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Supporting Information

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Hydrogel-Stamping of Arrays of Supported Lipid Bilayers with Various Lipid Compositions for Screening of Drug-Membrane and Protein-Membrane Interactions

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Fabrication of Agarose Stamps

We prepared agarose stamps according to the procedure described by Mayer *et al.*[1] Briefly, we heated an aqueous solution containing 4% (w/v) of high-gel strength agarose (OmniPur; Merck, Darmstadt, Germany) in 0.15 M KCl to the boiling point and cast it onto a patterned PDMS master at room temperature. Then we allowed the solution to gel at room temperature and peeled off the PDMS master to obtain the agarose stamps (Figure S1a). Depending on the desired dimensions of the agarose stamps, we used different PDMS masters to mold the stamps. The PDMS master for stamps with posts with 1 mm diameter was a replica (positive) of a PDMS replica (negative) of a standard 1536-well plate (polystyrene) with flat bottoms (Corning, Cambridge, MA, USA).[1] We also used masters prepared by photolithography[1] for stamps with posts with diameter of 200 and 700 µm. Depending on the PDMS master used for casting, arrays of posts on the agarose stamp, were (i) 200 µm in diameter, 130 µm in height, and spaced 200 µm from each other, (ii) 700 µm in diameter, 700 µm in height, and spaced 300 µm from each other; or, (iii) 1 mm in diameter, 1.5 mm in height, and spaced 1 mm from each other.

Preparation of Liposomes

Lipid mixtures used to prepare liposomes were: 99% L-a-phosphatidylcholine form chicken egg (egg PC; Sigma Aldrich) and 1% (w/w) 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine–rhodamine B sulfonyl) (rh-PE; Avanti Polar Lipids); 97% egg PC and 3% NBD-labled PE (NBD-PE; Avanti Polar Lipids); mixtures of egg PC / rh-PE or NBD-PE / 1,2-dioleoyl-*sn*-glycero-3-[phospho-L-serine] (DOPS; Avanti Polar Lipids), and mixtures of egg PC / rh-PE / cholesterol (Avanti Polar Lipids). The respective mixing ratios are mentioned in the main text. Small unilamellar liposomes were produced by tip sonication using a Branson Sonifier 150 (Branson Ultrasonics Corporation, Danbury, USA) of 1 mg lipid in 500 µL of an aqueous solution containing 0.15 M KCl, for 2-6 minutes (with ~ 5 watts output energy). Before sonication, the lipids were dissolved in chloroform and 100 µL of a 10 mg/mL lipid solution in chloroform was used to deposit a lipid film on the wall of a 5 mL round bottom flask using a rotatory evaporator under vacuum (starting form -300 torr and going up to -740 torr). Residual traces of chloroform were removed by desiccation under vacuum (~ -740 torr) for at least 1 hour.

Cleaning of Microscope Glass Slides

Microscope glass slides (Microslides, No. 2974, Corning, N.Y.) were cleaned with fresh piranha solution (mixture of concentrated sulfuric acid and 30 % hydrogen peroxide) followed by washing with deionized water at least eight times and drying at 180° C for 2 hours.

