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Modeling the interplay between protein and lipid aggregation in supported membranes

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ABSTRACT

We present a theoretical model that deals with the complex interplay between lipid segregation and the self-aggregation of lipid-attached proteins. The model, in contrast to previous ones that consider proteins only as passive elements affecting the lipid distribution, describes the system including three terms: the dynamic interactions between protein monomers, the interactions between lipid components, and a mixed term considering protein–lipid interactions. It is used to explain experimental results performed on a well-defined system in which a self-aggregating soluble bacterial cytoskeletal protein polymerizes on a lipid bilayer containing two lipid components. All the elements considered in a previously described protein model, including torsion of the monomers within the filament, are needed to account for the observed filament shapes. The model also points out that lipid segregation can affect the length and curvature of the filaments and that the dynamic behavior of the lipids and proteins can have different time scales, giving rise to memory effects. This simple model that considers a dynamic protein assembly on a fluid and active lipid surface can be easily extended to other biologically relevant situations in which the interplay between protein and lipid aggregation is needed to fully describe the system.

1. Introduction

Biological membranes are constituted by a complex mixture of diverse lipids and proteins. Their organization in space and time plays an essential role in a wide range of cell functions. Since the proposal of the fluid mosaic membrane model in the early 1970s (Singer and Nicolson, 1972), experimental evidence pointing toward a more complex membrane architecture has accumulated. Instead of the initial picture in which a mostly fluid lipid matrix provided dynamic support for membrane proteins, the current prevailing image is that of a membrane surface segregated laterally into protein and lipid domains of different sizes and life times (Lingwood and Simons, 2010; Nicolson, 2013). The spatial and dynamic regulation of these domains plays an important role in signaling pathways, endocytosis, cell polarity and migration, neuronal growth and a variety of disease settings (Head et al., 2014). Both lipids and proteins, depending on their individual characteristics, have their own tendency to self-aggregate. The resulting structural organization of the complex lipid–protein structure is then the balance of the interplay between these two trends. Biomembranes have a high protein content, so it is important to include their contribution in physical models that aim to explain their behavior (Jacobson et al., 2007; Yethiraj and Weisshaar, 2007; Fischer and Vink, 2011; Fischer et al., 2012; Dmitrieff and Sens, 2013).

Different atomistic (Pandit et al., 2004a,b) and coarse-grained (Faller and Marrink, 2004; Rabinovich and Lyubartsev, 2013) models have been used to study phase separation in binary and tertiary lipid mixtures (Reigada et al., 2008; Honerkamp-Smith et al., 2008; Gómez et al., 2009; Fan et al., 2010). It has been proposed that the presence of membrane bound proteins can account for the observed

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differences observed in vivo and in vitro (Fan et al., 2008; Ehrig et al., 2011a; Witkowski et al., 2012) in the stability, size and shape distribution of lipid domains. The proteins are considered either as a quenched disordered background in which the lipid separation has to evolve, or as a component inserted or adsorbed to (Loew et al., 2009; Witkowski et al., 2012; Gómez et al., 2010) the lipid bilayer, which may be preferentially segregated into one of the lipid phases. Lipid dynamics, with and without protein insertions, has been explored as thermal equilibrium distributions, considering the exchange of lipid molecules with the bath (Palmieri and Safran, 2013), or considering out-of-equilibrium processes (Ehrig et al., 2011b). In particular, the coalescence of lipid domains and the stability of the rafts have been shown to be affected by membrane bound or integral membrane proteins (Fan et al., 2008; Ehrig et al., 2011a; Witkowski et al., 2012).

In eukaryotic cells the most relevant membrane lipid domains are referred to as rafts (Simons and Ikonen, 1997). They are rich in sphingolipids and sterols (Simons and Sampaio, 2011) and some proteins are specifically tagged, through palmitoylation or the addition of a glycophosphatidyl-inositol (GPI) anchor, to localize within these domains (Levental et al., 2010). In recent years there has been increasing evidence that cytoskeletal proteins are also associated with rafts and that they can play an important role in regulating lateral diffusion of membrane proteins and lipids in response to extracellular events (Head et al., 2014; Kusumi et al., 2012; Ehrig et al., 2011a; Chichili and Rodgers, 2009; Liu and Fletcher, 2006). Both cytoskeletal proteins and lipids have a tendency to interact amongst themselves, so it is of interest to study how their interactions are interconnected.

