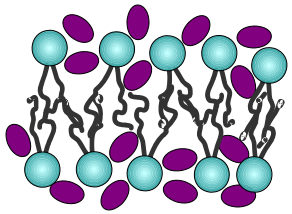
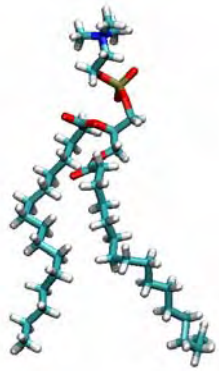


The influence of trehalose on melting and dynamics in dehydrated phospholipid bilayers

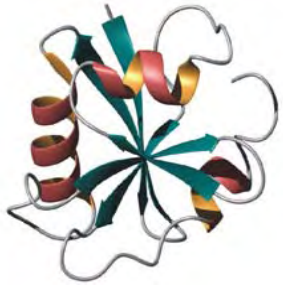
Janna K. Maranas

PENNSTATE
DEPARTMENT OF CHEMICAL ENGINEERING
Designing Molecular Technology for the 21st Century with Biology & Chemistry



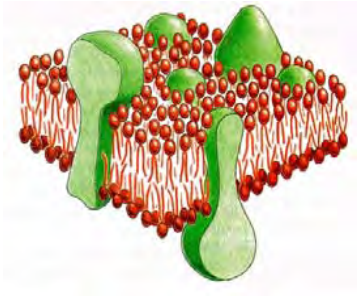
User friendly packages are available for simulations in biology

Proteins



Nucleotides

Membranes



PACKAGES

AMBER
Assisted Model Building with Energy Refinement

http://amber.scripps.edu/

NAMD
Nanoscale Molecular Dynamics

www.ks.uiuc.edu/Research/namd/

GROMACS
Groningen Machine for Chemical Simulations

www.igc.ethz.ch/GROMOS

FORCE FIELDS

AMBER
all-atom

ff94 ff96
ff99 ff99SB
ff02 ff03

CHARMM
all-atom

CHARMM22
CHARMM27

united atom

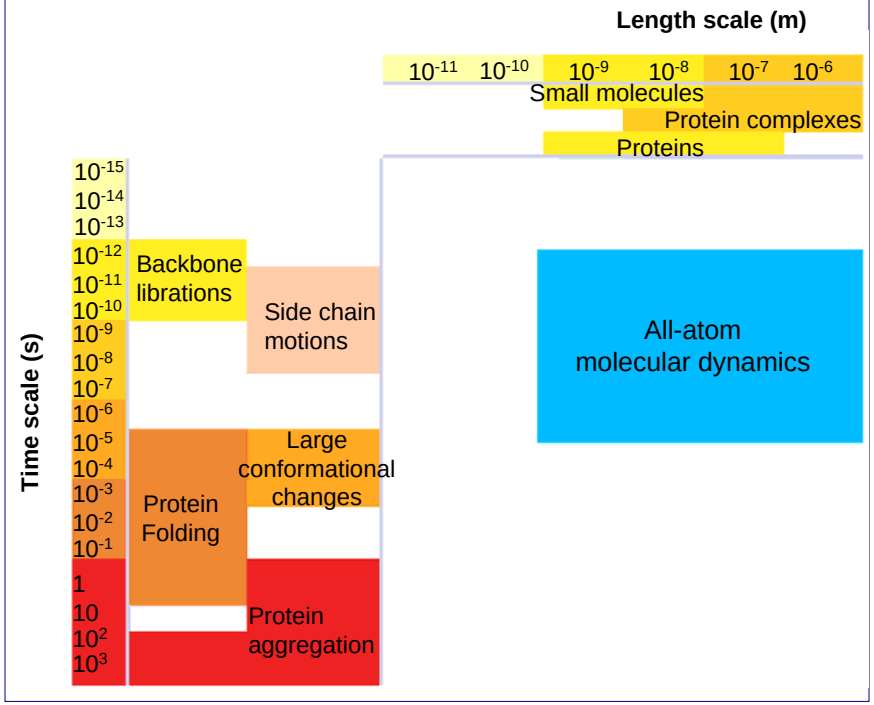
CHARMM19

GROMOS
united atom

GROMOS87
GROMOS96

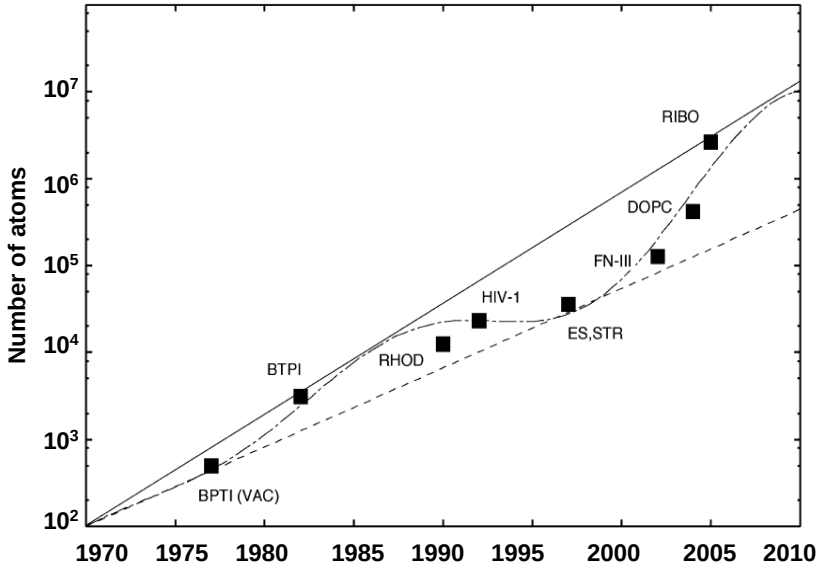
Accessible properties are similar to polymers; presence of water and initial configuration are different

Complete dynamics are difficult to access



Adapted from Ding et al., *Trends Biotechnol,* 2005. 23,9:450-455

Simulation size of biological systems



Sanbonmatsu K et al. *J.Struc.Biol.* 2007. 15,470-480

Modeling solvent effects

water models: TIP3P, TIP4P, SPC/E,F3C

Initial protein structures

Experimentally determined NMR structures

RCSB PDB PROTEIN DATA BANK

www.rcsb.org

Computational methods for structure prediction

Rosetta@home Protein Folding, Design, and Docking

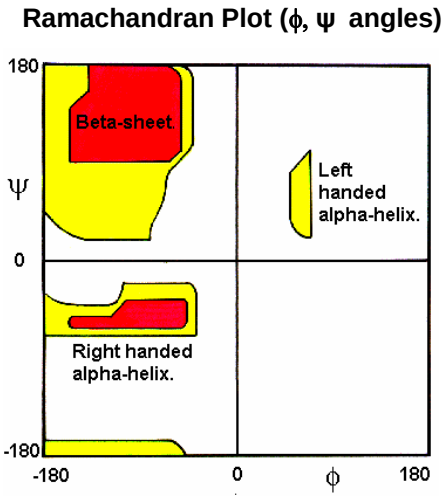
http://boinc.bakerlab.org/rosetta/

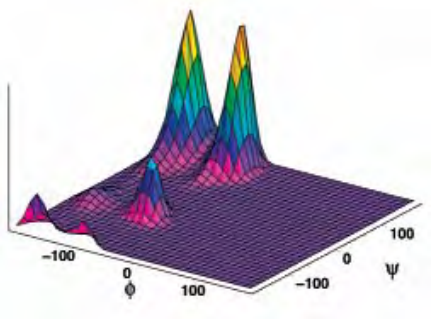
Educate yourself when choosing a force field

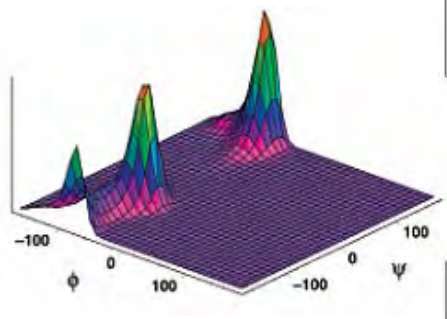
Conformational dynamics of trialanine in water

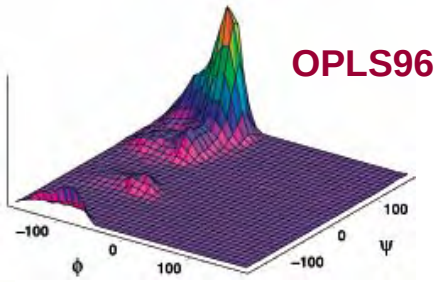
Mu et al., *J.Phys.Chem B* 2003, 107:5064-5073

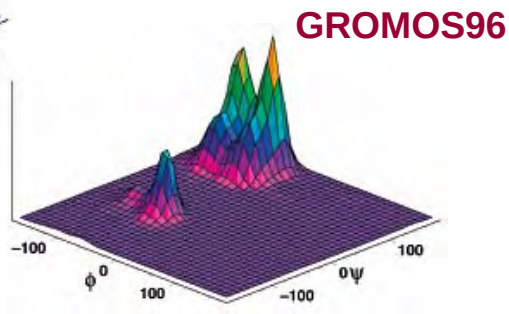
Possible conformations for trialanine from simulation







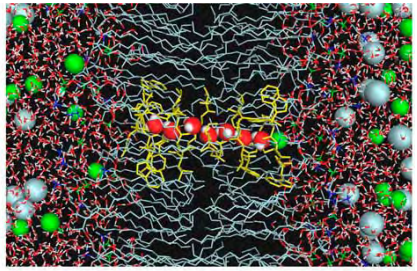




Ion permeation through a narrow membrane channel

Allen et al., *Biophys. J.* 2006, 90:3447-3468

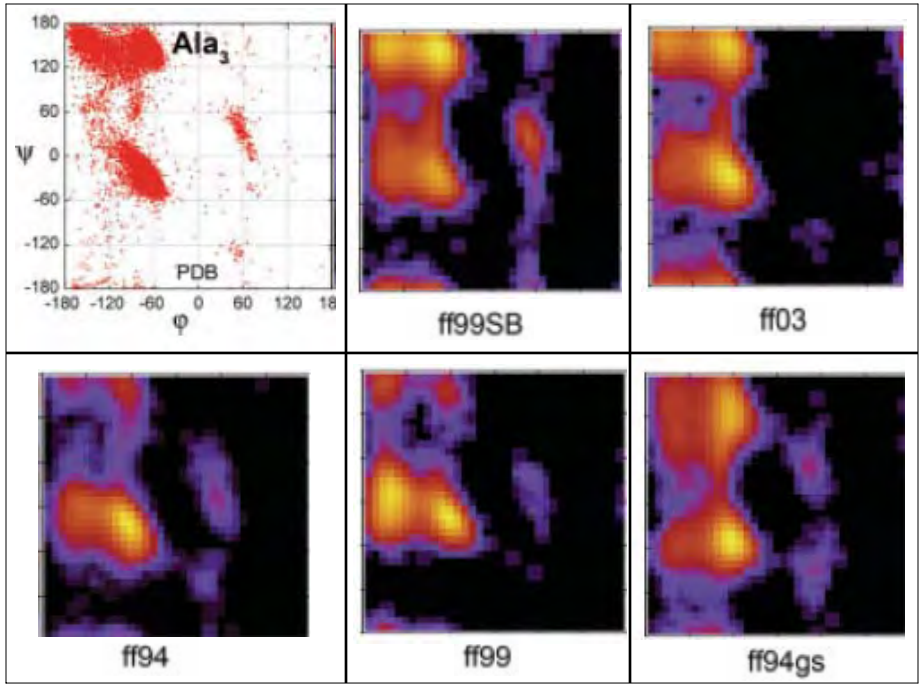
Conductance prediction across membrane (pS)			
Experimental	AMBER94	CHARMM27	GROMOS87
21	6.9	0.81	0.30