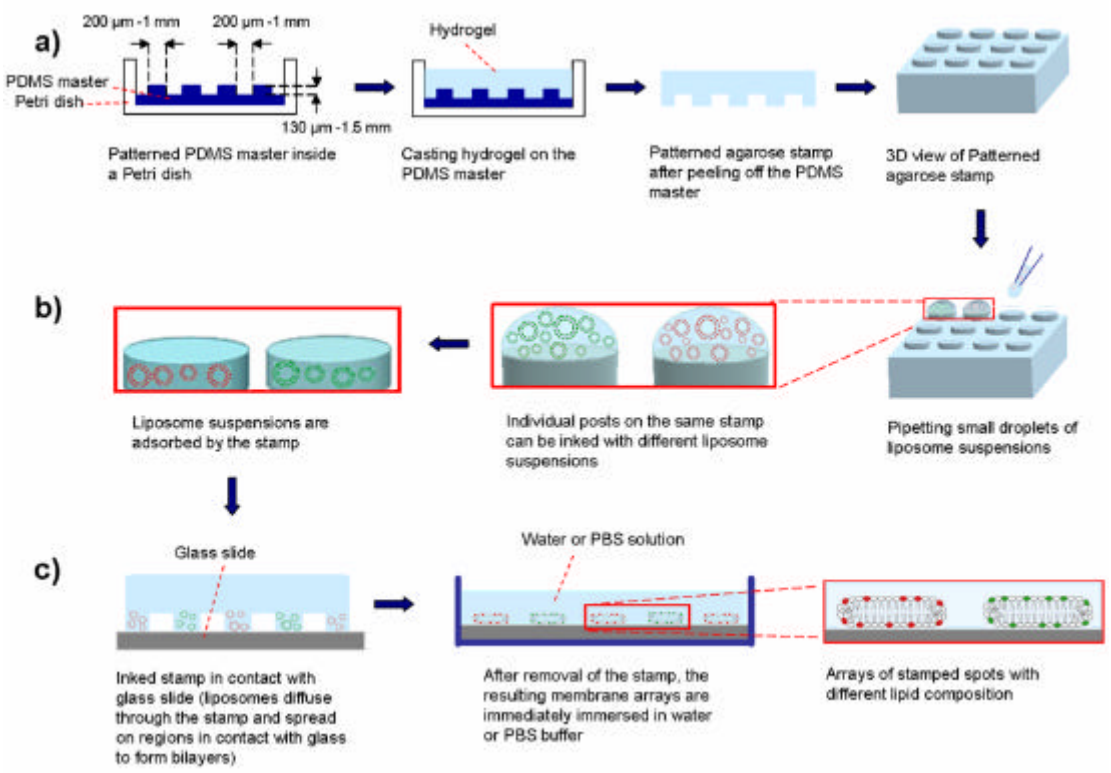

Figure S1. Schematic representation of the fabrication of agarose stamps, the inking process, and the stamping procedure. (a) Casting agarose gel onto a patterned PDMS master and peeling off the PDMS master from the agarose gel resulted in a topographically patterned agarose stamp. (b) In order to ink the posts of the stamp manually, the stamps were turned upside down such that posts were facing upwards and small droplets (~ 0.2 µL) of liposome suspensions were added on top of each post. Small liposomes inside the droplet diffused into the agarose gel and the solution of the liposome suspension was absorbed by the gel. (c) Supported lipid bilayer spots were formed by diffusion of liposomes through the gel and subsequent spreading of these liposomes onto the glass slide at the areas of contact between the stamp and glass slide. Stamped

membrane arrays on glass slides were immersed in water or PBS buffer immediately after removal of the stamp from the substrate. The membrane arrays were then ready for inspection, binding assays, or storage.

Inking and Stamping Procedure

To ink the agarose stamps with 1 mm or 700 μm posts, we turned the stamp upside down in a Petri dish containing a solution of 0.15 M KCl, such that ~ ¾ of the thickness of the stamp was immersed the KCl solution and the posts (which were facing upwards) were out of the KCl solution. We inked these posts individually by pipetting ~ 0.2 μL of liposome suspension on top of each post (Figure S1b). Neighboring posts on the same stamp could be inked with different liposome suspensions. Once the solution was adsorbed by the hydrogel (typically after ~ 4 minutes), we added another droplet of ~ 0.2 μL of solution on top of each post and this process was repeated for 4 or 5 times. In case of stamps with smaller posts (200 μm in diameter), we inked the agarose stamp by immersing the posts in a solution of liposomes for ~ 30 min. After inking we turned these stamps upside down (200 μm posts facing upwards) and after the stamp adsorbed all solution, we used it for stamping. In the beginning of a stamping series, we stamped 4-7 times on clean glass slides to remove excess solution of liposomes from the stamp.

To form arrays of lipid bilayers, we placed the inked agarose stamp in contact with clean glass slides for 5-10 sec (Figure S1c). After removing the stamp from the slides, the glass slides were immediately immersed in water or PBS solution. All stamping procedures were carried out at room temperature in a small room with = 55 %

humidity. We found that carrying out the stamping procedure in humidity < 50 % resulted in supported bilayers with reduced fluidity. The stamped spots of lipid bilayers retained their fluidity even after storing them for two weeks in buffer solution.

