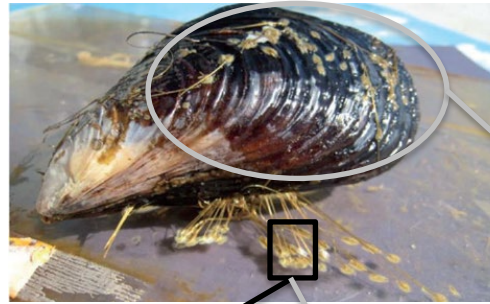
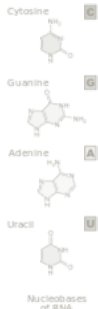


# Course outline

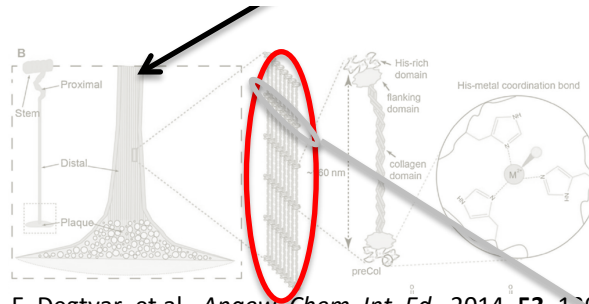


## Introduction



## Ordered materials

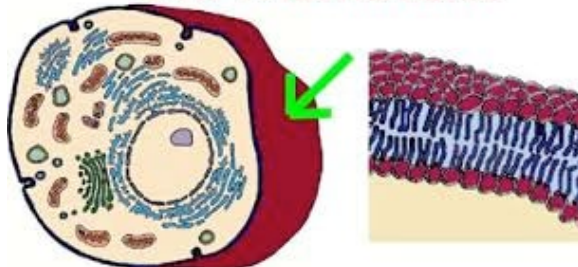
Thermotropic liquid crystals



E. Degtyar, et al., *Angew. Chem. Int. Ed.*, 2014, **53**, 12026-12044

Lytotropic liquid crystals

Cell Membrane

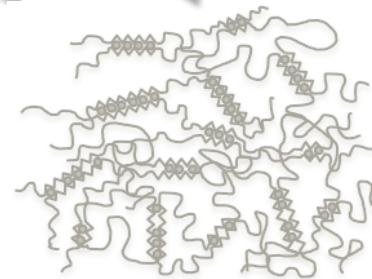


## Disordered materials

Polymers



Gels



## Colloids

Nanoparticles



Emulsions



# Outline

- Introduction: Vesicles
- Liposomes
- Polymersomes
- Vesicle production
- Characterization
- Applications

# Outline

- Introduction: Vesicles
- Liposomes
- Polymersomes
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# Vesicles

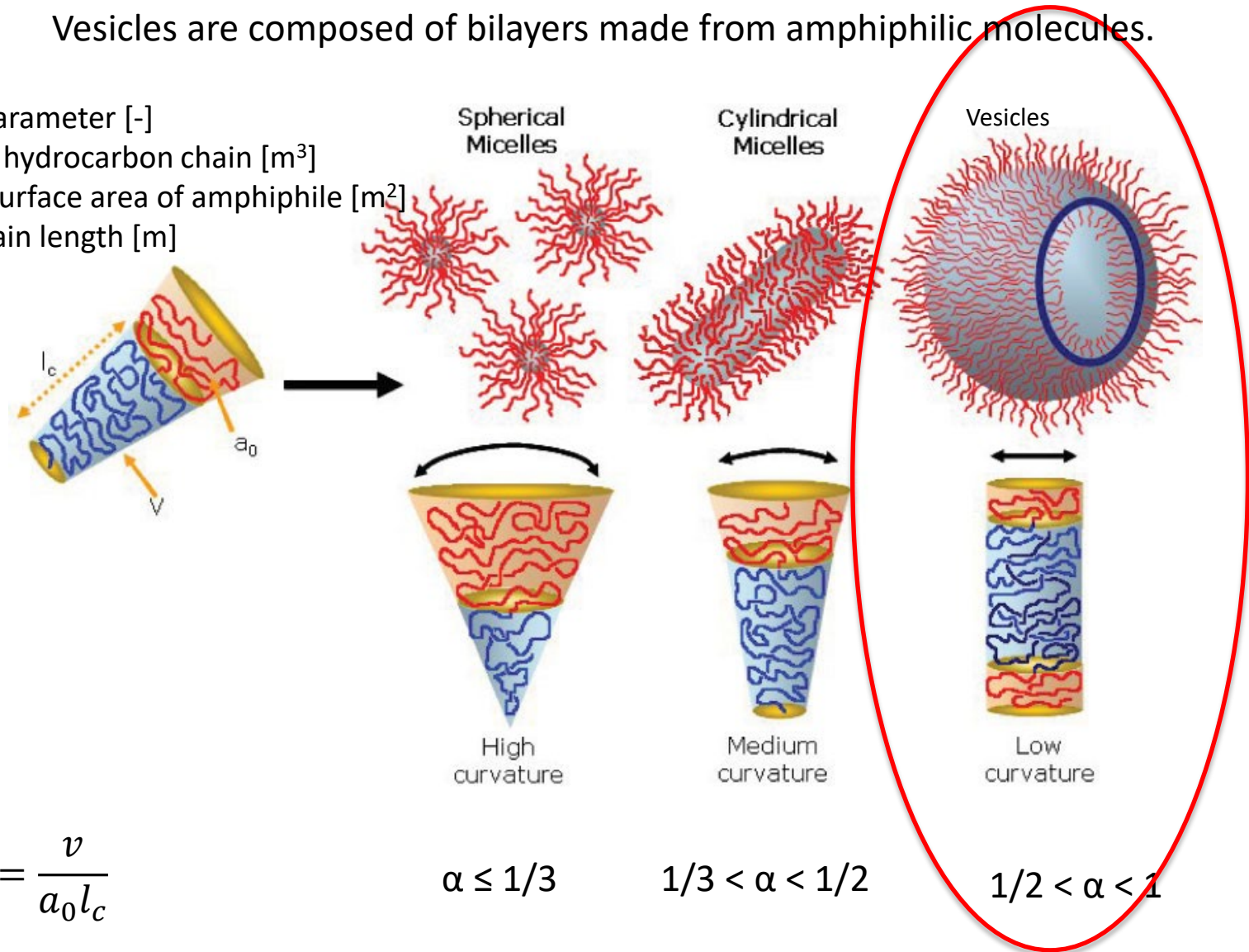
Vesicles are composed of bilayers made from amphiphilic molecules.

$\alpha$ : packing parameter [-]

$v$ : volume of hydrocarbon chain [m<sup>3</sup>]

$a_0$ : optimal surface area of amphiphile [m<sup>2</sup>]

$l_c$ : critical chain length [m]



$$\alpha = \frac{v}{a_0 l_c}$$

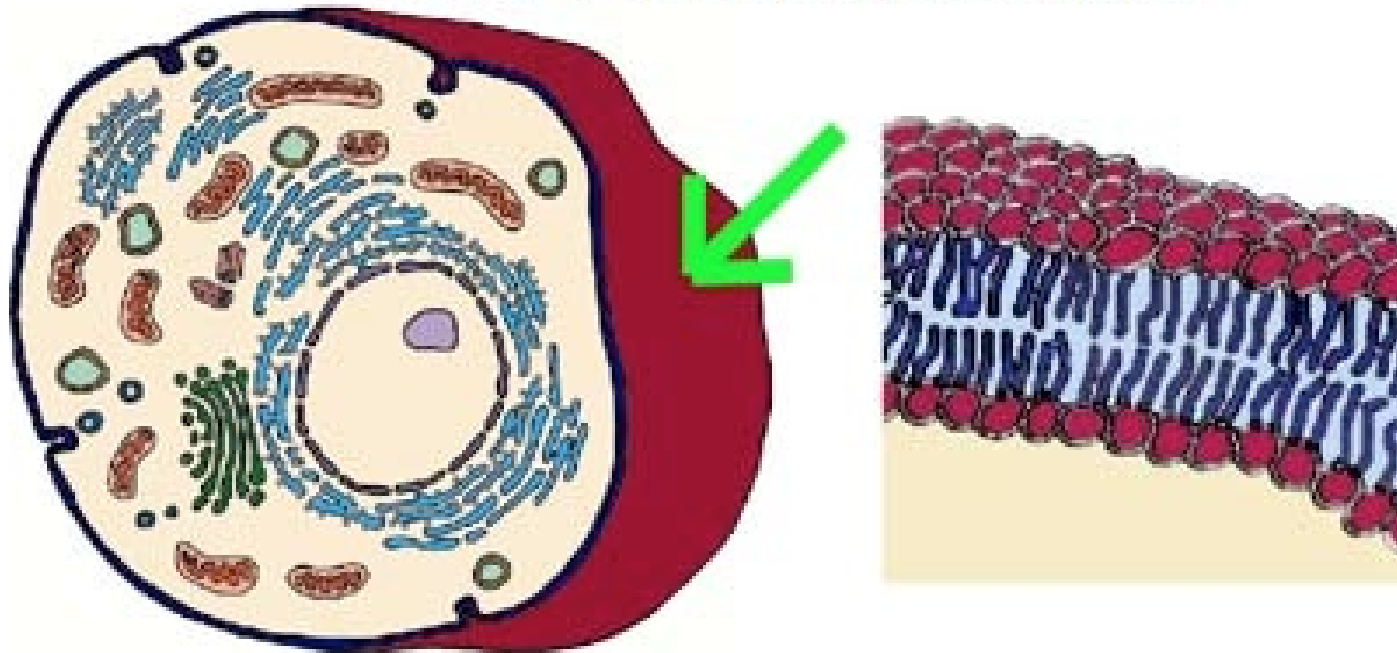
$$\alpha \leq 1/3$$

$$1/3 < \alpha < 1/2$$

$$1/2 < \alpha < 1$$

# Vesicles in nature

## Cell Membrane



<http://battleoftheorganelles.blogspot.ch/2013/10/the-celebratory-cell-membrane-cause-its.html>

# Outline

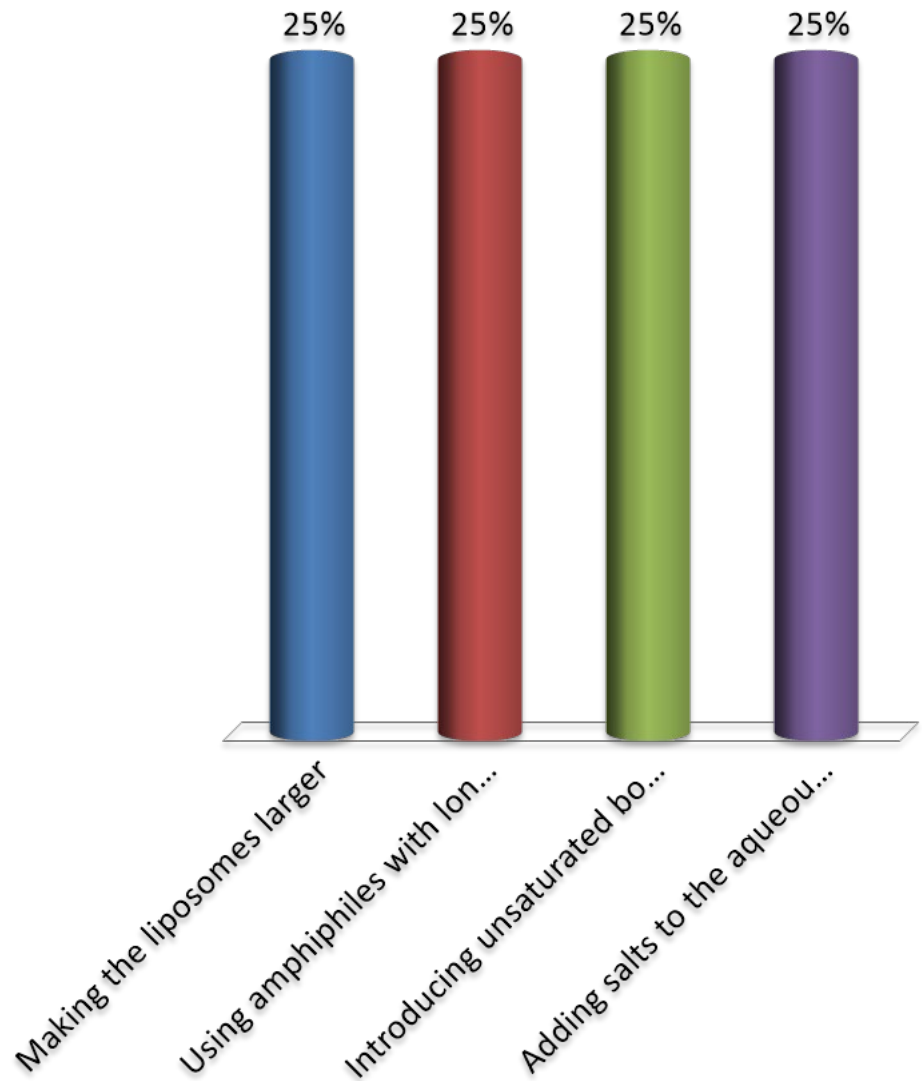
- Introduction: Vesicles
- **Liposomes**
- Polymersomes
- Vesicle production
- Characterization
- Applications



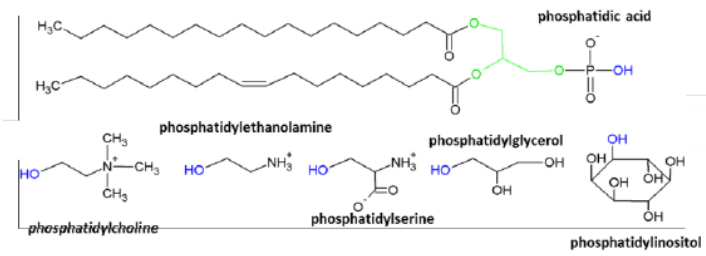
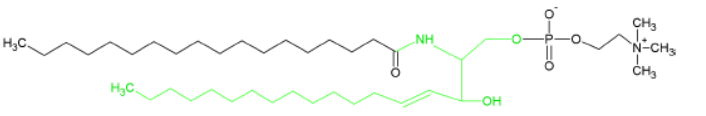
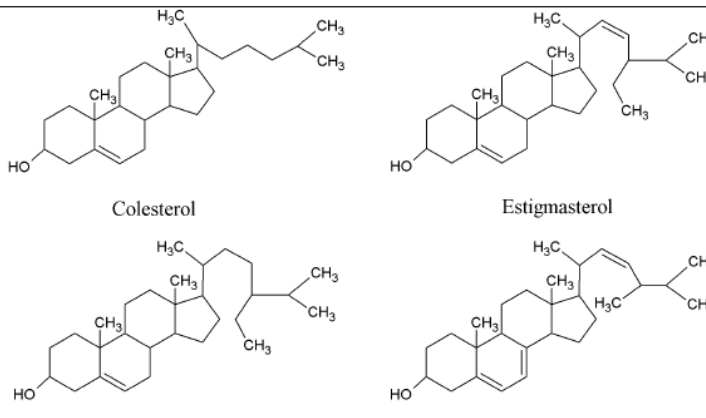


# Which factor determines the shape of vesicles?

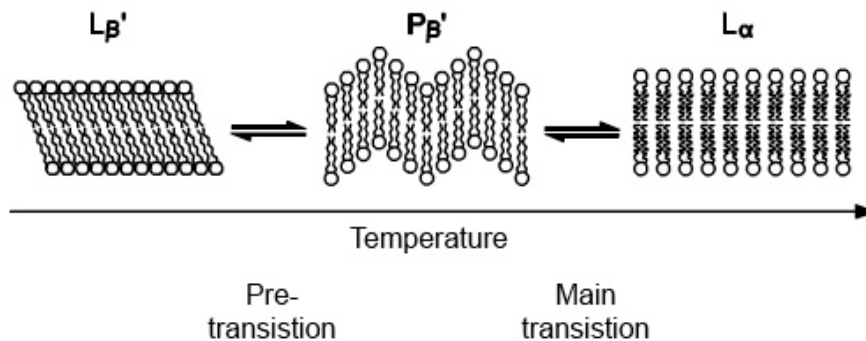
- A. Hydrophobic tail volume
- B. Critical chain length
- C. Optimal surface area of amphiphiles
- D. All of the above



# Liposomes consist of lipids

| lipid type     | chemical formula   | examples  |
|----------------|--|---|
| phospho-lipids |  <p>The diagram shows the general structure of a phospholipid with two fatty acid chains (one saturated, one monounsaturated) attached to a glycerol backbone. Below this, several head groups are shown: phosphatidylethanolamine (with an amino group), phosphatidylcholine (with a trimethylammonium group), phosphatidylserine (with a serine residue), phosphatidylinositol (with an inositol ring), and phosphatidylglycerol (with three hydroxyl groups).</p> | <p>1,2-dipalmitoylphosphocholine (DPPC)</p> <p>1-palmitoyl-2-oleylphosphocholine (POPC)</p>     |
| sphingo-lipids |  <p>The diagram shows a sphingolipid structure consisting of a sphingosine backbone with a long-chain fatty acid attached via an amide bond and a phosphate group linked to a trimethylammonium group.</p>   | <p>sphingomyelins</p> <p>cerebrosides</p> <p>gangliosides</p>                                   |
| steroids       |  <p>The diagram shows four steroid structures: Cholesterol (with a hydroxyl group and a branched hydrocarbon side chain), Estigmasterol (with a hydroxyl group and a branched hydrocarbon side chain), Sitosterol (with a hydroxyl group and a branched hydrocarbon side chain), and Ergosterol (with a hydroxyl group and a branched hydrocarbon side chain).</p>  | <p>cholesterol</p> <p>estigmasterol</p> <p>sitosterol</p> <p>ergosterol</p> <p>phytosterols</p> |

# Phase transition



<https://avantilipids.com/tech-support/physical-properties/phase-transition-temps/>

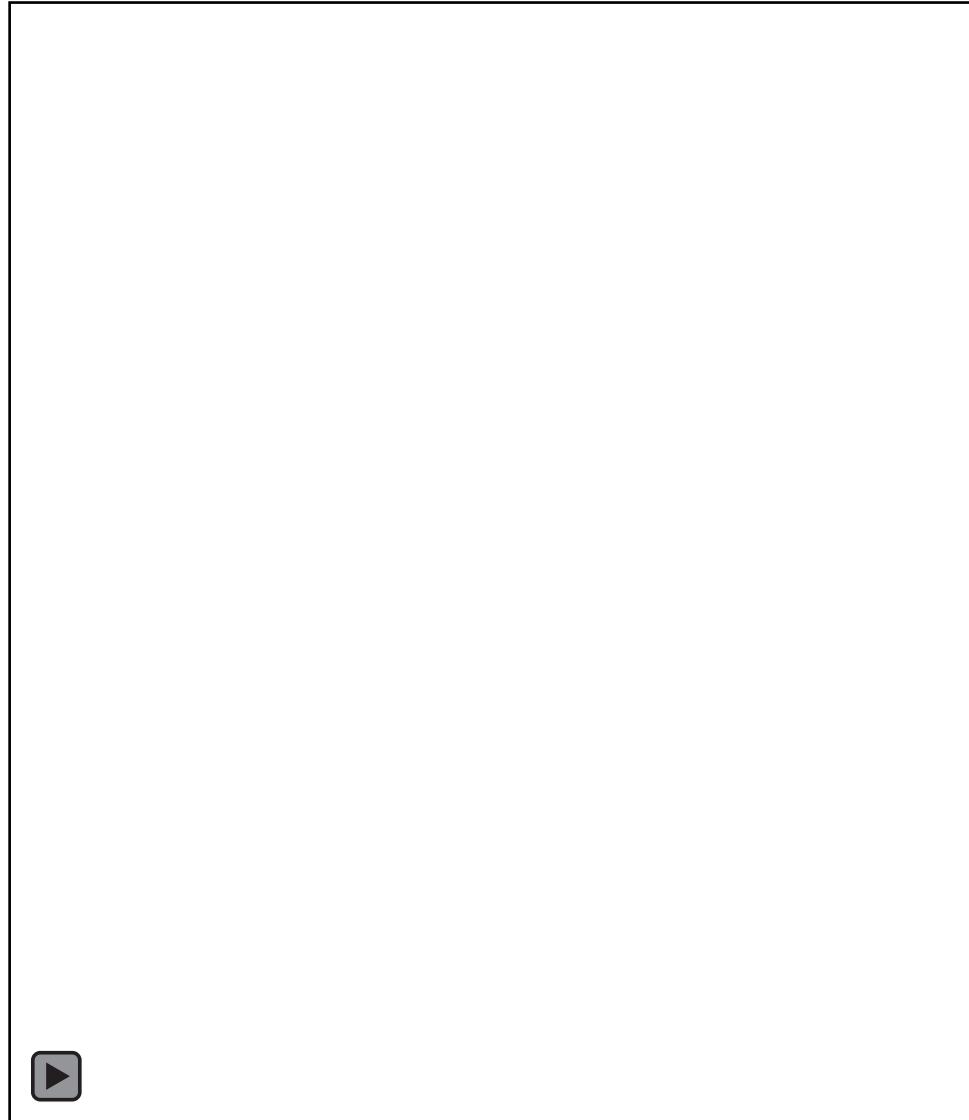
|            | Subgel ( $L_c$ ) | Gel ( $L_{\beta'}$ ) | Ripple ( $P_{\beta'}$ ) | Fluid ( $L_{\alpha}$ ) |
|------------|------------------|----------------------|-------------------------|------------------------|
| Experiment |                  |                      |                         |                        |
| DPD        |                  |                      |                         |                        |
| MARTINI    |                  |                      |                         |                        |

# Liposomes consist of lipids

| lipid type     | chemical formula   | examples  |
|----------------|--|---|
| phospho-lipids | <p>The diagram shows the chemical structures of several phospholipids. At the top, phosphatidic acid is shown with two fatty acid chains (one saturated, one monounsaturated) and a phosphate group. Below it, phosphatidylethanolamine is shown with a saturated fatty acid chain and a phosphate group linked to an ethanolamine head group. Further down, phosphatidylcholine is shown with a saturated fatty acid chain and a phosphate group linked to a choline head group. Other structures include phosphatidylserine (with a serine head group), phosphatidylinositol (with an inositol head group), and phosphatidylglycerol (with a glycerol head group).</p>             | <p>1,2-dipalmitoylphosphocholine (DPPC)</p> <p>1-palmitoyl-2-oleylphosphocholine (POPC)</p>     |
| sphingo-lipids | <p>The diagram shows the chemical structure of a sphingolipid. It features a long hydrocarbon chain (H<sub>3</sub>C) attached to a sphingosine backbone. The sphingosine backbone has a hydroxyl group (OH) and a phosphate group (P=O) linked to a choline head group (N(CH<sub>3</sub>)<sub>3</sub>).</p>  | <p>sphingomyelins</p> <p>cerebrosides</p> <p>gangliosides</p>                                   |
| steroids       | <p>The diagram shows the chemical structures of four steroids. Cholesterol is shown with a hydroxyl group (HO) at C3, a double bond at C5, and a branched hydrocarbon side chain at C17. Estigmasterol is shown with a hydroxyl group (HO) at C3, a double bond at C5, and a branched hydrocarbon side chain at C17 with a methyl group at C24. Sitosterol is shown with a hydroxyl group (HO) at C3, a double bond at C5, and a branched hydrocarbon side chain at C17 with a methyl group at C24. Ergosterol is shown with a hydroxyl group (HO) at C3, a double bond at C5, and a branched hydrocarbon side chain at C17 with a methyl group at C24 and a double bond at C22.</p> | <p>cholesterol</p> <p>estigmasterol</p> <p>sitosterol</p> <p>ergosterol</p> <p>phytosterols</p> |

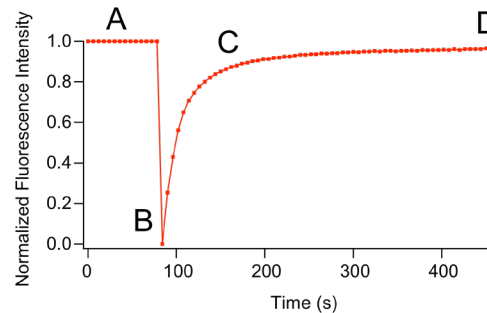
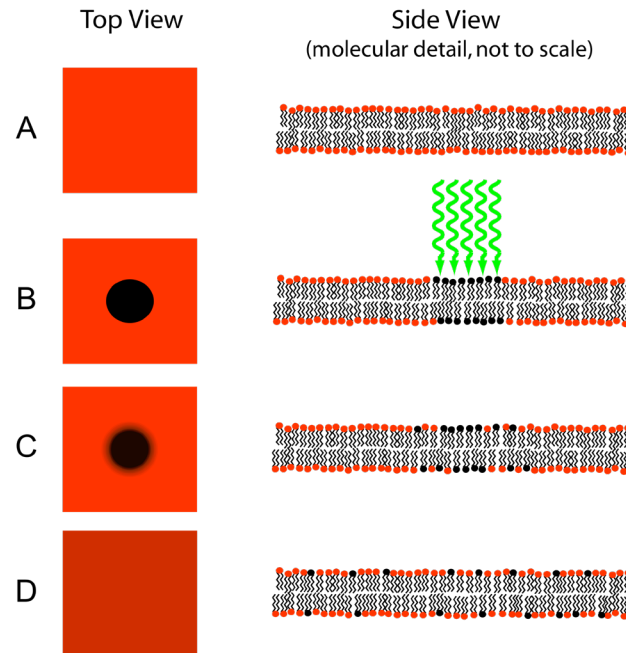


# Mobility of phospholipids in bilayers



# Diffusion coefficient

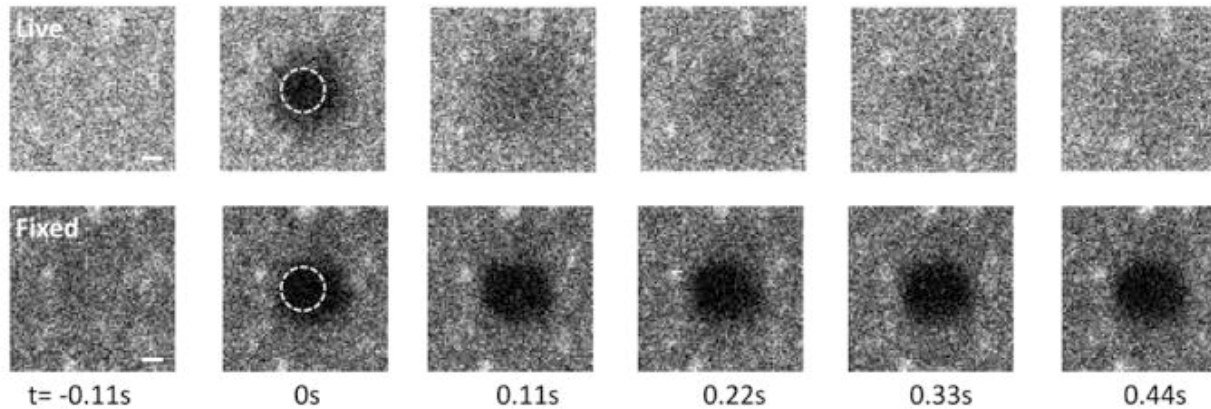
FRAP : fluorescence recovery after photobleaching



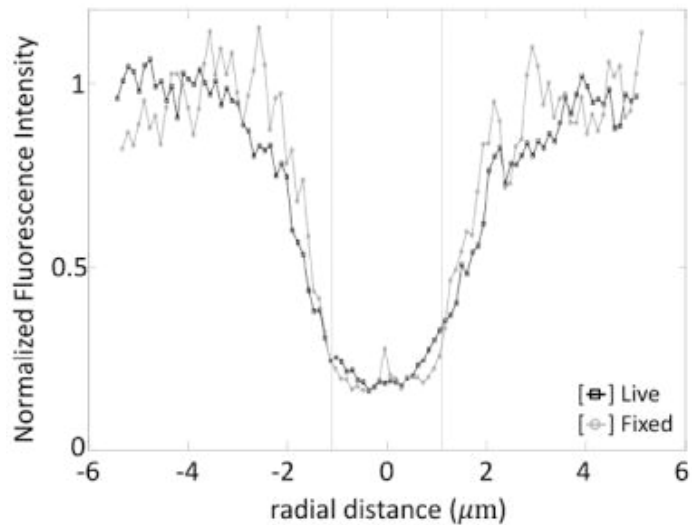
<https://www.youtube.com/watch?v=CfRvmtBdZ9I>

