Molecular secrets of membrane barrier modulators

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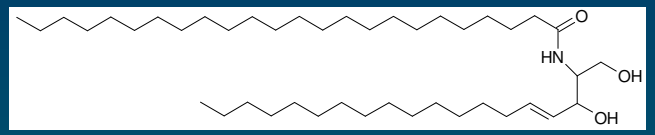
Molecular secrets of membrane barrier modulators

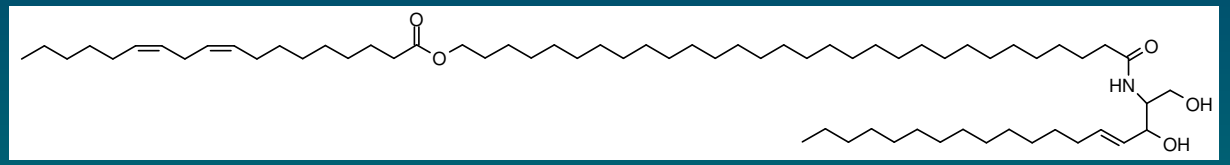
- Significance
  Drug & gene delivery; Membrane-lipid therapy; Cryoprotection
- Molecular dynamics simulation
- Interaction of small molecules with DPPC bilayers
  Oleic acid, DMSO, Ethanol, Urea
- Interaction of DMSO with ceramide bilayers
  Free energies of pore formation
- Concluding remarks

Skin lipids

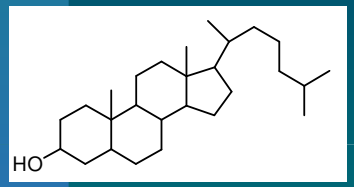
- Ceramide (~50%) (9 types in skin)
- Cholesterol (~25%)
- Free fatty acids (~10-20%)



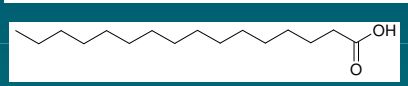
Ceramide 2



Ceramide 1

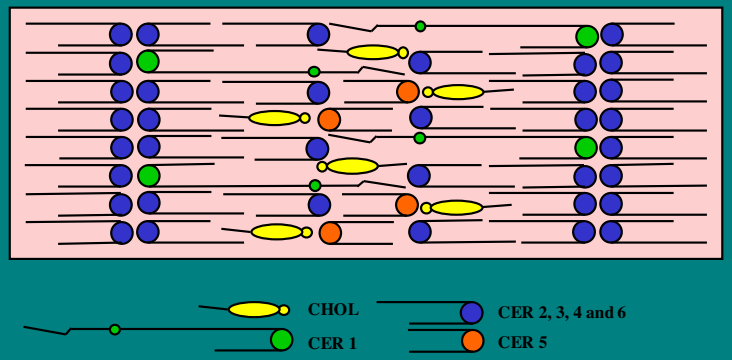


Cholesterol



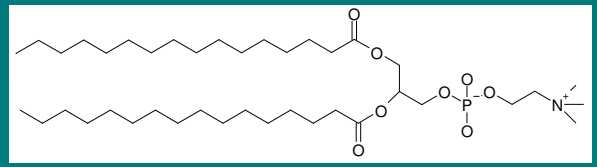
Stearic Acid

Sandwich model



Bouwstra et al. (2000) Acta. Derm. Venereol. Supp 208, 23

Extracted skin lipid behaviour is qualitatively similar to dipalmitoylphosphatidylcholine (DPPC)

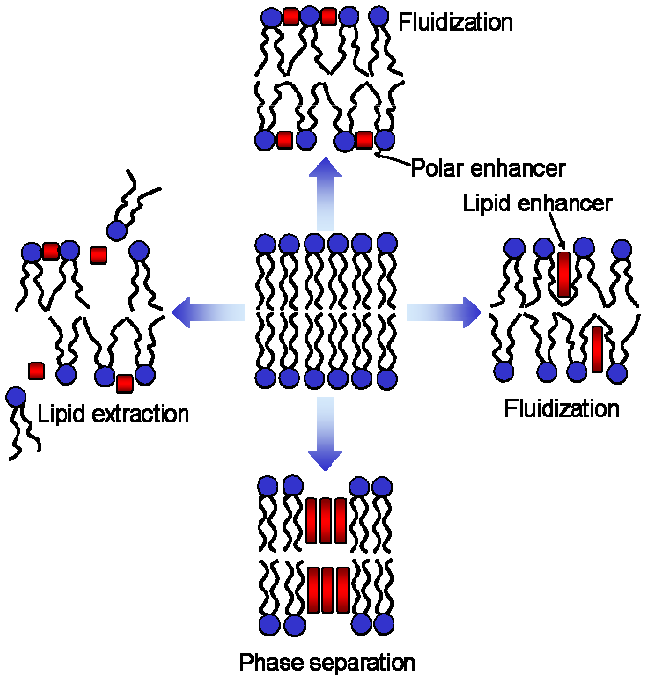


DPPC

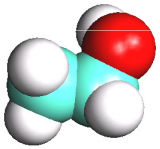
Chemical penetration enhancers

- Interact with the skin lipids
- Increase the permeability of membranes to drug molecules
- Mechanism of action is not fully understood at the molecular level

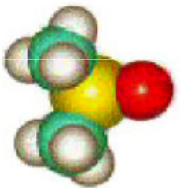
Proposed Mechanisms of Action



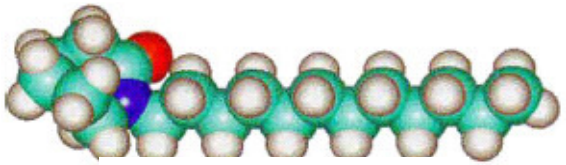
Common Penetration Enhancers



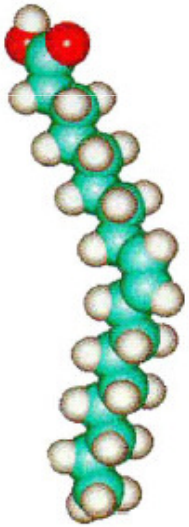
Ethanol



Dimethyl-sulphoxide (DMSO)



Azone



Oleic Acid

Molecular simulation

Reductionism

'Nothing exists except atoms and
empty space; all else is opinion'
Democritus 430-380 BC

Molecular level understanding

Why simulate?

Cannot do experiments that we would like to do
(Molecular level resolution)
Predict behaviour

Molecular simulations

Atomistic; Input is atom-atom interactions only
No 'fudge' factors

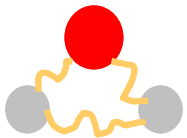
Interaction potential (molecular mechanics)

$$U = \sum_{i<j} \sum 4\varepsilon_{ij}\left[\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right] + \frac{q_i q_j}{4\pi\varepsilon_0 r_{ij}}$$

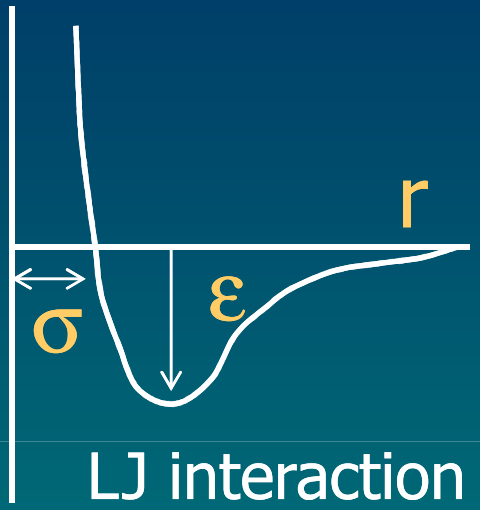
$$+ \sum_{bonds} \frac{1}{2} k_b (r - r_0)^2$$

$$+ \sum_{angles} \frac{1}{2} k_a (\theta - \theta_0)^2$$

$$+ \sum_{torsions} k_\phi [1 + \cos(n\phi - \delta)]$$



Ball & spring



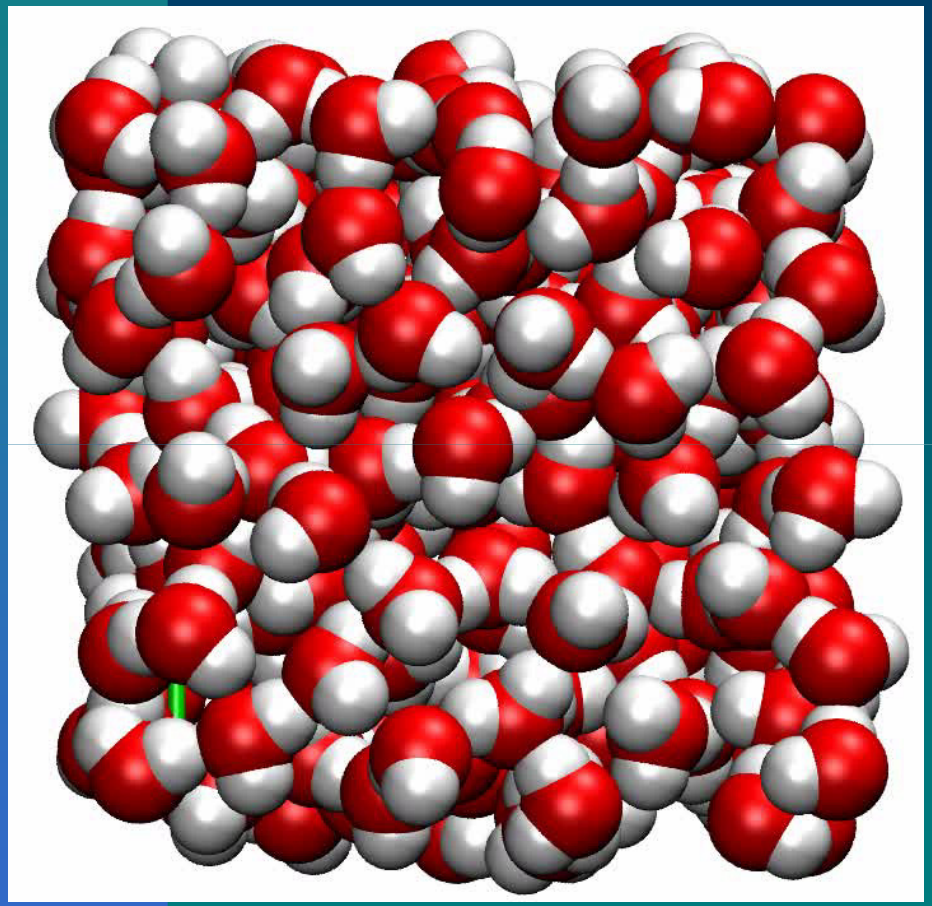
LJ interaction

Parameters empirical;
from experiment and optimised

Pair-interactions
N(N-1)/2

LJ: short ranged R_c
qq: long-ranged
(Ewald summation)

