Answers to Question Set 5

Membrane biophysics

Note that it is not necessary to read the papers beyond what is indicated by the questions and what is covered by the lecture notes.

Remember to draw figures to explain your answers. This is often more efficient than just using words.

If you have any questions feel free to send an email: diogo.volpati@ftf.lth.se

Questions for Cell membranes models & paper (Brogden and Singer & Nicolson)

1. Describe the evolution of Cell Membrane Models until our currently most accepted model proposed by Singer & Nicolson.

In 1984 Overton working with vegetable cells suggested that cell membranes had "lipids" in its composition. In 1917 Langmuir proposed that cell membranes could be composed by amphiphilic molecules like phospholipids, which could be organized in one monolayer. However, in 1925 Gorter and Gandel working with blood red cells concluded that there were enough lipids to form a bilayer, where hydrophobic chains were pointing to the interior and the hydrophilic heads were exposed to the environment. Davson and Danielli proposed two advances on the model saying later in 1954 that the phospholipid bilayer was enveloped by a layer of proteins. Here, the presence of proteins on the cell membrane were known. In 1960, Robertis and Robertson reinforced the tri-lamellar structure (phospholipid bilayer plus one layer of proteins) aided by the electronic microscopic images. However, in 1973 Singer and Nicholson proposed the fluid mosaic model which is currently the most accepted model, where all elements (lipids, several proteins, cholesterol, etc.) are on a fluid membrane that moves very fast.

2. Antimicrobial peptides can be considered an alternative for conventional antibiotics. Please, describe their three proposed mechanism of action to form pores into membrane bilayers.

The barrel-stave model: the attached peptides aggregate and insert into the membrane bilayer so that the hydrophobic peptide regions align with the lipid core region and the hydrophilic peptide regions form the interior region of the pore, allowing for leaking cytosol.

The carpet model: the peptides disrupt the membrane by orienting parallel to the surface of the lipid bilayer and forming an extensive layer or carpet.

The toroidal model: in this model the attached peptides aggregate and induce the lipid monolayers to bend continuously through the pore so that the water core is lined by both the inserted peptides and the lipid head groups.

3. What is the difference between a lipid molecule and detergent molecule? How would the structure of a lipid molecule need to change to make it detergent?

Lipid molecules are approximately cylindrical in shape. Detergent molecules are conical or wedge shaped. A lipid molecule with only one hydrocarbon tail would be a detergent. To make a lipid molecule into detergent, one would need to make the polar hear larger or remove one of its tails so that could form a micelle. Detergent molecules usually also have short hydrocarbon chains, which makes them slightly soluble in water.

4. Lipid molecules exchange places with their lipid neighbor every 10^{-7} sec. A lipid molecule diffuses from one end in a 2μm long bacterial cell to another in about 1 second. Are these two number in agreement (assume that a lipid head group is about 0.5 nm)? If not, can you think of a reason for the difference?

When lined up, we have about 2μm/0.5nm = 4000 lipid molecules. If the molecules change place every 10^{-7} sec, then would take only (4000 lipids x 10-7 sec) = 4 x 10-4 seconds to reach the other end. In reality, the lipid moves in random path rather linear direction, so there comes the difference between times.

5. To get an appreciation for the speed of molecular motions, assume a lipid head group is about a size of ping-pong ball (4cm diameter) and that the floor of your living room (6 m x 6 m) is covered wall to wall with these balls. If two neighboring balls exchange position every 10^{-7} sec, what would be the speed in kilometers per hour? How long would take a ball to move from one end of the room to another?

If one 4cm ping-pong ball exchange places with each neighbor every 10^{-7} sec, it would travel at a speed of 1,440,000 km/h (= 4cm / 10^{-7} sec). If it was in linear trajectory, would take 1.5 x10^{-5} sec; in random walk, it would take considerably ~ 2msec

6. What role does water play in determining the molecular organization of cell membranes?

The nonpolar fatty acid chains of the phospholipids are sequestered together away from contact with water, thereby maximizing hydrophobic interactions. the ionic

and zwitterionic groups are in direct contact with the aqueous phase at the exterior surfaces of the bilayer, thereby maximizing hydrophilic interactions.

7. How are vesicles formed? How can we define their size?

Vesicles are spontaneously formed when lipid film is hydrated, driven by a balance between hydrophobic and hydrophilic regions on the molecule. However, some methods like electro-formation or extrusion can control the size of the vesicles and its characteristics (unilamellar or multilamellar).

8. What are the main differences between Langmuir and Langmuir-Blodgett membranes?

Langmuir films are supported by water, with the head groups of phospholipids being anchored at the water interface and the hydrophobic tail pointing towards the air. By transferring Langmuir films to a solid substrate, we create solid-supported bilayers so called Langmuir-Blodgett films.

