Supporting Materials

Investigating ligand-receptor interactions at bilayer surface using electronic absorption spectroscopy and Fluorescence Resonance Energy Transfer

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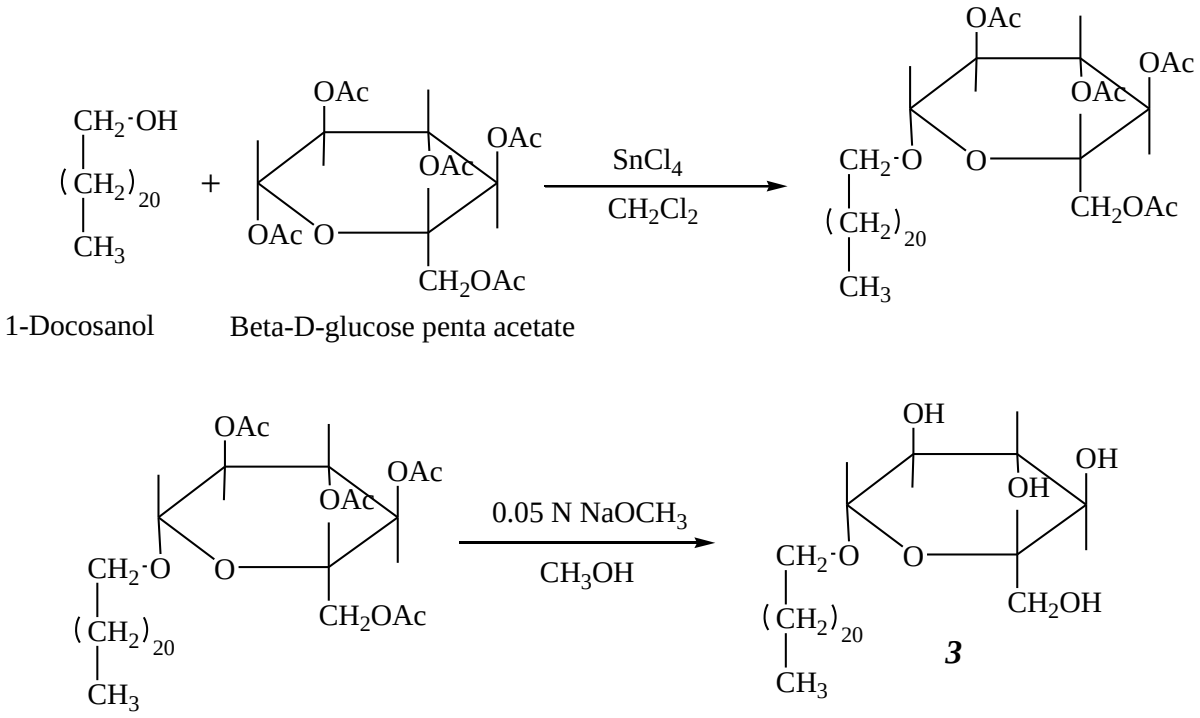
Fluorescence micrograph studies and their analysis. Glass slides were cleaned by immersion in "piranha" solution consisting of 3 : 1 ratio of aqueous solutions of 50% v/v of sulfuric acid and 30% w/v of hydrogen peroxide for 30 min (*Caution: this mixed solution is extremely energetic and reacts violently with organic materials. This solution must be handled with extreme caution and must be used in a ventilated hood*). Next, the glass slides were thoroughly rinsed with deionized water and dried under nitrogen. The glass slides were then placed in a solution containing 3-aminopropyltriethoxysilane (10 % v/v) diluted in 95% ethanol for 1 hour, rinsed thoroughly with DI water, and cured for 4-5 hours at 90 °C. Amino-functionalized glass slides were stored at 4 °C until further use.

The liposome-*E. Coli* interaction was visualized using fluorescence microscope for two different liposome sizes. In the first case, SR-101 tagged GUVs (large liposomes with diameter in 10-60 μm range) were incubated with excess *E. Coli* and the mixture was observed under microscope after evaporation of water in a desiccators. In the second case, *E. Coli* were spotted on glass slides and were then incubated with excess amount of SR-101 tagged liposomes prior to

fluorescence analysis. All the fluorescence micrographs were obtained using an inverted optical microscope (Lieca DMIRB) equipped with a QImage (Cooled Mono 12-bit) CCD camera. The red emission was obtained using a 41004 Texas Red filter (exciting and emitting band widths of the filter used were 527-567 nm and 605-682 nm respectively). The blue emission was obtained using a DAPI filter (excitation and emission band widths were (349 $\pm$ 25) nm and (459 $\pm$ 25 nm). All the dichroic filters were purchased from Chroma Technology Corporation.

