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Supporting Information

Chemoselectively Surface Funtionalizable Tethered Bilayer Lipid Membrane for Versatile Membrane Mimetic Systems Fabrication

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Experimental Methods

X-ray photoelectron spectroscopy

X-rayphotoelectron spectroscopy (XPS) spectra of azide modified glass slide and lipid tethered glass slide were recorded and analyzed with a PHI 5000 VersaProbe XPS system (SEMTech Solutions, Inc., North Billerica, MA). Monochromated Al Kα radiation was used to excite photoelectrons. 75 watts was selected for 300 micron spot size. The pass energy was 93.9 eV. As shown in Figure 1, the main component at C1s peak at 286.0 eV is assigned to PEG, and another typical component at 133.5 eV is assigned to the phosphate ester group of the lipid (1B), which indicated that the glass slide was successfully modified with anchor lipid.

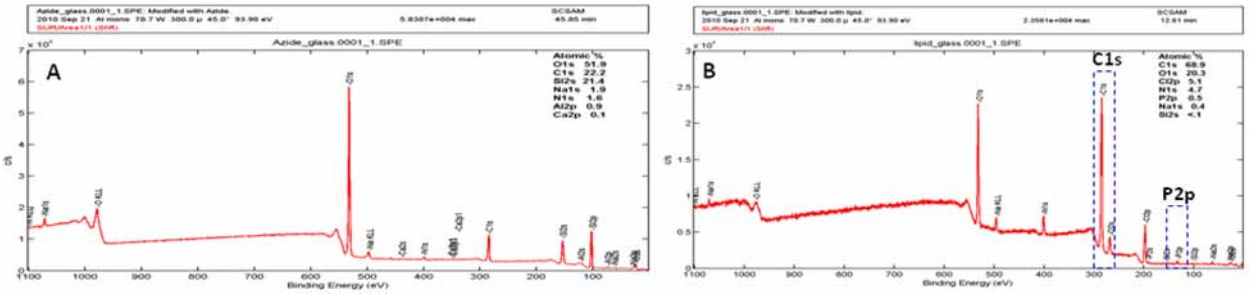

Figure 1. X-rayphotoelectron spectra (XPS) of azide-glass slide (A) and DSPE-PEG-TP tethered glass slide (B)

Biotin functionalization of tBLM

Liposomes containing DSPE-PEG_{2000}-TP (DPPE:CHL:DSPE-TP 2:1:1 mol%) were used to fabricate TP functional t-BLM as above. Then, the tBLM was modified with azide-containing lactose and biotin through Staudinger ligation, respectively. Briefly, the TP-functional tBLM-covered slide was incubated with 40 mg/mL azide-PEG_6-biotin in PBS buffer (pH 7.4) at room temperature for 2 hrs, the excess solution was removed from the surface by flushing with copious amounts of PBS buffer (pH 7.4) three times. To avoid the oxidation of TP, all operations were performed in a sealed container filled with argon.

Streptavidin binding assay: Streptavidin binding assay was examined to confirm the biotinylation and whether the grafted biotin residues are easily accessible at the surface of t-BLM. The glass slide was incubated in streptavidin-FITC (Sigma) PBS buffer solution (pH 7.4, 20 μg/mL) for 30 minutes. Excess streptavidin solution was removed and then washed with PBS buffer (pH 7.4) three times. After the unreacted streptavidin-FITC was removed and t-BLM washed completely, the fluorescence image was obtained by fluorescence image scanning with Typhoon 9410 Variable Mode Imager (Amersham Biosciences, USA). Two control experiments were operated at the same time, one is tBLM without biotinylation incubated with streptavidin-FITC, and the other one is biotinylated tBLM incubated with biotin pre-saturated streptavidin-FITC.

All tBLM covered glass slide surfaces were handled horizontally to avoid excessive force from liquid fronts running across the surface. Most washing/rinsing steps were carried out with the tBLM covered surfaces facing up at the bottom of the glass dishes and with buffer solutions added and withdrawn at localizations away from the sample surfaces.

Fluorescence Image: As shown in Figure 2, biotinylated tBLM (Figure 2B) yielded fluorescence image of bound streptavidin-FITC, while there was no apparent fluorescence image observed for the precursor non-biotinylated tBLM (Figure 2A). Furthermore, no apparent fluorescence image showed when free biotin pre-saturated streptavidin-FITC was used (Figure 2C). These results indicated that the tBLM surface was biotinylated successfully and the grafted biotin was easily accessible.