Molecular dynamics simulation



1. Initial configuration
2. Equilibration
3. Production (averages)

NPT ↓G

Simulate time evolution of a system of atoms/molecules

$$\mathbf{f}_i = -\sum_j \nabla_{\mathbf{r}_i} U(\mathbf{r}_{ij})$$

$$\mathbf{r}_i(t+\Delta t) = 2\mathbf{r}_i(t) - \mathbf{r}_i(t-\Delta t) + \frac{\mathbf{f}_i(t)}{m_i}\Delta t$$

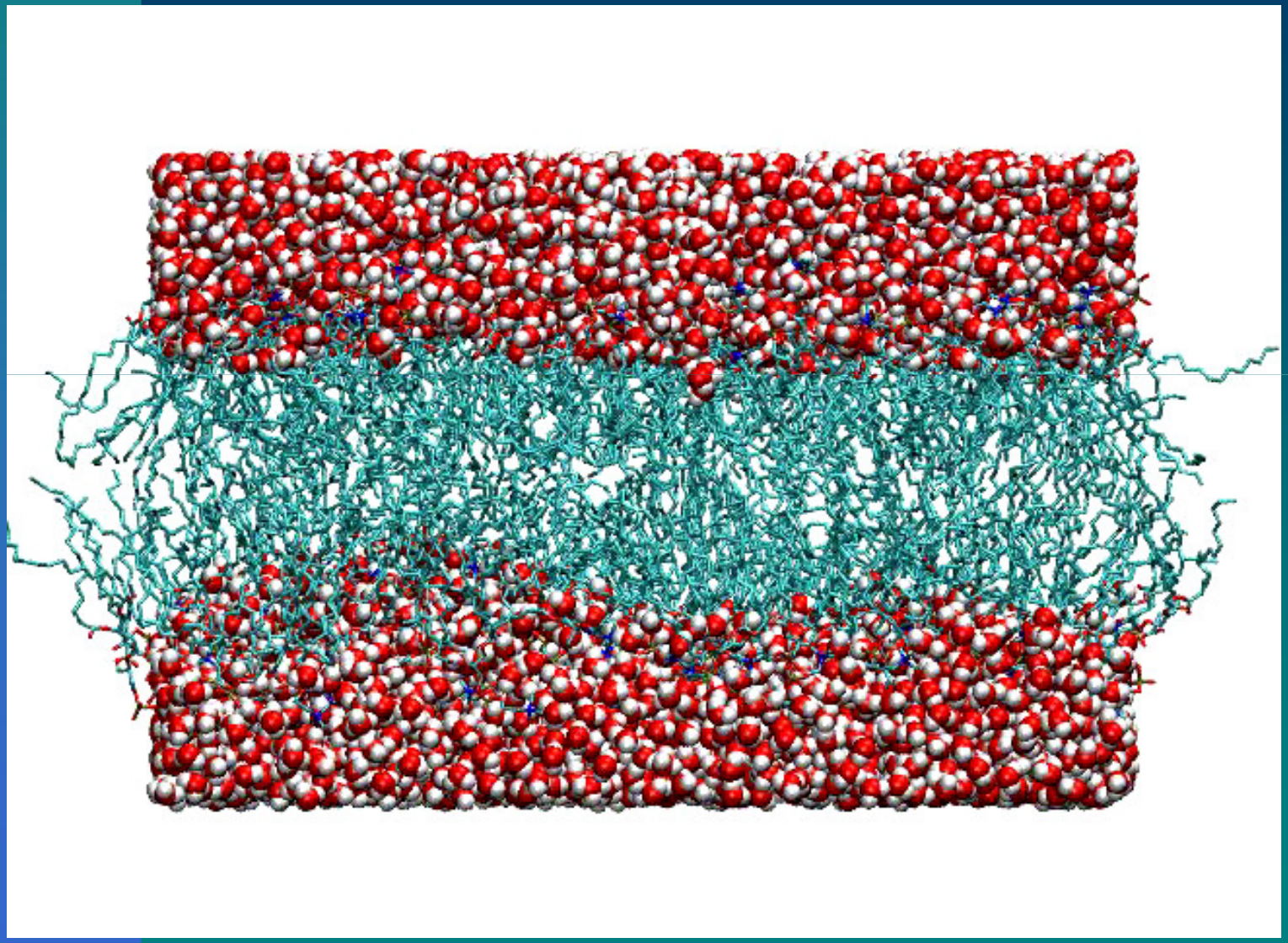
Periodic boundaries & minimum image convention

NVE, NVT, NPT & NσT
Employ extended Lagrangian
e.g. L(**r**,**p**,**H**,s)

Limitations

Limited system size
Cpu time (100ps -> 10ns)
Accuracy of interaction potential

Molecular dynamics simulation of dipalmitoylphosphatidylcholine (DPPC)

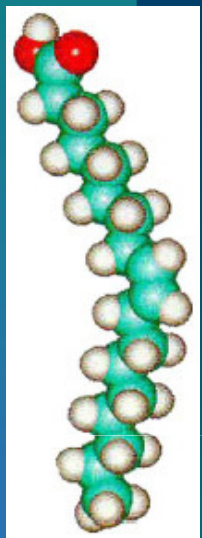


256 DPPC
1 ns

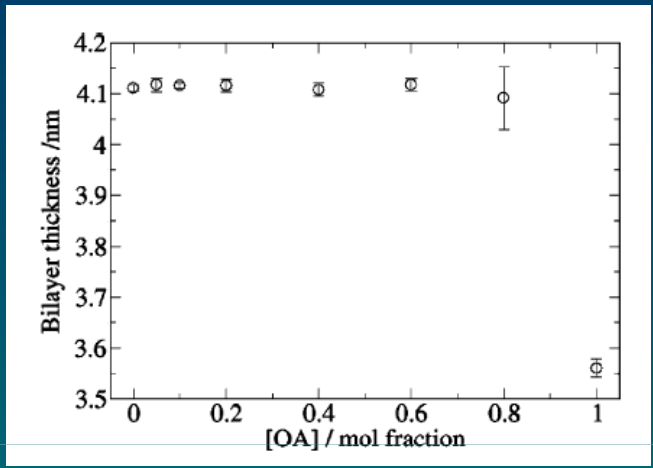
How faithful are molecular simulation? Comparison with experiment

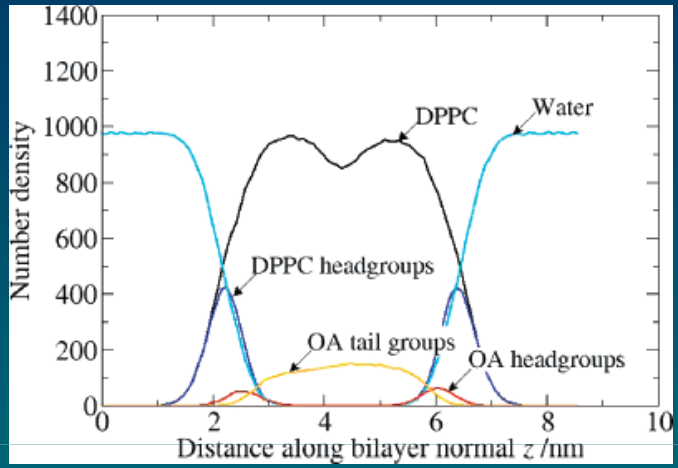
- Electron density profile
- Bilayer repeat L_z
- Volume per lipid V_L
- Area per lipid A_L
- Water penetration (depth)
- Conformation of molecules
- Order parameter for alkyl chain
- Diffusion constants
- Bending modulus
- Area compressibility modulus

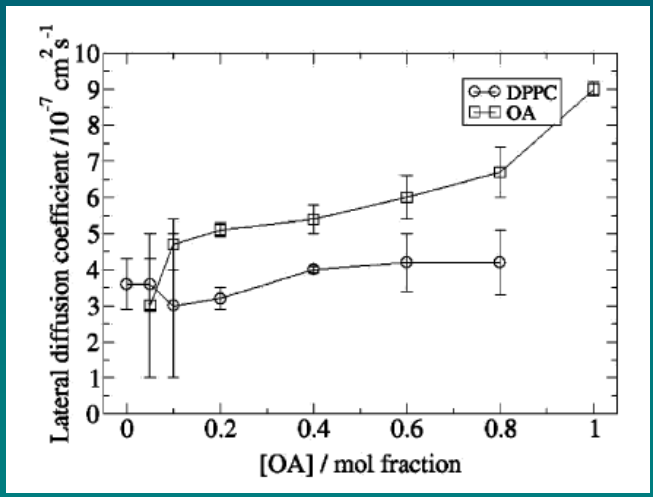
Interaction of oleic acid with DPPC bilayer

