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[Electronic supplementary information /8 Pages]

Dipolar Janus Liposomes: Formation, Electrokinetic Motion and Self-Assembly†

Zening Liu, Jinyan Cui and Wei Zhan*

Department of Chemistry and Biochemistry, Auburn University, Auburn, AL 36849

*Corresponding author:

E-mail: wzz0001@auburn.edu

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Experimental details

Reagents. All lipids, including 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol) (sodium salt) (DPPG), 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP), 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-*N*-(lissamine rhodamine B sulfonyl) (ammonium salt) (Rho-DOPE), and 23-(dipyrrometheneboron difluoride)-24-norcholesterol (Bodipy-chol), were obtained from Avanti Polar Lipids (Alabaster, AL) and used as received. Aqueous suspensions of green fluorescent polystyrene nanospheres were obtained from Bangs Laboratories, Inc. (carboxylic acid terminated, mean diameter: 0.196 μm; excitation/emission maximum: 480/520 nm; charge density: ~0.5 milliequivalents/g) and Thermo Fisher Scientific (amine-terminated, mean diameter: 0.19 μm; excitation/emission maximum: 505/515 nm; charge density: 0.1-2.0 milliequivalents/g). Other chemicals, including poly(vinyl alcohol) (PVA, MW: 145,000), cholesterol, chloroform, methanol were obtained from Sigma-Aldrich. Deionized water of 18.2 MΩ·cm (Millipore) was used throughout this work.

Liposome Preparation. Giant liposomes of various compositions and configurations were prepared via gel-assisted hydration[1] as previously detailed[2] with minor modifications. To start, lipids were first combined in chloroform to form stock solutions of desired compositions; the total lipid concentration in the final solutions was 1 mM. Separately, poly(vinyl alcohol) (PVA) films were prepared by spreading a 5 wt% PVA aqueous solution on precleaned glass slides, followed by drying at 50 °C for 0.5 h. To form a lipid precursor film, a 5-μL of the above stock solutions was then cast on the pre-dried PVA gel film using a microsyringe, which is further dried under vacuum for overnight in the dark at room temperature. The final hydration step was carried out by incubating these dried lipid films in 1 mL DI water at 45 °C for 2.5 h. Thus-produced liposomes were harvested with a pipette and stored at room temperature. Incubation at higher temperatures

(50 or 60 °C) was found to yield liposome products of similar yields and quality, and thus was not pursued further.

Confocal Fluorescence Microscopy. Fluorescence images and videos of giant liposomes were acquired on a Nikon A1+/MP confocal scanning laser microscope (Nikon Instruments, Inc. Melville, NY) using 10x objective and excitation laser lines at 488 and 561 nm. The corresponding green and red emission signals were filtered at 525 $\pm$ 25 and 595 $\pm$ 25 nm, respectively, before being detected by photomultiplier tubes. The confocal pinhole was typically set at 50 μm. As imaging cells, home-prepared poly(dimethylsiloxane) (PDMS, Sylgard 184, Dow Corning) microwells (diameter: 3 mm; depth: 2 mm) fixed on glass slides were employed. All liposome samples were given 0.5 h to settle in the PDMS cells under ~100% humidity before being imaged.

Zeta Potential Measurement. Zeta potential values of liposomes and polystyrene nanospheres suspended in DI water were obtained from a Malvern Zetasizer (Nano-ZS90, Malvern Instruments, Worcestershire, UK) using capillary cells (DTS1060/DTS1061) operated under a 150-V bias. For liposomes, the measurements were made on diluted samples of freshly prepared liposomes with a total lipid concentration of ~1 μM. The final concentrations of nanospheres were controlled to be ~1×10^9 beads per mL. In both cases, typically five parallel readings were taken for each sample.

General Characterization of Giant Liposomes Produced by Gel-Assisted Hydration. The liposome size distribution reported in Figure 1 was based on size analysis of 500 Janus liposomes arbitrarily sampled from multiple liposome batches. In order to reliably call their domain configuration, only liposomes with diameter >2-μm were included in this analysis. Similarly, the percent area occupied by the liquid-ordered lipid domain (%l_o) in these liposomes was determined by analyzing 100 such particles.

Characterization of Charge Distribution in DJLs. Placement of DOTAP and DPPG in DJLs was characterized by following their electrostatic binding with carboxyl- or amine-terminated, fluorescent polystyrene nanospheres (diameter: ~0.2 μm) with confocal fluorescence microscopy. To afford such binding, small quantities of these nanospheres were added into DJL solutions, briefly vortexed and subsequently incubated at room temperature in dark for 1-3 days. Typically, the total lipid and nanosphere concentrations in the final mixtures were controlled at 0.5 μM and 1 x 10^9 beads per mL, respectively.

