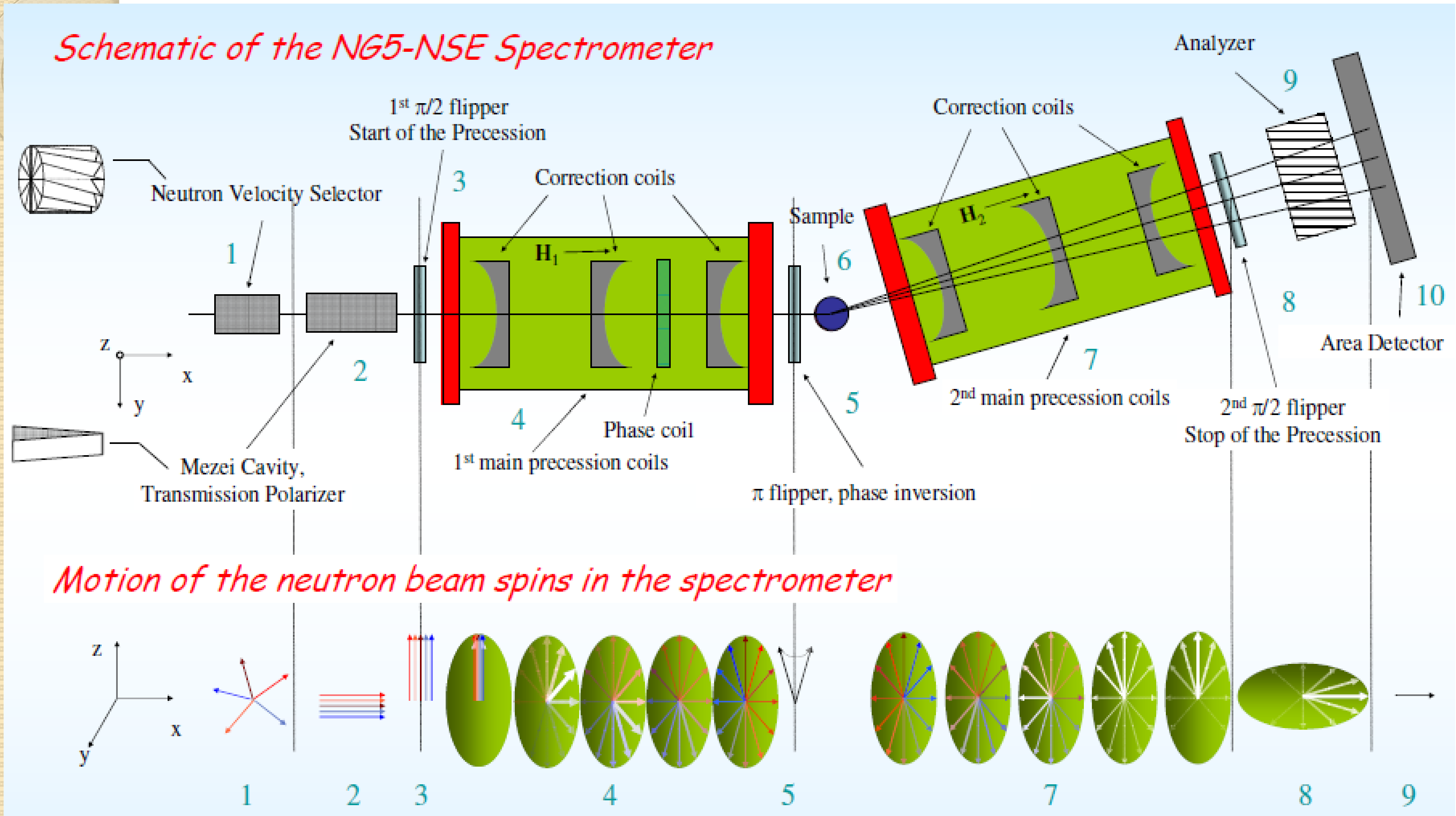

Protrusion


Thickness fluctuations

Techniques to Measure Dynamics



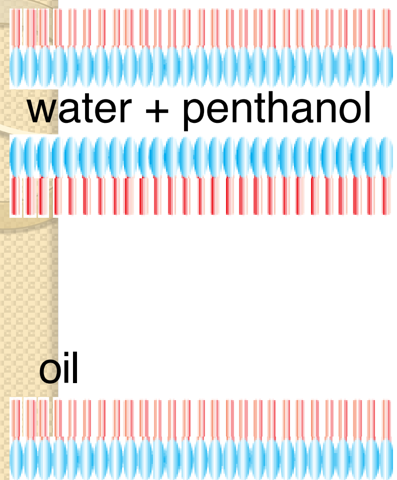
Neutron Spin Echo



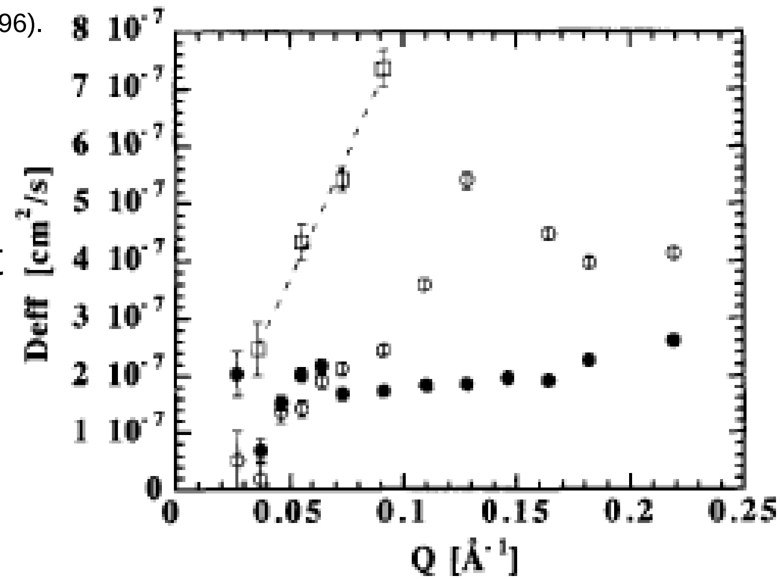
Elastic Scattering

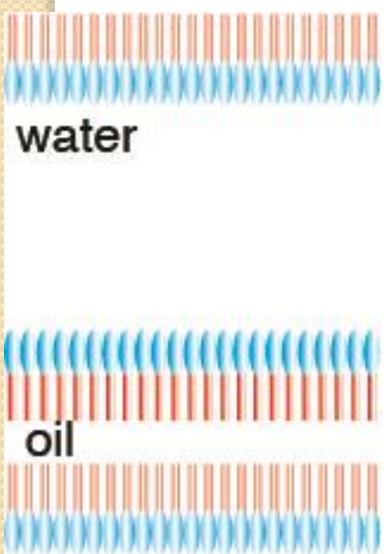
Surfactant Membranes

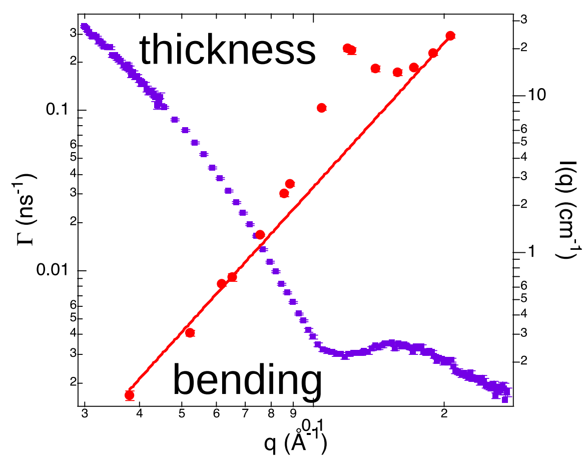
Farago et al, *Physica B* **213&214**, 712 (1995).; Farago, *Physica B* **226**, 51 (1996).

