3.052 Nanomechanics of Materials and Biomaterials : Spring 2007
Assignment #3

Due Date : Thursday 03.15.07

You are encouraged to use additional resources (e.g. journal papers, internet, etc.) but please cite them (points will be deducted for not doing so). You will need to research additional sources to answer some questions.

1. Heparin Biosensors Podcast: Milovic, *et al.*, "*Monitoring of heparin and its low-molecular-weight analogs by silicon field effect*." 13374–13379, *PNAS*, 2006103 (36)

a. Research the primary sequence of a protamine and of the chemistry of the sensor surface and based on this information explain in detail all of the possible nonspecific interactions involved in the physisorption process used for sample preparation.

ANS. The primary sequence of protamine can be found at http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=protein&val=85543

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>gi|85543|pir||B02669 protamine (salmine) AII - chum salmon
PRRRRSSSRPIRRRRPRRASRRRRRRGGRRRR
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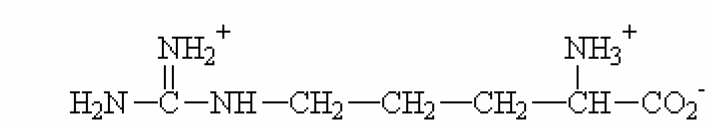
The amino acids appearing in the sequence are given below with their characteristics at physiological (neutral) pH:

R: arginine (66%) + charged
S: serine (12.5%) neutral
P: praline (9.4%) (hydrophobic, neutral)
G: glycine (6.3%) (hydrophobic, neutral)
I: isoleucine (3.1%) (hydrophobic, neutral)
A: alanine (3.1%) (hydrophobic, neutral)

The glass surface of the field effect sensor is negatively charged at neutral pH because the silicon dioxide is deprotonated at the surface (≡Si-O$^-$). [Hau *et al.* "Surface-chemistry technology for microfluidics" *J. Micromech. Microeng.* **13** (2003) 272-278.]

The most prevalent attractive interaction will be ionic bonding between arginine and ≡Si-O$^-$



$$H_2N-\overset{\overset{NH_2^+}{\|}}{C}-NH-CH_2-CH_2-CH_2-\overset{\overset{NH_3^+}{|}}{CH}-CO_2^-$$

b. Physisorption of biomacromolecules to surfaces can be highly variable and uncontrolled; name 5 important physical properties of the coating to consider.

The following are 5 important physical properties to consider; ^{1}uniformity of the coating (spatial density and homogeneity), ^{2}thickness of the coating (monolayer or multilayer), ^{3}conformation of coating molecules (molecules will need sufficient mobility in order to bind the target, hence "loopy" conformations are more desirable than completely flat adsorbed ones) ^{4}accessibility of functional groups involved, and ^{5}stability of the coating in the aqueous environment of the biomacromolecules (kinetics of exchange and desorption).

c. On page 13378, the text says "AT-III was covalently attached to the sensor surface by..." Some scientists could disagree with this point, why?

The surface chemistry sequence for attachment of AT-III is given as follows (Figure 4), glass substrate-silane-avidin-biotin-AT-III. Avidin is covalently bound to the surface through an aldehyde-mediated reaction, where free amines on the avidin react with the surface silane aldehydes. However, the AT-III attachment occurs through an avidin-biotin bond which is not a covalent bond.

d. Why does Jon Behr say that current clinical tests for heparin levels measure heparin activity, not actual concentration? Why are current tests for heparin levels not appropriate for clinical use? How is a clinical measure of concentration a major advantage in the case of heparin?

The current clinical tests generally measure a heparin effect, like activated clotting time. Colorimetric assays in the laboratory give a more accurate concentration measure but are too complicated for the clinic, in terms of precision and liquid handling, and they take too long. Clinical concentration measure will be a major advantage in the case of heparin because the samples are generally heterogeneous and polydisperse, causing differing levels of activity from sample to sample. This means that an antidote dosage based on activity might be severely mismatched to the actual amount of heparin in a patient's circulation. Clinicians would be best served by information on both absolute concentration and activity.