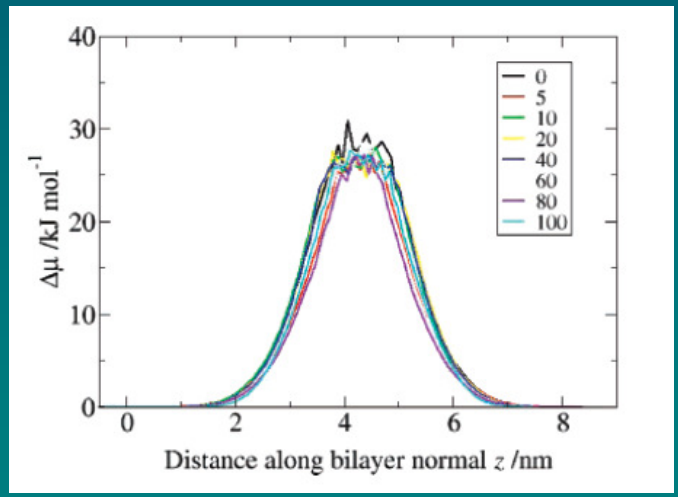


Oleic Acid



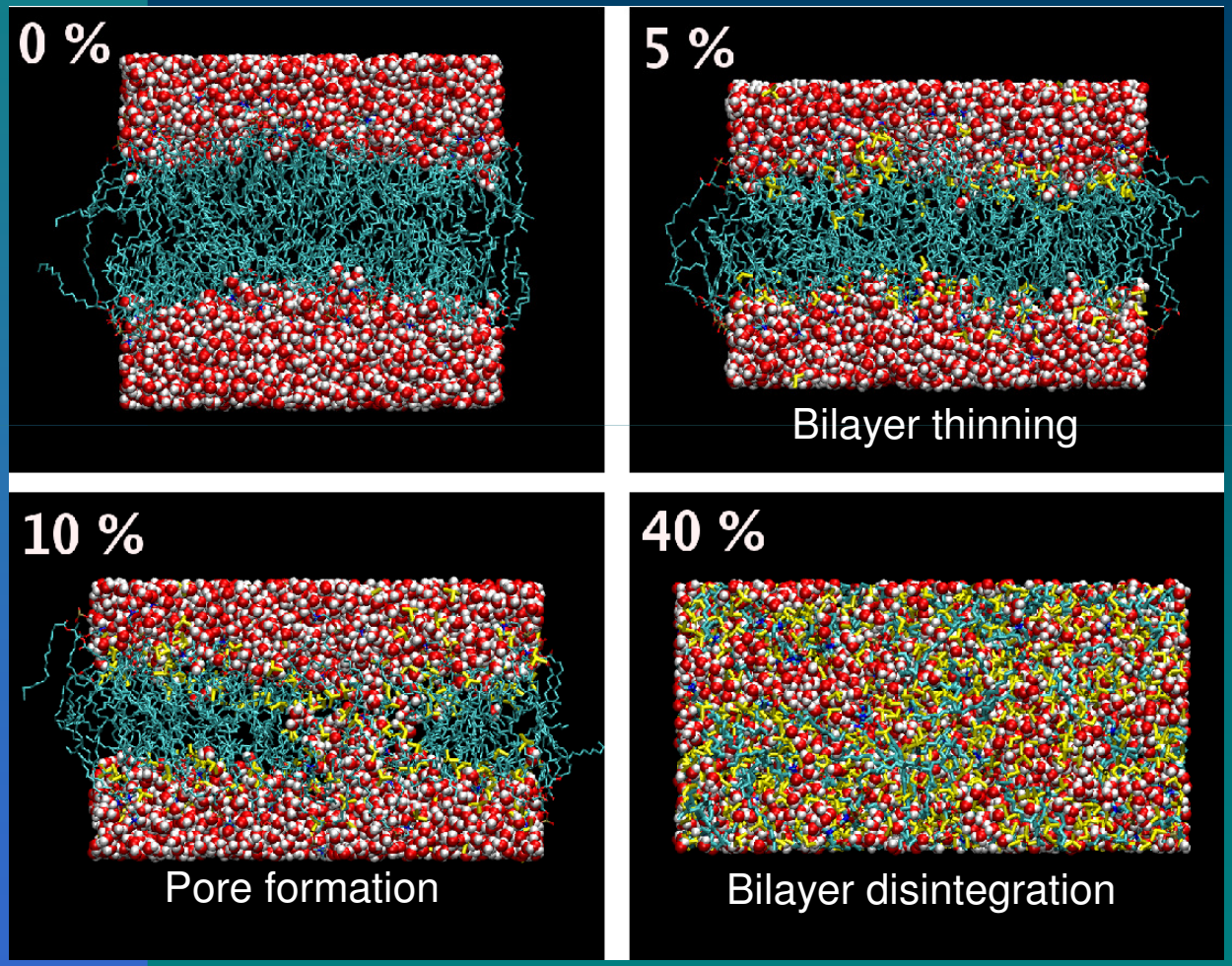






Oleic acid easily accommodated by the DPPC lipids;
No phase separation Notman, Noro & Anwar (2007) J Phys Chem B

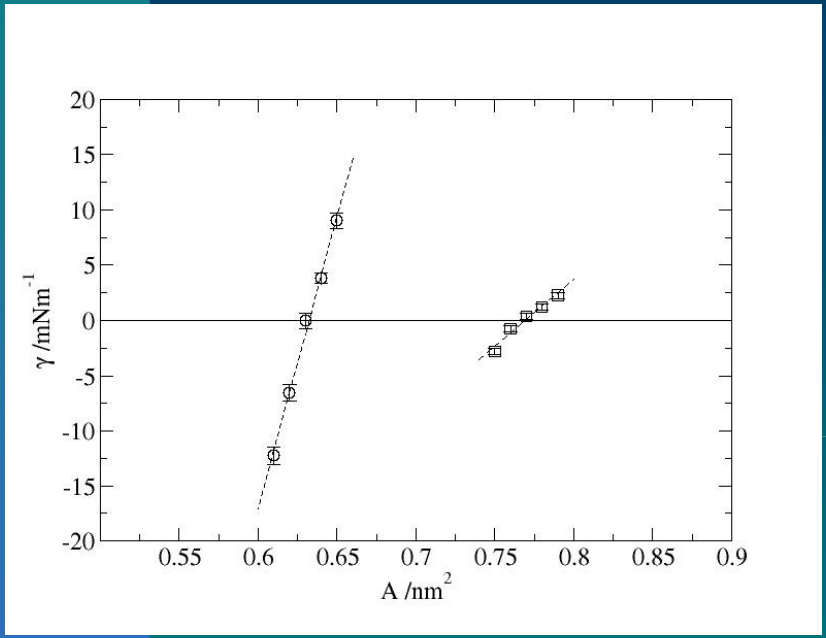
Interaction of DMSO with DPPC bilayers



350K

Notman et al. (2007) J Amer Chem Soc
Gurtovenko & Anwar(2007a) J Phys Chem B

DMSO makes DPPC membrane floppy



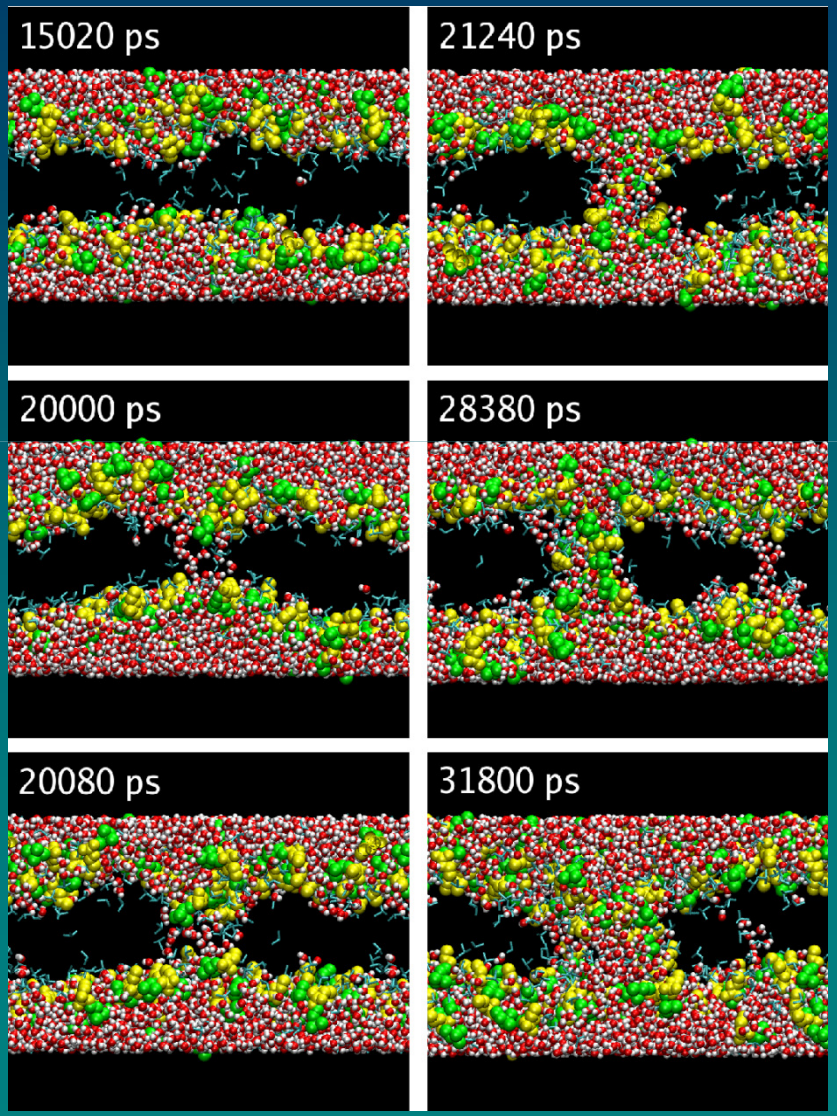
Surface tension v. area per lipid
Circles: without DMSO; Squares: 20% DMSO