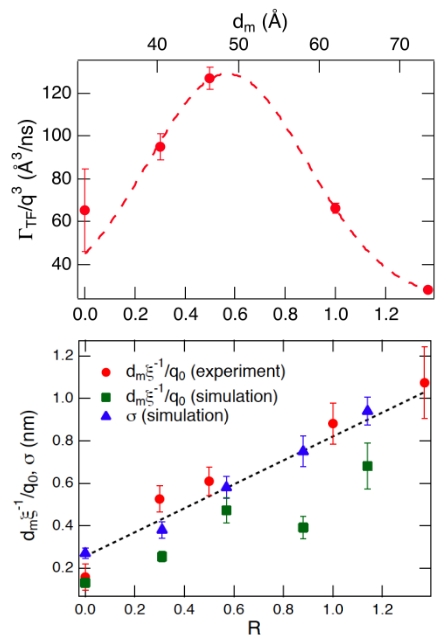


- NSE measured Q dependence of D_{eff}
- showing an increase at the membrane thickness length scale







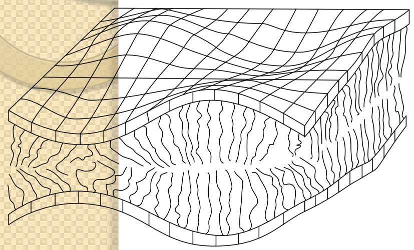


Nagao, *Phys. Rev. E* **80**, 031606 (2009).; Nagao et al., *Soft Matter* **7**, 6598 (2011).; Nagao, *J. Chem. Phys*. **135**, 074704 (2011).

Thickness fluctuations in lipid bilayers (theoretical studies)

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 ≈ 15 Å or more from the geometrical constraints (volume conservation)

Thickness fluctuations by Hladky and Gruen

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

Thickness fluctuations occur, but the amplitude is small.

Long wavelength fluctuation amplitude is negligible
Short wavelength fluctuations (< 30 Å) are severely limited
Intermediate wavelength fluctuation amplitude < 10 Å

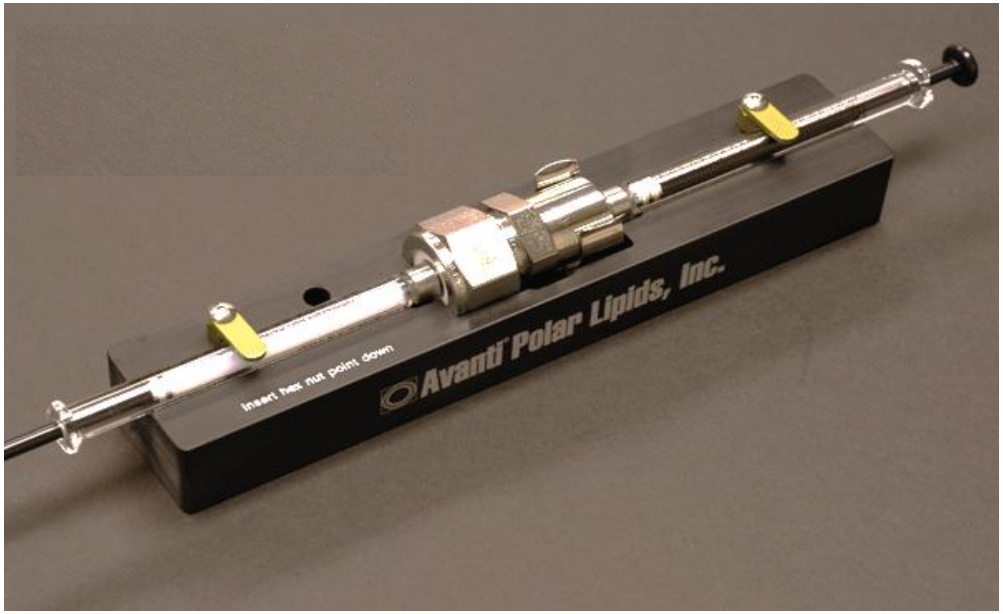
Deformation free energy of bilayer membranes by Huang

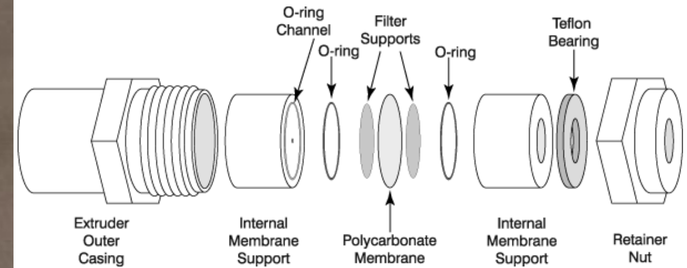
Huang, *Biophys. J*. **50**, 1061 (1986).

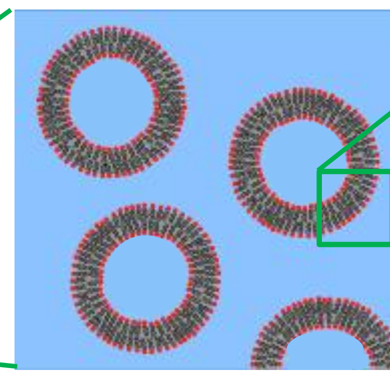
$$\sqrt{\langle \mathrm{Amplitude}^2 \rangle} = \frac{k_{\mathrm{B}}T}{\pi\gamma}\left\{\tan^{-1}\left[\frac{16\pi^2 K_1 + h\gamma}{2h\sqrt{K_1\bar{B}}}\right] - \tan^{-1}\left[\frac{\gamma}{2\sqrt{K_1\bar{B}}}\right]\right\} \approx 4.5\text{Å}$$

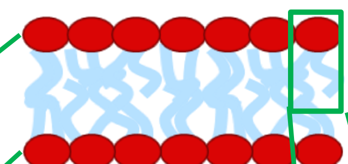
Theoretically, thickness fluctuations exist, their amplitude is very small

