MEMBRANE MEDIATED PROTEIN-PROTEIN INTERACTIONS

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In general, when most physicists think about the dynamics of a complex biological system, such as a cellular membrane, they are primarily considering the incoherent stochastic motions, or the local dynamics of molecules and functional groups. However, collective molecular motions are attracting increasing attention because of their potential impact on membrane and protein function. In the past lipid bilayers were considered to be homogeneous, largely passive fluid barriers, based on Singer and Nicholson's fluid mosaic model from 1972 [1]. Today, it seems to be increasingly accepted that the diversity and composition of lipids play an important role in the function of biological membranes. Not only does the membrane provide elementary and essential functionalities, such as permeability and elasticity, but the bilayer in particular mediates protein-protein interactions. Van der Waals and electrostatic interactions are usually relatively weak over the distance of typical protein-spacing in a biological membrane (~5 nm), therefore, the focus of this article will be largely centered on the role the membrane plays in mediating protein-protein interactions. The lipid bilayer composition and physical properties, such as elasticity, will obviously contribute to how the proteins are able to "feel" each other in the membrane; however, the membrane properties will also dictate whether or not the system will adopt an aggregated protein state, or whether the proteins will remain dispersed in the membrane.

To understand how the proteins will interact with each other, as well as how they will be influenced by the membrane, we need to consider the free energy per lipid molecule of a system of lipids and proteins, which can be modeled as [2,3]

$$f(u,a) = \gamma a + G(u) + K(a)\,(\nabla^2 u - \kappa(a))^2.$$

In this expression the free energy is a function of the monolayer thickness *(u)* and the surface area per phospholipid head group *(a)*. For a flat monolayer, the properties that will contribute to the free energy are the surface tension, γ, and the compression and expansion coefficient of the lipid molecules, *G(u)*. However, any deformation causing the membrane to have a non-zero curvature ($\nabla^2 u$) will have a free energy cost associated with it. This energy of curvature will depend on the bending stiffness of the monolayer, *K(a)* (see Fig. 1a)). The bending of the monolayer will arise, to some extent, due to thermal fluctuations in the membrane, but the most dominant energy cost associated with bending arises when there is an inclusion, such as a protein, in the membrane. This is particularly evident in the case of hydrophobic mismatch, as shown in Fig. 1b). Hydrophobic mismatch occurs when the hydrophobic region of the protein is larger, or smaller, than the bilayer thickness [4,5]. This subsequently causes each monolayer leaflet to distort in order to ensure that the entire hydrophobic region of the protein is contained within the

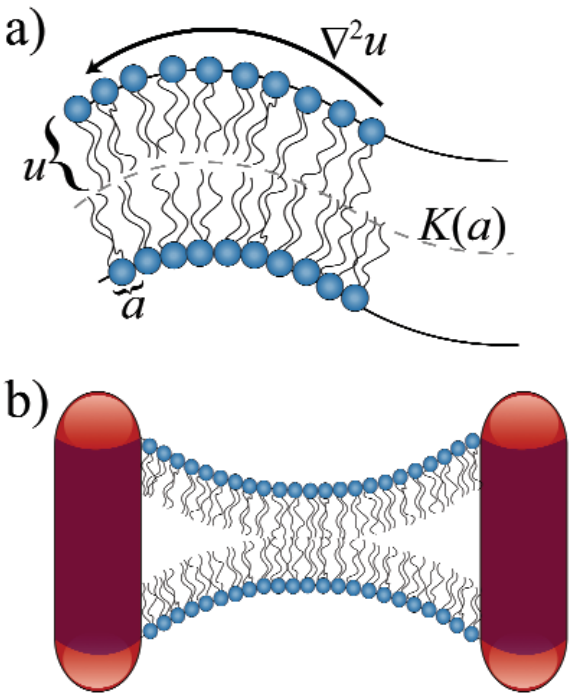

Fig. 1 a) Lipid bilayer displaying the parameters contributing to the free energy. b) Hydrophobic mismatch of a protein in a membrane, where the hydrophobic region of the protein is shown in purple and the hydrophilic region in red.

SUMMARY

This article addresses the theory behind membrane mediated protein-protein interactions and motivates the application of inelastic neutron scattering to study the dynamics of biologically relevant protein systems.