# Quantification of the diffusion coefficient

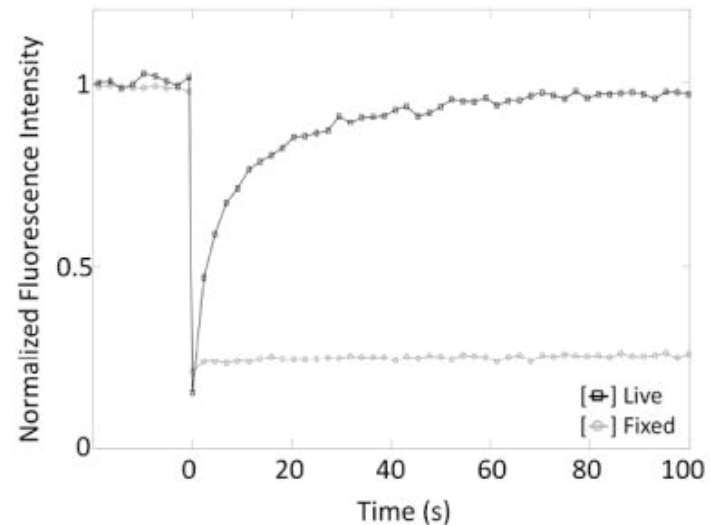
**A**



**B**

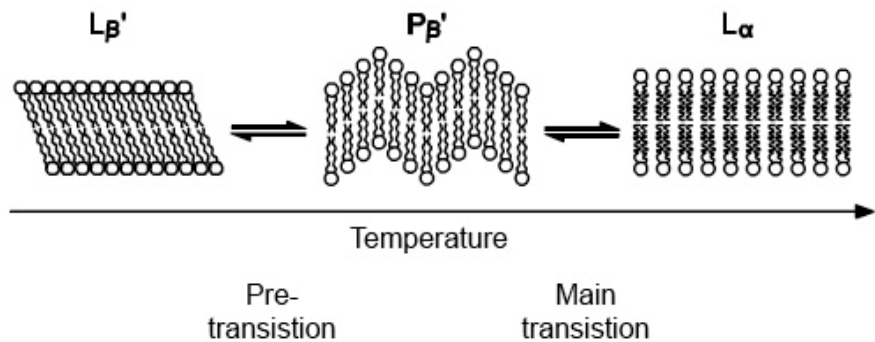


**C**



$$D_{rh} = 0.224 \frac{r_n^2}{\tau_{1/2}}$$

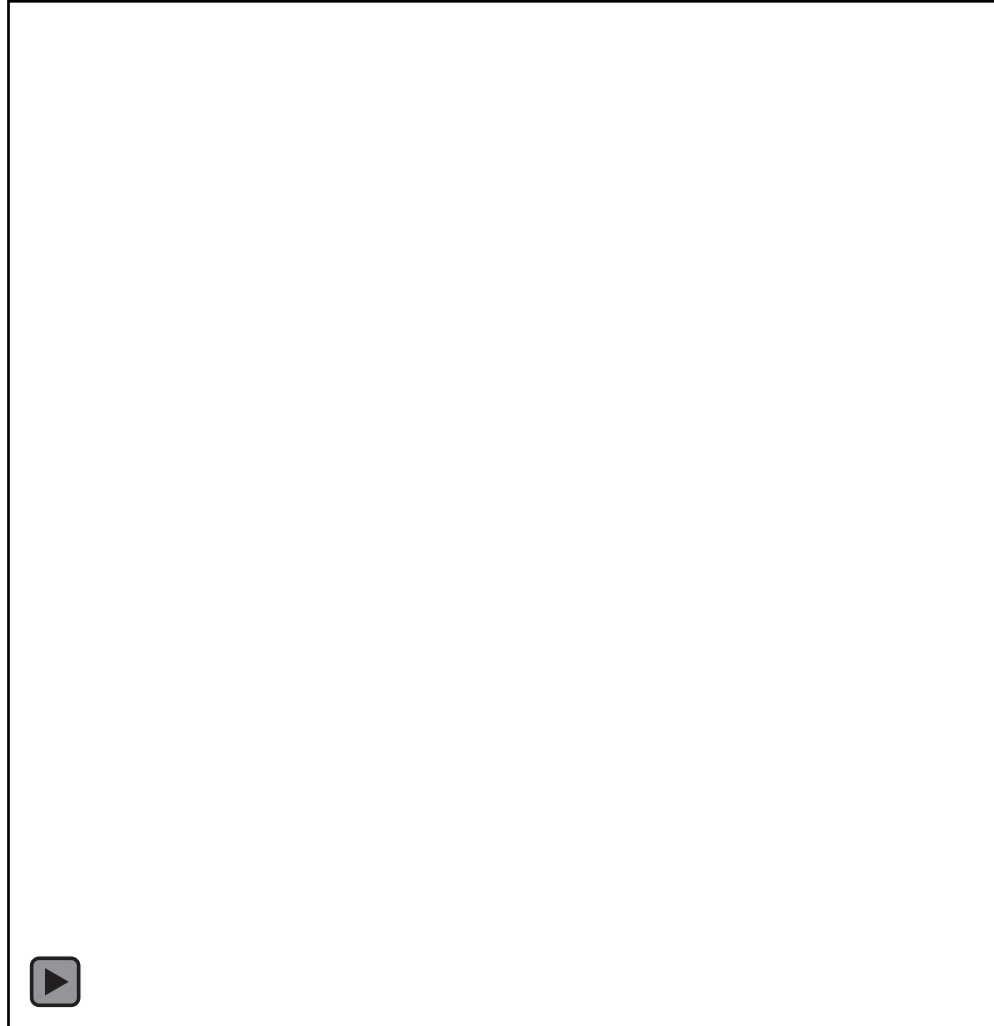
# Phase transition



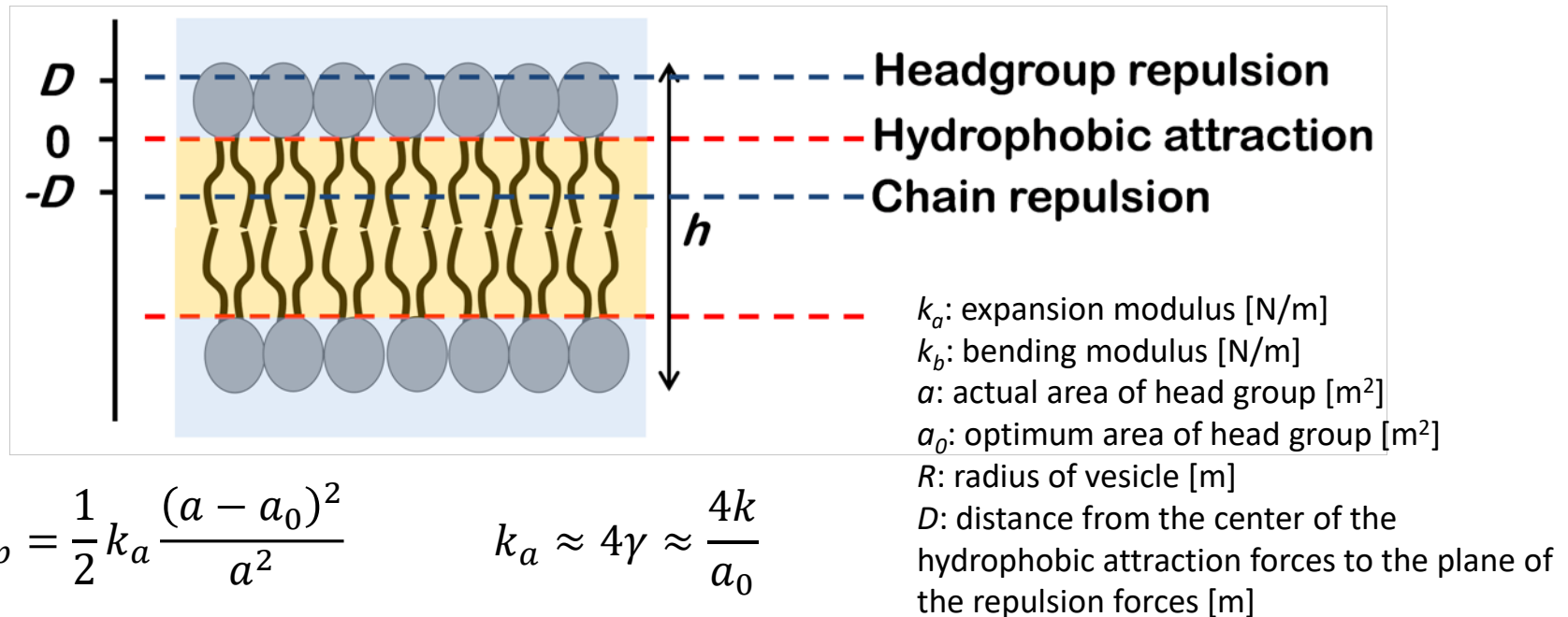
<https://avantilipids.com/tech-support/physical-properties/phase-transition-temps/>

|            | Subgel ( $L_c$ ) | Gel ( $L_{\beta'}$ ) | Ripple ( $P_{\beta'}$ ) | Fluid ( $L_{\alpha}$ ) |
|------------|------------------|----------------------|-------------------------|------------------------|
| Experiment |                  |                      |                         |                        |
| DPD        |                  |                      |                         |                        |
| MARTINI    |                  |                      |                         |                        |

# Vesicles can fluctuate



# Mechanical properties of membranes






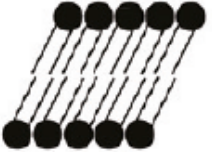
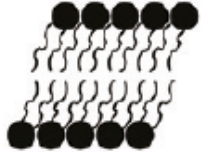
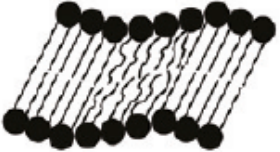

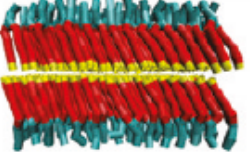
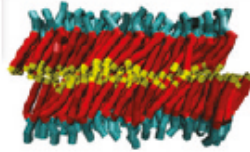
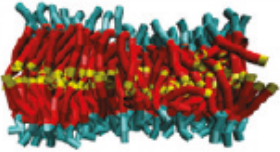
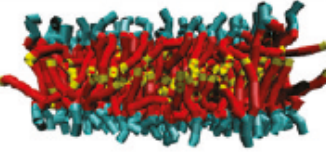
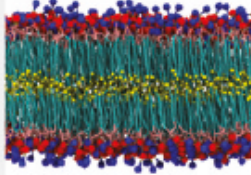
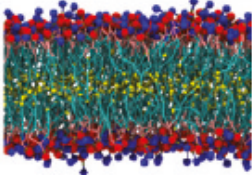
$$E_{comp} = \frac{1}{2} k_a \frac{(a - a_0)^2}{a^2} \quad k_a \approx 4\gamma \approx \frac{4k}{a_0}$$

Assuming  $\gamma$  is between 20 and 50 mN/m,  $k_a$  ranges from 80 to 200 mN/m.

$$E_{bend} = \frac{1}{2} \frac{k_b}{R^2} \quad k_b = -4\gamma h D$$

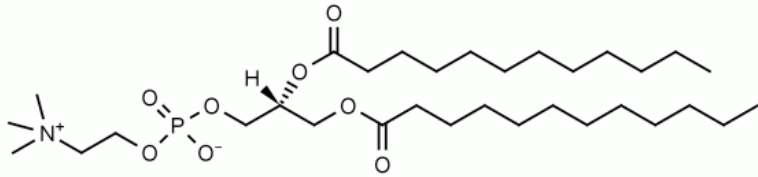
$k_b$  is typically 1-20  $k_B T$

# Phases of lipids in liposomes

|            |  |  |  |   |
|------------|---|---|---|---|
|            | Subgel ( $L_c$ )  | Gel ( $L_{\beta'}$ )  | Ripple ( $P_{\beta'}$ )   | Fluid ( $L_{\alpha}$ )  |
| Experiment |  |   |  |  |
| DPD        |  |   |  |  |
| MARTINI    |  |   |  |   |

M. Rodgers, J. Sorensen, F. J. M. de Meyer, B. Schiott and B. Smit, *J. Phys. Chem. B*, 2012, **116**, 1551-1569

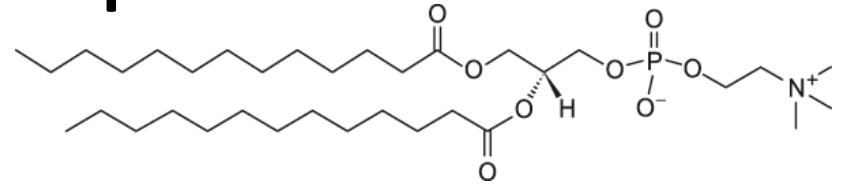
# Phospholipids



12:0 PC (DLPC)

1,2-dilauroyl-*sn*-glycero-3-phosphocholine

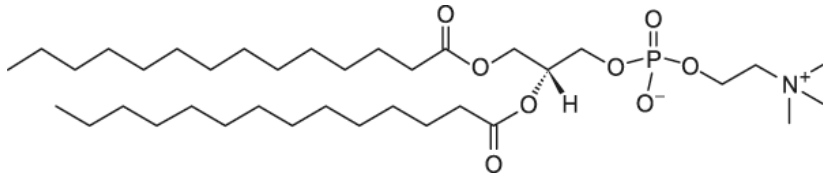
$$T_m \approx -2 \text{ }^\circ\text{C}$$



13:0 PC

1,2-ditridecanoyl-*sn*-glycero-3-phosphocholine

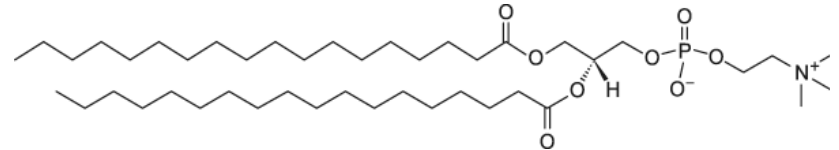
$$T_m \approx 14 \text{ }^\circ\text{C}$$



14:0 PC (DMPC)

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine

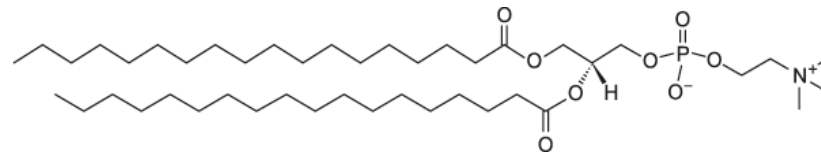
$$T_m \approx 24 \text{ }^\circ\text{C}$$



16:0 PC (DPPC)

1,2-distearoyl-*sn*-glycero-3-phosphocholine

$$T_m \approx 41 \text{ }^\circ\text{C}$$



18:0 PC (DSPC)

1,2-distearoyl-*sn*-glycero-3-phosphocholine

$$T_m \approx 55 \text{ }^\circ\text{C}$$

# Transition temperatures

## Phase Transition Temperatures for Saturated Diacyl Phospholipids

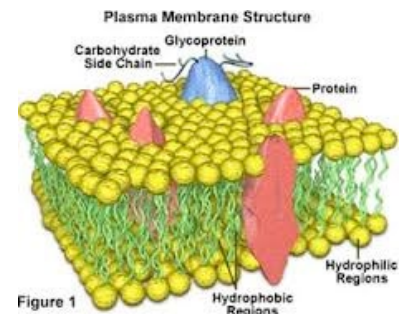
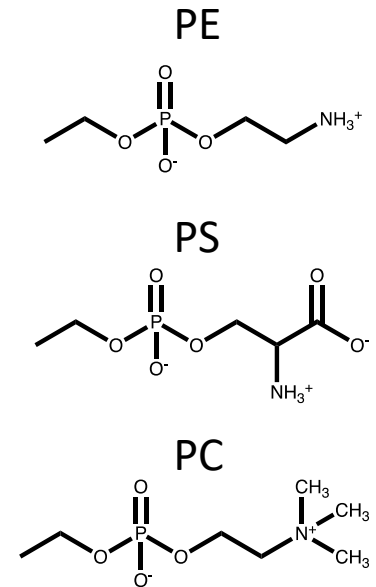
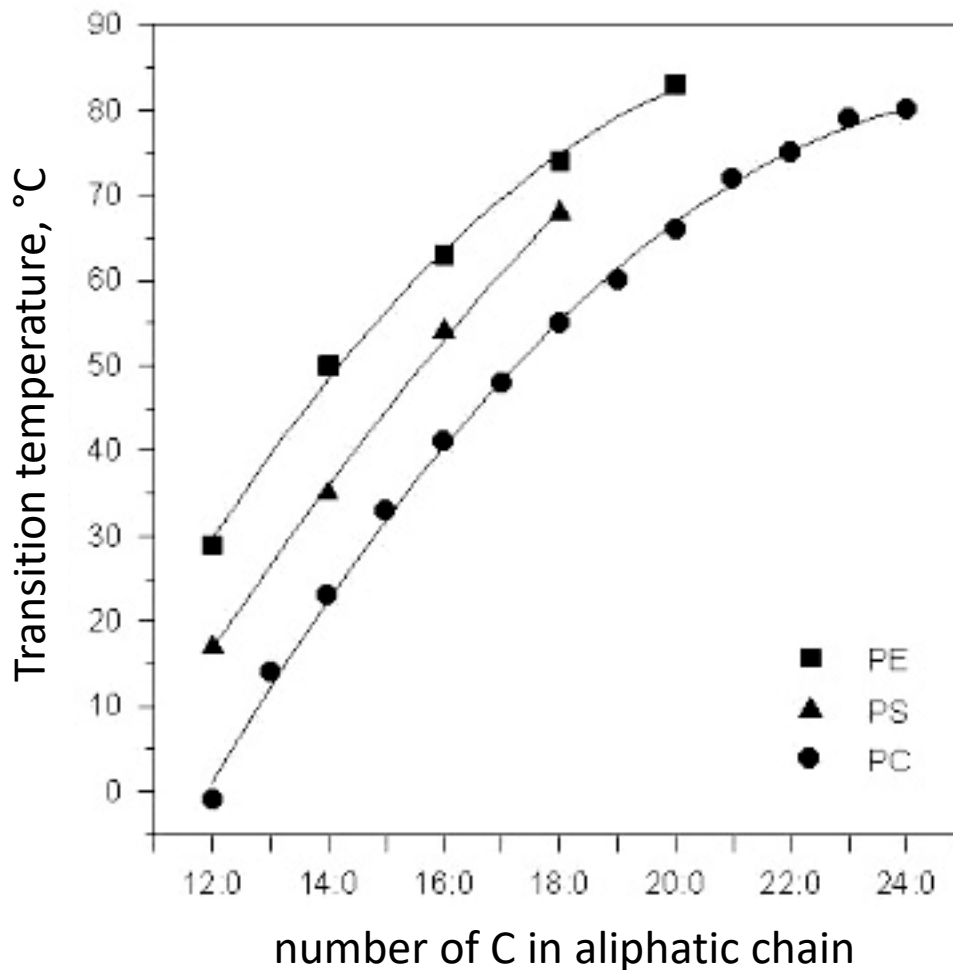
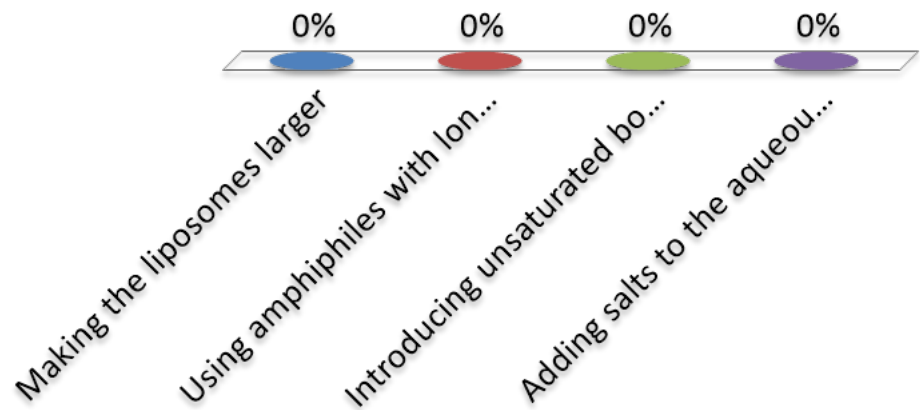


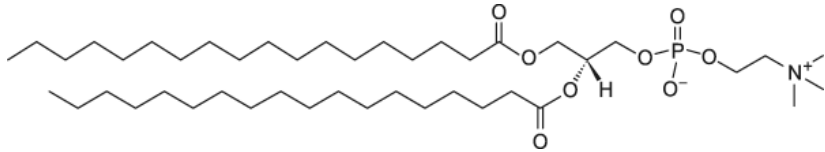
Figure 1

How can the transition temperature of lipids contained in liposomes be lowered?

- A. Making the liposomes larger
- B. Using amphiphiles with longer hydrophobic chains
- C. Introducing unsaturated bonds
- D. Adding salts to the aqueous solution



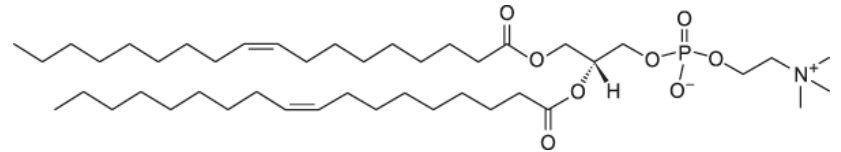
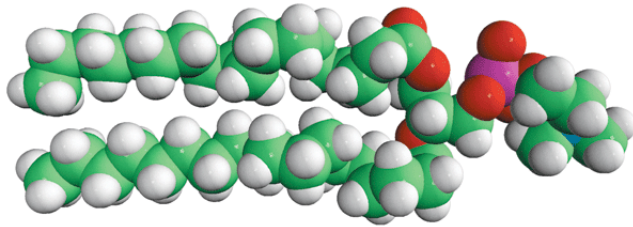
# Phospholipids



18:0 PC (DSPC)

1,2-distearoyl-*sn*-glycero-3-phosphocholine

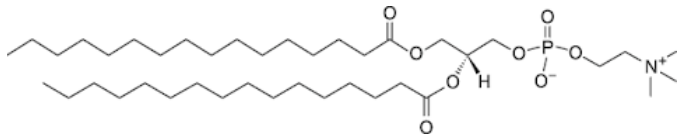
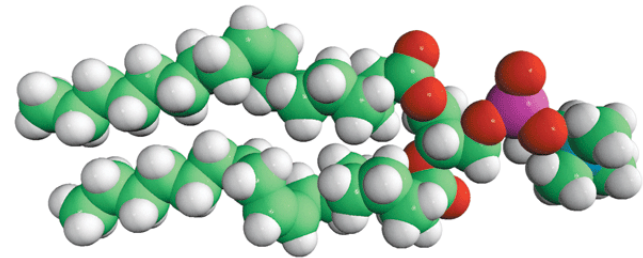
$T_m \approx 55 \text{ }^\circ\text{C}$



18:1 PC (DOPC)

1,2-dioleoyl-*sn*-glycero-3-phosphocholine

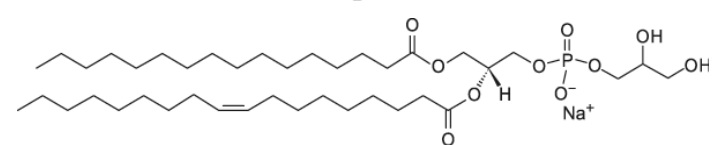
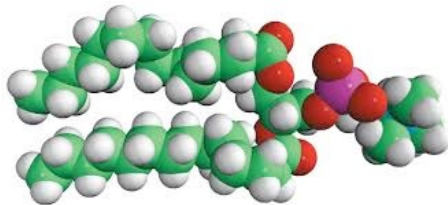
$T_m \approx -17 \text{ }^\circ\text{C}$



16:0 PC (DPPC)

dipalmitoylphosphatidylcholine

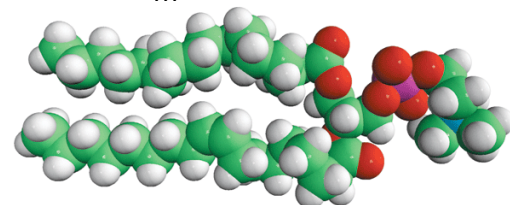
$T_m \approx 41 \text{ }^\circ\text{C}$



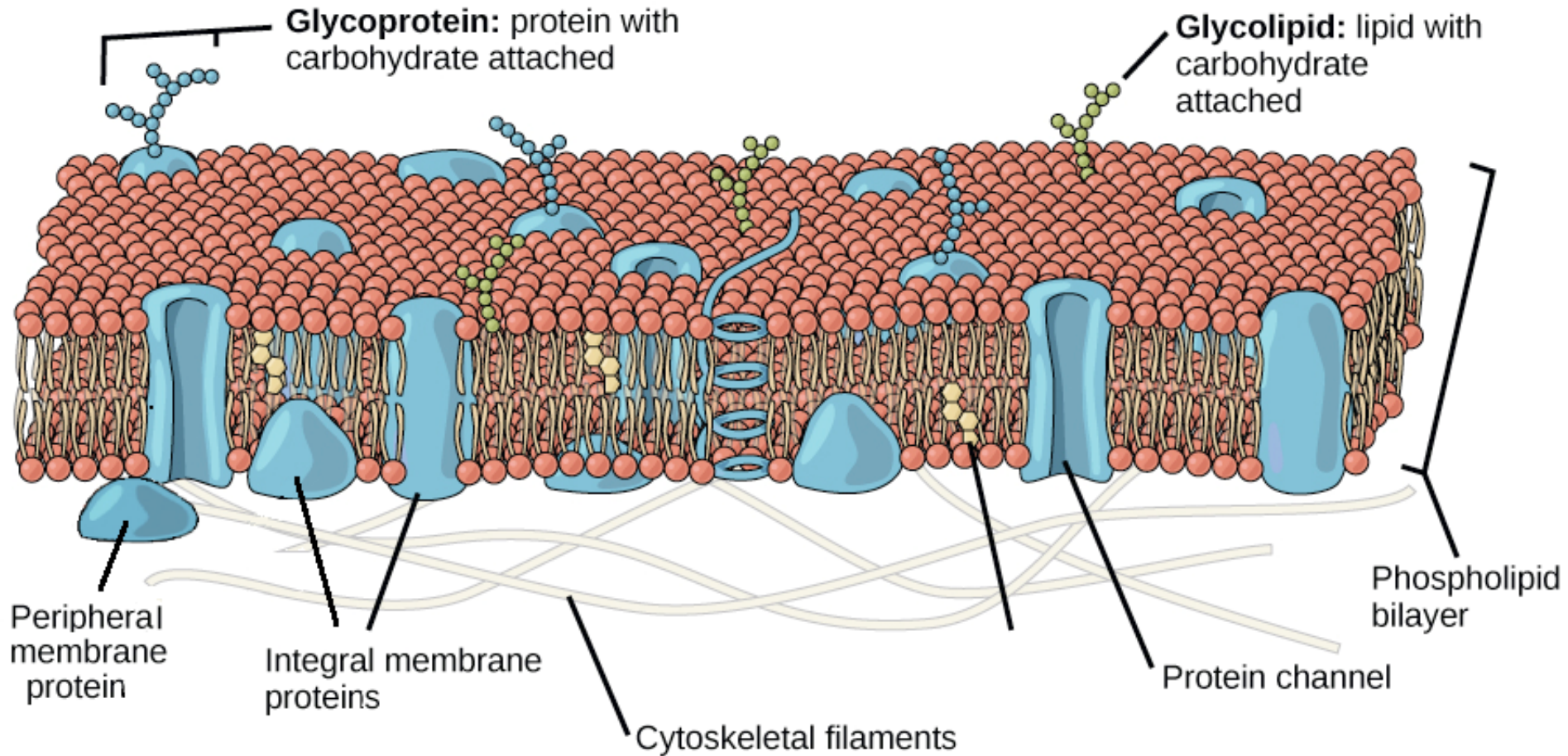
16:0-18:1 PC (POPC):

1-Palmitoyl-2-Oleoyl-*sn*-Glycero-3-Phosphocholine

$T_m \approx -2 \text{ }^\circ\text{C}$



# Cell membranes

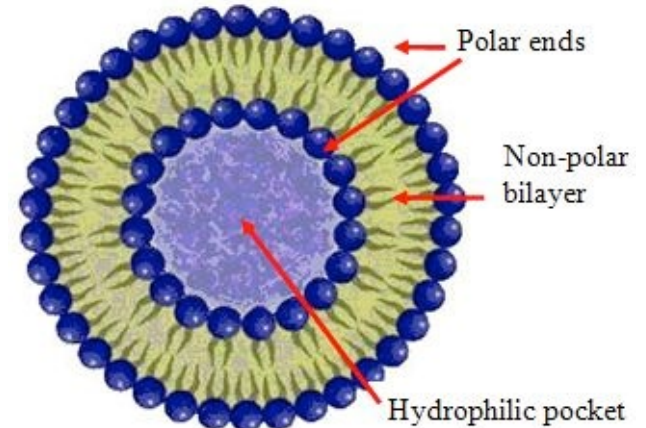
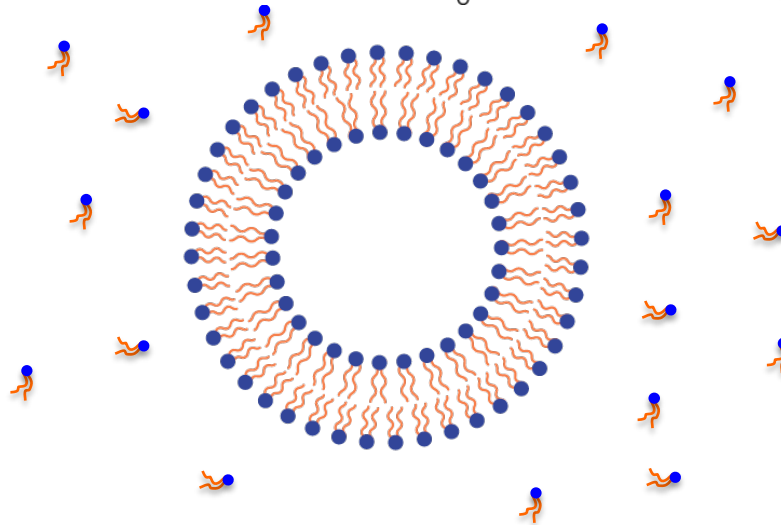
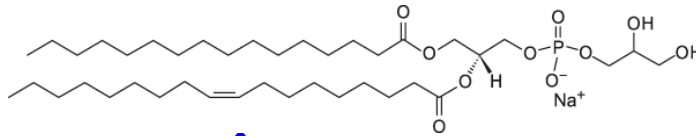


# Stability of liposomes

Liposomes are often made from phospholipids.