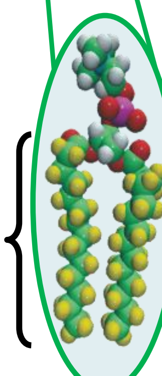
Bilayer Preparation











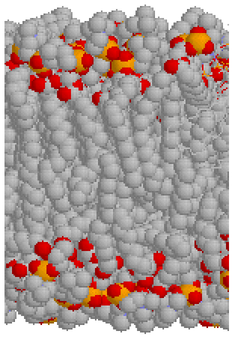
(D54)DMPC = C14

(D62)DPPC = C16

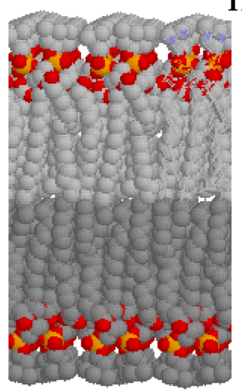
(D70)DSPC = C18

Phospholipid Melting Temperature

Fluid phase above T_m

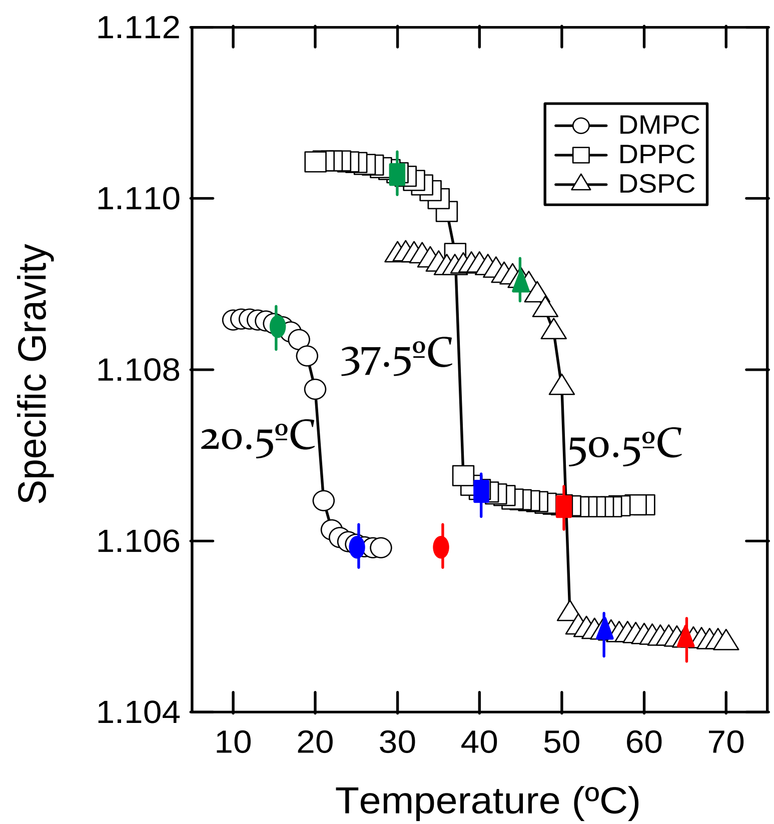


Gel phase, below T_m

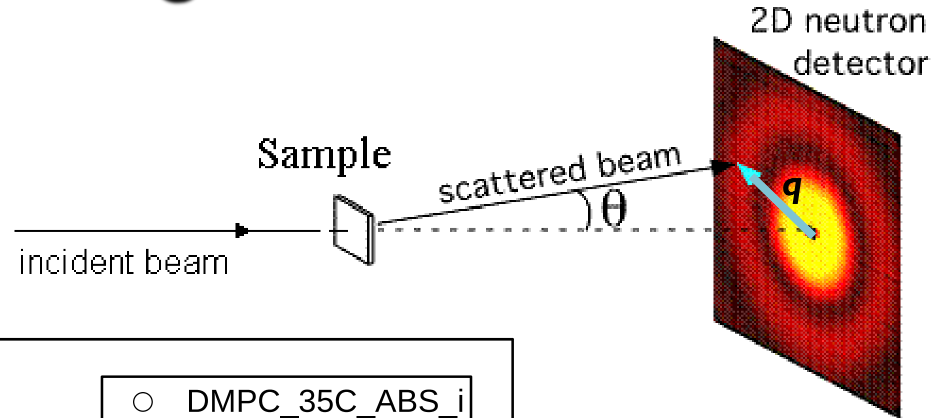


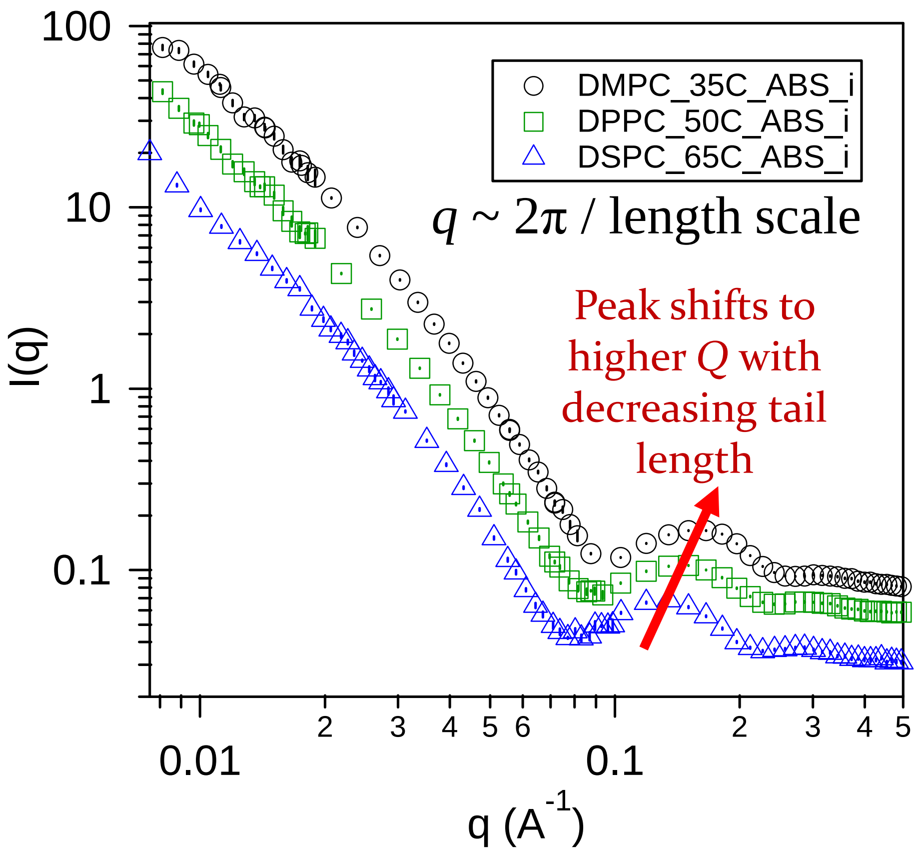
Above T_m lipid tails highly fluid, disordered and in constant motion

Below T_m transition to a gel state, tails are fully extended with highly ordered packing



Small Angle Neutron Scattering





*Scattering intensity is offset to highlight peak shift

SANS and NSE are complimentary techniques

SANS

static "snapshot"
elastic scattering

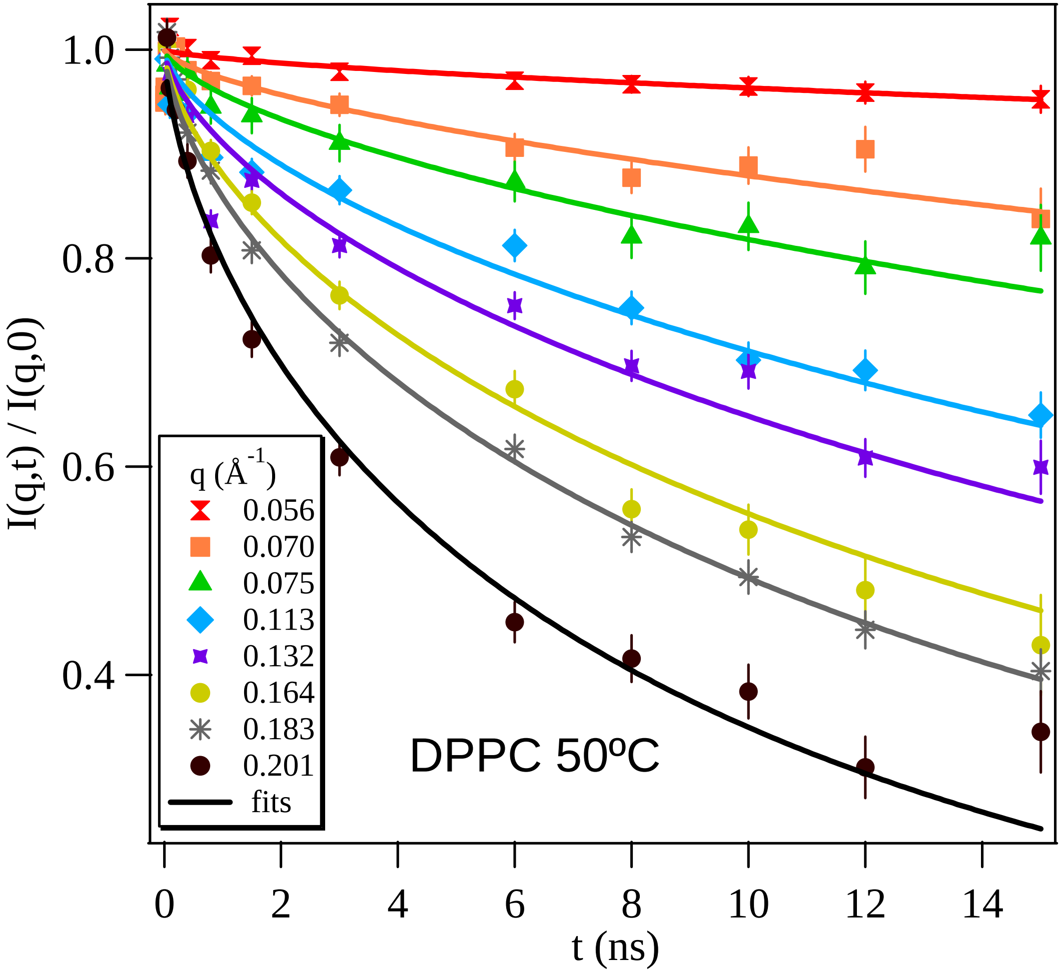
$$I(q) = \int S(q,\omega) d\omega$$

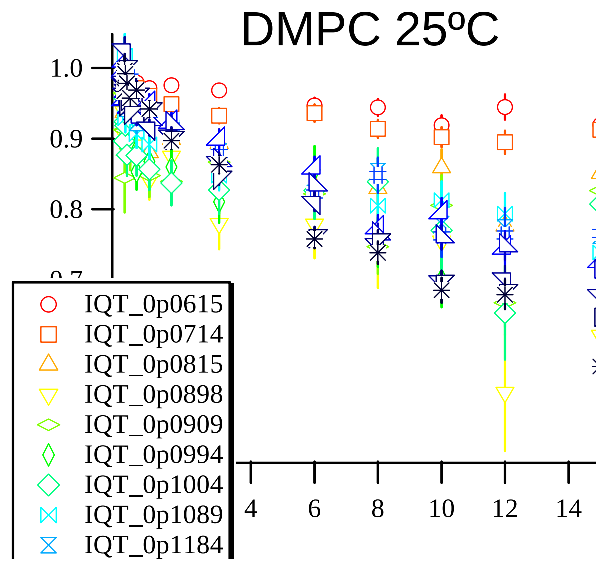
NSE

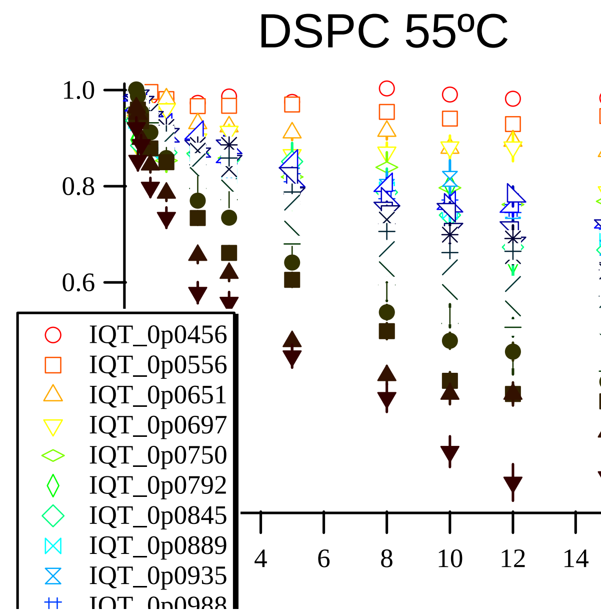
dynamic "snapshot"
quasielastic scattering

$$I(q,t) = \int S(q,\omega)\cos(\omega t) d\omega$$

NSE : *I(q,t)*







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

Surfactant Membrane Dynamic (bending)