Simplified lipid–cytoskeletal protein complexes system have been reconstituted in model membrane systems as GUVS, liposomes and supported lipid membranes (Merkle et al., 2008; Cabré et al., 2013; Osawa and Erickson, 2013; Pontani et al., 2009; Mateos-Gil et al., 2012a) using different ways to anchor the proteins to the membranes. Modeling the segregation of lipid phases under the presence of cytoskeletal proteins is challenging because it is important to include the self-assembling properties of the proteins. The typical length, spontaneous curvature and flexibility of the filaments bound to a membrane may set very distinct length scales for the lipid segregation. Additionally, the effects of the time scale set by the dynamic equilibrium of the lipids will affect the formation and breaking of longitudinal bonds along the filaments, as well as the formation of bundles or other aggregates driven by lateral attraction between the proteins.

What we present in this paper is a combination of experiments and modeling of a well-defined experimental system that includes two elements: a self-aggregating soluble bacterial cytoskeletal protein and a lipid bilayer with two lipid components. The soluble bacterial protein is artificially bound with a known orientation to the head of one of the lipids, which has a tendency to segregate from the other lipid component. The theoretical model presented is able to explain the experimental results observed and can serve as a useful platform to study more complex situations in which both lipid and protein dynamics contribute to the lateral separation of membrane components.

The bacterial cytoskeletal protein studied is FtsZ, a soluble globular protein with GTPase activity that attaches to the inner cytoplasmic membrane through its interaction with other proteins, such as FtsA or ZipA in *Escherichia coli* (Mingorance et al., 2010). It polymerizes into a ringlike structure and plays an important role acting as a scaffold to recruit other proteins forming the division ring and exerting force to drive cell division. In vitro, the isolated protein forms linear polymers in the presence of guanosine-5-triphosphate (GTP) that can condense into a large variety of higher-order structures depending on the medium conditions(Popp et al., 2010).

The detailed structure and dynamics of individual filaments observed with atomic force microscopy studies have been compared with theoretical models that describe the behavior of interacting particles in two dimensions (Mingorance et al., 2005; Hörger et al., 2008; Mateos-Gil et al., 2012b; Páez et al., 2009a,b). In more recent work directed toward understanding the role played by filament curvature (González de Prado Salas et al., 2013, 2014; Encinar et al., 2013), we have used a lipid platform to covalently attach proteins containing a single cysteine to a reactive lipid molecule present on the membrane. The native *E. coli* FtsZ protein does not contain cysteines, therefore the position of this amino acid in the mutant protein determines the way it orients on the lipid surface. We observed that filament orientation had a strong influence in the shape of the structures formed (Encinar et al., 2013). The combination of molecular dynamic simulations and theoretical modeling led us to propose a model in which a preferential curvature of the filaments, together with the presence of a twist, determines the way proteins are oriented and attached to the surface. This plays an important role in determining the shape of the filaments and on the amount of contractile force exerted on the surface (González de Prado Salas et al., 2014). The observed polymorphism of the protein filament aggregates observed when the protein is attached to the surface with different orientations can be understood if filament twist and curvature are both taken into consideration.

The maleimide lipid used to anchor the protein (DSPE-mal), due to its high melting temperature determined by the presence of two C-18 saturated fatty acid chains, has a tendency to segregate from DOPC lipid, (with two C-18 unsaturated fatty acids) used as the predominant lipid component. When experiments are performed at low temperatures (10–15 °C or at linker lipid concentration $\geq$10%), we observe that protein filaments aggregate adopting unexpected surface distributions that are dependent on the orientation of the monomer on the surface. Lipid segregation can also be observed with the AFM (GIocondi et al., 2010 and see Section 3.1). The theoretical model presented here accounts both for the effect of the lipid segregation and the contribution of monomer orientation and attachment strength and explains experimental observations.

Although the model is tuned to fit this particular experimental case, the formulation and analysis could be easily extrapolated to study other systems and protein–lipid platforms with rich phenomenological behavior in which both lipids and proteins contribute to lateral segregation.

2. Experimental

The preparation and characterization of the mutant FtsZ proteins was described previously (Encinar et al., 2013). The mutant proteins from *E. coli* used in this work have the cysteines located in amino acid 2 (F2C, referred to as Z2) and in amino acid 93 (E93C, referred to as Z93).

Fig. 1 shows the position of the mutated residues with respect to the carboxi–amino terminal domains and polymerization axis of the monomers within the filament. Attachment of the protein to the lipid surface through aa 93, located at the end of α-helix 3, provides a tight binding to the surface. The monomers are oriented on the surface with the axis connecting the carboxi and amino ends perpendicular to the lipid surface, that is, with the preferential filament curvature facing upward ($\psi_0 = 90^\circ$ as indicated in Fig. 2). In contrast, aa 2 is located at the end of a non-structured region of the protein that provides a loose attachment to the lipid surface. Although the orientation is also expected to have the C–N axis standing perpendicular to the membrane, the loose protein attachment imposes less restrictions to monomer orientation.