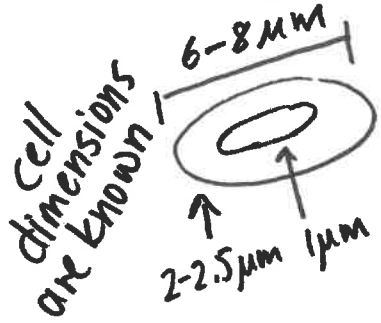
**Problem Set - Membranes & Membrane Proteins**

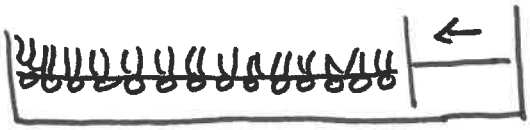
1. Consider the experiment conducted by Gorter and Grendel in 1925. Describe the experimental procedure, and what experimental variables could affect the interpretation of their results.




⇒ $SA_{cell}$

→ extracted lipid membranes of erythrocytes using acetone. Number of cells is known.

→ spread lipid extract onto water layer of a langmuir trough.



→ measured Area of monolayer

→ calculated the ratio of the measured area to the predicted cell surface area. ($R = A_M / SA_{cell}$)

→ measured 2:1 ratio indicating bilayer structure.

Factors affecting monolayer area ($A_M$)

↓ extraction efficiency ⇒ ↓R
ie. loss of lipids, or incomplete extraction.

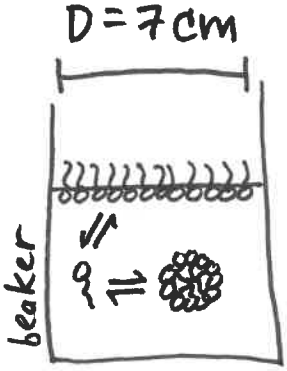
- surface pressure dependencies.

Factors affecting cell surface area ($SA_{cell}$)

↑ cell area is not just membrane, but protein too. ⇒ ↓R

2. A 200 mL solution of n-Decyl-β-D-Maltopyranoside is prepared in a standard 250 mL beaker at a concentration of 10 mM in 150 mM NaCl, 20 mM MOPS, at pH 7.0.

(a) Calculate the concentration of free detergent monomers in solution.
(b) What is the fraction of detergent molecules in the monolayer?
(c) What is the fraction of detergent molecules in the micelle form?




a) n-Decyl-β-D-Maltopyranoside
i.e. Decylmaltoside (DM)
CMC = 1.8 mM
∴ free monomer concentration is equal to the CMC, ⇒ **1.8 mM**

b) Number of DM molecules in total, $N_{DM}$:

$N_{DM} = 10\,mM * 200\,mL = 2\times10^{-3}\,moles = 2\times10^{-3}\times 6.022\times10^{23}\,molecules = 12.0\times10^{20}\,molecules.$

$A_{monolayer} = \pi r^2 = \pi(3.5\times10^{-2}\,m)^2 = 0.0039\,m^2 = 4\times10^{-3}\,m^2$

approximate the area of DM by $SA_{lipid}$:

$SA_{DM} < SA_{lipid} \sim 0.6\,nm^2 = 60\,\text{Å}^2 = 6\times10^{-19}\,m^2$

$N_{monolayer} \geq \frac{4\times10^{-3}\,m^2}{6\times10^{-19}\,m^2} = 0.7\times10^{16}$ molecules.

$\Rightarrow F_{monolayer} = \frac{7\times10^{15}}{12\times10^{20}} = 0.000004 = 4\times10^{-6} \sim$ **0**

c) $N^{DM\,in}_{micelle} = N_{DM\,Total.} - N_{DM\,monolayer} - N_{DM\,free}$

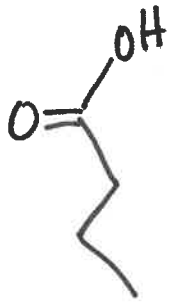
$N_{DM\,free} = 1.8\,mM \times 200\,mL = 360\,\mu moles = 0.36\times10^{-3}\,moles = 0.36\times10^{-3}\times6.022\times10^{23}\,molecules = 2.2\times10^{20}\,molecules.$

$\Rightarrow N_{DM\,in\,micelles} = 12\times10^{20} - 7\times10^{15} - 2\times10^{20} = 10\times10^{20}$ molecules.