Helfrich bending energy

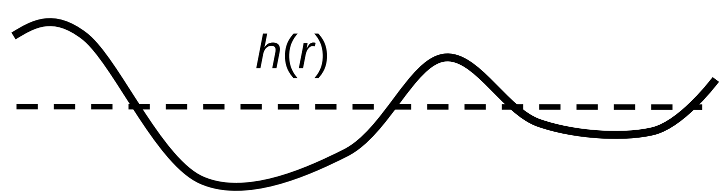
Helfrich, *Z. Natureforsch*. **28**, 693 (1973).

Assuming the membrane is thin enough sheet, which is undulating

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



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

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

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

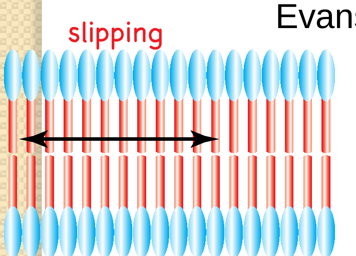
κ: bending modulus
η: solvent viscosity

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**, 7



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

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

Fitting I(*q*,*t*)

Bending motion is explained as a single membrane dynamics model

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

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

Γ: decay rate, β=2/3

$$\frac{\Gamma_{\mathrm{Bend}}}{q^3} = 0.025\alpha\sqrt{\frac{k_{\mathrm{B}}T}{\tilde{\kappa}}}\frac{k_{\mathrm{B}}T}{\eta_{\mathrm{D_2O}}}$$

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

Considering slipping friction

$$\tilde{\kappa} = \kappa + 2d^2 k_{\mathrm{m}}$$

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

$$k_{\mathrm{m}} = \frac{24\kappa}{d_{\mathrm{t}}}$$

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

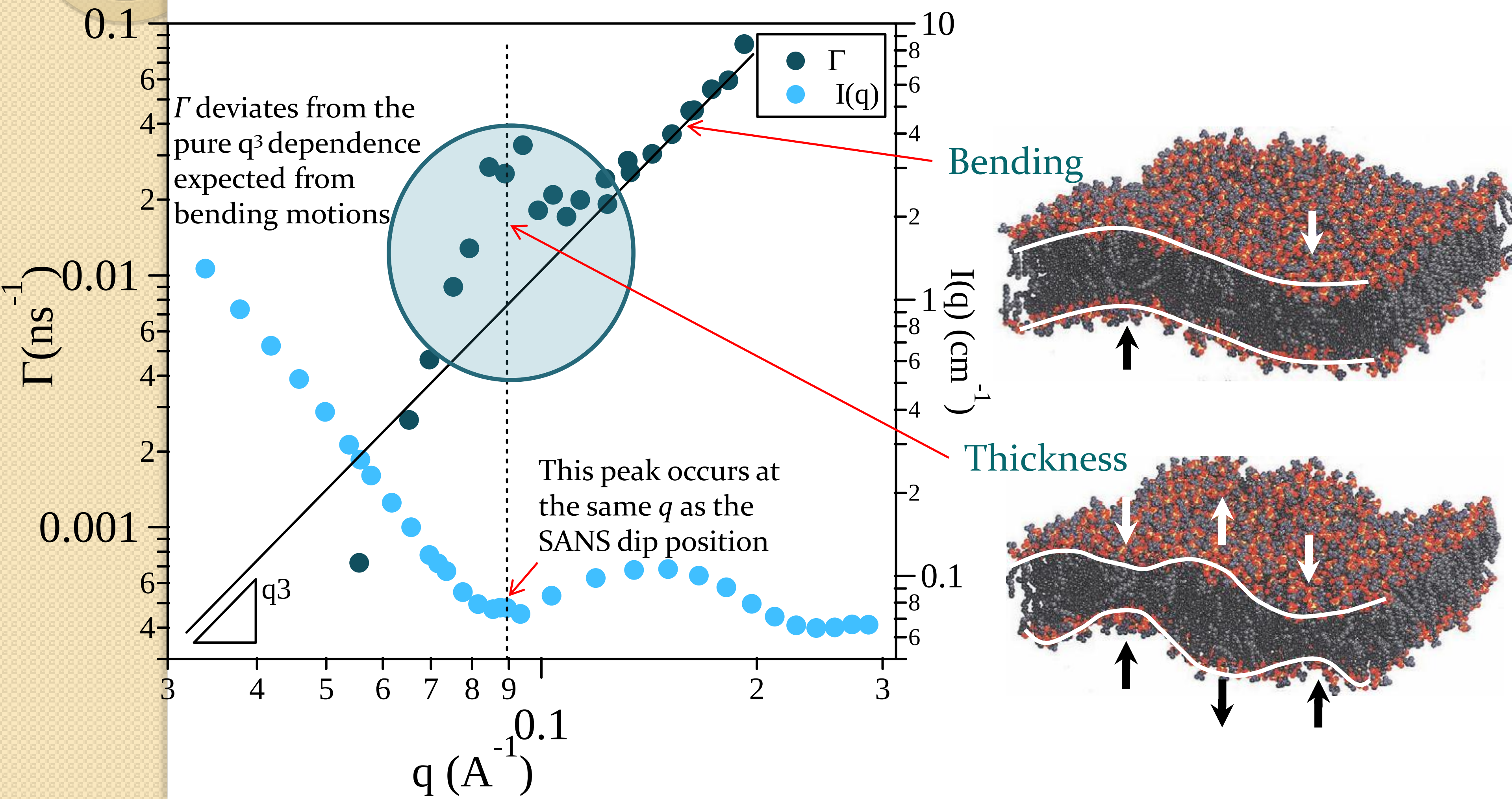
$$\frac{\Gamma_{\mathrm{Bend}}}{q^3} = 0.0058\sqrt{\frac{k_{\mathrm{B}}T}{\kappa}}\frac{k_{\mathrm{B}}T}{\eta_{\mathrm{D_2O}}}$$

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

NSE : *Γ vs. q*   *DPPC @ 50ºC*

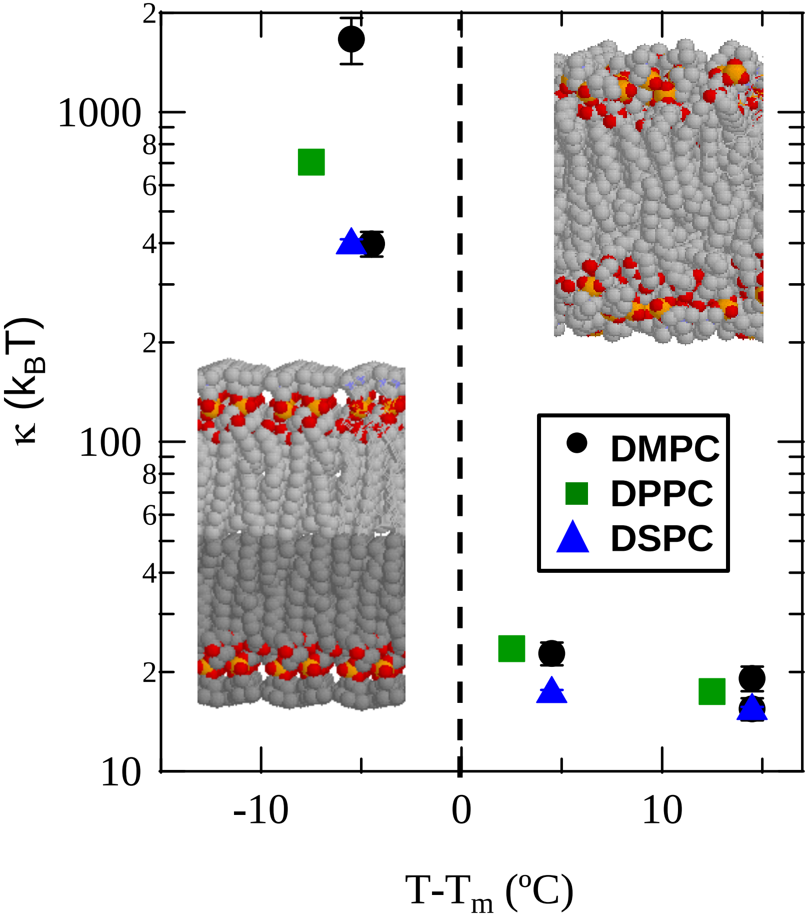
$$\Gamma_{BEND} = 0.025\left(\frac{k_B T}{\kappa}\right)^{1/2} \frac{k_B T}{3\eta_{D_2O}} q^3 \rightarrow \frac{\Gamma}{q^3} = \frac{\Gamma_{BEND}}{q^3} + \frac{\Gamma_{TF}}{q^3} \frac{1}{1+(q-q_0)^2\xi^2}$$