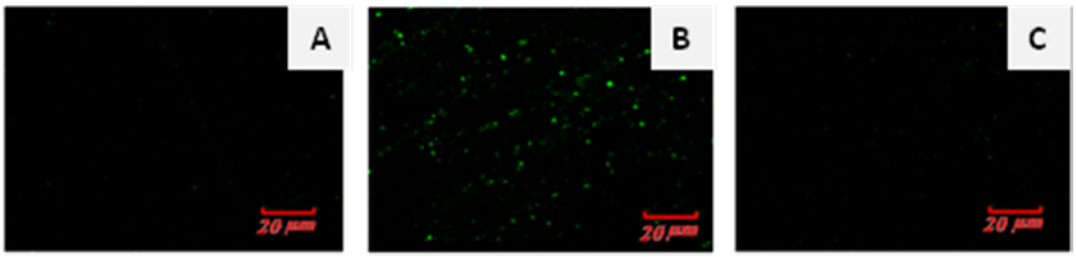

Figure 2. Fluorescence scanning image of streptavidin binding onto the bioinylated tBLM: unmodified tBLM (**A**), bioinylated tBLM (**B**), and bioinylated tBLM treated with free biotin pre-incubated lectin (**C**).

AFM Image: AFM experiments were carried out by Veeco Nanoscope Multimode AFM (Germany), the AFM samples were performed using the tapping mode. AFM image (Figure 3B) showed apparent streptavidin binding comparing to the non-biotinylated liposomes

(Figure 3A). Taken together, these results indicated that the tBLM could be chemically selective functionalized *via* Staudniger ligation.

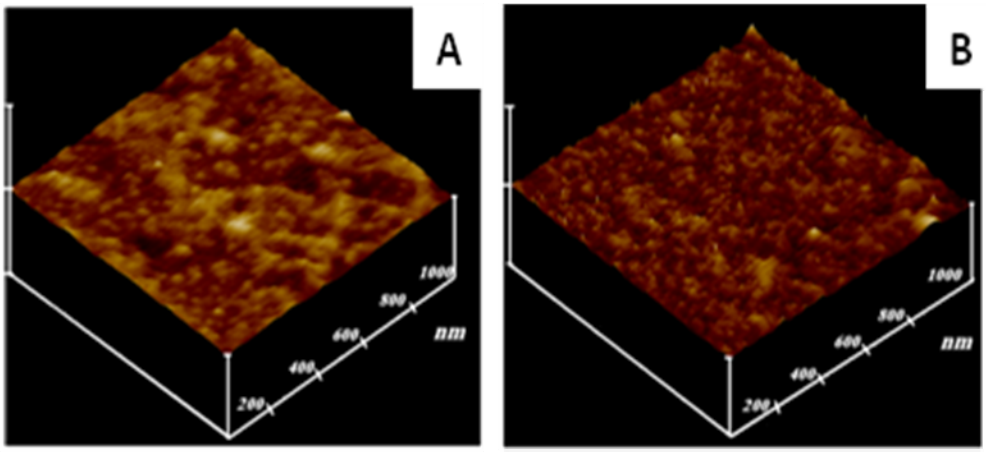

Figure 3. AFM image of bioinylated tBLM (**A**) and streptavidin binding onto the bioinylated tBLM (**B**).

Protein C activation assay

The catalytic efficiency of the TM reconstituted tBLM was accessed *via* the activation of human protein C by human thrombin as described in the literature with some modification.[3] Briefly, activation was performed at 37 °C in 20 mM Tris-HCl buffer, pH 7.5, containing 100 mM NaCl, 0.1% BSA, and 5 mM Ca^{2+}. A typical activation mixture contains 5 nM of thrombin, and 800 nM of protein C in the presence of TM-tBLM glass slide. Activation was terminated by the addition of antithrombin III (300 μg/mL final concentration). Activated protein C concentration was determined using the Spectrozyme PCa substrate (absorbance 405 nm). K_m and K_{cat} values were calculated assuming Michaelis-Menton reaction kinetics.

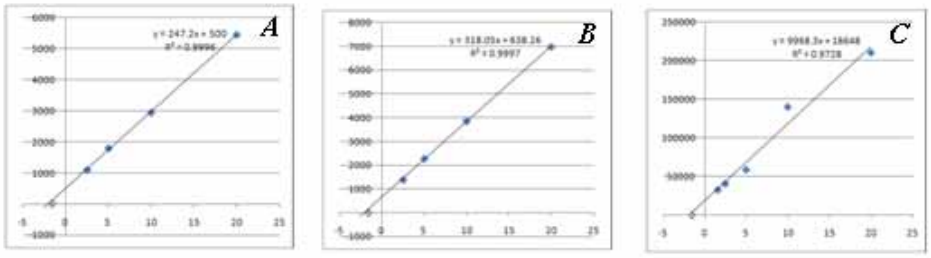

Figure 4. The double-reciprocal curve of free TM (**A**), liposome-TM (**B**) and tBLM-TM (**C**)