Electronic Supplementary Material (ESI) for Chemical Communications.

Supporting Information

Highly Sensitive Turn-on Biosensors by Regulating Fluorescent Dye Assembly on Liposome Surfaces

Sungbaek Seo‡, Min Sang Kwon‡, Andrew W. Phillips‡, Deokwon Seo, and Jinsang Kim*

Materials preparation. 10,12-pentacosadiynoic acid (PCDA) was obtained from GFS Chemicals. Rhodamine 6G (R6G) was purchased from Acros Organics. 1,2-dimyristoyl-*sn*-glycero-3-phosphate (DMPA) and phosphatidylinositol 4,5-bisphosphate (PIP_2) were obtained from Avanti Polar Lipids. Hexadecyltrimethylammonium bromide (CTAB), sodium dodecyl sulfate (SDS), polyoxyethylene (20) sorbitan monolaurate (Tween 20), potassium phosphate monobasic (KH_2PO_4), neomycin, gentamycin, tobramycin, streptomycin, oxytetracycline were purchased from Sigma–Aldrich. All chemicals were used without further purification.

Spectroscopic Characterization. UV-visible absorption spectra were measured on a Varian Cary50 UV/Vis spectrophotometer. Photoluminescence (PL) were obtained using PTI Quanta Master™ spectrofluorometers equipped with an integrating sphere and an laser excitation system.

Preparation of the turn-on type sensing platform for neomycin detection. PIP_2 (70 μg) and PCDA (20 μg) were dissolved in 0.2 ml of chloroform. The solution was placed in the bottom of a 50 ml Erlenmeyer flask and was dried by blowing with N_2. Next, 20 ml of deionized water were added and the resulting solution was placed in an 80 ^oC water bath and was dispersed well by shaking for 10 min to produce the final concentration of the liposomes (5×10^{-5} M). After filtration through a 0.8 μm cellulose acetate syringe filter, the resulting liposome solution was cooled at 5 ^oC for at least 2 hours. The liposomes were then

photopolymerized using a 254 nm UV lamp for 30 seconds. Lastly, we added 2.2 molar equivalents of R6G solution (in DI water) to the PCDA:PIP_2 (1:1) co-assembled liposome solution to make the turn-on type sensing platform.

Dialysis of PBS Buffer and Resulting "Turn-On" Sensor Signaling as Compared to Undialyzed PBS Buffer and Deionized Water. Approximately 3 mL of 1X PBS were dialyzed against approximately 1000 mL of deionized water, with stirring, using a regenerated cellulose membrane (Spectrum Labs) with a molecular weight cutoff of 100-500 Da. Aliquots of 400 uL were taken from the dialysis membrane at times 1 h (day 1), 2 h (day 1), overnight (day 2), overnight (day 3), overnight (day 4). Dialysis water was exchanged with new deionized water each time an aliquot was taken. Liposomes co-assembled of 75 percent PCDA and 25 percent DMPA were prepared. Sensors were prepared by the combination ofthese liposomes (800 uL, 0.05 mM) andrhodamine 6G (40 uL, 1E-4 M). Amount of R6G added to make the previously determined maximum quenching ratio of 0.4 equivalents of R6G to phospholipid. To the prepared sensor, 20 uL aliquots of samples were added. Seven samples were used: 1X PBS without dialysis (positive control), deionized water (negative control), 1X PBS with dialysis at the following times: 1 h (day 1), 2 h (day 1), overnight (day 2), overnight (day 3), overnight (day 4). Absorbance spectra were obtained with 0 uL, 20 uL, 40 uL, 60 uL, 80 uL, and 100 uL of each sample added to prepared sensors (*vide supra*). Note that this procedure could be faster, as dialysis rounds 3-5 could have been done at 8-hour intervals, instead of 24 h intervals. Note also that Desalting columns would provide removal of salts within minutes, as opposed to days. Lastly, note that products exist for fast, parallel dialysis using a 96 well plate. As the turn-on sensor is compatible with 96 well plates, the standard equipment for ELISA, this is not a huge deal that dialysis needs to be done. Examples of such products with relevant MWCO affording faster dialysis: http://www.nestgrp.com/protocols/AmiKa/biodialyzer96.pdf