Synthesis of glucose-tagged lipid *3*. The synthesis procedure for ***3*** is shown in Scheme 2, which is a modified method published in the literature.[1] Briefly, 6.5 mmole of β-D-glucose-penta-acetate (β-D-cellobiose octaacetate) was mixed with 6.5 mmole of $SnCl_4$ in 75 ml anhydrous dichloromethane (CH_2Cl_2) and 6 mmole of the docosanol was added to the mixture. The mixture was then refluxed for 8 hr. After cooling the mixture, it was treated with 100 ml 15% $NaHCO_3$ solution, and the organic layer was washed twice with brine, dried over anhydrous $CaCl_2$. The solvent was evaporated under vacuum. Deacetylation was performed by refluxing the residue with 150 ml 0.05 N sodium methanolate in methanol for 4 hr. The crude products are purified by flash chromatography ($CHCl_3$/MeOH, gradient from 20:1 to 5:1). The major fraction was collected and evaporated in vacuum to a white pure solid of ***3*** (1.17 g, 40%).

^{1}H NMR (300 MHz, DMSO) δ (ppm): 0.82 (t, 3H), 1.10-1.38 (m, 38H), 1.47-1.54 (m, 2H), 3.35-3.39 (m, 1H), 3.42(m, 1H), 3.49-3.68(m, 5H), 3.78 (t, 1H), 4.98 (d, 1H).



Scheme 1S. Schematically synthesis processes of glucose-tagged lipid (***3***).

Synthesis of glucose-tagged diacetylene monomer (*4*). The synthesis procedure for monomer ***4*** is a modified method from several literatures[2,3,4] (Scheme 3).

Synthesis of ***4a***. N-Boc-L-threonine (2.65 g, 12.0 mmol), acetobromoglucose (2.46 g, 6.0 mmol) and potassium carbonate (1.24 g, 9.0 mmol) were dissolved in 40 mL of dry acetonitrile (MeCN) under Ar atmosphere with stirring for 10 minutes. Iodine (2.28 g, 9.0 mmol) was then added against a flow of Ar. The glassware was then sealed and stirred at room temperature with the exclusion of light for 6 hours. A saturated sodium thiosulfate aqueous solution was added to the above stirring solution, until the deep red color had disappeared leaving a slightly yellow solution. The insoluble residual potassium carbonate was removed by filtration. The filtrate was concentrated to ¼ of its original volume under reduced pressure on a rotary evaporator. DCM (40 mL) was added to the mixture, and the solution was extracted with sodium bicarbonate

aqueous solution (5% w/v, 50 mL) one time, followed by brine (50 mL) two times, and the organic layer was dried over magnesium sulfate. The crude products were purified by flash chromatography (gradient Hexane/EtOAc 10:1 to 1:4). The major fraction was collected and evaporated in vacuum to a white solid ***4a***, yield (1.75 g, 53%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 1.25 (d, 3H), 1.46 (s, 9H), 2.02-2.08 (m, 12H), 3.84 (ddd, 1H), 4.09 (dd, 1H), 4.13 (dd, 1H), 4.28 (dd,1H), 4.33 (m, 1H), 5.10 (t, 1H), 5.14 (dd, 1H), 5.24 (t, 1H), 5.28 (d, 1H), 5.75 (d, 1H).