Questions for Probing Membrane Models at molecular Level

1. What are the main differences between FTIR and Raman Spectroscopies techniques? Fundamentally, what are the processes involved in both methods since they can probe exactly the same information (defined frequencies of the molecular vibrations)?

FTIR involves the process of “absorption of light”, where energy of the light shun into a sample are on the same magnitude of the energy involved in molecular vibrations.
The Raman process involves the “scattering of light”, and the light shun in the sample is one order of magnitude higher than that energy of the molecular vibrations.

Fundamentally, both techniques access the same information using different physical principles of absorption or scattering.

2. Describe what are the selection rules for a vibrational mode be active in FTIR spectroscopy. Also, describe the same selection rules for Raman Spectroscopy.

A certain vibration is only FTIR active (or allowed) if the molecular dipole moment changes during the vibration. For instance, O_2 cannot be probed with FTIR since no induced dipole moment can be generated. Molecules with different atoms can

interact with incident radiation, and even if a dipole moment is not present in the beginning it can be induced due to antisymmetric displacement of the center of charge (e.g., CO_2).

The polarizability is a measure for the electron cloud's ability to deform in contrast to the atomic nuclei. For vibrational Raman spectroscopy, the gross selection rule is that the polarizability of the molecule should change as it vibrates.

3. The vibrational frequency of a harmonic oscillator formed by two spheres of mass *m* can be calculated by $\upsilon_m = \frac{1}{2\pi}\sqrt{\frac{\kappa}{\mu}}$ where $\mu = \frac{m_1 m_2}{m_1+m_2}$. Please explain how we can use these equations to understand how different molecules like H_2, O_2, CO, HF have their well-defined and unique fundamental frequencies of vibration. Remember to consider the strength of the bonding between the atoms.

Two atoms linked by an electronic bond can be thought as the frequency of the harmonic oscillator. We attribute different mass to atoms (e.g. H=1u, N= 14u, Si=28u where 1u= 1,66054e-27 Kg), so that the heavier the atoms, the lower the frequency of vibration (and vice-versa). Also, the strength of the bond between the atoms is associated to the spring constant *K*. In the same way, stronger bonds (covalent) will increase the frequency of the vibration.

4. Given the phospholipid 1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), please find some of the fundamental vibrations associated with (a) PO_x group, (b) NH_3, CH_3 and CH_3. State your answers in cm^{-1} and then convert it to eV.

PO_2 – It vibrates around 1230 cm^{-1}.

CH_2 and CH_3 stretches are found around 2800-3100 cm^{-1}. C-H bends around 1100-1300 cm^{-1}

N-H stretches around 3400-3250. N–H bend (primary amines only) from 1650-1580 cm^{-1}

Questions for sensing with SPR and QCM

1. What is a plasmon? Which metals are most commonly used to support Plasmon's? Which characteristics of these metals are essential?

Plasmon is the collective oscillation of electrons on a surface of coin metals. Metals such as gold, silver, and aluminum support Plasmon's on the surface. They exhibit a negative real part of permittivity in the visible and near-infrared regions of the spectrum. These metals also exhibit a considerable imaginary part of the permittivity, which causes the propagation constant of a surface plasmon to have a nonzero imaginary part.

2. In the paper J. Am. Chem. Soc. 2000, 122, 4177-4184, please describe how phospholipid vesicles were used to build a SPR sensing unit to monitor the amount of PLA2 which bound to these vesicles. Please, describe the main strategy of the authors, and not the details.

Phospholipid vesicles containing 0.3% biotin-functionalized headgroups were bound to a streptavidin monolayer to make a high-density, planar layer of intact vesicles on top of surface plasmon resonance (SPR) sensor. The absolute amount of protein binding to the vesicle layer could be monitored by SPR in real time to extract equilibrium and kinetic information under flowing solutions.

References

i. *The Fluid Mosaic Model of the Structure of Cell Membranes*, S. J. Singer and Garth L. Nicolson, Science, **175**(4023), 720-731 (1972)

ii. *Antimicrobial Peptides: Pore Formers Or Metabolic Inhibitors In Bacteria?*, Kim A. Brogden, Nature Reviews Microbiology volume **3**, 238–250 (2005)

iii. *Surface Plasmon Resonance Sensors for Detection of Chemical and Biological Species*, Jiří Homola, Chem. Rev. **108**(2), 462-493 (2008)

iv. *Quantification of Tight Binding to Surface-Immobilized Phospholipid Vesicles Using Surface Plasmon Resonance: Binding Constant of Phospholipase A2*, Linda S. Jung et al. J. Am. Chem. Soc. **122**, 4177-4184 (2000)