R6G quenching ratio of lipid-R6G complexes against pure R6G solution. We prepared aqueous solutions of lipids (DMPA, CTAB, Tween 20, SDS, KH_2PO_4) at a high concentration, and subsequently added 1 molar equivalent of R6G to the lipid molecules. The integration of the emission spectrum for the final lipid-R6G complexes solution was measured (integration of the emission spectrum, I_f) with serial dilutions of a lipid. We additionally prepared pure R6G aqueous solutions and the integrations of the emission spectra were measured at the aforementioned concentrations with serial dilutions of a lipid (integration of the emission spectrum, I_i). We collected relative values of I_f/I_i, the quenching ratio, at each lipid concentration.

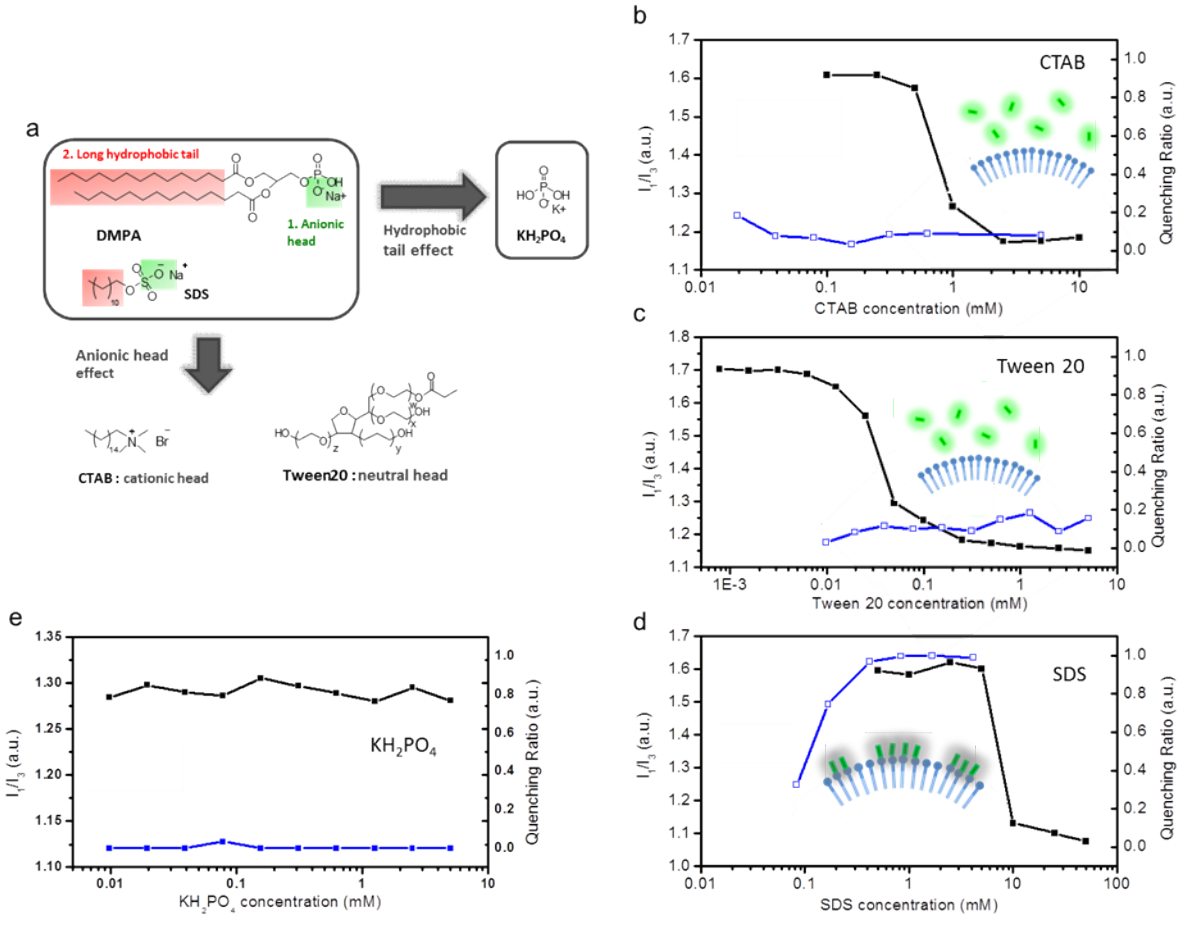

Figure S1. (a) Schematic illustration of molecular origin of H-type aggregation on liposome surface. Quenching ratio of R6G emission (blue sqaure) and I_1/I_3 value (black sqaure) were plotted versus (b) CTAB, (c) Tween 20, (d) SDS, and (e) KH_2PO_4 concentration (blue). I_1/I_3 value was used for determining critical micelle concentrations of various surfactants using pyrene fluorescence probing (see details in **Figure S3**).

Scanning electron microscopy (SEM) and dynamic scattering studies of mixed solution of DMPA /R6G. High-resolution SEM images were obtained from the mixed aqueous solution of R6G and DMPA at selected concentrations of DMPA, i.e. region A, B, C, and D in the Scheme S2. Spherical particles having a 50-500 nm diameter (region B) and colony-type aggregates (region C and D) were observed after the transition point, while no particles and/or aggregates were observed before the