3.052 Nanomechanics of Materials and Biomaterials :
Spring 2007 Final Exam

1. **Single Macromolecule Force Spectroscopy**. (Data taken from: Oesterhelt, et al. *New J. Physics* 1 (**1999**) 6.1-6.11)). Figure 1 shows four different single molecule stretching experiments (each colored line is a different experiment) for poly(ethylene glycol) (PEG) chemically end-grafted to a planar surface taken with a "bare" unfunctionalized silicon nitride atomic force microscope in aqueous solution. L_f is the bridging polymer chain length = tip-sample separation distance at which the polymer detaches from the probe tip.
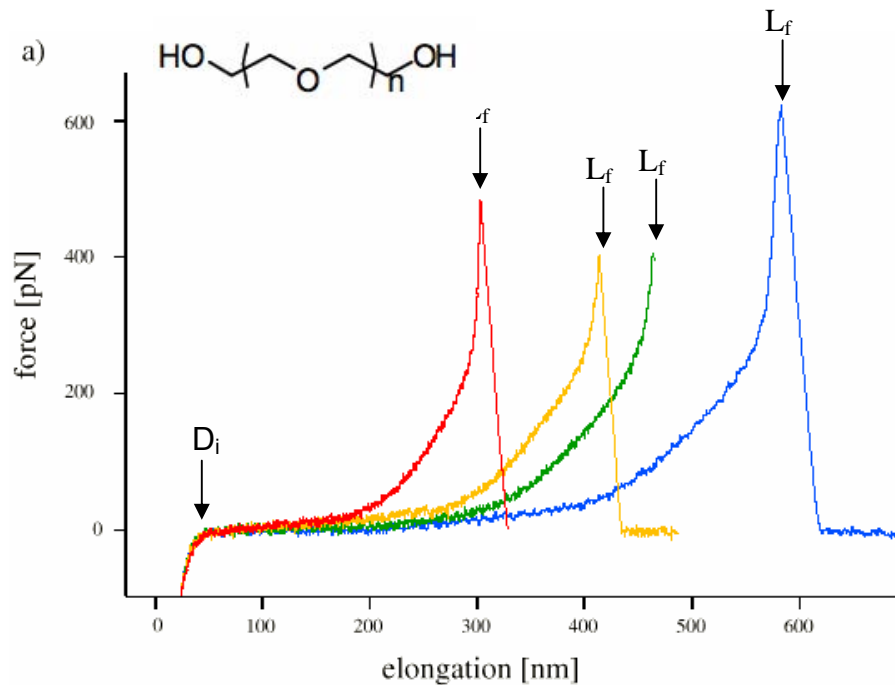

Figure 1.

(a) **Molecular Fingerprint of PEG (15 pts)**
(i) Does the inextensible FJC (a=7Å, L_f=0.99$L_{contour}$) overestimate or underestimate the experimentally measured single molecule stretching force at intermediate elongations? (quantitative numerical calculations are needed).
ANS. The most accurate form of the inextensible FJC is the hyperbolic cotangent function which is valid for a full range of elongations (the Gaussian expression can not be used for intermediate extensions, it is only valid for D< 1/3$L_{contour}$).

$$r(f) = na\left(\coth(x) - \frac{1}{x}\right) \text{where} : x = \left(\frac{fa}{k_B T}\right) \text{ where na = } L_{contour} \text{ (1)}$$