Variations within force field families and with water are less important

AMBER force fields variants simulation of alanine tetrapeptide

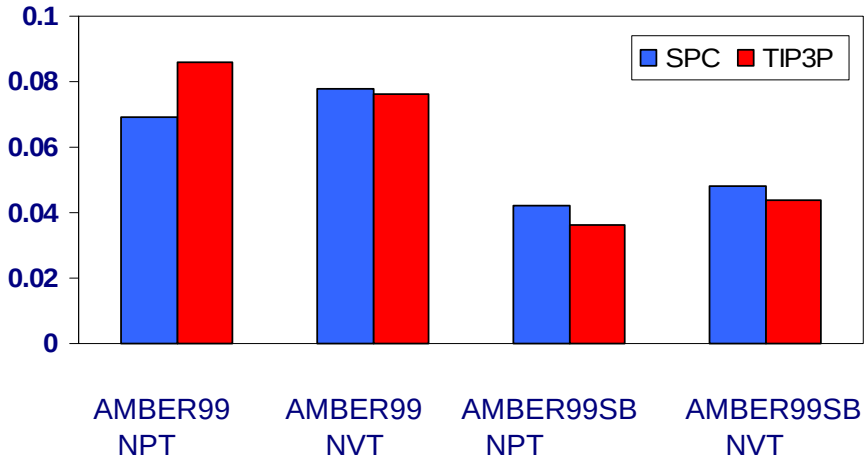
Ramachandran plots



Hornak et al., *Proteins: Struct. Funct. Bioinf.* 2006,65: 712-725

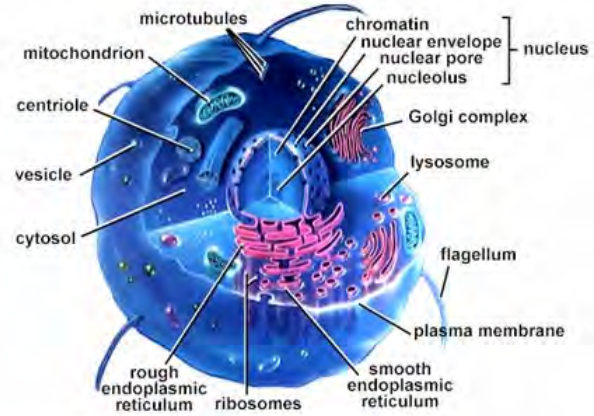
Models for water simulation of ubiquitin

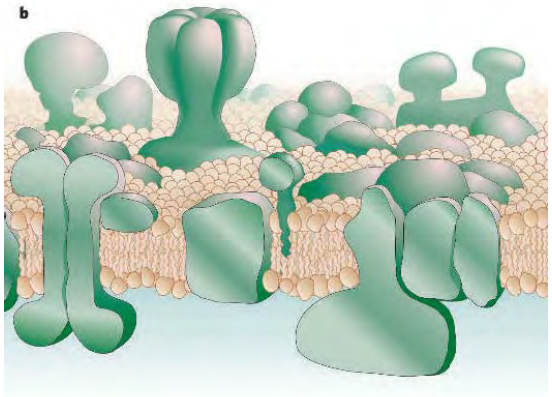
Showalter et al., *J. Chem. Theory Comput.* 2007, 3(3):961-975

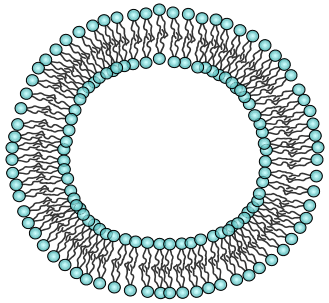


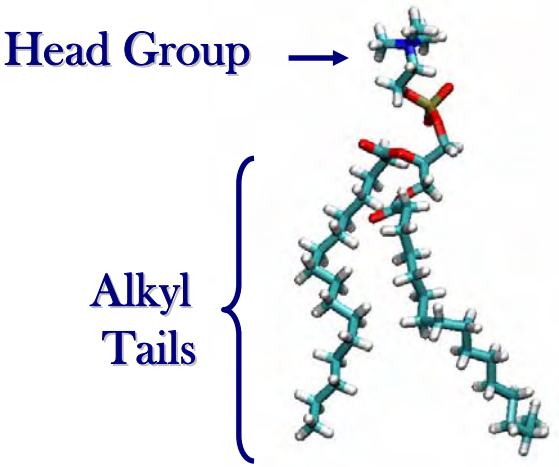
Nature uses sugars to survive dessication

Resurrection Plant (Selaginella)







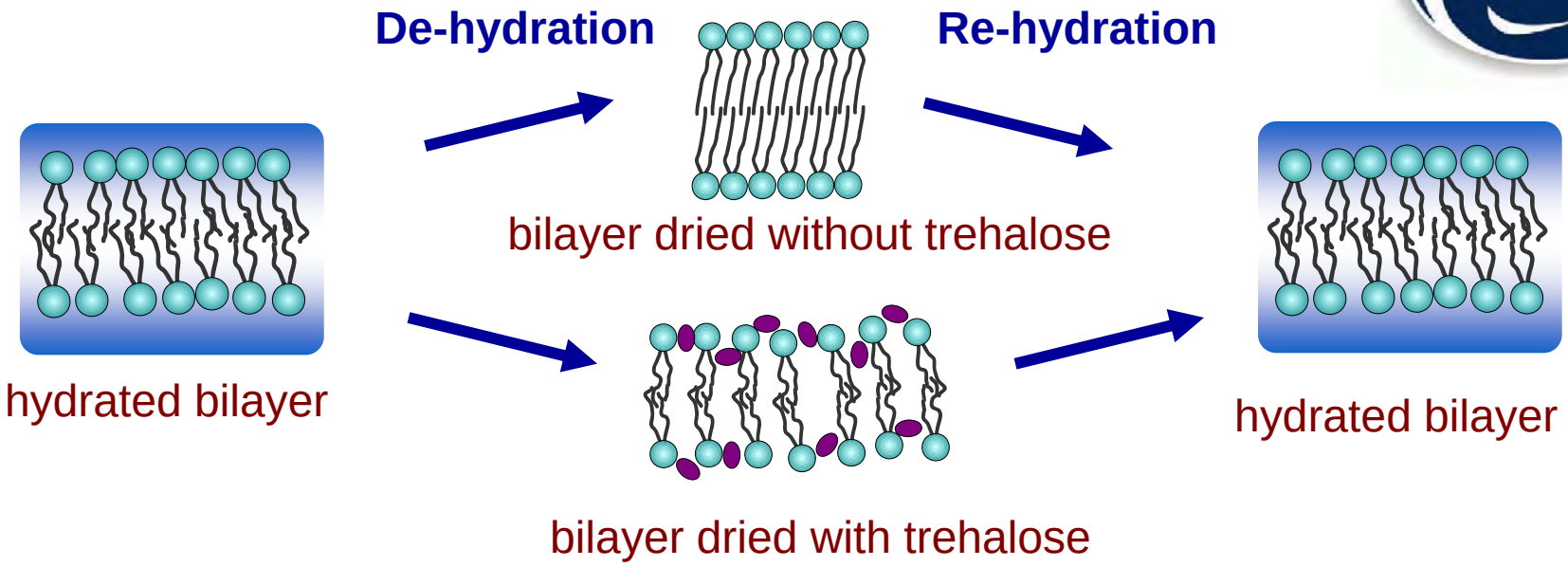


Phospholipid

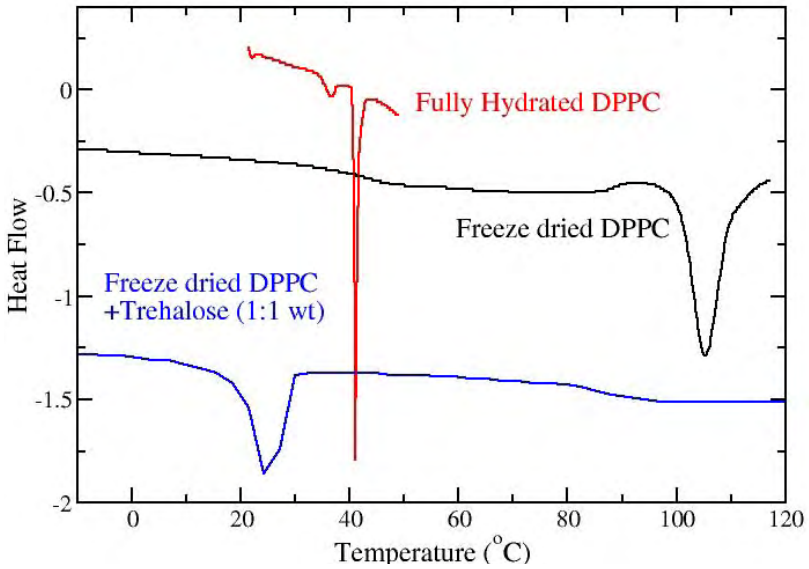
Trehalose and sucrose: preservation of biologicals

- Proteins: enzymes, antibodies
- Cells: stem cells, platelets, bacteria, sperm, seed embryos

Trehalose stabilizes membranes during re-hydration

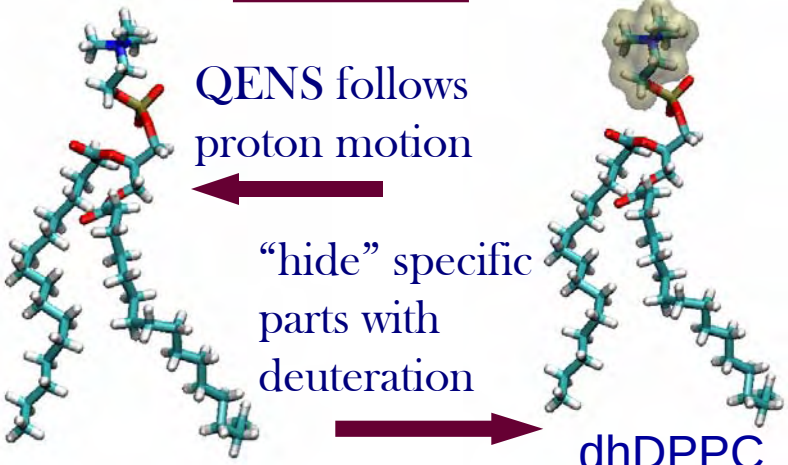


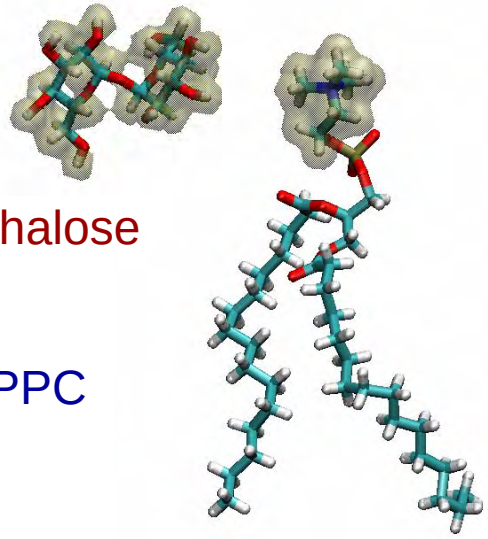
- What drives melting of freeze-dried liposomes: tails & heads?
- What is the nature of the transition and "liquid crystalline" state with trehalose?
- How does trehalose affect the dynamics of lipid heads & tails?



This study combines simulation with QENS

QENS

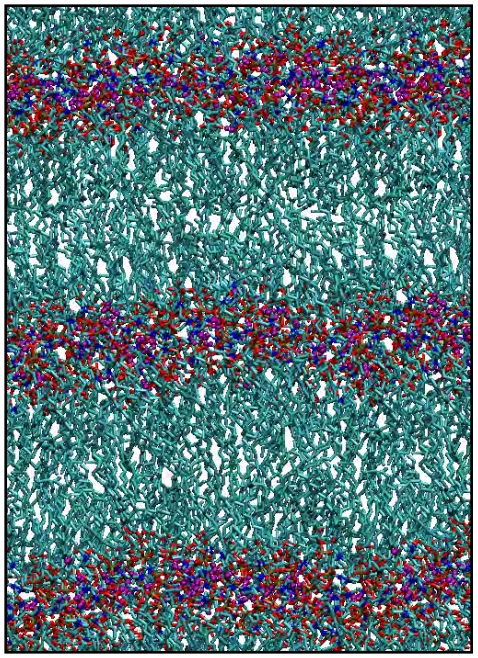




Systems investigated

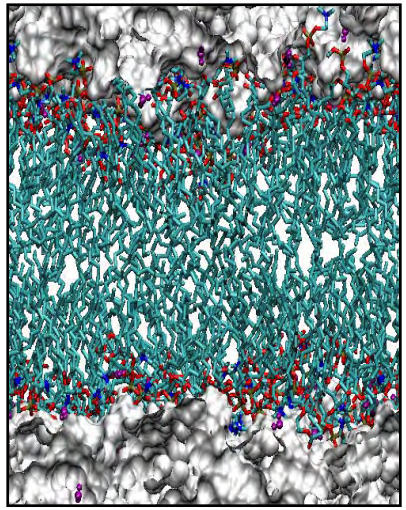
- hTrehalose
- dhDPPC
- hdDPPC
- hhDPPC+dTrehalose
- ddDPPC+hTrehalose
- dhDPPC+dTrehalose
- hdDPPC+dTrehalose