transition point (region A). Note: Colony-type aggregates in region C and D may appear during the sample preparation for SEM measurement. Dynamic light scattering results (Figure S2, insets) indicate that colony-type large aggregates are initially spherical particles having average diameters of about 100 nm in regions C and D, respectively). Furthermore, the fact that the transition concentration is far below the CBC of DMPA (around 0.3 mM; Figure S3) implies that the DMPA-R6G complexes resulting from Coulombic interactions are more hydrophobic than pure DMPA and so the complexes can form liposomes even at much lower concentrations of DMPA than the CBC of DMPA.

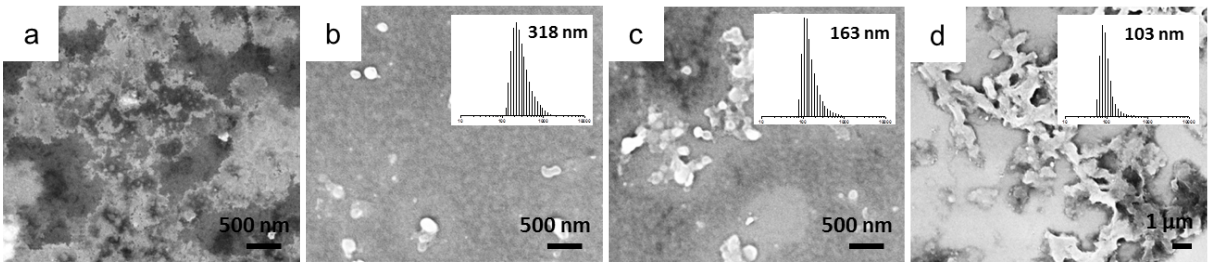

Figure S2. SEM images and dynamic light scattering studies of region A, B, C, and D in Scheme 2b.

Determination of CBC (or CMC) values for phospholipids liposomes by using pyrene fluorescence probing [Ref: K. Kalyanasundaram, J. K. Thomas, *J. Am. Chem. Soc.* **1997**, 99, 2039] 5 µl of a pyrene solution (2 mM) dissolved in methanol, solution A, was placed into a 20 ml vial and the solvent was evaporated by N_2 blowing. Serial dilutions of the lipid solution,

solution B, were added to the evaporated residue and the resulting solution were sonicated in a water bath at 25 °C for 10 min. Next, fluorescence spectra of each final solution were obtained. The excitation wavelength was 320 nm and the emission wavelength range was between 350 and 450 nm. The fluorescence intensities of the peaks at ~375 nm (I_1) and ~385 nm (I_3) were extracted from the spectra, and the I_1/I_3 values are plotted against the serially diluted lipid concentration to get the inflection point.

