

Supporting Information for

Lipid Bilayer Disruption Induced by Amphiphilic Janus Nanoparticles: The Non-Monotonic Effect of Charged Lipids

Kwahun Lee and Yan Yu*

Department of Chemistry, Indiana University, Bloomington, IN 47405, USA

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EXPERIMENTAL METHODS

1. Materials

Amine-functionalized silica particles (100 nm) were purchased from Nanocomposix (San Diego, CA). Octadecanethiol (ODT) were obtained from Sigma Aldrich (St Louis, MO). Chromium (99.99% purity) and gold (99.99% purity) pellets were purchased from Kurt J. Lesker (Jefferson Hills, PA). Cyanine5 N-hydroxysuccinimide ester (Cy5 NHS ester) was obtained from Lumiprobe Corporation (Hunt Valley, MD). Succinic anhydride (99% GC) was purchased from AK Scientific, Inc. (Union City, CA). Phospholipids, 1,2- dioleoyl - sn -glycero -3-phosphocholine (DOPC), 1,2- dipalmitoyl- sn -glycero -3-phosphoethanolamine -N-lissamine rhodamine B sulfonyl (RhB-PE), 18:1 PA, 1,2-dioleoyl-sn-glycero-3-phosphate (sodium salt) (DOPA) and 18:1 TAP, 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP) were purchased from Avanti Polar Lipid (Alabaster, AL). N-(4, 4-difluoro-5, 7-dimethyl-4-bora-3a, 4a-diaza-s-indacene-3-propionyl)-1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine, triethylammonium salt (BODIPY-DHPE) was purchased from Thermo Scientific (Waltham, MA). Ultrapure water (resistivity of 18.2 M Ω ·cm) was used for all experiments.

2. Methods

Fabrication of Janus particles. Glass microscope slides were cleaned in piranha solution (H₂SO₄: H₂O₂ = 3:1 v: v) for 15 minutes. Sub-monolayers of amine-modified silica nanoparticles were made on glass microscope slides by using solvent evaporation method with slight modification.¹ Briefly, 50 μ L of 0.1 wt% silica particles in ethanol was slowly cast on a glass substrate while ethanol evaporated, to reduce the coffee-ring effect. Thin films of chromium (5 nm) and gold (25 nm) were deposited sequentially onto the particle sub-monolayer using an Edward Thermal Evaporator (Nanoscale Characterization Facility at Indiana University). After coating, the glass slides were immediately immersed in ethanol containing 2 mM octadecanethiol (ODT) until further usage. Prior to bilayer experiments, particles were sonicated off glass slides and cleaned in ethanol and then DI water prior to experiments. Several rounds of centrifugation (100 rcf for 30sec three times, and then 500 rcf for 30 sec three times) was used further remove particle aggregates. To fluorescently label Janus nanoparticles, ticles, Cy5 (0.5 mg/mL in DMSO) was mixed with amine-functionalized particles in 1 \times PBS (pH 7.4). The final concentration was 80 pM for particles and 0.8 μ M for Cy5. The reaction proceeded overnight at room temperature. Particles were washed with DI water after the reaction and stored in ethanol.

Characterization of particles. Hydrodynamic size and zeta potential of particles were measured in DI water using Zetasizer. Morphology of Janus particles was characterized using scanning electron microscopy (SEM). Concentration of particles was measured using ZetaView Nanoparticle Tracking Analyzer.

Preparation of supported lipid bilayers. 100 nm unilamellar vesicles were prepared by extrusion method. Briefly, DOPC, Rhd-DHPE (0.2 mol%) and DOPA or DOTAP were mixed at various ratios in chloroform in a round bottom flask. The lipids were dried under nitrogen flow. . Dried lipid films were hydrated in 1 \times PBS (lipid final concentration: 1mg/mL), underwent five repeated freeze-and-thaw cycles, and then passed through 100 nm filter membranes using a mini-extruder (Avanti Polar Lipids, Inc.). To prepare supported lipid bilayer, lipid vesicles were diluted with 1 \times PBS to a final concentration of 0.2 mg/mL and added to a pre-cleaned glass-bottom imaging chamber. After incubation for 30 minutes, the formation of lipid bilayer was complete and excess lipid vesicles were gently rinsed away with DI water thoroughly.

Fluorescence imaging and fluorescence recovery after photobleaching (FRAP). Wide-field epifluorescence images were acquired on a Nikon Eclipse Ti microscope equipped with an Andor iXon3 EMCCD Camera and a Nikon Plan Apo 100×/1.49 N.A. objective was utilized in wide-field epifluorescence imaging mode. All imaging experiments were done at room temperature. Each sample was repeated at least twice on different days to ensure reproducibility. In FRAP measurements, a circular region of the bilayer in the field of view was photobleached using a 562 nm laser and the recovery of fluorescence was recorded every 2 seconds for a total of 240 seconds after photobleaching. For each particle interaction experiment, FRAP was measured before and 70 minutes after the addition of particles. "simFRAP", a built-in plugin of ImageJ, was used to process the data.² Janus particle orientation and particle-bilayer association rate constant were quantified as described previously.³