Atomistic MD



- 384 DPPC molecules
- 300 water molecules

- 128 DPPC molecules
- 100 water molecules
- 256 trehalose molecules

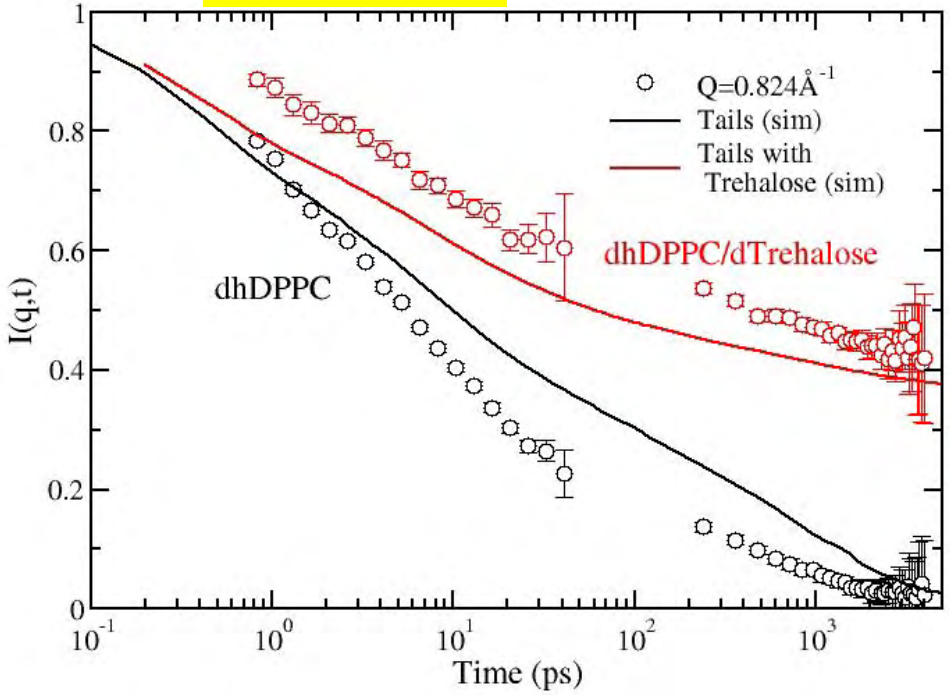


The GROMOS force field is accurate for this system

PENNSTATE

Hydrated bilayers

Dry bilayers

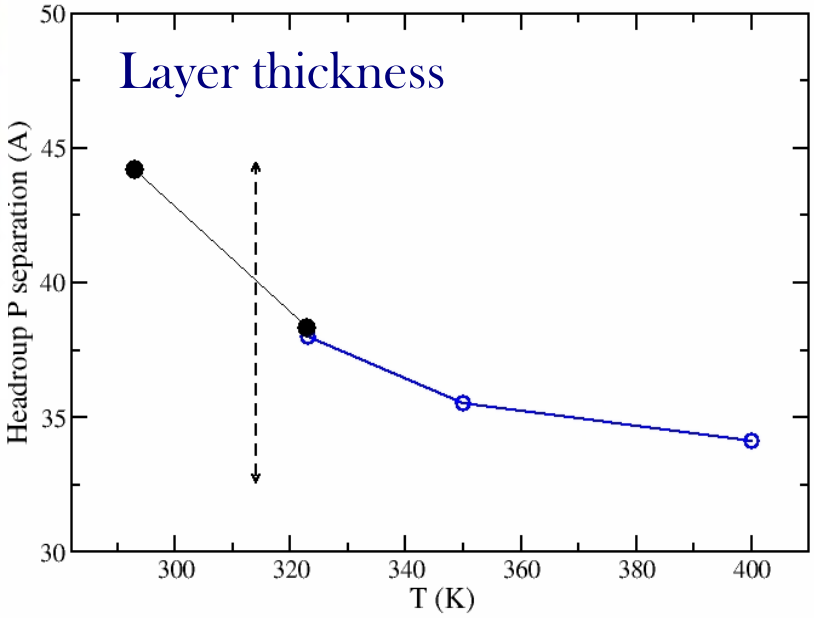


Area per lipid, 320 K

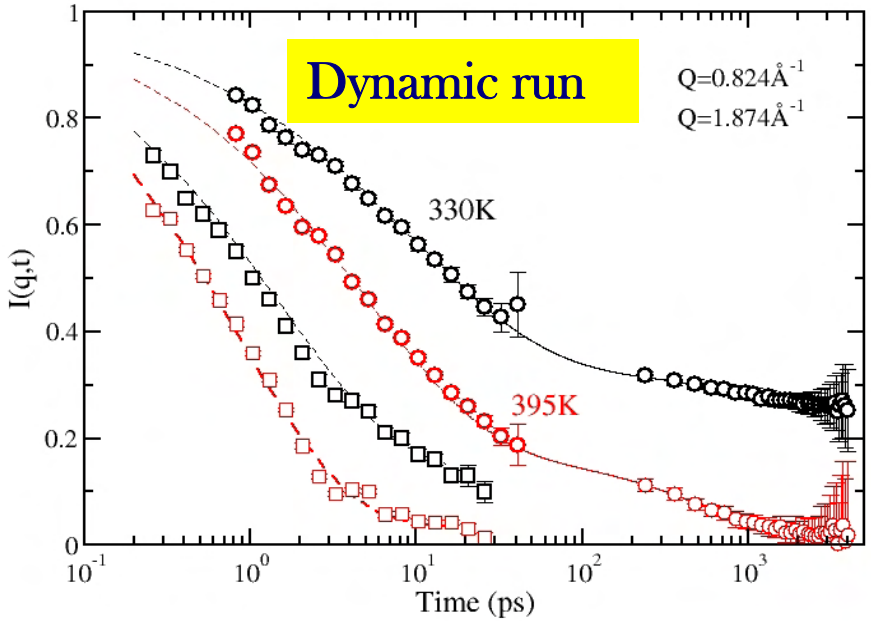
Expt: 64 Å^2

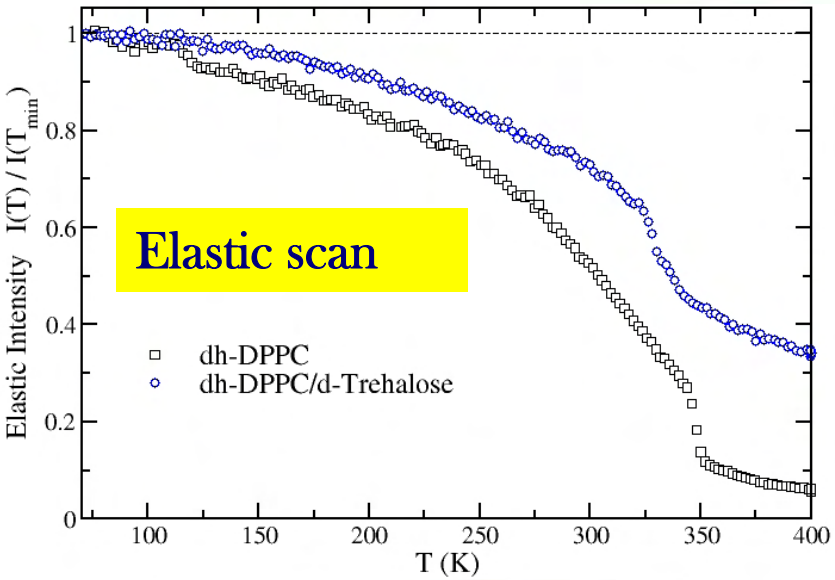
[ave. value over xray, NMR, neutron]

Sim: 60 Å^2

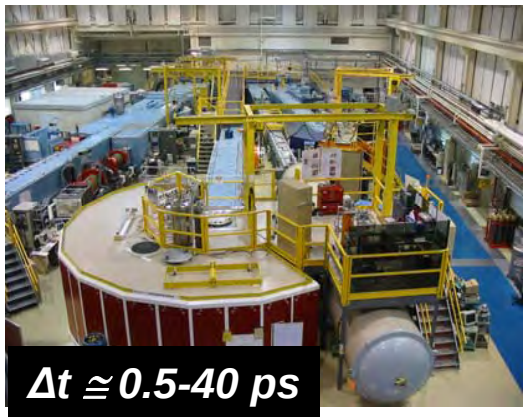


We combined HFBS and DCS dynamic runs and used HFBS for elastic scans

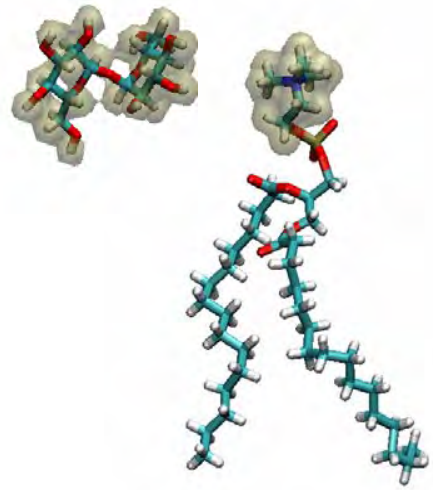




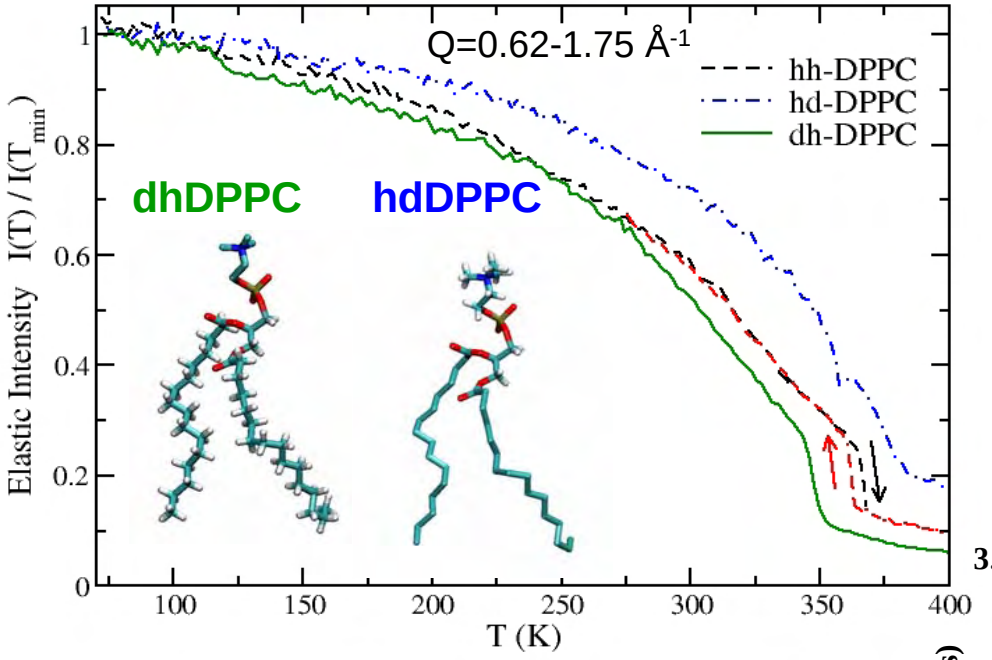
NG4 Disk-chopper time-of-flight spectrometer



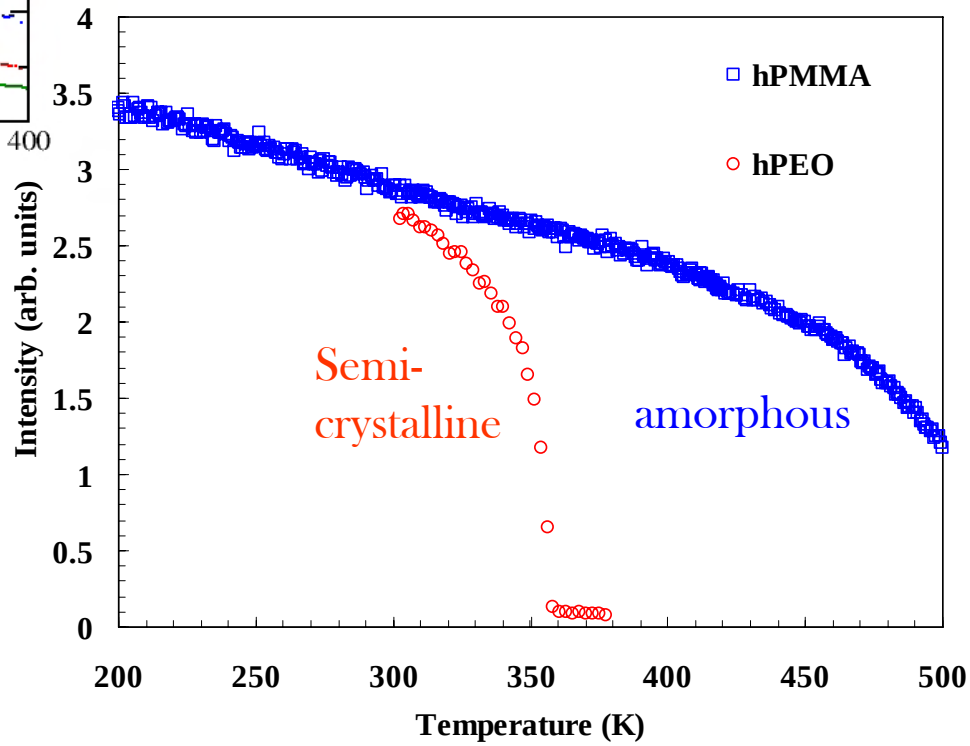
NG2 Backscattering spectrometer



Melting in dry bilayers without trehalose is driven by lipid tails



Elastic Scans: melting of Tails, Heads and Whole Lipid



Melting: lipid tails

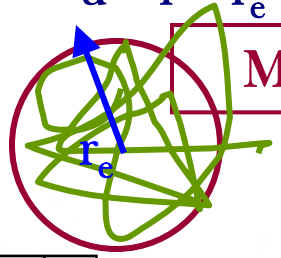
- melting transition: lipid tails
- Significant mobility before T_m