L_f is read off the chart for a given peak
L_f=0.99$L_{contour}$ *(2)*
Let's choose the first peak :
L_f = 315 nm
From (1) $L_{contour}$=317 nm
For the exam, any intermediate force value (e.g. 200 pN, e.g. intermediate elongations are >0.5$L_{contour}$ or so) could have been substituted into equation (1) to obtain the corresponding distance and then compare to the data in Figure 1.
The full FJC is plotted below for clarity :
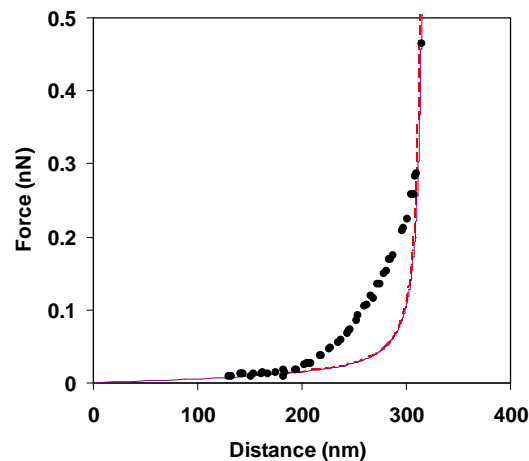
One can see that the inextensible FJC underestimates the force for the intermediate force range.

(ii) Pose a possible explanation for the deviation observed in (a) based on the known conformation of PEG in water. Clarify whether the origin of this deviation is expected to be entropic or enthalpic. (**10 pts**)
ANS. So the result from (i) suggests that some extra energy beyond entropy is needed to extend the chain. If we look at the known structure of PEG in water : Lecture 18 : Nanomechanics and Biocompatibility : Protein-Biomaterials Interactions 2 Slide 8, we see a huge amount of hydrogen bonding with water. Hence, upon extension extra enthalpic energy is needed to break these hydrogen bonds and the force needed for a given extension increases. More on this topic is given at the end of the mp3 of Lecture 18.
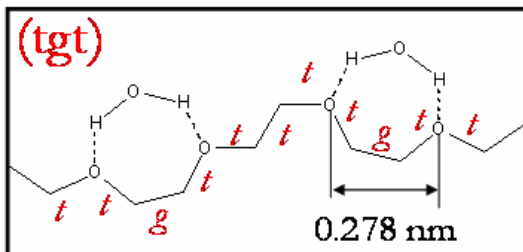
(b) **Short Answer** : **Properties obtained from Force-Elongation curve (answer questions based on the data provided in Figure 1).**
(i) Calculate a numerical value for the average adhesion force between the probe tip and a single molecule of PEG. Explain how this value was obtained.
ANS. The adhesion force between the single polymer chains and the probe tip is the maximum peak detachment force for each plot, $F_{adhesion}$ (pN) ≈ 474, 400, 400, 600. Average = 468 pN (**5 pts**)

(ii) What is the height of the undeformed PEG layer? Explain how you obtained this value. What is the primary source of error in this value? (**5 pts**)
ANS. This is D_i (shown in plot above) which is the position a repulsive force is first observed to rise above the noise. The primary source of error is the incompressible layer height- To obtain an accurate value for the undeformed height one would have to shift the data by this value (measured for example by microcontact printing) which has not been done (remember discussion of incompressible aggrecan height).

(iii) Why is there a large distribution of values for L_f observed for different experiments? (**5 pts**)

ANS. There are two possible reasons (1) polydispersity of the polymer sample which results in variable n in the chemical structure above and (2) the nonspecial adsorption mechanism to the tip picks up the polymer chain at variable locations along its length.

