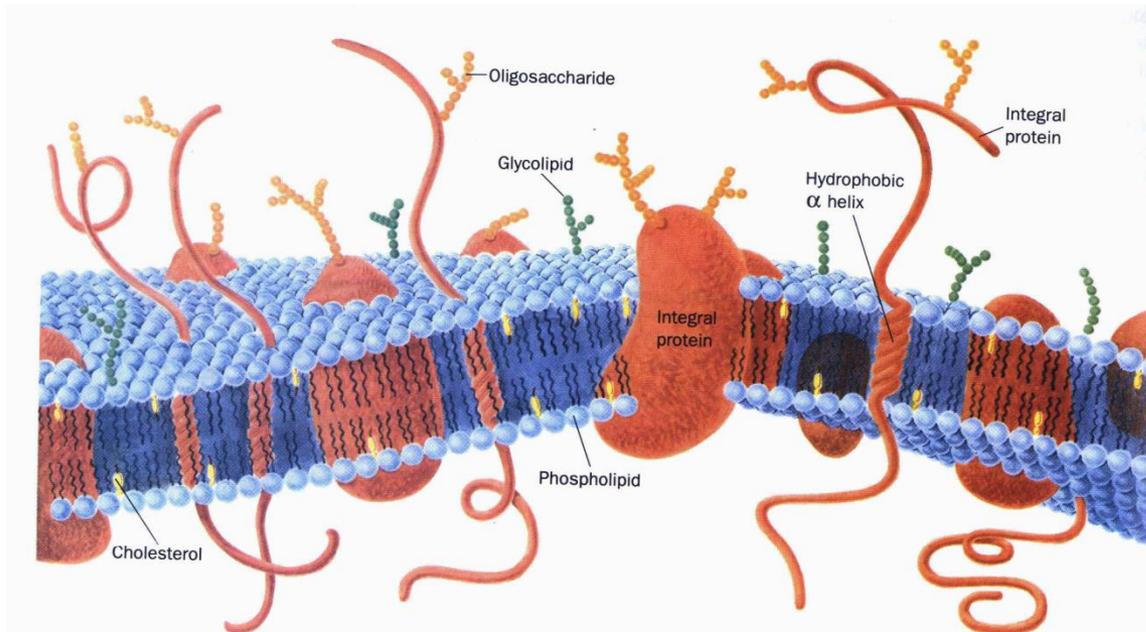


# Introduction to lipid biomembranes



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Ben Gurion University

# OUTLINE

1. Basic concepts
2. Elasticity of membranes
3. Computer simulations of membranes and more complex systems

# Chapter 1- Basic concepts

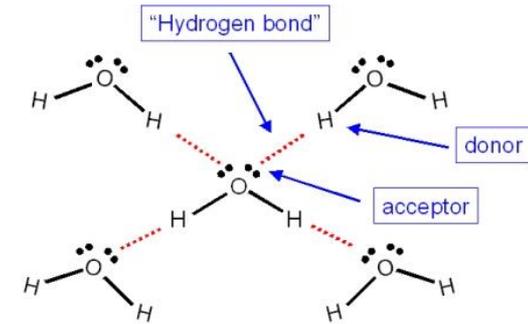
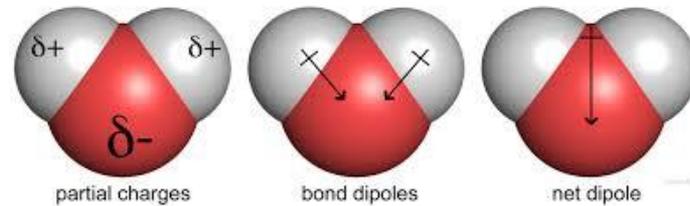
# 1.1 The concepts of hydrophobicity and hydrophilicity

Everything in biology happens in water.



Water is a unique solvent because:

- Very polar molecule
- Form hydrogen bonding network



Unique properties of water

- High melting temperature
- Liquid water is denser than solid ice

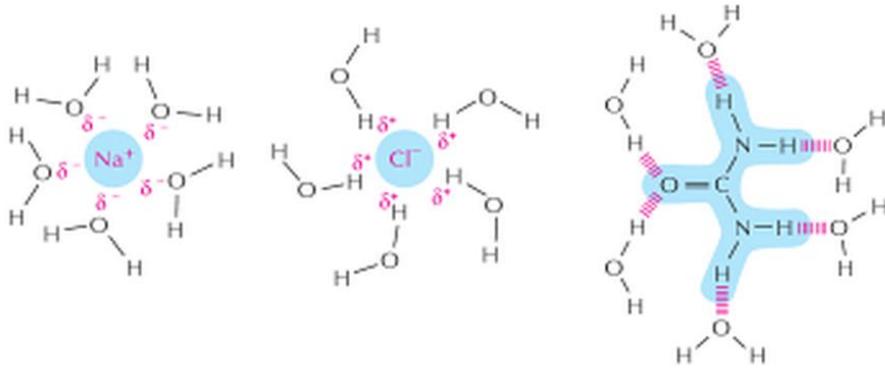


A molecule whose interaction with water is thermodynamically **more** favorable than water-water interactions are water-soluble or **hydrophilic**.

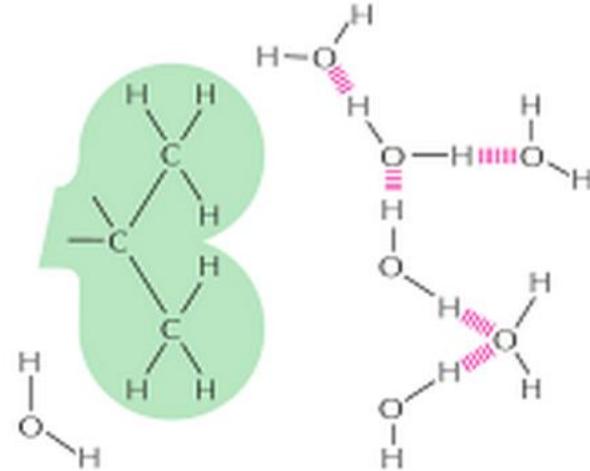
A molecule whose interaction with water is thermodynamically **less** favorable than water-water interactions are water-insoluble or **hydrophobic**.

# 1.2 The concepts of hydrophobicity and hydrophilicity

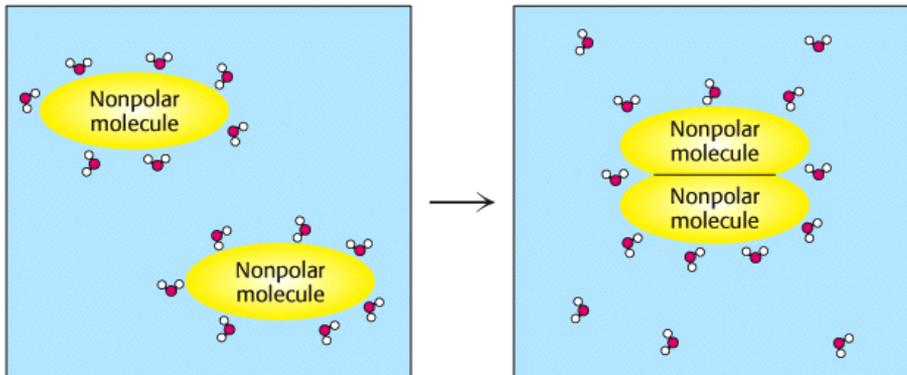
Ionic and polar molecules that are hydrophilic



Hydrocarbons are hydrophobic



The hydrophobic effect – hydrophobic molecules tend to aggregate in order to minimize their contact with water

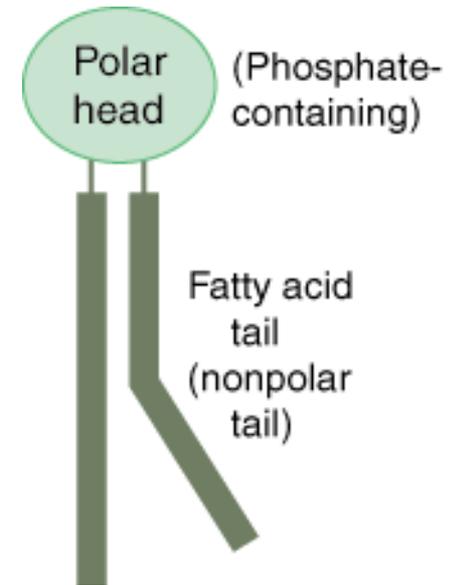
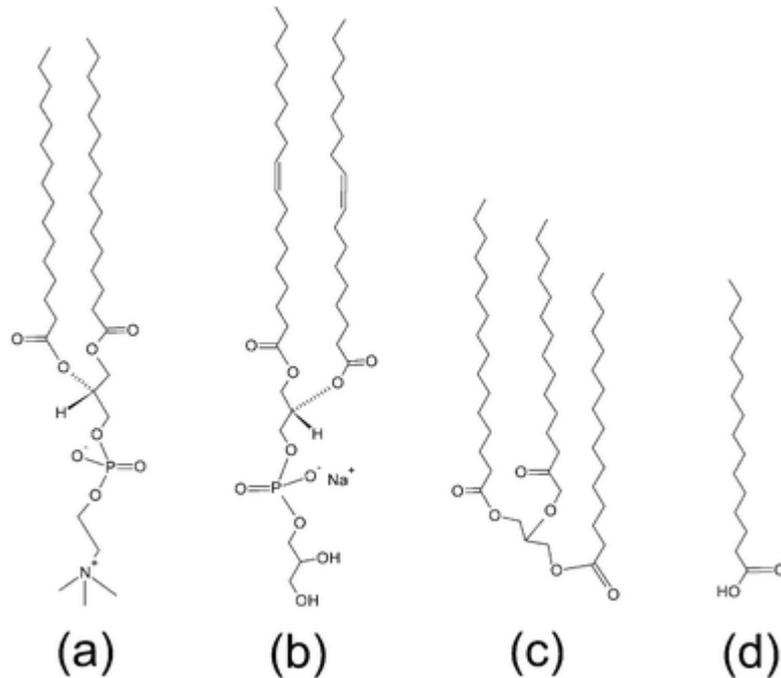


$$G = \gamma_{ow} A \quad ; \quad \gamma_{oA} \sim 0.05 \frac{N}{m}$$

# 1.3 Amphiphilic molecules

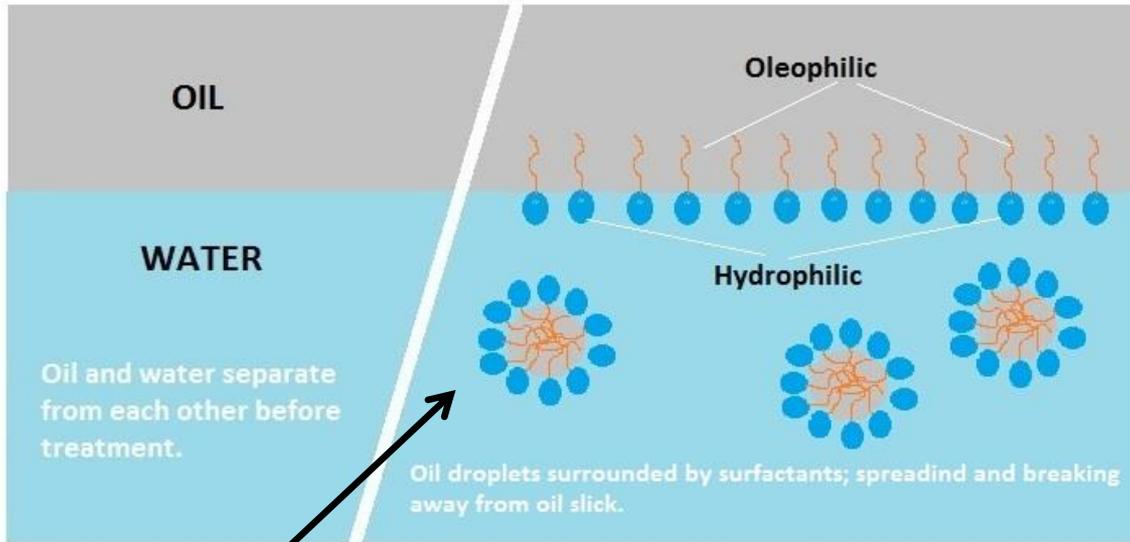
Amphiphiles are molecules with covalently-bonded hydrophilic and hydrophobic groups.