$\therefore F_{DM,micelle} = \frac{10\times10^{20}}{12\times10^{20}} =$ **0.83**

3. Find the common names for the following fatty acids chains:

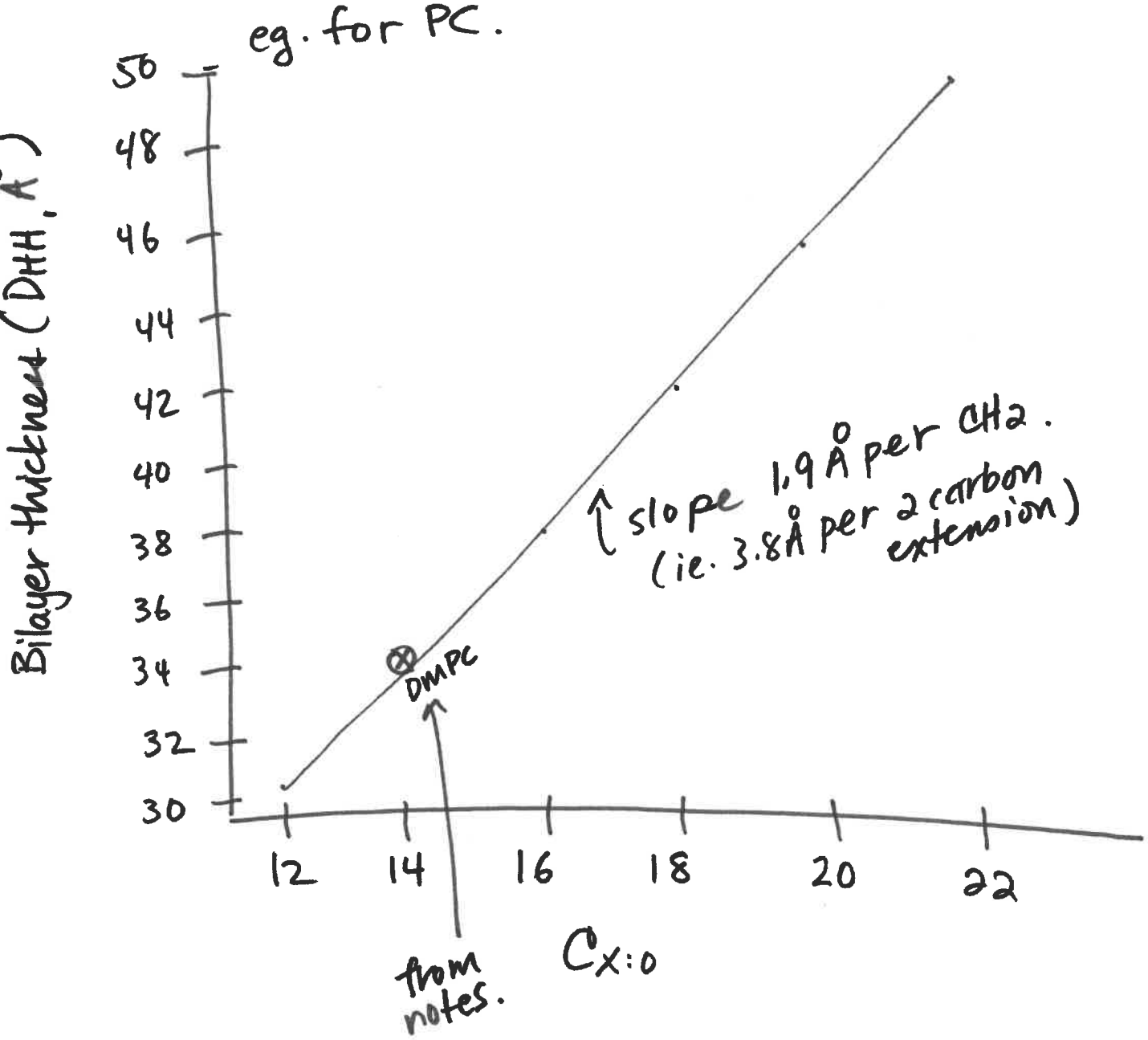
a. C4:0 butyric
b. C6:0 caproic
c. C8:0 ~~caprylic~~ caprylic
d. C10:0 capric
e. C12:0 lauric
f. C14:0 myristic
g. C16:0 palmitic
h. C18:0 stearic
i. C20:0 arachidic
j. C22:0 behenic