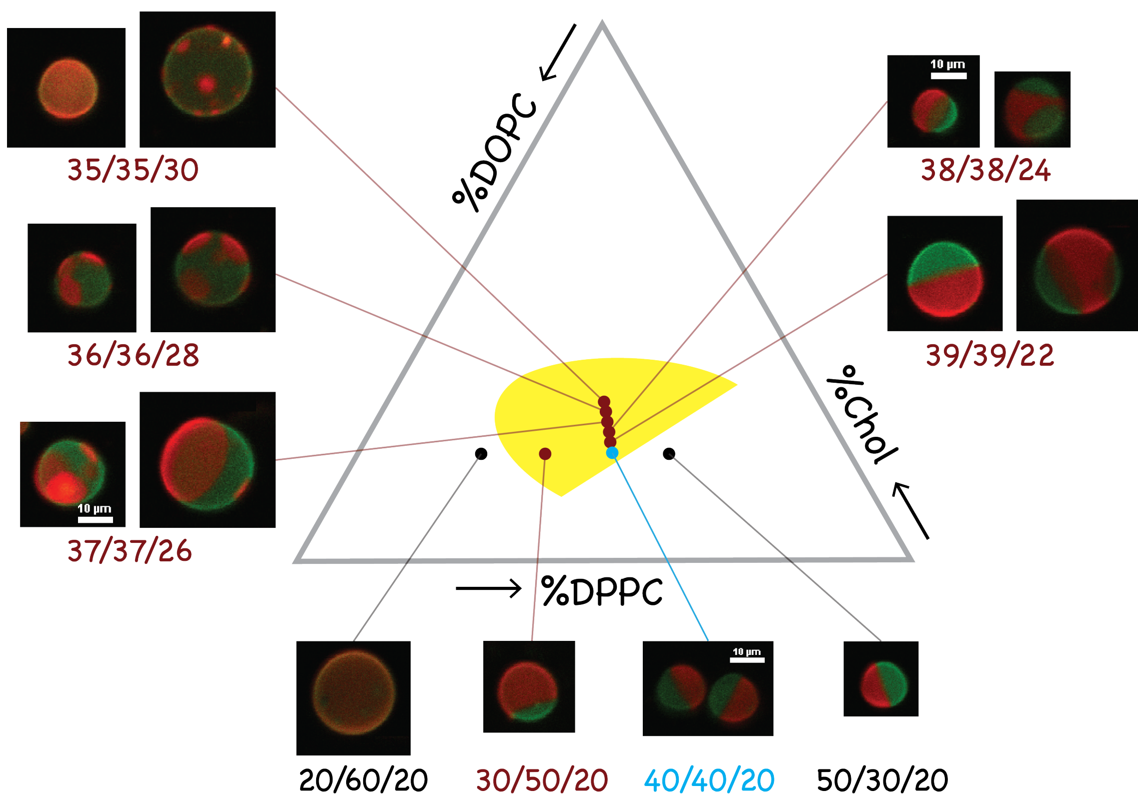
Self-Assembly of DJLs. Electrostatic self-assembly events of DJLs in DI water were followed in liposome-density and time-dependent manner using confocal fluorescence microscopy. Of the former condition, liposome interactions were allowed to proceed either at the as-prepared density, i.e., with a total lipid concentration of ~5 μM in the final liposome products, or at a 4-time dilution of the original. Before imaging, these samples were incubated at room temperature in dark for various time periods specified in the Results section.

Electrokinetic Motion of Janus Liposomes. Electrokinetic motion of various Janus liposomes under *DC* electric field was conducted in cyclic olefin copolymer microfluidic channels (thinXXS 100182 microfluidic linear channel slide; dimensions: 200 μm x 200 μm x 18 mm; Cole-Parmer, Vernon Hills, IL) and recorded by the same confocal fluorescence microscope. Before each measurement, the microchannel was first cleaned by sonication in methanol and then DI water, 30 min each, rinsed with methanol and DI water, and blow-dried with a nitrogen stream. Following sample injection, two platinum wires (diameter: 0.5 mm) were inserted into the inlets of the microchannel and subsequently fixed in position with parafilm, which in turn seals the liposome solution inside the channel. The magnitude and polarity of applied potential were controlled by a *DC* power supply (BK Precision 9122A) connected directly to the platinum wires.