2. Theoretical Modeling of the Mechanics of Single Cells. In Section 3.1 of Dao, et al. 2003 51 *J. Mech. Phys. Solids* 2259-2280 (posted under "Supplementary Resources" on Stellar) an equation is presented for the membrane shear modulus, μ, which is not constant but is dependent upon the degree of stretch.

a. Starting from the neo-Hookean strain energy potential function (equation (2), derived in recitation on 03.02.07), present the full derivation of equation (5). Show all steps in between equations (2)-(4). Be sure to justify all assumptions and equations used, and define all symbols used. Include an explicit derivation for the constant volume constraint, $\lambda_1\lambda_2\lambda_3=1$. Note that the symbol T is the membrane "stress" which is equal to the Force per unit length in units of N/m.

N.B.: There is a typo in equation (3) and it should read :

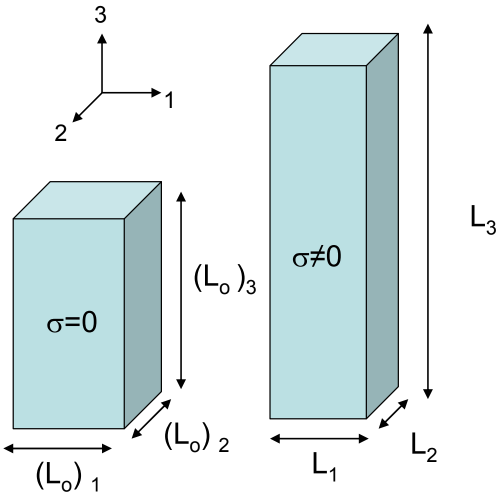
$$T_1 = h\lambda_1 \frac{\partial U}{\partial \lambda_1} = G_0 h_0 \left(\lambda_1^{1.5} - \lambda_1^{-1.5}\right).$$

There is also a mistake in equation (5); it should read :

$$\mu(\lambda_1) = \frac{1}{2}\frac{\partial T_s}{\partial \gamma_s} = \frac{3G_0 h_0 (\lambda_1^{0.5} + \lambda_1^{-2.5})}{2(2\lambda_1 + \lambda_1^{-2})}$$

ANS.

First derive constant volume constraint equation by considering an arbitrary deformation of a cube of material.



$(L_o)_1$,$(L_o)_2$,$(L_o)_3$ = the initial side lengths of the cube at zero stress in the 1,2, and 3 directions respectively

L_1,L_2,L_3 = the final or instantaneous side lengths of the cube at a constant stress state in the 1,2, and 3 directions respectively

From Lecture 7, by definition: λ = extension or stretch ratio; $\lambda_1 = \frac{L_1}{(L_o)_1}, \lambda_2 = \frac{L_2}{(L_o)_2}, \lambda_3 = \frac{L_3}{(L_o)_3}$

The volume of the cube before and after deformation is

$V(\sigma=0)= (L_o)_1(L_o)_2(L_o)_3$, $V(\sigma \neq 0)= L_1 L_2 L_3$

The change in volume can be set equal to zero: $\Delta V = L_1 L_2 L_3 -(L_o)_1(L_o)_2(L_o)_3=0$

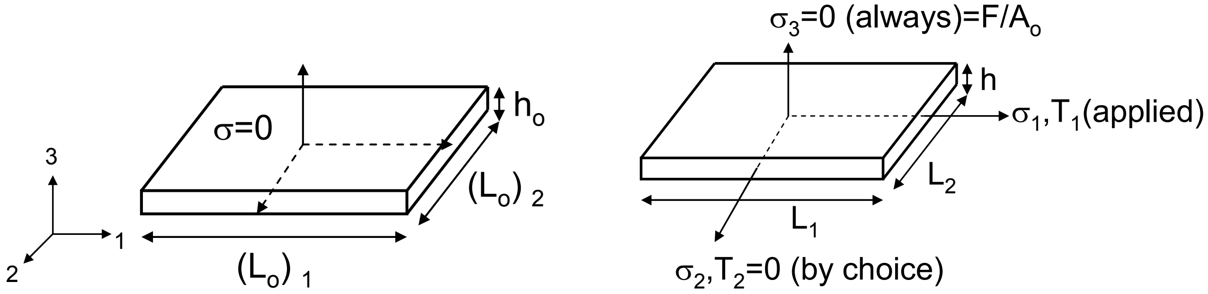
Then $L_1 L_2 L_3 = (L_0)_1 (L_0)_2 (L_0)_3$