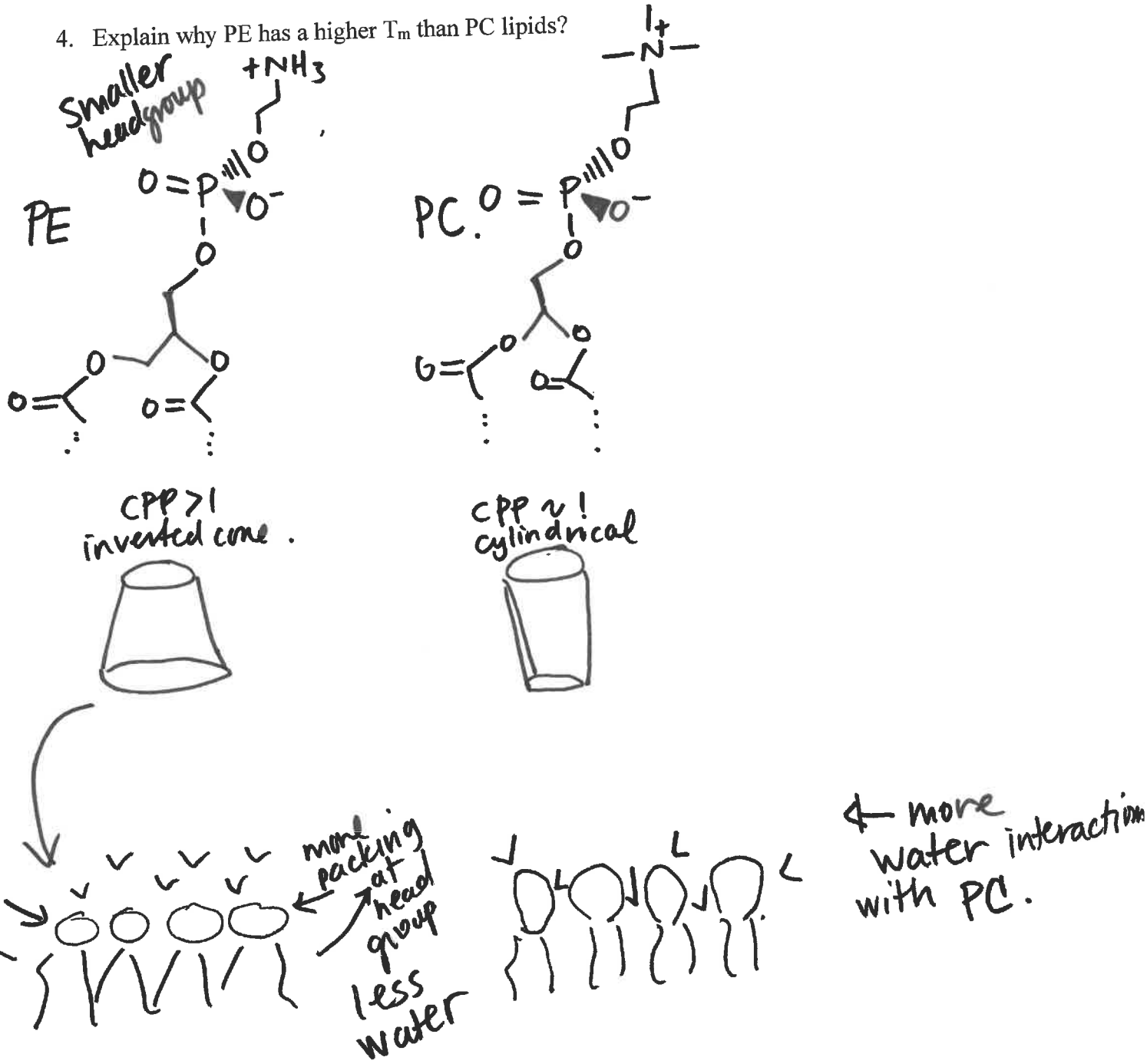

butyric acid

C4:0

3. Draw a graph that shows the change in bilayer thickness as a function of increasing tail length from C12:0 to C22:0.

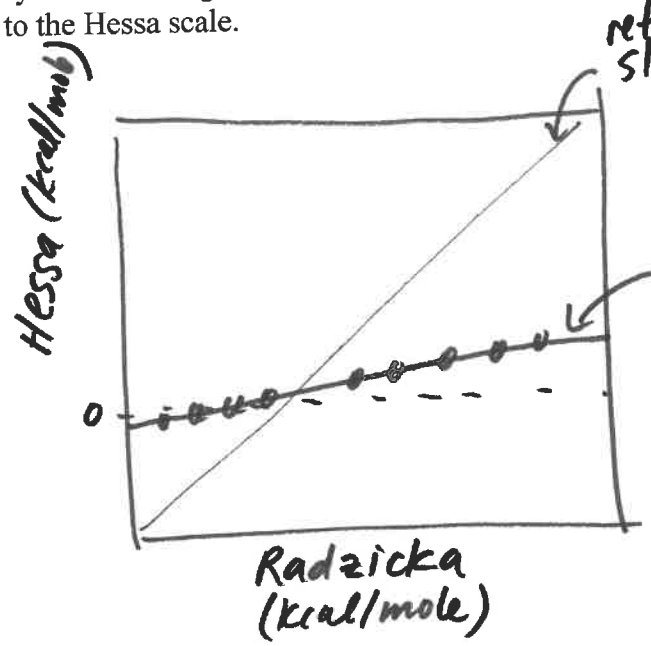


4. Explain why PE has a higher $T_m$ than PC lipids?

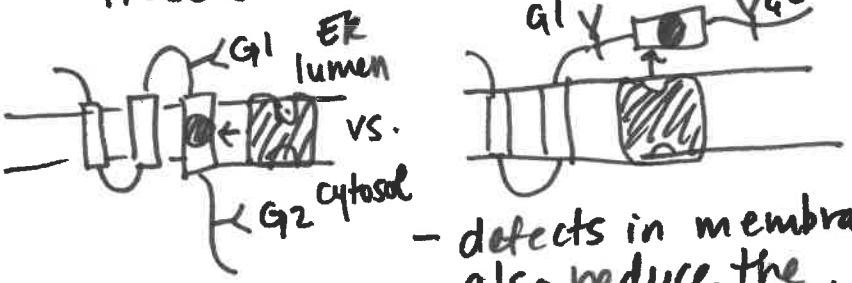



⇒ increased stability with respect to temperature.

5. Explain why the free energies for amino acid partitioning are larger for the Radzicka scale compared to the Hessa scale.

