Specific protein binding on fluidic lipid bilayer microarray corralled by well-defined polymer brushes

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Introduction: Cellular membranes consisting of phospholipid bilayers have functions of molecular recognition and transportation through the action of intrinsic glycolipids and membrane proteins. Substrate-supported phospholipid bilayers (SPBs) are being studied as models of cellular membranes. One of the important features of SPBs is the possibility of generating micropatterned membranes on the substrate, which enables the creation of designed arrays of biological materials for various applications. We have developed a new method for creating phospholipid bilayer microarrays (PLBMAs) *via* site-specific adsorption of phospholipid vesicles on a silicon surface bearing prepatterned, well-defined poly(2-methacryloyloxyethyl phosphorylcholine) (PMPC) brushes prepared by surface-initiated atom transfer radical polymerization (SI-ATRP).

Experiments: A sufficient amount of the suspension containing small unilamellar vesicle (SUV) was placed on the surface prepatterned PMPC brushes[1] and incubated for 1 h at room temperature. Excess vesicles were removed from the surface by rinsing with copious amounts of PBS buffer and deionized water. To observe the bilayer formation, a given amount of Texas Red® 1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt, (TR-DHPE) was added to the 1,2-dioleoyl-sn-glycero-3-phosphorylcholine (DOPC). The adsorption of phospholipid vesicles on the silica and PMPC polymer surfaces was also determined by a surface plasmon resonance sensor. Lateral mobility of the lipid molecules was confirmed by fluorescence recovery after photobleaching (FRAP) experiments.

PLBMAs containing 2% ganglioside GM_1 were prepared by the addition of these functional lipids to DOPC. Cholera toxin subunit B (CTB) labeled with Alexa Fluor 488 was used as model proteins to evaluate protein/lipid specific binding on the PLBMA. CTB was separately dissolved in PBS buffer or blood plasma and placed in contact with the PLBMA surfaces having different lipid fractions at room temperature. The surfaces were then rinsed with PBS buffer and purified water; the adsorption protein on the PLBMAs was observed by fluorescence microscopy. In addition, the GM_1/CTB binding was evaluated by changing the concentration of CTB.

Results and Discussion: The phospholipid vesicles selectively adsorbed on the silicon surface and homogeneous membranes were observed in array form. This phenomenon coincided well with the SPR. FRAP experiments were preformed to validate the fluidity of PLBMA using PMPC brushes. The fluorescence images and graphs of fluorescence intensity correspond to times subsequent to the photobleaching. The results demonstrate that the intrinsic fluidity of the bilayer is maintained during array formation with essentially no fraction indicated by nearly full recovery of fluorescence.

Phospholipid bilayers have long been known to be biomimetic substrates that are ideally suited for performing biological assays. We incorporated a glycolipid containing the oligosaccharide unit for specific binding by cholera toxin B (CTB) subunits into phospholipid bilayers having 2 mol% ganglioside GM_1. The fluorescence microscopic image of Figure 1 shows pure DOPC PLBMAs (a) for controls and PLBMA containing GM_1 (b) after contact with the green dye-tagged 25 nM CTB. As expected, the CTB binds specifically to GM_1 containing PLBMAs (b). More importantly, nonspecific protein binding were observed to lipid bilayer elements not containing a ligand other than the one required by the protein–ligand pair of interest. We performed a quantitative analysis of CTB binding on the PLBMAs with GM_1 using a competition assay. Below a CTB concentration of 25 nM, the fluorescence intensity increases with the CTB concentration. This relationship between CTB concentration and fluorescence intensity demonstrates the quantitative capability of CTB binding on the PLBMAs with GM_1. Moreover, the quantitative capability was observed regardless of the presence of high concentrations of plasma proteins in the media (>1 mg/mL). In diagnostic applications, nonspecific protein adsorption on a solid surface is always an unfavorable factor for reliable analysis. Molecular recognition on PLBMA-corralled PMPC brushes is performed precisely and the surface may have a suitable design for directing biological responses and useful as matrices for use in diagnostic and pharmaceutical fields.

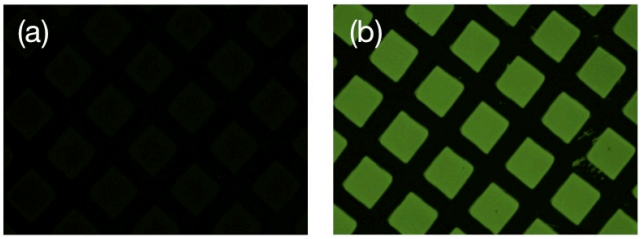

Figure 1 Fluorescence micrographs of PLBMA containing (a) DOPC and (b) 2.0 mol% GM_1 in DOPC after contact with green dye-tagged 200 nM CTB.

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References:

[1] Iwata R, Iwasaki Y et al., *Biomacromolecules* **2004**, 5, 2308-2314.