Area compressibility modulus K_A
330±20 mN/m pure DDPC
90±10 mN/m 20% DMSO

Bending modulus κ
5.6×10^{-21} J pure DPPC
1.1×10^{-21} J for 20% DMSO

Notman et al. (2007) J Amer Chem Soc

DMSO-induced water pore formation

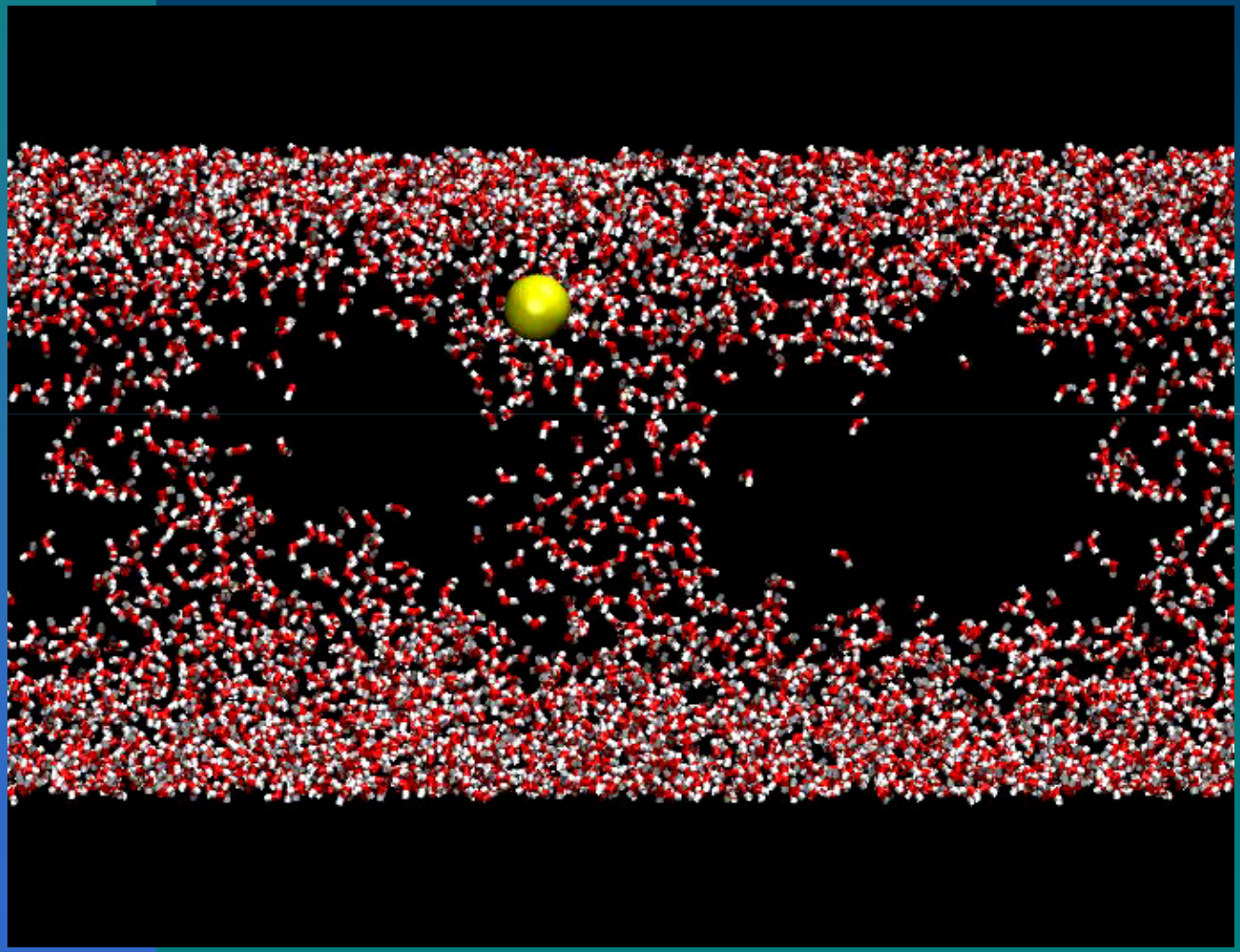


Pore formation timescale
Dependent on [DMSO]
Typically > 5 -10 ns

Pores stable for
up to 80 ns

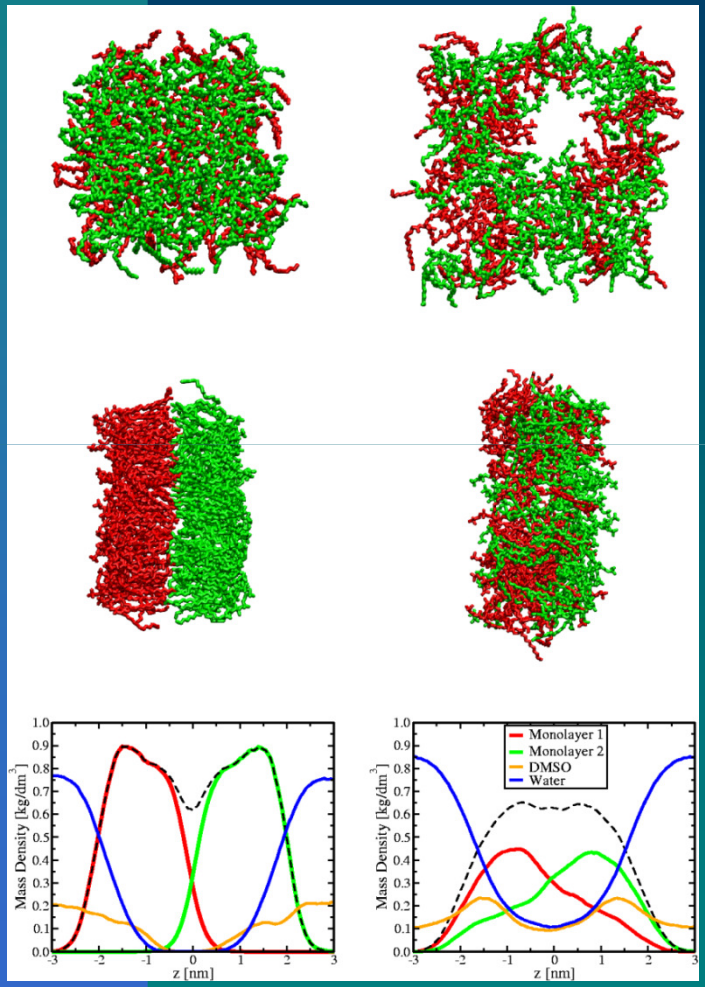
Explains for the first time
permeability enhancement
of hydrophillic molecules

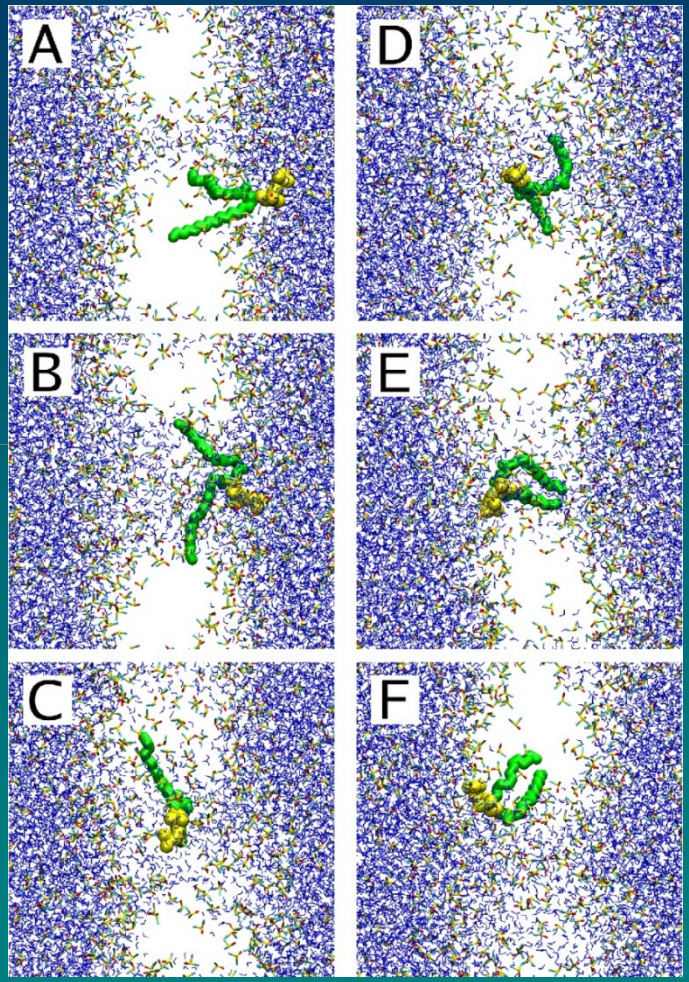
Permeability enhancement in action: Ion leakage across DMSO-induced water pores



Gurtovenko & Anwar (2007b), J Phys Chem B

DMSO induces translocation (flip-flops) of lipids





Gurtovenko & Anwar (2008) Langmuir

Rationalization of DMSO's pharmacological effects

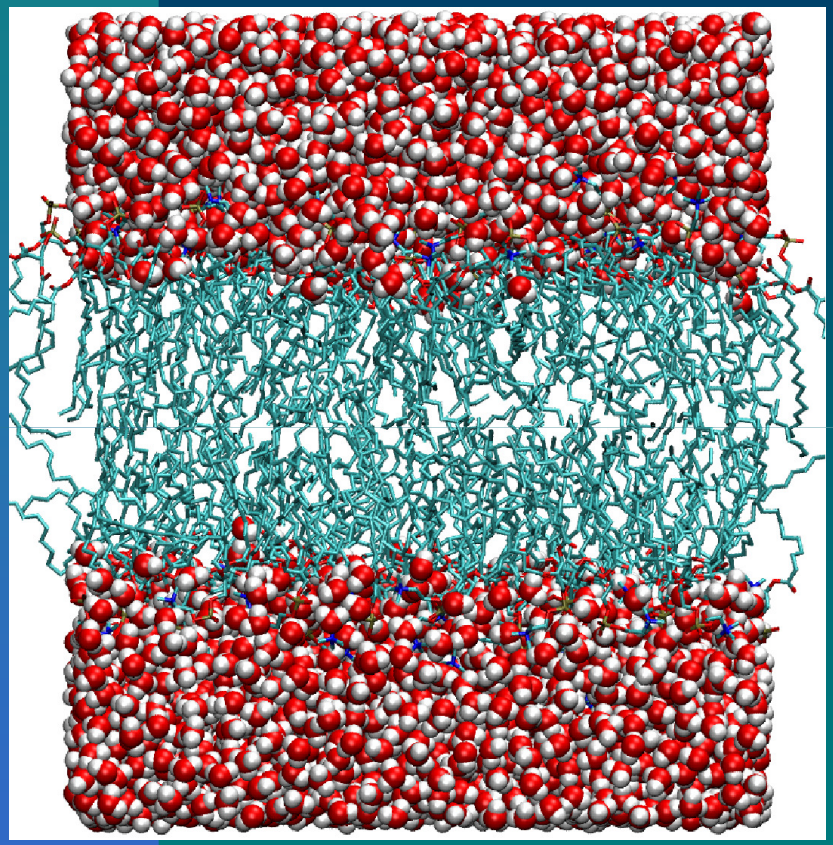
Insights from simulations

- Makes membrane more floppy
- Induces water pores; enhances permability of ions and water
- Destroys bilayer structure at high [DMSO]
- Induces flip-flops (induces programmed cell death?)

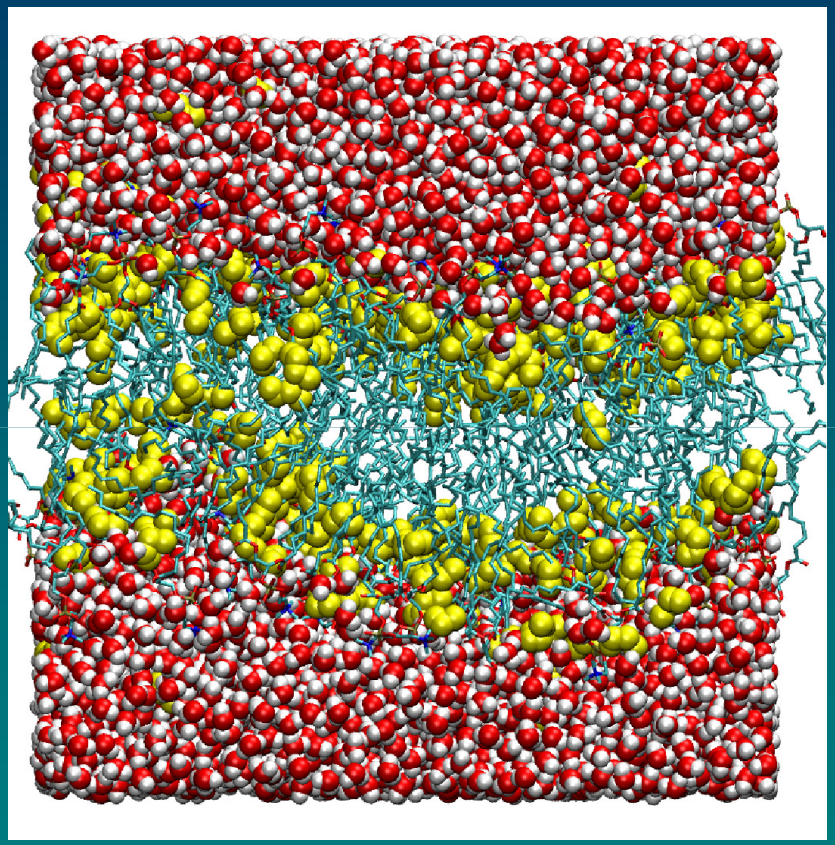
Applications of DMSO

- Permeability enhancement
- Cryoprotection
  Floppier membrane enable better accommodation of mechanical stress during ice formation & melting; Water pores control osmotic imbalance
- Local analgesic
  Disrupts action potential by allowing ion leakage in nerves
- Cell fusagen in cell biology
  Reduction in compressibility modulus and bending rigidity + pore formation