Divide both sides by $(L_o)_1(L_o)_2(L_o)_3$:

$$\frac{L_1 L_2 L_3}{(L_0)_1 (L_0)_2 (L_0)_3} = \frac{(L_0)_1 (L_0)_2 (L_0)_3}{(L_0)_1 (L_0)_2 (L_0)_3}$$

Substitute the definition of each extension ratio into the equation to get $\lambda_1 \lambda_2 \lambda_3 = 1$

Now set up the membrane geometry for zero (left Figure below) and applied uniaxial stress (right Figure below), following Dao, et al. and Lecture 7:



h_o, h = initial (σ=0) and final ($\sigma=\sigma_1$) thickness of membrane

$(L_o)_1$,$(L_o)_2$, L_1, L_2= initial (σ=0) and final ($\sigma=\sigma_1$) dimensions of bilayer in 1 and 2 directions

T_1, T_2 = in-plane principal membrane stresses = force per current transverse unit length (N/m)

T_s = membrane shear stress (N/m)

λ_1, λ_2, λ_3 = principal stretch ratios; e.g., $\lambda_1 = L_1/(L_o)_1$, etc.

Start with equation (2), derived in recitation on 03.02.07:

$$U = \frac{G_0}{2}\left(\lambda_1^2+\lambda_2^2+\lambda_3^2-3\right) \qquad \text{equation (2) Dao}$$

where G_o is the shear modulus. Constant volume constraint: Since $\lambda_1 \lambda_2 \lambda_3 = 1$, $\lambda_1 = \lambda$ and $\lambda_2 = \lambda_3$ (for an isotropic material with a positive Poisson's ratio, there is an equal contraction in the transverse direction), we have $\lambda_2 = \lambda_3 = \frac{1}{\sqrt{\lambda_1}}$, substitute into equation (2) ;

$$U = \frac{G_0}{2}\left(\lambda_1^2+\frac{2}{\lambda}-3\right). \qquad (2.1)$$

Nominal stress is found from the derivative of the energy function with respect to λ_1:

$$\left(\sigma_{\mathrm{n}}\right)_1 = \frac{\partial U}{\partial \lambda} = G_0\left(\lambda_1-\frac{1}{\lambda_1^2}\right). \qquad (2.2)$$

Note that by definition, $\sigma_{\mathrm{n}} = \frac{\mathrm{F}}{\mathrm{A}} = \frac{F_1}{\left(L_o\right)_2 h_0}$, $h = h_0 \lambda_3 = h_0 \frac{1}{\sqrt{\lambda_1}}$ and $T_1 = \frac{F_1}{L_2}$. So

$$T_1 = \frac{F_1}{L_2} = \frac{F_1}{\lambda_2\left(L_o\right)_2} = \sqrt{\lambda_1}\frac{F_1}{\left(L_o\right)_2} = h_0\sqrt{\lambda_1}\sigma_{\mathrm{n}} = h\lambda_1\sigma_{\mathrm{n}}, \qquad (2.3)$$

and consequently ;

$$T_1 = h\lambda_1\left\{G_0\left(\lambda_1-\frac{1}{\lambda_1^2}\right)\right\} = h_0\sqrt{\lambda_1}\left\{G_0\left(\lambda_1-\frac{1}{\lambda_1^2}\right)\right\} = G_0 h_0\left(\lambda_1^{\frac{3}{2}}-\lambda_1^{-\frac{3}{2}}\right). \qquad (2.4)$$

We arrive at equation (3) in Dao *et al.*:

$$T_1 = h\lambda_1\frac{\partial U}{\partial \lambda_1} = G_0 h_0\left(\lambda_1^{1.5}-\lambda_1^{-1.5}\right). \qquad \text{equation (3) Dao}$$

Equations (1a) and (1b from Dao, *et al.*) give the membrane shear stress, T_s and shear strain, γ_s:

$$T_s = 2\mu\gamma_s = \frac{1}{2}(T_1-T_2) \quad \text{and} \quad \gamma_s = \frac{1}{4}\left(\lambda_1^2-\lambda_2^2\right) \qquad \text{equation (1a,1b) Dao}$$

$$T_s = \frac{G_0 h_0}{2}\left(\lambda_1^{1.5}-\lambda_1^{-1.5}\right) \qquad \text{equation (4) Dao}$$

For uniaxial tension: $\gamma_s = \frac{1}{4}\left(\lambda_1^2-\frac{1}{\lambda_1}\right) = \frac{1}{4}\left(\lambda_1^2-\lambda_1^{-1}\right)$

