Transport of Biomolecules on Lipid Membranes

by

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ABSTRACT

Biological membranes, including the plasma membrane and the internal membranes of the eukaryotic cells, have a common overall structure of a lipid bilayer embedded with proteins. Structural distribution of membrane lipids into different phase types and lateral diffusion of proteins in the plane of the cell membrane are critical for cellular functions including cell growth, signal transduction in response to extra cellular stimuli, cell adhesion, homeostasis and cell death. Consequently, there has been significant interest in understanding the heterogeneity of biological membranes and transport properties of membrane constituents.

The overall theme of this thesis work is to use phase separated model membrane systems to investigate the transport of adsorbed DNA and interpret the motion of macromolecules in real cell membranes. In this work, we describe the influence of membrane heterogeneity on the adsorption and diffusion of single stranded DNA (ssDNA). Cellular membranes are believed to contain domains – lipid rafts – that influence processes ranging from signal transduction to the diffusion of membrane components. By analogy, we demonstrate that the formation of raft-like domains in supported lipid bilayers provides control over the adsorption and diffusion of ssDNA. We also observed that the immobile phase-separated lipid domains acted as obstacles to the lateral diffusion of adsorbed ssDNA. Fluorescence recovery after photo bleaching (FRAP) analysis revealed that the diffusivity (D) of the adsorbed ssDNA tracked that of the underlying lipid, although the lipid diffusivity changed by an order of magnitude with changes in bilayer composition. Further, motivated by the increasing interest in developing strategies for the separation of DNA, we investigated the influence of chain length (N) on the diffusion and electrophoresis of ssDNA adsorbed on heterogeneous cationic supported lipid bilayers. FRAP measurements revealed that the diffusivity of adsorbed ssDNA varied with N as $D \sim N^{-1}$, similar to a trend previously observed for the diffusion of double stranded DNA on homogenous supported lipid bilayers. In contrast, the electrophoretic mobility of the adsorbed ssDNA in the presence of an applied tangential electric field was independent of N. Our results also suggest that the use of asymmetric diffusion barriers or other tunable obstacles could assist DNA separation on

supported lipid bilayers. In addition to providing fundamental insight into the adsorption and diffusion of DNA on heterogeneous surfaces this study could also be useful for the design of novel techniques for the size-based separation of DNA.

On basis of the knowledge gained from our work on transport of macromolecules on heterogeneous bilayers we explored diffusion and electrophoresis of receptor clusters in biological membranes. In this study, we observed that epidermal growth factor receptors form mobile clusters in lung cancer cells and described the diffusion of epidermal growth factor receptor (EGFR) clusters in the apical membrane. We detected that EGFR clusters display several modes of motion within membrane similar to individual membrane-anchored proteins. From electrophoresis of EGF-bound EGFR clusters, we observed that even if the cluster is primarily comprised of EGF bound EGFR with a high negative charge, a fraction of clusters could move in the opposite direction towards the negative electrode instead of moving towards the positive electrode. In order to understand the composition of these clusters, we characterized the clusters using fluorescently labeled ligands, antibody fragments and gained insight on the minimum number of receptor molecules present in individual clusters using brightness analysis. Subsequently, we studied the role of cluster charge in electrophoretic motion of the ligand-bound receptor clusters by treating the cells with BTC and MβCD. From the preliminary results, we deduce that there could also be other charged species present within the cluster or specific interactions of the cluster with the surrounding membrane that dictate the direction of motion of these clusters in the presence of an electric field. Since EGFR over expression and cluster formation is widely reported in cancerous cells, insights on dynamics and composition of such clusters could lead to the development of novel diagnostic strategies in oncology.