Effect of ethanol on lipid bilayers: Low concentrations (up to 10 mol %)

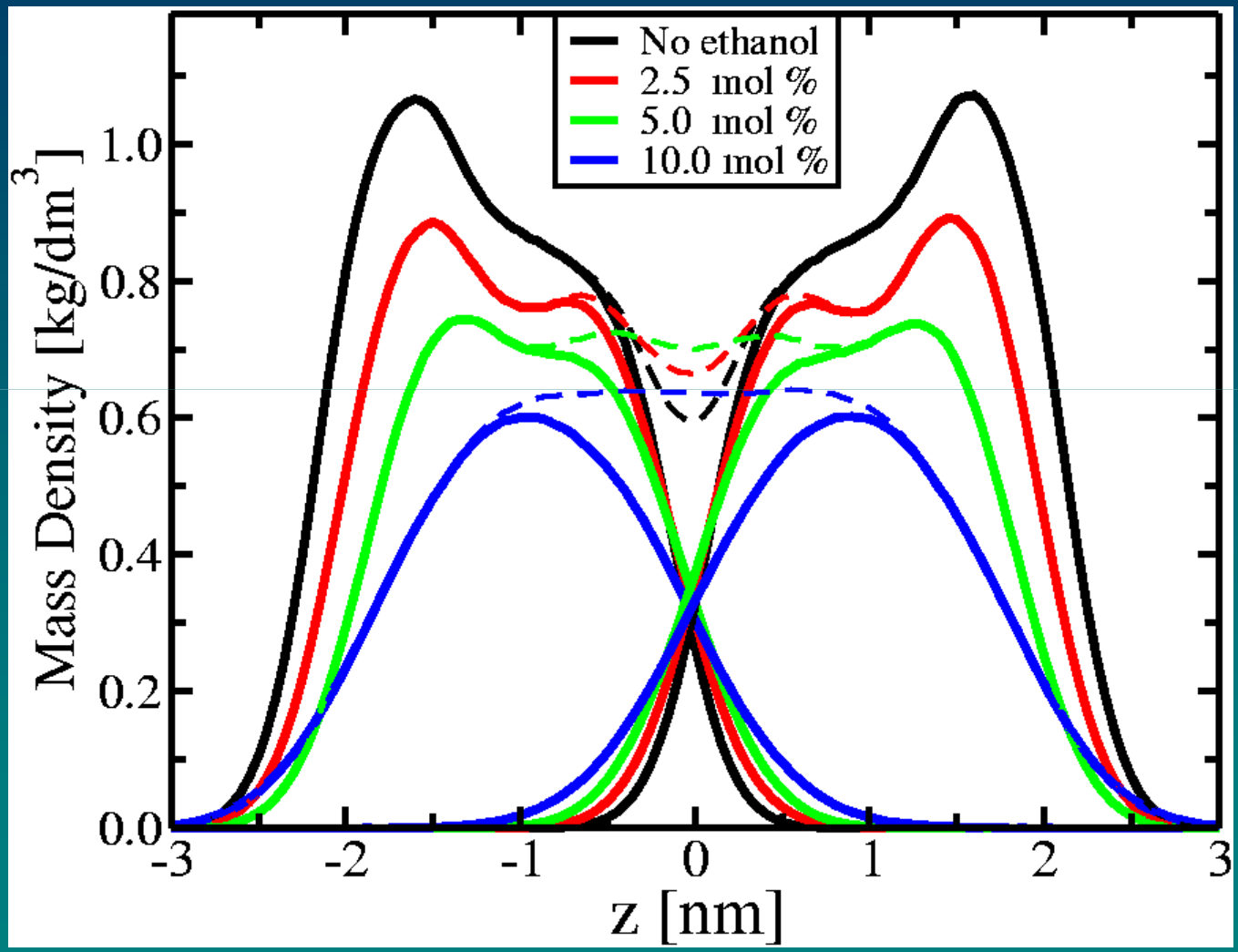


No ethanol

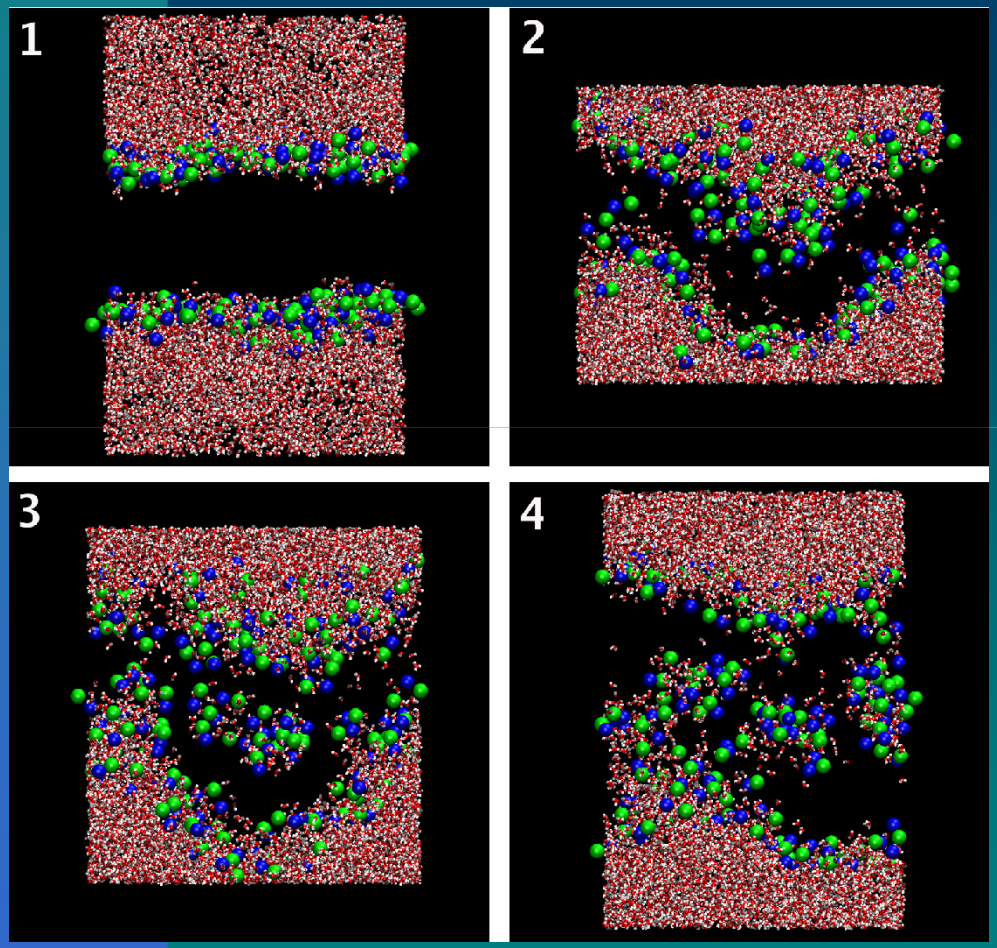


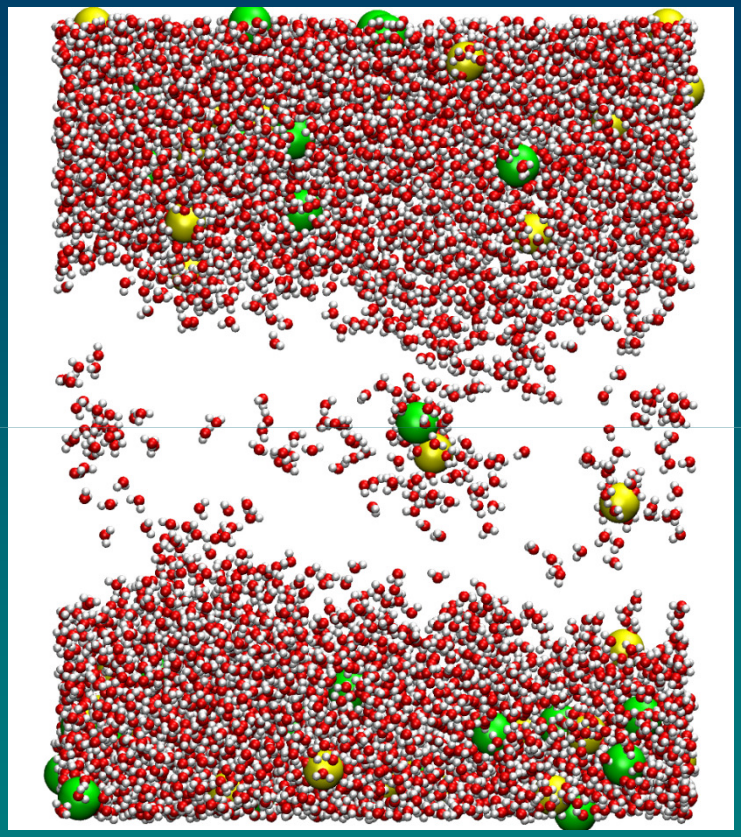
5 mol % ethanol

Effect of ethanol on lipid bilayers:
Interdigitation of lipid hydrocarbon chains



Effect of ethanol on lipid bilayers: High concentrations (~15 mol%)