- The hydrophilic group (“head group”) is usually charged or polar.
- The hydrophobic part is usually a long hydrocarbon chain(s) (“tail”).



# 1.4 Surface active agents (surfactants)

Amphiphilic molecules accumulate at water-oil interfaces and lower the surface tension between them. This is how detergents work.



$$G = \gamma_{OW} A - \Pi A = \gamma_{\text{eff}} A$$

$\gamma_{OW}$  - oil-water surface tension

$\Pi$  - surfactants spreading pressure

$$\gamma_{\text{eff}} = (\gamma_{OW} - \Pi) \sim 0$$

(Schulman & Montagne, 1961)

Microemulsion - oil droplets dispersed in water (microphase separation)

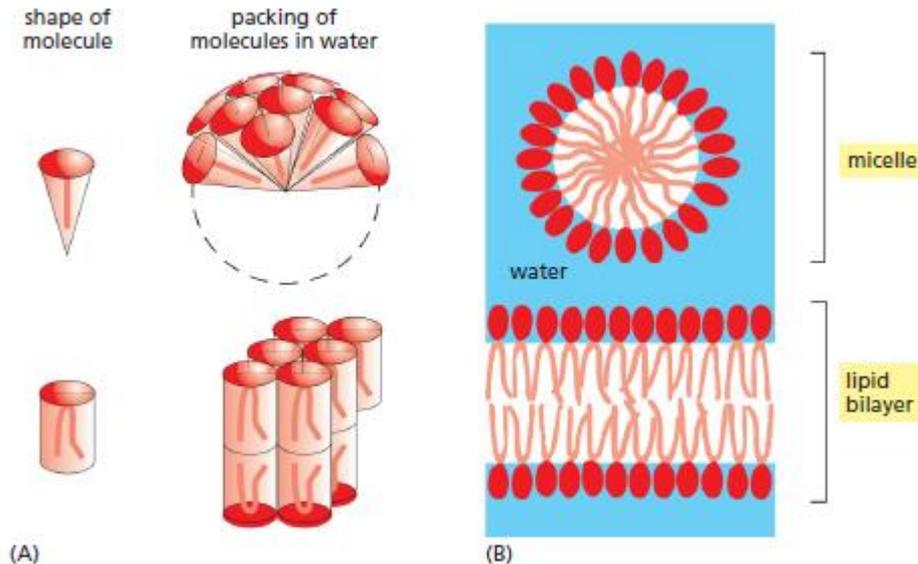
phase coexistence



# 1.5 Amphiphilic molecules in water

In aqueous solution, amphiphilic molecules form aggregates that protect their hydrophobic tails from contact with water. This is the hydrophobic effect in action.

- Molecules with a single tail, like detergents, tend to form micelles.
- Molecules with a double tail, like biological lipids, tend to form bilayers



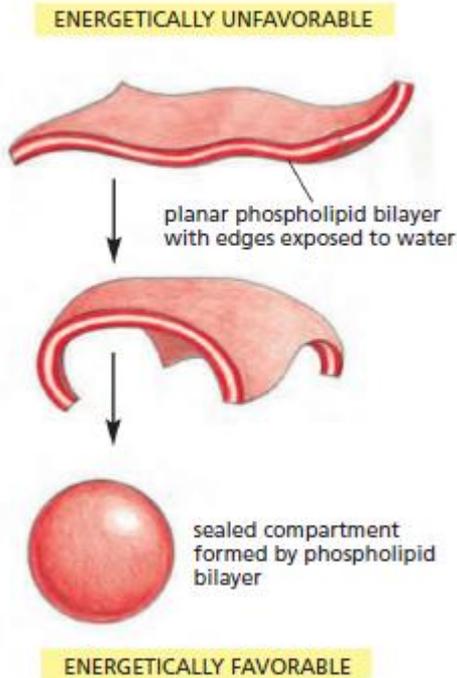
The shape of the aggregate is determined by packing considerations.

Packing parameter	Shape	Structures formed	
$< 1/3$ Cone		Micelle	
$1/3 - 1/2$ Truncated cone		Cylindrical micelle	
$1/2 - 1$ Truncated cone		Vesicle bilayer	
$\sim 1$ Cylinder		Planar bilayer	
$> 1$ Wedge		Inverted micelle	

$$\text{Packing Parameter} = \frac{v}{al}$$

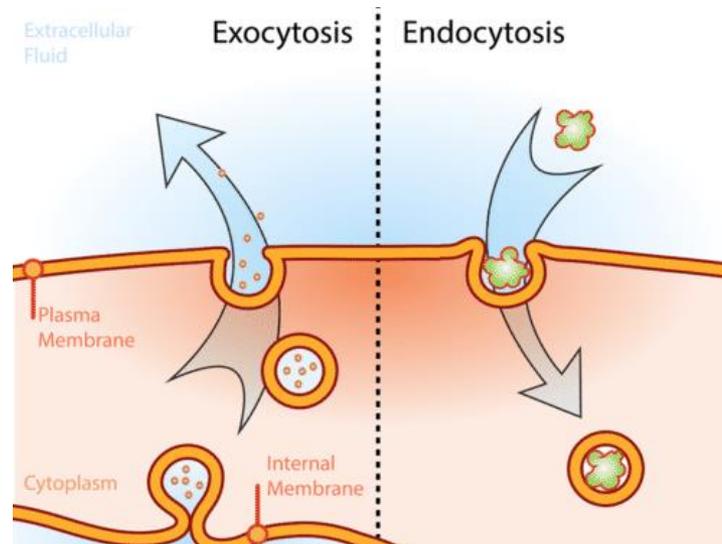
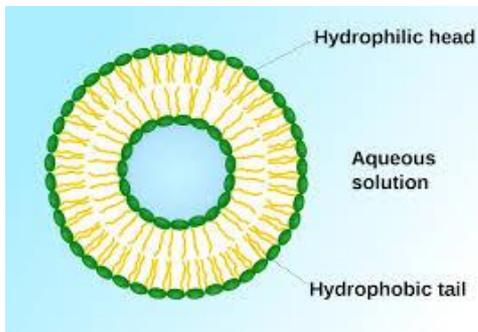
# 1.6 Amphiphilic molecules in water

Due to the hydrophobic effect, open bilayers close on themselves in order not to expose their edges to water. They form vesicles.



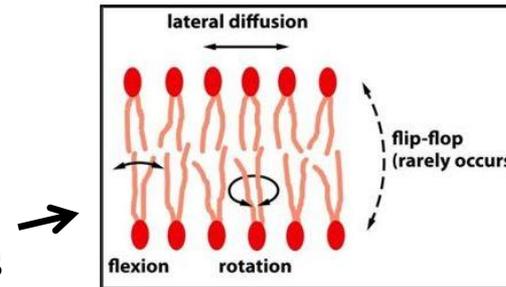
The self-sealing property of membranes is essential for the existence of biological cells. The membrane defines the boundary of the cell, and regulates the exchange of material with the surrounding.

Vesicles (“liposomes”) are formed in endo- and exo-cytosis processes



# 1.7 The lipid bilayer is a two-dimensional fluid

The lipids and the membrane proteins can diffuse and rearrange themselves. This is critical for proper biological function.

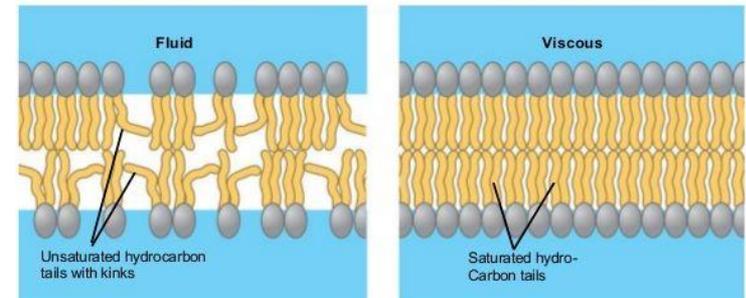
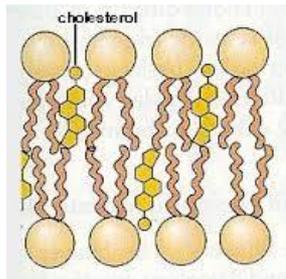


Diffusion and other types of lipid movements

The membrane is fluid because the “hydrophobic interactions” between the lipids are “not very strong” (physical interactions  $\sim k_B T$ ).

The membrane fluidity can be controlled by:

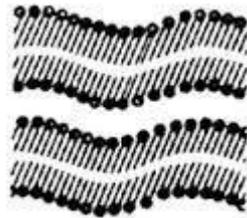
- Temperature (higher temperature – higher fluidity)
- Composition – biological membranes contain hundreds (!) types of lipids:
- Chain length (shorter chains - weaker hydrophobicity – higher fluidity)
- Unsaturated lipids are harder to pack and, therefore, tend to increase fluidity
- Cholesterol prevents “over fluidity” and “over rigidity”



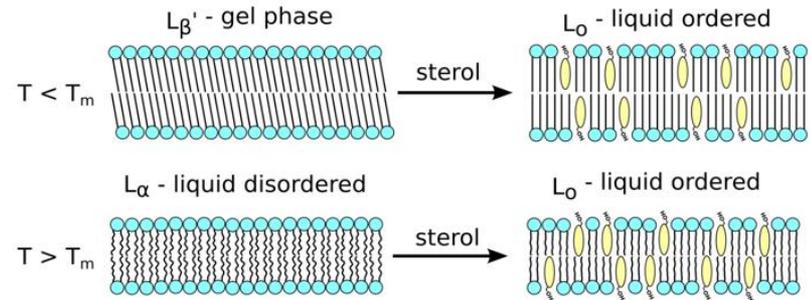
# 1.8 The thermodynamics of membranes is complex

Changes in temperature and composition (especially amount of cholesterol) can induce phase transitions:

- Fluid (fast diffusion, disordered chains)
- Liquid-ordered (fast diffusion, ordered chains)
- Gel (low mobility, ordered chains, hexagonal order)



- Ripple phase

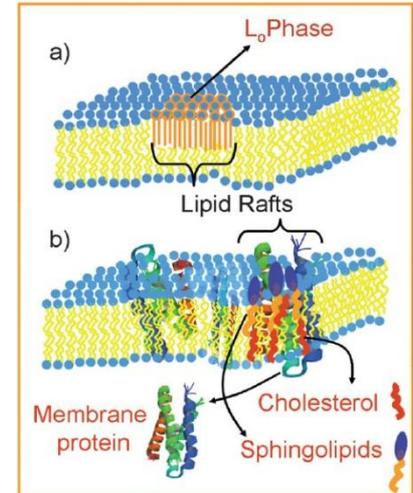


Membranes are heterogeneous. They feature domains with different lipid-sterol-protein compositions that are typically more ordered and tightly packed than the fluid membrane.