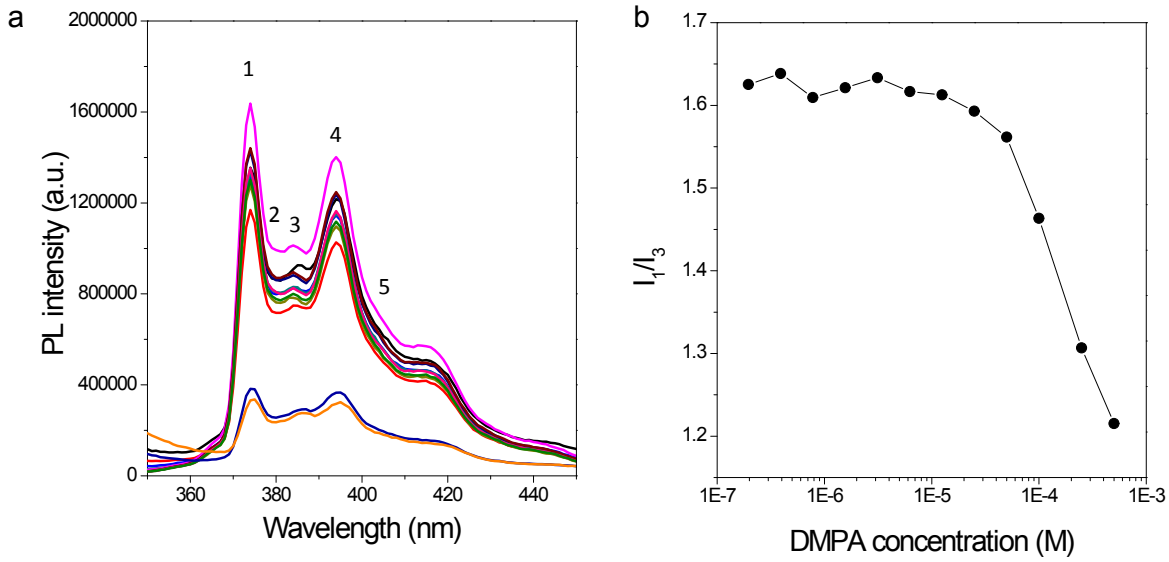

Figure S3. (a) PL spectra of pyrene at various concentration of DMPA. (b) I_1/I_3 value was plotted versus DMPA concentration. The curve shows a typical sigmoid shape.

Titration study of R6G solution with DMPA. During a fluorescence titration, 1 ml of an aqueous solution of R6G (1×10^{-5} M) was placed in a quartz cuvette ($10 \times 10 \times 40$ mm) and the initial emission spectrum was recorded with excitation at 420 nm using a PTI Quanta Master™ spectrofluorometer. 20 µl aliquots of a DMPA solution in deionized water (1×10^{-4} M, 0.2 molar equivalents to the R6G) were introduced to the cuvette until reaching 10 molar equivalents to the R6G. After each addition, a fluorescence spectrum was recorded. In the

same way as the fluorescence titrations, the absorption titrations were conducted in a UV-transparent cuvette using a Varian Cary 50 UV-Vis spectrophotometer.

