Communication

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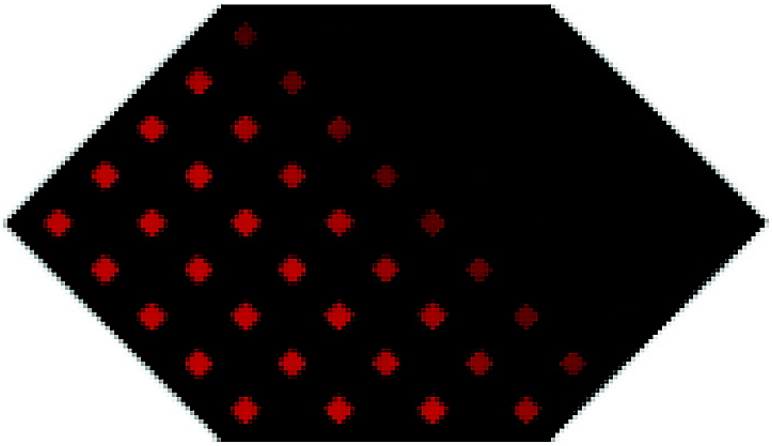

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Lipid Mobility and Molecular Binding in Fluid Lipid Membranes

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Innumerable biochemical events, ranging from intercellular signaling to viral infection, involve receptor−ligand engagement on the cell membrane surface. It is becoming increasingly evident that spatial rearrangement of receptors and signaling molecules within the fluid membrane environment is a broadly significant aspect of these processes. Polyvalent ligands, for example, induce co-localization of their target receptors, thus encoding collective properties that are appreciatively different from individual binding events.[1] Correspondingly, the ability of target receptors to move and adopt complementary configurations is intimately associated with the overall affinity of the molecular recognition event.[1−3] In other examples, such as G-protein coupled receptor (GPCR) and integrin signaling, ligand binding triggers a conformational change in the receptor protein which, in turn, alters its association state with other membrane-localized signaling molecules.[4,5] In each case, changes in the organization and mobility of membrane components occur in conjunction with signaling and recognition events.

Here, we examine the mobility of nonparticipating background lipid in conjunction with ligand binding membrane-associated receptors in a fluid lipid bilayer membrane. Despite the fact that the background lipid is not directly involved in the ligand binding interaction, binding-induced mobility changes are clearly discernible for the two systems studied: cholera toxin binding membrane-associated monosialoganglioside G_{M1} and antibody binding to a glycanphosphatidylinositol (GPI)-linked form of intercellular adhesion molecule (ICAM-1). A useful corollary of this third-party effect is that binding can be detected without labeling the ligand or the receptor of interest.

Experiments were performed using supported membrane microarrays.[6−9] Membrane arrays were assembled on silica substrates, which had been photolithographically patterned with chrome grids. The chrome creates surface barriers that isolate the individual membrane corrals. Robotic direct dispensing methods with Cartesian MicroSys model 4100-2SQ were employed to deposit 40 nL droplets of vesicle suspension into the prepatterned 500 × 500 μm corrals. Vesicle fusion occurs within seconds of deposition, forming fluid-supported membranes that continuously fill each corral (Figure 1A). Mobility of fluorescently labeled components was monitored by fluorescence recovery after photobleaching (FRAP). FRAP measurements were performed using a ∼100 μm diameter bleaching spot and a 60 s bleach exposure time. For this spot size, observations of diffusive recovery were made several minutes after the bleach exposure.

Results characterizing molecular mobility within the supported membrane during cholera toxin binding are summarized in Figure 1. Cholera toxin, which is naturally secreted by *Vibrio cholerae*, exists as a hexamer involving two different types of subunits in an AB_5 configuration. The B subunits (CTB) organize into pentamers with each subunit specifically binding the ganglioside G_{M1} pentasaccharide headgroup.[10,11] Binding of CTB to G_{M1} containing supported membranes is readily confirmed using fluorescently labeled CTB (Alexa Fluor 594 conjugate) (Supporting Information).