**Membrane rafts** are small (10-200 nm), heterogeneous, highly dynamic, sterol- and sphingolipid enriched domains that compartmentalize cellular processes. Small rafts can sometimes be stabilized to form larger platforms through protein-protein and protein-lipid interactions.

The lipid compositions at both leaflets are different.

Biological membranes are NOT at thermodynamic equilibrium. They are living systems.

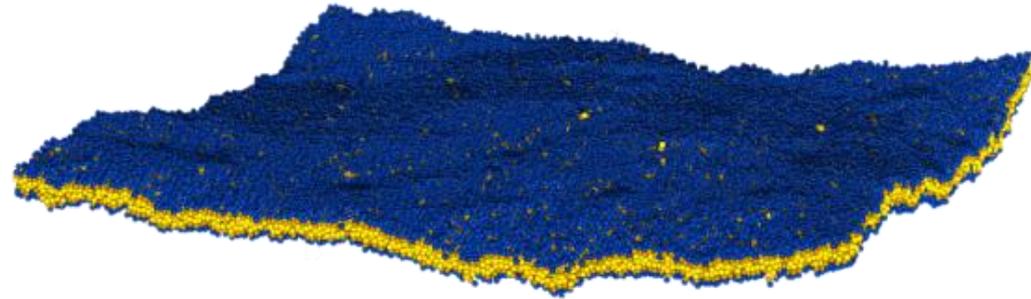


# Chapter 2- Elasticity of Membranes

## 2.1 Membranes as elastic sheets

Membranes are thin and long:

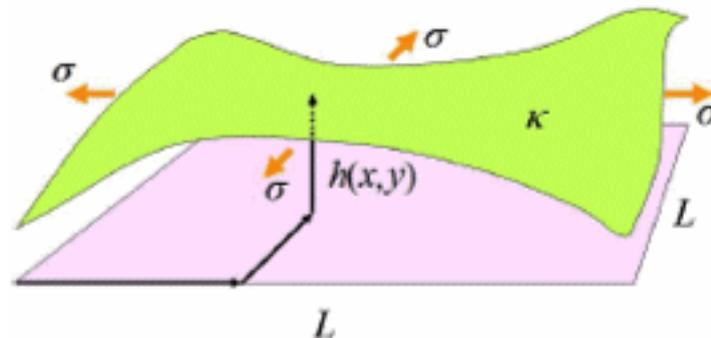
- Thickness  $\sim 5$  nm
- Lateral size  $\gtrsim 10$   $\mu\text{m}$



Because they are fluid and thin, they have unique elastic properties:

- Resist in-plane compression/stretching.
  - Bulk Modulus  $\gtrsim 0.1 \frac{\text{Joule}}{\text{m}^2}$  ; rupture strain  $\lesssim 0.05$  ; area per lipid  $\sim 0.5 - 0.7$   $(\text{nm})^2$
- Bend easily (out-of-plane deformations).

What is the elastic energy of a curved surface (continuum model)?



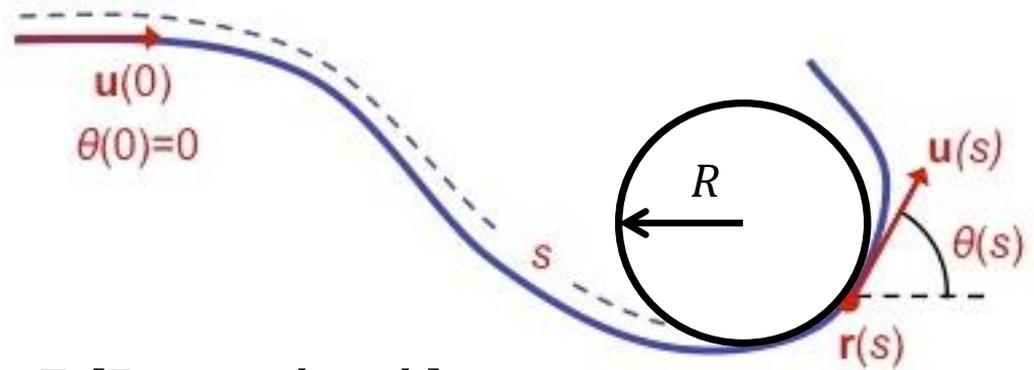
## 2.2 Curvature energy

A little bit of polymer physics – The Worm-Like-Chain model

Local curvature

$$c = \frac{d\theta}{ds} = \left| \frac{d\mathbf{u}}{ds} \right| \quad c = \frac{1}{R}$$

$$E_{\text{bend}} = \int_0^L \frac{1}{2} \kappa c^2(s) ds \quad \kappa = \xi \cdot k_B T \text{ [Energy * length]}$$



A surface has two local principal curvatures

Sphere



$$c_1 = c_2 = 1/R$$

$$J = 2/R$$

$$K = 1/R^2$$

Cylinder

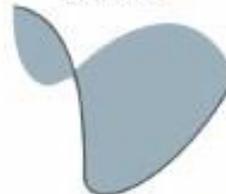


$$c_1 = 1/R \quad c_2 = 0$$

$$J = 1/R$$

$$K = 0$$

Saddle

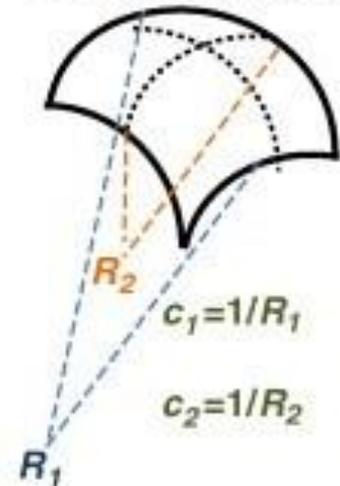


$$c_1 = -c_2 = 1/R$$

$$J = 0$$

$$K = -1/R^2$$

Surface element



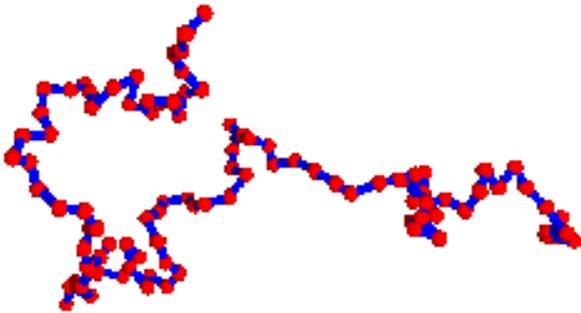
## 2.3 The Helfrich Hamiltonian

$$E_{\text{bend}} = \int \left[ \frac{1}{2} \kappa (c_1 + c_2 - 2c_0)^2 + \kappa_G c_1 c_2 \right] dA \quad (\text{Helfrich, 1973})$$

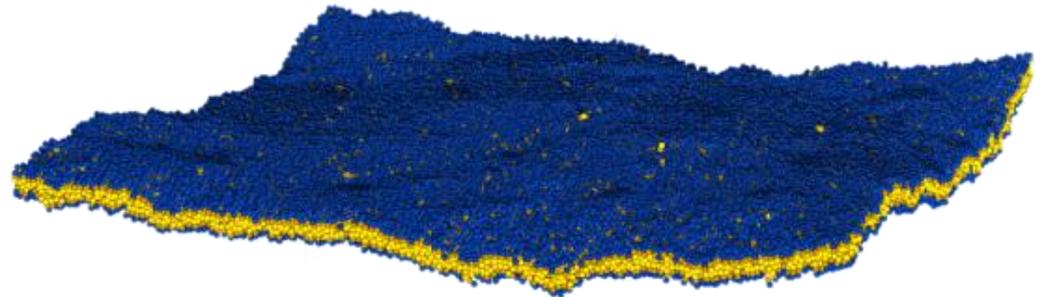
$\kappa$  - bending modulus (rigidity) ;  $\kappa_G$  - saddle-splay modulus ;  $c_0$  - spontaneous curvature

$\kappa$  [Energy] ; Phospholipid bilayers  $\kappa \sim 20 - 50 k_B T$  ; Red blood cell membrane  $\kappa \sim 10 k_B T$

Polymers are coiled – Membranes are flat.

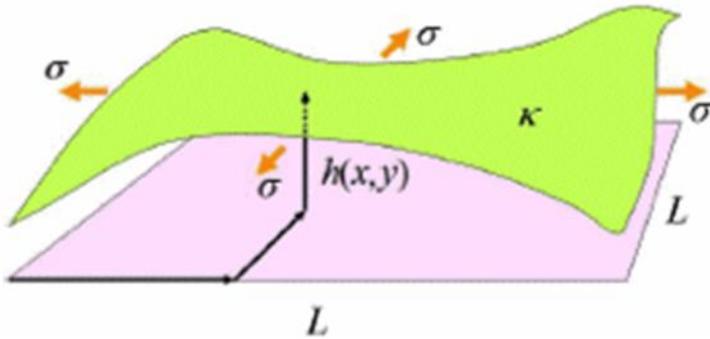


$$\xi_{\text{DNA}} \simeq 50 \text{ nm} ; \xi_{\text{F-Actin}} \simeq 10 \text{ } \mu\text{m}$$



$$\xi_{\text{lipid membrane}} \rightarrow \infty$$

## 2.4 Nearly-flat membranes



$$E_{\text{bend}} = \int \left[ \frac{1}{2} \kappa (c_1 + c_2 - 2c_0)^2 + \kappa_G c_1 c_2 \right] dA$$

$c_0 = 0$  – symmetric membrane

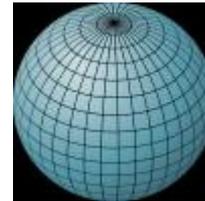
$$\int c_1 c_2 dA = \text{Const} = 4\pi(1 - g) ; g = \text{Genus}$$

$$E_{\text{bend}} = \int \frac{1}{2} \kappa (c_1 + c_2)^2 dA$$

$$h(x, y) ; -\frac{L}{2} < x, y < \frac{L}{2} \quad \text{Monge parametrization}$$

$$|\vec{\nabla} h| \ll 1 \Rightarrow (c_1 + c_2) \simeq \nabla^2 h(x, y) \\ \Rightarrow dA \simeq dx dy$$

$$E_{\text{bend}} = \int \frac{1}{2} \kappa [\nabla^2 h(x, y)]^2 dx dy$$



$g = 0$



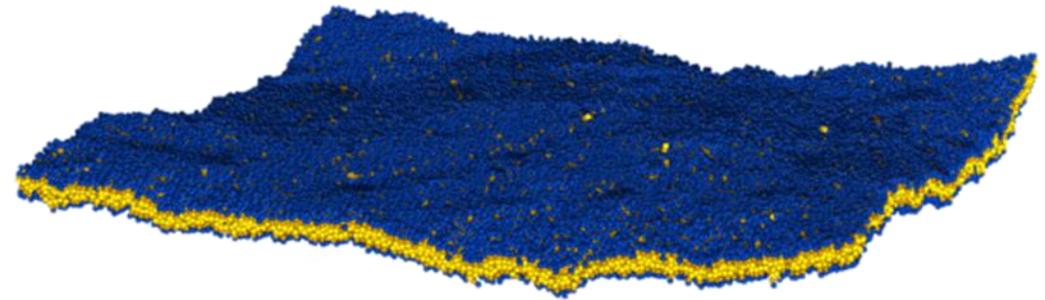
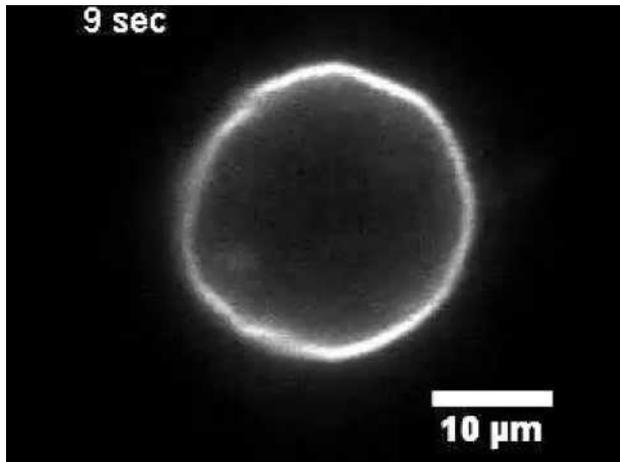
$g = 1$