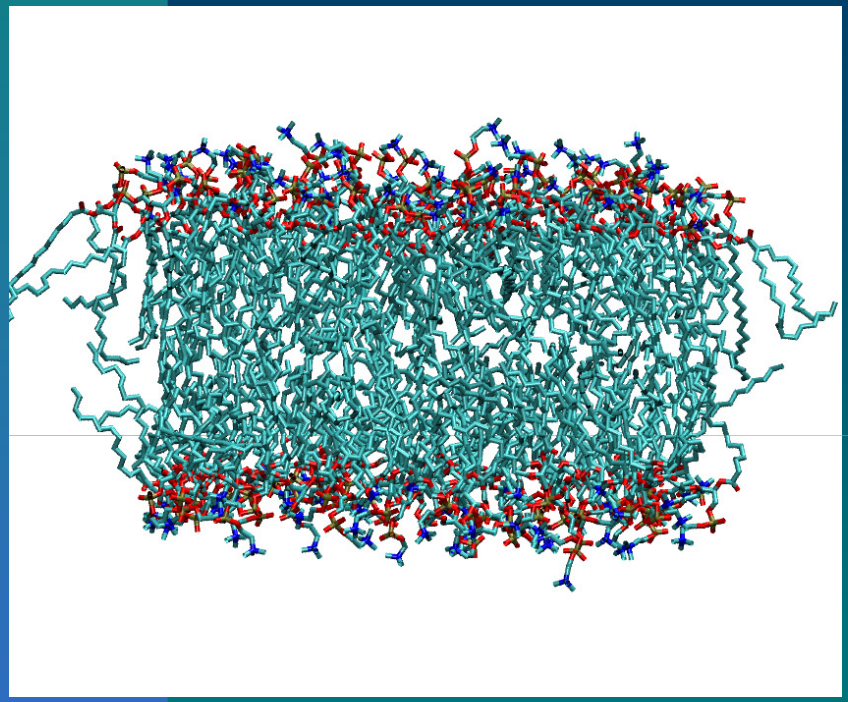




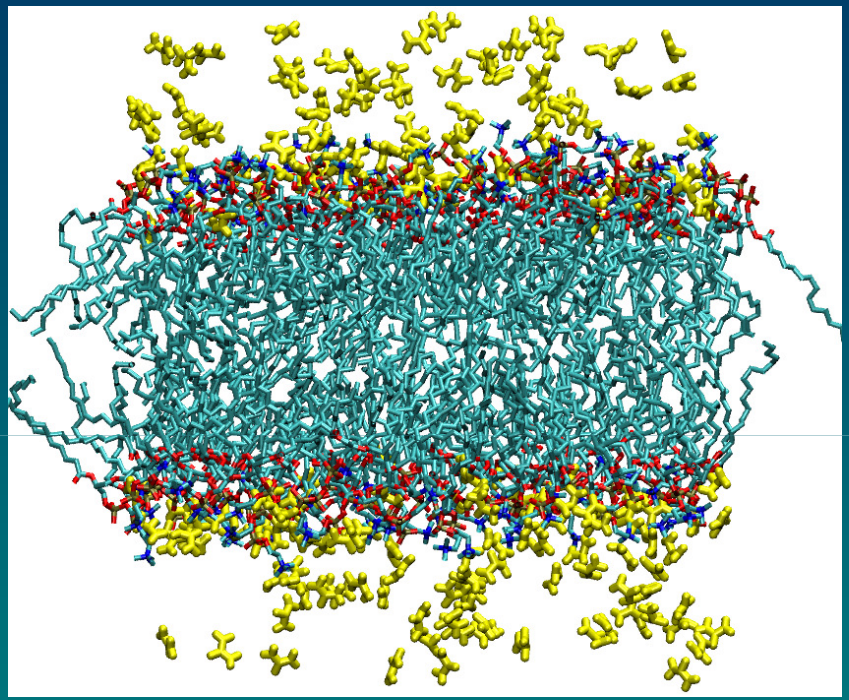
Micelle-like structures in the bilayer interior