2. **Nanoindentation.** Below is a plot of AFM-based nanoindentation data taken on bone which appears today(!) online in the journal *Nature Materials* (Tai, Ortiz, et al.- see press release on MIT News website). Each colored set of symbols is a different nanoindentation experiment on the same sample.
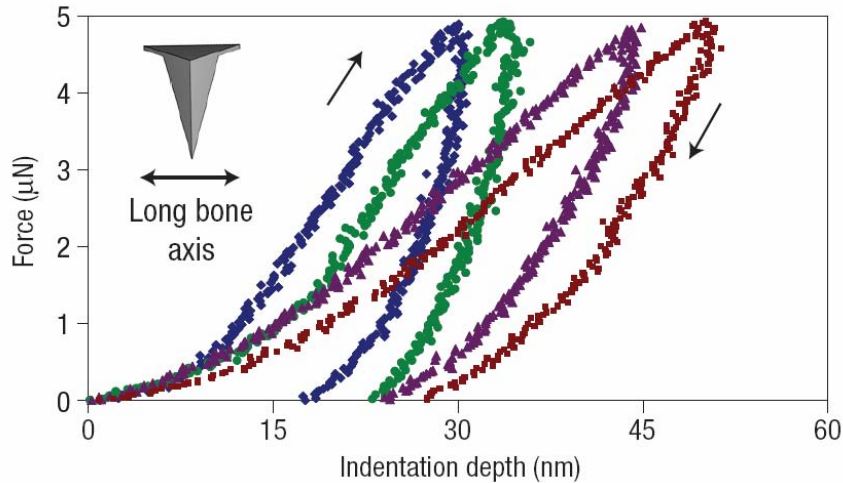
Figure 2.

(a) Estimate the average value of the modulus for the four experiments presented in Figure 2, you can approximate the geometry of the probe tip as a cone with an included conical angle, α = 24.5°. The contact area for a conical indenter is defined as $A=\pi h_c^2 \tan^2\alpha$.(**15 pts**)

ANS. The appropriate equation to use is the Oliver-Pharr equation :

$$E_r = \frac{\sqrt{\pi}}{2\sqrt{A(h_c)}}S$$

h_c can be read directly off the plots as the intercept of the tangent to the unloading curve as shown below, S is the slope of the unloading curve.
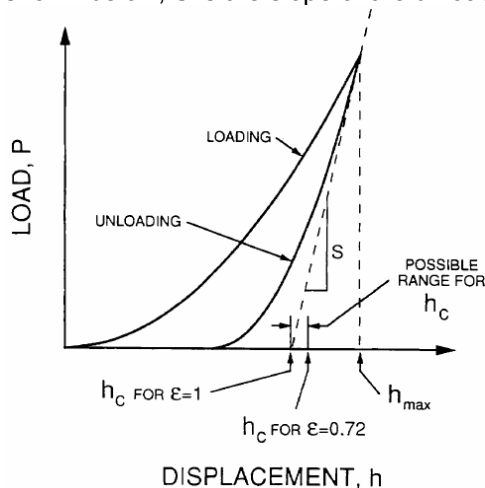
Experiment (left to right)	h_c(nm)	$A=\pi h_c^2 \tan^2\alpha$ (nm^2) $\tan^2\alpha=0.20768$	$\sqrt{A(h_c)}$ (nm)	S (μN/nm)	E_r(GPa)
1	23	345	18.6	0.8	38
2	27	475	21.8	0.75	30

3	32	667	25.8	0.33	11
4	37.5	917	30.3	0.32	9.4
AVERAGE					22.18

(b) Why is the value obtained for (a) not equivalent to the elastic Young's modulus (as defined by the slope of the traditional stress versus strain curve)? (**5 pts**)

ANS. The indentation modulus is a multiaxial measurement while the traditional stress versus strain curve is uniaxial. Also, the length scale probed is much smaller in nanoindentation. Either of these answers was acceptable.

(c) Does this sample undergo inelastic deformation? Why or why not? (**5 pts**)

ANS. Yes, this indicated by the finite values of a residual depth upon unloading, h_r.

(d) One important experimental parameter to consider is the spacing between neighboring indents when carrying out multiple nanoindentation experiments. Explain why in one or two sentences based on the data in Figure 2. (**5 pts**)

ANS. If there is residual plasticity (as seen by the finite value of residual depth), then the indents need to be spaced sufficiently far away from each other to avoid overlap of residual plastic zones.

(e) How is the zero position (force, displacement) determined in AFM-based nanoindentation?