$g = 2$

Nearly-flat membranes (Gaussian approximation)

## 2.5 Membrane thermal fluctuation

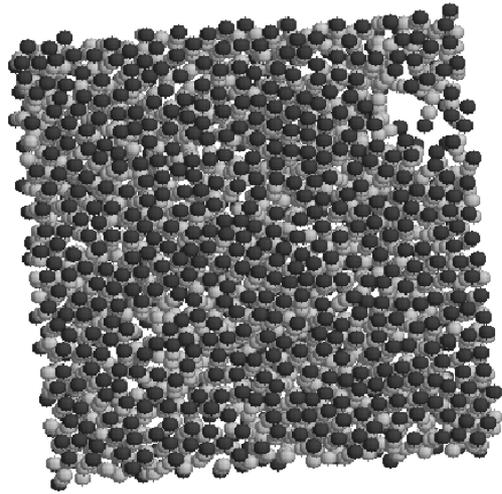


$$E_{\text{bend}} = \int \frac{1}{2} \kappa [\nabla^2 h(x, y)]^2 dx dy$$

$\langle h(x, y) \rangle = 0$  (symmetry) ;  $\langle h^2(x, y) \rangle = ?$



## 2.6 Membrane rupture



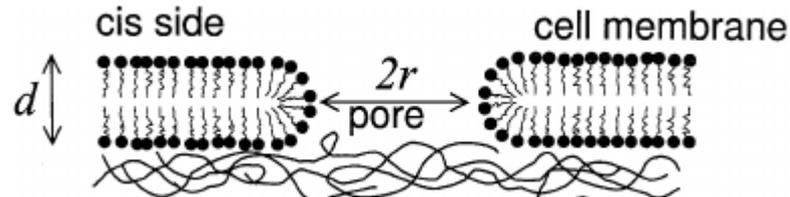
The pore (hole) relieves the surface tension energy, at the cost of exposing lipids to water.

Nucleation of a circular pore (Litster, 1975)

$R$  – Pore radius

$$E = -\sigma A + \lambda P = -\sigma(\pi R^2) + \lambda(2\pi R)$$

$\sigma$  - surface tension ;  $\lambda$  – line tension



- When is a hole formed?



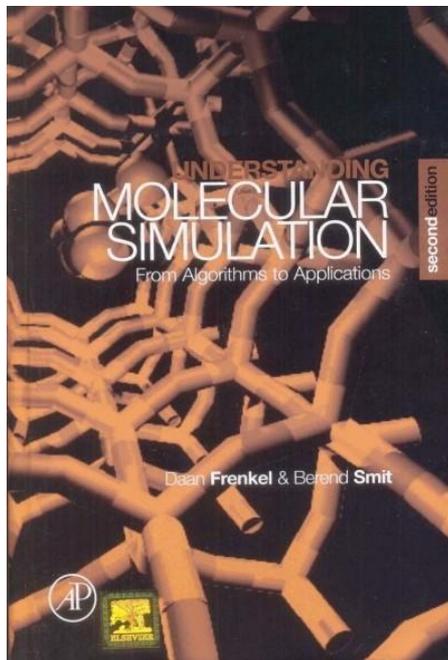
# Chapter 3- Computer simulations of membranes and more complex systems

## 3.1 Molecular simulations

Molecular simulations – investigations, using computational tools, of systems consisting of particles that interact with each other and with their environment.

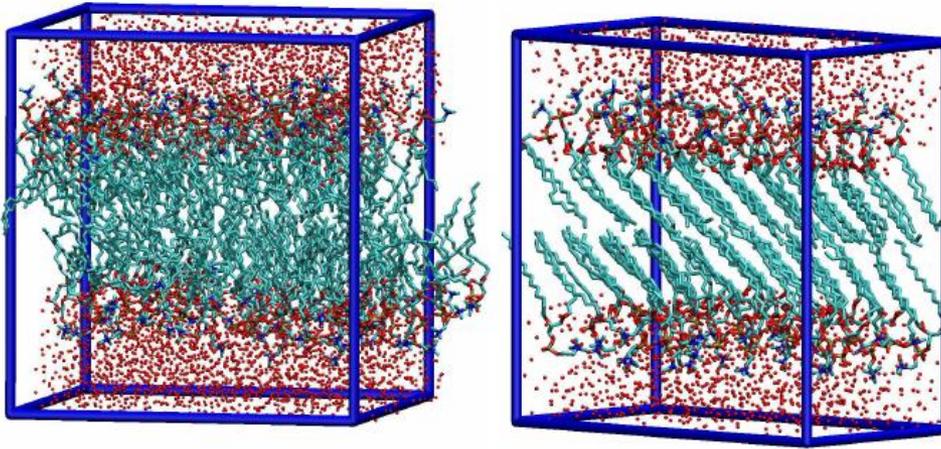
There are two basic approaches to molecular simulations:

- Molecular dynamics - We follow the trajectory of the particles by integrating their (classical) equations of motion (Newton's second law).
- Monte Carlo – We generate stochastic trajectories that sample the phase-space of the system.

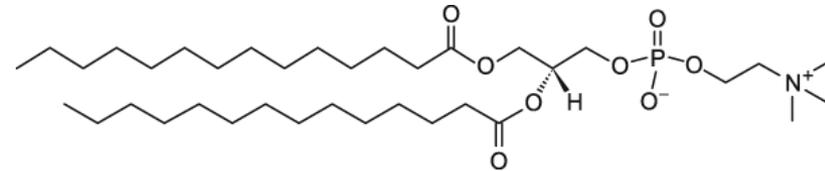


Understanding Molecular Simulations /  
Daan Frenkel & Berend Smit

## 3.2 All-atom simulations of lipid membranes

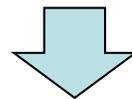


Simulations of DMPC lipids in the fluid and gel phases.



Require very large computer power:

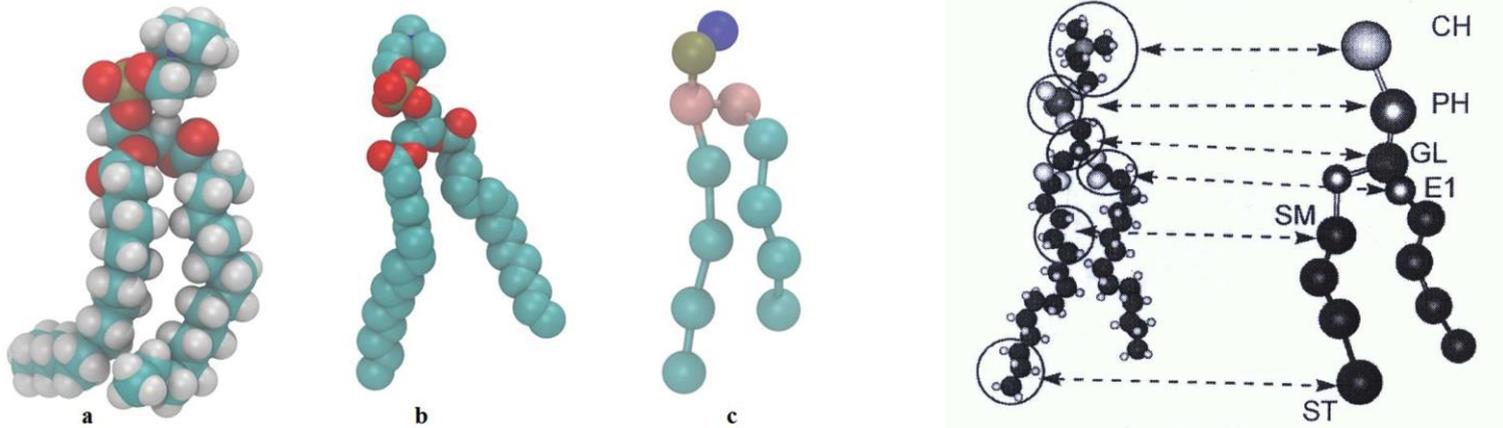
- A large number of atoms (>150 atoms per lipid)
- Even larger number of water molecules (10-50 per lipid)
- Many force/energy calculations: covalent bonds, bond angles, non-bonded interactions (LJ), electrostatics
- Simulation time step ~ 1fs



Simulated system size ~ 100 – 1000 lipid (~ 10-20 nm)

Simulated process duration ~ 100 ns – 1  $\mu$ s

## 3.3 Coarse-grained modeling



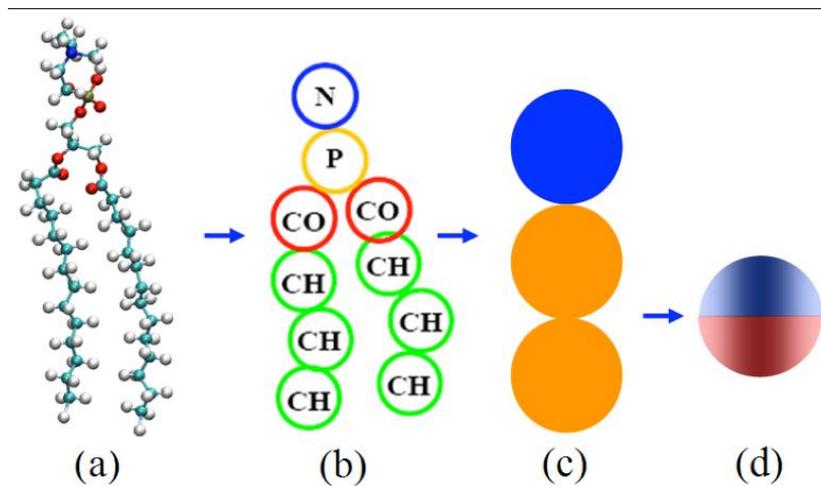
The idea: Use simpler models, where several atoms (chemical units) are represented by a single particle. Water is also represented via a single solvent particle.

The challenge: To design effective interactions (force fields) that reproduce the microscopic properties of the membrane.

Many CG models have been developed in the past 15 years:

- Klein model – the classical one
- Martini model – a very popular modular model (different types of lipids, cholesterol, CG proteins ...)