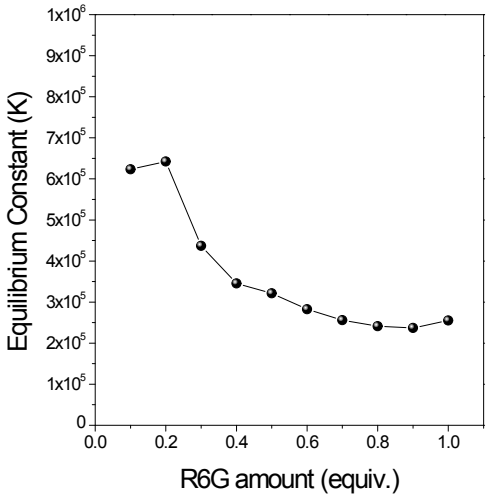

Figure S4. Equilibrium constant (K_b) of DMPA/R6G system was plotted against the amount of R6G.

K_b values were determined by following equation.

$$\text{DMPA} + \text{R6G} \underset{}{\overset{K_b}{\rightleftharpoons}} \text{DMPA-R6G}$$

$$K_b = \frac{[\text{DMPA-R6G}]_{eq}}{[\text{DMPA}]_{eq}\,[\text{R6G}]_{eq}}$$

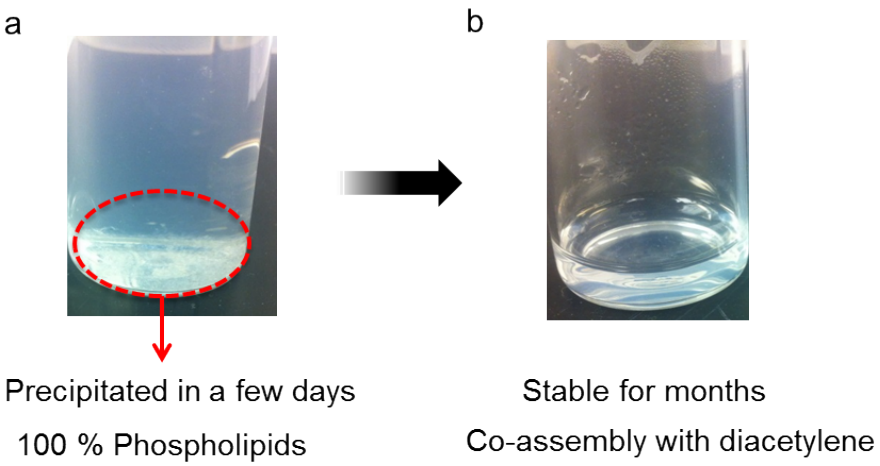

Figure S5. Pictures of (a) DMPA based liposome after few days from the initial preparation; (b) PIP_2/PCDA based liposome after several months from the initial preparation.

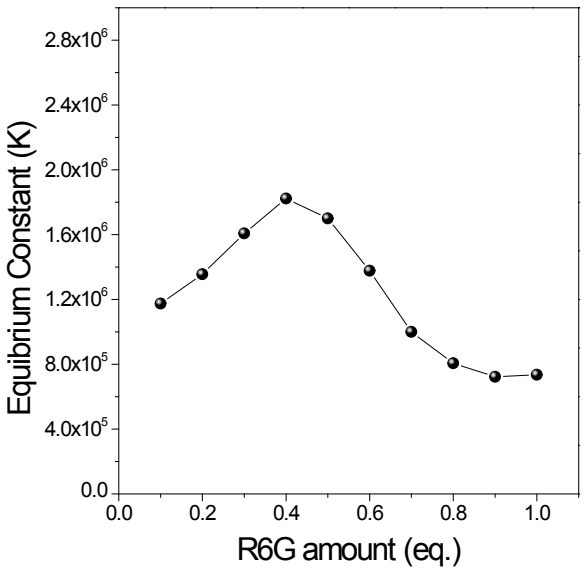

Figure S6. Equilibrium constant (K_b) of DMPA/PCDA/R6G system was plotted by the amount of R6G.