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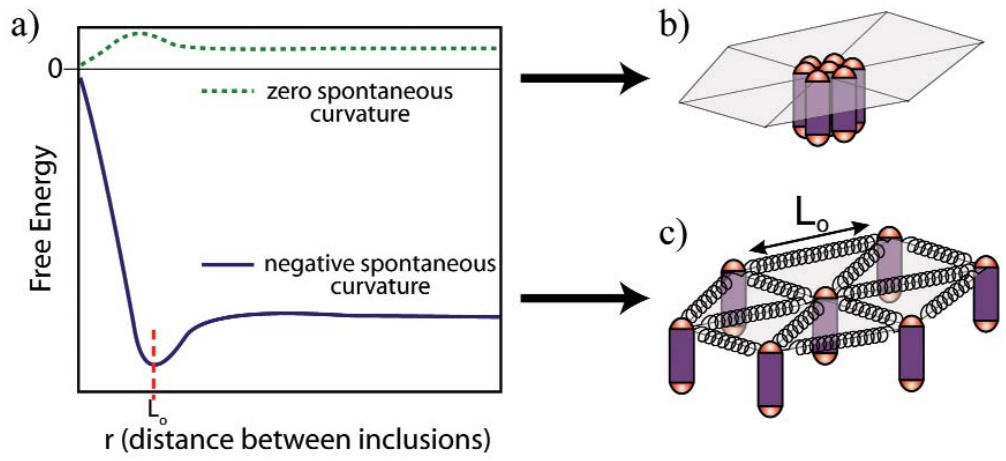

Fig. 2 a) Free energy curve for the zero (dashed) and negative spontaneous (solid) curvature bilayers (Adapted from Aranda-Espinosa, *et al.* 1996) [2], b) Lowest free energy state of the zero spontaneous curvature system is the aggregate protein state, c) The lowest free energy state for a non-zero spontaneous curvature system is a dispersed protein state. This system can be modelled by assuming all of the proteins are connected by a spring-like interaction force.

hydrophobic core of the membrane.

In the case of the flat lipid bilayer the lowest free energy state will always tend towards protein aggregation, as can be seen by the free energy plot in Fig. 2a). Because there is an energy cost associated with the protein boundary, the system will try to minimize that protein-membrane interface (Fig. 2b)). In nature, however, we know that proteins do not always aggregate in membrane systems and, in fact, are often require dispersion throughout the membrane in order to function properly.

Another factor to consider in the free energy is the spontaneous curvature of the membrane ($\kappa(a)$), which allows for the addition of an inclusion to lower the free energy of the membrane system [2,6]. A monolayer may adopt different spontaneous curvatures depending on its composition. The size of the phospholipid head group, as well as additional molecules incorporated into the monolayer, can influence whether or not the monolayer assumes a positive or negative spontaneous curvature. It can, therefore, be speculated that the spontaneous curvature of the monolayer plays an important role in protein dispersion. A system with a non-zero spontaneous curvature has a free energy minimum when the proteins are dispersed at some finite spacing, creating a protein lattice-like formation, as sketched in Fig. 2c).

When studying protein aggregation, this dispersed state is very important. It enables the study of the interaction and determining the interaction force in a "frozen", stable intermediate state. One way to model the forces proteins experience in the dispersed state is to think of all of the proteins being connected by springs (Fig. 2c)). In this model, the information regarding inter-protein interactions, which are mediated by the membrane, as well as the physical properties of the membrane itself, are encompassed in the dynamics of the springs. One of the challenges of trying to experimentally study these biological processes and interactions is that they occur on very small, nanometer length scales, and very fast, pico- to nanosecond time scales. Inelastic neutron scattering provides an ideal tool for studying such processes, as it is capable of accessing the relevant length and time scales on which these dynamics occur.

