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Directed Formation of Lipid Membrane Microdomains as High Affinity Sites for His-Tagged Proteins

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Microdomain formation in lipid membranes is the basis for important cellular events, including signaling, material uptake or release, and pathogen invasion.[1] Membrane domains are metastable structures that rapidly alter in composition and physical properties dependent upon cellular needs and environmental conditions.[2] Phase separation based on lipid structure or protein annular effects alone seem unlikely to produce such rapid reorganization of the membrane. Ion binding to membrane components, on the other hand, offers a mechanism for rapid and reversible structure formation. Cation interactions with lipids are well-known for their effect on membrane structure and phase transition temperature (T_g).[3] Localized concentration of ions within or about the cell is a potential route for temporal and precise control of domain formation and functional processes. Attaining a high level of control over domain formation in synthetic systems could similarly enable the development of switchable surfaces for the capture and display of biomolecules or construction of nanoscale features in 2D and 3D architectures.

Previous work has shown that synthetic lipid membranes can be designed for a highly specific and sensitive response to transition metal ions resulting in rapid dispersion of phase separated structures.[4] Biomolecule affinity to such membranes was observed, but the random dispersion of receptors impedes multivalent ligation.[5] Phase separated domains of a nitrilo triacetic acid (NTA) lipid in a DMPC film, on the other hand, have exhibited selective affinity for peptides, but the actuated formation/dissolution of those domains was not observed.[6]

Herein, we report on the development of a synthetic membrane with rapid and reversible formation of microdomains through the selective binding of metal ions. Further, we show the function of those domains as high affinity sites for proteins. The membranes were composed of a synthetically prepared lipid with an iminodiacetic acid headgroup (either as DSIDA or DOIDA; see Figure 1)[7] and 1-palmitoyl-2-oleoyl phosphatidylcholine (POPC). In the case of DSIDA, formation of the microdomains was observed via fluorescence microscopy using BODIPY 530/550 HPC (Invitrogen) as a membrane probe, and protein affinity was monitored using TAMRA-labeled maltose binding protein with a 6-His tag (6-His MBP). Rapid reversibility of the system was achieved by EDTA sequestration of metal ion from the membrane surface resulting in the release of bound protein and dissolution of the membrane domains.

Supported lipid bilayers of DSIDA/POPC were prepared on Piranha cleaned glass coverslips and imaged with a custom confocal spectral and lifetime imaging microscope.[8] The bilayers were prepared by incubating a 120 μL solution of small unilamellar vesicles prepared by sonication (~50 nm diam, 2 mM lipid in MOPS buffer, pH 7.4), within a 1 cm diameter hole in a silicon gasket fixed against the coverslip. Following incubation, the sample was liberally rinsed leaving 120 μL of fresh buffer on the coverslip. The fluorescence image revealed a supported bilayer with uniform coverage and no observable defects over the entire surface (Figure 2A).

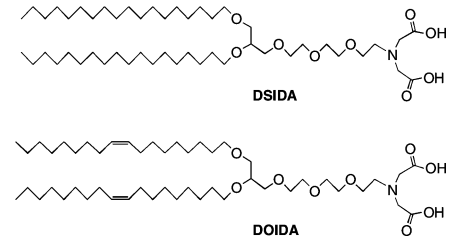

Figure 1. Structures of IDA-functionalized lipids.

Addition of $CuCl_2$ (10 μM) induced the formation of dark circular regions (Figure 2B) within several minutes. The domains were stable over a period of hours with little if any coalescence occurring or evidence of Oswald ripening. For bilayers composed of 10% DSIDA/POPC the dark spots had a size range of 0.5−3 μm. The dark patches were found to increase in size and contrast with increasing mole fraction of DSIDA (data not shown).

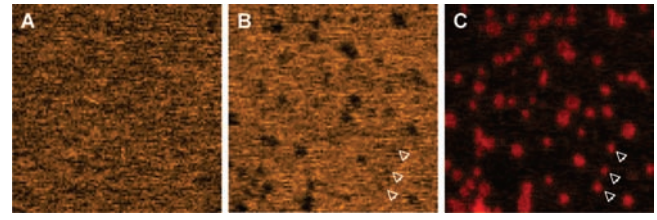

Figure 2. Confocal microscopy images of 10% DSIDA/POPC with 0.003% BODIPY 530/550 HPC bilayer on glass (A) before and (B) after $CuCl_2$ addition (10 μM) and (C) following incubation with 6-His MBP (15 nM). White arrows identify low contrast domains that became distinct with protein affinity (Image size = 10 μm).