## 3.4 Implicit-solvent models (more coarse-grained)



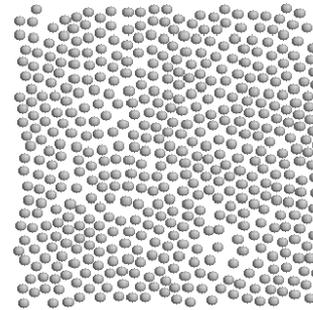
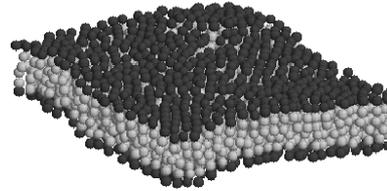
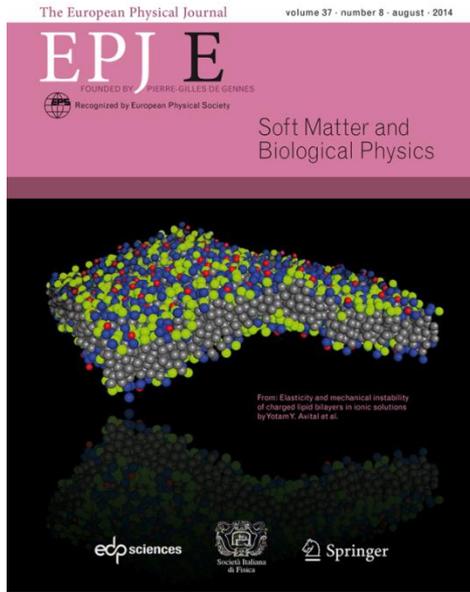
The principles of the model:

- Lipids are modeled via extremely simple **generic** structures (e.g., a trimer with one hydrophilic and two hydrophobic beads).
- **No water!** The hydrophobic attraction is introduced via effective potentials between the hydrophobic beads.

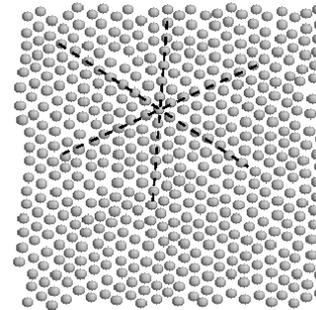
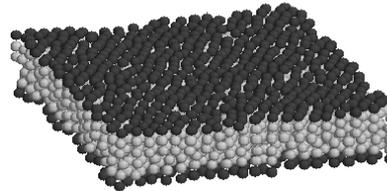
The “trick”:

- Lennard-Jones pair potentials with a shallow potential well (create a relatively smooth energy landscape that allow in-plan diffusion of lipids) – **Farago, Branningan & Brown, Cooke & Deserno**
- Many-body attractive potential (mimics hydrophobicity by defining energy that depends on the local density of the hydrophobic beads) - **Noguchi**

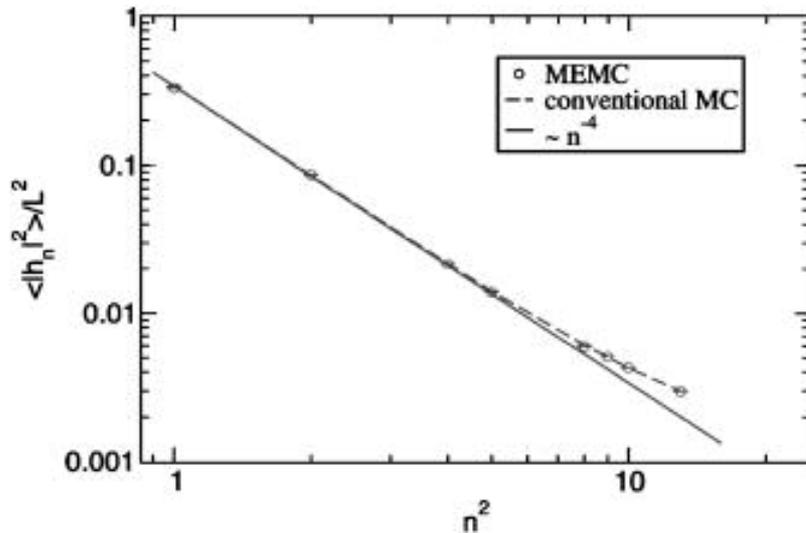
# 3.5 Implicit-solvent models (more coarse-grained)



Fluid



Solid



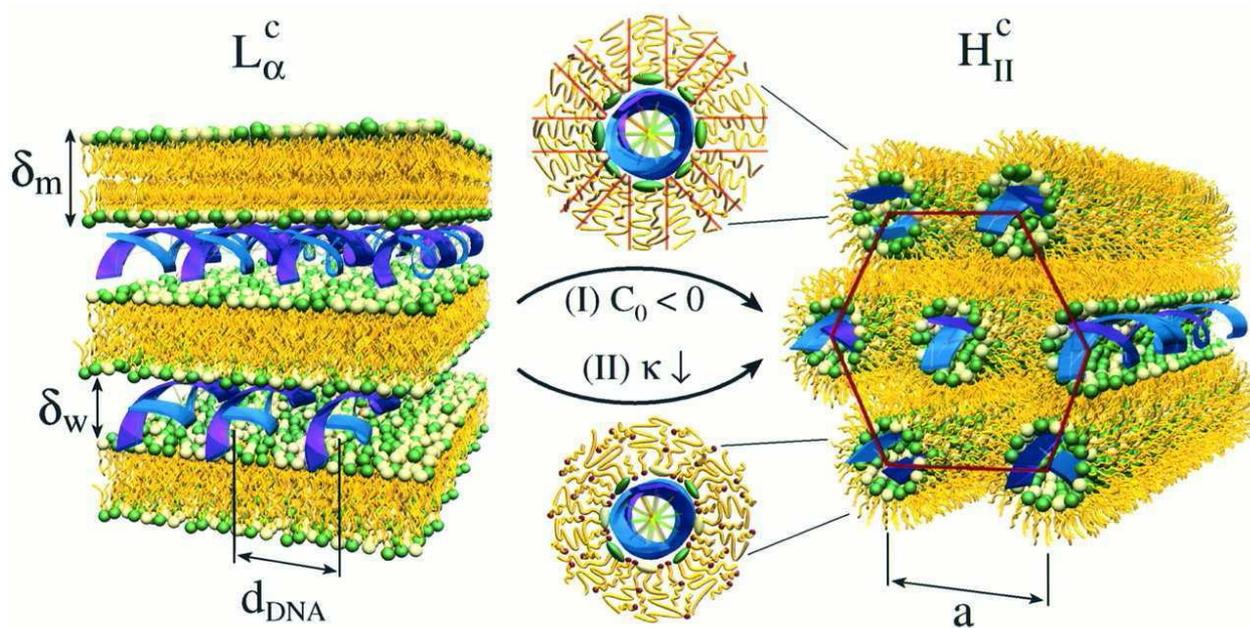
Fluctuation spectrum:

$$\langle |h_q|^2 \rangle \sim \frac{1}{q^4} \quad (\text{valid on length scales } \gtrsim 10 \text{ nm})$$

$\kappa \sim 10 - 50 k_B T$  (typical to phospholipid bilayers)

# 3.6 Implicit-solvent simulations of biological complexes

Self assembly of cationic lipid-DNA complexes for gene therapy



J. O. Radler *et al.*, Science **275**, 810 (1997).

I. Koltover *et al.*, Science **281**, 78 (1998).

Flat bilayer membranes =  
lower bending energy

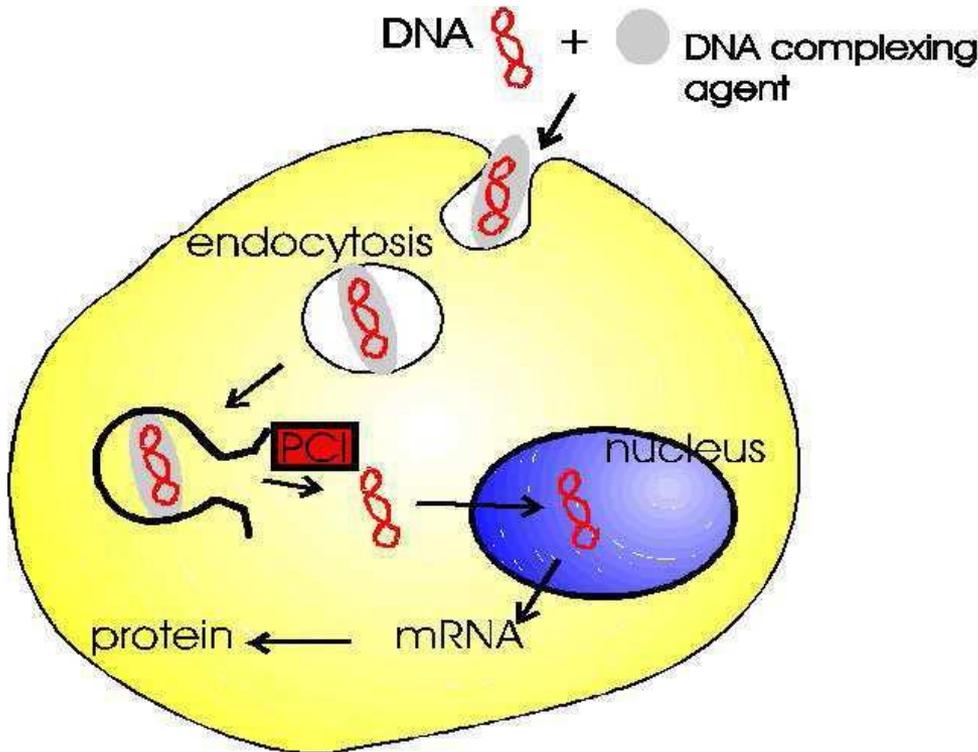
Local charge neutralization =  
lower electrostatic energy

# 3.7 Non-viral (synthetic) vectors for gene delivery

Advantages: easy mass production, lack of immune response, allow transport of unlimited DNA segments

Shortcomings: low efficiency

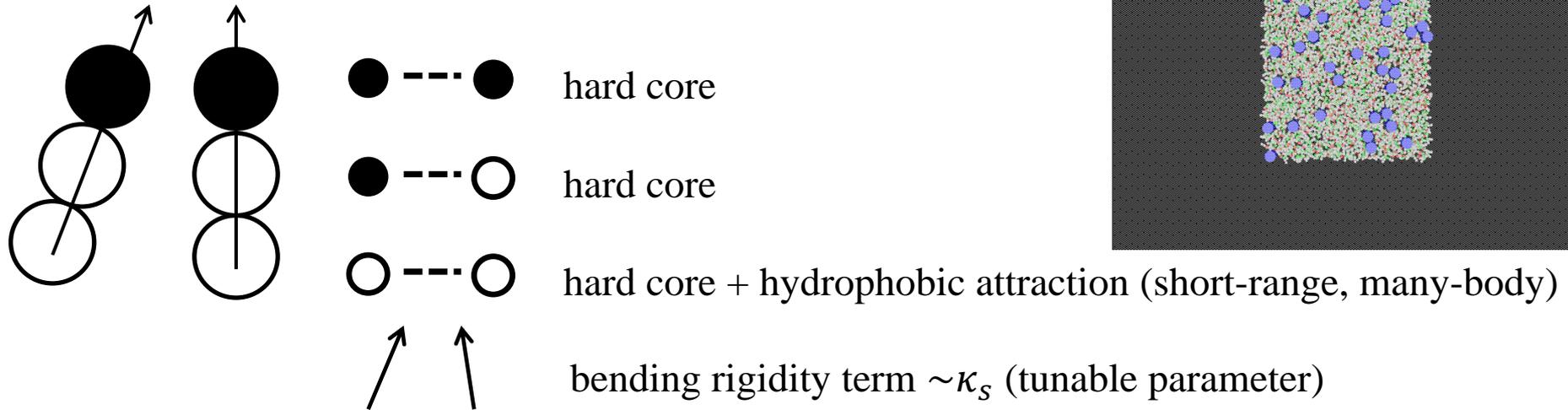
Transfection (transfer + expression)



1. Spontaneous self-assembly
2. complex-cell adherence (electrostatics / specific interactions)
3. Endocytosis
4. escape from endosomes (fusion with the endosomal membrane)
5. DNA release
6. transport into the nucleus