SI Figures

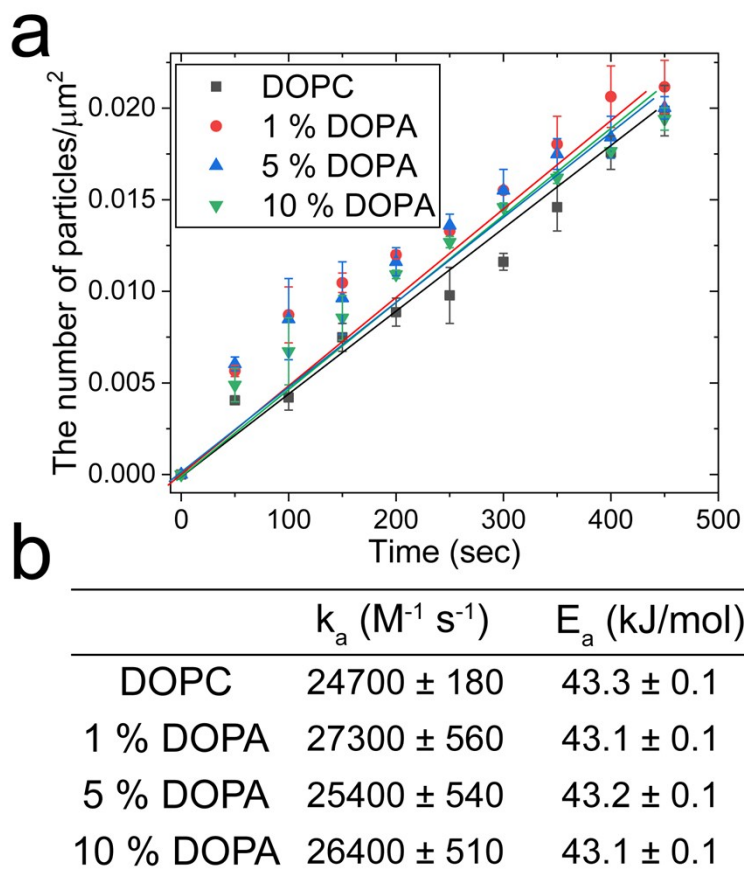


Figure S1. (a) Plots showing the number of particles adsorbed on bilayers as a function of time. Solid lines are linear fit to individual data set shown in the same color. (b) Association rate constant (k_a) and activation energy (E_a) of Janus particle adsorption on DOPA and pure DOPC lipid bilayers.

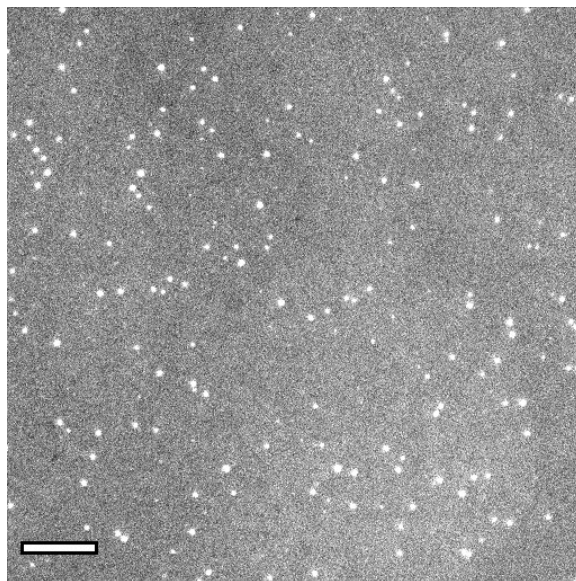
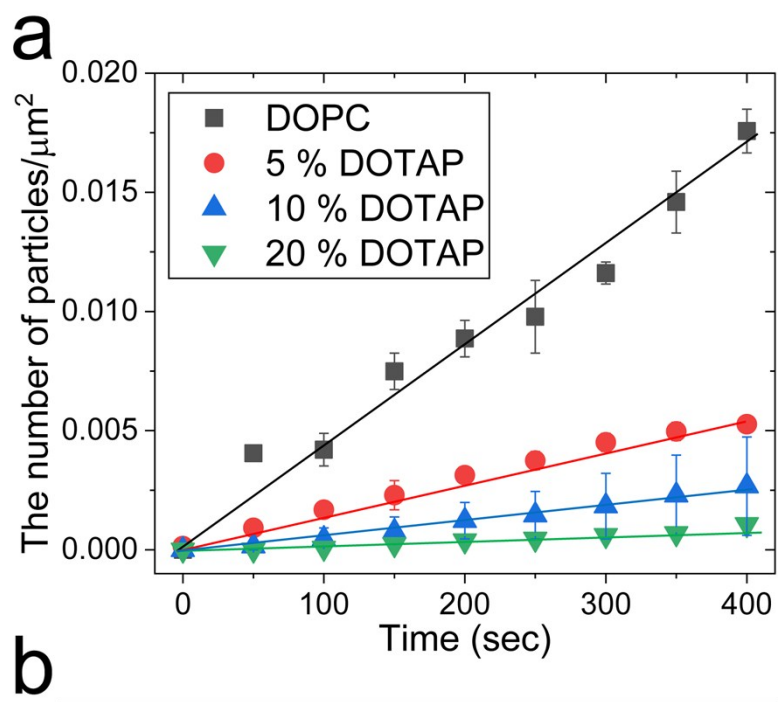


Figure S2. Fluorescence images showing the morphology of 5% DOPA bilayers 70 minutes after the addition of 20 pM cationic uniform nanoparticles. Scale bar: 10 μm



b

	k_a ($\text{M}^{-1} \text{s}^{-1}$)	E_a (kJ/mol)
DOPC	24700 ± 180	43.3 ± 0.1
5 % DOTAP	5770 ± 30	46.9 ± 0.1
10 % DOTAP	2970 ± 220	49.5 ± 0.1
20 % DOTAP	1170 ± 90	50.8 ± 0.2

Figure S3. (a) Plots showing the number of particles adsorbed on bilayers as a function of time. Solid lines are linear fit to individual data set shown in the same color. (b) Association rate constant (k_a) and activation energy (E_a) of Janus particle adsorption on DOTAP and pure DOPC lipid bilayers.

References

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3. K. Lee, L. Y. Zhang, Y. Yi, X. Q. Wang and Y. Yu, *ACS Nano*, 2018, **12**, 3646-3657.