Gurtovenko & Anwar (2009) J. Phys. Chem. B

Effect of urea on lipid bilayers

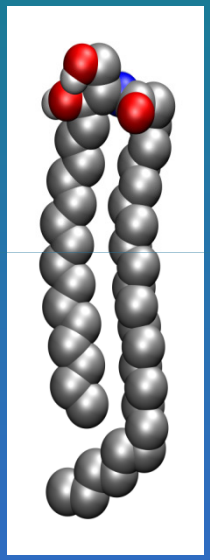


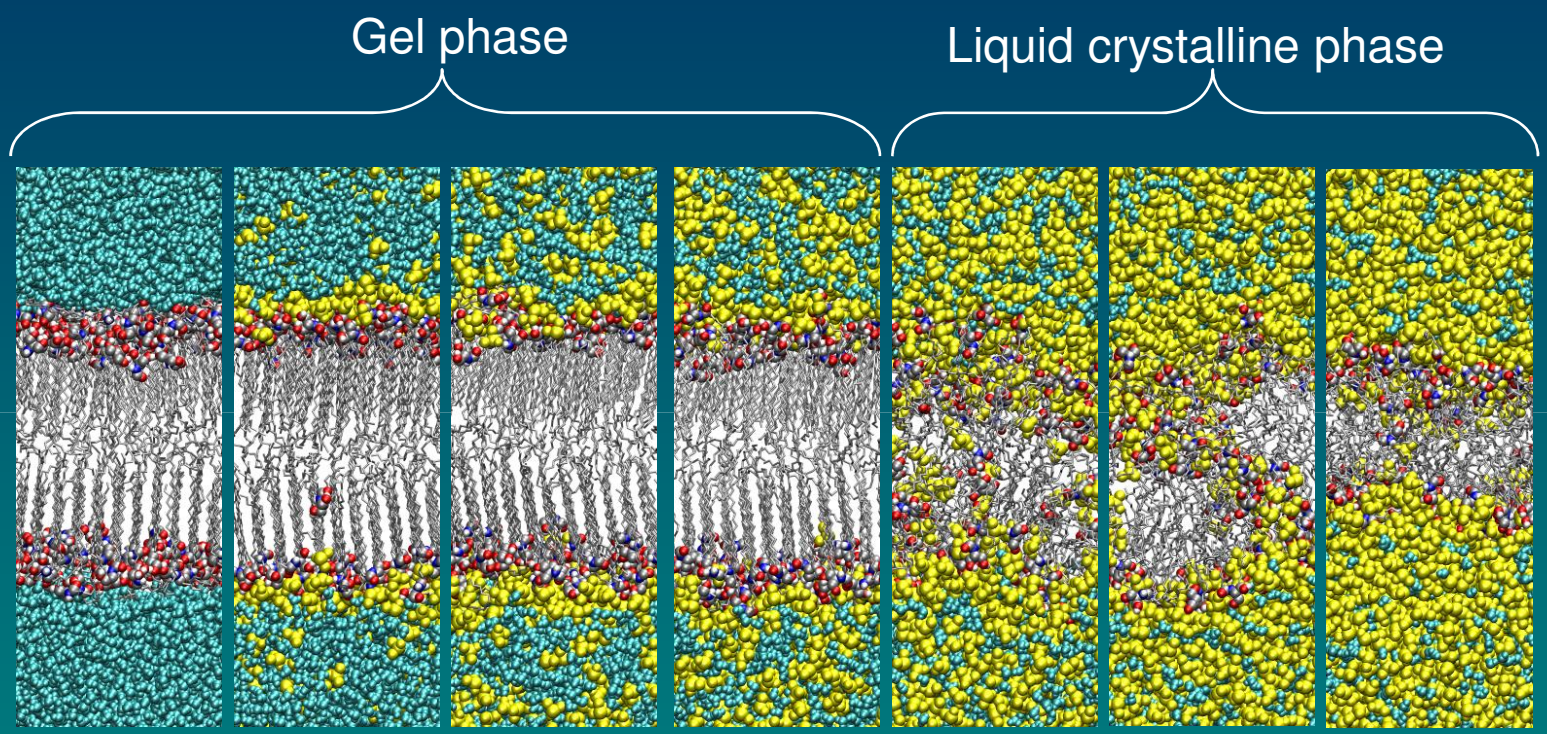
No urea

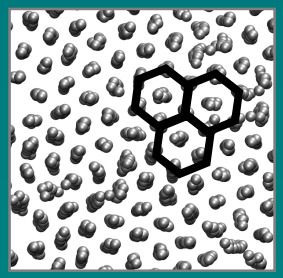


2M of urea

Structure of ceramide bilayer as a function of DMSO concentration in the solvent



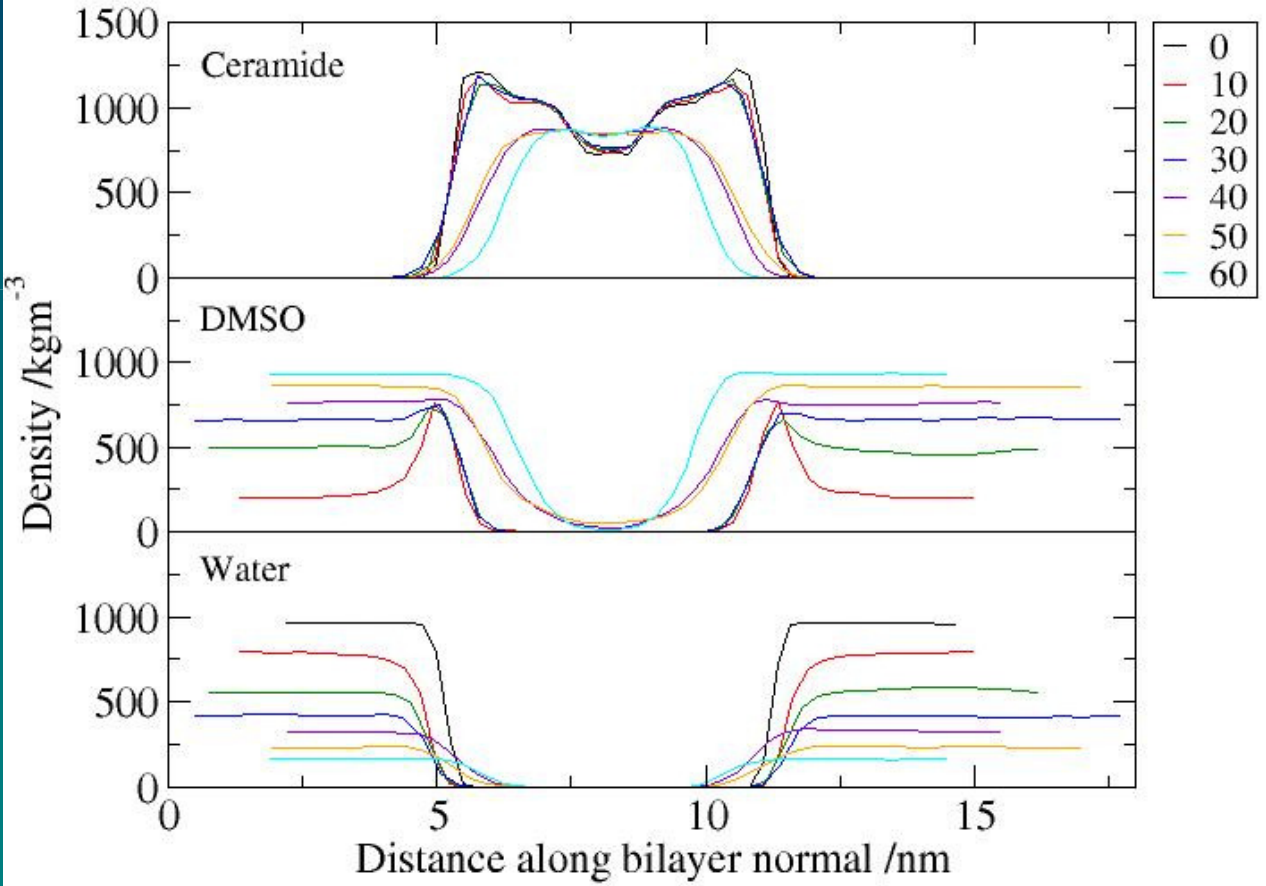




323K

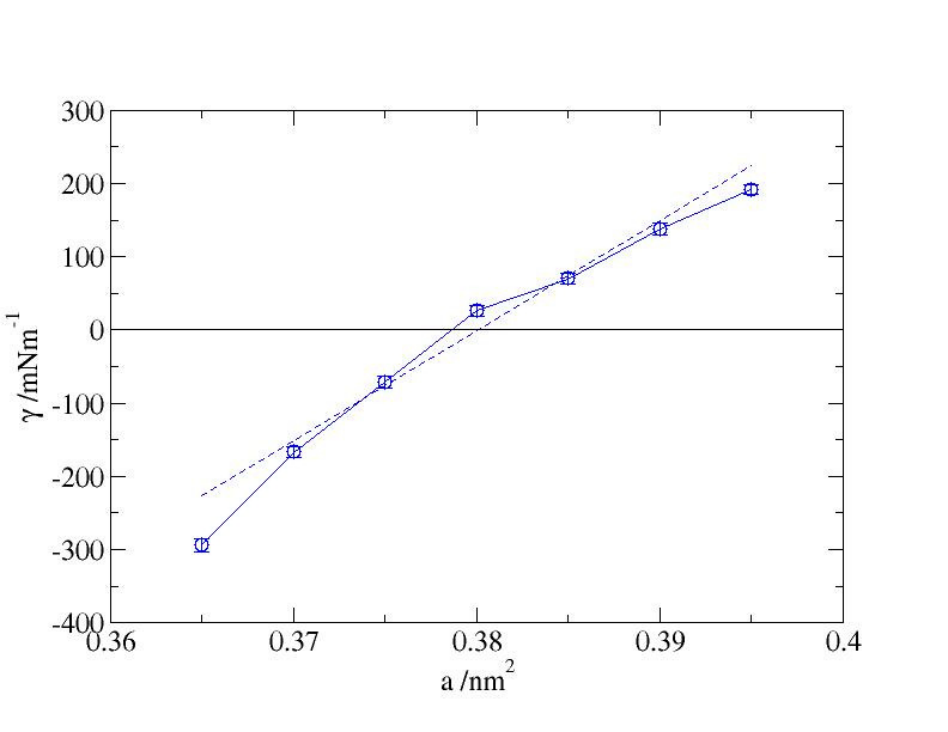
Notman et al (2007) Biophys. J

Ceramide bilayers containing DMSO



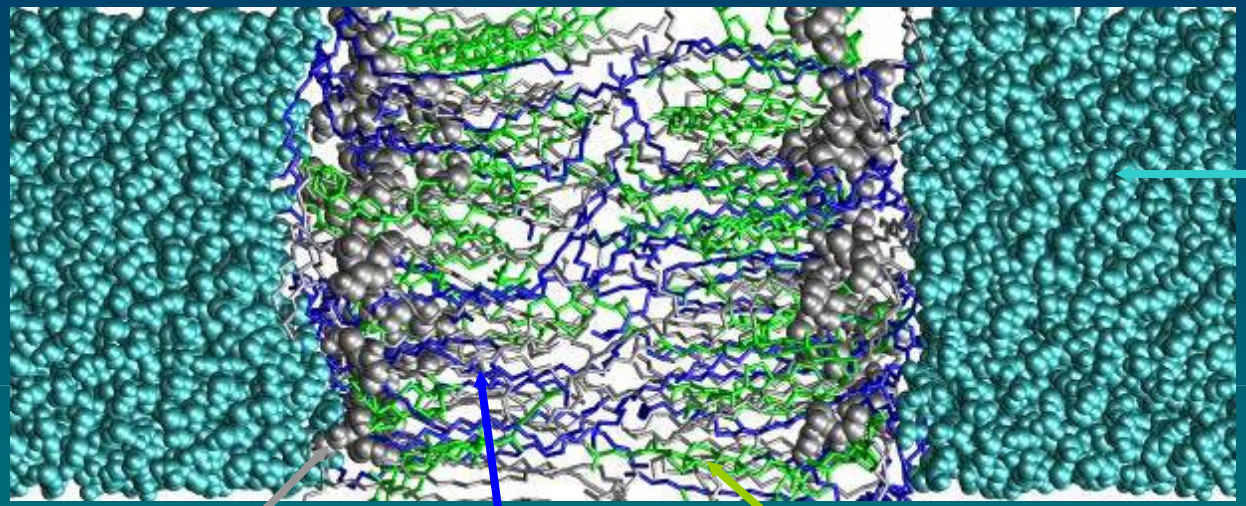
Notman et al (2007) Biophys. J

Ceramide bilayers containing DMSO



$$K_A = \begin{cases} 7900\,\mathrm{mJ/m^2} & 0\% \\ 3700\,\mathrm{mJ/m^2} & @\ 10\% \\ 190\,\mathrm{mJ/m^2} & 60\% \end{cases}$$

Ceramide/fatty acid/cholesterol bilayer (1:1:1)



Free energy of pore formation

Pores

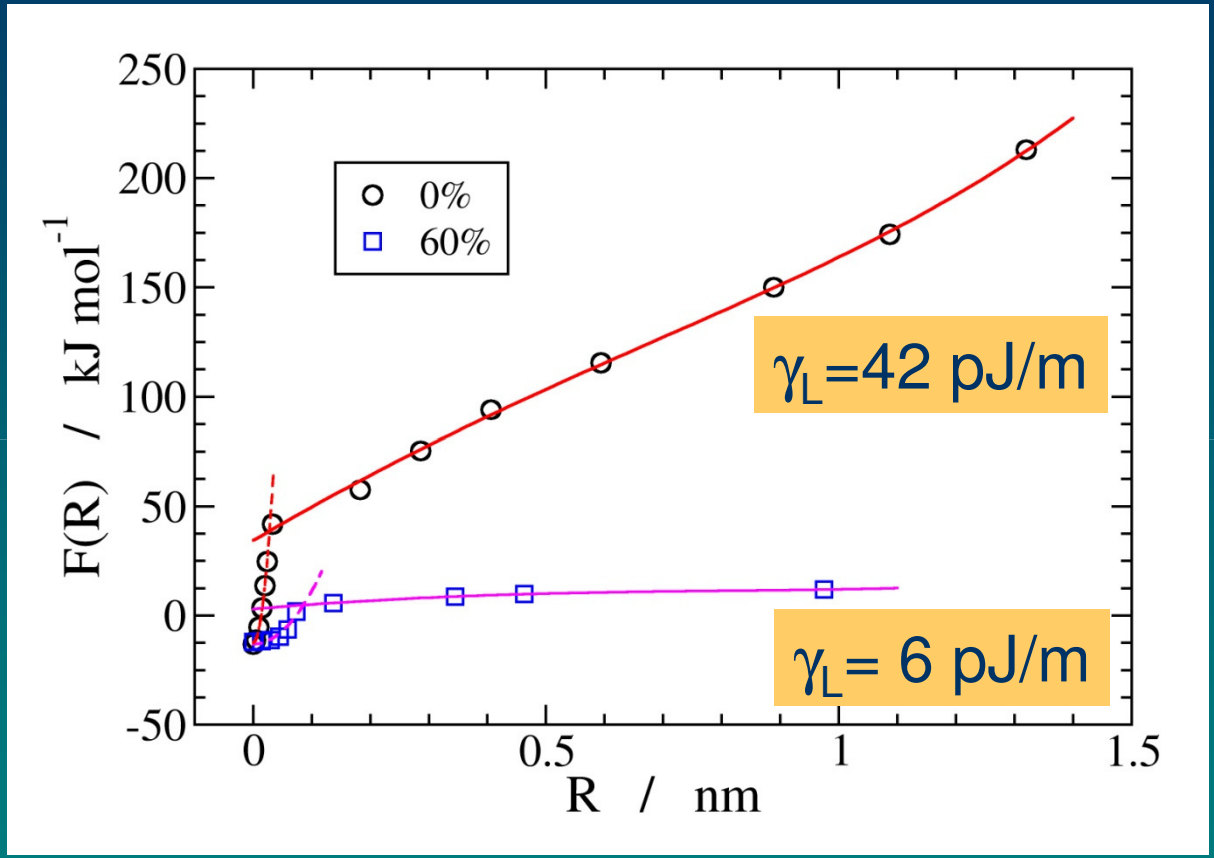
Cell death; Genes/drug delivery (eg electroporation);
Lipid asymmetry, Flip-flops & signalling