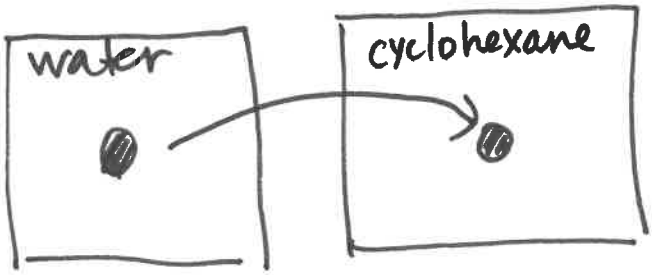



Hessa → translocon scale.



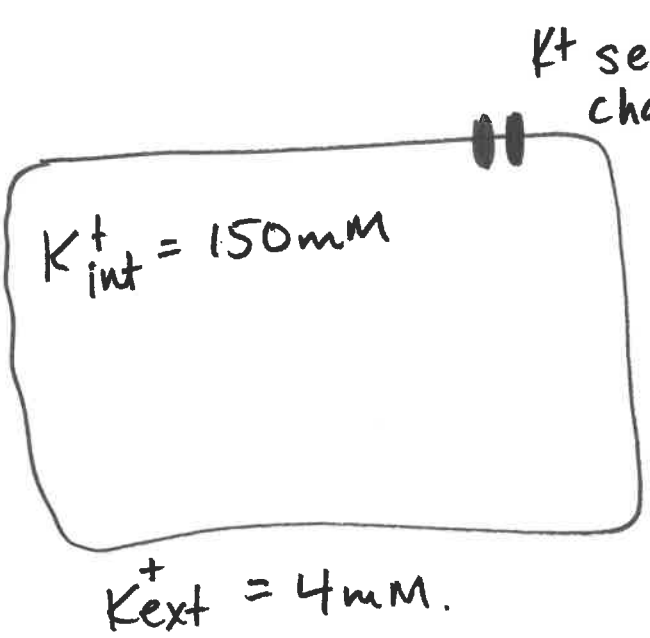

- captures free energy of partitioning into lipid bilayer.
- we do not know the orientation of the helix or other stabilizing factors (interactions w/ other proteins).
- defects in membrane also reduce the free energy penalty.

Radzicka → water to cyclohexane.




- captures free energy of transferring to isotropic hydrophobic core.
- no defects. pure low dielectric solvent.