The membrane shear modulus will depend on the stretch and is determined from

$$\mu(\lambda_1) = \frac{1}{2}\frac{\partial T_s}{\partial \gamma_s} \qquad \text{1st two terms equation (5) Dao}$$

where

$$\frac{dT_s}{d\lambda_1} = \frac{G_0 h_0}{2}\left(\frac{3}{2}\lambda_1^{0.5} + \frac{3}{2}\lambda_1^{-2.5}\right) \quad \text{and} \quad \frac{d\gamma_s}{d\lambda_1} = \frac{1}{4}\left(2\lambda_1 + \lambda_1^{-2}\right) \qquad (5.1)$$

so that

$$\mu(\lambda_1) = \frac{3G_0 h_0 (\lambda_1^{0.5} + \lambda_1^{-2.5})}{2(2\lambda_1 + \lambda_1^{-2})} \qquad \text{corrected equation (5) Dao}$$

Note: there was a typo in the equation in the assignment handout whereby the numerator had a subtraction instead of an addition. No points were taken off if you plotted this equation.

b. Recreate Figures 3a and 3b with the horizontal axis covering up to a maximum principal stretch of λ_1 = 3.

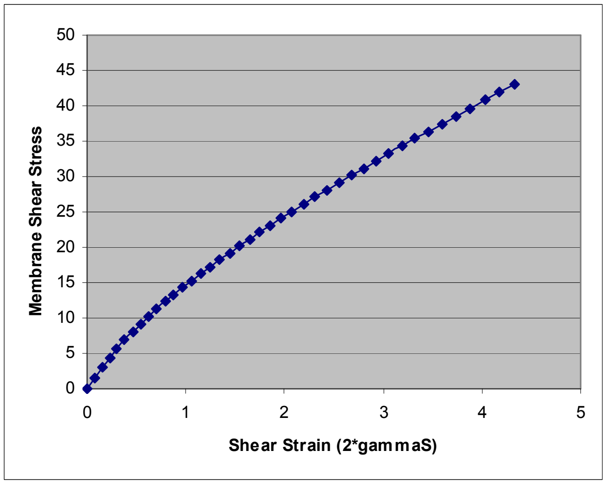
First, make sure all material parameters are known. On page 2268, Dao *et al.* state that they used an initial value of the shear membrane modulus of μ_0=22.5 μN/m. In other words,

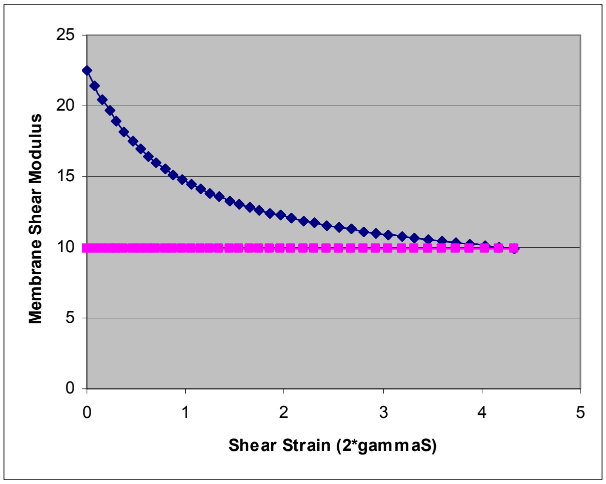
$$\mu_0 = \mu(\lambda_1 = 1) = G_0 h_0 = 22.5\ \mu\text{N/m}$$

This value can be used to graph the shear stress and shear modulus functions as done in the attached excel file. Alternatively, you can use the value of $\mu_1(\lambda_1=3)$ = 13.3 μN/m which is cited as the corresponding μ_1 value to the μ_0 above but no longer holds true due to the equation errors in the paper. Using $\mu_1(\lambda_1=3)$ = 13.3 μN/m yields μ_0 = 30.2 μN/m. For the range $1 < \lambda_1 < 3$, compute twice the shear strain to form the x axis of both figures:

$$2\gamma_s = \frac{1}{4}\left(\lambda_1^2 - \lambda_1^2\right)$$

Then calculate the shear modulus according to the corrected equation (5) above; plot the modulus on the y axis with $2\gamma_s$ on the x axis to recreate figure 3b. Next, calculate $T_s = 2\mu\gamma_s$ and plot on the y axis with $2\gamma_s$ on the x axis to recreate figure 3a.





c. In Lecture, we discussed a secondary strain hardening term added to the the neo-Hookean strain energy potential function. Explain one interpretation of the physical origin of this term.

