Phospholipid-Membrane Protein Selectivity: AFM-FS and Fret Studies

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Since transmembrane proteins (TMPs) crystals are more frequently obtained with enough purity for x-ray diffraction studies than decades ago, their action mechanisms may become elucidated. One of the pending issues is the actual interplay between transmembrane proteins and membrane lipids. There are strong evidences on the involvement of specific lipids on membrane proteins function, as the potassium channel KcsA or the secondary transporter LacY, which activities are related with the presence of anionic phospholipids as phosphatidylglycerol (PG) and phosphatidylethanolamine (PE), respectively. We have approached this issue by implementation of atomic force microscopy (AFM), AFM in force spectroscopy (FS) mode and Förster resonance energy transfer (FRET). We will present in this communication the observations performed with the AFM on the distribution of LacY in binary supported lipid bilayers (SLBs) of PE and PG. The preference of LacY for fluid phases in SLBs as observed by AFM will be discussed under the basis of FS measurements. Single molecule force spectroscopy (SMFS) will be used to characterize the unfolding force (F_u) required to extract LacY from different lipid environments. FRET measurements between pyrene labeled phospholipids and the single tryptophan mutant (LacY-C154G) will evidence the selectivity between specific PE species and the protein. The body of results will be discussed within the framework of the flexible surface model (FSM) of the membrane.