ANS. From Lecture 22 : The zero position is where the repulsive force starts to rise above the noise. For extremely small load nanoindentation experiments, sometimes it may be possible to determine the contact point from the jump-to-contact position. (**5 pts**)
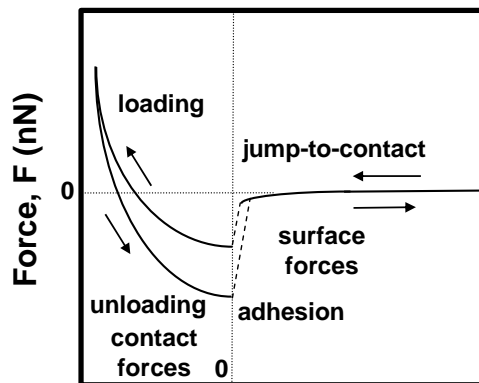
Tip-Sample Indentation Depth or Separation Distance, D (nm)
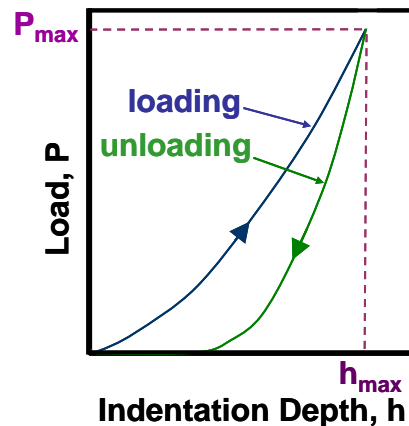
Indentation Depth, h

3. **Electrical Double Layer and Cartilage.**
   (a) Below is a Figure from the podcast paper Dean, et al. *J. Biomech.* **2006**. These are AFM height images taken with a bare unfunctionalized silicon nitride probe tip of a microcontact printed surface of cartilage aggrecan in aqueous salt solution. The salt concentration for each image is given underneath the image. Explain the trends observed in terms of the EDL. (**10 pts**)
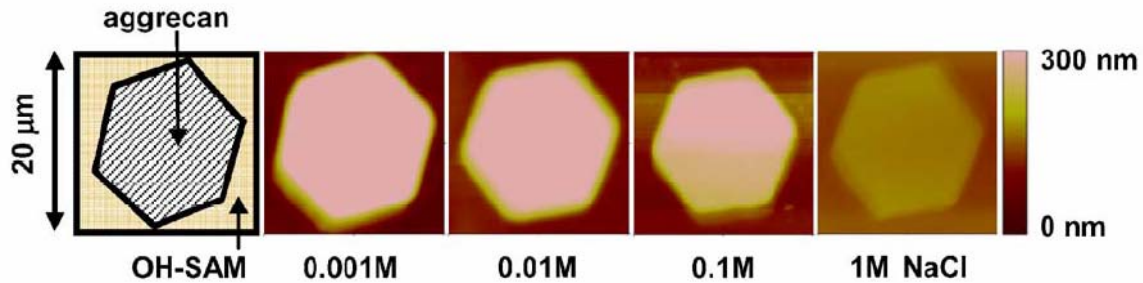
Figure 3.

ANS. By comparing the images with the color bar, one observed that the height of the aggrecan layer decreases with increasing salt concentration. This is due to screening of the electrostatic double layer and intra-molecular charge-charge repulsion of the side chain GAGs within the brush.

(b) Derive a theoretical equation (**force** versus distance) for the compression of a cartilage aggrecan brush taking into account van der Waals interactions, steric repulsion, and EDL defining all parameters. Explain which physical parameters in your equation are the most important for controlling each constituent interaction. (**15 pts**)

ANS. For sphere-surface geometry :

$$F_{total} = \underbrace{R\kappa Z e^{-\kappa D}}_{EDL} - \underbrace{\frac{AR}{6D^2}}_{VDW} + \underbrace{\frac{\partial}{\partial D}\left(\frac{F_0 R L_0}{\sigma} \left[\left(\frac{L_0}{D}\right)^{-1/4} - \left(\frac{L_0}{D}\right)^{11/4} \right] \right)}_{Steric}$$

Most important parameters :
EDL : Electrical Debye length, κ^{-1} and surface charge density (σ) or surface potential (ψ)
VDW : Hamaker constant, **A**
Steric : grafting density (σ) and height of polymer brush (L_0)

HAVE A GREAT SUMMER!

