Supporting Information

LIPID AND PROTEIN TRANSFER BETWEEN NANOLIPOPROTEIN PARTICLES AND SUPPORTED LIPID BILAYERS

Amanda T. Dang[1], Wei He[2], Daniela B. Ivey[3], Matthew A. Coleman[2], and Tonya L. Kuhl[3]

[1]Department of Materials Science and Engineering, University of California, Davis CA 95616
[2]Lawrence Livermore National Laboratory, Livermore, CA 94550
[3]Department of Chemical Engineering, University of California, Davis CA 95616

PAA Cushion Preparation (Detailed Protocol)

PAA cushions were prepared using spin-coating methods described by El-Khouri et al. [1]. PAA (450k MW, 0.1% cross-linked, Sigma-Aldrich, Inc.) was dissolved in methanol (≥ 99% purity, Sigma-Aldrich**)** at a concentration of 1 mg/mL, stirred overnight (<16 hr), and subsequently passed through a 0.2 µM Whatman PFTE filter. Next, coverslips were functionalized with aminopropyltriethoxysilane (APTES) by immersion in a gently stirred solution of ~1 mM APTES (Gelest Inc.) in toluene (≥ 99% purity, Sigma-Aldrich) for one 1 hr. Silanized samples were then rinsed with toluene, dried under a light stream of nitrogen (specialty grade, 99.998% pure), and cured for 2 hr at 100 °C.

Once samples had cooled to room temperature, PAA was deposited by gently injecting dissolved PAA on the silanized surface and spin-coating for 2 min at 2000 RPM. Covalent linkage between the PAA and APTES was promoted through subsequent curing for 2 hr at 200 °C. Finally, PAA substrates were immersed in Tris buffer (pH 9) to convert anhydrides to carboxylates and relieve mechanical stresses in the polymer layer. Areas exposed during photolithography were subsequently treated with AquaSil siliconizing fluid (Thermo Fisher Scientific) per manufacturer instructions to render them resistant to nonspecific protein adsorption.

MSP1D1-NLP Assembly (Detailed Protocol)

NLPs stabilized with membrane scaffold protein MSP1D1 (MSP1D1-NLPs) were assembled using methods adopted from protocols described by Zeno et al. [2]. A stoichiometric excess (4.3 mg) of DMPC containing 2 mol% Rhodamine-DHPE was dried in a glass vial with nitrogen (specialty grade, 99.998% pure) and placed under mild vacuum for at least 6 hr. The lipid mixture was then rehydrated in reconstitution buffer consisting of 17 mg of sodium cholate hydrate added to 1 mL of Tris buffer (pH 7.4), transferred to a plastic centrifuge tube, mixed using a vortex shaker at room temperature for 30 min, and then sonicated for 10 min.

2 mg of lyophilized apolipoprotein MSP1D1 (Cube Biotech, Inc.), resuspended in 0.5 mL of MilliQ deionized water, was then added to the lipid mixture such that the molar ratio of lipid-protein

was 80:1. The lipid-protein mixture was subsequently incubated for 2 hr, alternating between light shaking at 4 °C and 37 °C every 20 min. After incubation, the mixture was transferred to a 10 kDa MWCO Slide-A-Lyzer dialysis cassette (Thermo Fisher Scientific) and dialyzed at 4 °C, in Tris buffer (pH 7.4) at over 300 times the sample volume (4 buffer exchanges over 48 hr) to promote cholate removal and NLP assembly.