6. Calculate the resting membrane potential for a cardiac myocyte (look up the appropriate ionic concentrations).




assuming $K^+$ is the only permeating species at rest (ie. non-excited state).

$$V_{rest} = E_{K^+} = \frac{RT}{zF} \ln \frac{[K^+]_{ext}}{[K^+]_{int}}$$

$\frac{RT}{F} \approx 25mV$ at room temp.

$$\Rightarrow E_{K^+} = \frac{25mV}{+1} \ln\left(\frac{4}{150}\right) = -91mV.$$

$\therefore$ the resting membrane potential of this cardiac myocyte is $-91mV$.

7. Explain how the selectivity filter of the potassium channel achieves selectivity for $K^+$ over $Na^+$.

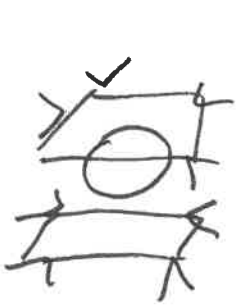
$$\Delta\Delta G_{Na,K} = \left(G_{Na}^{site} - G_{Na}^{bulk}\right) - \left(G_{K}^{site} - G_{K}^{bulk}\right)$$

$$= \Delta G_{Na}^{site} - \Delta G_{K}^{bulk}$$

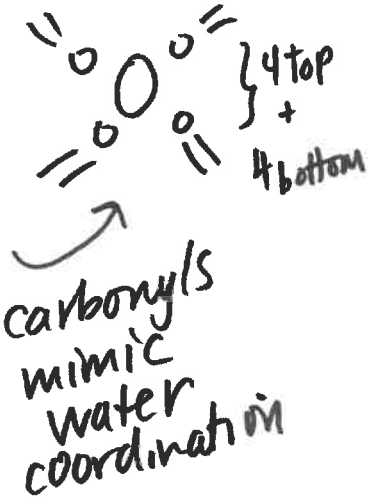
$\Delta\Delta G_{Na,K} > 0 \rightarrow$ K+ selective

$< 0 \rightarrow$ Na+ selective

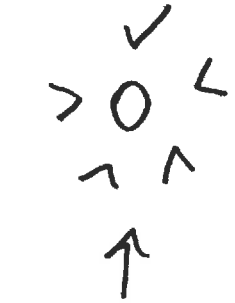
$= 0$ non-selective



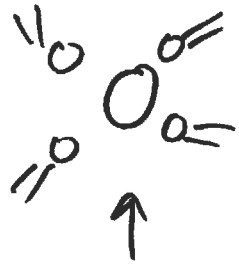
K+ well coordinated by ~8 waters in bulk.




8 carbonyls mimic water coordination



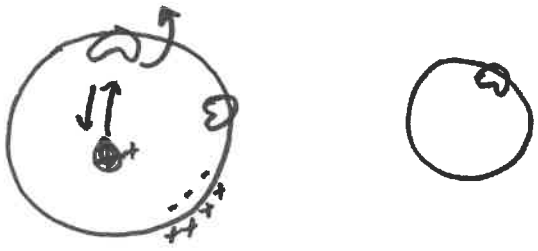
Na+ coordinated by <8 waters in bulk.



cannot be optimally coordinated ~~solvated~~ in site.

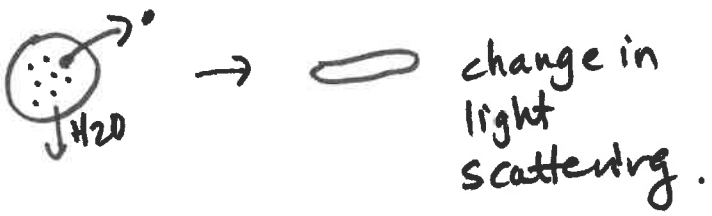
8. Describe three factors that affect transport kinetics in an ion efflux measurement.

→ unitary transport turnover rate. (half cycle & full cycle).
→ concentration of substrate.
→ concentration/density of active transporters per liposome
→ size of liposomes.
→ electrogenic transport depends on membrane potential.



9. Describe a method for measuring transport activity for non-electrogenic transport.

- fluorescence detection of substrate.
- radioactivity → ie. radioactive uptake of substrate into liposomes.
- vesicle scattering light.



10. Describe the DOs and DON'Ts of secondary active transport

- Neither the substrate or driving ions are transported alone
- binding of both is required for conformational change (OR binding of none for resetting the transporter).
- binding can be cooperative.
- thermodynamically reversible. directionality is set by the concentration of the driving substrate.