**Phospholipids** have usually two hydrophobic chains connected to one head group.

$$\alpha = \frac{v}{a_0 l_c} \approx 1$$



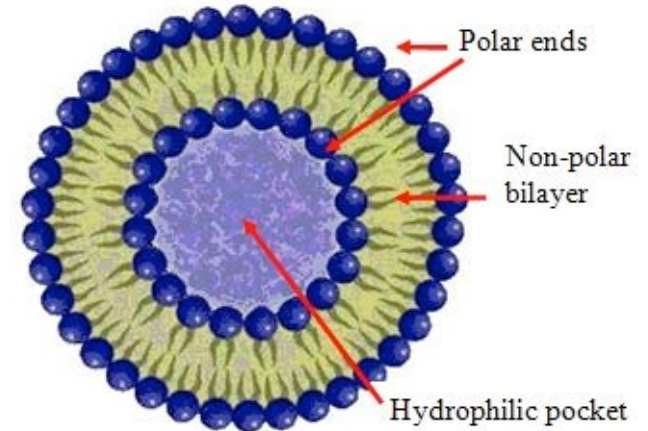
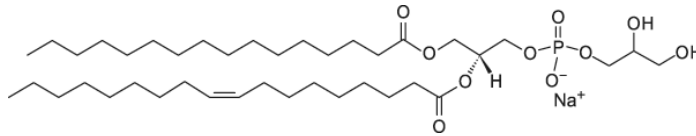
$v$ : volume of hydrocarbon chain [m<sup>3</sup>]  
 $a_0$ : optimal surface area of amphiphile [m<sup>2</sup>]  
 $l_c$ : critical chain length [m]

# Stability of liposomes

Liposomes are often made from phospholipids.

**Phospholipids** have usually two hydrophobic chains connected to one head group.

$$\alpha = \frac{v}{a_0 l_c} \approx 1$$



$$CMC \approx \exp \left[ -\frac{\mu_1^0 - \mu_N^0}{k_B T} \right]$$

Phospholipids have a much lower CMC than molecules with only one hydrocarbon chain such that less free phospholipids are in solution.

The probability for a molecule to leave the bilayer  $\propto e^{-\frac{\Delta E}{k_B T}}$

where  $\Delta E = \mu_1^0 - \mu_N^0$

$v$ : volume of hydrocarbon chain [m<sup>3</sup>]

$a_0$ : optimal surface area of amphiphile [m<sup>2</sup>]

$l_c$ : critical chain length [m]

# Lifetime of molecule in aggregates

The mean lifetime of molecule in bilayer:  $\tau_R = \frac{\tau_0}{e^{-\frac{\Delta E}{k_B T}}}$

The probability that molecules that hit the interface will leave the bilayer.

$\tau_R$ : time for an exchange to take place [s]

$\tau_0$ : motional correlation time for amphiphiles in micelles or bilayers [s]

$\tau_0 \approx 10^{-9} - 10^{-7} \text{ s}$

$\mu$ : chemical potential [J/molecule]

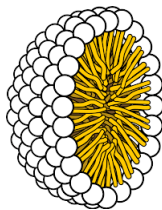
$N$ : number of molecules contained in the aggregate [-]

$P$ : constant that depends on the dimension of the aggregate [-]

$$\tau_R = \frac{\tau_0}{e^{-\frac{\mu_1^0 - \mu_N^0}{k_B T}}} \approx \frac{55\tau_0}{CMC}$$

For micelles

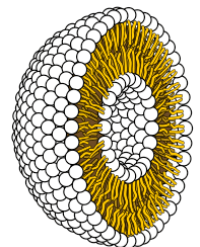
$$\tau_R \approx 10^{-4} \text{ s}$$



For vesicles

$$\tau_R \approx 10^4 \text{ s}$$

$$\tau_R \approx 3 \text{ h}$$



How much does the exchange rate decrease if one CH<sub>2</sub> group is added to the hydrophobic tail of amphiphiles containing 8 C atoms in its chain?

- A. 10%
- B. factor 2
- C. factor 7
- D. factor 10
- E. factor 100

CMC of amphiphile with 8 C atoms:  $2.2 \times 10^{-7}$  M

$r \approx 0.2$  nm

CH<sub>2</sub>-CH<sub>2</sub> bond length = 0.154 nm

angle between C-C bonds: 109°

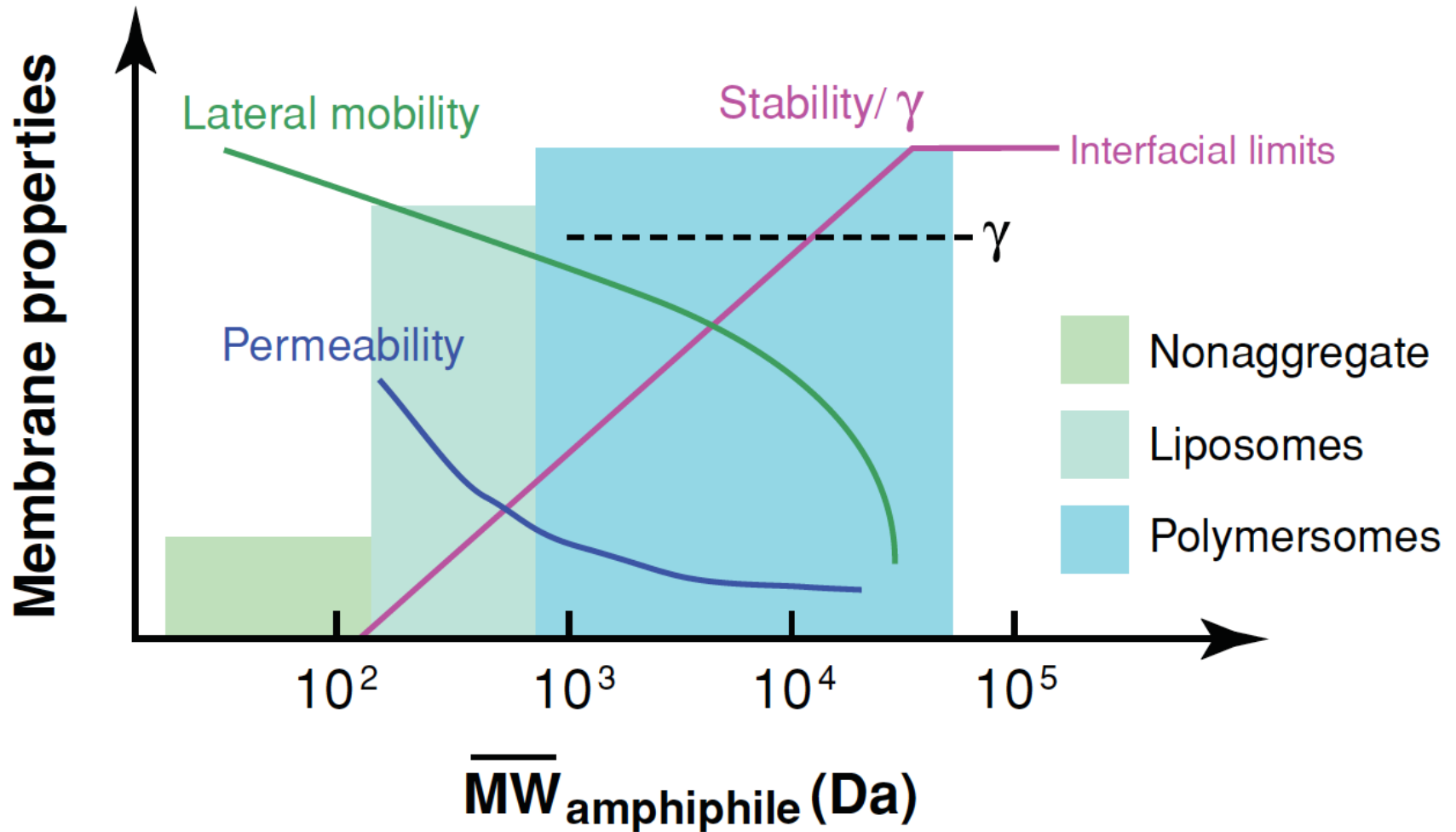
$\gamma = 50$  mJ/m<sup>2</sup>

$k_B = 1.38 \times 10^{-23}$  J/K

$T = 298$  K

$$CMC \approx e^{-\alpha} \approx e^{-\frac{\pi r l \gamma}{k_B T}}$$

# Membrane properties

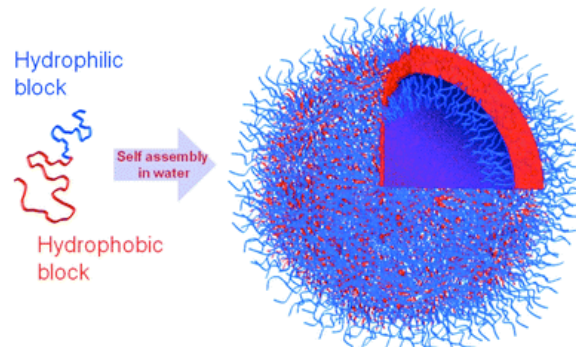


# Outline

- Introduction: Vesicles
- Liposomes
- **Polymersomes**
- Vesicle production
- Characterization
- Applications

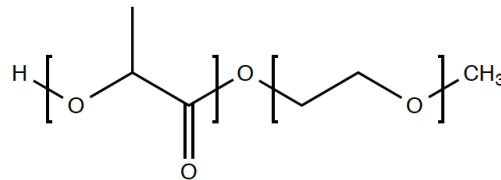
# Polymersomes

**Polymersomes** are vesicles made from block-copolymers.



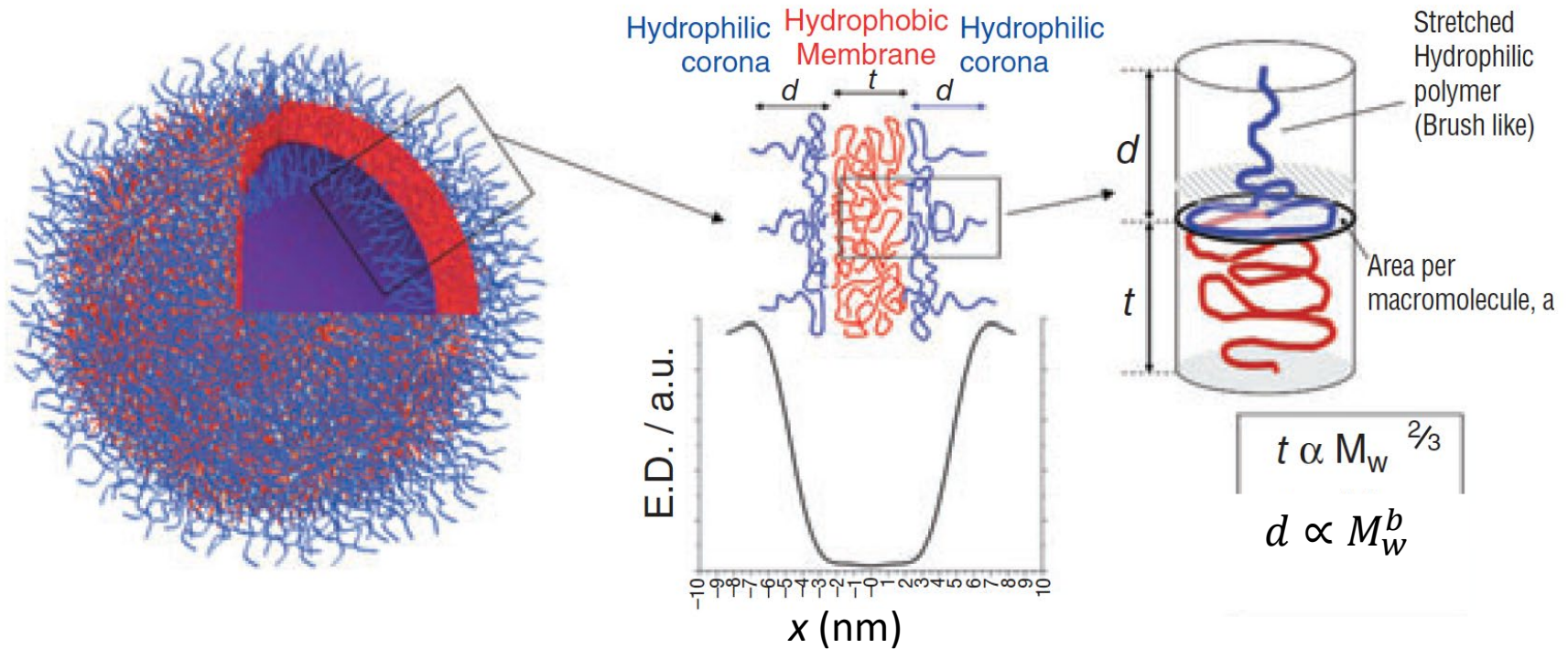
LoPresti, C.; Lomas, H.; Massignani, M.; Smart, T.; Battaglia, G.  
*Journal of Materials Chemistry* **2009**, *19* (22), 3576-3590.

Example of a block-copolymer:



# Polymersomes

Polymersomes are vesicles made of block-copolymers.



$b = 0.5$ : random Gaussian coil  
 $b = 1$ : fully stretched chain  
 $b \approx 0.55$ : experimentally determined value

$$d_{\text{membrane}} \approx 5-18 \text{ nm}$$

# Block-copolymer mobility

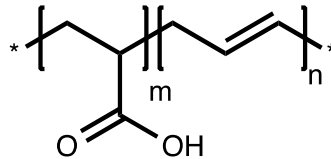
The mobility of block-copolymers in polymersome membranes is more than one order of magnitude lower than that of phospholipids in liposome membranes.

## Comparison of diffusion coefficients in vesicles

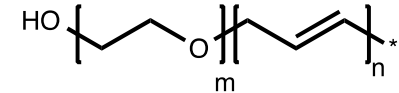
block-copolymers:  $D \approx 0.1 \mu\text{m}^2\text{s}^{-1}$

phospholipids:  $D \approx 1 \mu\text{m}^2\text{s}^{-1}$

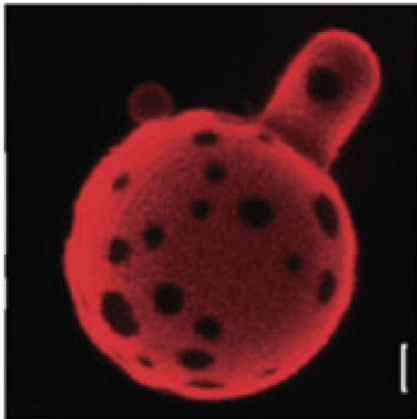
poly(acrylic acid)-  
poly(butadiene)  
(PAA-PBD)



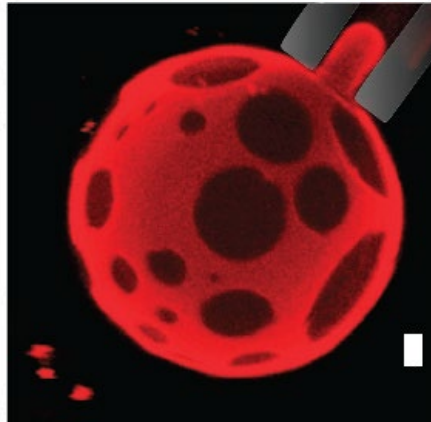
poly(ethylene oxide)-  
poly(butadiene)  
(PEO-PBD)



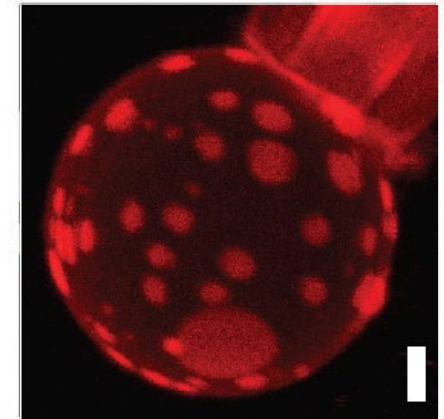
25% PAA-PBD 75% PEO-PBD



50% PAA-PBD 50% PEO-PBD

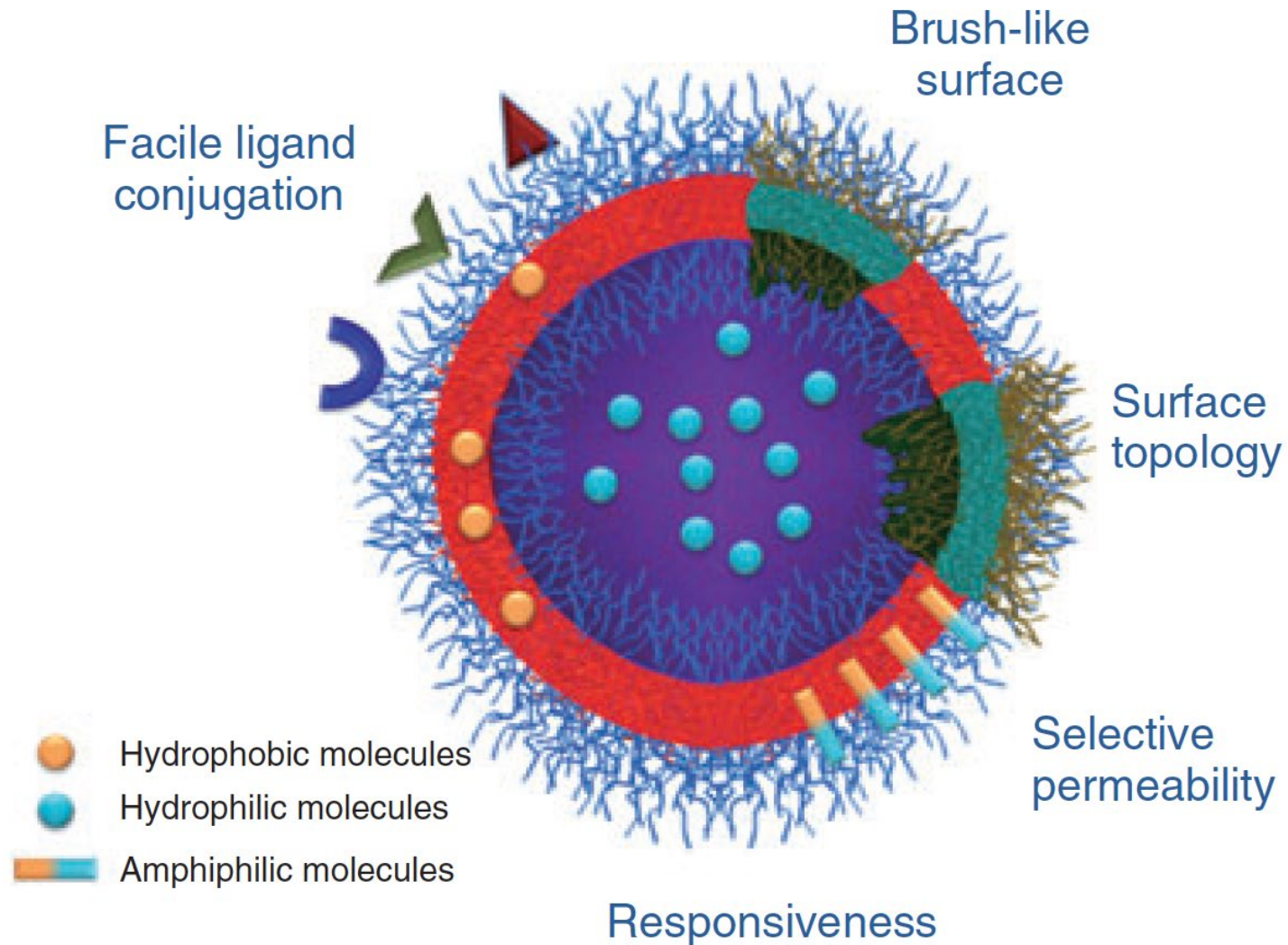


75% PAA-PBD 25% PEO-PBD



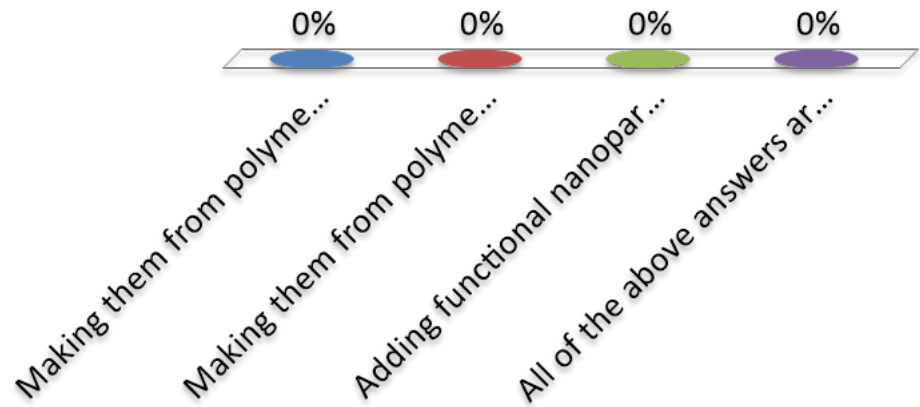
scale bars: 2  $\mu\text{m}$

# Functionalization of Polymersomes

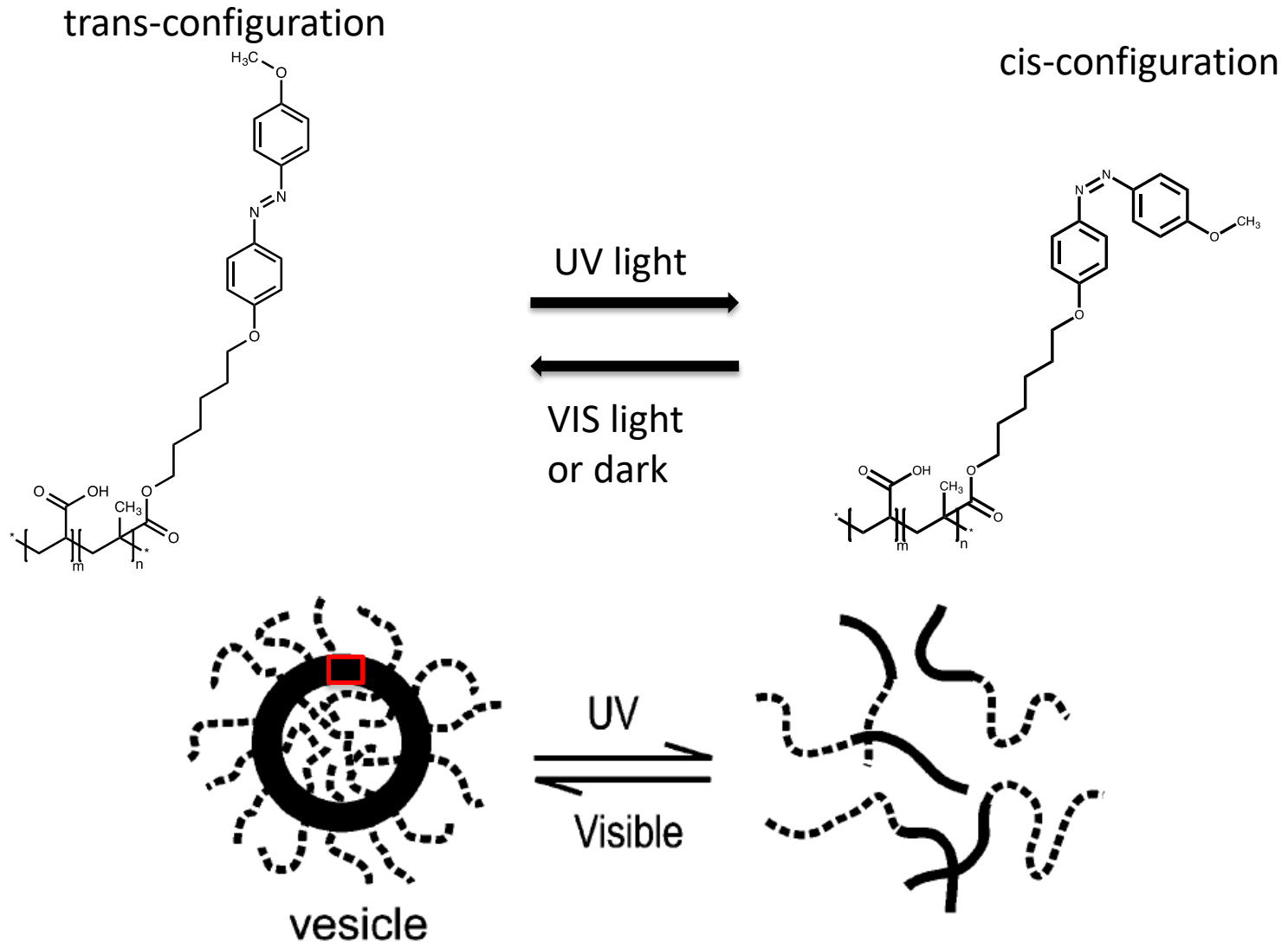


# How can polymersomes be made responsive?

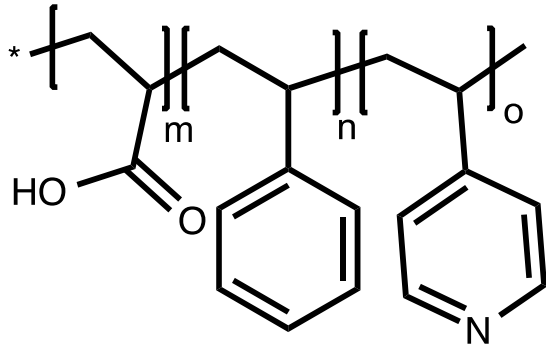
- A. Making them from polymers that have responsive hydrophilic blocks.
- B. Making them from polymers that have responsive hydrophobic blocks.
- C. Adding functional nanoparticles into their membranes.
- D. All of the above answers are correct.