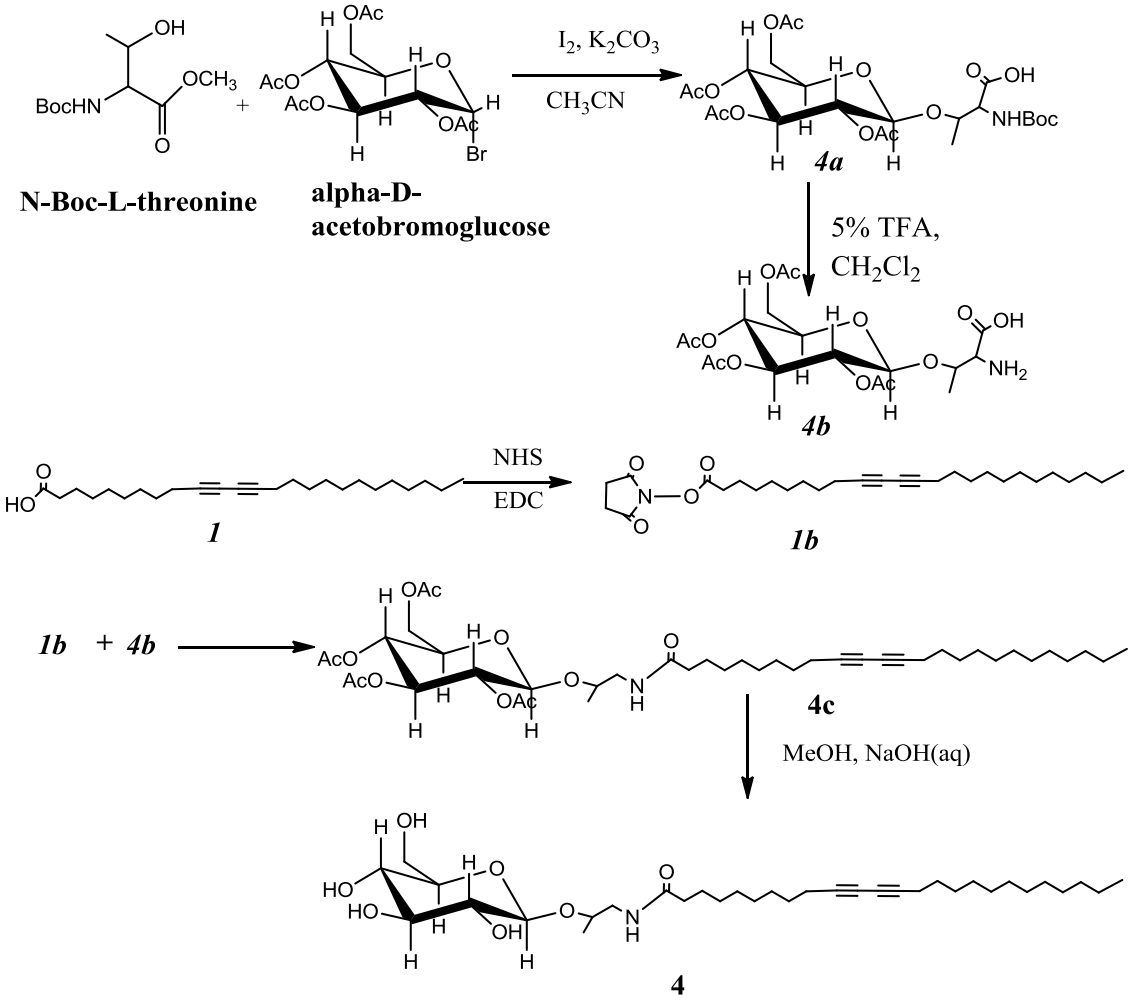
Synthesis of ***4b***. ***4a*** (1.5 g, 2.73 mmol) was dissolved in 20 mL of anhydrous DCM under Ar. TFA (1.0 mL, 13.01 mmol) was then added dropwise to it, and the solution was stirred at room temperature for 3 hours. The process was monitored by TLC until no starting materials was detected. The solvent was evaporated under reduced pressure to give a viscous liquid ***4b***.

Synthesis of ***1b***. To a solution of 10,12-pentacosadiynoic acid (1.00 g, 2.7 mmol) in anhydrous dichloromethane (DCM, 20 mL), N-hydroxysuccinimide (0.348 g, 3.0 mmol) and 1-(3-dimethylamino-propyl)-3- ethylcarbodiimide hydrochloride (0.570 g, 3.1 mmol) were added. The solution was stirred at room temperature for two hours followed by rotary evaporation of the DCM. The residue was extracted with diethyl ether and water for three times. The organic layer was dried over magnesium sulfate for half an hour, filtered, and the solvent is removed by rotary evaporation to give white solid ***1b*** (1.18g, 93%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88 (t, 3H), 1.22-1.45 (m, 26H), 1.45-1.52 (m, 4H), 1.80 (m, 2H), 2.22 (t, 3H), 2.42 (t, 3H), 2.78 (t, 2H).