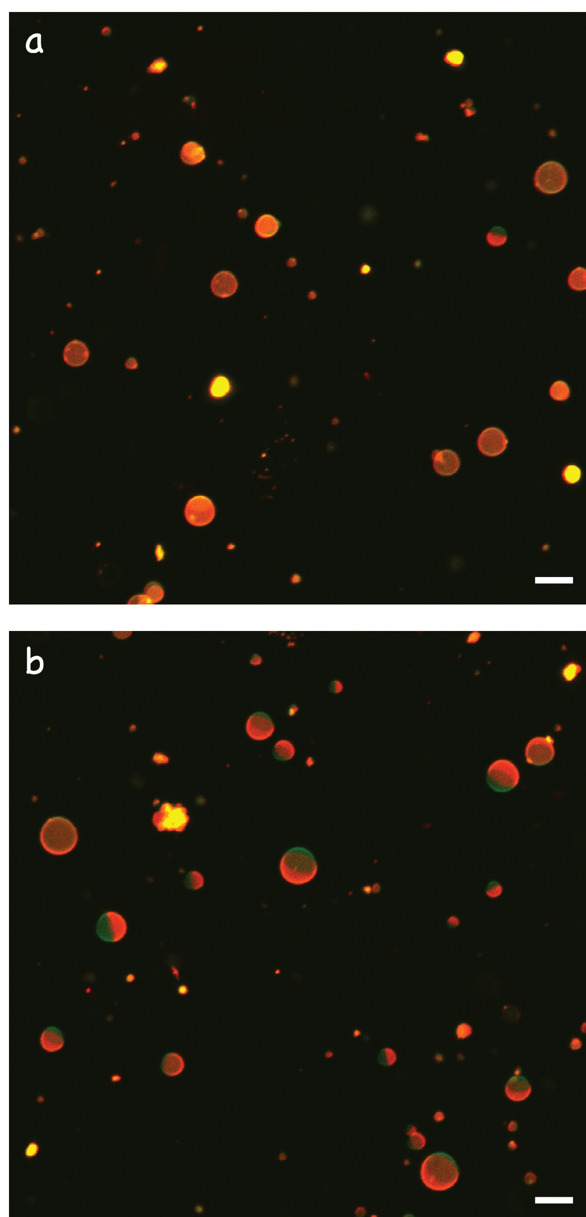
1. Weinberger, A.; Tsai, F.-C.; Koenderink, G. H.; Schmidt, T. F.; Itri, R.; Meier, W.; Schmatko, T.; Schoder, A.; Marques, C. Gel-Assisted Formation of Giant Unilamellar Vesicles. *Biophys. J.* **2013,** *105,* 154-164.

2. Wang, M.; Liu, Z.; Zhan, W. Janus Liposomes: Gel-Assisted Formation and Bioaffinity-Directed Clustering. *Langmuir* **2018,** *34,* 7509-7518.

ESI Figure 1. Optimization of lipid composition for production of Janus liposomes containing 2 mol% DPPG and DOTAP. (Center) Generic triangle phase diagram of DPPC/DOPC/cholesterol system at room temperature; for clarity, only the liquid/liquid coexistence region is marked out (in yellow). Images surrounding the phase diagram are confocal fluorescence micrographs of liposomes produced at different lipid compositions, which are specified below images in ratios of total saturated lipids vs. total unsaturated lipids vs. cholesterol. All samples in addition include 0.2 mol% Bodipy-chol and Rho-DOPE; the scale bars included in the images are 10 μm and apply to all samples.



ESI Figure 2. Effect of the charge loading level on Janus liposome production. a) Fluorescence micrograph of liposomes prepared from a lipid precursor of DPPC/DOPC/Chol/DPPG/DOTAP/Bodipy-chol/Rho-DOPE mixed at 30/30/20/10/10/0.2/0.2 mole ratio. b) Fluorescence micrograph of liposomes prepared from a lipid precursor of DPPC/DOPC/Chol/DPPG/DOTAP/Bodipy-chol/Rho-DOPE mixed at 35/35/20/5/5/0.2/0.2 mole ratio. In both cases, the total lipid concentration in the final liposome product is ~5 μM. Scale bar: 20 μm.



Estimation of surface charge density (C/m^2) due to 2% DPPG located in the l_o domain of a DJL. This exclusive arrangement in effect doubles DPPG's concentration; fractions of all other components are taken from ref. 38.

	DPPC	DPPG	DOPC	chol
Lipid composition in lo domain (mol%)	55	4	15	31
Area occupied per lipid (nm^2, same unit below)	0.64	0.48	0.7	0.37
Area occupied by 105 lipids according to above composition	35.2	1.92	10.5	11.47
Total area occupied by 105 lipids	59.09			
Elemental charge	1.60E-19			
Area unit conversion (nm^2 to m^2)	1.00E-18			
Lo domain charge density due to DPPG (C/m^2)	5.20E-03			