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Revealing Transient Interactions between Phosphatidylinositol-specific Phospholipase C and Phosphatidylcholine–Rich Lipid Vesicles[1]

BOQIAN YANG, UMass-Amherst, TAO HE, Boston College, CÉDRIC GRAUFFEL, NATHALIE REUTER, Univ. of Bergen, MARY ROBERTS, Boston College, ANNE GERSHENSON, UMass-Amherst — Phosphatidylinositol-specific phospholipase C (PI-PLC) enzymes transiently interact with target membranes. Previous fluorescence correlation spectroscopy (FCS) experiments showed that *Bacillus thuringiensis* PI-PLC specifically binds to phosphatidylcholine (PC)–rich membranes and preferentially interacts with unilamellar vesicles that show larger curvature. Mutagenesis studies combined with FCS measurements of binding affinity highlighted the importance of interfacial PI-PLC tyrosines in the PC specificity. All-atom molecular dynamics simulations of PI-PLC performed in the presence of a PC membrane indicate these tyrosines are involved in specific cation-pi interactions with choline headgroups. To further understand those transient interactions between PI-PLC and PC-rich vesicles, we monitor single fluorescently labeled PI-PLC proteins as they cycle on and off surface-tethered small unilamellar vesicles using total internal reflection fluorescent microscopy. The residence times on vesicles along with vesicle size information, based on vesicle fluorescence intensity, reveal the time scales of PI-PLC membrane interactions as well as the curvature dependence. The PC specificity and the vesicle curvature dependence of this PI-PLC/membrane interaction provide insight into how the interface modulates protein-membrane interactions.

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