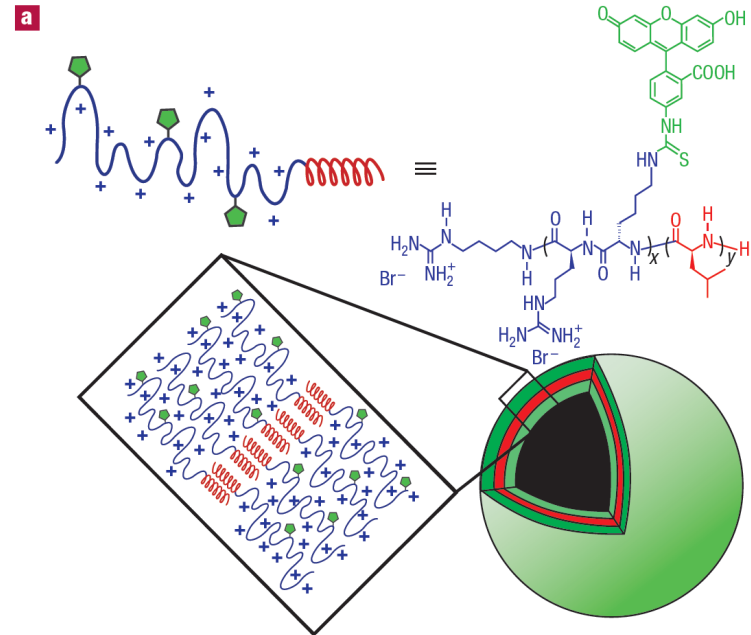
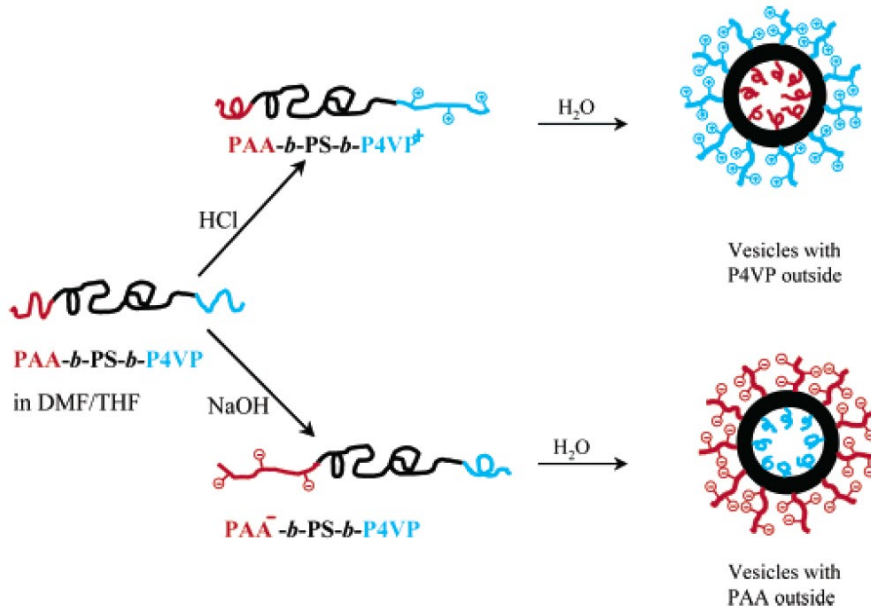
# UV-responsive polymersomes



# pH-Responsive polymersomes



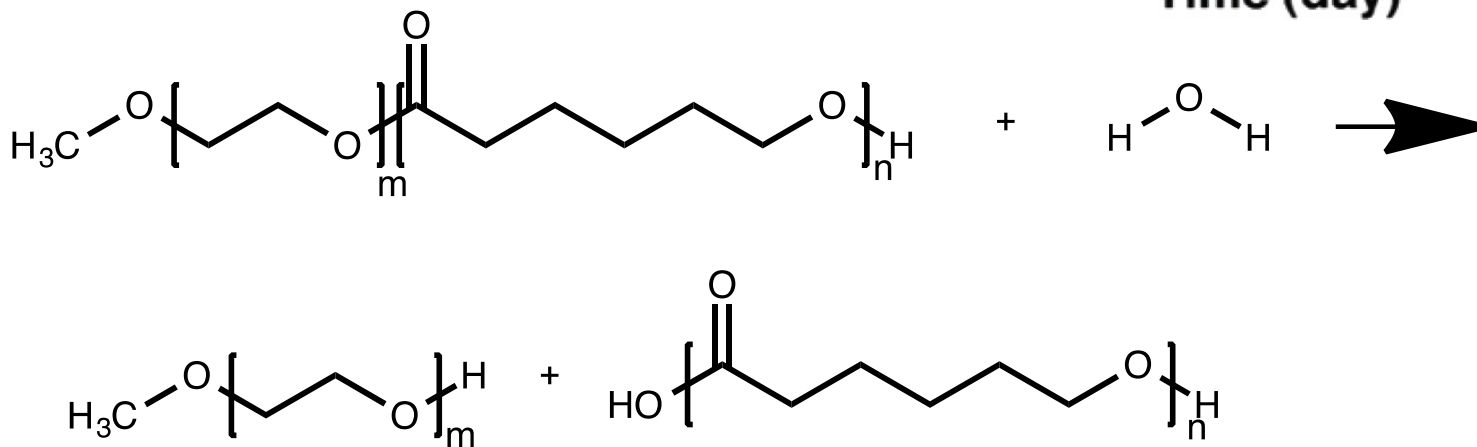
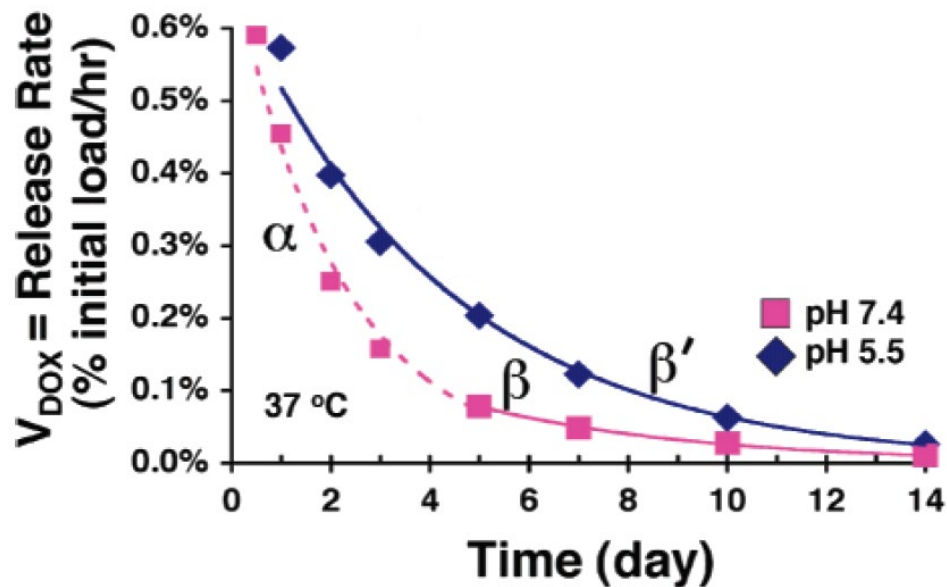
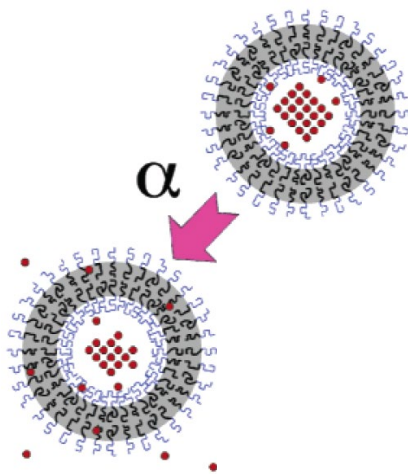
poly(acrylic acid)-polystyrene-poly(4-vinyl pyridine)



poly(arginine)-poly(L-leucine)

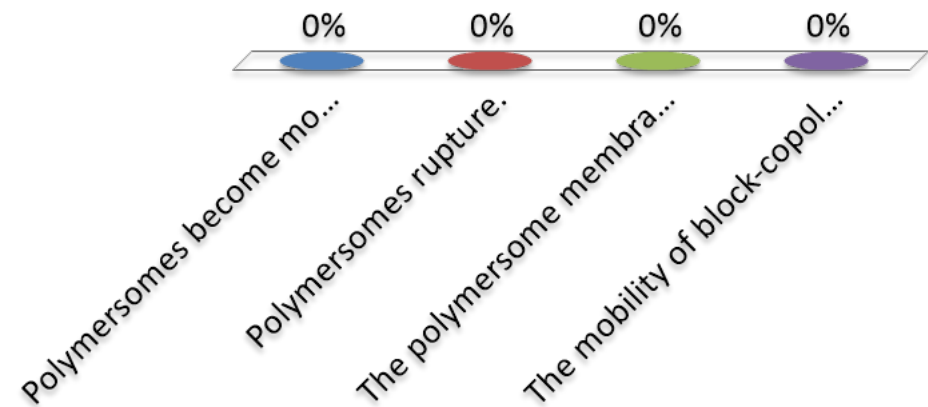
# Polymersomes that can undergo hydrolysis

lower pH  $\rightarrow$  hydrolysis



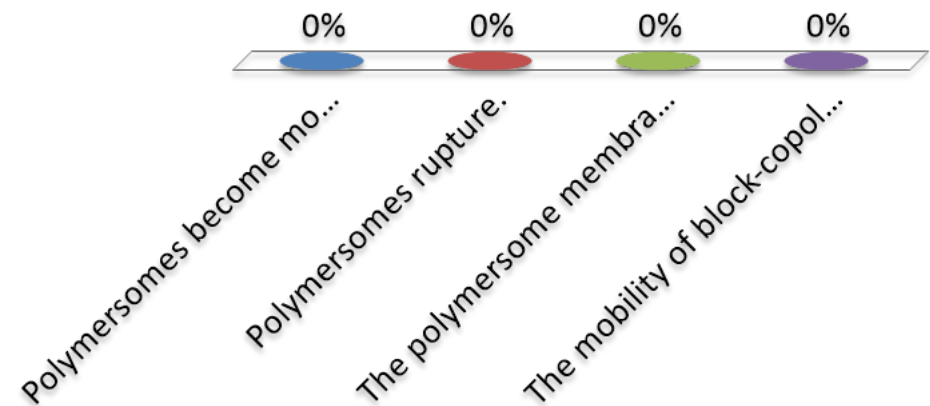
Polymersomes are made from block-copolymers whose hydrophilic block is temperature-responsive. What happens if the temperature is increased such that the hydrophilic block collapses?

- A. Polymersomes become more permeable but remain intact.
- B. Polymersomes rupture.
- C. The polymersome membrane becomes more rigid.
- D. The mobility of block-copolymers in the membrane increases.



Polymersomes contain very small domains that are made of block-copolymers whose hydrophilic block is temperature-responsive. What happens if the temperature is increased such that the hydrophilic blocks contained in the small domains collapse?

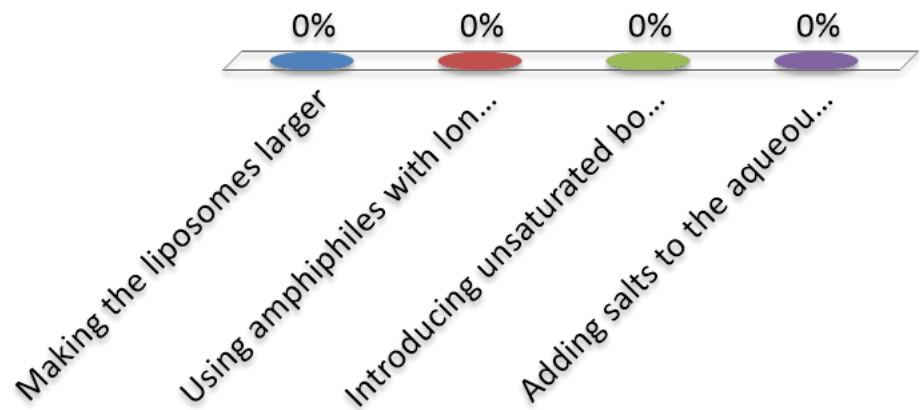
- A. Polymersomes become more permeable but remain intact.
- B. Polymersomes rupture.
- C. The polymersome membrane becomes more rigid.
- D. The mobility of block-copolymers in the membrane increases.





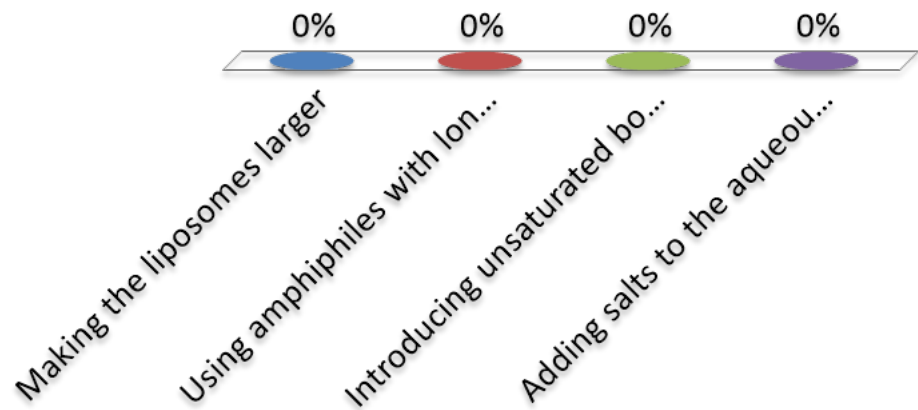
# What is a vesicle?

- A. A single-layered aggregation of amphiphilic molecules
- B. A bilayer structure formed from amphiphilic molecules
- C. A polymeric micelle
- D. A solid-state nanoparticle



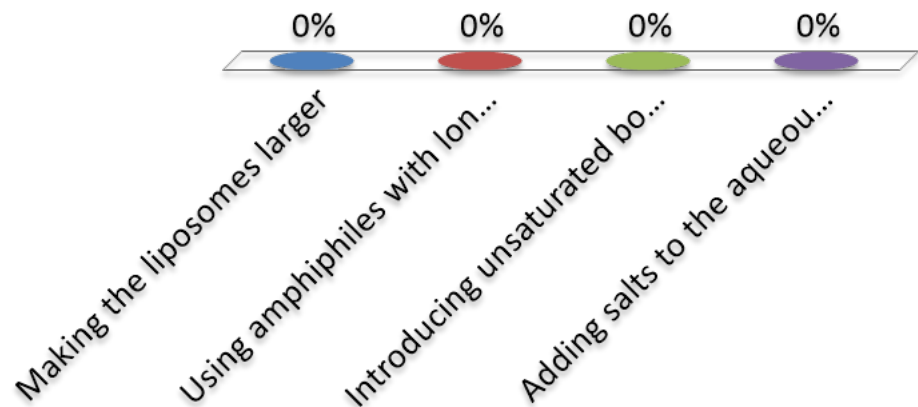
# Which of the following best describes a liposome?

- A. A vesicle made from lipids
- B. A vesicle made from block-copolymers
- C. A monolayer structure of amphiphilic molecules
- D. A surfactant-free colloidal system



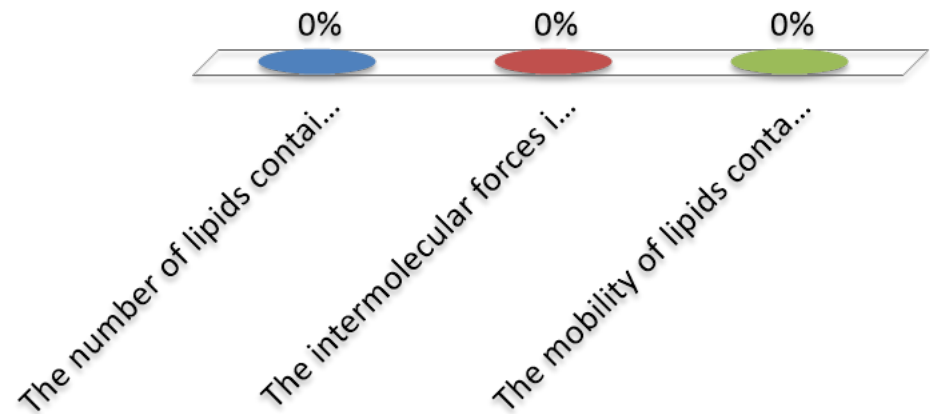
# What is the main difference between liposomes and polymersomes?

- A. Polymersomes are composed of lipids, while liposomes are composed of block-copolymers
- B. Polymersomes are more stable and mechanically robust compared to liposomes
- C. Liposomes are always larger than polymersomes
- D. Polymersomes are more permeable than liposomes



# Liposomes can heal defects much faster than polymersomes can. Why?

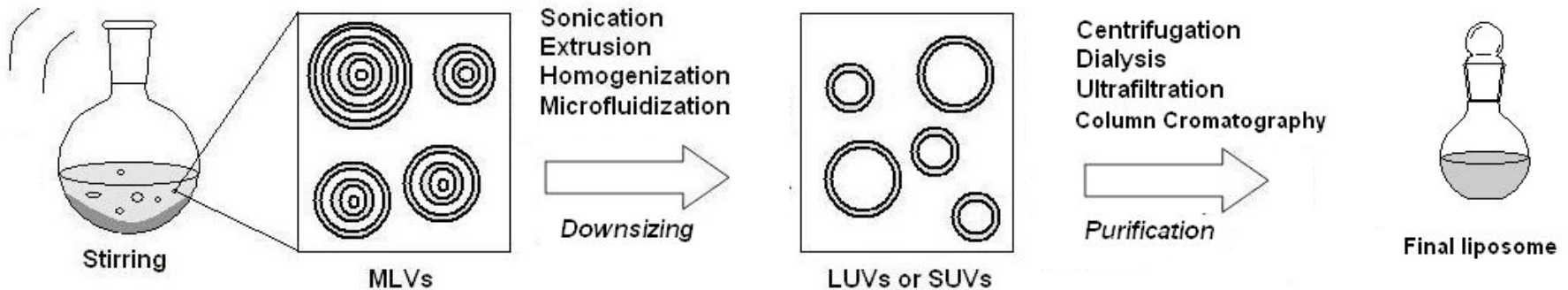
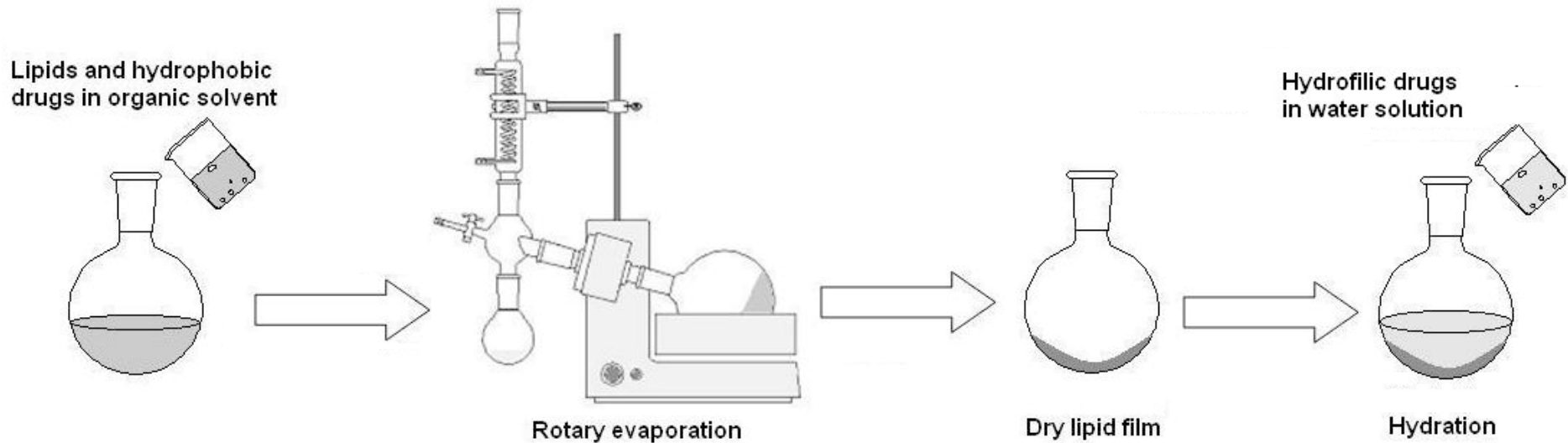
- A. The number of lipids contained in liposomes is larger than the number of block-copolymers contained in polymersomes
- B. The intermolecular forces in bilayers of liposomes are stronger than those in bilayers of polymersomes.
- C. The mobility of lipids contained in bilayers is higher than that of polymers.



# Outline

- Introduction: Vesicles
- Liposomes
- Polymersomes
- **Vesicle production**
- Characterization
- Applications

# Vesicle formation: Rehydration



# Sonication

1

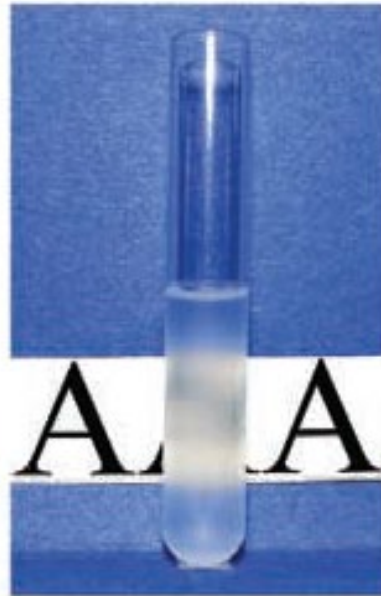


Lipid film  
No chloroform

Dissolve  
in 5 mM  
Tris pH 8,  
300  $\mu$ l SDS



2

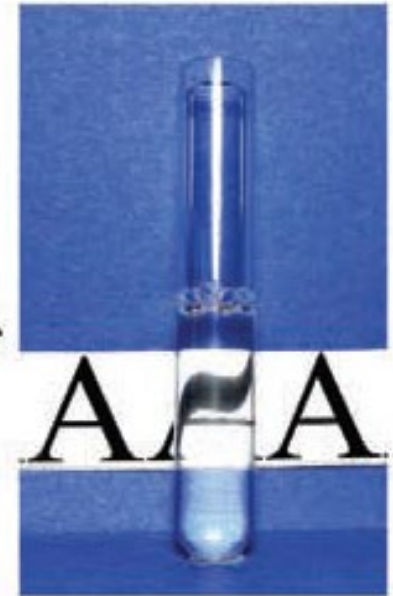


Translucent liposome

Bath  
sonicate  
10 min



3

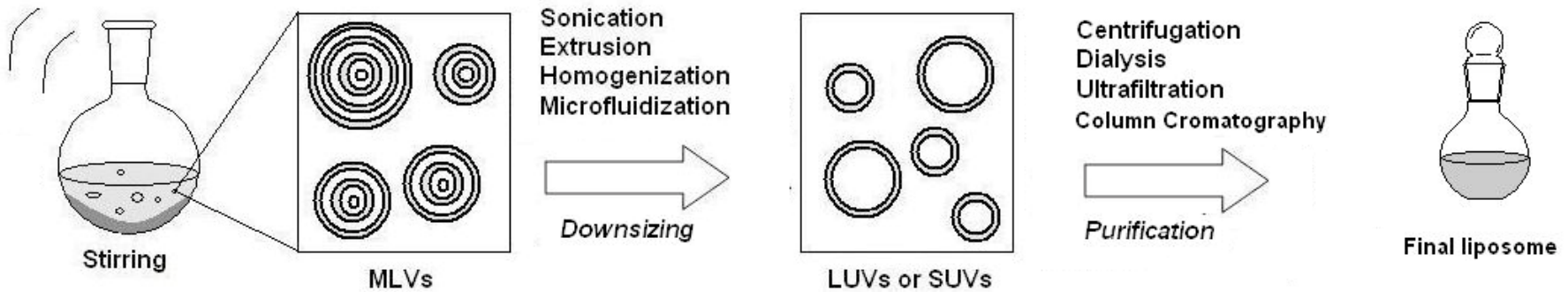
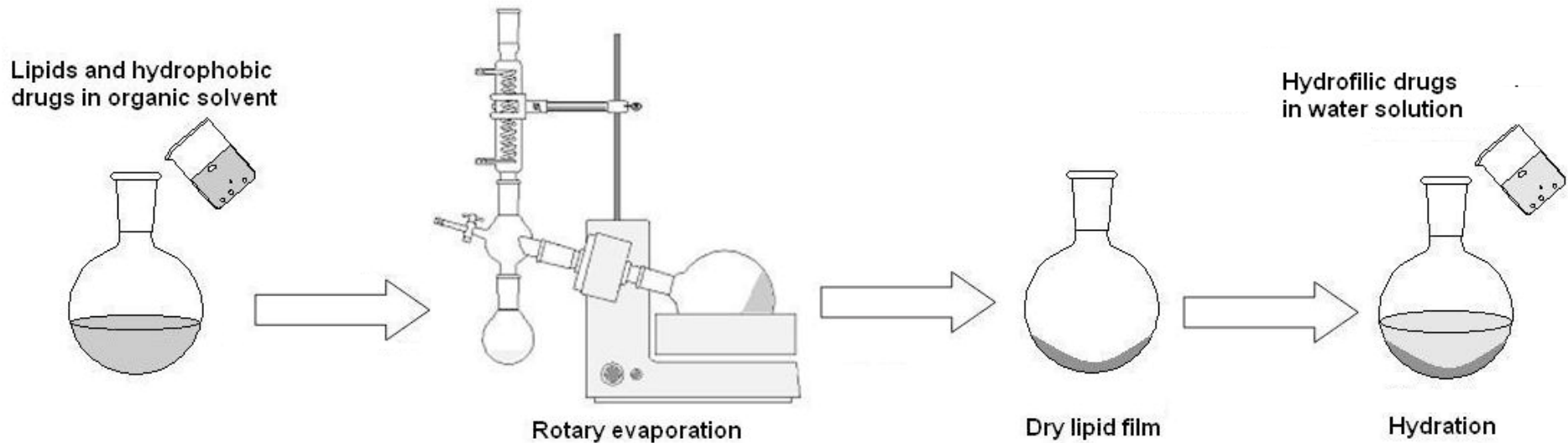


Clear liposome

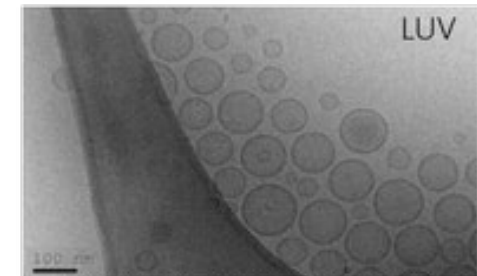
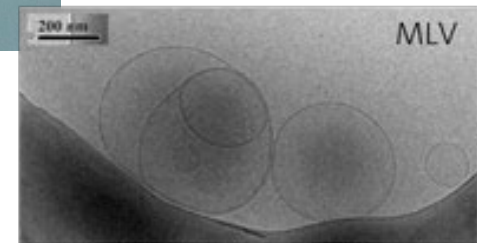
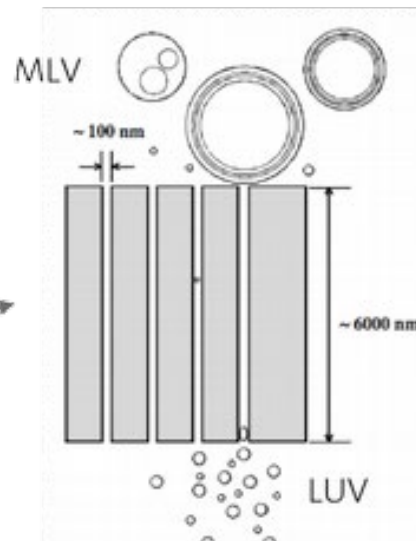
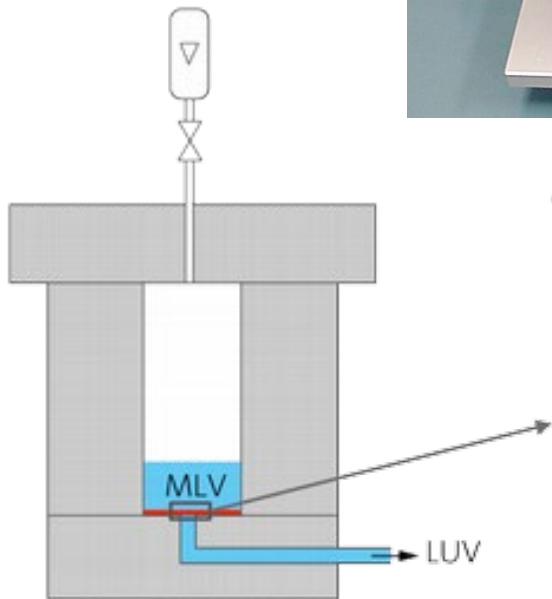
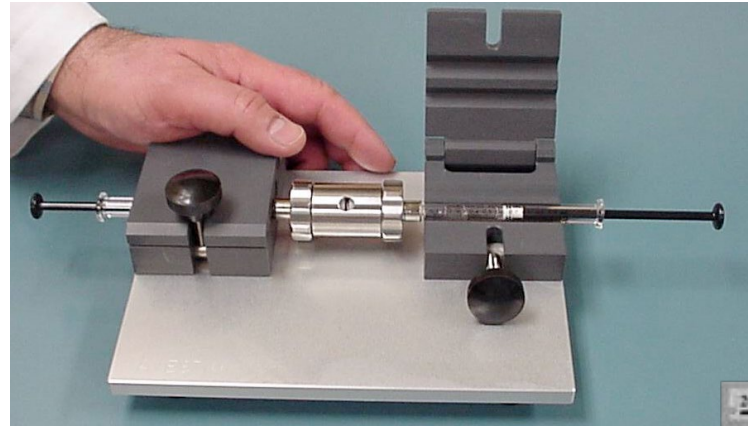
Das, N.; Murray, D. T.; Cross, T. A. *Nat. Protocols* **2013**, 8 (11), 2256-2270.