Zilman-Granek   M. Nagao

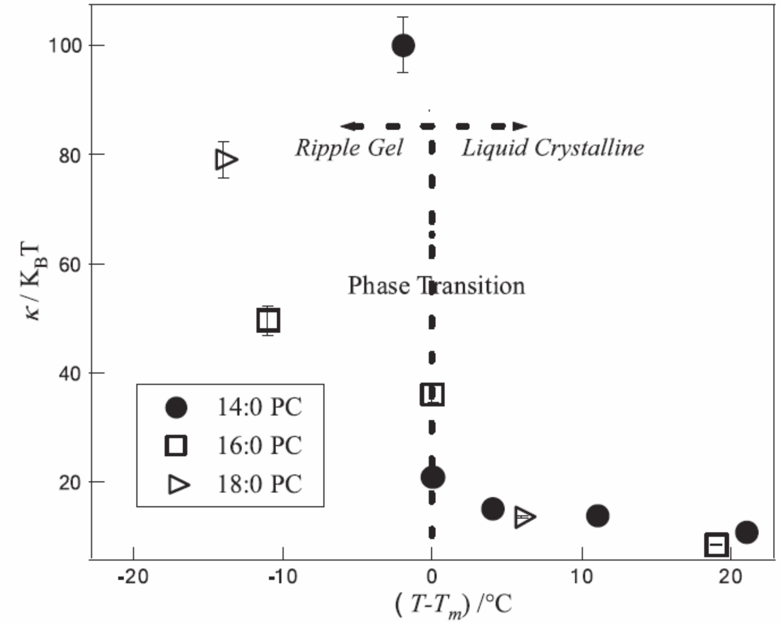


NSE : *Bending Modulus*

Experiment



Literature

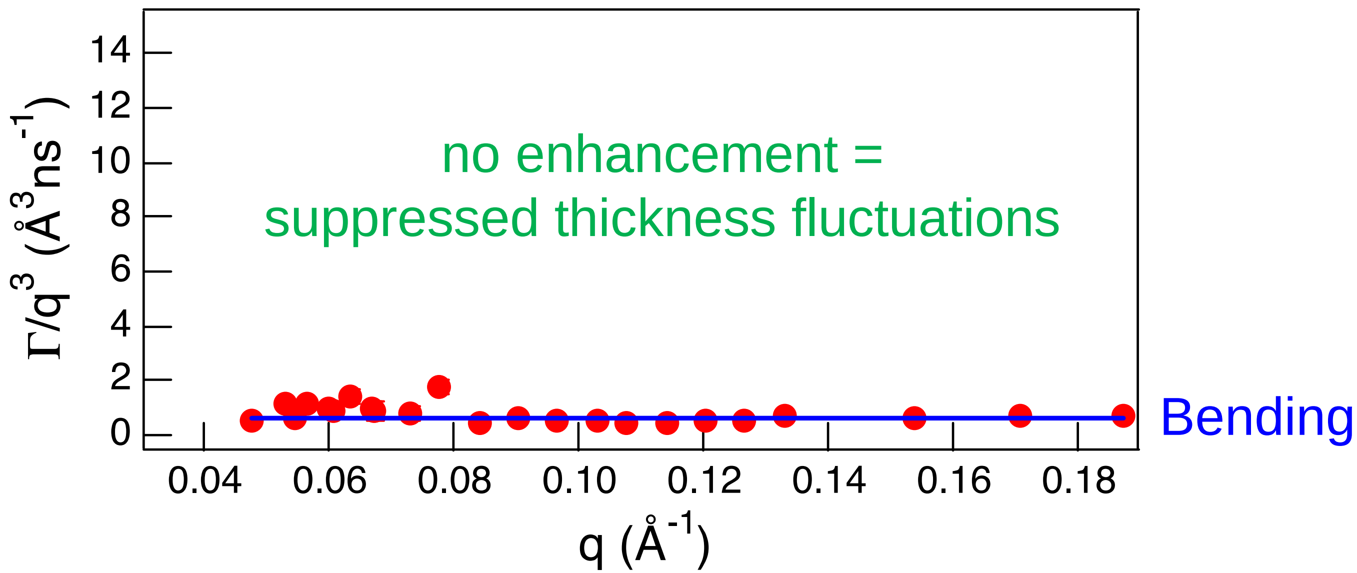


Z.Yi, et. al, J. Phys. : Cond. Mater, **21**, 155104 (2009).

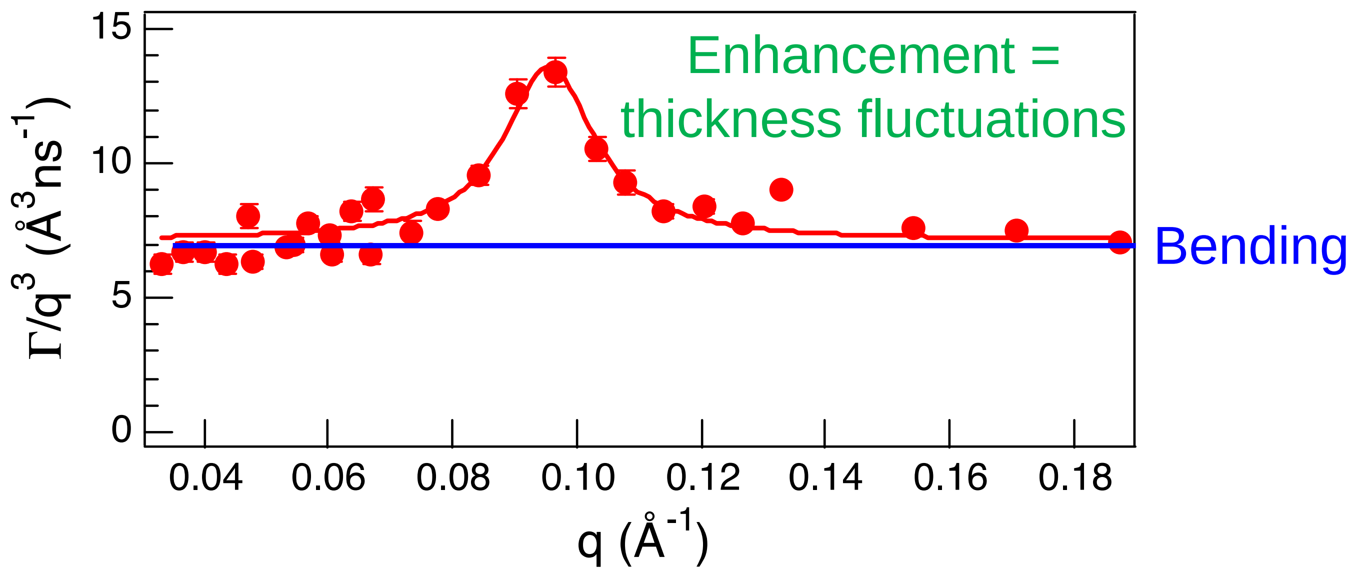
Lipid	T_m (°C)	T (°C)	$(T - T_m)$ (°C)	$\kappa_c / k_B T$
14:0 PC	24	22	−2	100.0 ± 4.99
		24	0	20.9 ± 0.61
		28	+4	13.9 ± 0.24
		35	+11	15.3 ± 0.31
		45	+21	13.9 ± 0.44
		60	+36	8.2 ± 0.12
16:0 PC	41	30	−11	49.6 ± 2.78
		41	0	36.1 ± 1.49
		60	+19	9.5 ± 0.18
18:0 PC	54	40	−14	79.1 ± 3.23
		60	+6	13.6 ± 0.24