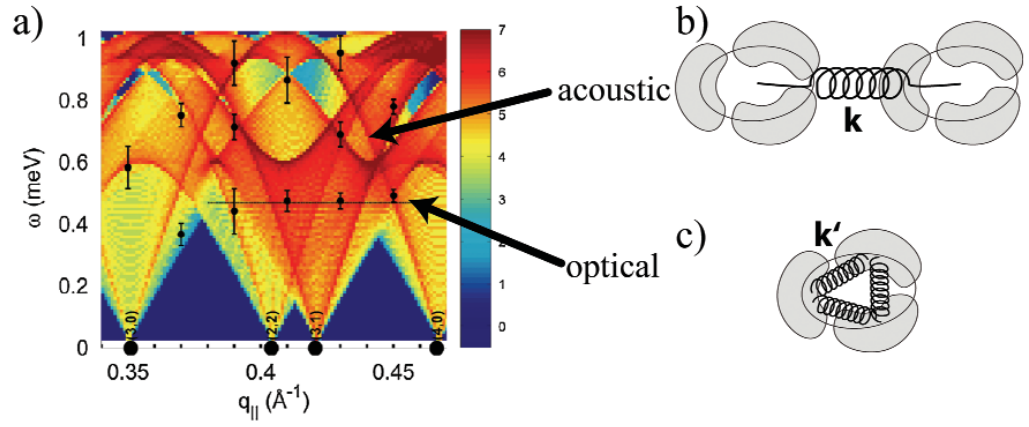

Fig. 3 a) Theoretical phonon dispersion curves with experimentally determined neutron excitation peaks superimposed (black dots) (Adapted from Rheinstädter, *et al*. 2009) [7], b) Model of trimer-trimer interaction resulting in the acoustic phonon mode, c) Model of monomer-monomer interaction resulting in the optical phonon mode.

To study membrane mediated protein interactions we have chosen to examine Purple Membrane (PM). PM, which is composed of phospho- and glycolipids as well as the protein bacteriorhodopsin, is one of nature's simplest bioenergetic devices. The bacteriorhodopsin monomers form trimer hexagonal unit cells, which can combine together to create PM patches in the form of a 2-dimensional hexagonal protein lattice. This high degree of order makes PM the perfect model system for the study of protein interactions.

We model PM by treating each protein monomer as a point mass with a spring like interaction force acting between them. Different force constants are used to include intra-protein (k') and inter-protein interactions (k), as sketched in Figs. 3b) and c). All of the information regarding how strongly the proteins "feel" each other, how they interact with the membrane, as well as the membrane properties like elasticity, is contained within the spring constants k and k'. The entire system of hexagonally arranged trimers essentially behaves as a system of coupled oscillators.

This model was used to generate a theoretical phonon dispersion curve for the system. Because PM forms in patches, there are inevitably many different lattice orientations within the plane of the membrane, so the theoretical phonon dispersion curve is displaying a statistical average of all of these lattice orientations. The experimentally determined neutron excitation peaks were superimposed on the theoretical phonon curves, as shown in Fig. 3a). Data and calculations provide experimental evidence for acoustic and optical phonons in the 2D protein lattice. We believe that the acoustic phonon mode corresponds to the trimer-trimer interaction (Fig. 3b)), while the optical phonon mode is related to the monomer-monomer interaction (Fig. 3c)). The trimer-trimer interaction in native PM was determined to be $k = 52$ N/m. The protein-protein interaction in PM is, therefore, about 2-3 orders of magnitude weaker than a C-C bond (10,000 N/m), but 2-3 orders of magnitude stronger than a pure van der Waals interaction (0.1 N/m). This first experiment proves that protein-protein interactions in biological membranes can be studied in-situ under physiological conditions, and the corresponding interaction constant can be determined quantitatively.

Understanding the role of the membrane in mediating protein-protein interactions is a vital component of understanding protein function. The properties of the lipid bilayer, such as its elasticity and bending stiffness, will contribute to how the proteins are able to communicate with each other in the membrane and may lead to concerted protein dynamics. The spontaneous curvature of the membrane will dictate whether the lowest free energy state results in protein aggregation or dispersion. Establishing what influences protein aggregation, or how proteins are able to remain dispersed in membrane, is a topic which is expected to have a high impact in the biomedical field as a number of neurodegenerative disorders, such as Alzheimer's and Parkinson's disease, are related to protein aggregation. The neutron scattering experiments we have performed have shown first experimental evidence for collective protein-protein interactions in the model purple membrane system. The data obtained is described well by the theoretical spring model. We will elucidate the role of the membrane in protein-protein interaction using neutron scattering to examine how these interactions are affected by changes to the protein density, as well as changes to the physical properties of the membrane. This will be an important step towards determining how protein-protein interactions ultimately affect protein function.

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