Atomic Force Microscopy (AFM) Experiments

We performed AFM experiments to examine the structure of the stamped lipid bilayers on the glass slides.[2] These experiments were carried out on a Nanoscope IIIA Multimode AFM (Veeco Metrology) in the tapping mode. All AFM experiments were performed in deionized water at room temperature using a commercially available fluid cell, sealed by an O-ring. The images were collected at 256 × 256 pixel resolution at a scan rate of 2.39 Hz using the Olympus Biolevers tips (Asylum Research). The spring constant of the cantilever was 0.027 N/m and the imaging frequency was 7 - 9 kHz.

Figure 2Sa shows an AFM image of a stamped bilayer composed of 99% (w/w) egg PC and 1% (w/w) rh-PE on a glass substrate. Figure 2Sb shows a height profile of the stamped bilayer using a defect site as a reference.[3]

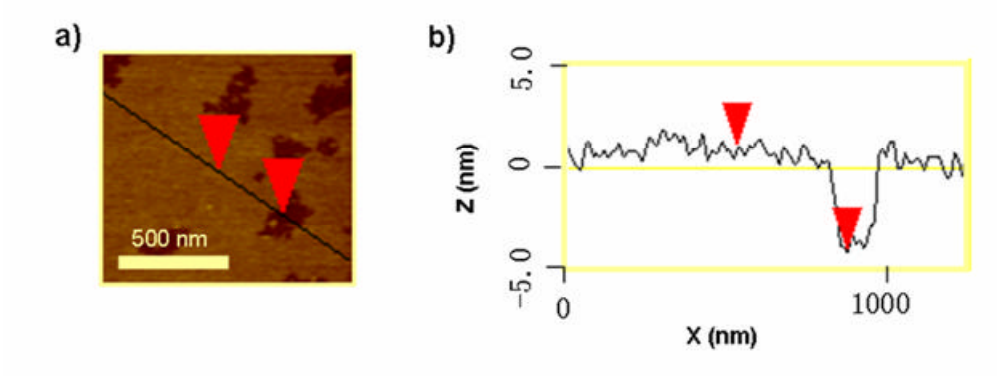

Figure S2. (a) AFM image of a potion of a stamped spot of bilayer composed of 99% (w/w) egg PC and 1% (w/w) rh-PE. The area of defects within this image is ~ 14%. (b) Height profile of the supported lipid bilayer along the black line in (a). The step height indicated by the markers is 4.5 nm.

Fluorescence Intensity after Multiple Stamping without Re-Inking

Using an agarose stamp which was inked once, we patterned 100 membrane arrays. The mean fluorescence intensity of the supported lipid bilayers was measured and Figure 1a shows the fluorescence intensity as a function of the number of stamping events. The error bars in Figure 1a, represent the standard deviation of the fluorescence intensity of all spots in each array and the dashed line represents the mean fluorescence intensity of the background. The standard deviation of the fluorescence intensity within any individual spot was less than 9.5% and from spot to spot in an array it was less than 9%.

Fluorescence Recovery after Photobleaching (FRAP) Experiments

FRAP experiments were performed on stamped supported lipid bilayers on glass slides (immersed in water or PBS) using a Nikon E600FN epifluorescence microscope equipped with an Evolution MP (Media Cybernetics, Canada) camera. The rhodamine label of lipids was excited using standard filter settings for rhodamine. Using a 40× water-immersion objective a spot with a diameter of ~ 30 μm was photobleached for 6-9 minutes with the aperture of the microscope closed as much as possible and all neutral density filters taken out of the light path. Fluorescence recovery of the bleached spot was then monitored with the same 40× objective. The recovery process was imaged with the aperture open and a ND4 neutral density filter in the light path to minimize further photobleaching. FRAP experiments were carried out in a dark room and the shutter for excitation was open only during image acquisition to minimize further photobleaching.

Images were analyzed by calculating the difference between the mean fluorescence intensity of the photobleached spot and a fluorescent spot in the same bilayer (a spot within the bilayer that had not been bleached) and then normalized to the maximum difference between these two intensities. Diffusion coefficients of stamped membranes were compared to the diffusion coefficient of a control bilayer prepared on the same glass slides by the established vesicle fusion method.[4] Diffusion coefficients were calculated by the equation, D ($cm^2\ s^{-1}$) = 0.224 w^2 $(cm)^2$ / $t_{1/2}$ (s), where w is the radius of the bleached spot and $t_{1/2}$ is the half time of the fluorescence recovery.[5,6] We obtained the value of $t_{1/2}$ from an exponential curve fit through the data.