## 3.8 Coarse-grained implicit-solvent model

### 1. Solvent-free membrane model (Noguchi-Takasu model, 2001)



2. A fraction  $\phi_c$  of the head-groups carry charge  $+e$

3. DNA is modeled as a rigid rod of diameter  $\sim 25\text{\AA}$  and uniform charge density  $-e/(1.7\text{\AA})$

4. Periodic boundary conditions along the rods axes

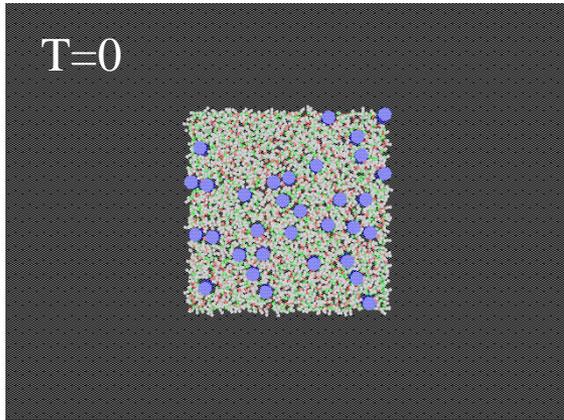
5. Long-range electrostatic interactions are treated by Lekner summation

Large scale simulations: 800 CLs, up to a few thousands of NLs, 32 DNA rods

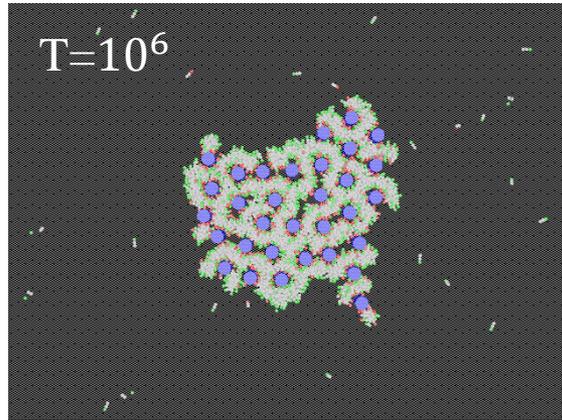
The model retains the essential physical features:  
hydrophobicity, electrostatics, entropy, and molecular geometry.

# 3.9 Self-assembly

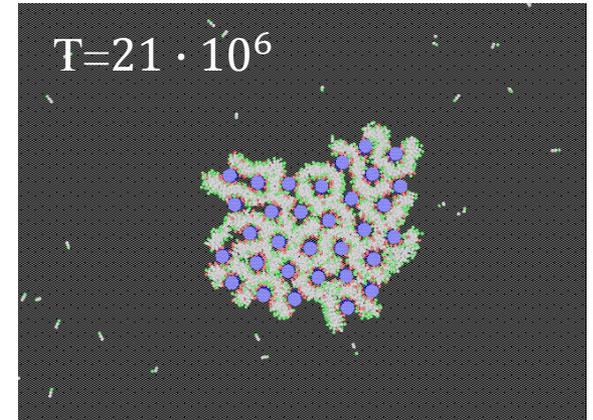
$\phi_C = 1/3$  (moderate charge density) ;  $\kappa_s = 0$  (soft membranes)



random initial configuration



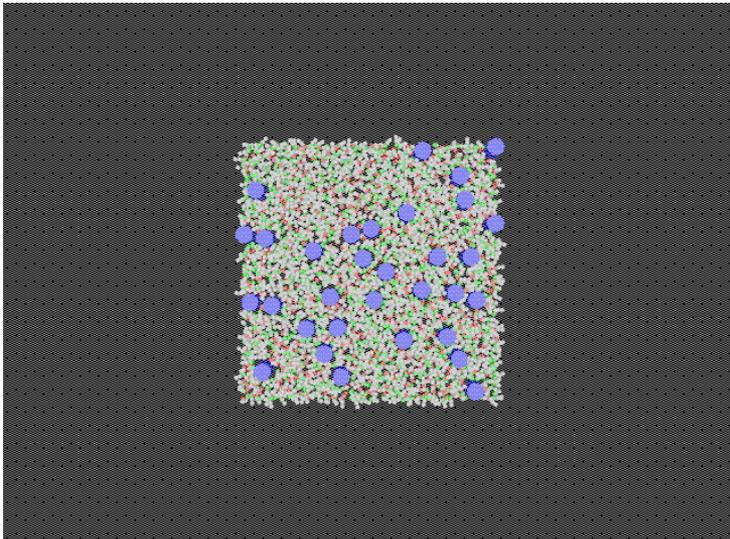
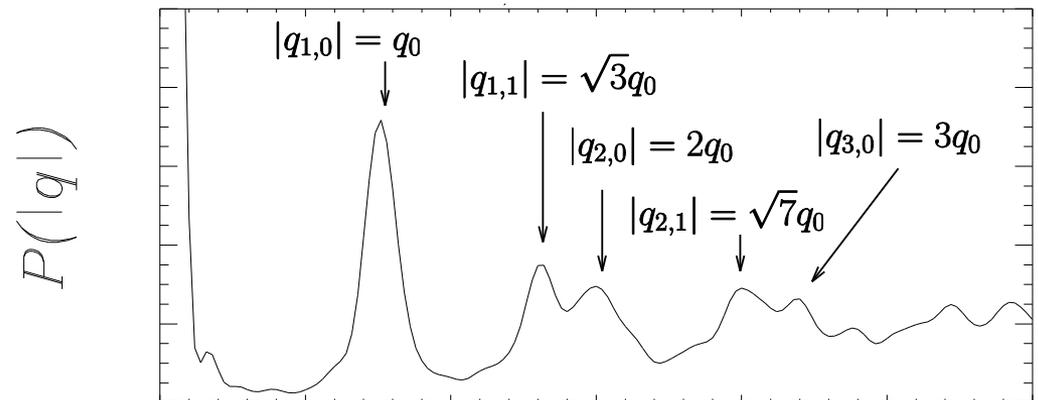
disordered complex:  
hydrophobic shielding  
electrostatic self-screening



inverted hexagonal complex

$$P(q) = \left\langle \left| \sum_{j=1}^{N_{DNA}} e^{i\vec{q} \cdot \vec{r}_j} \right|^2 \right\rangle_{\theta(2D)}$$