Hydrophilic Chemical Groups

hydroxyl groups -OH

carbonyl groups

carboxyl groups -   can ionize to COO⁻
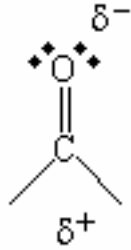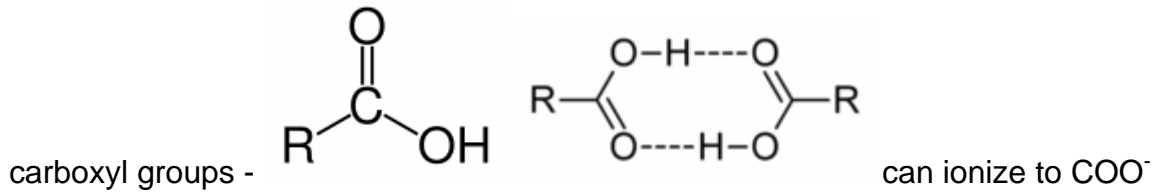
sulfates : SO_4^-
sulfonates : SO_3^-

aldehydes

ketones

alcohols
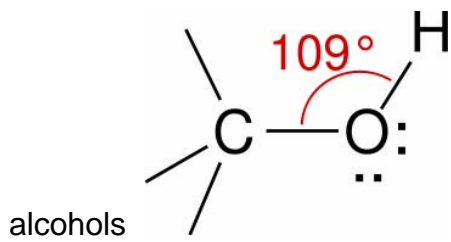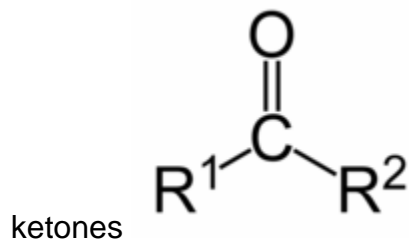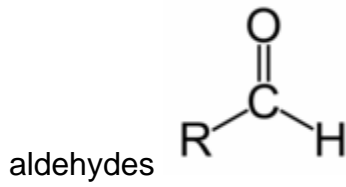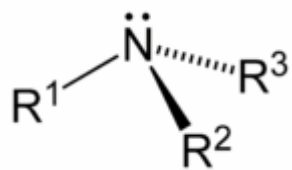
amines
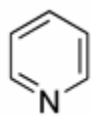
N-heterocyclic rings

Amides
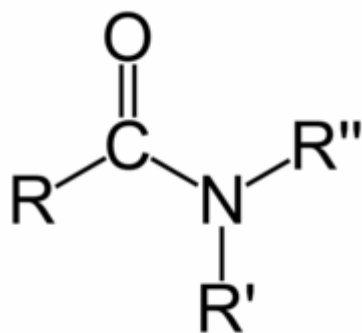
O-heterocyclic rings
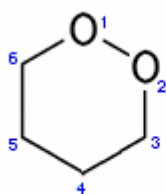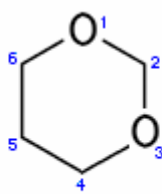
| 1,2-dioxane | 1,3-dioxane | 1,4-dioxane |
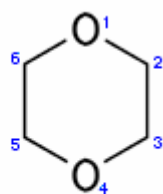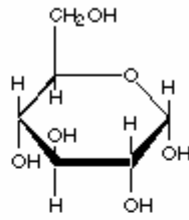
Monosaccharides
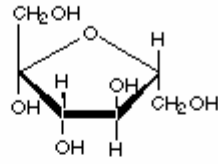
α-Glucose