Mobility varies between heads and tails and with tail position

No translational motion:

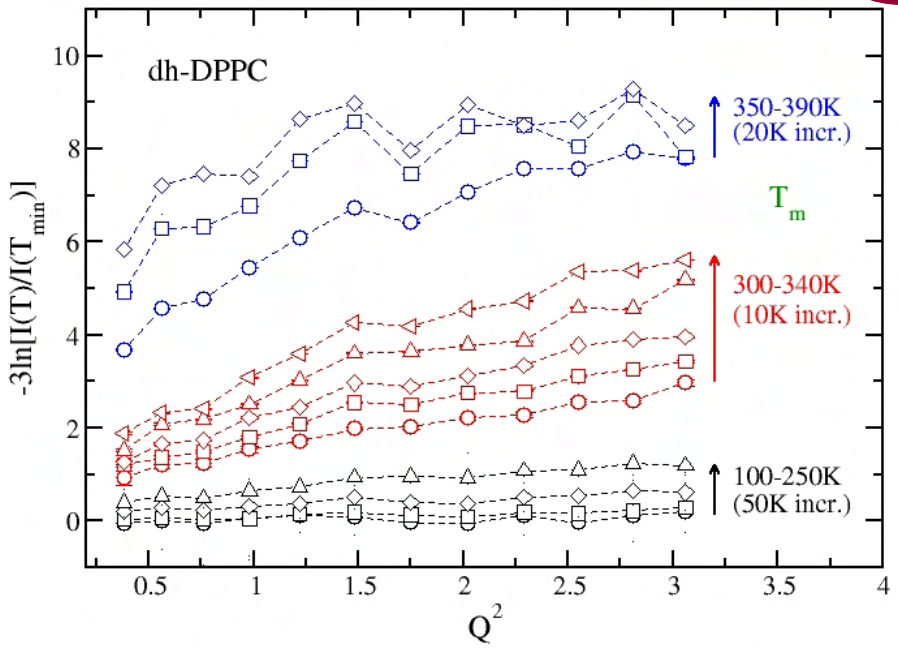
$$I \propto \exp\left(-Q^2 \left\langle u^2 \right\rangle / 3\right)$$

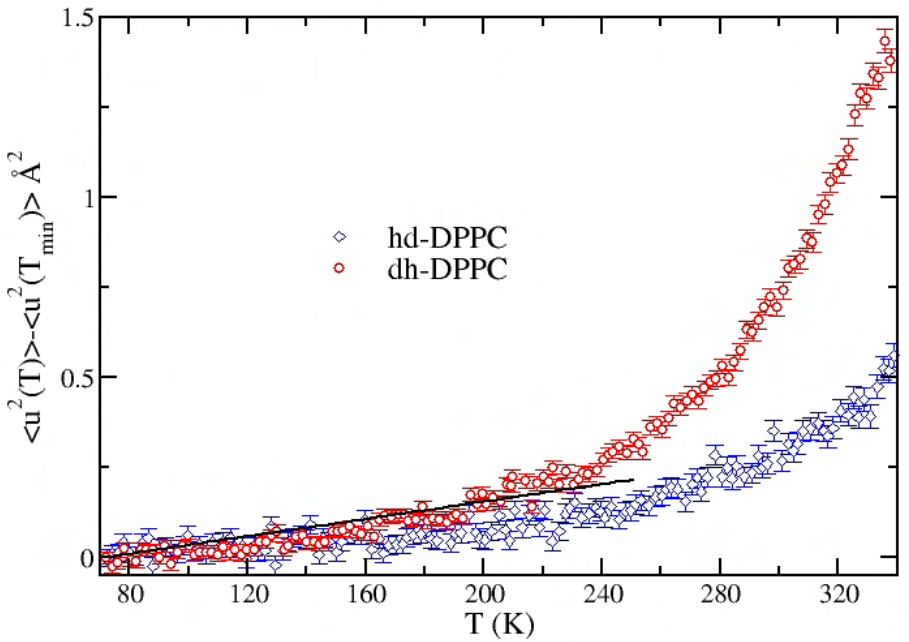
$u = r - r_e$



Molecular Simulations

Head and **Tail** mean displacement



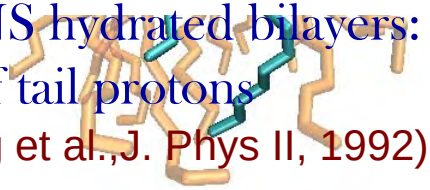


Three regimes in tail dynamics:

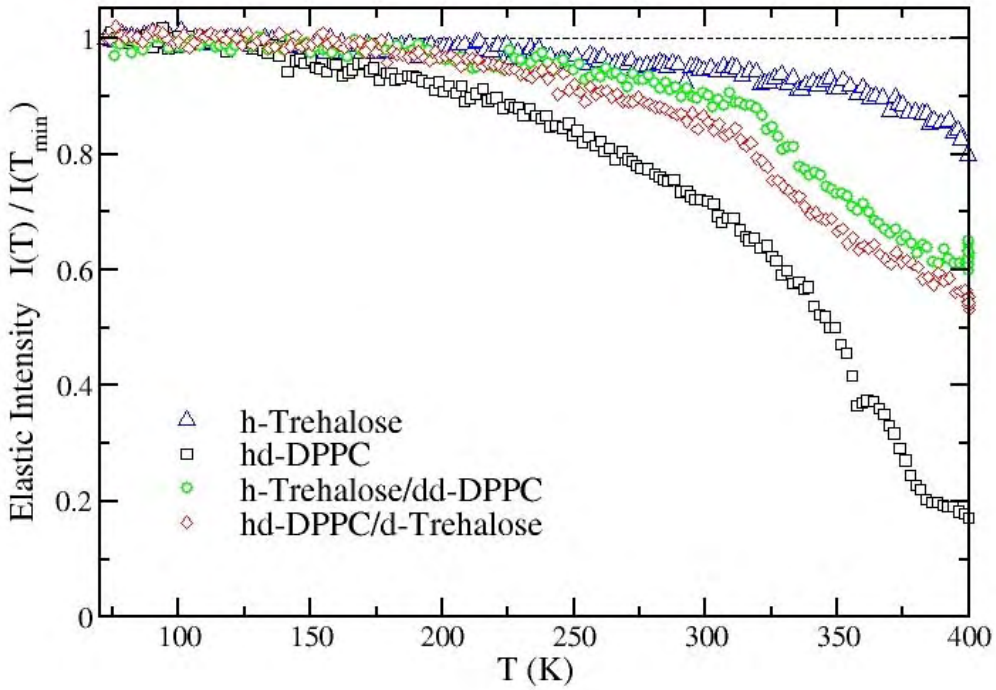
1. Vibrational motion
2. "Localized" motion
3. Translational motion

Previous QENS hydrated bilayers: equivalence of tail protons

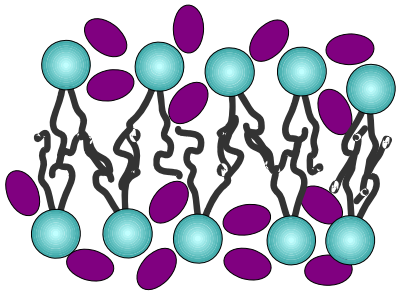
(König et al., J. Phys II, 1992)



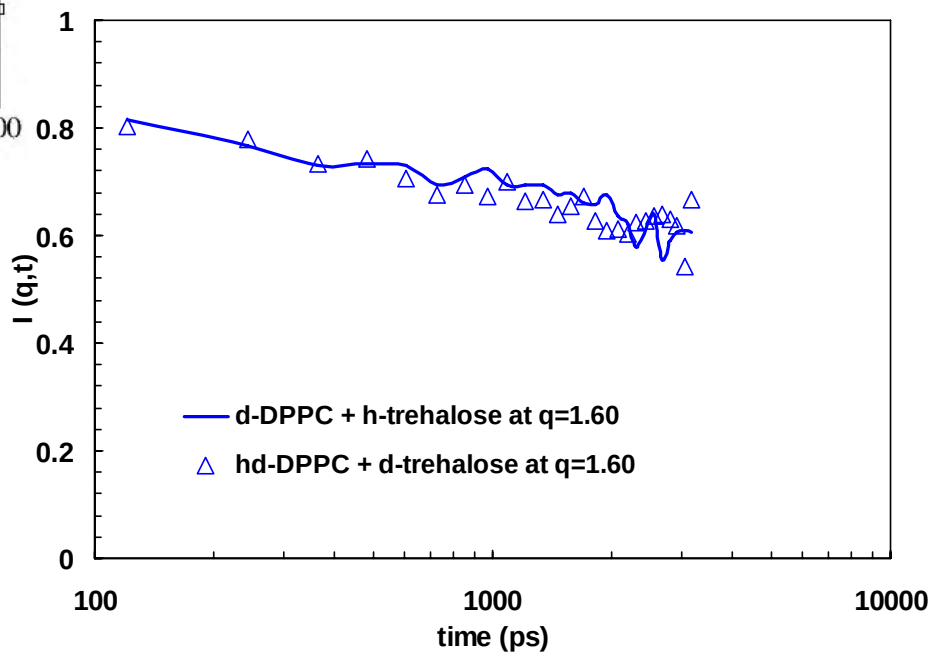
Trehalose restricts headgroup mobility



QENS: elastic scans



QENS: dynamics at 395 K

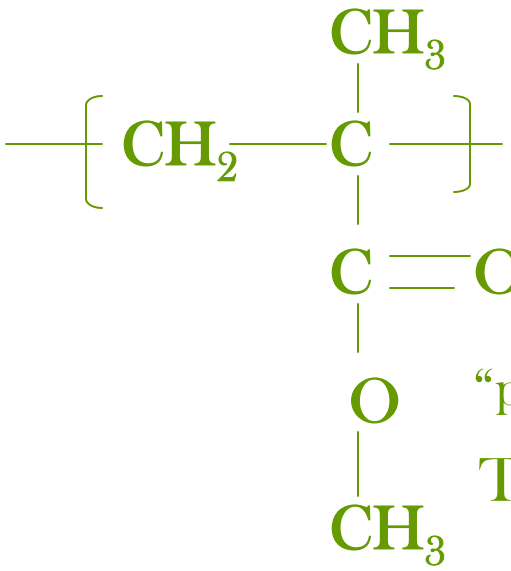


- Trehalose restricts headgroup mobility
- Trehalose and phospholipid headgroups: similar mobility

This behavior is encountered in polymer mixtures

PEO "poly ethylene oxide"

T_g ~ 220 K, more mobile

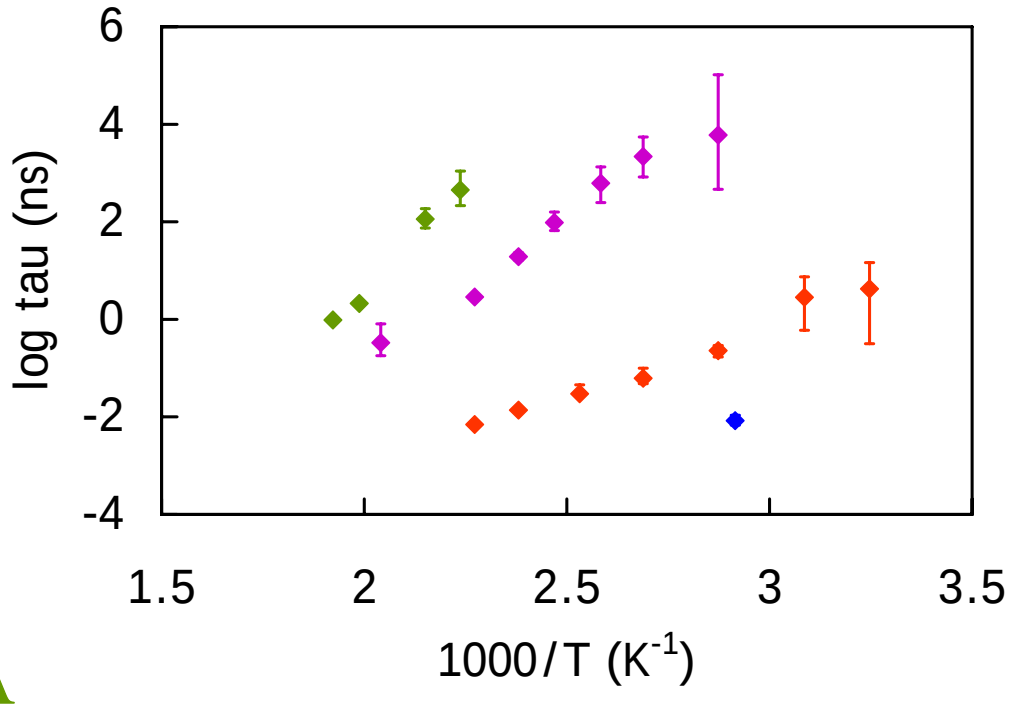


PMMA

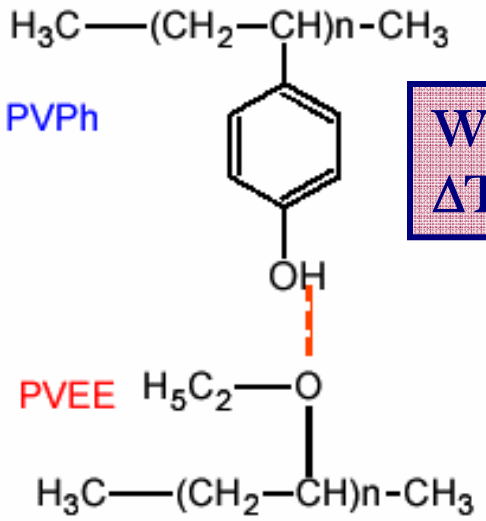
"poly methyl methacrylate"