The vesicle diameter after sonication is between 50 nm and a few 100 nm.

# Vesicle formation: Rehydration



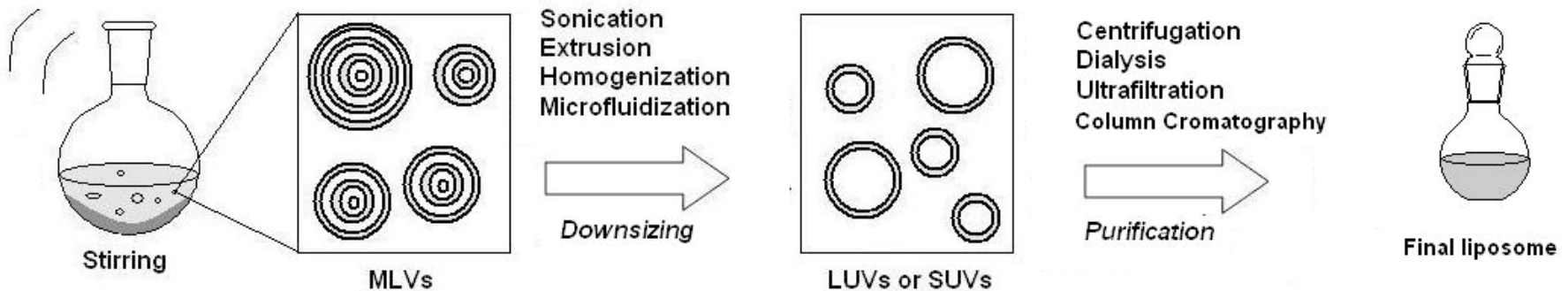
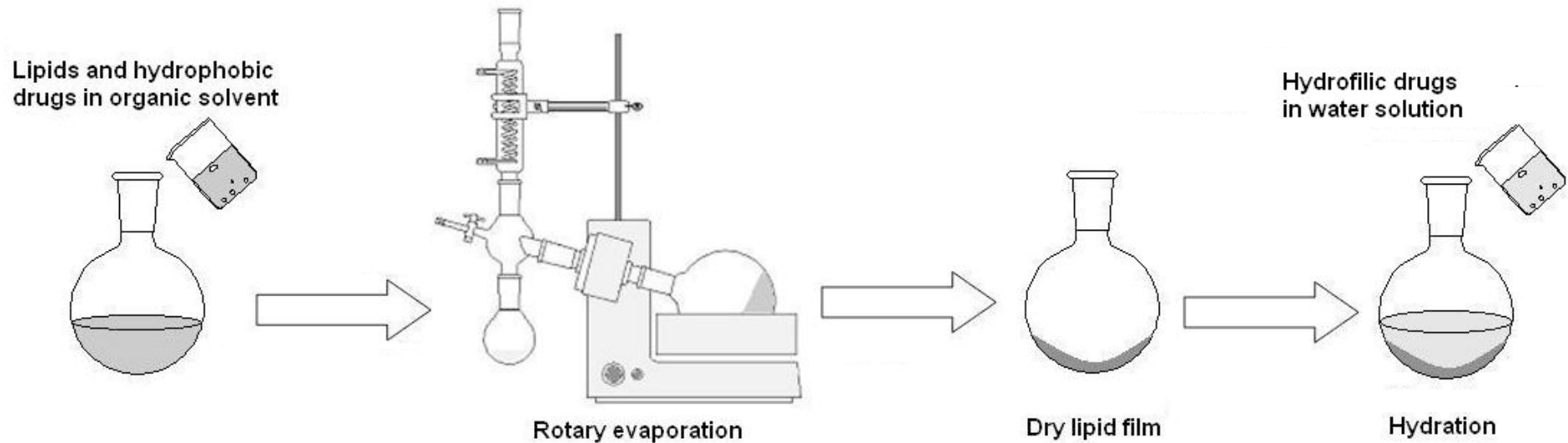
# Extrusion



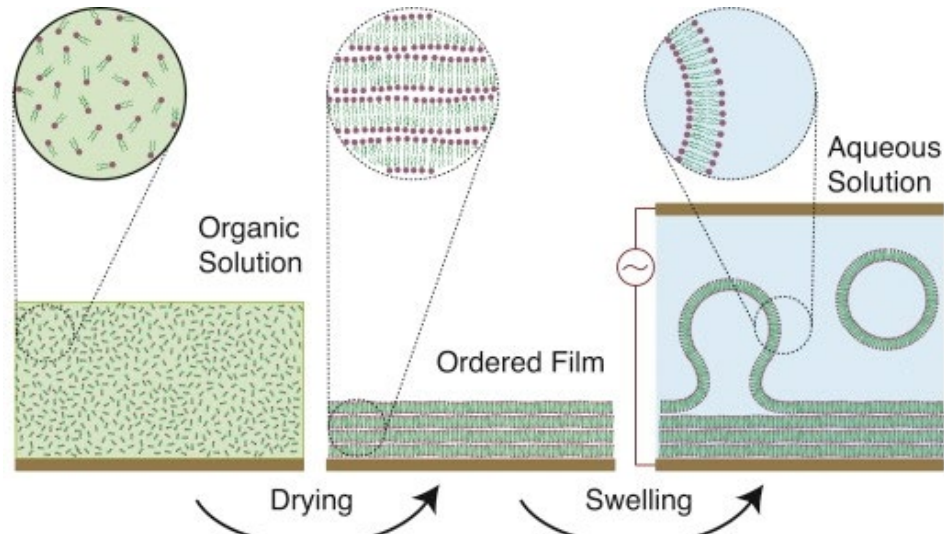
<http://www.ifnh.ethz.ch/vt/research/projects/engelh>

The vesicle diameter is similar to membrane pore size; it is usually between 50 nm to 500 nm.

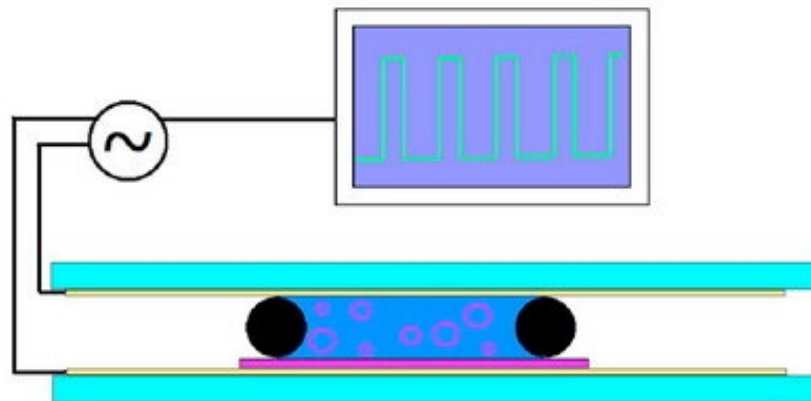
# Vesicle formation: Rehydration



# Electroformation



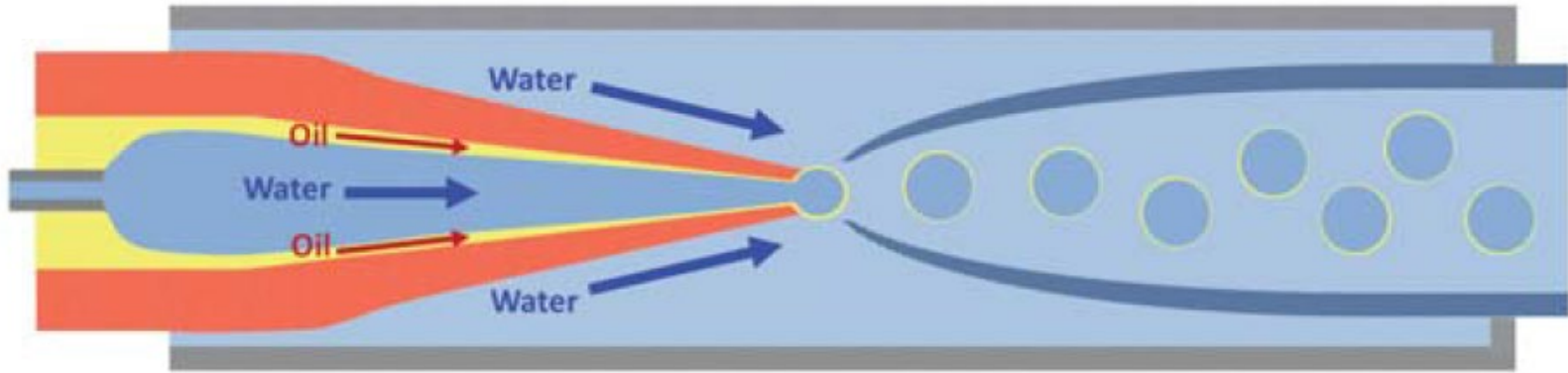
Mertins, O.; da Silveira, N. P.; Pohlmann, A. R.; Schroeder, A. P.; Marques, C. M. *Biophysical Journal* **2009**, 96 (7), 2719-2726.



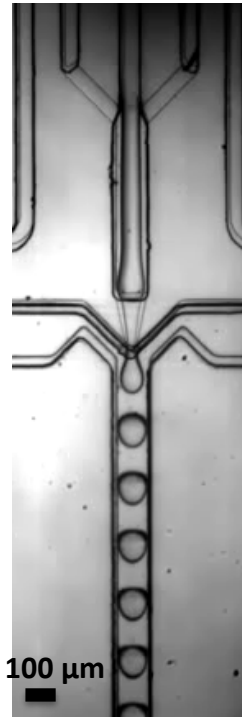
<http://www.rpgroup.caltech.edu/courses/PBL/lipids.htm>

The vesicle diameter ranges from a few up to a few 10s of  $\mu\text{m}$ .

# Microfluidics

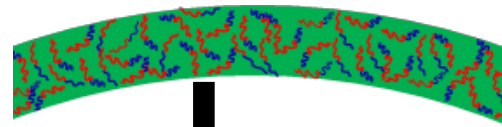
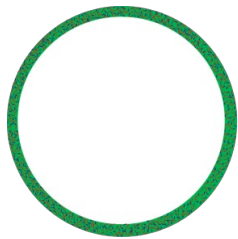


Kim, S.-H.; Kim, J. W.; Cho, J.-C.; Weitz, D. A. *Lab on a Chip* **2011**, *11* (18), 3162-3166.



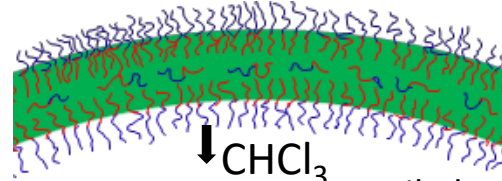
# Microfluidics

water/oil/water  
double emulsion



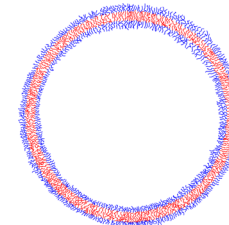
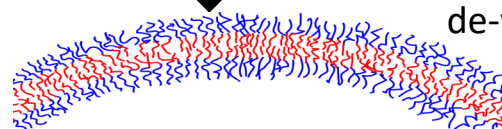
↑ CHCl<sub>3</sub>

molecules assemble at  
liquid/liquid interfaces

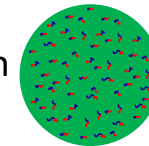


↓ CHCl<sub>3</sub>

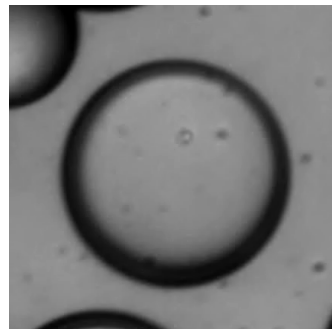
oil phase is enriched with  
hexane →  
de-wetting



vesicle



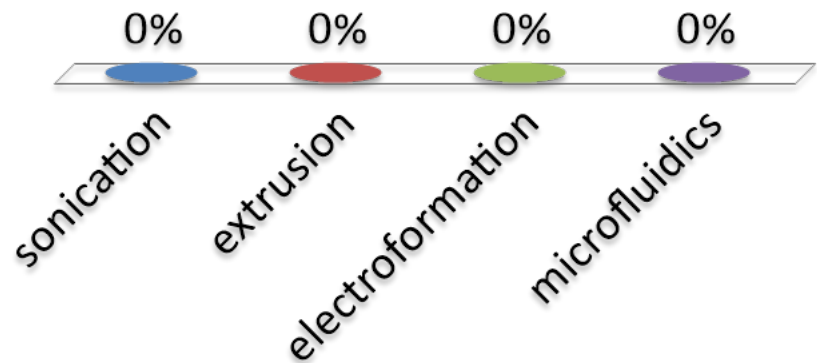
oil drop containing  
excessive molecules



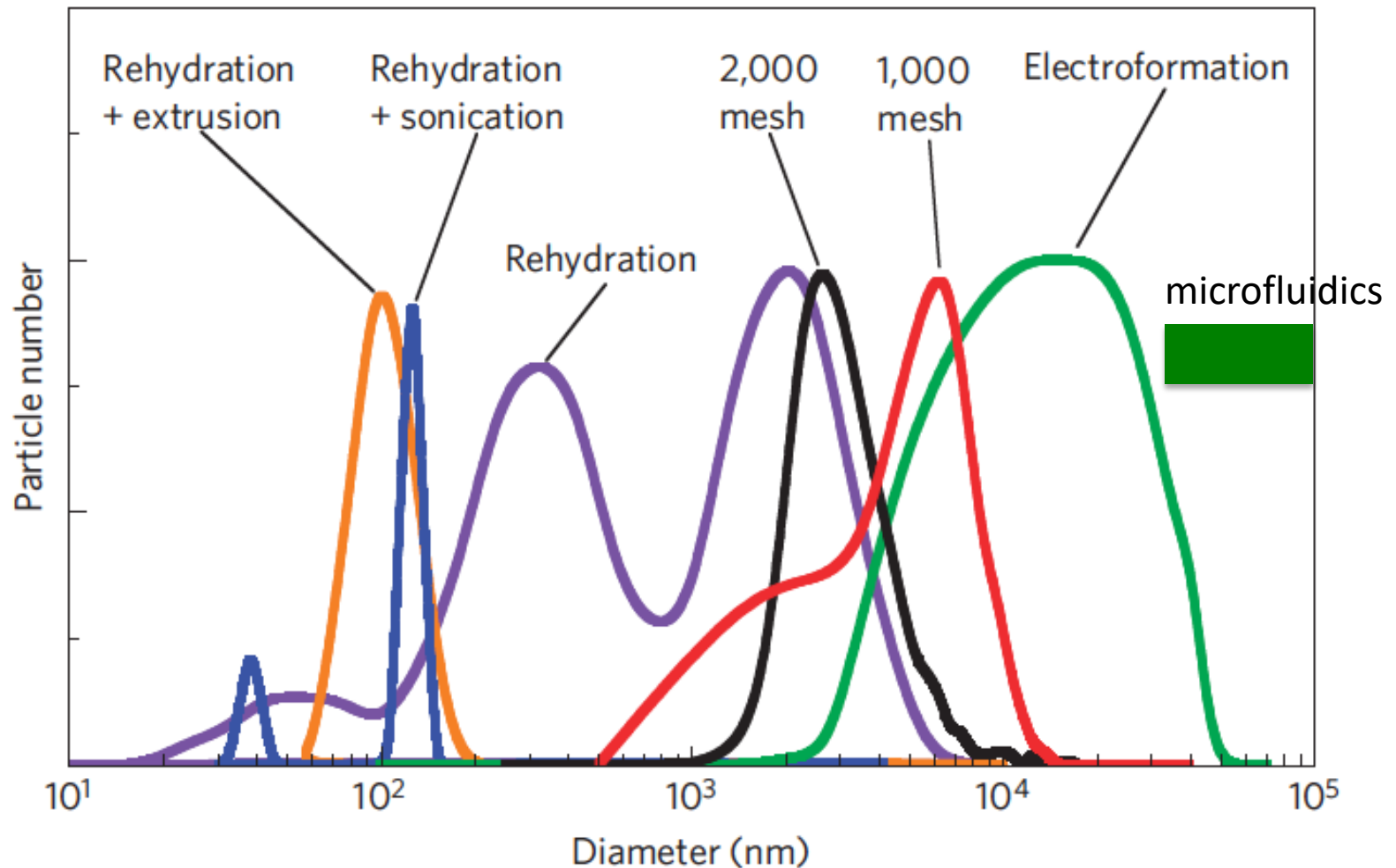
The vesicle diameter is similar to the diameter of double emulsion drops; it is usually between 50  $\mu\text{m}$  and 200  $\mu\text{m}$ .

You are asked to prepare vesicles with a diameter of 200 nm and an as narrow size distribution as possible. Which of these methods would you chose to prepare them?

- A. sonication
- B. extrusion
- C. electroformation
- D. microfluidics



# Influence of production method on vesicle size

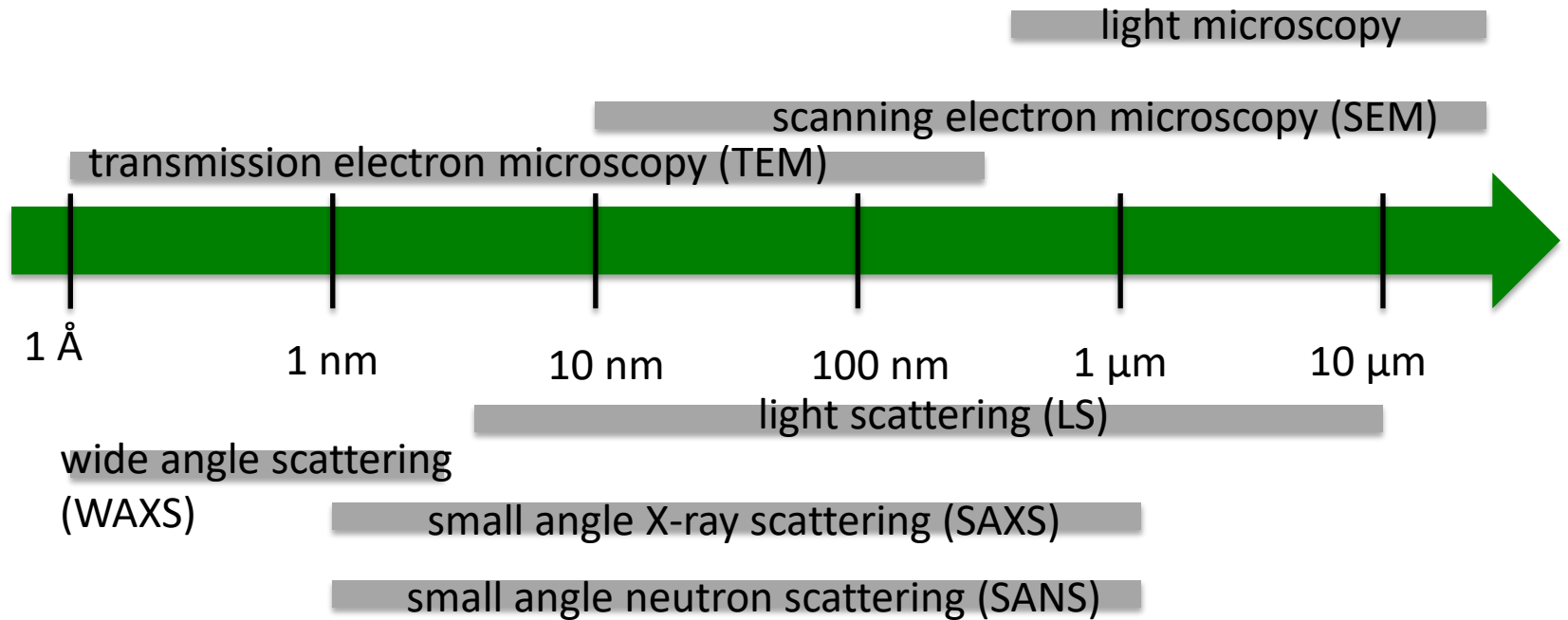


# Outline

- Introduction
  - Vesicles
  - Vesicle production
- Liposomes
- Polymersomes
- **Characterization**
- Applications

# Characterization of soft materials

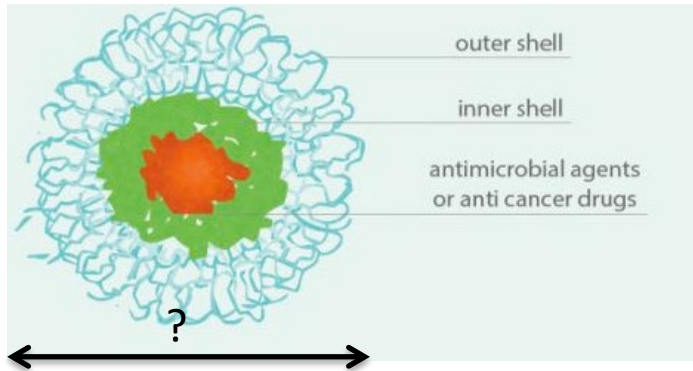
## I. Microscopy



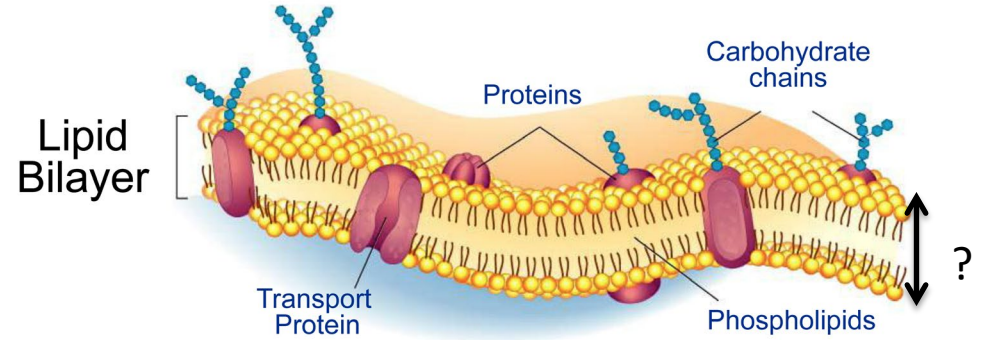
## II. Scattering

# How would you characterize the dimensions of these samples?

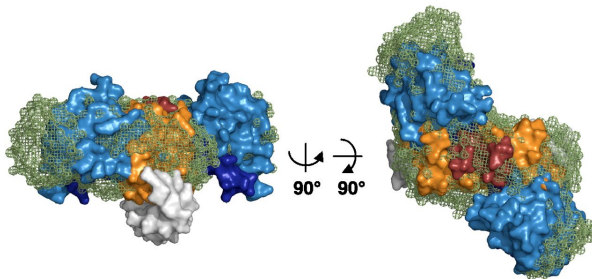
core-shell nanoparticles



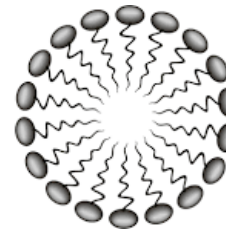
cell membrane



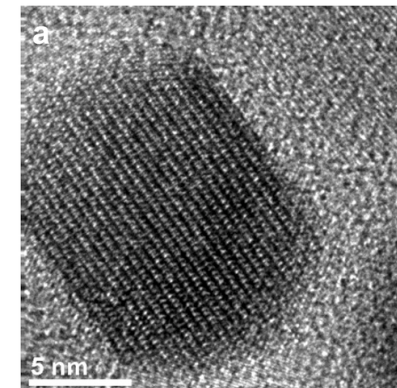
protein structure



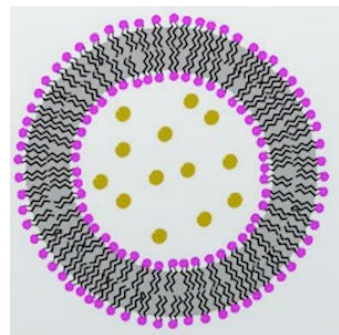
micelles



lattice spacing



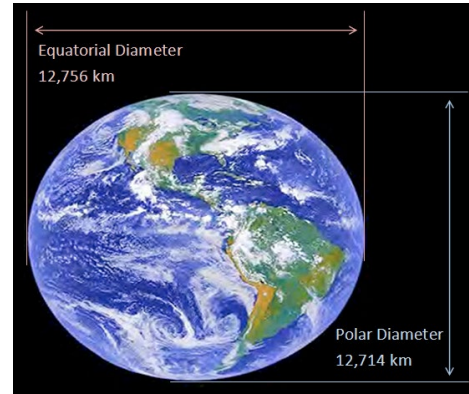
vesicles



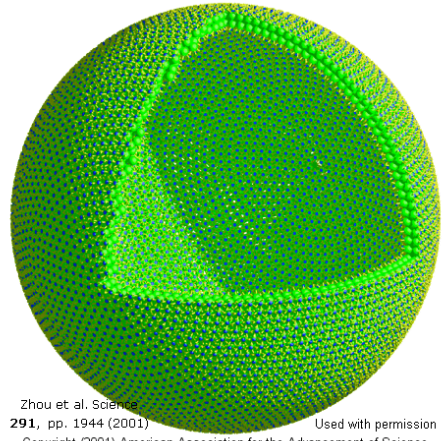
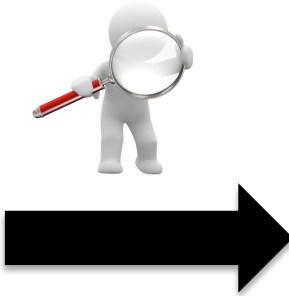
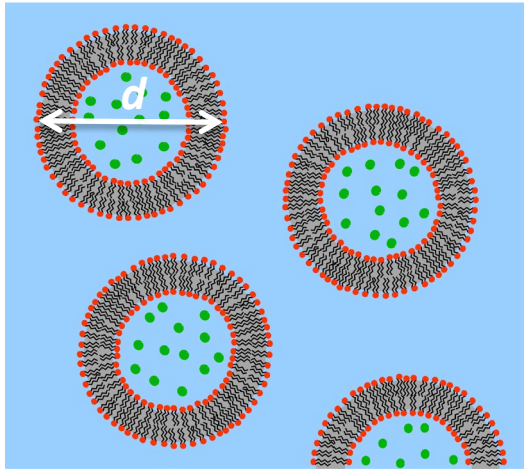
D. Strickland, K. Moffat and T. R. Sosnick, *PNAS*, 2008, **105**, 10709-10714.