Fructose

Disaccharides
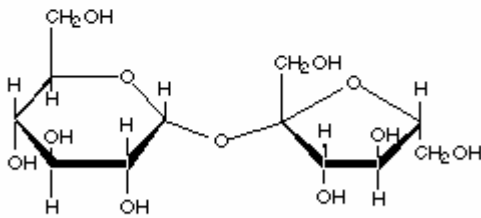
Sucrose
(glucose (α1-->2) fructose)
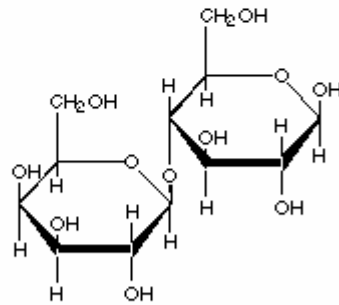
Lactose
(Galactose (β1-->4) Glucose)

Hydrophobic Chemical Groups

methyl -CH_3
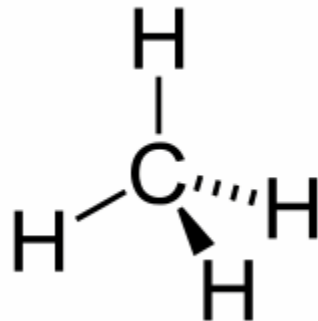
alkanes   Alkanes can be linear (general formula C_nH_{2n+2}) where the carbon atoms are joined in a snake like structure, cyclic (general formula C_nH_{2n}, n>2) where the carbon backbone is linked so as to form a loop, or branched (general formula C_nH_{2n+2}, n>3)
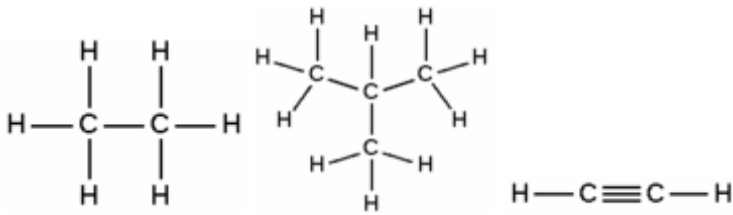
hydrocarbons

Fatty acids : mostly hydrophobic, but have hydrophilic COOH at end

Butyric	(butanoic acid):	$CH_3(CH_2)_2COOH$	C4:0
Caproic	(hexanoic acid):	$CH_3(CH_2)_4COOH$	C6:0
Caprylic	(octanoic acid):	$CH_3(CH_2)_6COOH$	C8:0
Capric	(decanoic acid):	$CH_3(CH_2)_8COOH$	C10:0
Lauric	(dodecanoic acid):	$CH_3(CH_2)_{10}COOH$	C12:0
Palmitic	(hexadecanoic acid):	$CH_3(CH_2)_{14}COOH$	C16:0
Stearic	(octadecanoic acid):	$CH_3(CH_2)_{16}COOH$	C18:0
Arachidic	(icosanoic acid):	$CH_3(CH_2)_{18}COOH$	C20:0
Behenic	(docosanoic acid):	$CH_3(CH_2)_{20}COOH$	C22:0

Amino acids with hydrophobic side groups
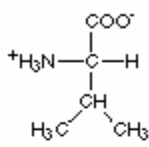
Valine
(val)
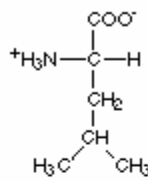
Leucine
(leu)
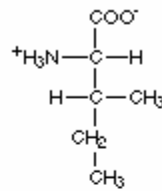
Isoleucine
(ile)
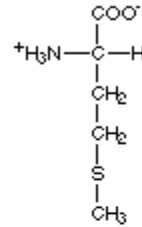
Methionine
(met)
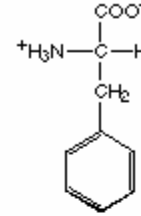
Phenylalanine
(phe)