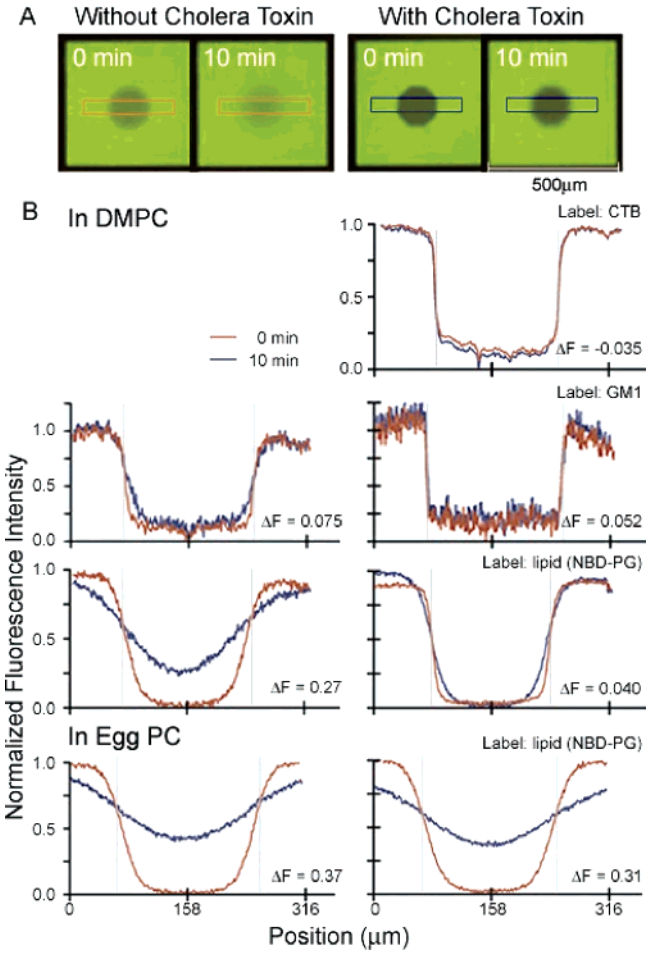

Figure 1. (A) Representative FRAP experiments on a pair of 500 × 500 μm membrane corrals containing unlabeled ganglioside G_{M1} (0.25 mol %) with background lipids consisting of DMPC (98.75 mol %) and NBD-PG (1 mol %). Experiments were performed before and after exposure to CTB (1.40×10^{-7} M), as labeled. The 0 min images depict the photobleached spots immediately after exposure to bleaching light. Images taken 10 min later reveal the extent of diffusive mixing. (B) Quantitative traces of fluorescence intensity across the bleach spot at 0 and 10 min for a series of FRAP experiments probing the change in mobility of each component upon CTB binding, as labeled. The parameter, ΔF, represents the linearly integrated and normalized difference between before and after fluorescence traces. A value of 0 indicates no diffusion, and a value of 1 indicates complete recovery.

FRAP mobility measurements of labeled CTB, labeled G_{M1}, and labeled lipid (NBD-PG) are summarized in Figure 1B. Observations of labeled CTB indicate that it is relatively immobile when bound to supported membranes. The large size, potential for oligomer-

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ization, and multivalent binding of CTB likely contribute to this reduced mobility. A corresponding set of experiments, utilizing labeled G_{M1} (BODIPY FL C5) and unlabeled CTB, was performed to characterize the mobility of G_{M1} during CTB binding. Before exposure to CTB, labeled G_{M1} exhibits lateral diffusion, though somewhat attenuated relative to other lipids, perhaps as a result of slight aggregation (Figure 1B). After CTB binding, a substantial reduction in the diffusion rate of labeled G_{M1} (now complexed with CTB) is observed.