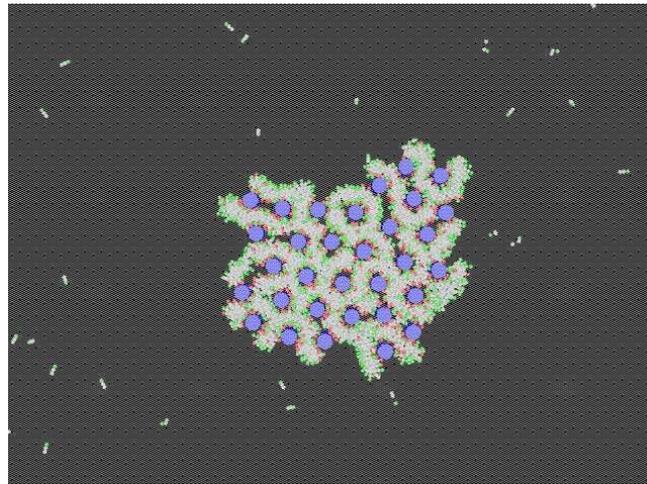
DNA scattering intensity



# 3.10 Changing the charge density

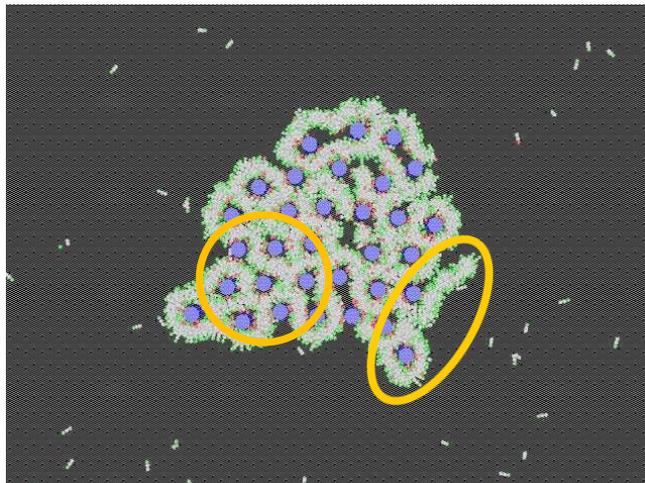
$$\phi_c = 1/3$$

inverted hexagonal complex



$$\phi_c = 4/17$$

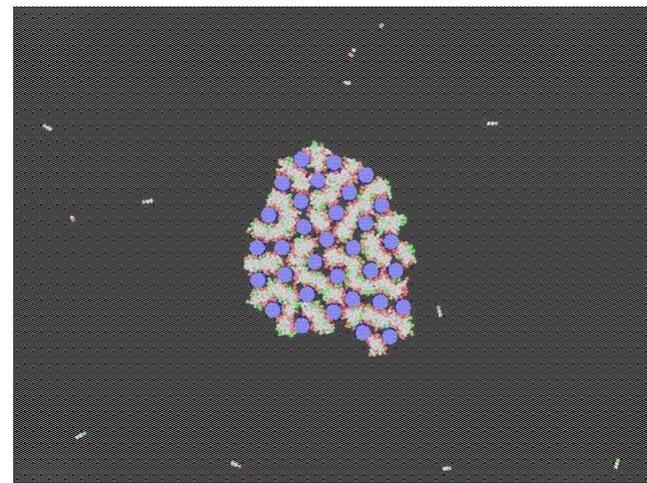
loss of long-range order



large amounts of neutral lipids =  
local hexagonal order  
+ defects

$$\phi_c = 2/3$$

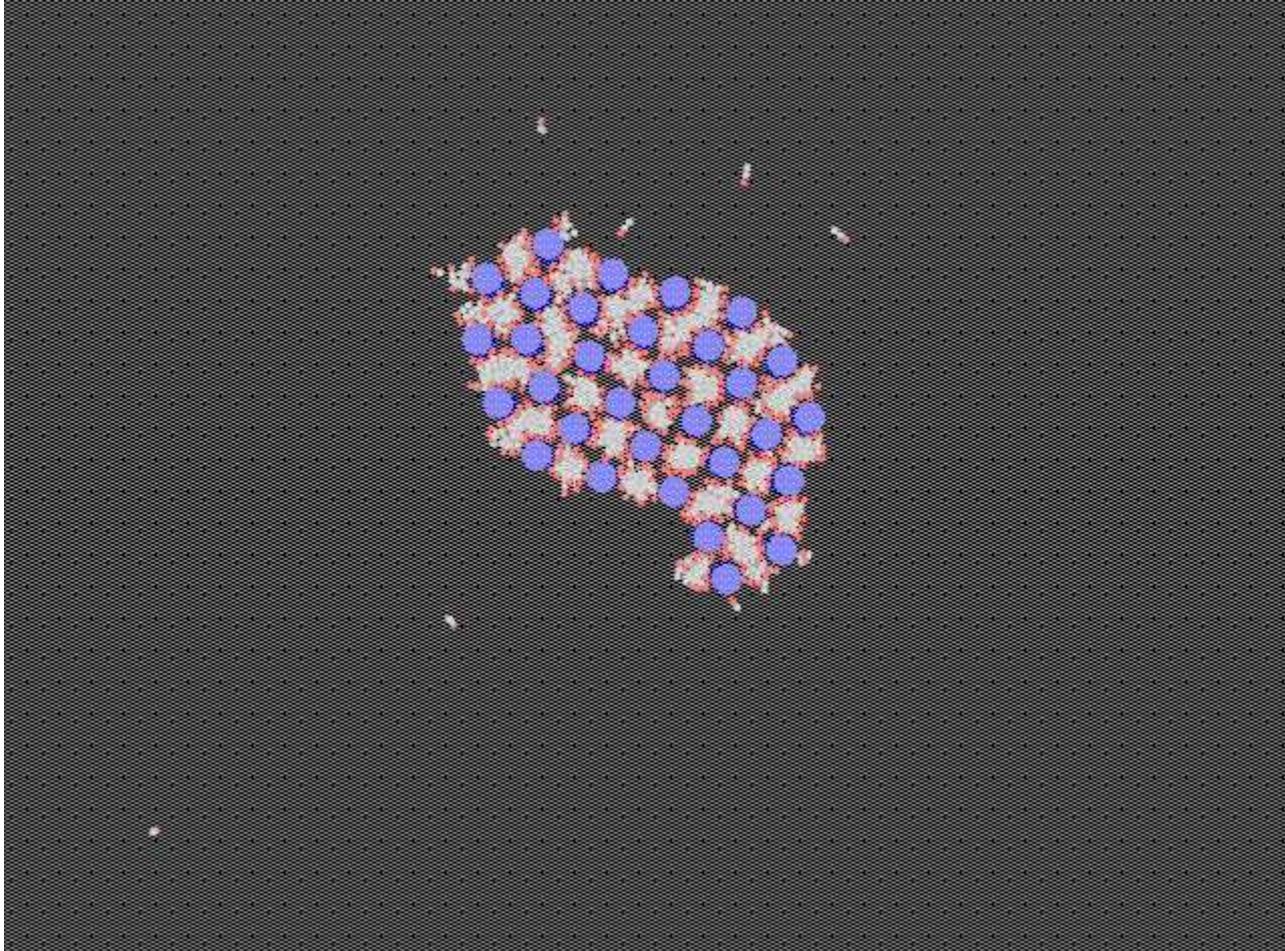
collapsed complex



small amounts of neutral lipids =  
large tensile stresses and membrane rupture

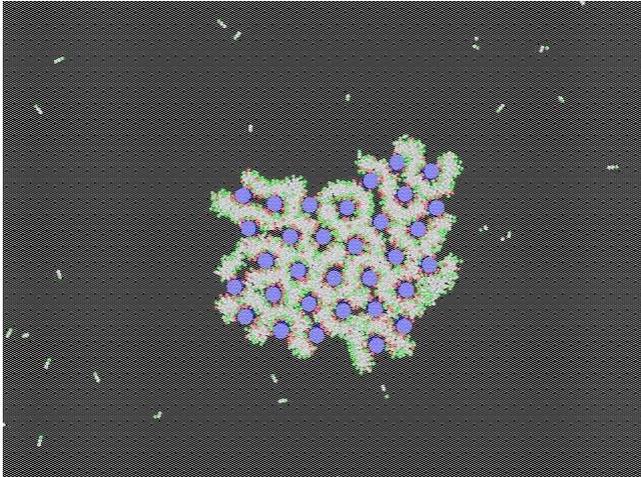
## 3.11 Novel structure (not seen experimentally yet)