Amino acids with hydrophilic side groups
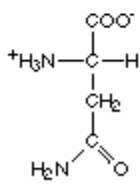
Asparagine
(asn)
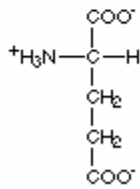
Aspartic acid
(asp)
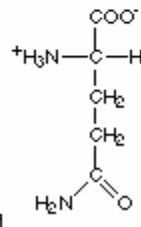
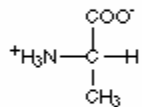Glutamic acid
(glu)

Glutamine
(gln)
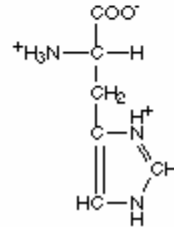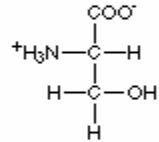
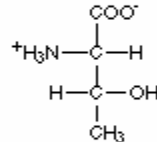Histidine
(his)
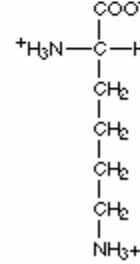
Lysine
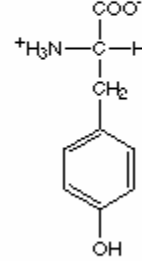(lys)
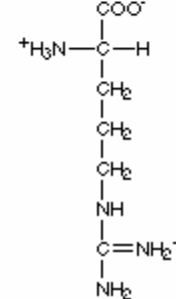
Arginine
(arg)
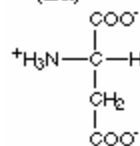
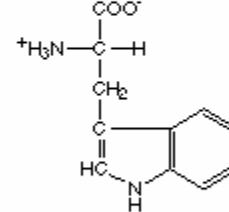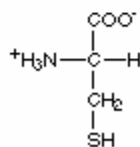Amino acids that are in between
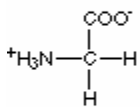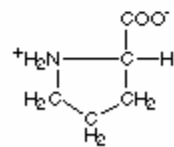
Glycine
(gly)

Alanine
(ala)

Serine
(ser)

Threonine
(thr)

Tyrosine
(tyr)

Tryptophan
(trp)

Cysteine
(cys)

Proline
(pro)

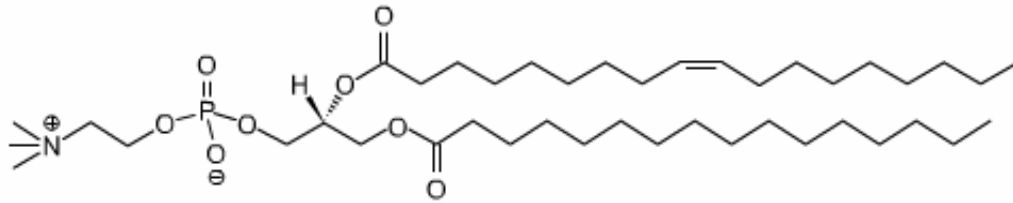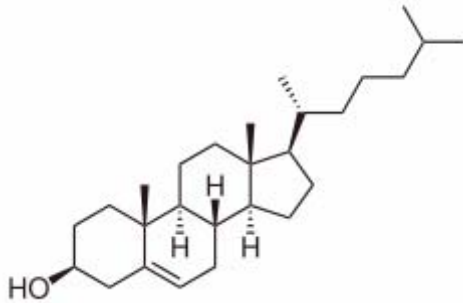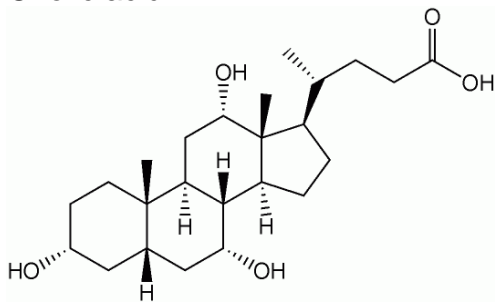# **Amphiphilic Molecules**

Lipids

Cholesterol

Cholic acid

Polyethylene glycol