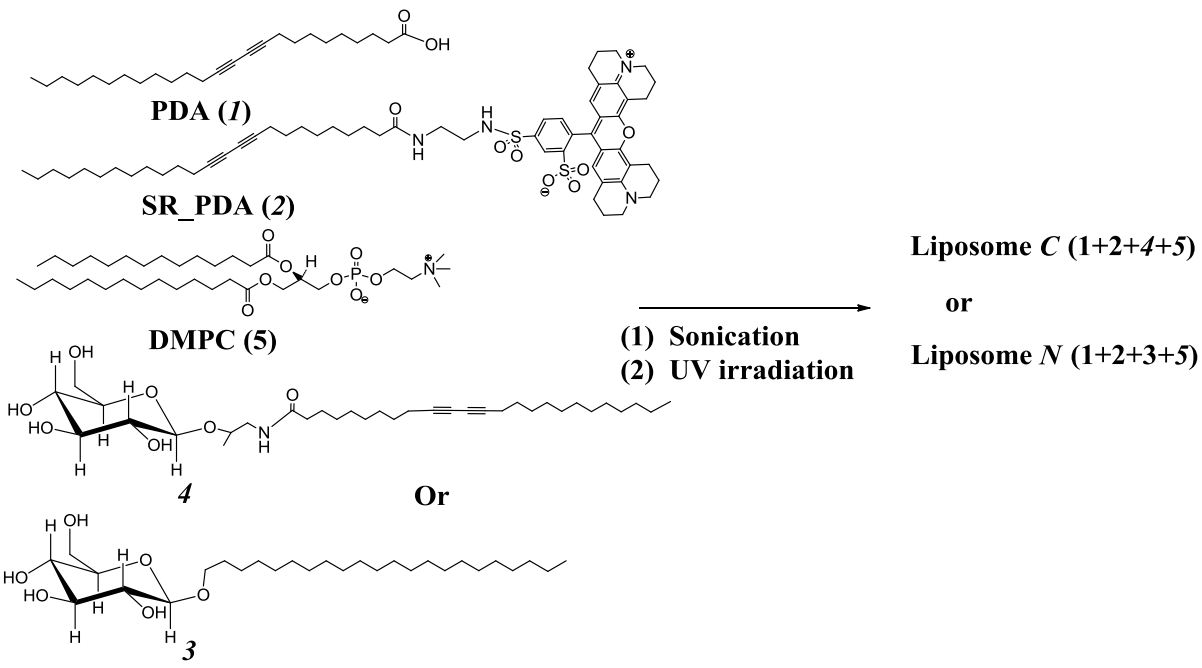
Synthesis of ***4c***. To a solution of ***1b*** (1.0 g, 2.12 mmol) in 20 mL anhydrous DCM, triethylamine (1.49 mL, 10.6 mmol) and a solution of ***4b*** (0.867 g, 1.93 mmol) in 10 mL anhydrous DCM was added. After stirring over 36 hours, the solvent was removed by rotary evaporation. The residue was redissolved in 20 mL DCM, and then was washed with following solutions: 1M HCl

aqueous solution (twice); saturated sodium bicarbonate solution (twice); and saturated sodium chloride solution (once). The organic layer was dried over with magnesium sulfate, filtered and evaporated to give crude semi-solid. The crude products are purified by flash chromatography (TCM/MeOH 20:1). Compound ***4c*** was obtained as a white powder (1.102 g, 75%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88 (t, 3H), 1.27-1.38 (m, 29H), 1.45-1.52 (m, 4H), 1.82-1.98 (m, 2H), 2.02-2.10 (m, 12H), 2.25 (t, 2H), 2.37 (t, 2H), 2.54 (m, 2H), 3.84 (ddd, 1H), 4.11 (dd, 1H), 4.23 (dd, 1H), 4.28 (dd,1H), 5.10 (t, 1H), 5.15 (dd, 1H), 5.25 (t, 1H), 5.28 (d, 1H), 5.76 (d, 1H).