The strain hardening term here comes from non-Gaussian finite extensibility of the network strands, i.e., the individual random coils do not behave as linear elastic springs that can be extended forever. The force rises nonlinearly and asymptotically as the contour length is approached. The origin of this is still entropic in nature (due to a reduction in the number of accessible conformations).

3. Intermolecular Potentials, Water, and H-Bonding. Consider the following contributing interactions between two water molecules; dispersion interactions, orientation polarization of freely rotating dipoles, and induced polarization (freely rotating dipole-induced dipole) which are collectively known as the van der Waals interaction, $w(r)_{VDW}$.

a. Plot $w(r)_{VDW}$ quantitatively for water in units of *w* (k_BT) and (r) nm. Include a short range Coulombic repulsion term; $w(r)_{\mathrm{repulsive}}=B/r^{12}$ with B=10^{-134} Jm^{12}.

ANS.

Van der Waals : $w(r)_{VDW} = w(r)_{orient} + w(r)_{induced} + w(r)_{dispersion}$

Keesom Energy : Freely rotating dipole-freely rotating dipole interaction :

$$w(r)_{orient} = \left(\frac{-u^4}{3k_{\mathbf{B}}T(4\pi\varepsilon_0)^2}\right)r^{-6} = -C_{orient}r^{-6}$$

Debye Energy : Freely rotating dipole- nonpolar (induced dipole) interaction :

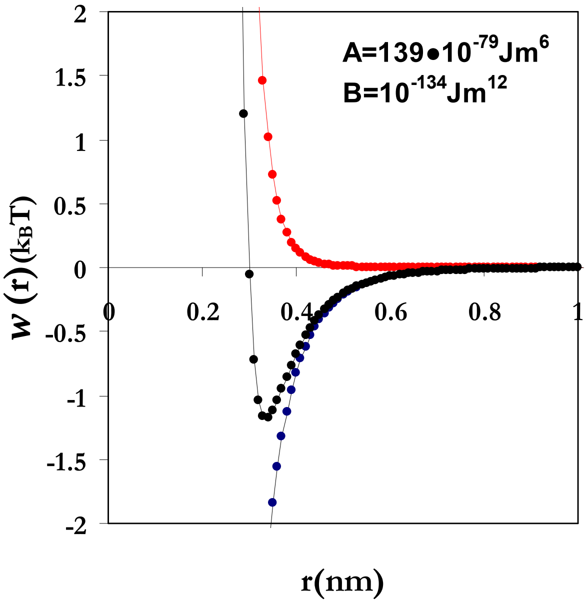
$$w(r)_{induced} = \left(\frac{-u^2\alpha}{(4\pi\varepsilon_0)^2}\right)r^{-6} = -C_{induced}r^{-6}$$

Dispersion Energy : Induced dipole-induced dipole :

$$w(r)_{dispersion} = \left(\frac{-3h\nu\alpha^2}{4(4\pi\varepsilon_0)^2}\right)r^{-6} = -C_{dispersion}r^{-6}$$

Q= electric charge (C)
ε_o= dielectric permittivity of free space = 8.854●10^{-12} $C^2J^{-1}m^{-1}$
α=electric polarizability ($C^2m^2J^{-1}$)
θ= dipole angle to horizontal
u= electric dipole moment (Cm)

From Israelachvili, *Intermolecular and Surface Forces,* 1992, page 95 (Table 6.3) for H_2O-H_2O interactions: $C_{induced}$ = 10, C_{orient}= 96, C_{disp}=33 (●10^{-79} Jm^6)

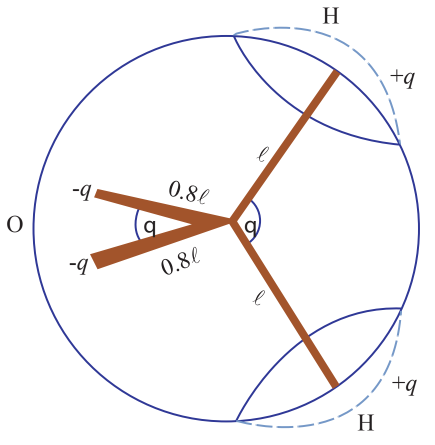


b. Compare the strength and equilibrium bond length of this van der Waals interaction estimation to a typical H-bond.