H.-M. Song, J. I. Zink and N. M. Khashab, *PCCP*, 2015, **17**, 18825-18833

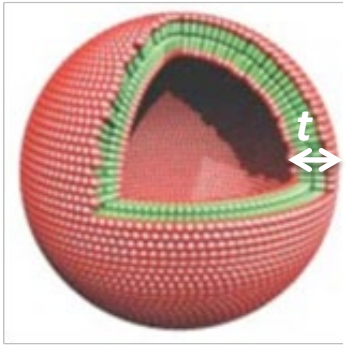
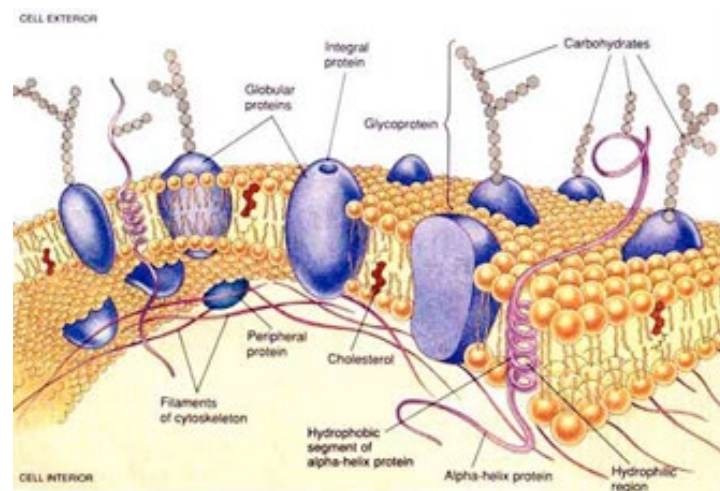
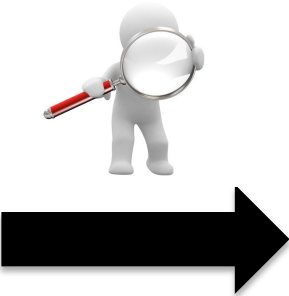
# Length scales



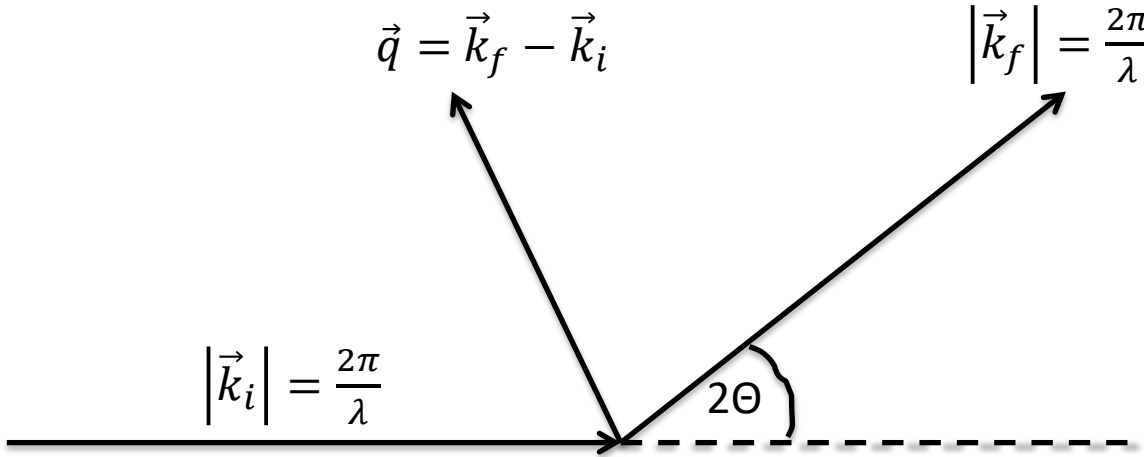
# Scattering



Zhou et al. Science 291, pp. 1944 (2001) Used with permission Copyright (2001) American Association for the Advancement of Science



# Scattering

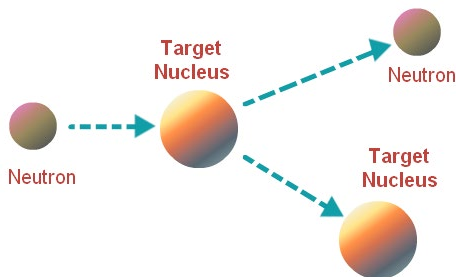


$$q = |\vec{q}| = \frac{4\pi \sin \theta}{\lambda}$$

$k_i$ : wave vector of incident beam [ $\text{m}^{-1}$ ]  
 $k_f$ : wave vector of scattered beam [ $\text{m}^{-1}$ ]  
 $q$ : scattering wave vector [ $\text{m}^{-1}$ ]  
 $\lambda$ : wavelength [m]

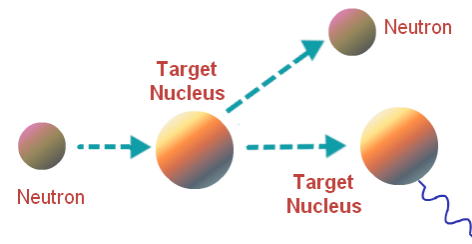
## Elastic scattering

energy of incoming wave/object =  
 energy of outgoing wave/object

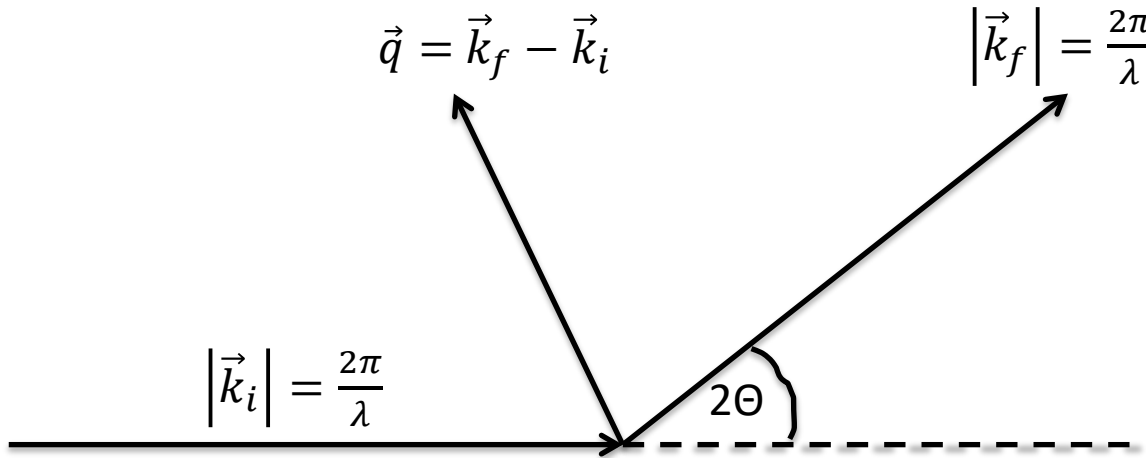


## Inelastic scattering

energy of incoming wave/object >  
 energy of outgoing wave/object



# Scattering



$$q = |\vec{q}| = \frac{4\pi \sin \Theta}{\lambda}$$

$k_i$ : wave vector of incident beam [ $\text{m}^{-1}$ ]  
 $k_f$ : wave vector of scattered beam [ $\text{m}^{-1}$ ]  
 $q$ : scattering wave vector [ $\text{m}^{-1}$ ]  
 $\lambda$ : wavelength [m]

## Light:

For the characterization of objects with sizes of a few nm up to approximately  $10 \mu\text{m}$ .

## X-rays:

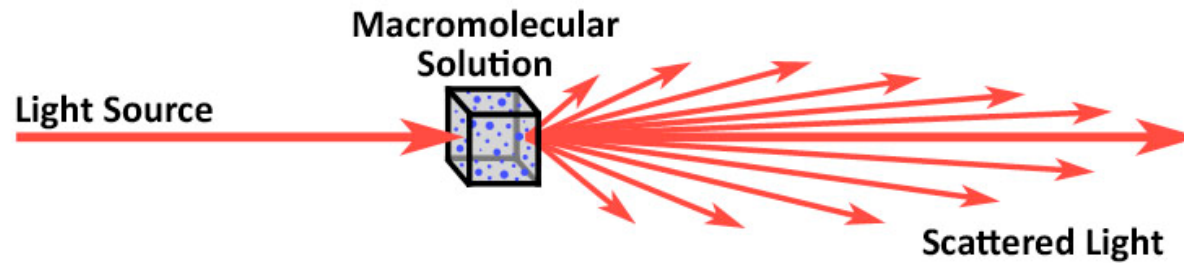
They interact with electrons and are good to visualize heavy atoms that give a strong response.

## Neutrons:

- They interact with atomic nuclei and
- are better suited to detect light atoms (H, O) than X-rays
  - can easily distinguish atoms with similar atomic numbers
  - can distinguish isotopes.

# Light scattering

$\lambda$ : 350 nm – 680 nm



<http://www.legendairy.com.au/dairy-foods/dairy-products/milk/how-milk-is-made>



<http://navanrfc.ie/blue-sky-desktop/>

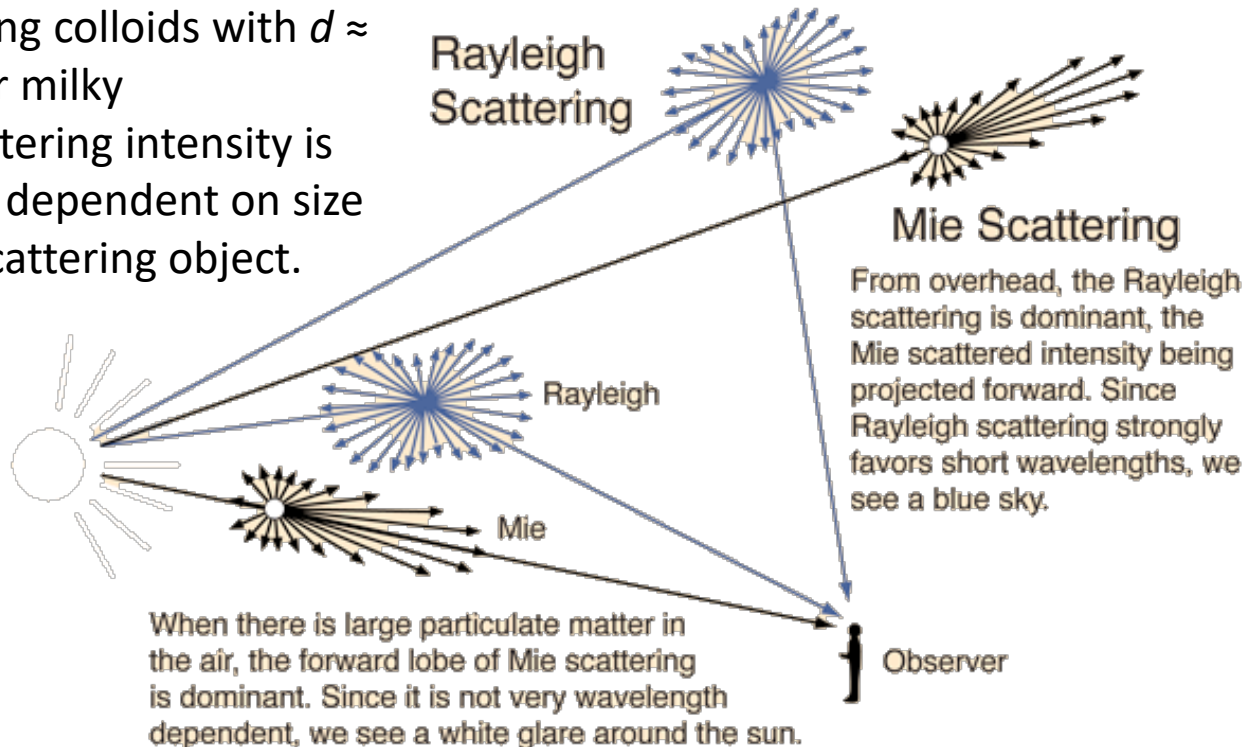
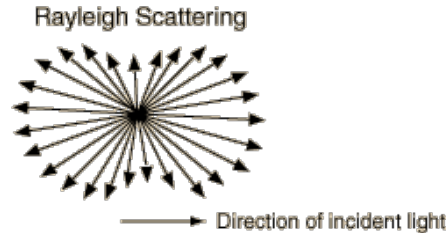
# Light scattering

$I$ : Scattering intensity [-]  
 $d$ : diameter of particle [m]  
 $\lambda$ : wavelength [m]

**Rayleigh scattering:**  $d < 0.1 \lambda$   $I \propto \frac{1}{\lambda^4}$

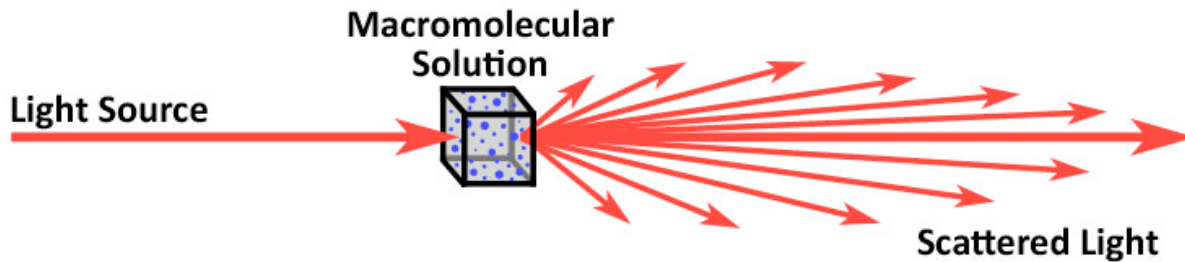
**Mie scattering:**  $d \approx \lambda$

- The scattering intensity is only weakly dependent on  $\lambda$  such that dispersions containing colloids with  $d \approx \lambda$  appear milky
- The scattering intensity is strongly dependent on size of the scattering object.

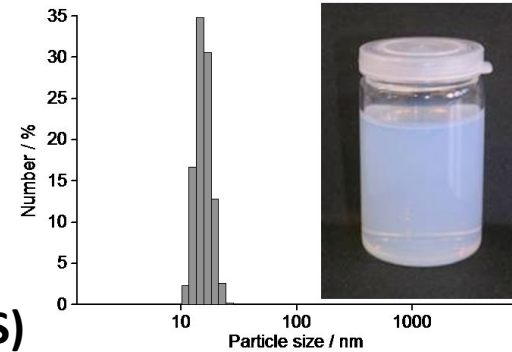


# Light scattering

$\lambda$ : 350 nm – 680 nm



example:



## Dynamic light scattering (DLS)

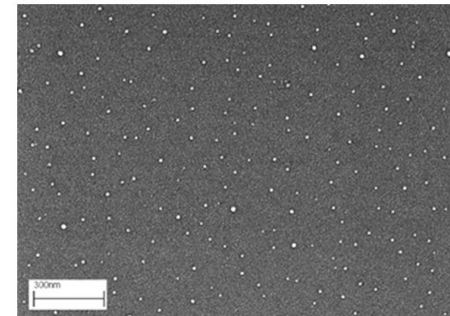
measures real-time intensities → dynamic properties

- diffusion coefficient
- hydrodynamic diameter

## Static light scattering (SLS)

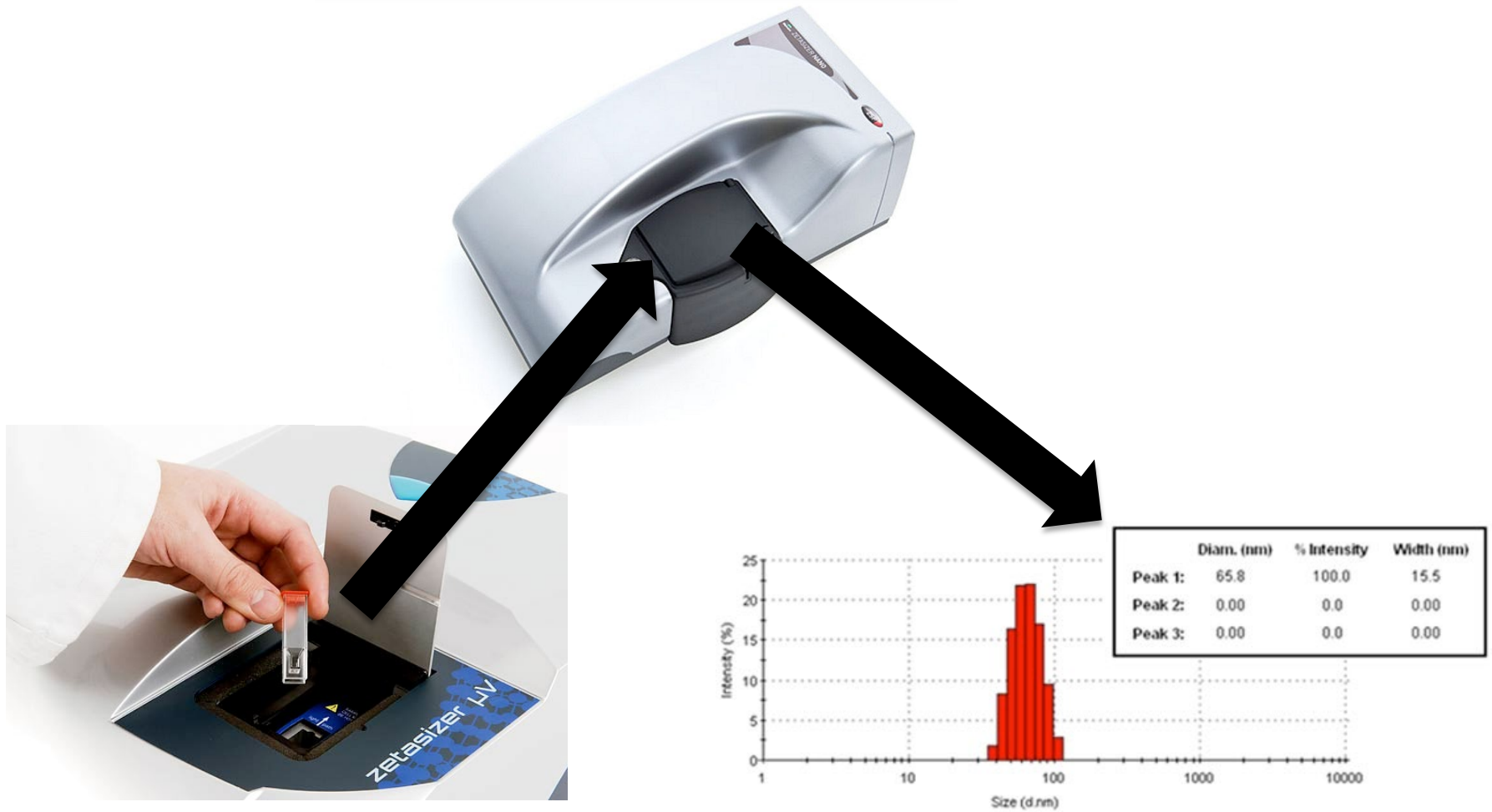
measures the time averaged scattering intensities

- particle morphology
- particle arrangement



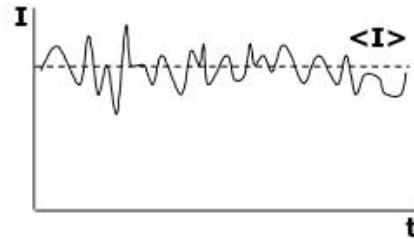
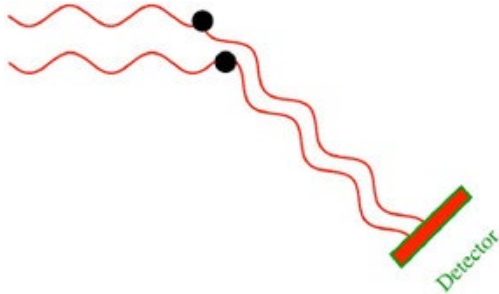
E. Hammarberg, *et al.*, *J. Colloid Interface Sci.* 2009, **334**, 29.

# Dynamic light scattering



# Dynamic light scattering (DLS)

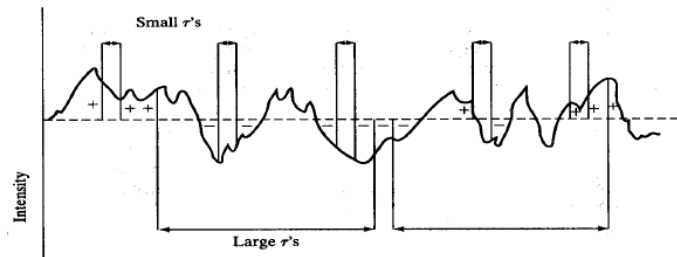
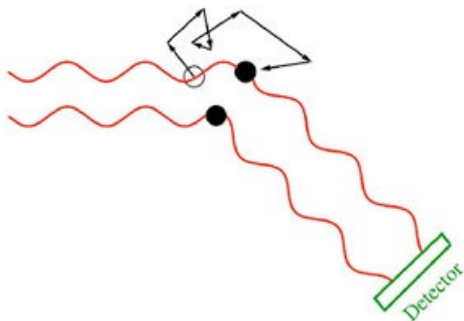
constructive interference



correlation function

$$G(\tau) = \sum I(t)I(t + \tau)$$

destructive interference



$$G(\tau) = G_0 e^{-Dq^2\tau}$$

Plot  $\ln(G(\tau))$  vs.  $\tau$

slope:  $Dq^2$

$$q = \frac{4\pi \sin \Theta}{\lambda}$$

$$R_H = \frac{k_B T}{6\pi\eta D}$$

# Dynamic light scattering (DLS)

## 1. Raw data

correlation function

$$G(\tau) = G_0 e^{-Dq^2\tau}$$

## 2. Fitting

deduce  $Dq^2$

## 3. Calculation

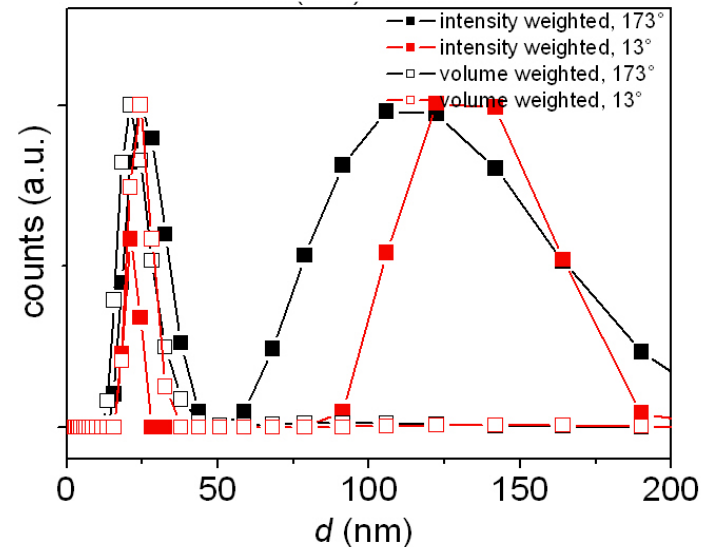
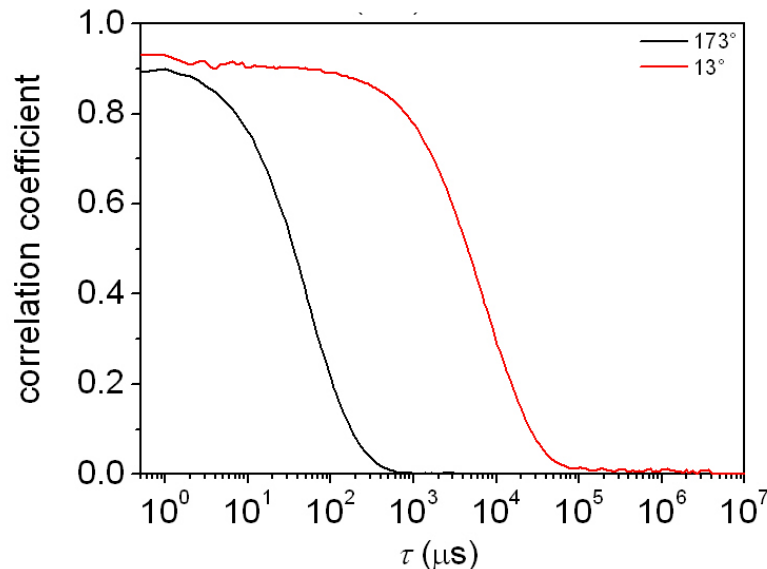
determine  $D$  using  
the known  $q$

$$q = \frac{4\pi \sin \Theta}{\lambda}$$

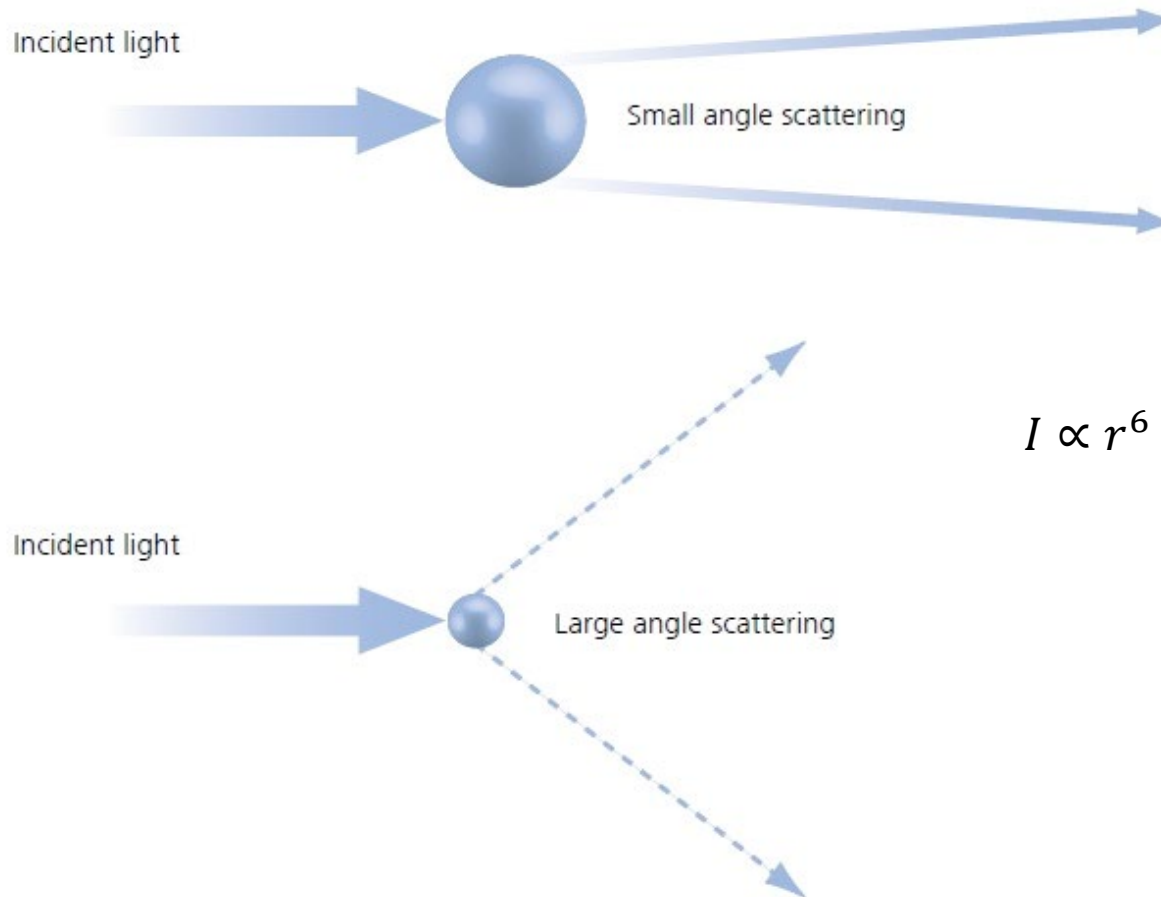
## 4. Calculation

intensity weighted  
hydrodynamic radius

$$R_H = \frac{k_B T}{6\pi\eta D}$$



# Influence of particle size



# Dynamic light scattering (DLS)

**1. Raw data**  
correlation function

$$G(\tau) = G_0 e^{-Dq^2\tau}$$

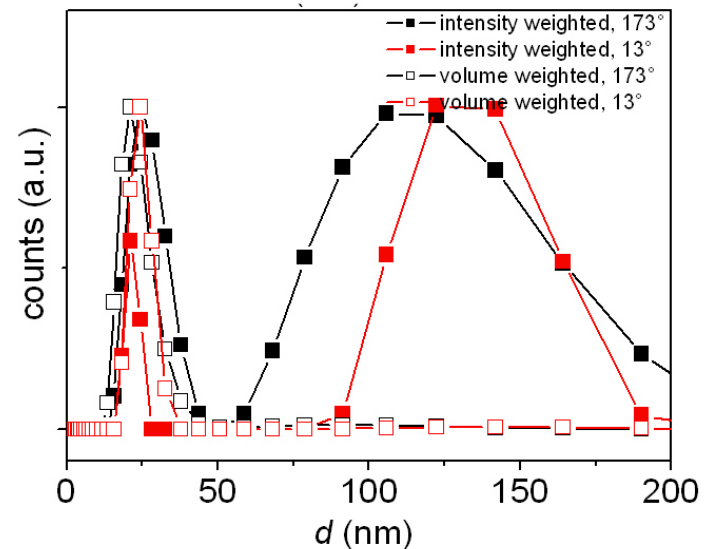
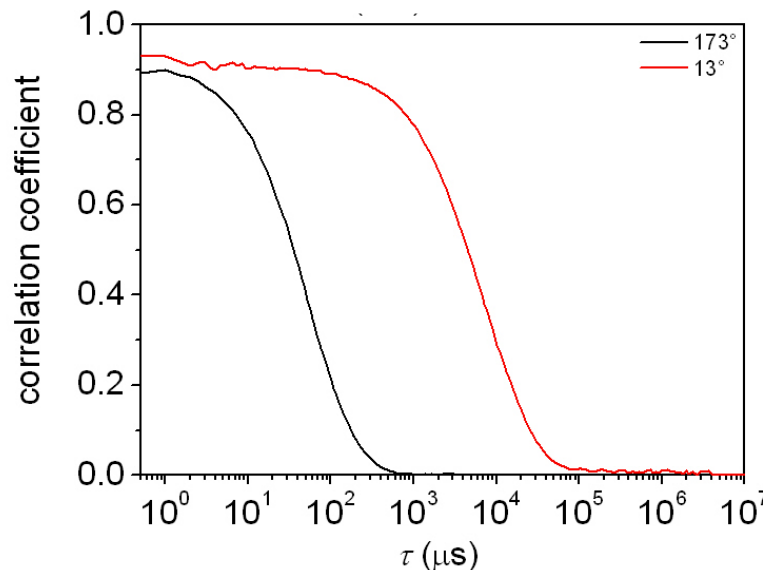
**2. Fitting**  
deduce  $Dq^2$