q dependence of the decay rate: *Below vs. Above T_m*

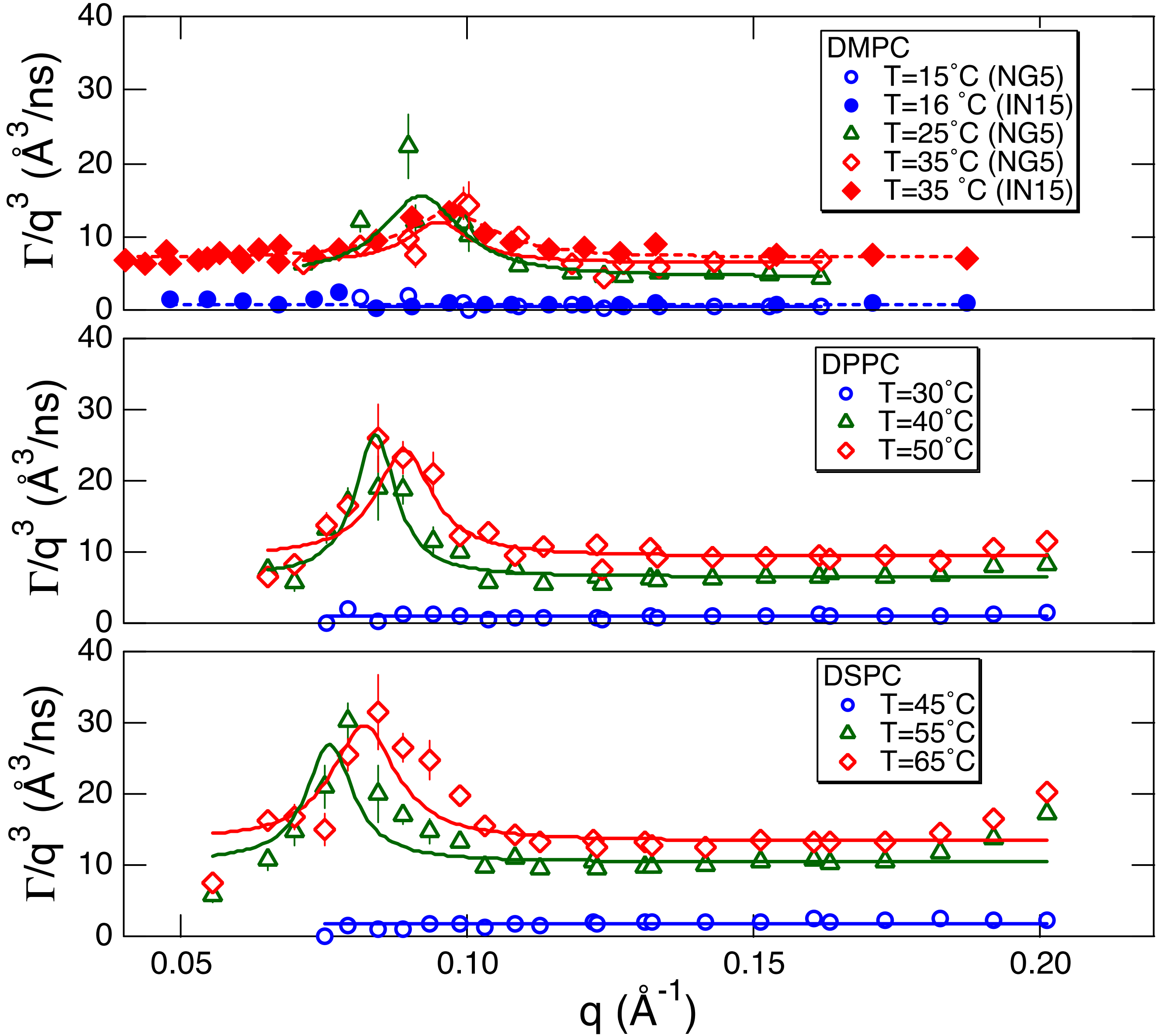
Below T_m
DMPC
T = 16 ˚C



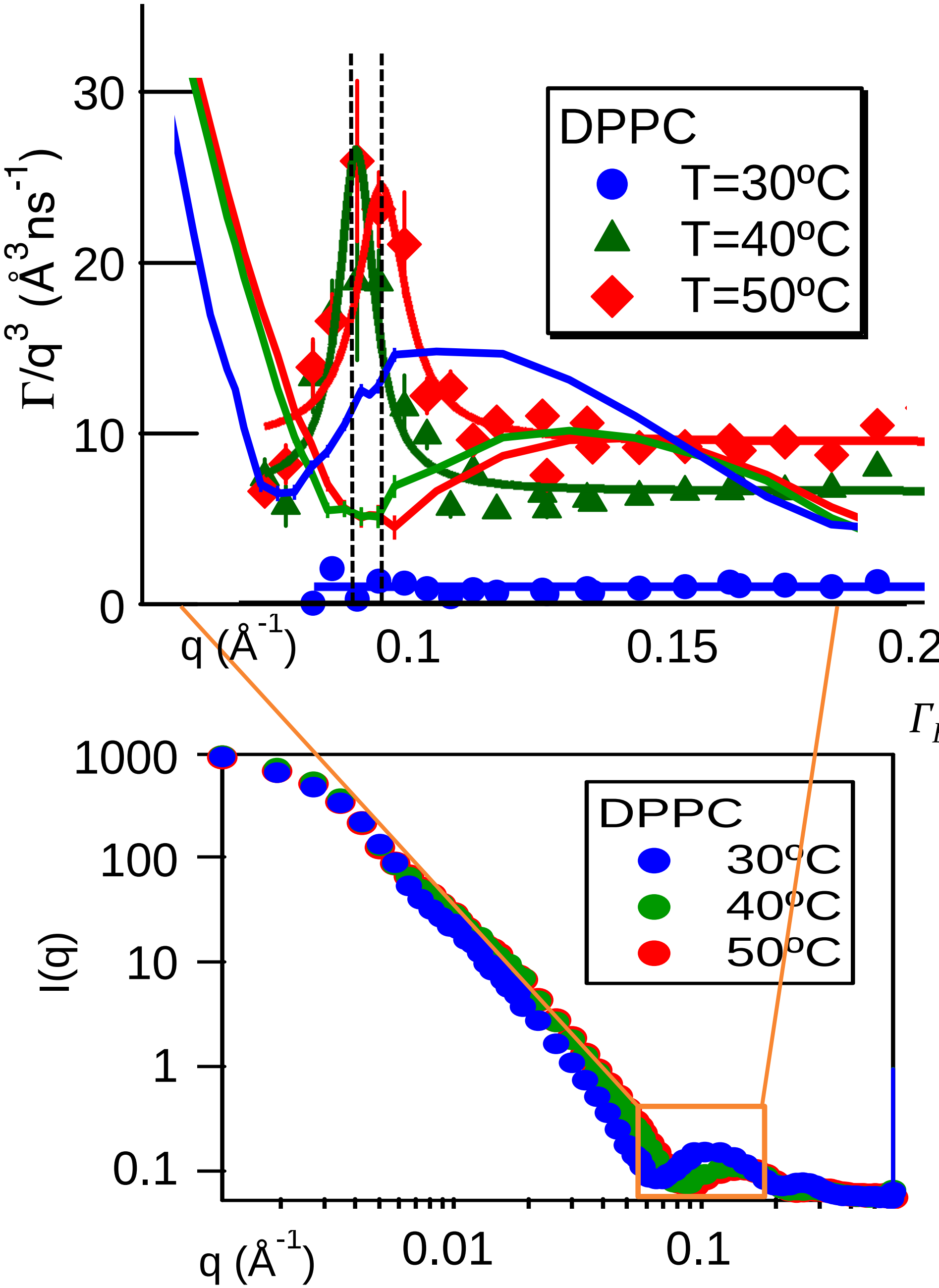
Above T_m
DMPC
T = 35 ˚C



q dependence of the decay rate: *Lipid Tail Length*



NSE : *Thickness fluctuations (Γ / q³)*



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

M. Nagao

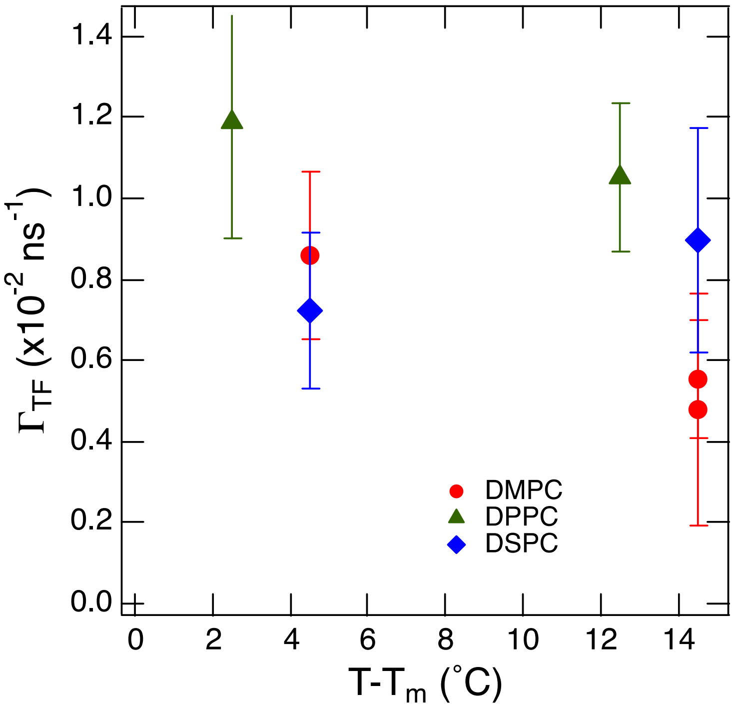
Γ_{BEND} / q^3 : accounts for bending motions