T_g ~ 400 K, less mobile

Without hydrogen bonds: ΔTg = 180K

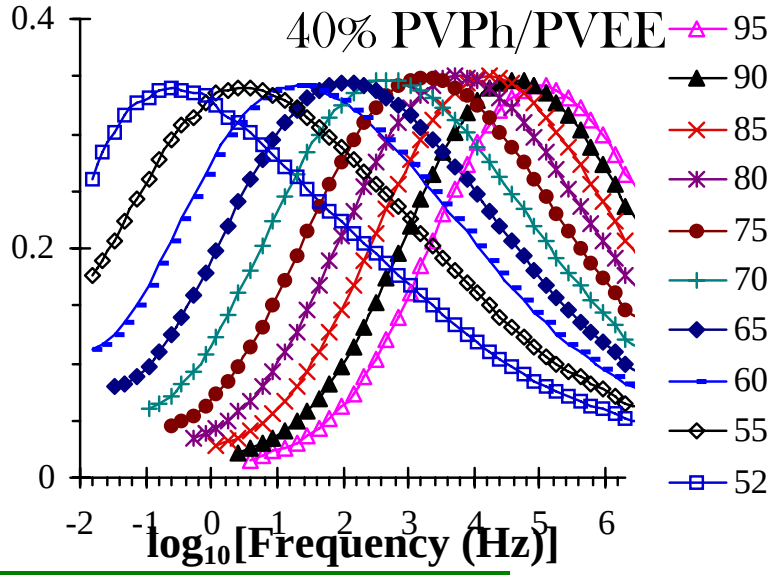


Significant sugar-headgroup hydrogen bonding is likely



With hydrogen bonds:
ΔTg = 185 K

One dynamic response:
30 - 70% PVPh

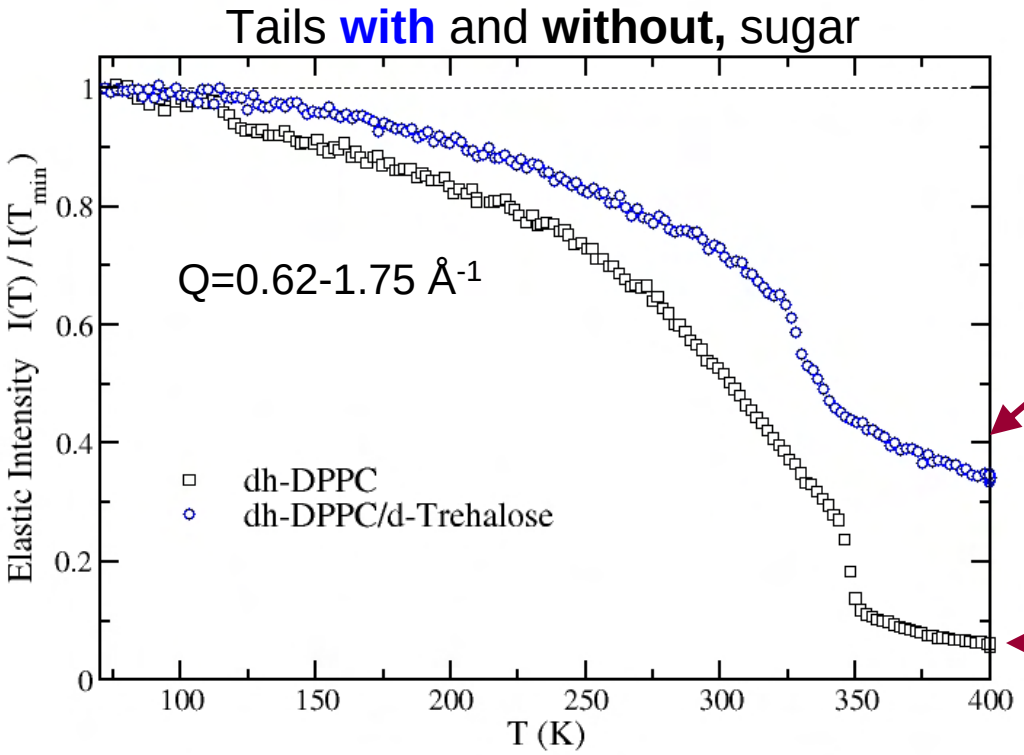


Hydrogen bonding = similar dynamics

Trehalose and lipid headgroups have significant hydrogen bonding

* Zhang, Runt, et al., *Macromolecules* (2004, 2003)

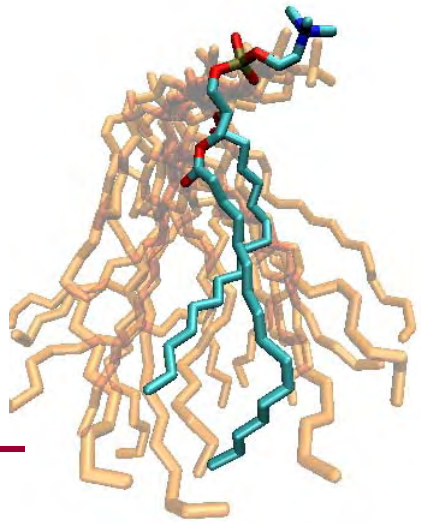
Trehalose also restricts mobility of lipid tails



Dry Bilayer with trehalose 395K

??

Dry Bilayer 395K



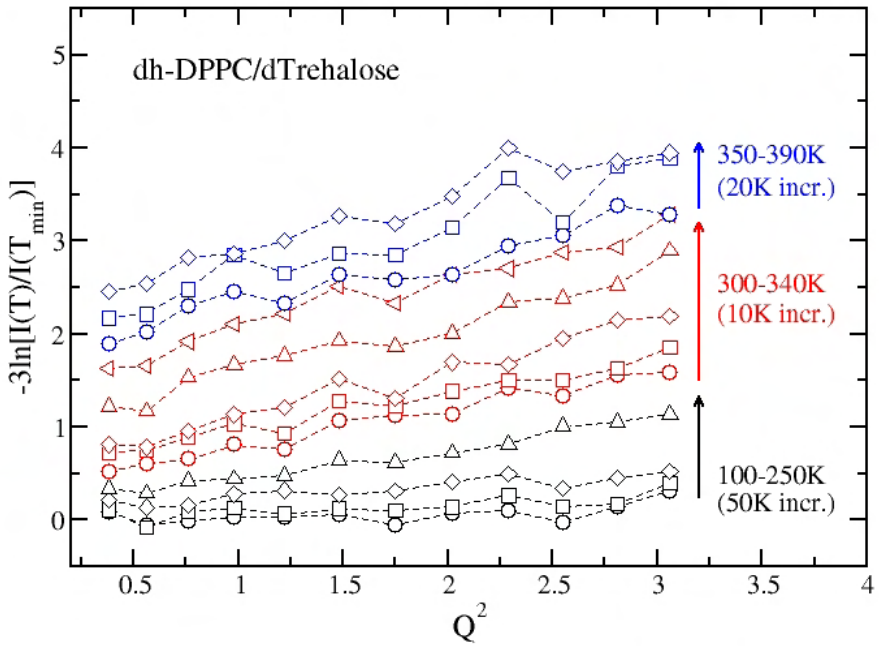
- Trehalose lowers melting transition and restricts mobility
  - Some differences below Tm
  - Large difference above Tm

Molecular nature of mobility is different when trehalose is present

Molecular Simulations

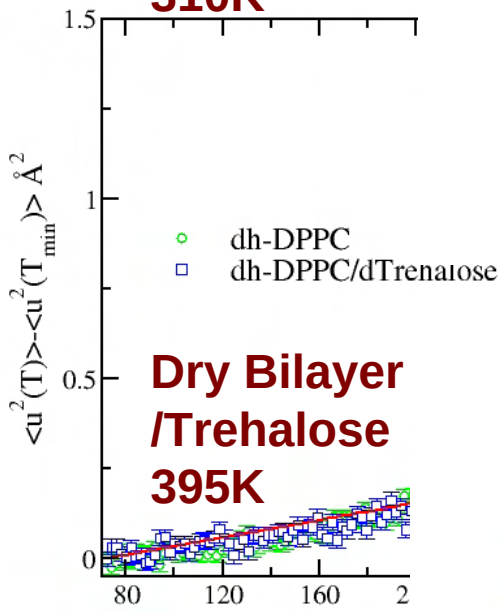
No translational motion:

$$I \propto \exp\left(-Q^2 \left\langle u^2 \right\rangle / 3\right)$$

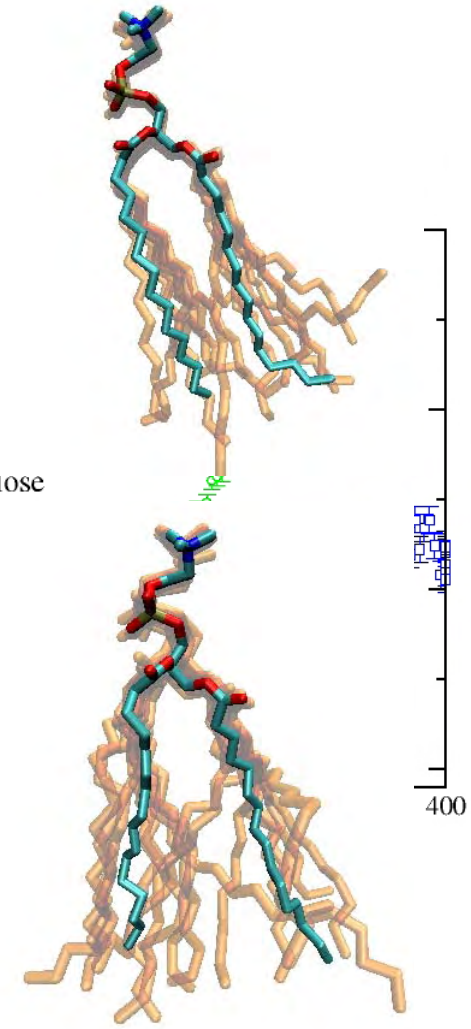


Tails mean displ
and without tr

Dry Bilayer /Trehalose 310K



Dry Bilayer /Trehalose 395K



Trehalose decreases the mean displacements and restrains the lipid mobility

The melting transition differs with trehalose

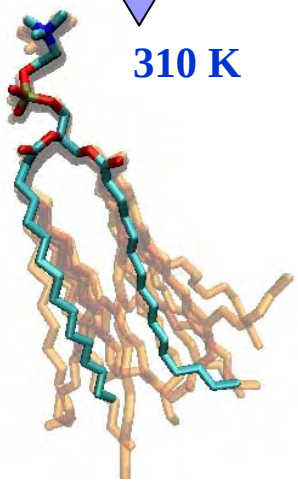
With trehalose

Without trehalose

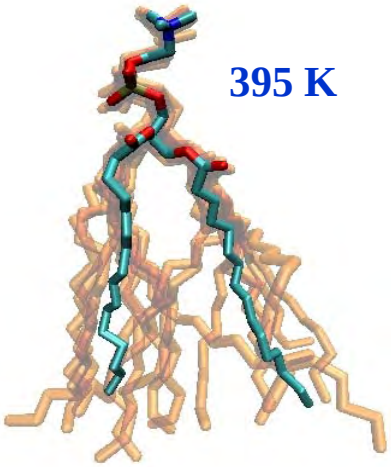
"melting" = bottom of tails

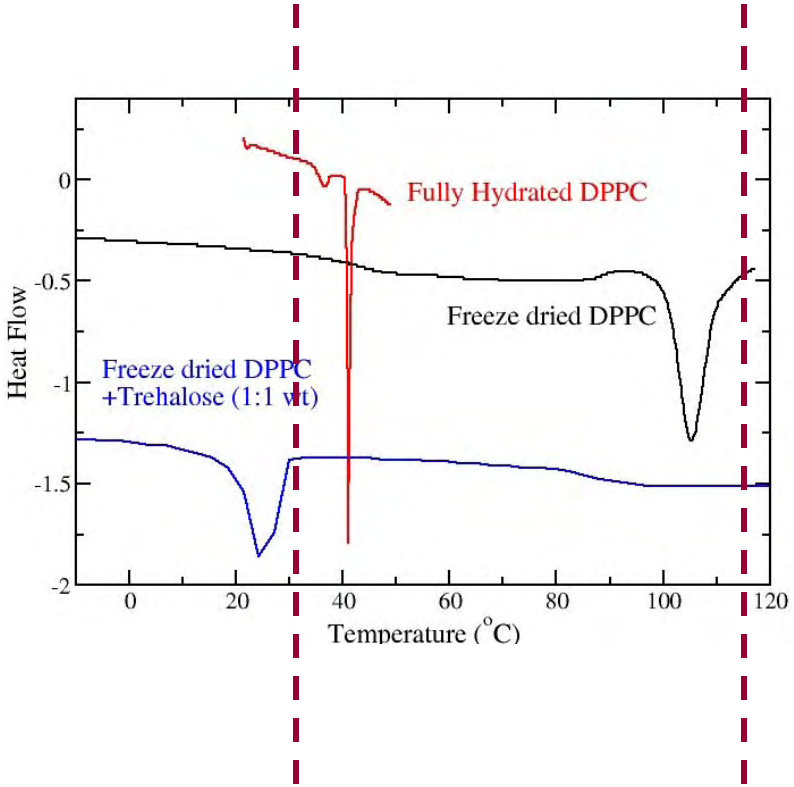
Tm

310 K



395 K



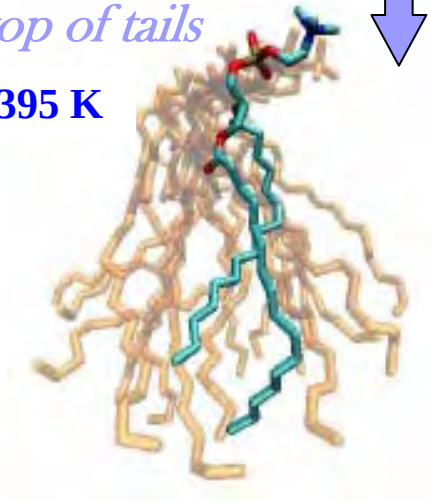


310 K

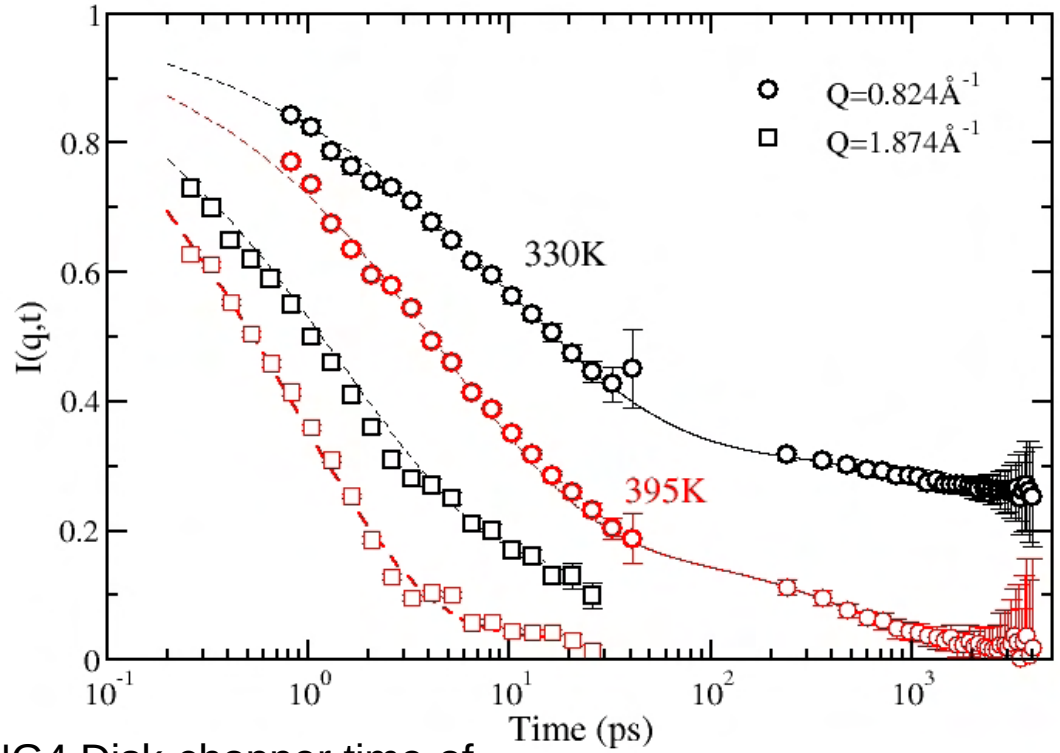
Tm

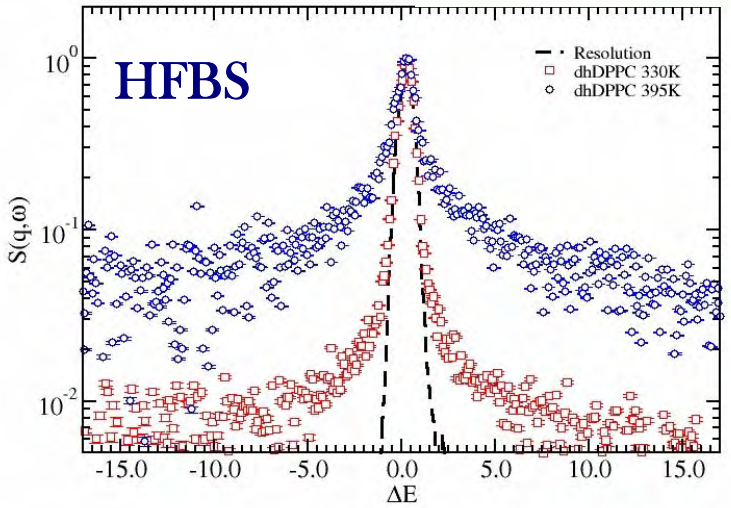
"melting" = top of tails

395 K

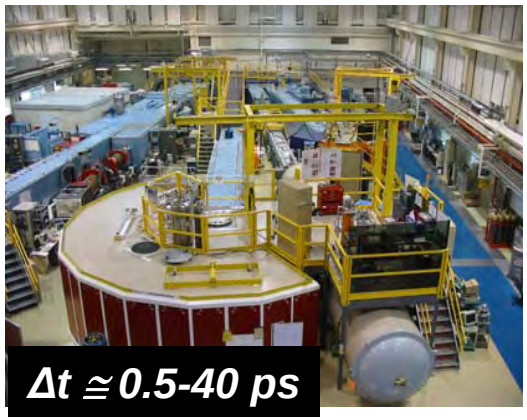


Dynamic runs were made on tail labeled samples

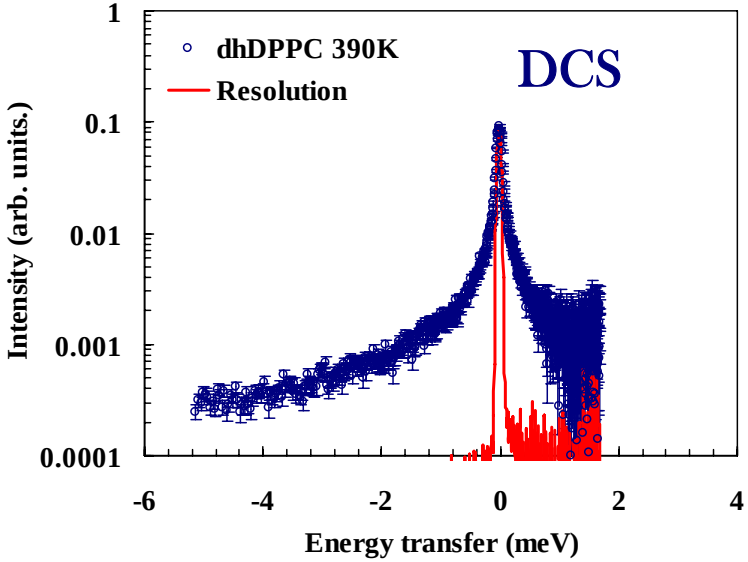




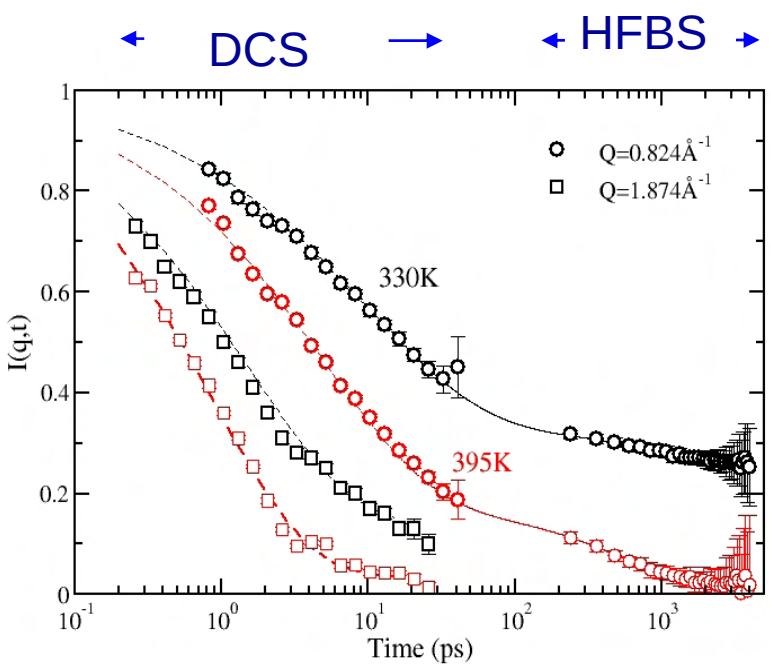
NG4 Disk-chopper time-of-flight spectrometer



NG2 Backscattering spectrometer

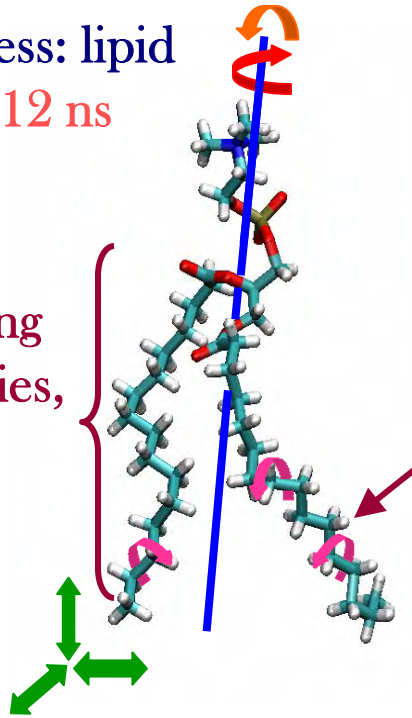


Tail motion in QENS window has two processes



Fast process: trans/gauche isomerization 7-45 ps

Slow process: lipid rotation 2-12 ns



If varying mobilities, $\beta < 1$

τ: typical time scale for trans/gauche isomerization

Fit lines

$$I(q,t) = A\,KWW_1\,KWW_2$$

$$KWW_i = E_i + (1 - E_i)\exp\left[(-t/\tau_i)^{\beta_i}\right]$$

- τ: characteristic time
- β: distribution of times
- E: motion in restricted geometry