Table S1: SLB Details

Imaging Method	Outer Leaflet	Inner Leaflet	Support	Preparation Method
FM	DOPC+ 5 mol% DOGS-NTA	DOPC+ 5 mol% DOGS-NTA	Glass Coverslip	Vesicle Fusion
FM	DPPE:DOPC (3:7)	DPPC	Glass Coverslip	LB-LB
FM	DPPE:DOPC + 5 mol% DOGS-NTA (3:7)	DPPC	Glass Coverslip	LB-LB
FM	DMPC + 5 mol% DOGS-NTA	DPPE	Glass Coverslip	LB-LB
FM	DMPC	DMPC	Glass Coverslip	LB-LB
FM	DMPC + 5 mol% DOGS-NTA	DMPC	Glass Coverslip	LB-LB
AFM	DMPC + 5 mol% DOGS-NTA	DPPE	Mica	LB-LB
AFM	DPPC:DOPC + 5 mol% DOGS-NTA:Chol (9:9:2)	DPPE	Mica	LB-LB
FM	DMPC + 5 mol% DOGS-NTA	DMPC + 5 mol% DOGS-NTA	PAA-Cushion	LB-LS

SDS-PAGE

SDS-PAGE on Δ49ApoA1-NLPs (Figure S1A) and CLIP-ErbB2-NLPs (Figure S1B) was performed using NuPAGE Novex™ 4-12% Bis-Tris protein gels (Thermo Fisher Scientific). Samples were heated to 98 °C for 5 min with NuPAGE LDS Sample Buffer and NuPAGE reducing Agent, mixed per manufacturer specifications. Gels were run at 200V for approximately 30 min. Bands were compared against NuPAGE Novex™ pre-stained standard to determine molecular weight.

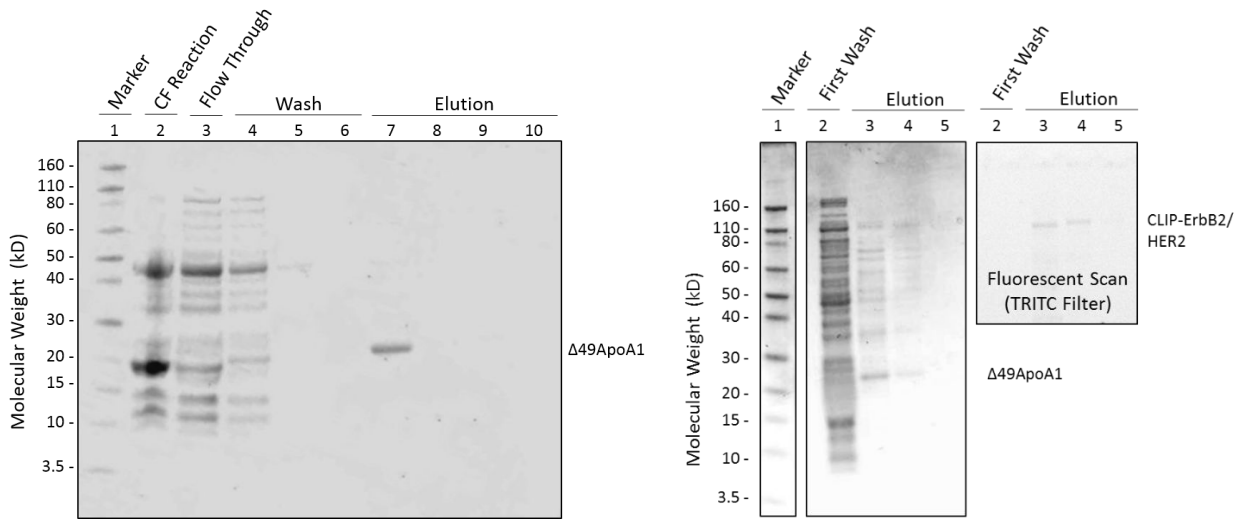

Figure S1: SDS-PAGE gels of (A) Δ49ApoA1-NLPs and (B) CLIP-ErbB2-NLPs. The sizes of CLIP-ErbB2/HER2 and Δ49ApoA1 (lacking post-translational modification) are ~120 kDa and ~26 kDa, respectively.