Fluorescence correlation spectroscopy (FCS) showed a marked difference in mobility within the dark domains compared to the bright areas. A diffusion constant of 0.04 $\mu m^2/s$, consistent with a gel phase membrane, was measured in the dark domains while the bright regions exhibited faster, albeit heterogeneous, diffusion with two constants of 4.7 and 0.1 $\mu m^2/s$, consistent with fluid phase regions measured in other multicomponent membranes.[9] DSC analysis for DSIDA found a T_g of 55 °C, which increased to 73 °C (67 °C pretransition) upon $CuCl_2$ presence. With mixed bilayers the phase separation of DSIDA from POPC was only observed in the presence of $CuCl_2$ (see Supporting Information (SI)). These

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results strongly suggest that the bright regions consist of fluid phase POPC while the dark regions are enriched in gel phase Cu^{2+}-bound DSIDA lipid. Liberal rinsing of the $CuCl_2$-containing solution from the supported bilayer with MOPS buffer resulted in the same dark spotted pattern in the membrane but with enhanced fluorescence from the fluid phase regions due to removal of fluorescence quenching Cu^{2+}. Stability of those domains over 20 h in buffer indicates a slow dissociation rate for Cu^{2+}-IDA. Experiments with $CaCl_2$ (10 mM), in place of $CuCl_2$, yielded similar dark patches confirming that the domain formation is directed by metal ion recognition. Addition of EDTA (100 μM) resulted in the rapid (<1 min) conversion back to a homogeneous fluorescent film.

Since the Cu^{2+}-IDA complex exhibits an affinity for imidazole ($\sim 10^{3.5}$ M^{-1}),[7] spatial selectivity of proteins to the membrane could be evaluated using a His-tagged protein. To a freshly prepared Cu^{2+}-DSIDA/POPC membrane were added 150 μL of a 15 nM 6-His MBP solution. Figure 2C shows the fluorescence image of the bilayer after incubation for 15 min with the protein followed by thorough rinsing with buffer. The red fluorescing TAMRA label on the protein was distinguished from the BODIPY label on the membrane with the spectral resolution of the microscope. Figure 2B and 2C are of the same area showing that the dark, and even faintly dark (white arrows), patches of Figure 2B possess high affinity for the protein as they become bright with high concentrations of TAMRA-labeled 6-His MBP. Affinity for MBP was observed to concentrations as low as 0.7 nM. Multiple-point binding interaction of the His-tag to aggregated Cu^{2+}-IDA lipids in the domains facilitates strong protein affinity. Accurate determination of the binding constant of 6-His MBP to the 10% DSIDA/POPC bilayer was beyond the limit of our setup; however, we were able to estimate a lower limit of $K_a > 10^8$ M^{-1} (see SI). Negligible affinity was observed for proteins lacking His-tags (BSA, streptavidin), or for His-tagged proteins (MBP, GFP, kinesin) in the absence of Cu^{2+}. Addition of EDTA to the Cu^{2+}-DSIDA/POPC membrane bound with 6-His MBP affected the rapid release of protein and recovery of the original membrane. Figure 3 shows a schematic of the reversible system.

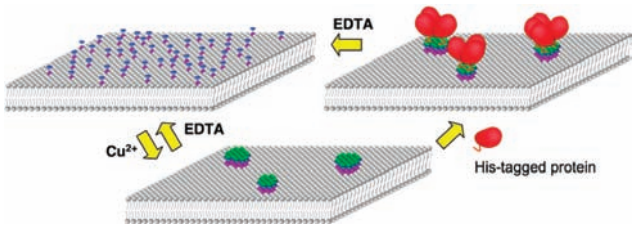

Figure 3. Schematic of reversible formation of microdomains driven by Cu^{2+} recognition followed by selective adsorption of His-tagged protein. EDTA releases protein and Cu^{2+} and restores the membrane.

Based on their structures and phase transition temperatures DSIDA and POPC should form phase separated structures at room temperature. Lack of visible DSIDA domains suggests that if domains exist they are smaller than the diffraction limit. Addition of Cu^{2+} facilitates the coarsening of the domains. Previous work with colloidal probe atomic force microscopy determined that the DSIDA lipid is negatively charged at neutral pH, while binding of Cu^{2+} produces a charge neutral complex.[10] Additionally, POPC bilayers containing DOIDA, a fluid phase analogue of DSIDA, yielded no observable domain formation upon Cu^{2+} addition. These results emphasize the importance of electrostatics and lipid structure in the directed formation and functional properties of membrane domains.

Binding of His-tagged proteins to lipid bilayers was also examined with 10% Cu^{2+}-DOIDA and 10% Ni^{2+}-NTA DOGS functionalized POPC membranes. These two bilayers remain homogeneous upon metal ion and protein addition under microscopic resolution. Estimated binding constants for these bilayers ($K_a \approx 10^7$ M^{-1}) were consistent with literature values.[11] The notably higher affinity observed with the domain-forming DSIDA/POPC bilayers highlights the importance of receptor aggregation that promotes facile multivalent binding interaction thereby amplifying ligand affinity.[12] For switchable surfaces it is also important to note that the IDA membranes affected release of bound proteins with $\sim$1000-fold reduction in EDTA concentration compared to the NTA membrane.[10a,12] This simple synthetic membrane demonstrates a first step in the generation of actuated materials that utilize ion recognition and protein complexation for temporal structure formation.

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Supporting Information Available: DSC measurements, FCS analyses for lipid mobility rates, additional images of Cu^{2+}-treated membranes with 6-His GFP, and protein affinity data. This material is available free of charge via the Internet at http://pubs.acs.org.

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