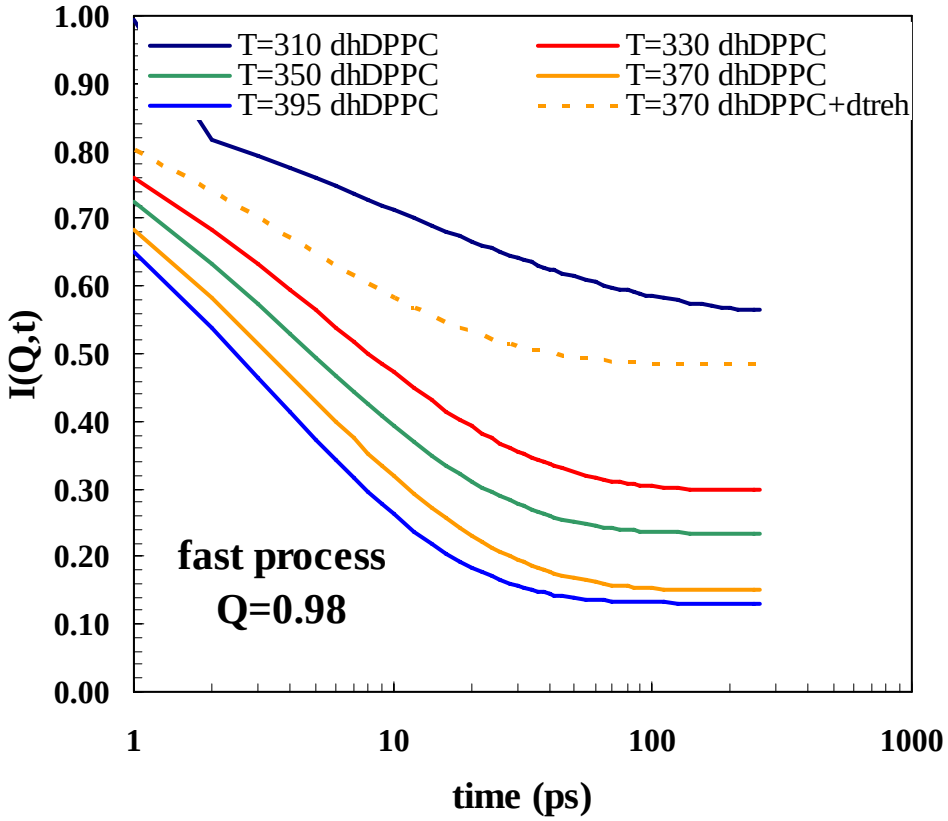
KWW Limiting cases

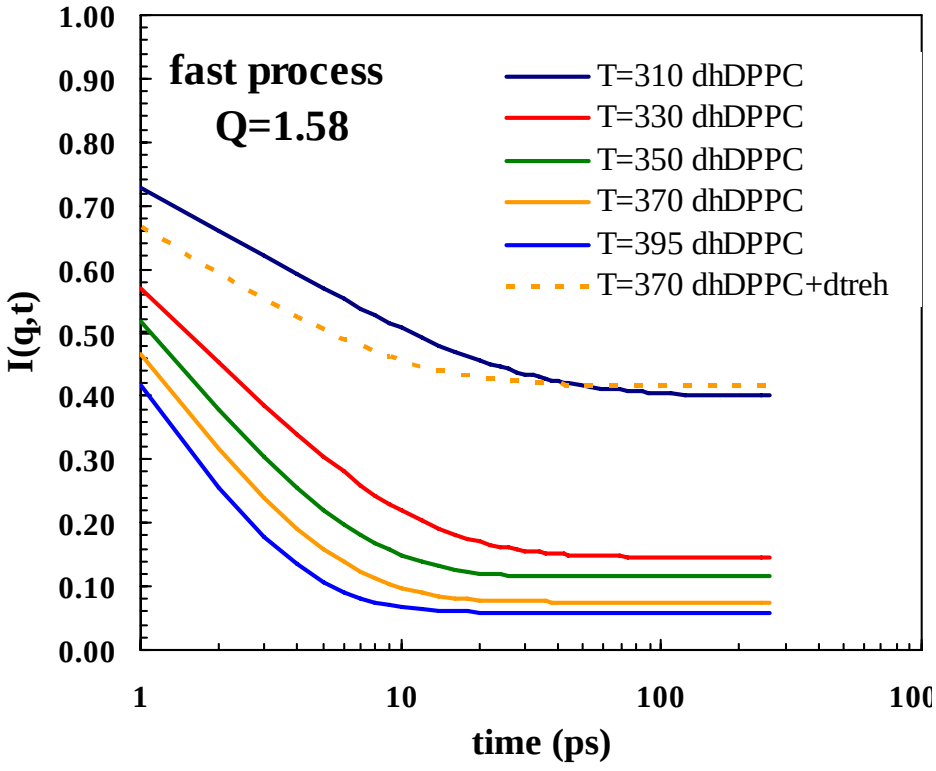
$\beta = 1$: same τ for all protons

E = 0: no spatial restriction

Isolating the fast process reveals conformational transitions

$$KWW_1 = E_1 + (1 - E_1)\exp\left[(-t/\tau_1)^{\beta_1}\right]$$



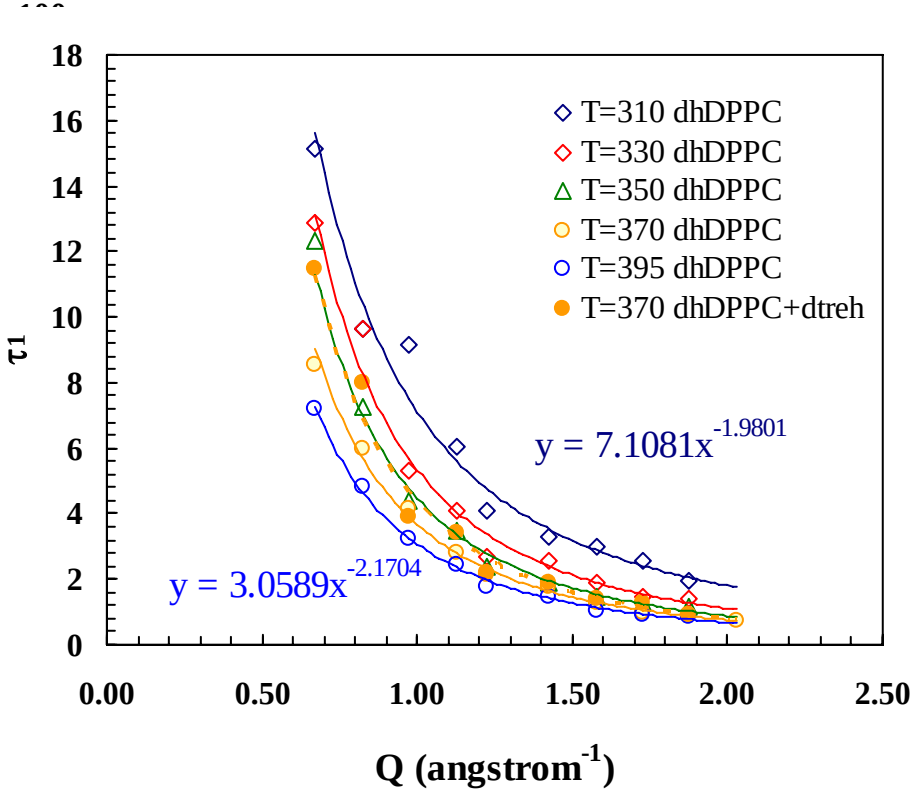


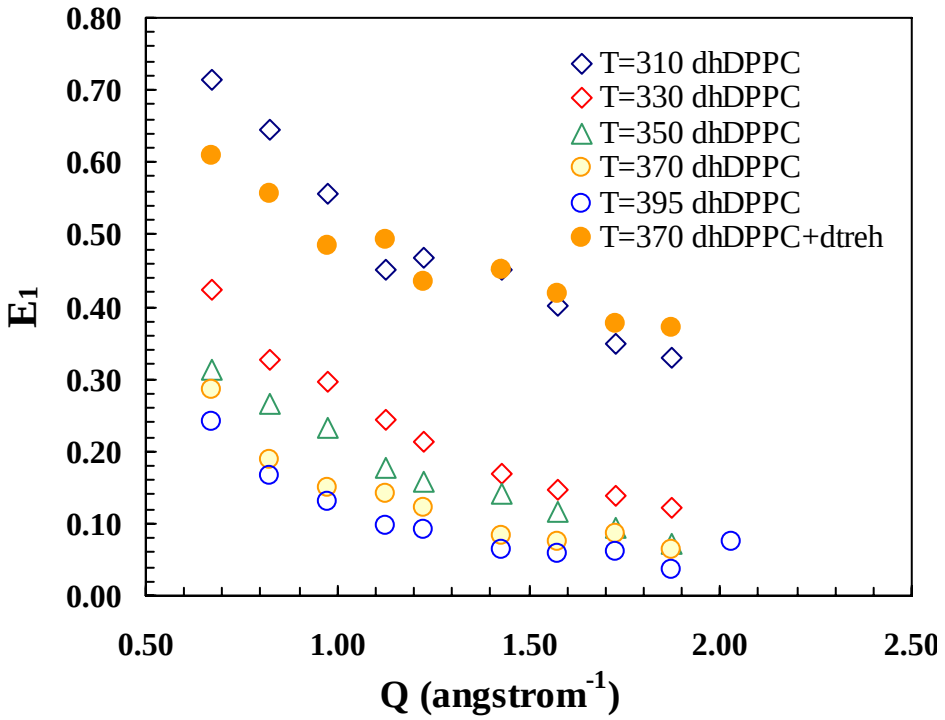
- trehalose decreases conformational transitions
- effect is similar to decreasing T to between 310 & 330 → below Tm
- larger spatial restriction of protons

Trehalose slows conformational transitions and increases spatial localization

Hydrated bilayers, T=333 K: τ_{conf} = 7-45 ps
Venable, et al, Science, 262, 223 (1993)

Characteristic times:
Slower with decreasing T
Little change with trehalose





Spatial localization
Larger with decreasing temperature AND with trehalose

Modeling of spatial localization quantifies the variation in mobility along lipid tails

Q dependence of EISF

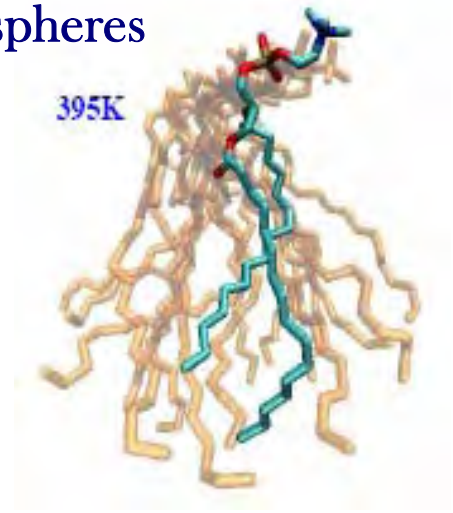
Single sphere size

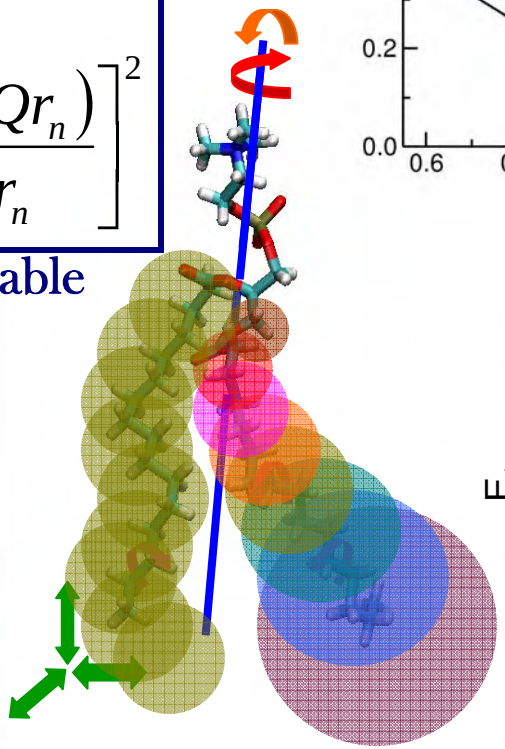
$$E(Q) = \left[\frac{3 j_1(Qr)}{Qr}\right]^2$$

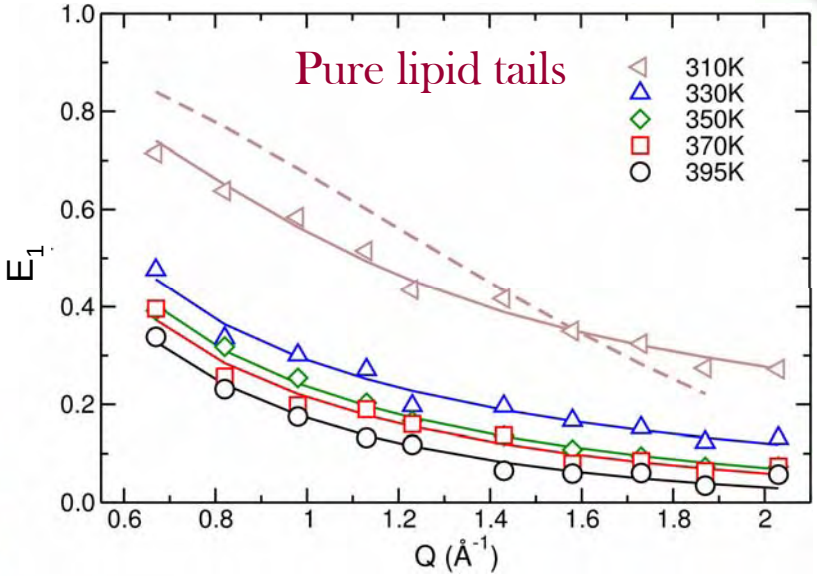
varying sphere sizes

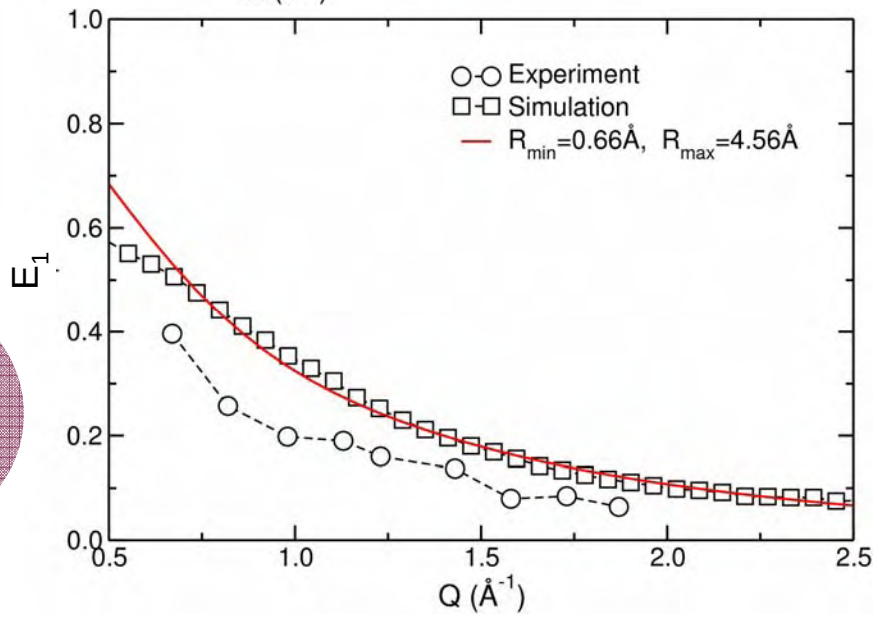
$$E(Q) = \frac{1}{N}\sum_{n=2}^{N}\left[\frac{3 j_1(Qr_n)}{Qr_n}\right]^2$$