Synthesis of ***4***. Compound ***4c*** (1.0 g, 1.31 mmol) was dissolved in 50 mL methanol containing 0.05 N sodium methanolate and was stirred at room temperature over night. Ion exchange resin was added to the solution until it was acidic to pH paper. The solution was filtered and the solvent was removed by rotary evaporation to get a white solid. The crude products are purified by flash chromatography (TCM : MeOH :: 2:1). Compound ***4*** was obtained as a white powder (0.389 g, 50%). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 0.88 (t, 3H), 1.23-1.38 (m, 29H), 1.45-1.54 (m, 4H), 1.62-1.70 (m, 2H), 2.21 (t, 2H), 2.34 (t, 2H), 2.54 (m, 2H), 3.29 (d, 1H), 3.35-3.38 (m, 1H), 3.42(m, 1H), 3.49-3.68(m, 3H), 3.78 (m, 2H), 4.68 (d, 1H).



Scheme 2S. Schematically synthesis processes of glucose-tagged PDA (***4***).



Scheme 3S. Preparation of covalently and non-covalently bound glucose receptors on the surface of liposomes.

Preparation of sulforhodamine-glucose-tagged liposomes. The self-assembled diacetylene liposomes were prepared by using a mixture of 10,12-penta-cosadiynoic acid (***1***), SR-101-tagged diacetylene (***2***), glucose-tagged lipid (***3*** or ***4***) and DMPC (***5***). The glucose residue was either covalently or non-covalently linked to the liposome surface when monomer ***4*** or ***3*** respectively was used in the liposome preparation. The liposomes were synthesized according to published literature procedure.[5,6,7]

Briefly, a mixture containing ***1***, ***2***, ***5*** and ***3*** (for ***N***) or ***4*** (for ***C***) in a desired ratio was dissolved in chloroform in a round bottom flask. The total concentration of all the monomers ([***1***+ [***2***] + [***5***] + either [***3***] or [***4***]) was 1 mM in the final solution. The concentrations of ***3*** and ***4*** were varied between 5 and 20 mole% of the total concentration to investigate the effect of glucose on the binding of *E. Coli* with liposomes (please see below). Our previous experiments

have shown that the incorporation of receptor molecules in the bilayer was almost quantitative.[43] The solvent was evaporated completely and deionized (DI) water or PBS buffer solution (0.01 mM, pH 7.4) was added to make liposome solution of a desired concentration (~1 mM). The resultant suspension was sonicated at 76 °C for ~15 minutes. The solution then was passed through a 0.8 μm nylon filter to remove the lipid aggregates, and is cooled at 4 °C for overnight. The resultant solution was optically clear. Polymerized diacetylene liposomes were prepared by exposing the liposome solutions to UV radiation of 254 nm for 2-5 minutes using a Pen Ray UV source (4.5 mW/cm^2). The resulting blue liposome solution was stored in the dark at 4 °C. Dialysis of the liposome was carried out with a membrane (M_w cut-off: 10,000) against deionized water.