**3. Calculation**  
determine  $D$  using  
the known  $q$

$$q = \frac{4\pi \sin \Theta}{\lambda}$$

**4. Calculation**  
intensity weighted  
hydrodynamic radius

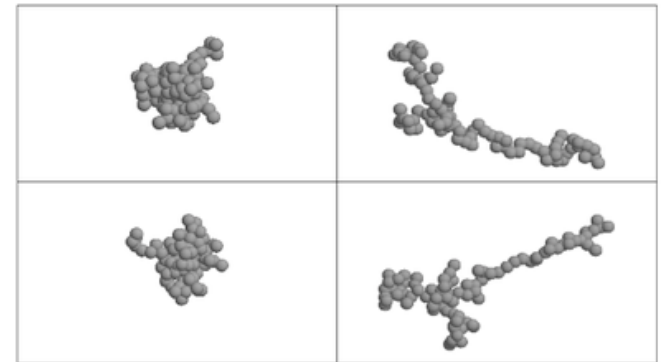
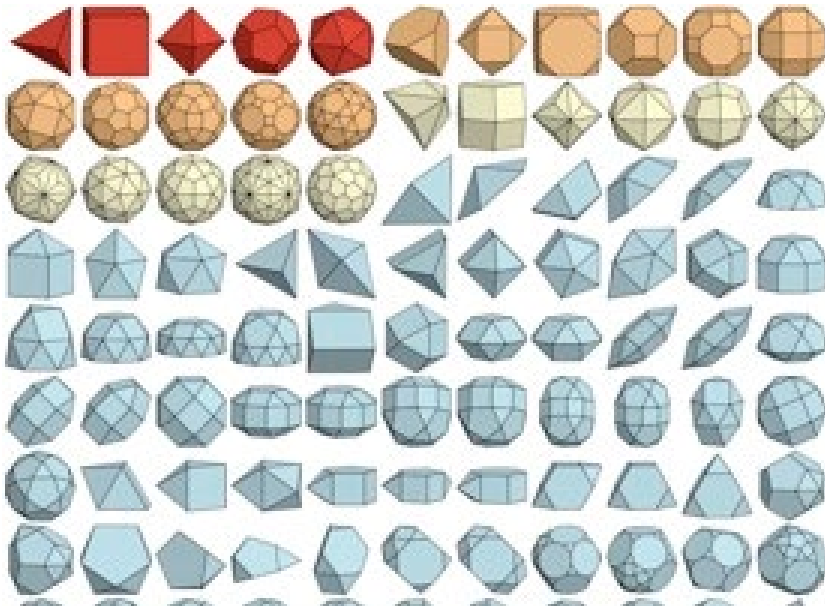
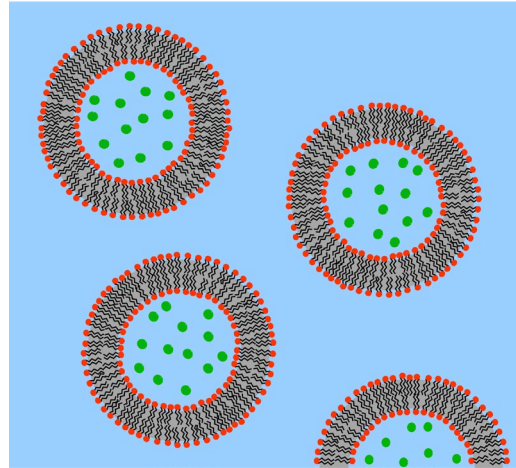
$$R_H = \frac{k_B T}{6\pi\eta D}$$



*Assumptions:*

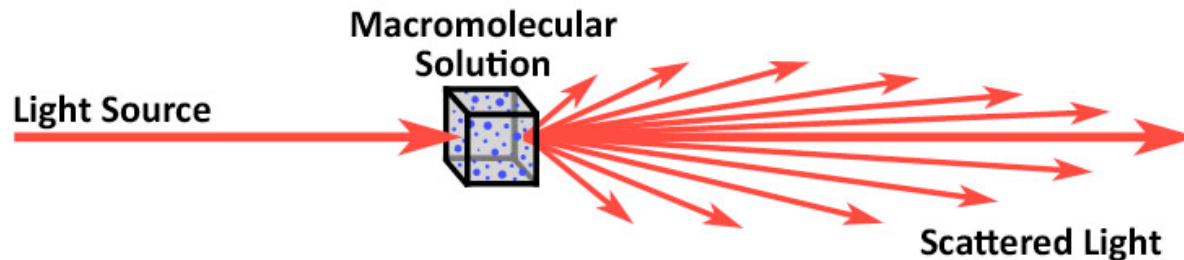
1. spherical particles
2. homogeneous mass/density of particles
3. known real and imaginary refractive index

# Scattering



# Light scattering

$\lambda$ : 350 nm – 680 nm



## Dynamic light scattering (DLS)

measures real-time intensities →  
dynamic properties

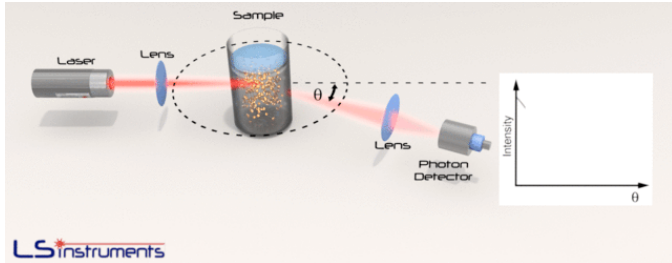
- diffusion coefficient
- hydrodynamic diameter

## Static light scattering (SLS)

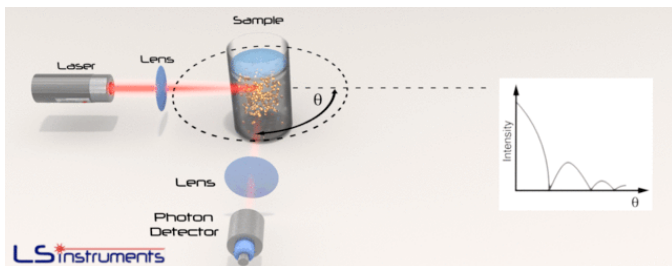
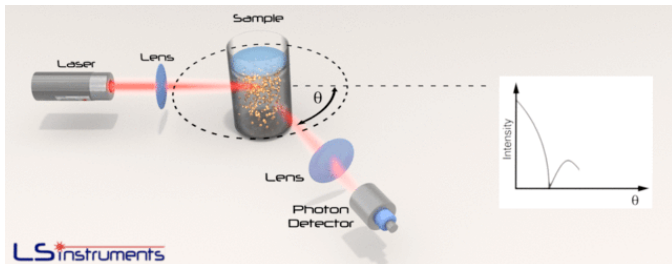
measures the time averaged  
scattering intensities

- particle morphology
- particle arrangement

# Static light scattering (SLS)



$$I(\vec{q}, c) = I_i \frac{f(\theta)}{R_0^2} c V_s \Delta\rho^2 V_p^2 P(\vec{q}) S(\vec{q}, c)$$



$I$ : scattering intensity [-]

$I_i$ : light intensity before impingement [-]

$f(\theta)$ : geometrical factor accounting for polarization for incident and collected light [-]

$R_0$ : distance between sample and detector [m]

$P$ : form factor [-]

$S$ : structure factor [-]

$c$ : concentration [ $\text{m}^{-3}$ ]

$V_s$ : molecular volume solvent [ $\text{m}^3$ ]

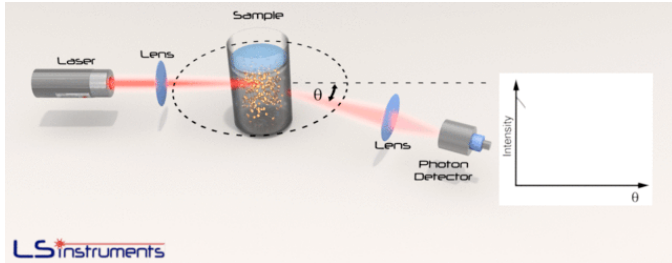
$V_p$ : molecular volume particle [ $\text{m}^3$ ]

$\Delta\rho$ : difference in scattering length density [ $\text{m}^{-2}$ ]

experimental parameters to be adjusted to get a sufficiently high scattering intensity

$$q = |\vec{q}| = \frac{4\pi \sin \Theta}{\lambda}$$

# Static light scattering (SLS)



$$I(\vec{q}, c) = I_i \frac{f(\theta)}{R_0^2} c V_s \Delta\rho^2 V_p^2 P(\vec{q}) S(\vec{q}, c)$$

$I$ : scattering intensity [-]

$I_i$ : light intensity before impingement [-]

$f(\theta)$ : geometrical factor accounting for polarization for incident and collected light [-]

$R_0$ : distance between sample and detector [m]

$P$ : form factor [-]

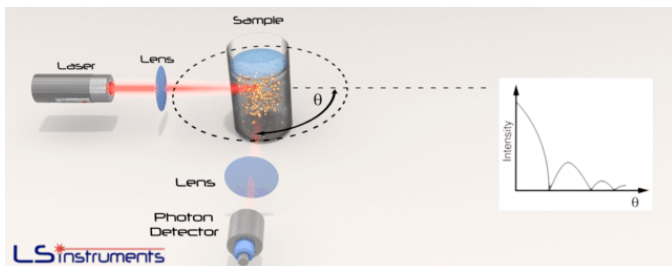
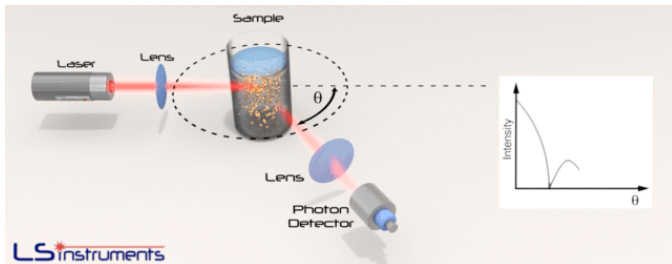
$S$ : structure factor [-]

$c$ : concentration [ $\text{m}^{-3}$ ]

$V_s$ : molecular volume solvent [ $\text{m}^3$ ]

$V_p$ : molecular volume particle [ $\text{m}^3$ ]

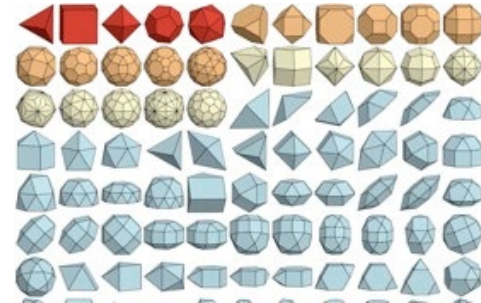
$\Delta\rho$ : difference in scattering length density [ $\text{m}^{-2}$ ]



$$q = |\vec{q}| = \frac{4\pi \sin \Theta}{\lambda}$$

# Form factor

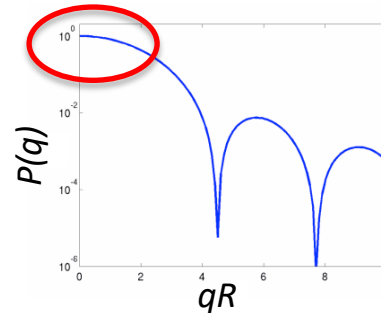
Tells us something about particle size and shape:



<http://www.scientificamerican.com/article/nanoparticle-bottom-up/>

For a sphere with radius  $R$ :

$$P(q) = \left[ \frac{3}{(qr)^3} (\sin(qr) - qr \cos(qr)) \right]^2$$



$P$ : form factor [1]

$r$ : position of particle time  $t$  [m]

$g$ : pair correlation function [-]

$q$ : scattering wave vector [ $\text{m}^{-1}$ ]

$R$ : radius of particle [m]

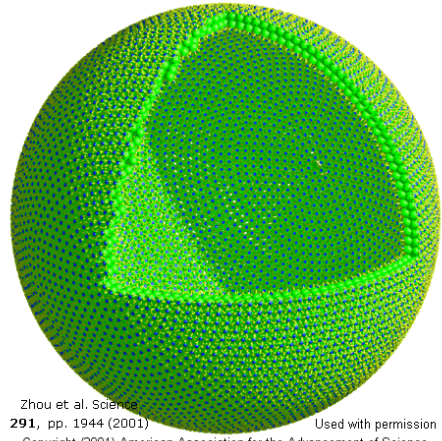
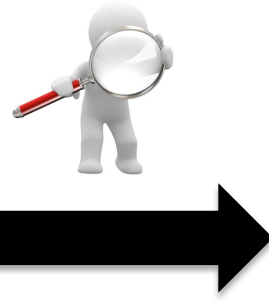
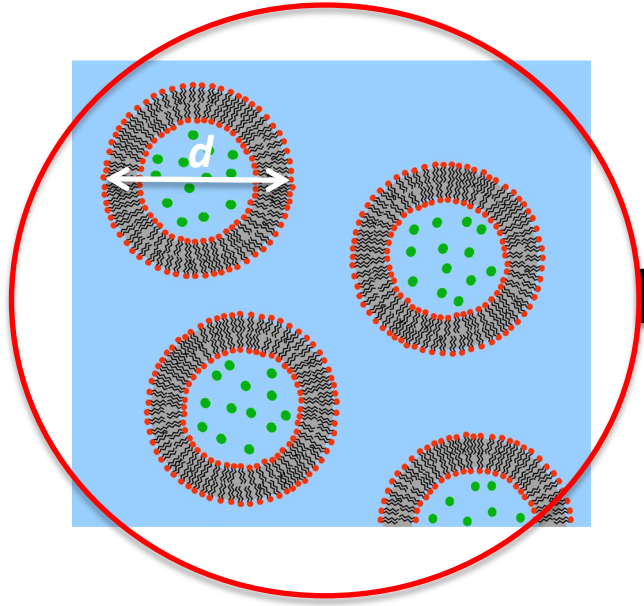
$R_g$ : radius of gyration [m]

To obtain a simpler, general formula for the form factor, we introduce a pair correlation function  $g(r)$ ; it describes the probability to find two points, that belong to a particle at a distance  $r$ .

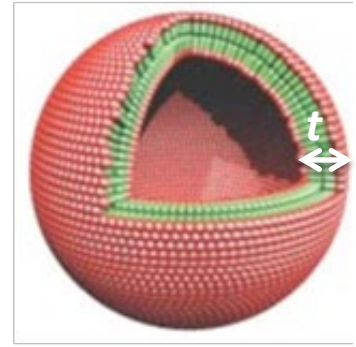
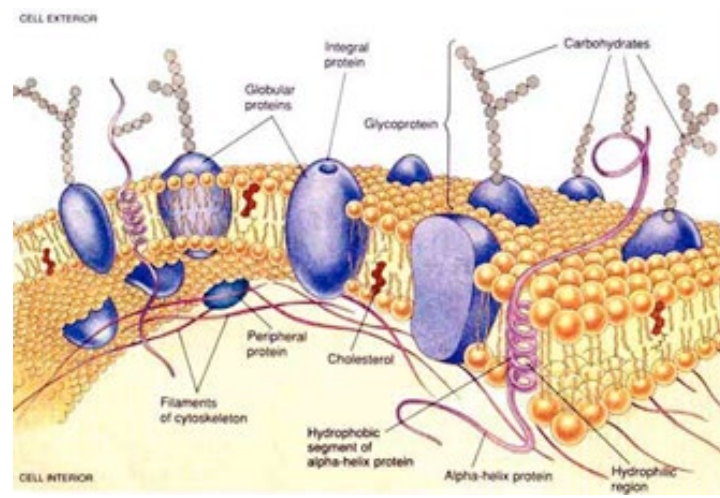
$$P(q) = \left[ \int_0^\infty r^2 g(r) \frac{\sin(qr)}{qr} dr \right]^2 \xrightarrow{\text{expanding}} \frac{\sin(qr)}{qr} \boxed{P(q) \approx 1 - \frac{(qR_g)^2}{3}} \quad \text{with} \quad R_g^2 = \int_0^\infty r^2 g(r) dr$$

[http://www.lsinstruments.ch/technology/static\\_light\\_scattering\\_sls/form\\_factor/](http://www.lsinstruments.ch/technology/static_light_scattering_sls/form_factor/)

# Scattering

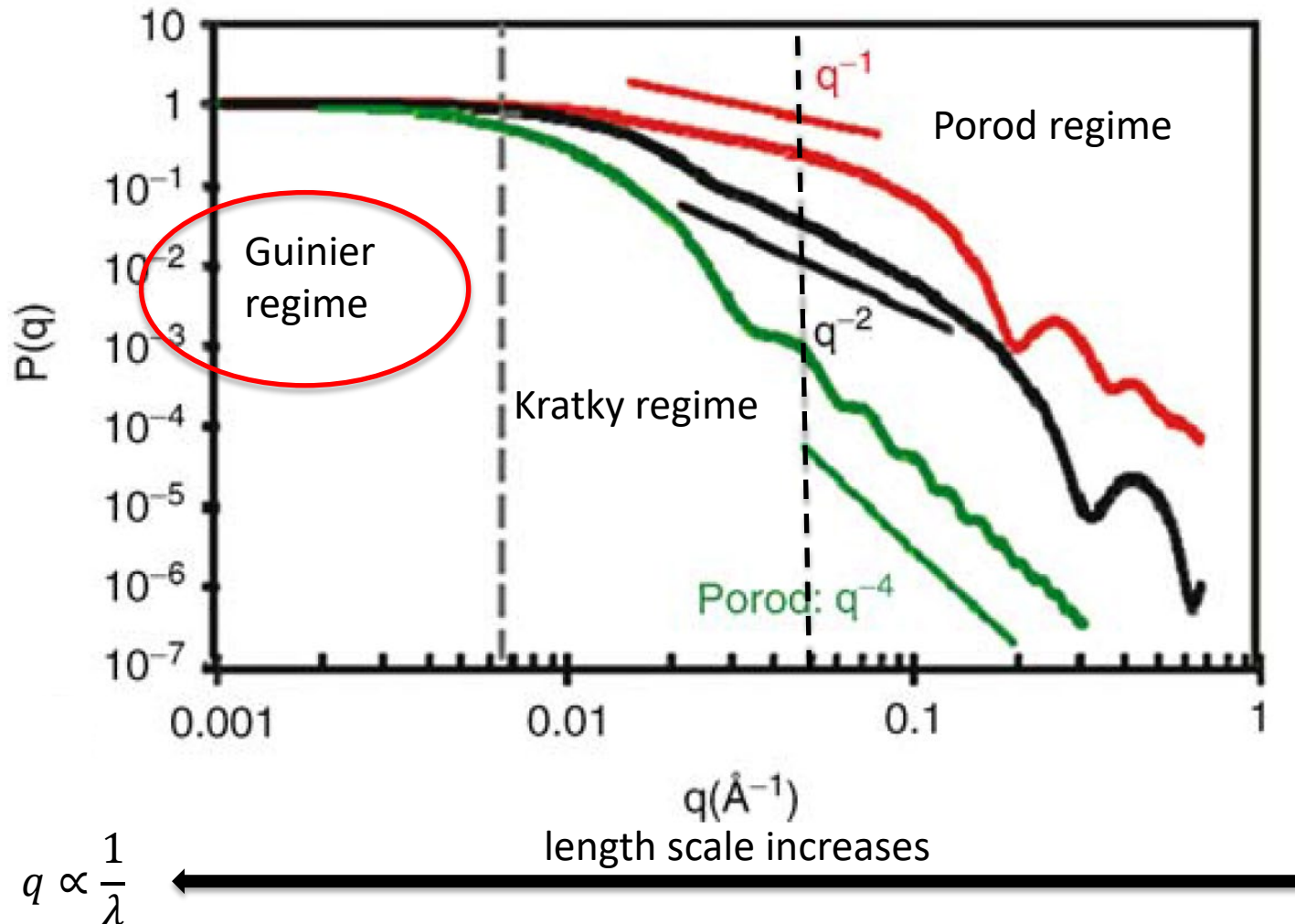


Zhou et al. Science 291, pp. 1944 (2001) Used with permission Copyright (2001) American Association for the Advancement of Science



# Small angle scattering data

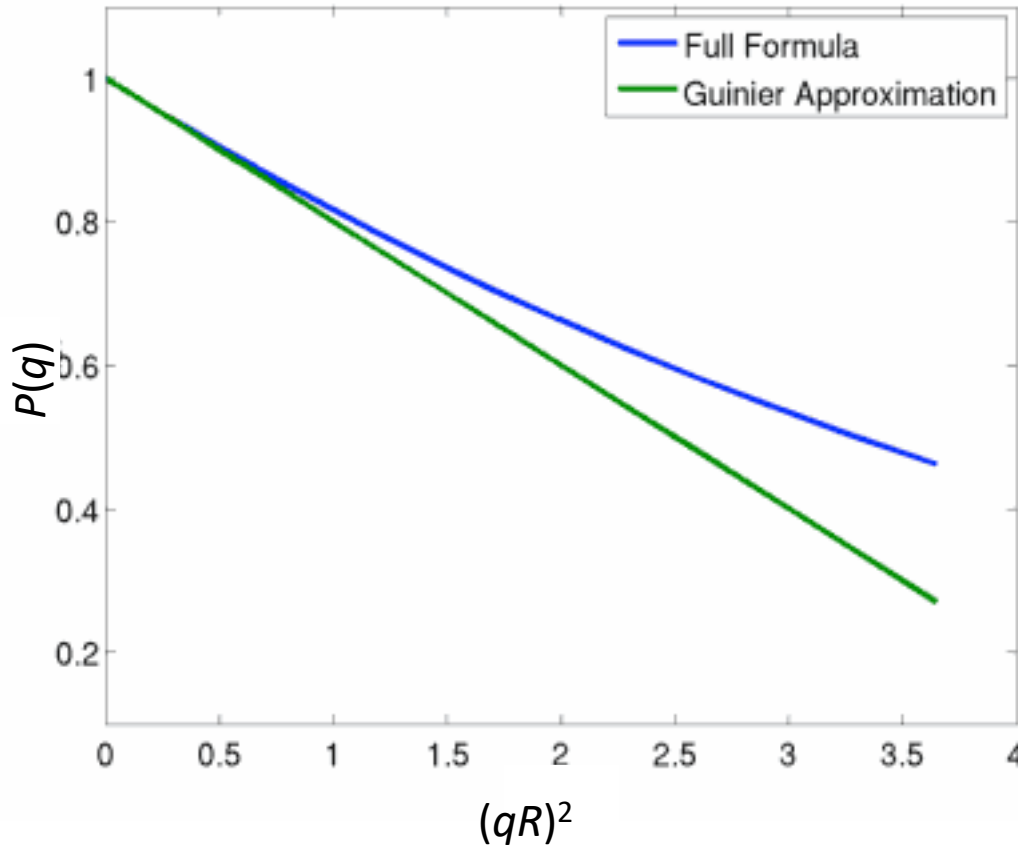
Different regimes give information about the sample structure on different length scales.