Γ_{TF} : damping frequency of thickness fluctuations

ξ^{-1} : Proportional to the amplitude of thickness fluctuations

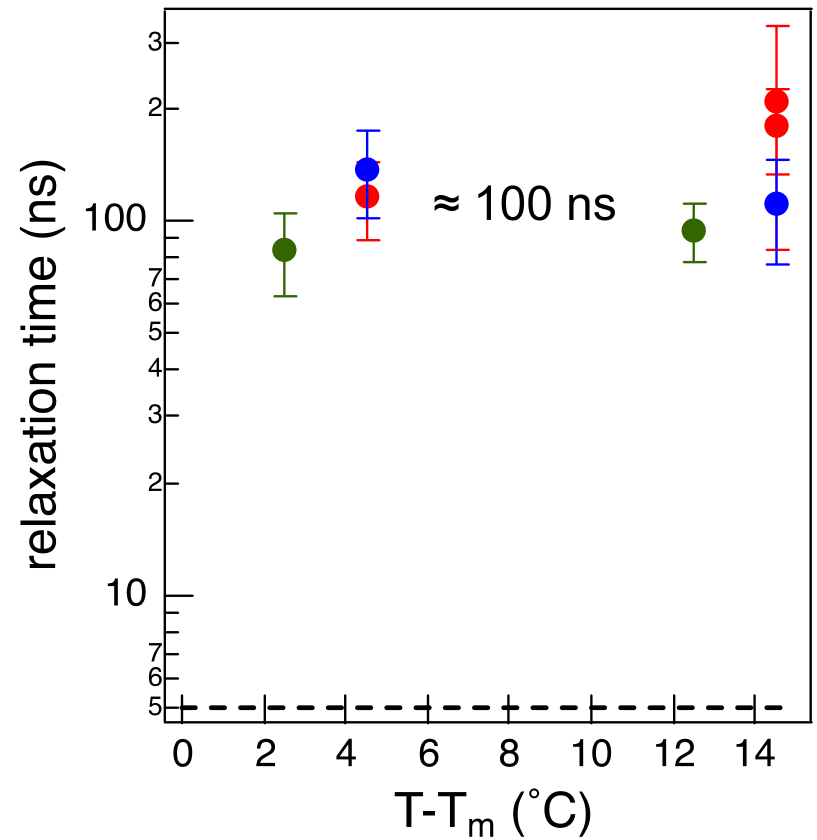
q_o : SANS dip position (Lorentzian peak position)

Membrane Thickness Fluctuations Time scale



Above T_m: independent of either temperature or tail length

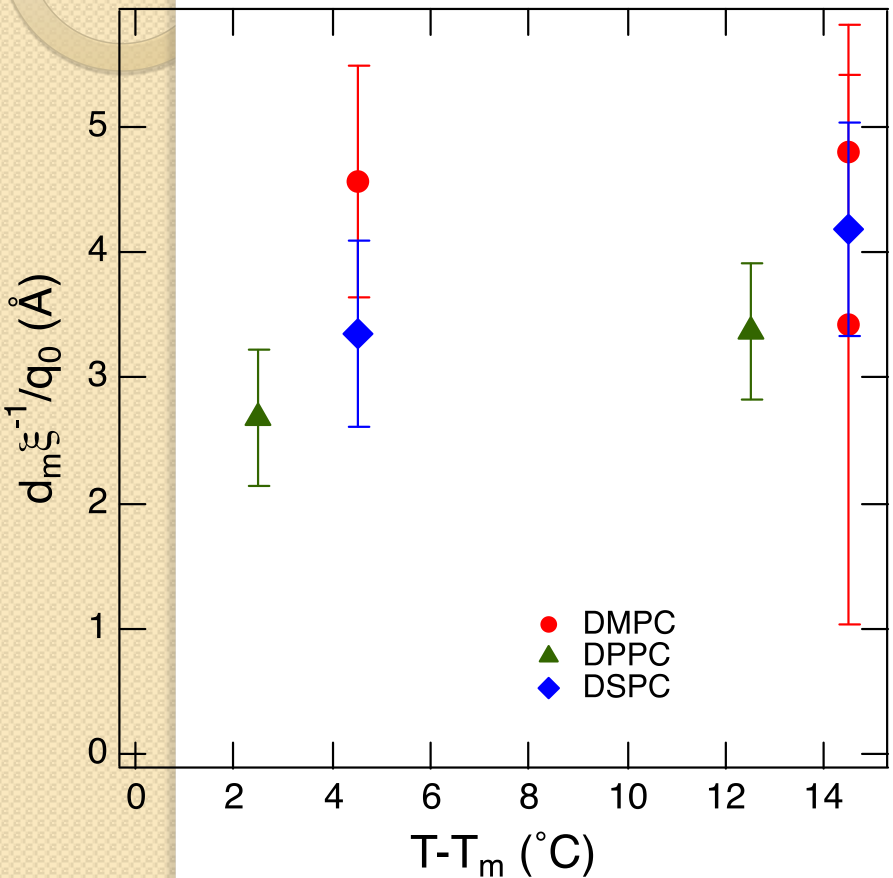
$$\frac{\Gamma}{q^3} = \frac{\Gamma_{BEND}}{q^3} + \frac{\Gamma_{TF}}{q^3}\frac{1}{1+(q-q_0)^2\xi^2}$$



An order of magnitude slower (than surfactant membranes)

Membrane Thickness Fluctuations Amplitude

Width of Lorentzian peak relates to the fluctuation amplitude[(1)]



Experiment:

Mean amplitude = 3.7 Å ± 0.7 Å

Theory:

Huang's mean amplitude ≈ 4.5 Å[(2)]

Simulation:

Lindahl & Edholm's amplitude ≈ 5 Å[(3)]

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

Suggests amplitude is defined by physical constraints, like volume conservation

(1) Nagao et al., *Soft Matter* **7**, 6598 (2011). (2) Huang, *Biophys. J*. **50**, 1061 (1986). (3) Lindahl and Edholm, *Biophys. J*. **79**, 426 (2000).

Thickness Fluctuation Theory

Although membrane thickness fluctuations have not been previously measured Huang[(7)] has proposed a theory for thickness fluctuations in a lipid bilayer under the consideration of deformation free energy:

$$\left\langle D^2 \right\rangle = \frac{k_B T}{2\pi a K_1 C_2} \left\{ \tan^{-1}\left[\left(\left(\frac{2\pi}{\lambda_0} \right)^2 + C_1 \right) \Big/ C_2 \right] - \tan^{-1}\left(C_1 / C_2 \right) \right\}$$

$$C_1 = \frac{\gamma}{2aK_1} \qquad C_2 = \left(\frac{\overline{B}}{a^2 K_1} \right)^{1/2}$$