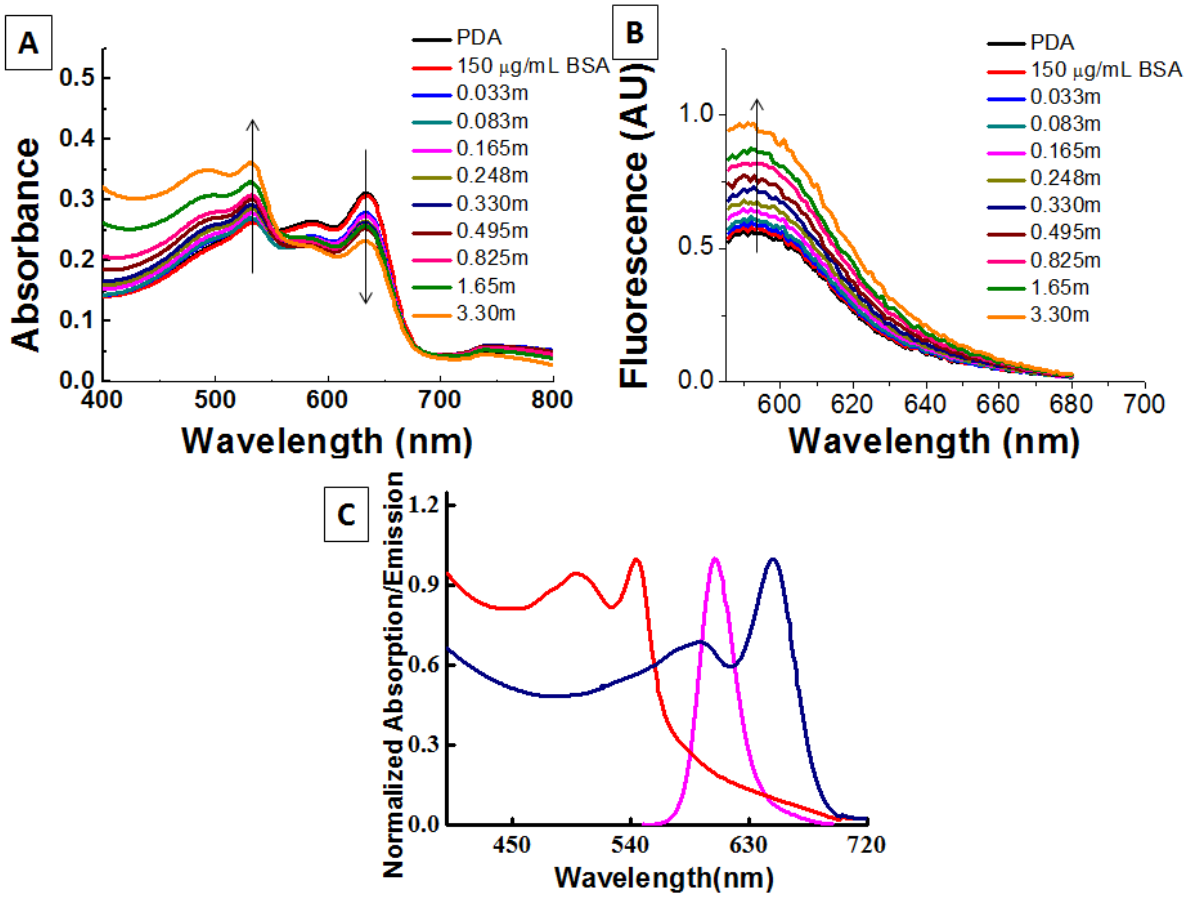


Figure 1S. Changes in the UV-Vis absorption spectra (A) and emission spectra of liposomes ***C*** after addition of *E. Coli* at different concentration to the solution. The concentration of *E. Coli* stock solution was 3.3 x 107 *E. Coli*/mL. The concentration of BSA was 150 µg/mL. (C) The spectral overlap (*J*) between SR-101 (donor) emission (pink curve) and PDA (acceptor) absorption in blue- (blue curve) and red-forms (red curve). The liposome solution was 5 mL for experiments. The excitation wavelength for FRET experiments was 560 nm.

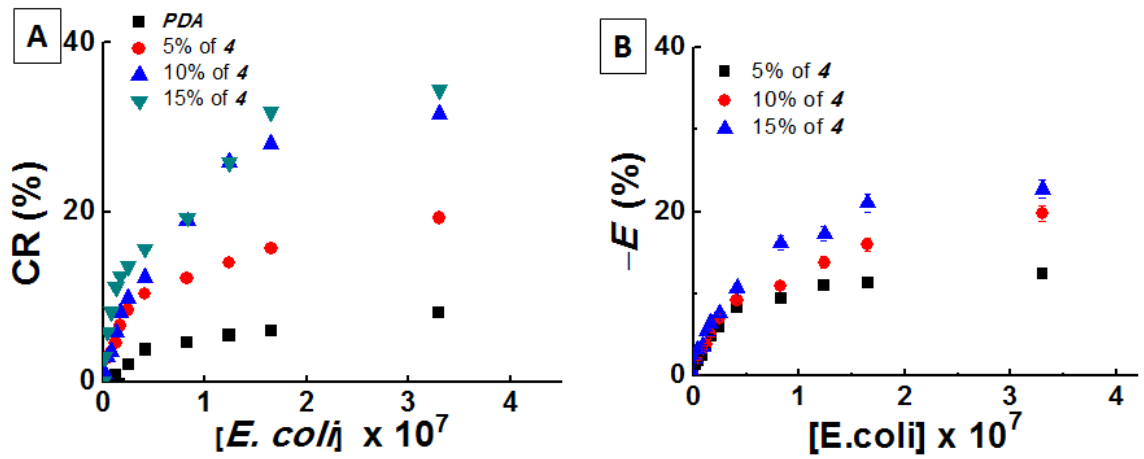


Figure 2S. (A) Colorimetric response (CR) of the liposomes-versus-*E. coli* concentration for liposomes ***C*** , (B) shows FRET efficiency for liposomes *C*. Minus sign in (B)denote a decrease in the FRET efficiency after addition of ***E***. *Coli* to the solution. CR and ***E*** were calculated using Eqs. 1 and 4 respectively.