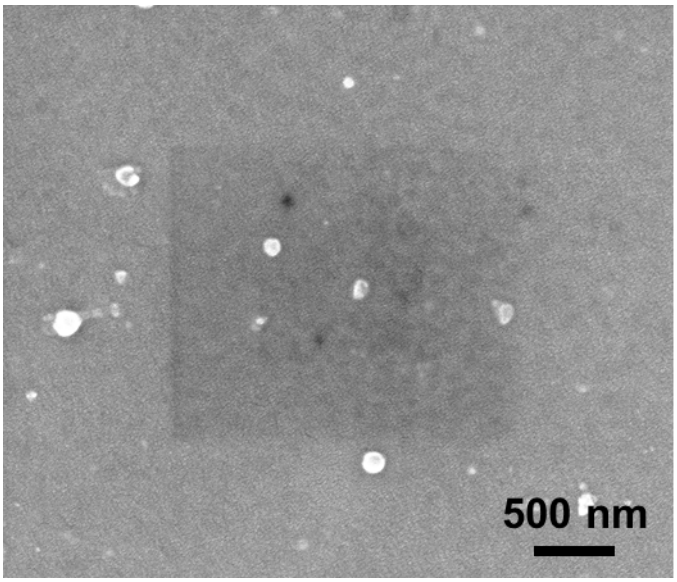

Figure S7. SEM image of PCDA/PIP_2/R6G based liposome.

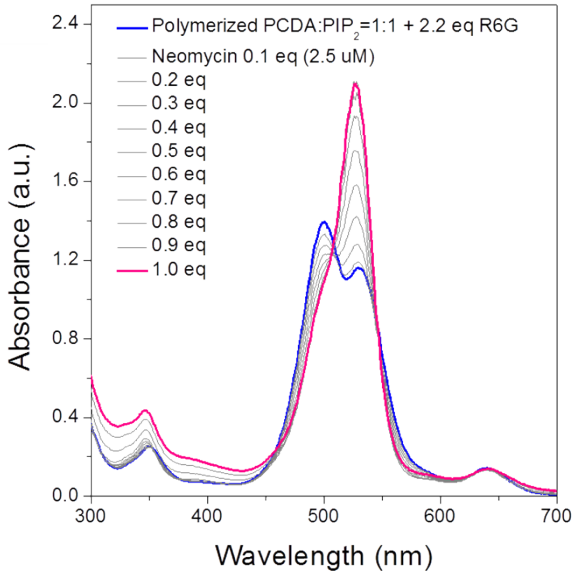

Figure S8. Changes in UV-Vis spectra of PCDA/PIP_2/R6G liposome based sensor by the addition of neomycin.

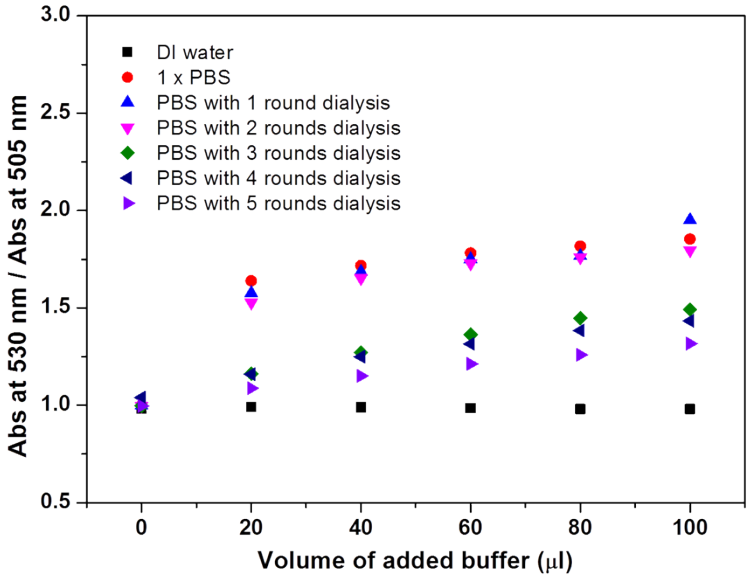

Figure S9. The absorption intensity ratio was calculated from UV-Vis spectra of PCDA/DMPA liposome-R6G complex in the presence of PBS buffer.