SDS-PAGE on MSP1D1-NLPs (Figure S2) was performed using Mini-PROTEAN® TGX Precast Gels (Bio-Rad Laboratories, Inc.). Samples were heated to 80 °C for 10 min with Laemmli sample buffer and 1-mercaptoethanol, mixed per manufacturer specifications. Gels were run at 200V for approximately 30 min. Bands were compared against Precision Plus Protein™ pre-stained standard to determine molecular weight.

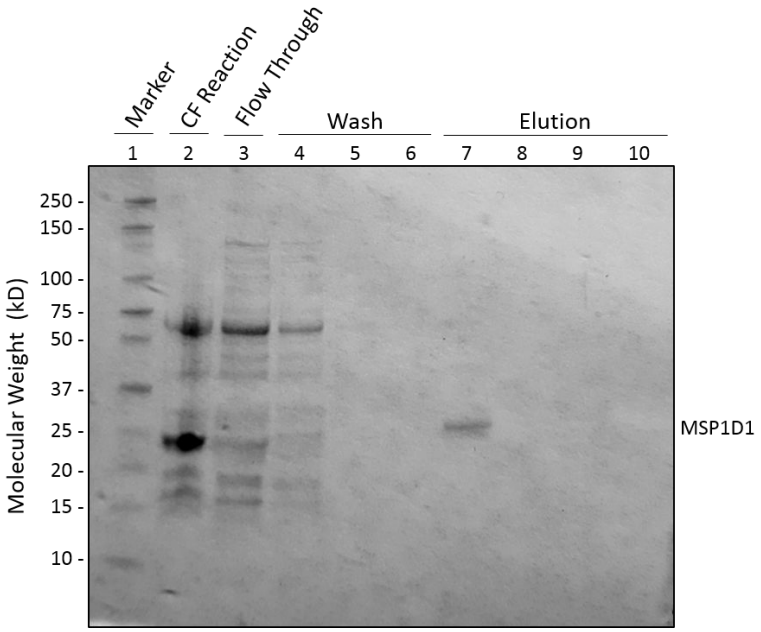

Figure S2: SDS-PAGE gel of MSP1D1-NLPs. The size of MSP1D1 is ~25 kDa.

UV-Vis Spectrophotometry

Protein concentration was quantified by measuring peak absorbance at 280 nm and applying the Beer-Lambert Law (Eqn. S1) where *A* is the measured absorbance, ε is the protein's intrinsic extinction coefficient, *b* is the path length (1 cm in this case), and *c* is the concentration.

$$A = \varepsilon * b * c \qquad \textbf{Eqn. S1}$$

The absorbance spectrum of a control solution with a known concentration of vesicles composed of DMPC containing 2 mol% Rhodamine-DHPE was recorded and used to calibrate for vesicle contributions to the spectra obtained for NLPs.

Particle Size Analysis of NLPs

The Stokes diameter, d_s reported using dynamic light scattering assumes a spherical particle. This value can be used to derive a discoidal diameter, d_D using the following equation Eqn. S2, which equates the area of a sphere to that of a cylinder [3]. The height of the disc, *h* is assumed to be 5 nm, the height of a typical bilayer in an NLP.

$$d_D = \left(\frac{2d_s^3}{3h}\right)^{1/2} \qquad \textbf{Eqn. S2}$$

Excluding large contaminants (> 1000 nm diameter), the size distribution measurements of MSP1D1-NLP mixtures, measured in terms of the Stokes diameter were bimodal. One peak, centered at 203.1 ± 57.0 nm, was attributed to residual vesicles in solution. The other, centered at 14.8 ± 2.7 nm, was ascribed to NLPs and corresponded to a discoidal diameter of 20.7 ± 1.6 nm.

Negative Control – CLIP-Cell TMR-Star

A negative control experiment was conducted to determine whether fluorescent substrate CLIP-Cell TMR-Star inserted into the hydrophobic domain of the NLP bilayer. Negative control NLPs were expressed using cell-free expression and subjected to the same CLIP-Cell TMR-Star conjugation protocol that was used to label CLIP-ErbB2-NLPs before purification. When these NLPs were incubated with unlabeled SLBs, no detectable fluorescence was imparting into the sample. This indicated CLIP-Cell TMR-Star did not insert into NLPs in the absence of a CLIP-tagged membrane protein and that the purification scheme was sufficient for removing unconjugated substrate from the final product.