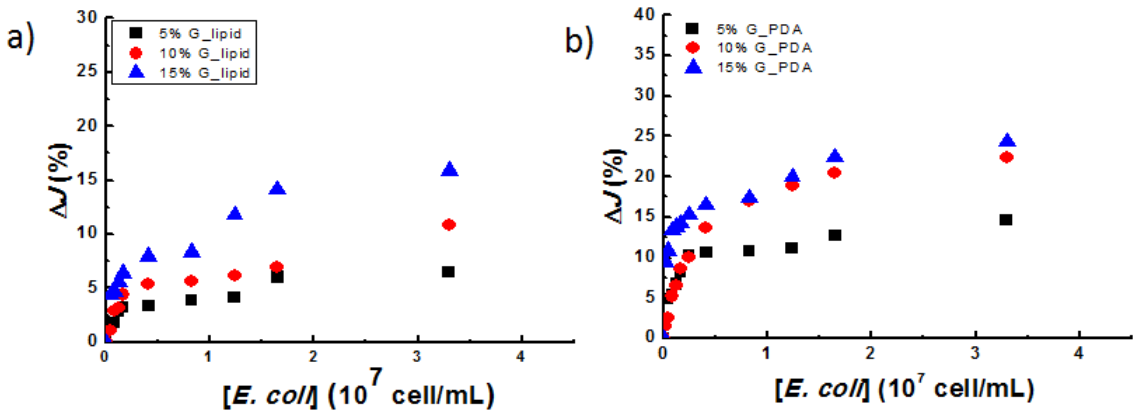


Figure 3S. Δ*J*-[*E. Coli*] for three different concentration of ***3*** and ***4***. Δ*J* represents changes in the spectral overlap between emission of SR-101 and absorption of PDA at different concentration of *E. Coli*.

