## SUPPORTING INFORMATION

## Lipid membrane formation on chemical gradient modified surfaces

Ying Zhang, Xuejing Wang, Shenghua Ma, Kunpeng Jiang, Xiaojun Han\*

State Key Laboratory of Urban Water Resource and Environment, School of Chemical Engineering and Technology, Harbin Institute of Technology, 92 West Da-Zhi Street, Harbin, 150001, China. Email:hanxiaojun@hit.edu.cn

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## Materials and methods:

**Materials:** The 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) was purchased from Avanti Polar Lipids (USA). Texas red-labeled 1, 2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (TR-DHPE) and N-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)-1,2- dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine, triethylammonium salt (NBD PE) were purchased from Molecular Probes (Eugene, Oregon, US). Chloroform (analytical grade, purity > 99.8 %) was purchased from Sigma-Aldrich. Sucrose and MgCl<sub>2</sub> • 6H<sub>2</sub>O were purchased from Xilong Chemicals (China). Ethanol and toluene were purchased from FuYu Chemicals (China). Coverslips (200 µm in thickness) was supplied by Fisher scientific. Silicon wafers from Rockwood Electronic Materials were cut in pieces with a size of 1 × 1 cm. Trimethoxy(octadecyl) silane (TODS) was purchased from Sigma (China). Deionized water with a resistivity of 18.2 MΩ·cm was used throughout.

**Preparation of chemical gradient substrates:** Glass substrates were cleaned by ultrasonication in ethanol and distilled water each for 10 min, respectively, and then dried with nitrogen, followed by 30 s air plasma treatment. After immersing in a 1% (v/v) solution of TODS in toluene for 4 h at room temperature, the substrates were washed with toluene and dried under a stream of nitrogen. The SAMs modified substrates were covered by a silicon wafer with an extremely two thin spacers (height = 0.18 mm) at the corners of one end to form a "wedge" shape space. This setup was loaded in a plasma system (Diener), and exposed to air plasma under power of 80 W RF for 2 min.

Wetting angle measurements: The measurement of contact angles were carried out on a JC2000D contact angle measurement equipment (Shanghai Zhongchen Digital Technology Apparatus Co. Ltd).

**Preparation of supported lipid membranes:** Giant unilamellar vesicles were prepared by the electroformation method. 5  $\mu$ L of lipid chloroform solution, composed of DOPC and NBD PE at a 95 : 5 mass ration (or TR-DHPE at a 99.5 : 0.5 mass ration), was deposited onto the ITO electrode surfaces. After drying the lipid films deposited on the electrode surfaces for 2 h under vacuum, a certain volume of 30 mM sucrose was gently dropped into the chamber between the two ITO electrodes. A sinusoidal AC electric field (5 V, 10 Hz) was applied to induce GUV formation.<sup>1, 2</sup> Within 5 min after the contact angle gradient was prepared, the substrate was cultivated in a flow cell with freshly prepared vesicles and 10 mM MgCl<sub>2</sub> solution at final lipid concentration of 0.01 mg mL<sup>-1</sup> for 2 h. After that, pump water through the cell (Fig. S4) at the flow rate of 3 mL min<sup>-1</sup> in amount of 200 mL to remove the residual vesicles.

**Fluorescence microscopy and fluorescence recovery after photobleaching(FRAP):** A Nikon Eclipse 80i fluorescence microscope equipped with a Nikon DS-Fi1 digital camera was used to image the lipid membrane on the gradient surfaces and to carry out FRAP measurements. Image J (Version 1.44p, USA) was used to analyze images. The lateral diffusion coefficient D was calculated from  $D=0.224w^2/t^{1/2}$ , where w is the radius of the bleached spot and  $t^{1/2}$  the half-life of fluorescence recovery.<sup>3</sup>

Atomic Force Microscopy (AFM): AFM images were collected using a Cypher S atomic force microscope (Oxford Instruments). Silicon nitride probes (RC8009SA, Asylum Research, Oxford Instruments) with a resonant frequency between 51 kHz

and 99 kHz and a spring constant between 0.49 N/m and 1.52 N/m were used for scanning in tapping mode in pure water.

The power of the plasma was fixed at 80 W and the exposure time of TODS SAMs modified substrates in air plasma was varied from 10 s to 160 s.



Fig. S1 Contact angle of TODS SAM modified substrate against plasma oxidized time.



Fig. S2 Freshly prepared GUVs. Scale bar 200 µm.



Fig. S3 Fluorescence microscopy images of lipid membranes on the contact angle gradient surface formed with LUVs. a) uniform lipid monolayer, b) lipid monolayer patches, c) LUVs attached onto the substrate and d) the lipid bilayer. The scale bar is 10 µm.





## Notes and references:

- 1. M. I. Angelova and D. S. Dimitrov, *Faraday Discussions*, 1986, **81**, 303-311.
- 2. H. M. Bi, B. Yang, L. Wang, W. W. Cao and X. J. Han, *Journal of Materials Chemistry A*, 2013, **1**, 7125-7130.

3. L. Q. Zhang, M. L. Longo and P. Stroeve, *Langmuir*, 2000, **16**, 5093-5099.