Solvent-Assisted Lipid Self-Assembly at Hydrophilic Surfaces: Factors Influencing the Formation of Supported Membranes

Supporting Information

Seyed R. Tabaei^{†,‡}, Joshua A. Jackman^{†,‡}, Seong-Oh Kim^{†,‡}, Vladimir P. Zhdanov^{†,‡, \parallel}, Nam-Joon Cho^{*,†,‡,§}

[†]School of Materials Science and Engineering, Nanyang Technological University, 50 Nanyang Avenue 639798, Singapore

[‡]Centre for Biomimetic Sensor Science, Nanyang Technological University, 50 Nanyang Drive 637553, Singapore

[§]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive 637459, Singapore

Boreskov Institute of Catalysis, Russian Academy of Sciences, Novosibirsk 630090, Russia



10 20 30 40 Distance (μm)

Figure S1. Observation of Membrane Fluidity in Microscopic Lipid Patches on Glass. Fluorescence micrographs were recorded for lipid layers formed on glass via the SALB procedure immediately (A) and at selected times (B-E) after photobleaching (the dark spot in the image center corresponds to the photobleached region; the scale bars are 30 μ m). The precursor mixture in isopropanol solution had 0.1 mg/mL DOPC lipid, and contained 0.5 wt% fluorescent Rhodamine-PE lipid. (F) The normalized intensity profiles of the bleached spots as a function of time after photobleaching are presented.



Figure S2. Observation of Fluidic Supported Bilayer on Glass. Fluorescence micrographs were recorded for lipid layers formed via the SALB procedure immediately (A) and at selected times (B-E) after photobleaching (the dark spot in the image center corresponds to the photobleached region; the scale bars are 30 μ m). The precursor mixture in isopropanol solution had 0.25 mg/mL DOPC lipid, and contained 0.5 wt% fluorescent Rhodamine-PE lipid. (F) The normalized intensity profiles of the bleached spots as a function of time after photobleaching are presented.



Figure S3. Adsorption of DOPC Lipid in Isopropanol onto a Silicon Oxide Surface. The QCM-D measurement values are presented as a function of time for eight lipid concentrations (after the moment marked by arrow 2 of the SALB experiment, as presented in Figure 1 in the main text).



Figure S4. Linear Dependence of QCM-D Equilibrium Frequency Shifts on Lipid Concentration. Data are based on the QCM-D frequency shifts curves shown in Figure S3.



Figure S5. FRAP Analysis of Lipid Layers on Glass Formed at High Lipid Concentration. Fluorescence micrographs were recorded for a supported lipid layers obtained via the SALB procedure immediately (t = 0 sec) and in 20-sec increments after photobleaching (the dark spot in the image center corresponds to the photobleached region; the scale bars are 30 μ m). The precursor mixture in isopropanol solution had 5 mg/mL DOPC lipid, and contained 0.5 wt% fluorescent Rhodamine-PE lipid.



Figure S6. QCM-D Detection of Adsorbed Vesicles on Silicon Oxide via Peptide-Induced Vesicle Rupture. Using QCM-D tracking, changes in (A) frequency (ΔF) and (B) dissipation (ΔD) were measured as functions of time for SALB experiments. The lipid concentration was 5 mg/mL DOPC lipid and one of three organic solvents was used in the SALB procedure: isopropanol, n-propanol, or ethanol. After completion of the SALB procedure, AH peptide was added to induce vesicle rupture (see arrow). For all experiments, measurement baselines were recorded in the same aqueous buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl).



Figure S7. Effect of Temperature on SALB Procedure with DMPC Lipid. Using QCM-D tracking, changes in frequency (ΔF) and energy dissipation (ΔD) were measured as functions of time for SALB experiments conducted at two temperatures. The lipid concentration was 0.5 mg/mL DMPC lipid in isopropanol. After completion of the SALB procedure, 0.2 mg/mL BSA was added (see arrow). For all experiments, measurement baselines were recorded in the same aqueous buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl). Note that the set temperature remained constant throughout the experiment.



Figure S8. Effect of Temperature on SALB Procedure Using DPPC Lipid. Using QCM-D tracking, changes in (A) frequency (ΔF) and (B) energy dissipation (ΔD) were measured as functions of time for SALB experiments conducted at two temperatures. The lipid concentration was 0.5 mg/mL DPPC lipid in isopropanol. After completion of the SALB procedure, 0.2 mg/mL BSA was added (see arrow). For all experiments, measurement baselines were recorded in the same aqueous buffer solution (10 mM Tris [pH 7.5] with 150 mM NaCl). For panel (A), the temperature experiment was initially conducted at 24 °C, and then exchanged to 50 °C at t = 45 min (it reached the set temperature by t = 75 min). It was again exchanged back to 50 °C at t = 80 min (it reached the final temperature by t = 95 min).

Substrate	ΔF on Bare Substrate (Hz)	ΔF on Bilayer-Coated Substrate (Hz)	Surface Coverage (%)
Chrome	19 ± 1	1.2 ± 0.3	94 ± 2
ITO	31 ± 4	5.0 ± 2.6	84 ± 9
TiO ₂	30 ± 3	4.0 ± 1.7	87 ± 6

Table S1. Summary Values for BSA Protein Attachment to Bare and Lipid Bilayer-Coated Substrates. 0.2 mg/mL BSA was added and the frequency shift was recorded by QCM-D measurement.

Supplementary Videos

Video S1. FRAP Analysis of a Lipid Layer on Silicon Oxide Formed via the SALB Procedure from 5 mg/mL DOPC Lipid in n-Propanol. An SALB experiment was performed as described in the Materials and Methods section. FRAP was recorded after a bleach pulse (at t = 0 min in the video) and it is observed that a fluidic layer is present along with an appreciable number of extended lipid structures (note the protrusions extended from the surface which are coming in and out of focus). The real-time duration captured in the video recording is two minutes.

Video S2. FRAP Analysis of a Lipid Layer on Silicon Oxide Formed via the SALB Procedure from 5 mg/mL DOPC Lipid in Ethanol. A similar experiment as described in Video S1 was conducted using ethanol. In this case, poor recovery of the bleached spot is observed along with a low fraction of extended lipid structures (note the lipid assembly appears granular, which is consistent with adsorbed vesicles).