Fluorescence Intensity Analysis

Mean fluorescence intensity was measured as the grayscale intensity (arbitrary unit) averaged across 10+ raw images recorded from across the surface of each sample. Illumination conditions and temperature (T = 25 ˚C) were kept consistent across all samples. Outliers, defined as images with intensity values that were more than three scaled median absolute deviations away from the median, were removed. Images from 3-5 samples, collected over at least 3 independent experiments were analyzed for each composition.

Normalized fluorescence intensity was calculated by dividing the mean fluorescence intensity by that of unlabeled SLBs.

Fluorescence Recovery after Photobleaching (FRAP)

FRAP was performed by closing the aperture to expose a small section of the field of view for 5 min, and then recording an image time-lapse of the entire area over 20-30 min. To minimize photobleaching throughout the session, the shutter was closed between image captures. Lateral mobility of fluorescent molecules in the SLB was confirmed by observation of recovery (brightening) of the photobleached area over time (Figure S3).

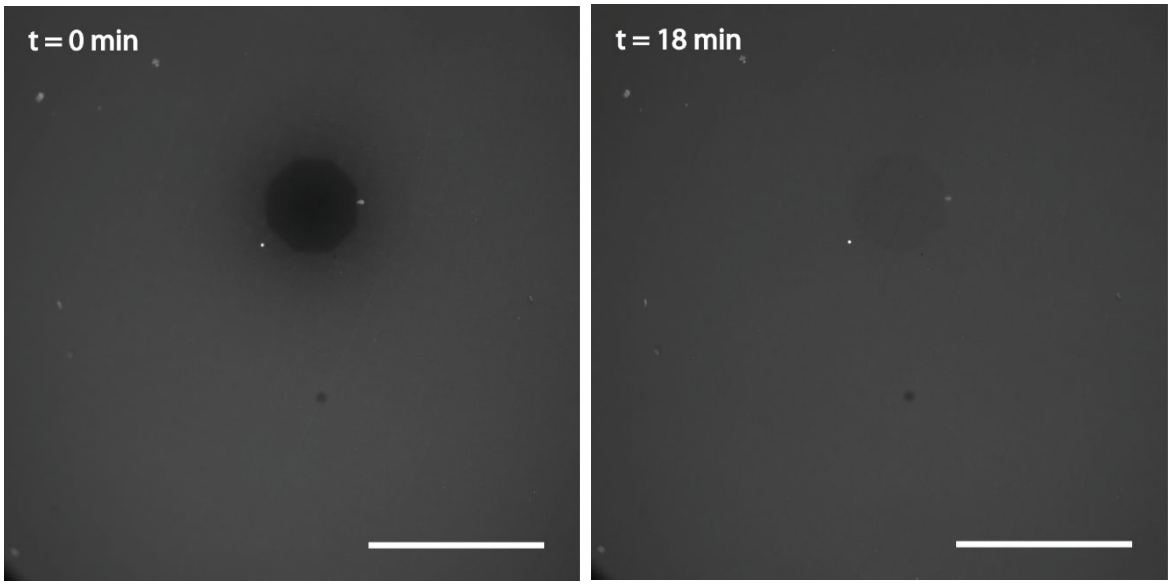

Figure S3: FRAP of a DMPC + 5 mol% DOGS-NTA (inner leaflet DMPC) SLB after incubation with MSP1D1-NLPs containing fluorescent lipid Rhodamine-B DHPE at T = 25 ˚C. More rapid recovery was observed when the temperature was raised well above the transition temperature of DMPC (T_m = 24 ˚C), to T = 33 ˚C. (scale bar = 100 µm)