$$\phi_c=1$$

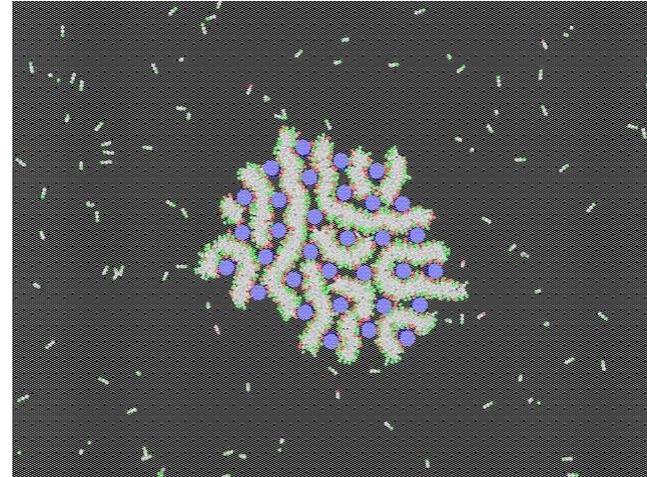


## 3.12 Changing the stiffness $(\phi_c = 1/3)$

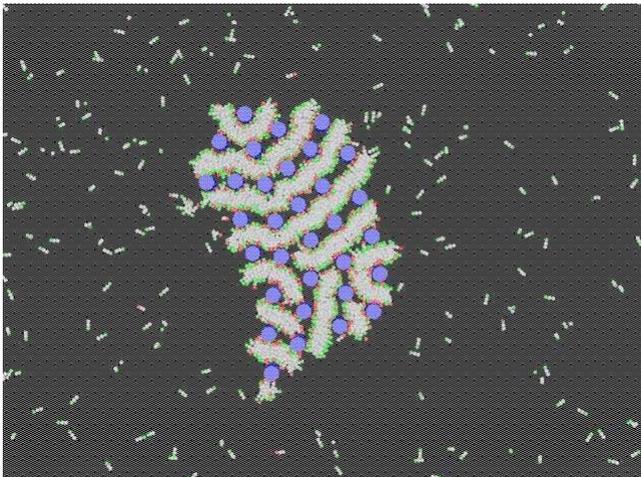
inverted hexagonal  $\kappa_S = 0$



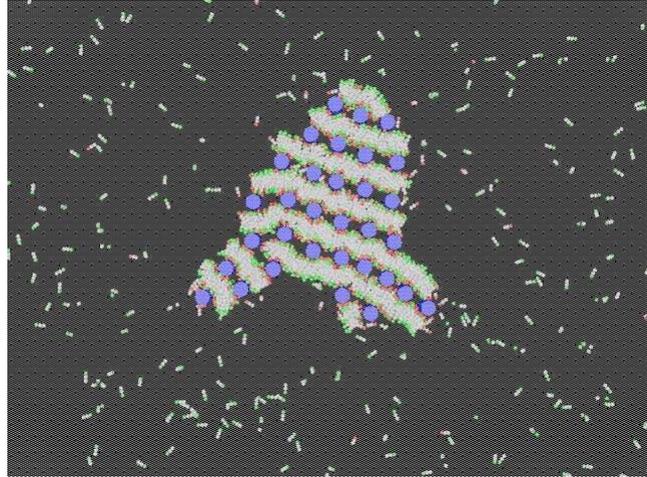
$\kappa_S = 2.5 k_B T$



$\kappa_S = 5 k_B T$



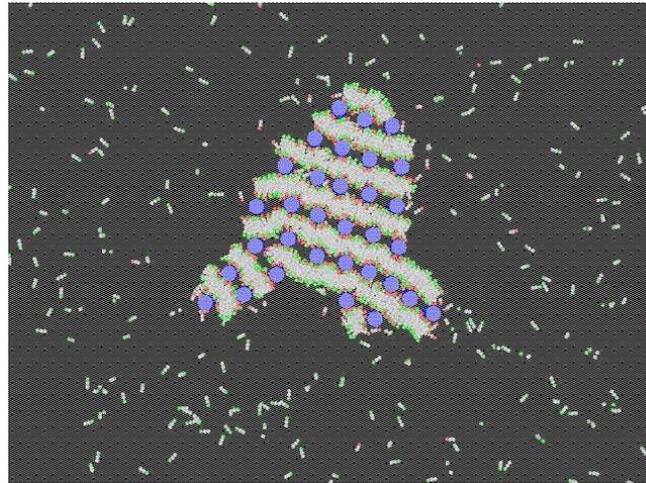
$\kappa_S = 10 k_B T$  lamellar



## 3.13 Changing the charge density

$$\phi_c = 1/3$$

lamellar complex



$$(\kappa_S = 10 k_B T)$$

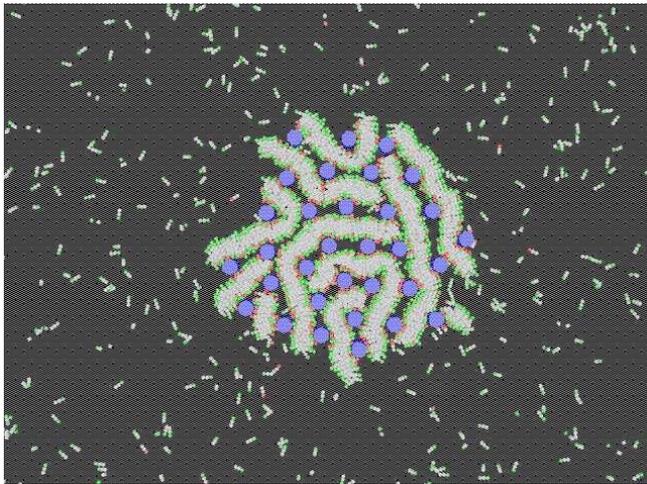
$$\phi_c = 4/17$$



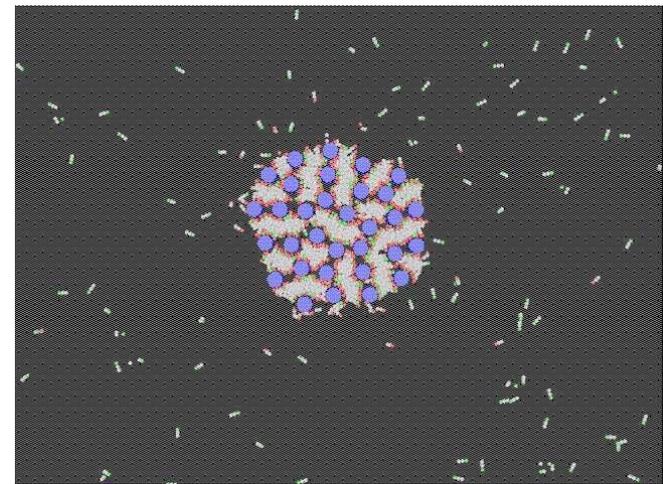
loss of lamellar ordering



$$\phi_c = 4/7$$



over-dilution by neutral lipids

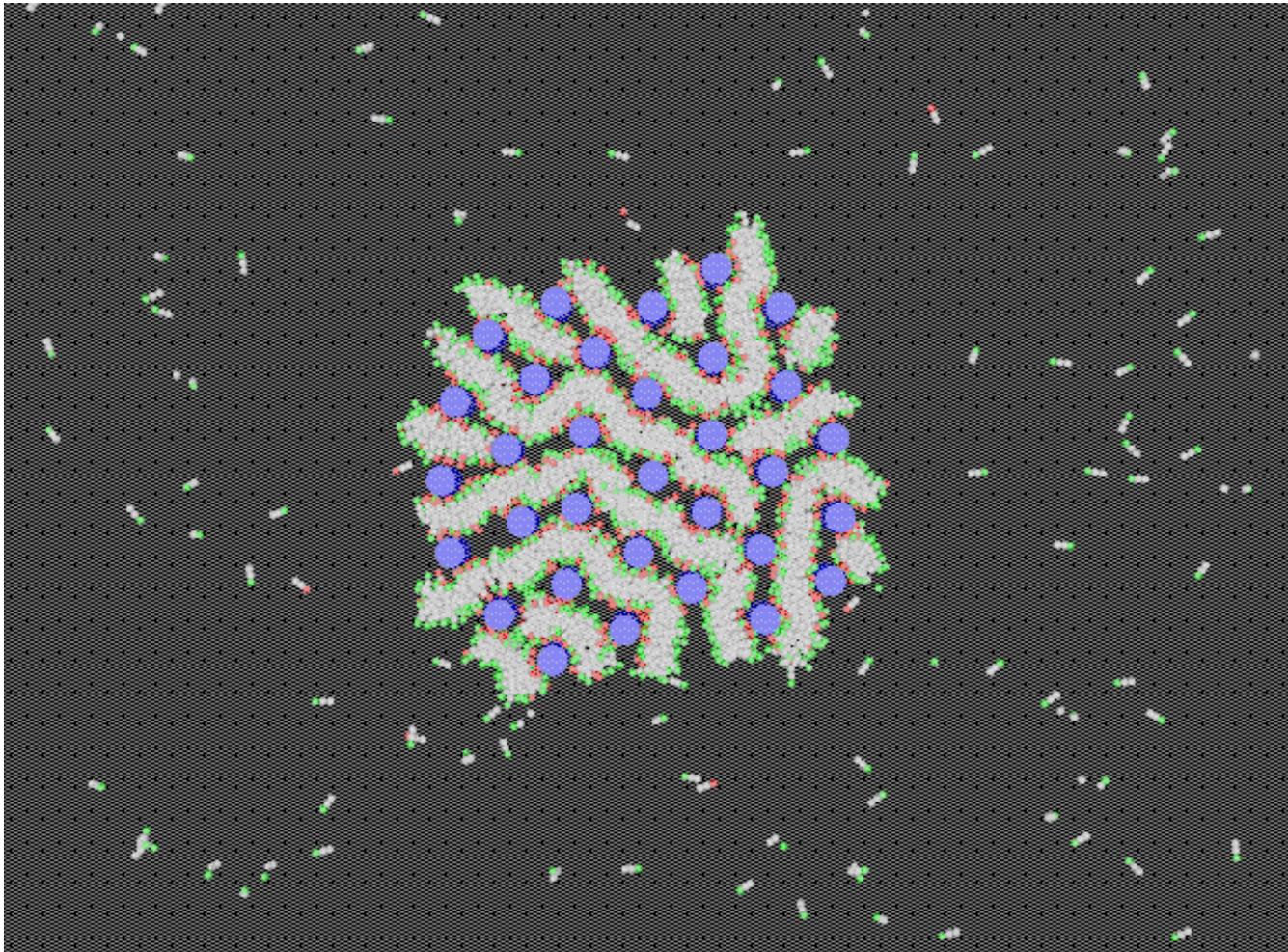


membrane rupture due to strong electrostatic stresses

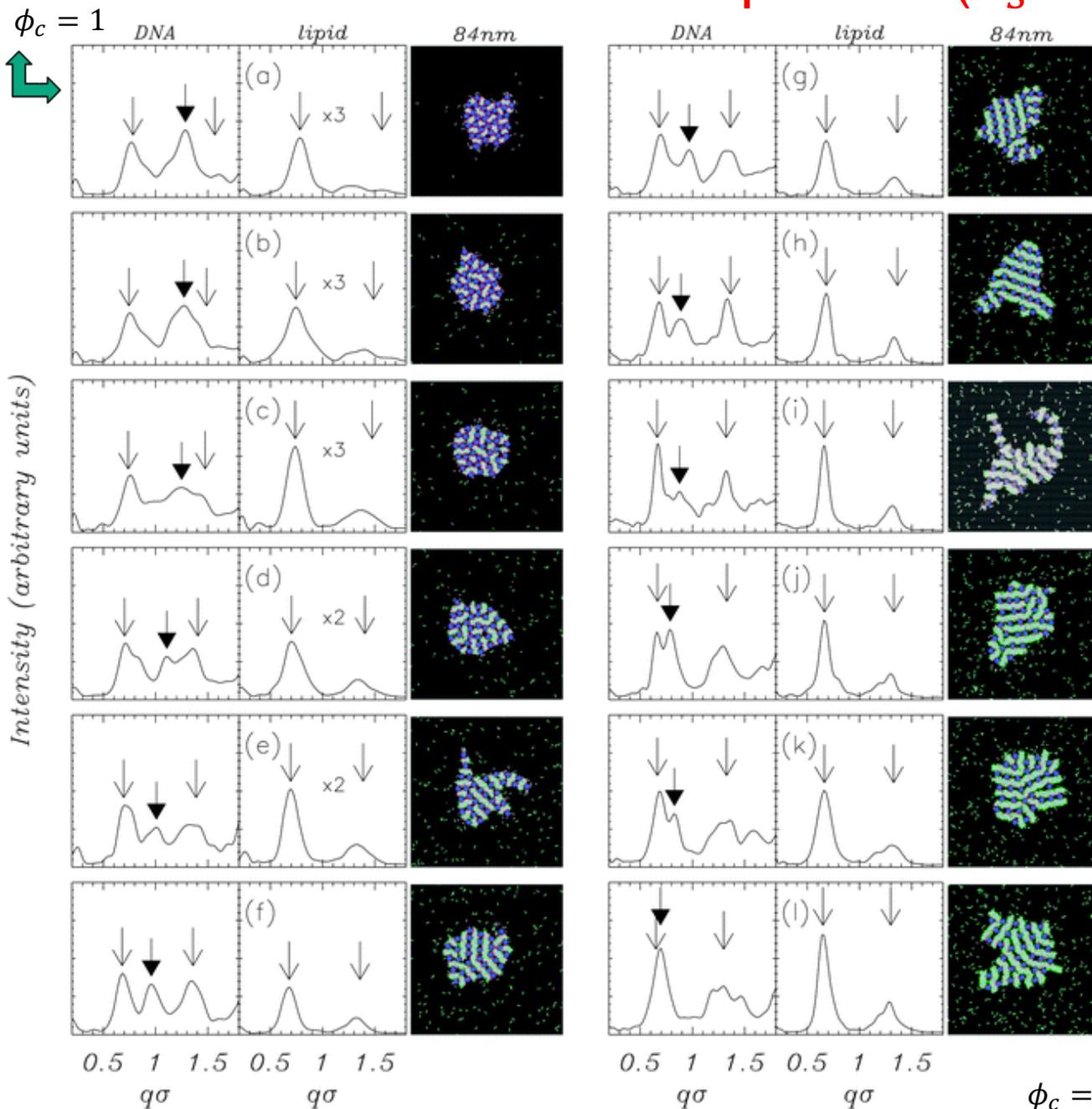
## 3.14 Lamellar or hexagonal?

$\phi_c=4/13$  (moderately charged complex)

$\kappa_s = 2.5 k_B T$  (moderate rigidity)



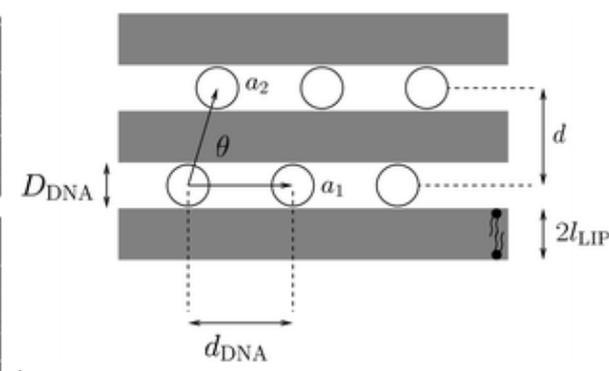
# 3.15 Diffraction patterns ( $\kappa_S = 10 k_B T$ )



$$P(q) = \left\langle \left| \sum_{j=1}^N \omega_j e^{i\vec{q} \cdot \vec{r}_j} \right|^2 \right\rangle_{\theta(2D)}$$

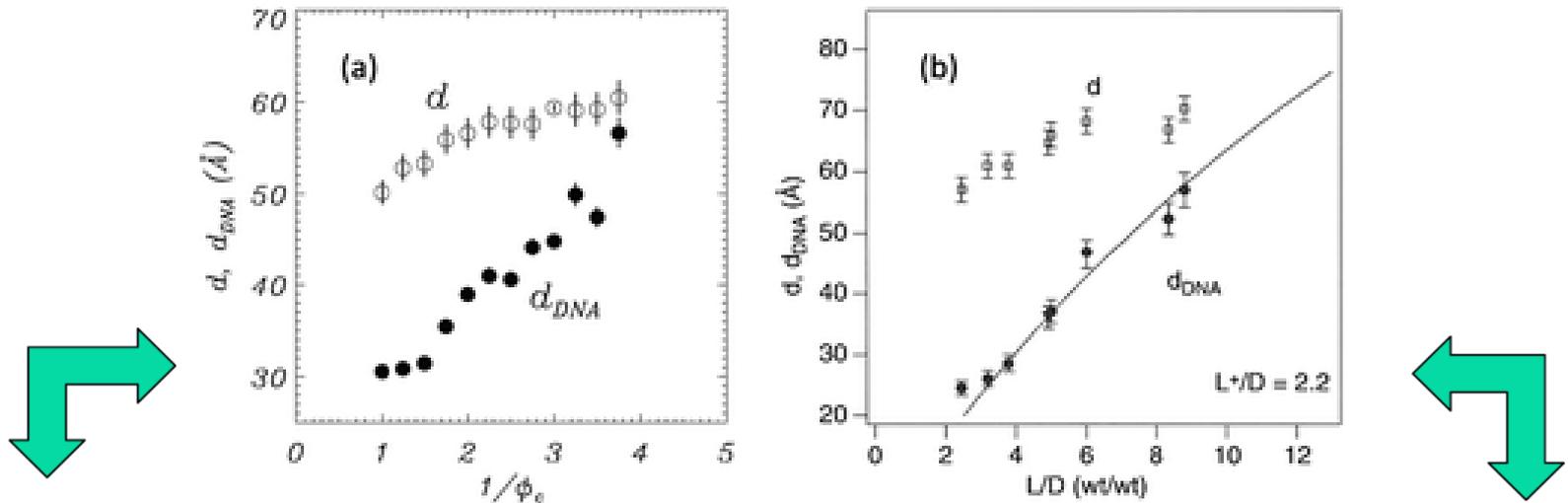
Lipid scattering:  
 $q_{LAM}, 2q_{LAM}; q_{LAM} = \frac{2\pi}{d}$

DNA scattering:  
 $q_{LAM}, 2q_{LAM},$   
 $q_{DNA}$  (strong  $\phi_c$ -dependent)  
 $q_{LAM} = \frac{2\pi}{d}; q_{DNA} = \frac{2\pi}{d_{DNA}}$



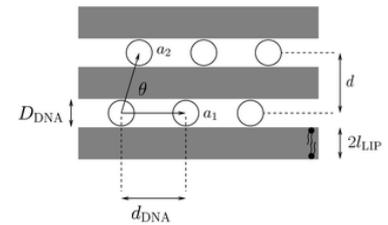
$\phi_c = 4/15$

## 3.16 Comparison with X-ray scattering



Farago & Gronbech-Jensen (computer simulations, 2011)      Safinya et al. (X-ray data, 1997)

1. Remarkable **quantitative** agreement
2. Low charge densities:  $d \approx 2l_{LIP} + D_{DNA} \approx 62\text{\AA}$ ;  $d_{DNA} \sim 1/\phi_c$
3. High charge densities:  $d < 2l_{LIP} + D_{DNA} \approx 62\text{\AA}$ ;  $d_{DNA} > 1/\phi_c$



Diffraction from disordered-fragmented structures being interpreted as if originating from lamellar complexes?

**Molecular simulations as a computational microscope**