Diffusion in impenetrable spheres

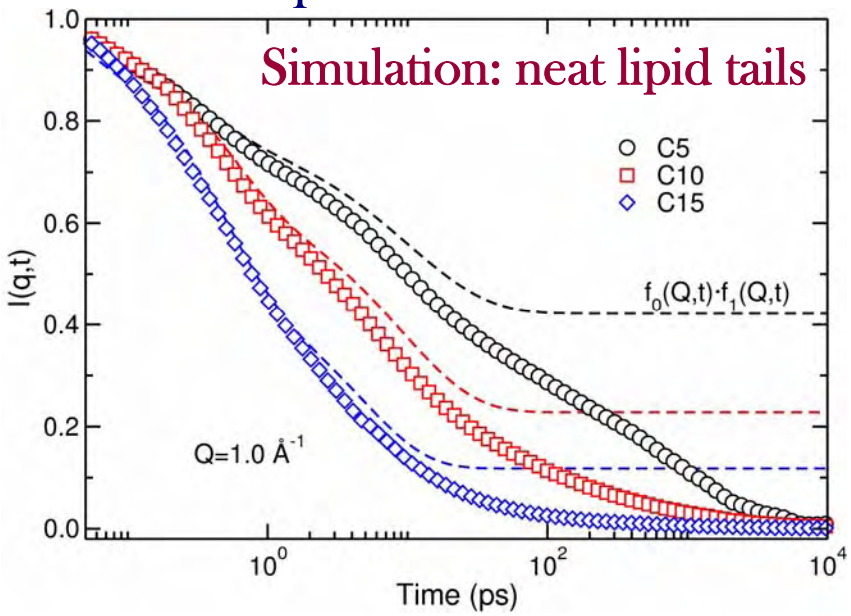


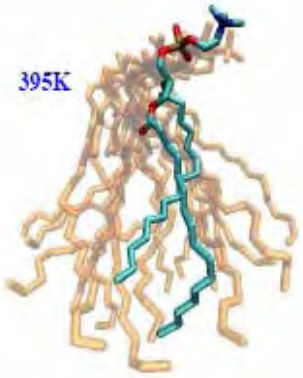


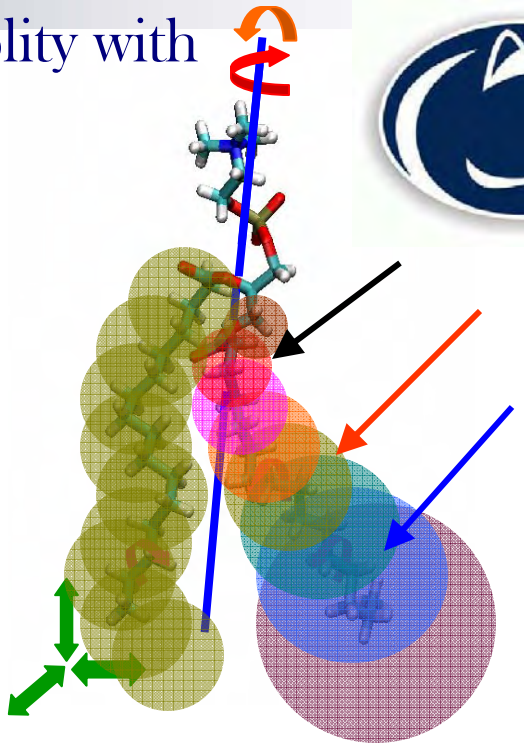


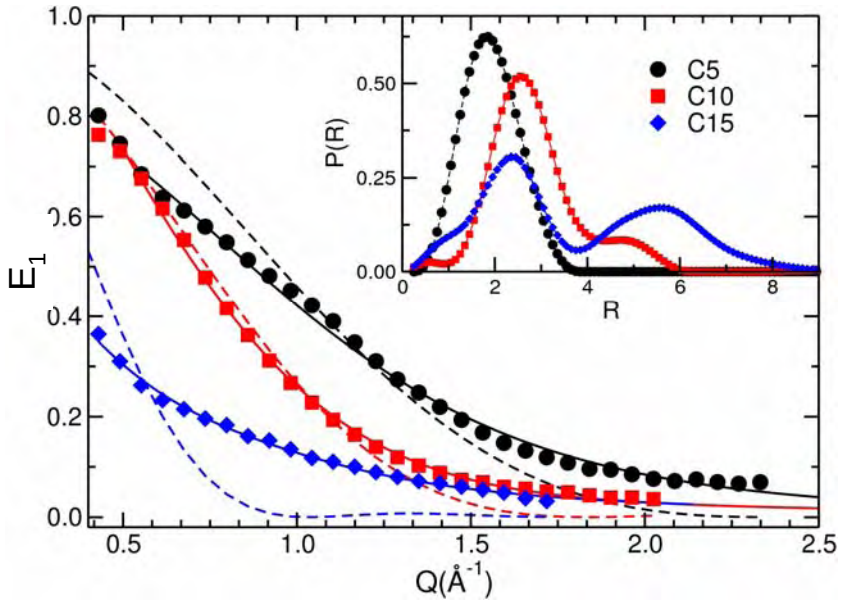


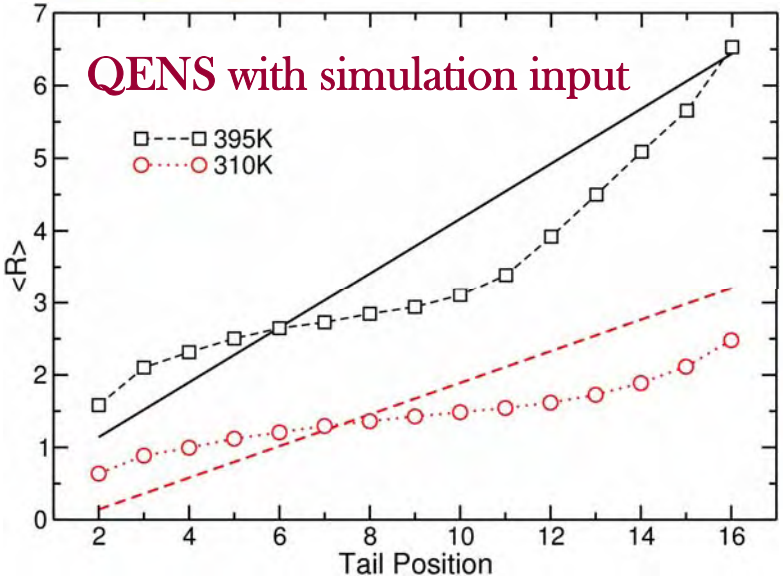
Simulation directly probes difference in moblity with carbon position





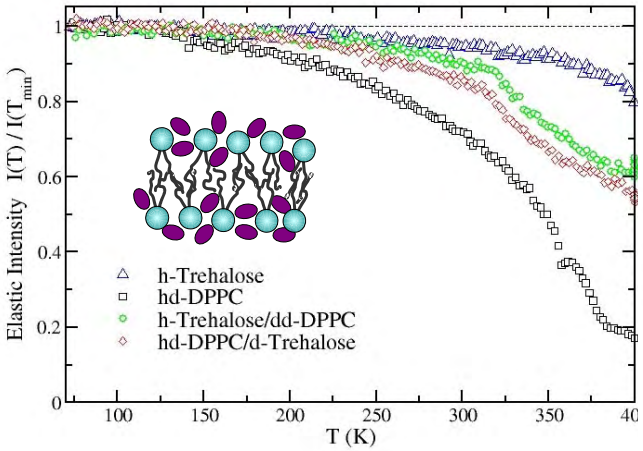




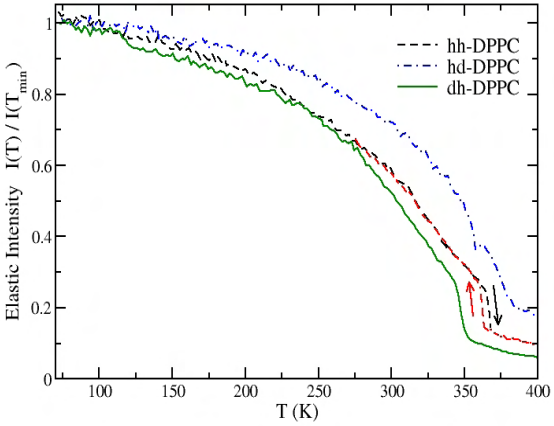


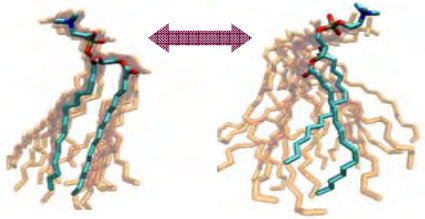
Five things to remember

lipid headgroups and trehalose: significant hydrogen bonding

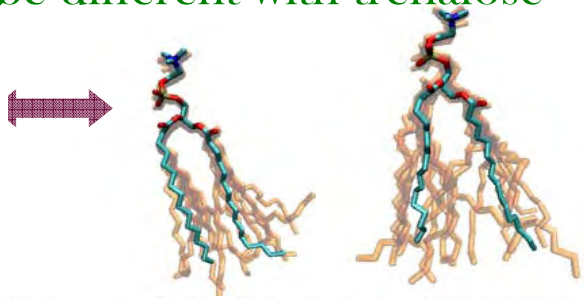


Melting: lipid tails

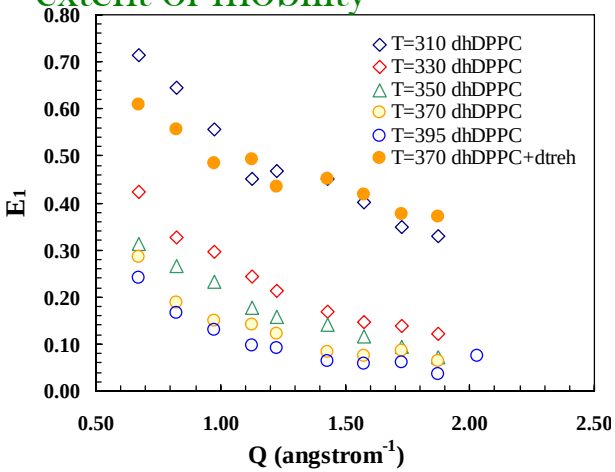


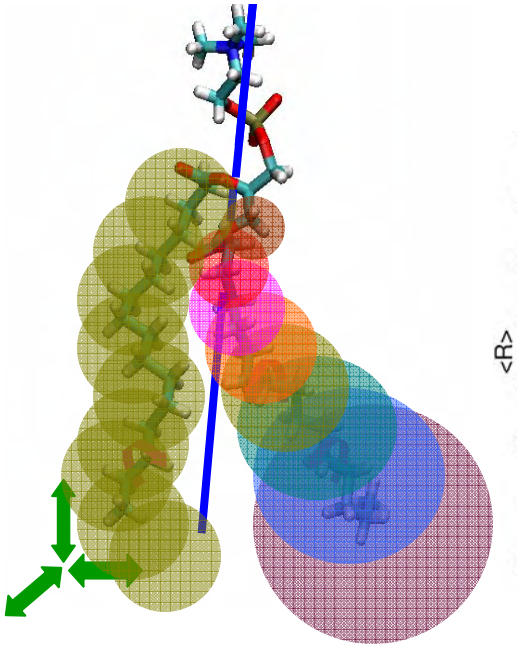


nature of main transition may be different with trehalose



Trehalose decreases spatial extent of mobility





Spatial extent of mobility varies with tail postion