A most interesting feature of these experiments is revealed when the mobility of the lipid probe (NBD-PG) is monitored during CTB−G_{M1} binding. Despite the fact that this lipid does not participate in the binding interaction, its mobility is markedly affected by CTB−G_{M1} binding. FRAP experiments on the 1 mol % NBD-PG in DMPC/G_{M1} (98.75/0.25 mol %) membranes reveal a drastic reduction in mobility in conjunction with CTB binding (Figure 1B). Similar experiments, performed using egg-PC (a natural mixture of PCs containing ∼50% unsaturated fatty acids) instead of the saturated DMPC, do not show a reduction in NBD-PG mobility associated with CTB−G_{M1} binding. The independence of NBD-PG mobility from CTB−G_{M1} binding in egg-PC membranes confirms that NBD-PG has no intrinsic interaction with CTB or G_{M1}. An important difference between egg-PC and DMPC membranes is the gel−fluid transition temperature of DMPC (23 °C), which is much higher than that of egg-PC (<10 °C). Proximity to a gel−fluid transition contributes to the mobility effect we observe in the DMPC system (Supporting Information).

We therefore suggest that protein binding modulates the gel−fluid transition temperature of the membrane. As a corollary of this, one can expect the miscibility phase transition temperature of a mixed membrane to be similarly modulated by protein binding. Preliminary studies indicate this is so.

To test the generality of using lipid mobility as a measure of receptor−ligand binding, antibody binding to the cell surface adhesion molecule, ICAM-1, was examined. A GPI-linked form of ICAM, which is known to be biologically functional in the supported membrane configuration,[12] was used for this study. This protein was expressed in CHO cells, purified, and reconstituted into preformed lipid vesicles (99% DMPC, 1% NBD-PG) by detergent dialysis (Supporting Information). A phycoerythrin-conjugated anti-ICAM-1 antibody (BD Biosciences) was utilized for direct fluorescence observation of the protein. FRAP images and compiled results from the lipid mobility assay (Figure 2) reveal an effective K_d of ∼0.8 nM for the antibody−ICAM-1 interaction, which is comparable to the K_d of ∼3.8 nM obtained from direct fluorescence measurements of the labeled antibody (Supporting Information).

A useful consequence of the mobility effects described here is that traces of fluorescent probe lipid, doped into the background of the membrane, can be utilized to detect binding of unlabeled ligands to unmodified membrane targets. Low concentrations of target (0.25 mol %) can trigger substantial mobility changes. The G_{M1} target concentration used in these experiments is 20-fold lower than the 5 mol % G_{M1} Kuziemko et al. report as the minimum required for analyzable kinetic data using a Biacore surface plasmon resonance system.[13] With use of a laser, diffusion measurements could be executed rapidly (seconds), enabling acquisition of kinetic

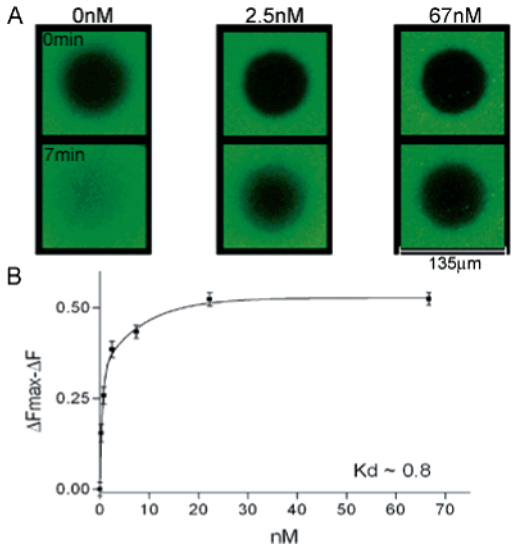

Figure 2. (A) Corrals of chips containing 0.25 mol % ICAM-1 in 98.75% DMPC with 1 mol % NBD-PG were exposed to increasing concentrations of anti-ICAM-1 antibody (BD Biosciences). FRAP was performed on at least 4 corrals for each concentration of antibody. (B) The average ΔF (as described in Figure 1) for each antibody concentration was subtracted from the average maximal ΔF (ΔF_{max}), which was obtained from corrals exposed to no antibody. K_d was determined with Prism 3.03.

binding data by lipid mobility analysis. This methodology may be extended to studies of fully transmembrane proteins, such as the GPCRs, by using polymeric layers on the supporting substrate to facilitate lateral mobility of the protein within the membrane.

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Supporting Information Available: Binding affinity on a chip, lipid mobility at 22 and 30 °C, and fluorescent anti-ICAM-1 antibody affinity (pdf). This material is available free of charge via the Internet at http://pubs.acs.org.

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