Fluorescence Analysis of Vesicle Control Samples

Control SLB samples incubated with vesicles exhibited noticeable variability in the measured fluorescence intensity across the surface area of individual samples. Qualitative analysis of FM images revealed that this was due to vesicles adsorbed unevenly across the surface (Figure S4). No recovery was observed during FRAP, but it is possible that recovery was not detected due to the low signal to noise ratio for these samples. Nonetheless, incubation with vesicles, unlike NLPs, did not result in even incorporation of fluorescence across the SLB. Given this crucial distinction in the interaction mechanism, it was not appropriate to quantify the degree of transfer for each experimental condition as a ratio of the average fluorescent signal of SLBs incubated with NLPs compared to vesicle controls.

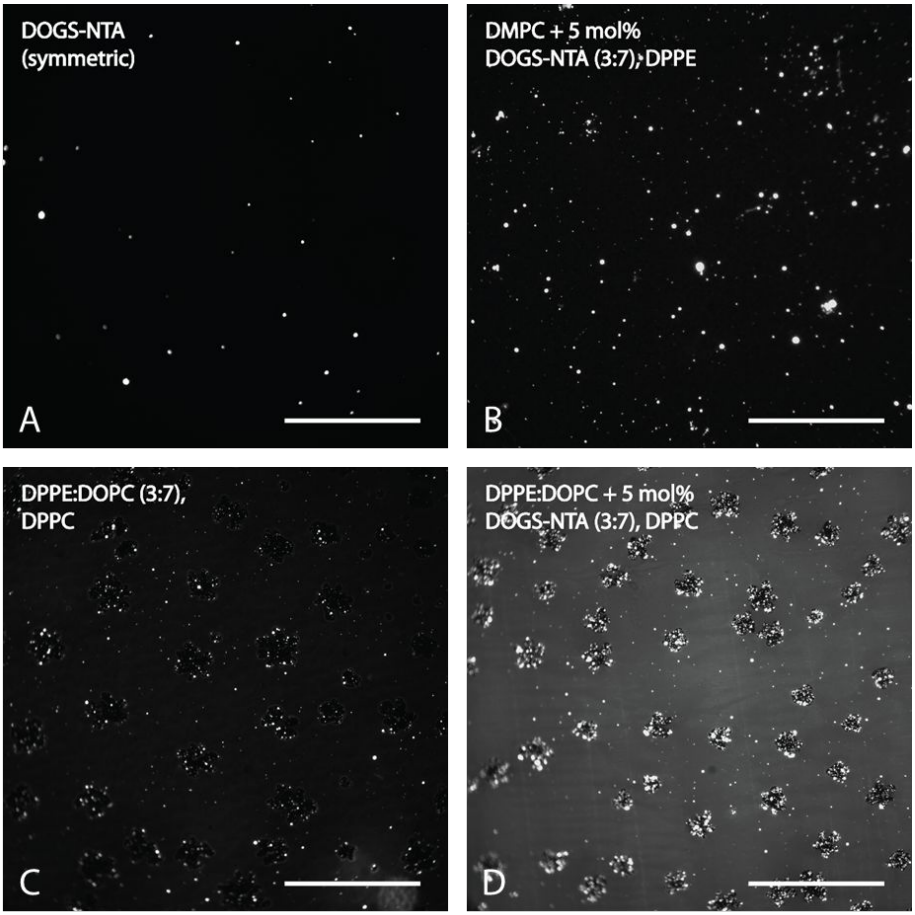

Figure S4: (A, B, C) Most FM images of SLBs after incubation with vesicles (composition: DMPC + 2 mol% Rhodamine B-DHPE) exhibited minimal fluorescence emission. (D) Rare occurrences of high fluorescence intensity in limited regions, usually in close proximity to where the vesicle solution was injected, were artificially enhanced by a large number of adsorbed vesicles on the surface. (scale bar = 100 μm)

References

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