Stamping Arrays of Bilayers with Different Lipid Compositions

We studied the correlation between the lipid composition of the liposomes in the inking solution and the composition of the resulting bilayers on the glass substrate. Liposomes composed of 99% (w/w) egg PC and 1% (w/w) rh-PE (population A) and liposomes composed of 50% (w/w) egg PC and 50% (w/w) DOPS (population B) were prepared separately and mixed in different ratios shortly before the inking procedure. Only liposomes of the population A were fluorescently labeled and therefore the fluorescence intensity of the resulting supported bilayers represented the percentage of the transferred liposomes from this population. Five different mixtures of the two aforementioned liposome populations were used to ink the posts on a stamp, the ratios were: 100% A, 0% B; 80% A, 20% B; 50% A, 50% B; 30% A, 70% B; 0% A, 100% B. Figure S3 shows fluorescent micrographs of the resulting array and the graph represents the mean fluorescence intensity in the bilayer spots which resulted from inking solutions with varying ratios of fluorescently-labeled liposomes.

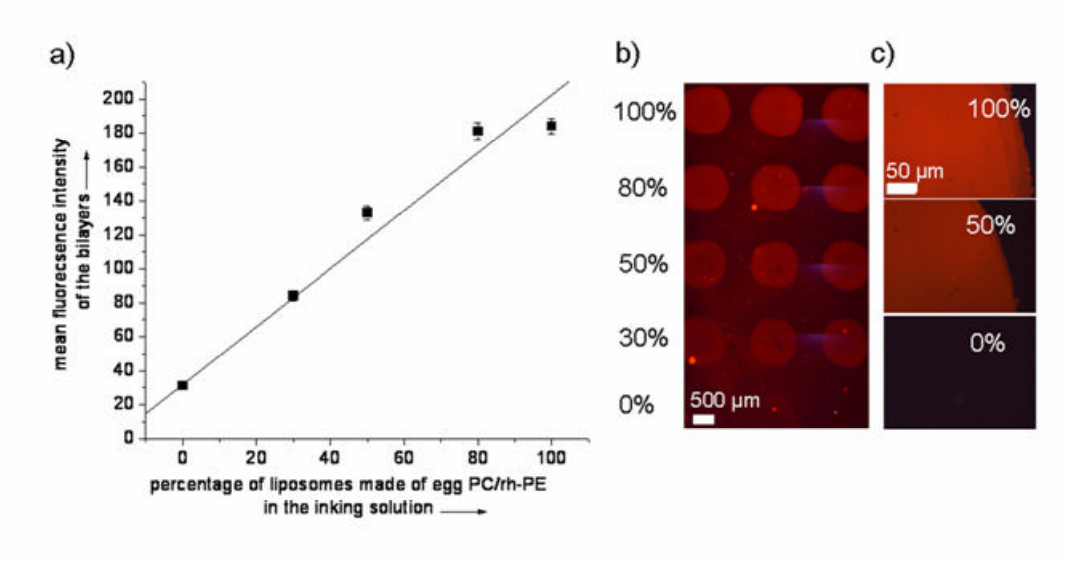

Figure S3. Transfer of liposomes from a mixture of liposome preparations which were used for inking of an agarose stamp. (a) Graph showing the mean fluorescence intensity of bilayers transferred from the posts which were inked with mixtures containing different ratios of fluorescent liposomes of population A and non-fluorescent liposomes of population B. Error bars represent standard deviations of fluorescence intensity. (b) Fluorescent micrograph of the array of bilayers with various compositions. Spots of each row have the same composition. The corresponding posts on the stamp were inked with (from top) first row: 100% population A, second row: 80% population A and 20% population B, third row: 50% population A and 50% population B, forth row: 30% population A and 70% population B, and fifth row: 100% population B. (c) Fluorescent images of a portion of bilayer spots of the array shown in (b) at higher (40×) magnification.

References:

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