# Guinier approximation

$$\text{For a sphere: } P(q) \approx 1 - \frac{(qR_g)^2}{3}$$

$P$ : form factor [-]  
 $q$ : scattering wave vector [ $\text{m}^{-1}$ ]  
 $R$ : radius of particle [m]  
 $R_g$ : radius of gyration [m]



The slope lets you determine the relation between  $R$  and  $R_g$ . In this case,  $R_g = \sqrt{\frac{3}{5}} R$

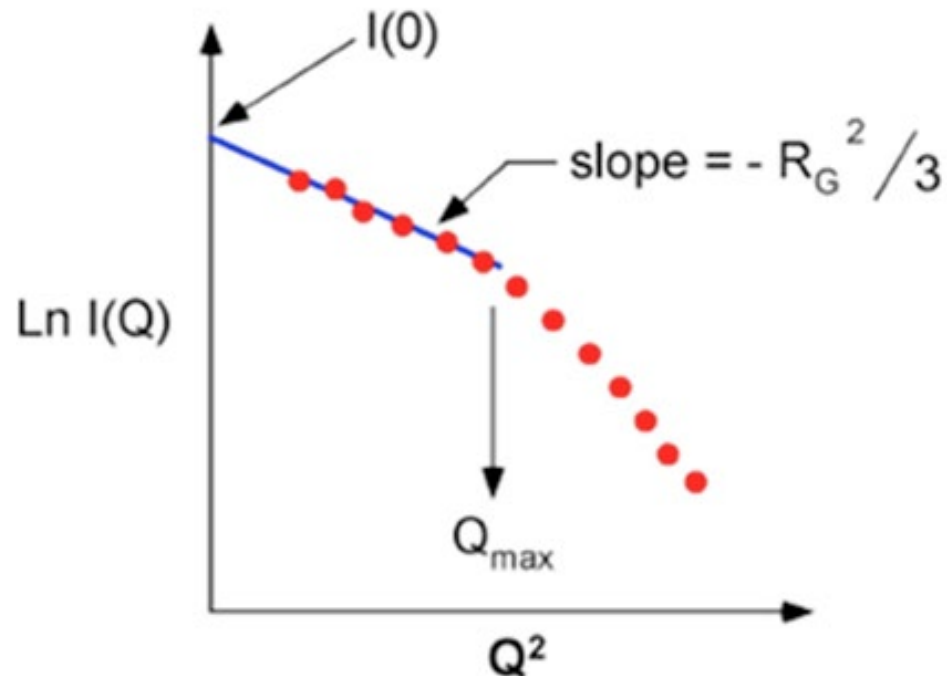
# Guinier Region

$$q \rightarrow 0$$

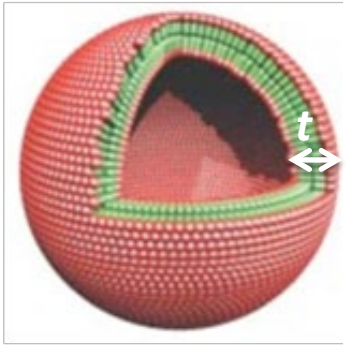
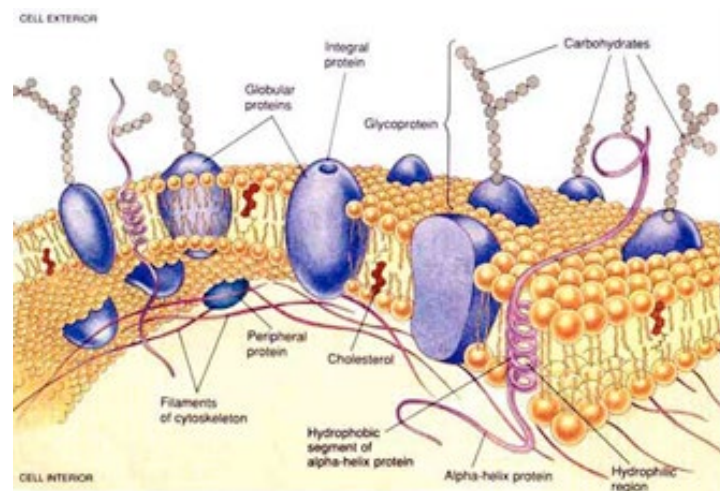
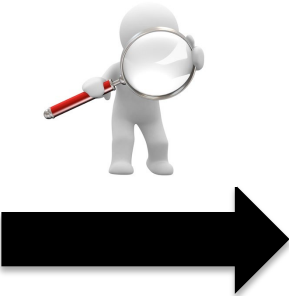
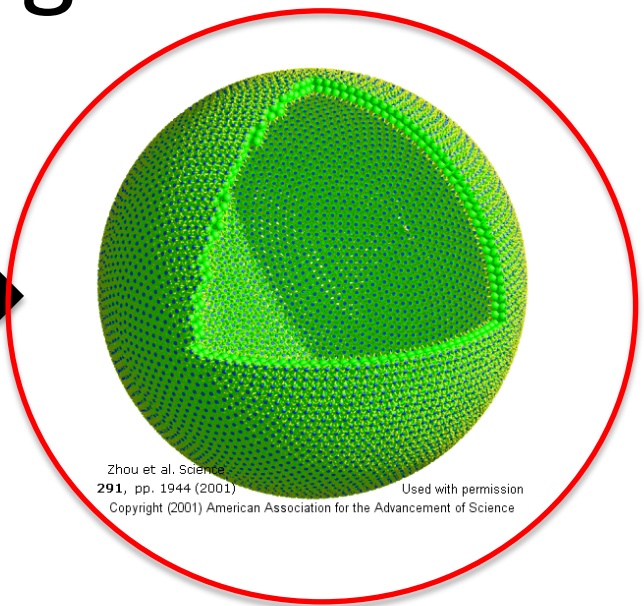
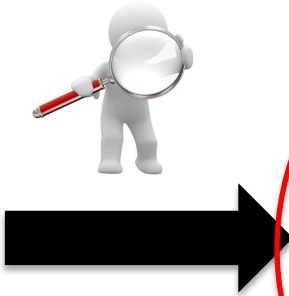
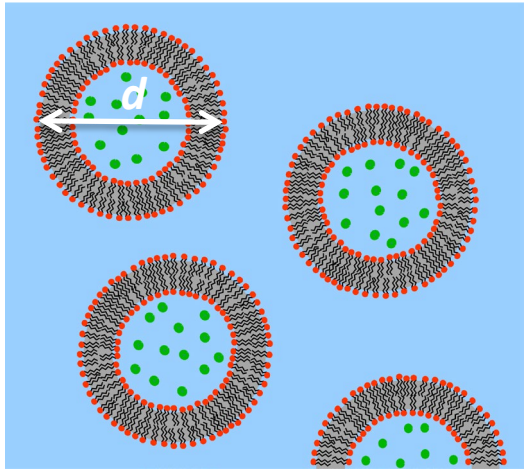
determine  $R_g$

$$I(q) = I(0)e^{-\frac{(qR_g)^2}{3}}$$

$$q = \frac{4\pi \sin\left(\frac{\theta}{2}\right)}{\lambda} \pi r^2$$

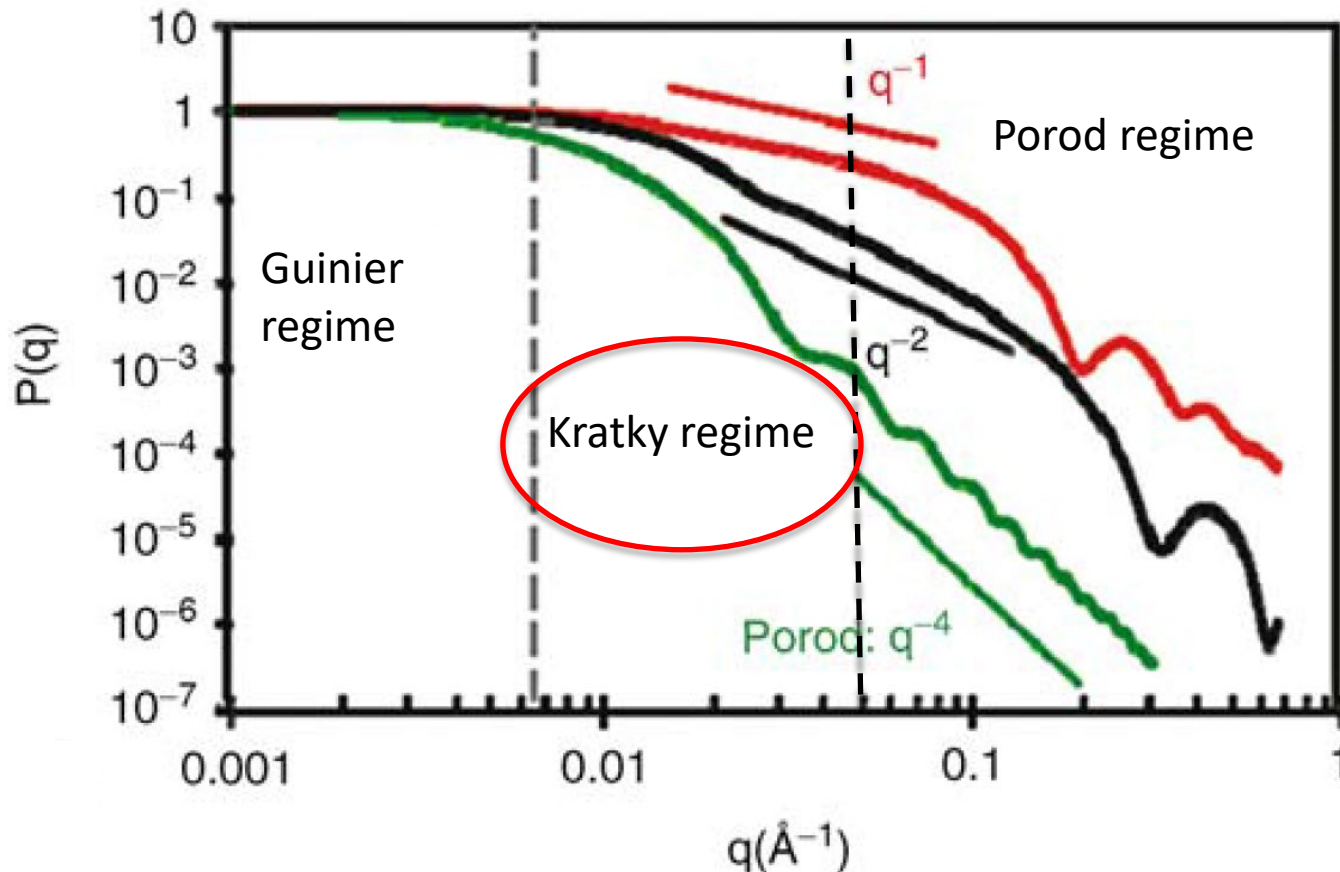


# Scattering



# Kratky Region

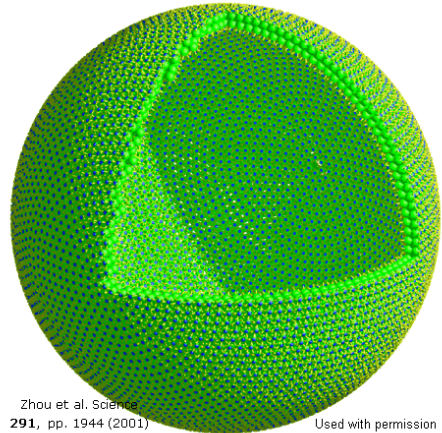
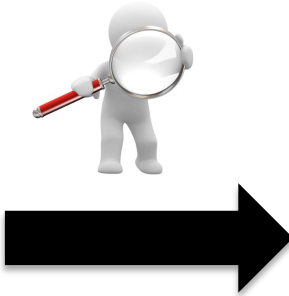
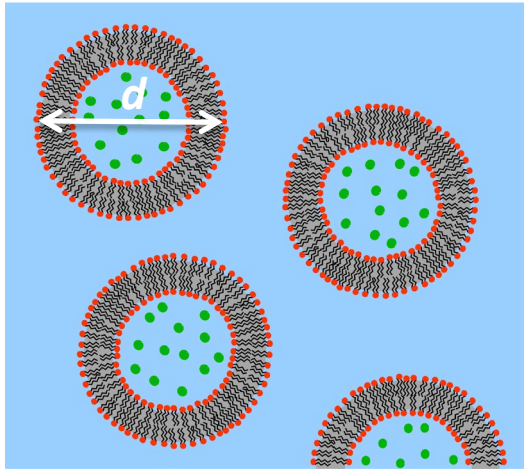
In certain  $q$ -ranges, the scattering behavior of polymers is like that of a Gaussian coil. This region has slopes typically between  $q^{-1}$  and  $q^{-3}$ .



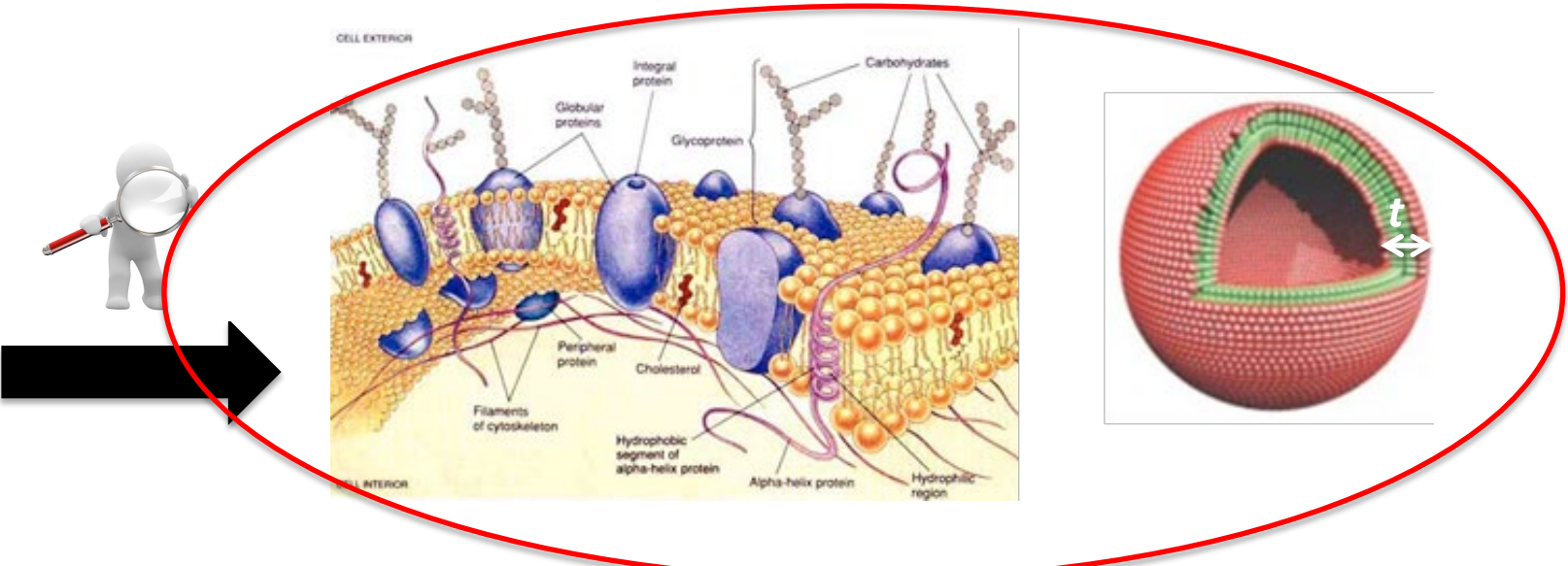
slope of  $q^{-1}$ : 1 D object e.g. rigid fiber

slope of  $q^{-2}$ : 2 D object or locally planar e.g. membrane

# Scattering

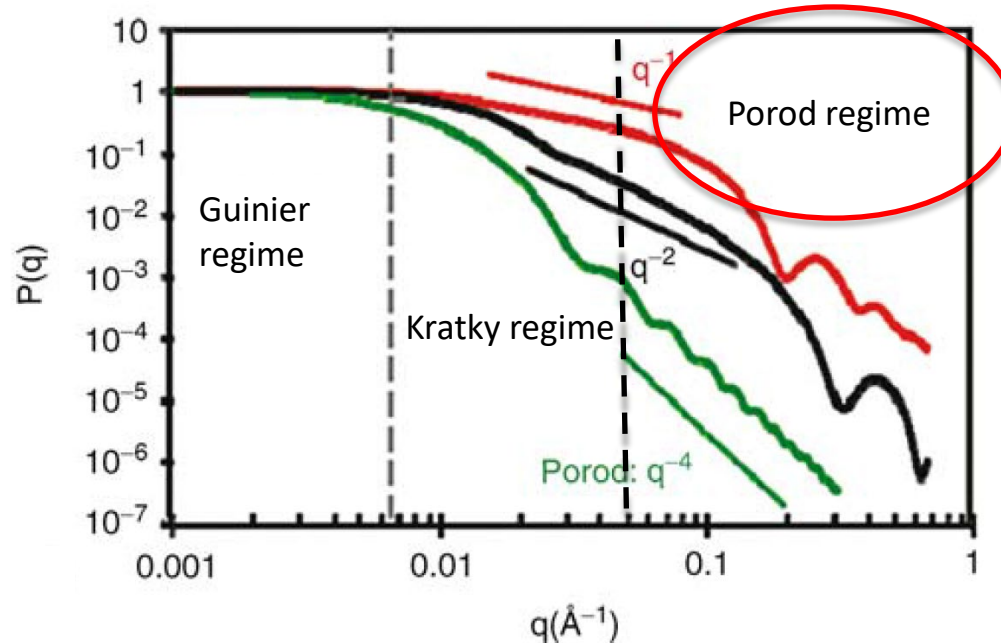


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# Porod Region

The minimum in  $P(q)$  at highest  $q$  value is related to the smallest dimension of the object.



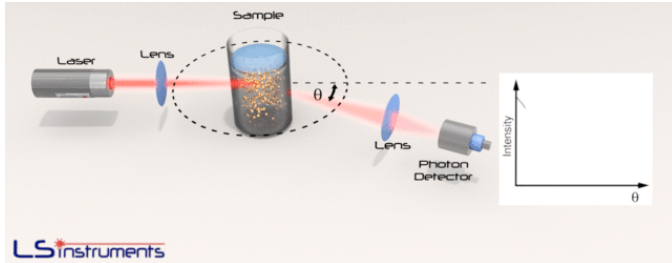
For sharp interfaces and large  $q$ 's:  $\Sigma = \frac{1}{2\pi(\Delta\rho)^2} \lim_{q \rightarrow \infty} I(q)q^4 = \pi r^2 \Sigma$   $\Sigma$ : specific surface area

$$I \propto \frac{1}{q^{-4}}$$

The Porod region can be used to

- determine shell thickness
- learn something about the surface roughness from the exact exponent of  $q$

# Static light scattering (SLS)



$$I(\vec{q}, c) = I_i \frac{f(\theta)}{R_0^2} c V_s \Delta\rho^2 V_p^2 P(\vec{q}) S(\vec{q}, c)$$

$I$ : scattering intensity [-]

$I_i$ : light intensity before impingement [-]

$f(\theta)$ : geometrical factor accounting for polarization for incident and collected light [-]

$R_0$ : distance between sample and detector [m]

$P$ : form factor [-]

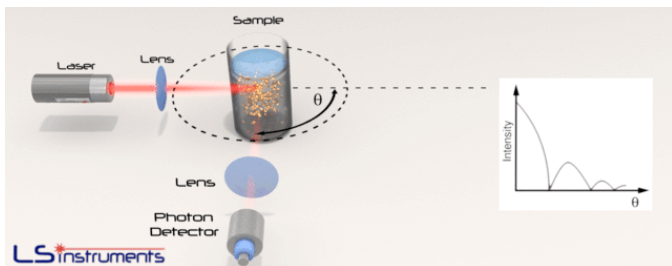
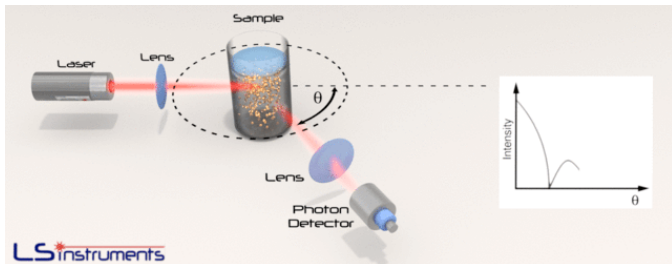
$S$ : structure factor [-]

$c$ : concentration [ $\text{m}^{-3}$ ]

$V_s$ : molecular volume solvent [ $\text{m}^3$ ]

$V_p$ : molecular volume particle [ $\text{m}^3$ ]

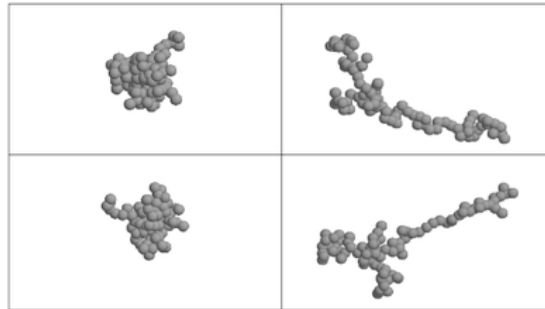
$\Delta\rho$ : difference in scattering length density [ $\text{m}^{-2}$ ]



$$q = |\vec{q}| = \frac{4\pi \sin \Theta}{\lambda}$$

# Structure factor

Tells us something about inter-particle interactions or particle arrangement within clusters.



$$S(\vec{q}, c) = \frac{1}{N} \sum_{j,k} \left\langle \exp \left[ -i\vec{q} \cdot (\vec{r}_j(t) - \vec{r}_k(t)) \right] \right\rangle$$

phase shift between light scattered by particle  $j$  and  $k$

$S$ : structure factor [1]

$N$ : number of scattering centers [1]

$r_x$ : position of particle  $x$  to time  $t$  [m]

# Structure factor

Introducing pair correlation function:

$$S(q) = 1 + c \int_0^{\infty} [g(r, c) - 1] \frac{\sin(qr)}{qr} 4\pi r^2 dr$$

for  $c \rightarrow 0$ :  $S = 1$

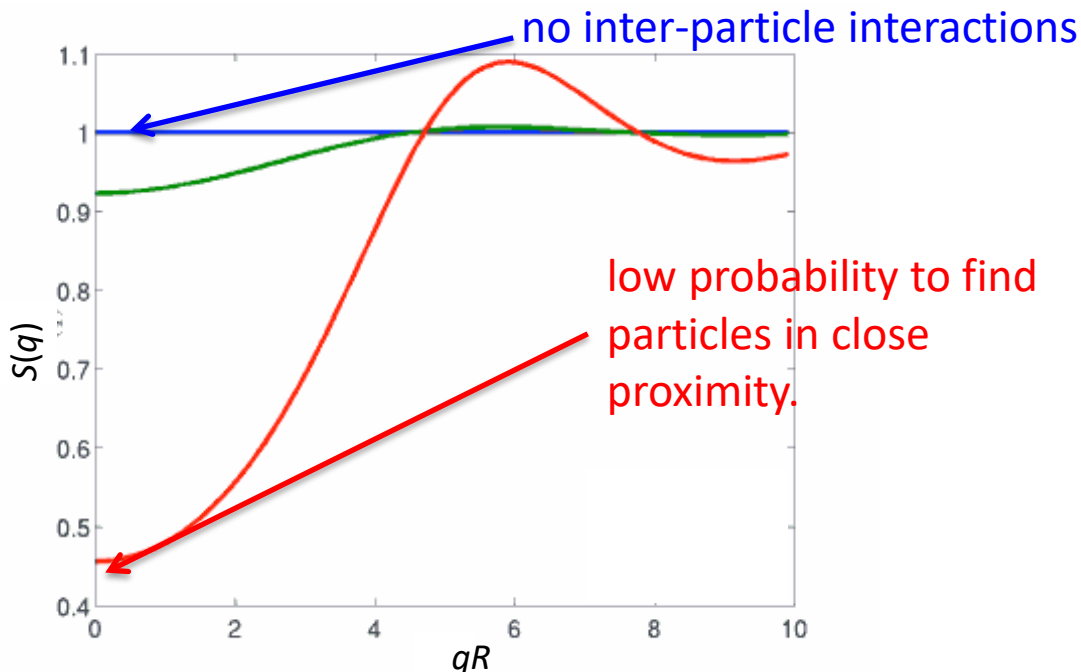
$S$ : structure factor [1]

$r_x$ : position of particle  $x$  to time  $t$  [m]

$g$ : pair correlation function [1]

$c$ : concentration [ $\text{m}^{-3}$ ]

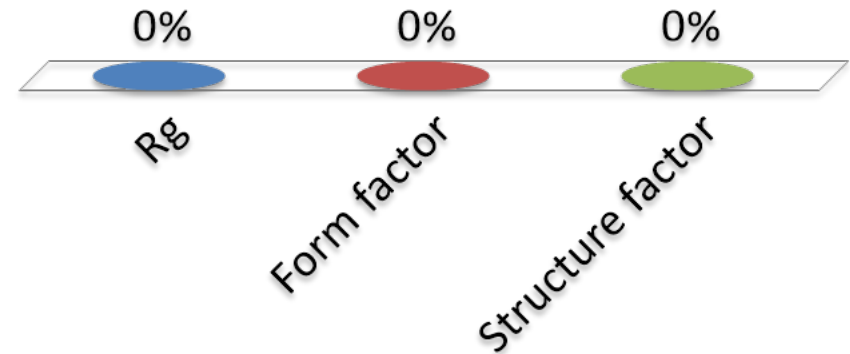
**example: colloidal dispersion**



For particle dispersions and if measured at low ( $qR$ ) values:  
 $S(q) < 1$ : particles are repellant  
 $S(q) > 1$ : particles are attractive

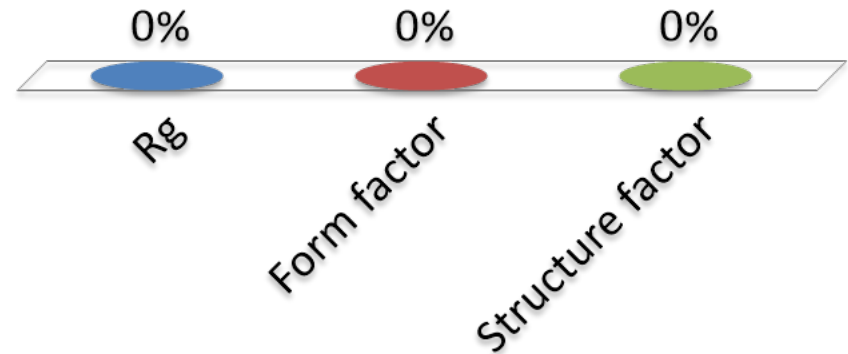
From what parameter can you extract information about the shape of a particle

- A.  $R_g$
- B. Form factor
- C. Structure factor



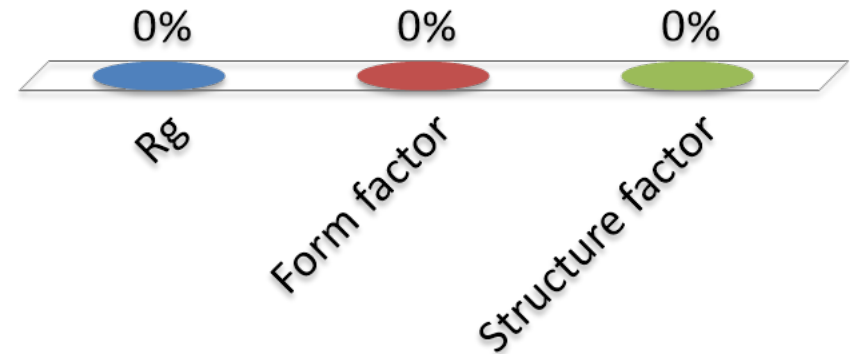
From what parameter can you extract information about the size of a particle

- A.  $R_g$
- B. Form factor
- C. Structure factor



From what parameter can you extract information about the aggregation state of a particle

- A.  $R_g$
- B. Form factor
- C. Structure factor

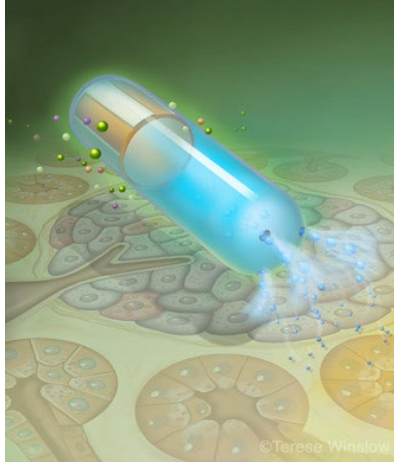


# Outline

- Introduction
  - Vesicles
  - Vesicle production
- Liposomes
- Polymersomes
- Characterization
- **Applications**

# Applications

drug delivery



cosmetics



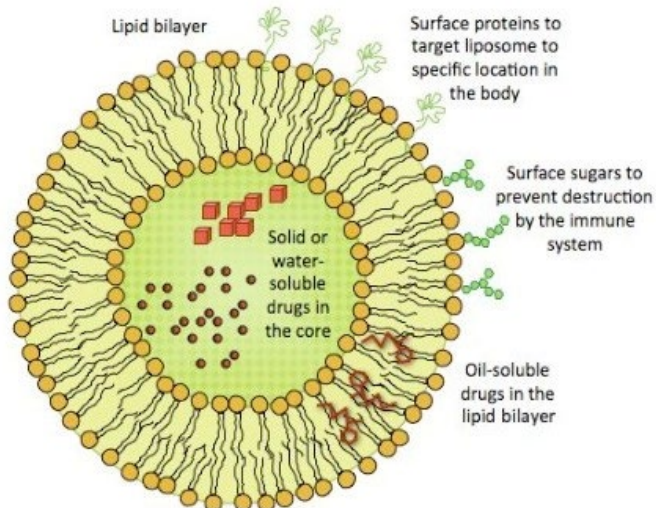
coatings



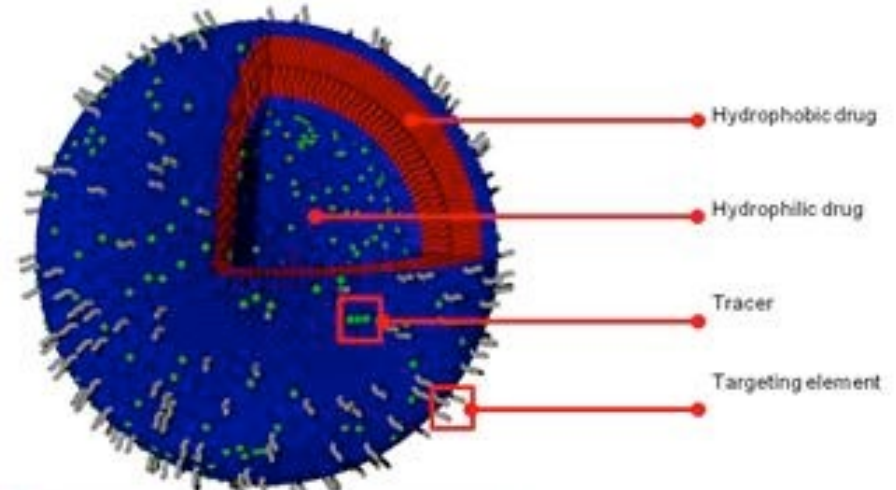
food



# Vesicles as delivery vehicles

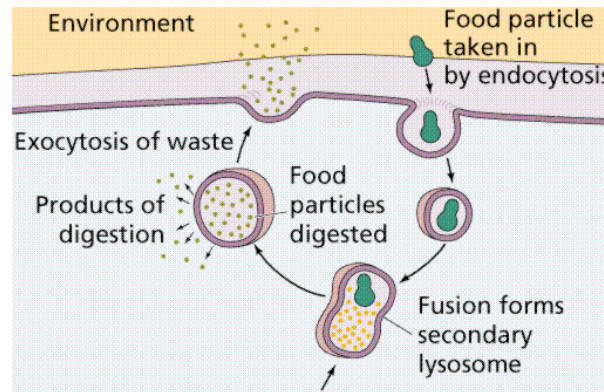


<http://sitn.hms.harvard.edu/flash/2011/materials-for-drug-delivery/>



<http://www.ru.nl/bio-orgchem/@789716/pagina/>

## Vesicles in the body



<http://www2.estrellamountain.edu/faculty/farabee/BIOBK/BioBooktransp.html>

# Rendering polymersomes permeable