$$K_1 = \frac{\kappa}{d_m}$$

$$d_m = 2a \qquad \lambda_0 \approx a$$

$D \approx 4.5\text{Å}$

D = thickness fluctuation amplitude

$B^{\#}$ = membrane compressibility

λ_0 = wavelength cut off

d_m* = membrane thickness

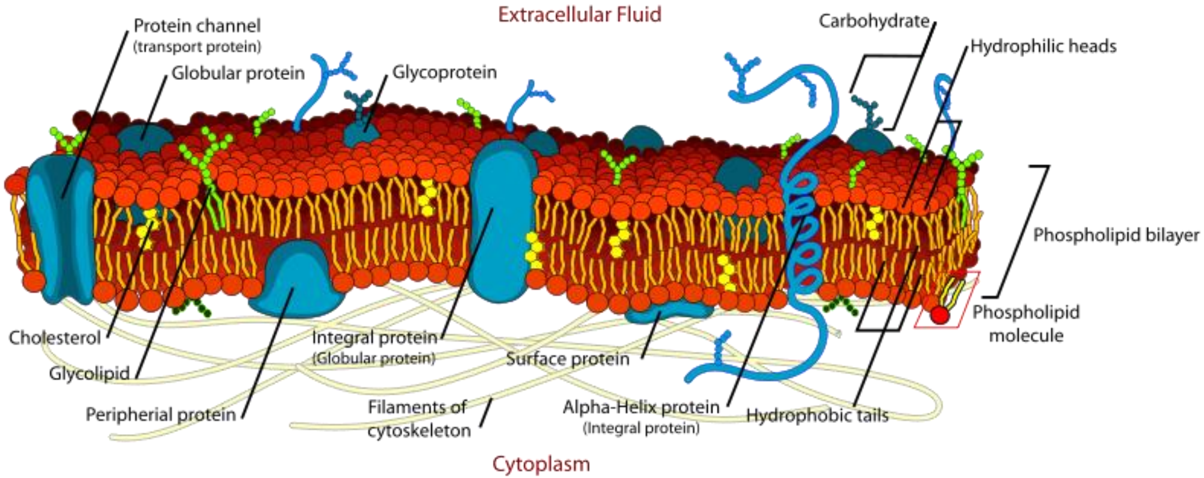
κ^{+} = bending modulus

$\gamma^{\#}$ = surface tension

+ from NSE measurements   * from SANS measurements   # from literature

Conclusions

- NSE was used to successfully measure lipid membrane thickness fluctuations
- From SANS it is clear that these fluctuations appear at the length scale of the membrane thickness.
- The relaxation time ≈100 ns and is independent of temperature and tail length.
  - An order slower than that observed in surfactant membranes
- Amplitude is ≈ 8 % of the thickness, consistent with surfactant membranes (12 %).
  - Volume conservation may define the fluctuation amplitude.
- Below T_m, thickness fluctuations are not observed, suggesting total suppression of the mode or much slower relaxation times which are not accessible by the current setup.
- The experimental amplitude agrees well with both theory and simulation
- FUTURE DIRECTION: What kind of effects do membrane associated molecules have on membrane dynamics such as thickness fluctuations?



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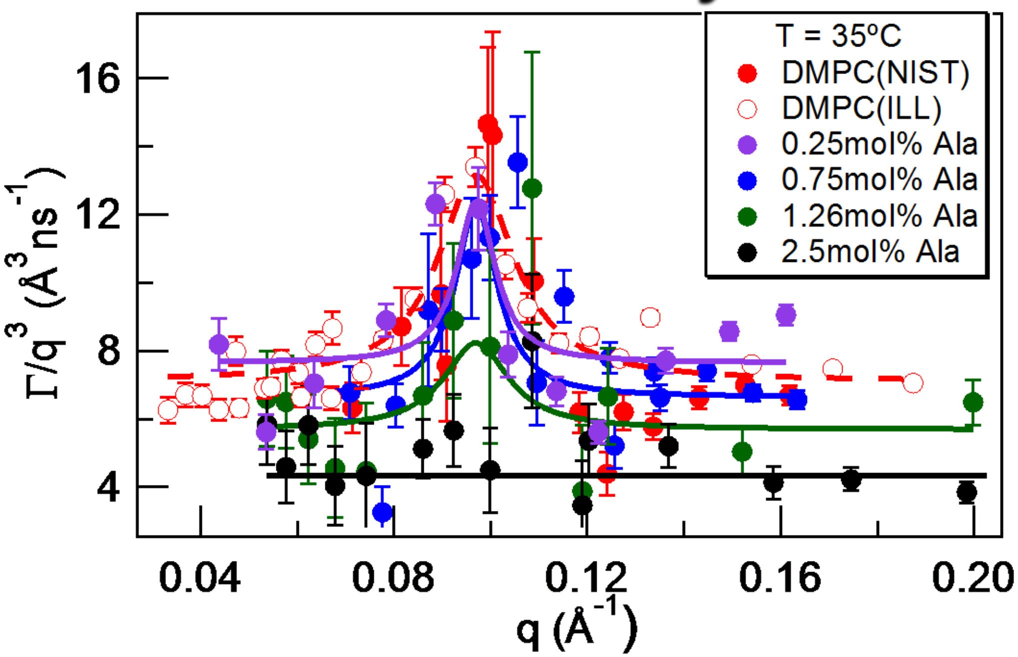
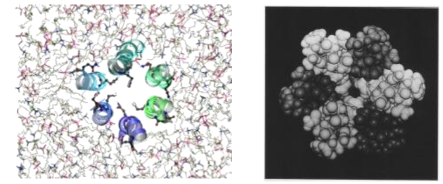
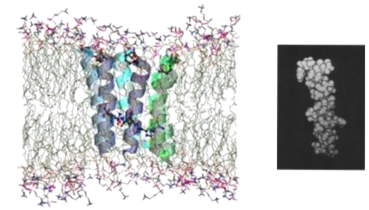
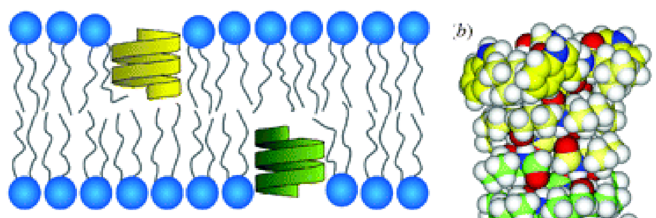


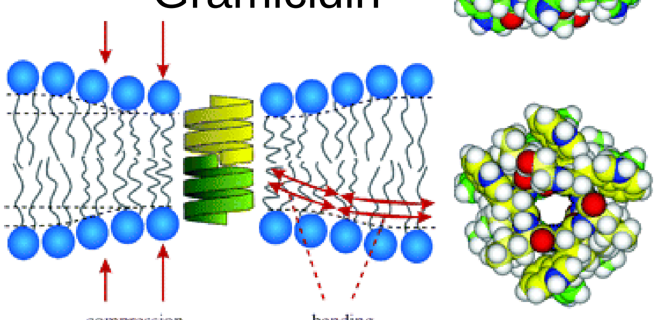


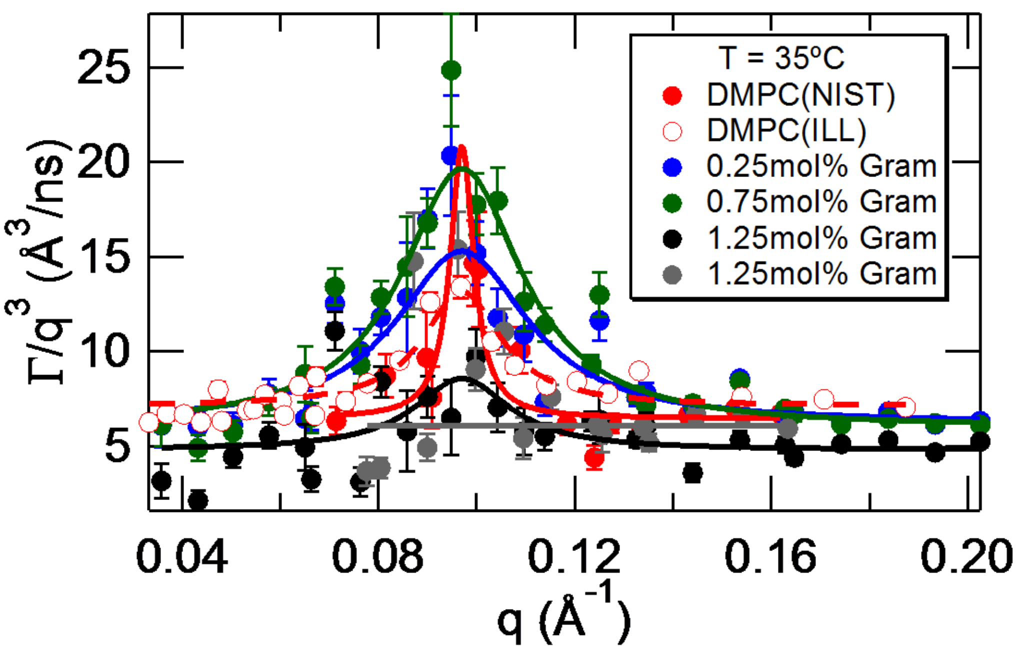
Alamethicin





Gramicidin





Preliminary Data w/ Protein