The equilibrium bond length, r_e and binding strength, E_B, can be read directly from the attractive minimum of the graph of *w(r)*; r_e=0.338 nm and E_B=1.01 k_BT. In Lecture 9, Slide 9, the interaction distance for an H-bond is given as 0.3 nm with a strength of 4-16 k_BT. Hence, this van der Waals force is quite a bit less than H-bonding, which dominates HOH-HOH interactions.

c. A better model (relative to part a) for the interaction between two water molecules is a Lennard-Jones potential plus 16 Coulombic terms. Explain the reasoning behind such a model. What would the latter terms correspond to?

The Lennard-Jones potential would be the van der Waals interaction calculated above. The additional 16 Coulombic terms would represent ionic interactions between each of the possible 4 point charge interactions, shown in Figure 8.3 from Israelachvili, *Intermolecular and Surface Forces,* 1992:



ST2 model of water molecule; $q = 0.24e$, $l = 0.1$nm, q = 109º

Figure by MIT OCW.

d. Estimate the van der Waals force of a water molecule approaching a layer of water on a surface at a separation distance of 3 nm.

In Lecture 10 we derived the general form of the molecule-surface interaction:

$$W(D)_{MOL\text{-}SFC} = \frac{-2\pi C\rho}{(n-2)(n-3)D^{n-3}}$$

$$\text{London Dispersion Interactions } n = 6\ ;$$

$$W(D)_{MOL\text{-}SFC} = \frac{-\pi C\rho}{6D^3}$$

$$F(D)_{MOL\text{-}SFC} = \frac{-dW(D)}{dD} = \frac{-\pi C\rho}{2D^4}$$

Number density of water molecules can be calculated from the known density of water:

$$\rho = 1.0\frac{g}{cm^3}\cdot\frac{1mole}{18g}\cdot\frac{6.023\cdot10^{23}\,molecules}{mole}\cdot\left(\frac{10^2\,cm}{1m}\right)^3 = 0.33\cdot10^{29}\,\frac{molecules}{m^3}$$

As above, the prefactor is a combination of the three attractive van der Waals interactions; C = A = $C_{dispersion}$+ $C_{dispersion}$ + $C_{dispersion}$ =139 x 10^{-79} Jm^6, so that

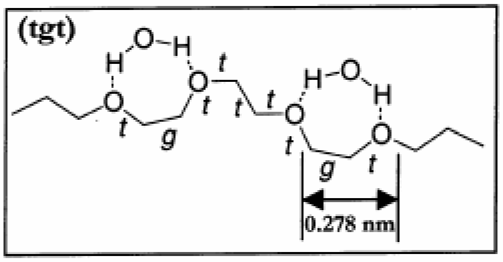
$$F(3nm) = \frac{-\pi(139\cdot10^{-79}\,Jm^6)(0.33\cdot10^{29}\,\frac{molecules}{m^3})}{2(3\cdot10^{-9}\,m)^4} = 17.78\,pN$$

e. The "range" of an interaction is an ambiguous concept. Why? Propose a reasonable mathematical definition for the range of an interaction.

ANS. The range of an interaction is ambiguous because the mathematical power law form, $w(r) = -A/r^n$ shows that the magnitude→0 only when r, D→∞. The range could be defined as the distance over which the interaction energy is within some percentage of the maximum interaction energy, for instance over the range of r where $w(r) > 0.01\ w(r)_{max}$. Generally, the range will be determined by the limit of force detection, i.e. when the force decreases below the instrumental noise, it is considered essentially = 0.

f. Polymethylene oxide -[CH_2-O]$_n$- is hydrophobic but polyethylene oxide -[CH_2- CH_2-O]$_n$- is hydrophilic. Explain why.

ANS. It is thought that PEO exists in a trans-gauche-trans conformation which can exactly accomodate the size of a water molecule via hydrogen bonding and hence is highly hydrated and hydrophilic.



The distance between oxygens in PMO is too short to be able to precisely fit with water.

+ 5 extra credit for posting a message on the podcast message board.