$$\Delta F = 2\pi r\,\gamma_L - \pi r^2 \gamma_S$$ 2-d nucleation

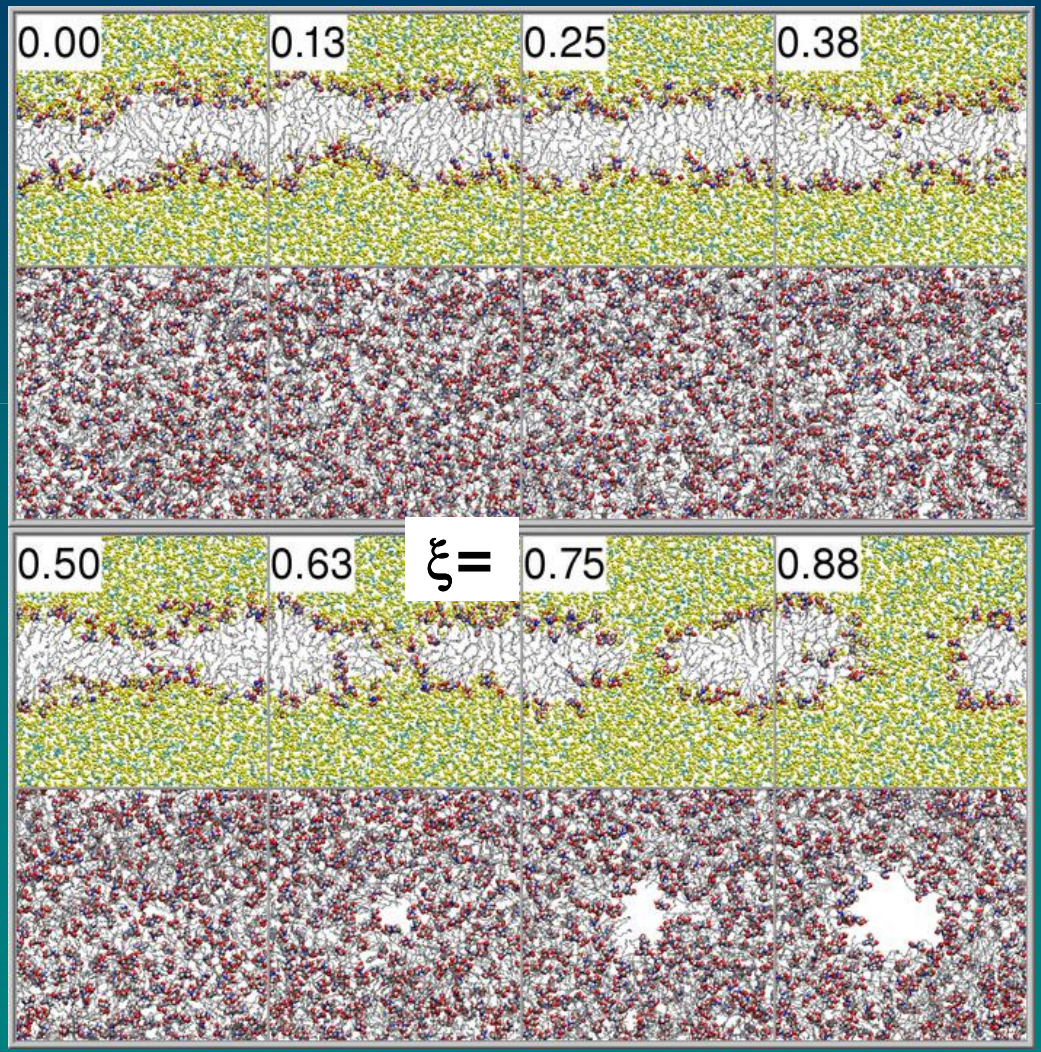
Where γ_S is the surface tension (surface energy)
γ_L is the line tension (edge energy)

Assumes surface lost due to lipids going into bilayer!

Free energy of pore formation in ceramide bilayers



Induced pore in ceramide bilayer containing 60 mol% DMSO



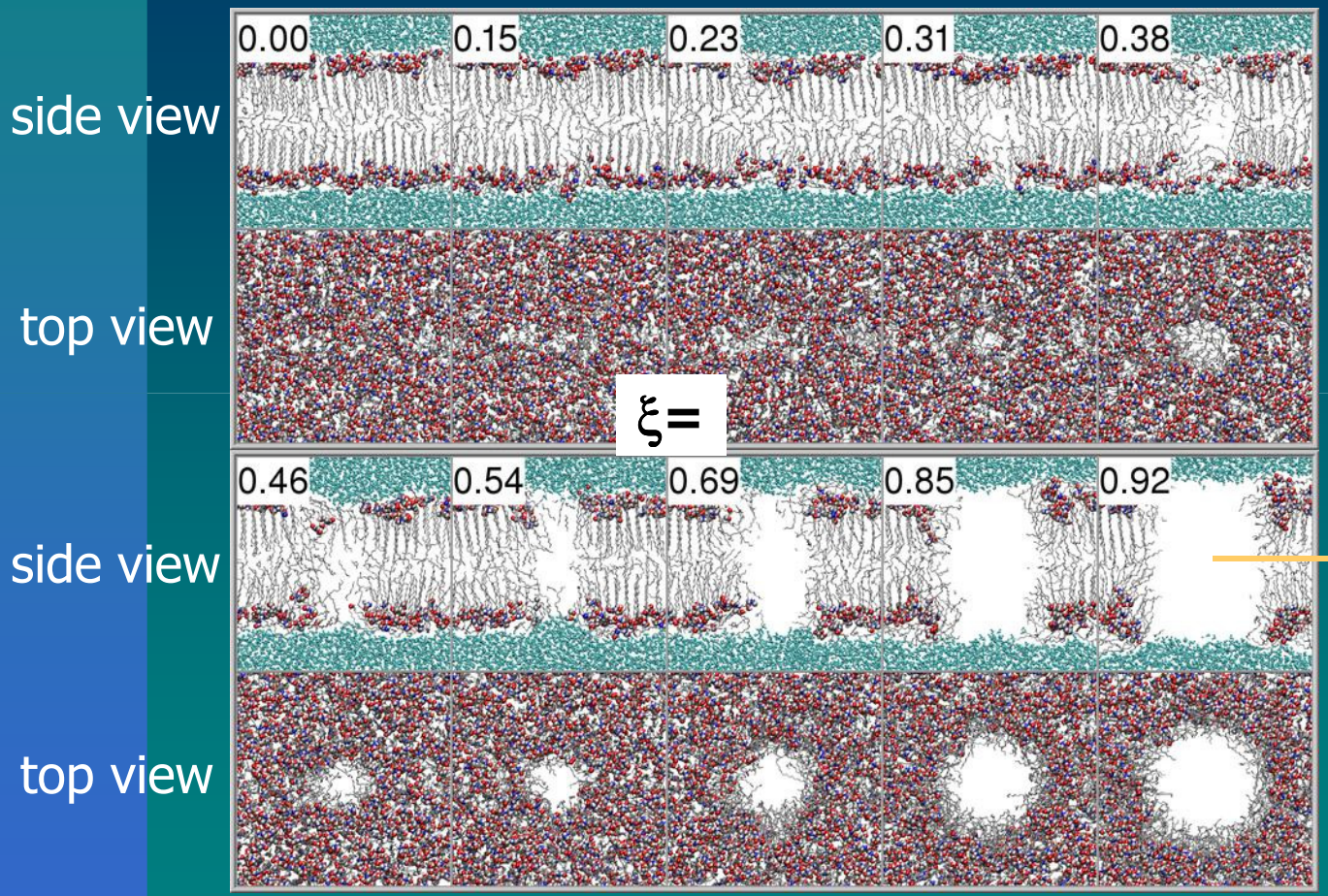
side view

top view

side view

top view

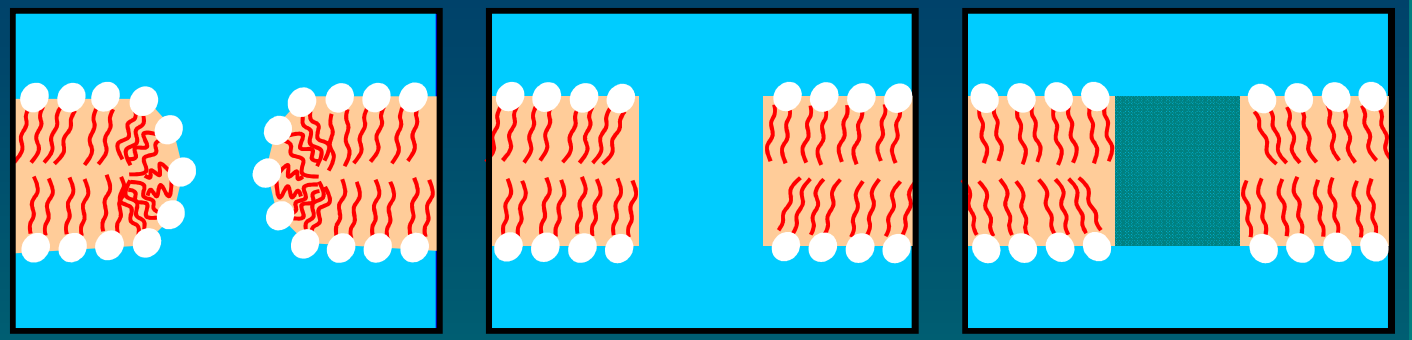
Induced pore in ceramide bilayers



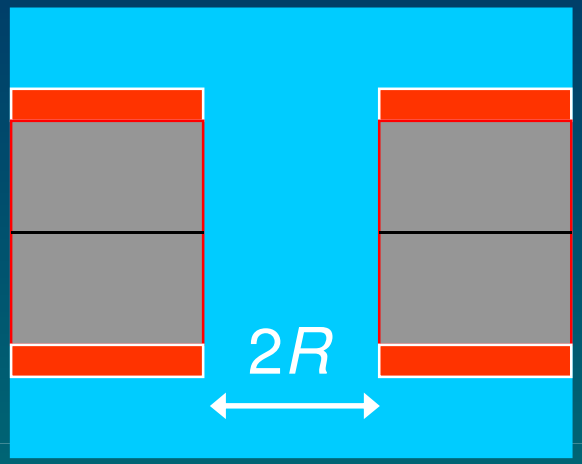
"hydrophobic pore"
but ....
where is the water?

Notman et al (2008) Biophys. J

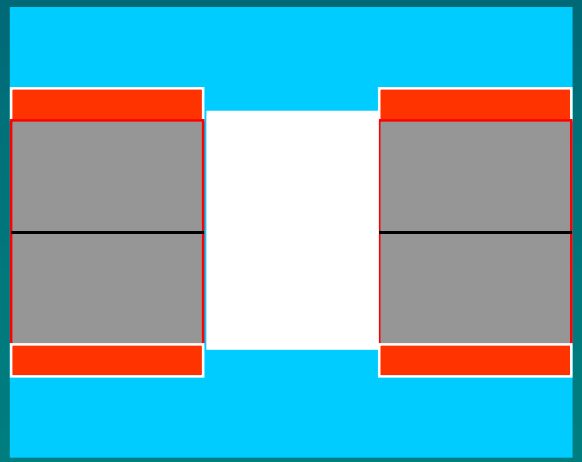
Schematic structures of pores



Should hydrophobic pores contain water?



$$F_{\text{edge}}(R) = 2\pi R h \gamma_{l/w}$$



$$F_{\text{edge}}(R) = 2\pi R h \gamma_{l/v} + 2\pi R^2 \gamma_{w/v}$$

$$\rightarrow R_{eq} = \frac{\gamma_{l/w} - \gamma_{l/v}}{\gamma_{w/v}} h \approx 2\text{nm}$$

Notman et al (2008) Biophys. J

Concluding remarks

DPPC bilayers

- Oleic acid: accommodated within bilayer without much perturbation
- DMSO induces water pores and makes membrane floppy; induces flip-flops
  → rationalization of DMSO's pharmacological activity
- Ethanol: high concentrations cause internalisation of H2O and ions into the centre of bilayer forming micelle-like structures that may serve as delivery packages
- Urea: replaces H2O at the interface; significance??

Ceramide bilayers

- DMSO induces gel→ fluid phase transition; Explains DMSO's role as a penetration enhancer in transdermal drug delivery; Significance of vapour pores in skin.

Future

- Impedance measurements & AFM; transport through bilayers
- In-silico screening of molecules that enhance &/or retard permeability