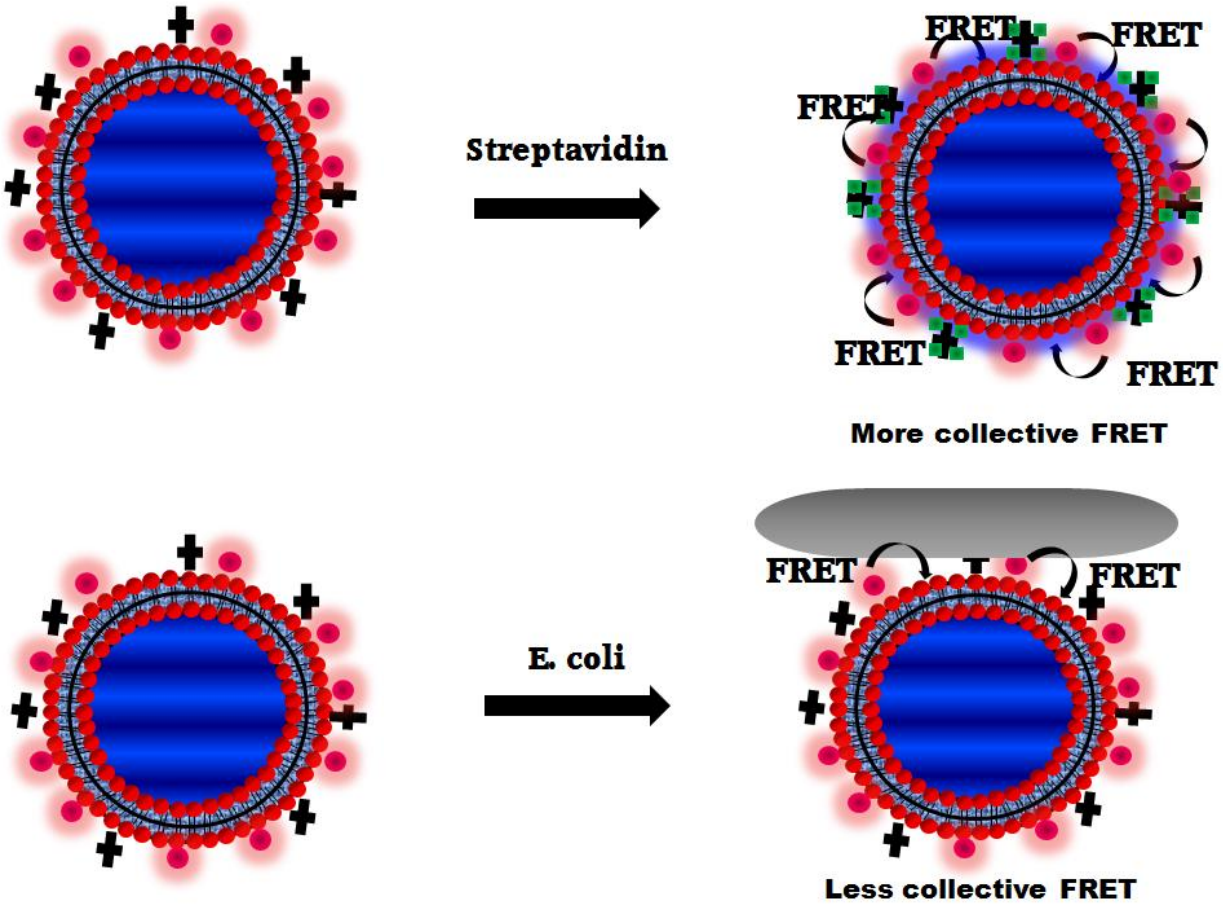


Figure 4S. A comparison of interaction of liposomes (biotin tagged) with *Streptavidin vs.* interaction of liposomes (Glucose tagged) with *E. coli* under our experimental conditions.

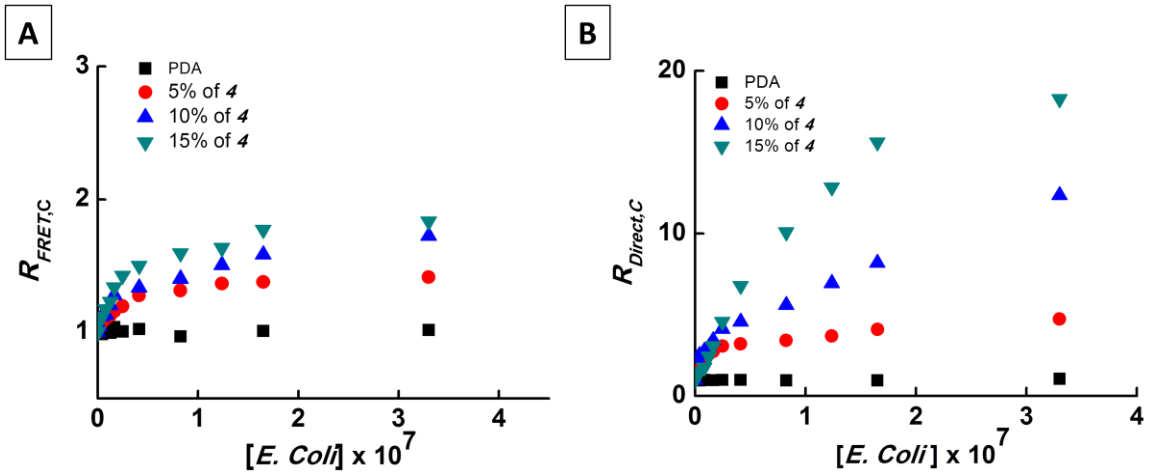


Figure 5S. (A) R_{FRET} for ***C*** series liposome at different *E. Coli* concentrations. R_{FRET} represents the ratio of SR-101 emission intensity (excitation wavelength was 560 nm) after addition of *E. Coli* of a given concentration to SR-101 emission intensity in the absence of *E. Coli*. (B) R_{Direct} at different *E. Coli* concentrations. R_{Direct} represents the ratio of PDA emission intensity (excitation wavelength was 490 nm) in the presence and absence of *E. Coli*. The excitation wavelengths for FRET and direct excitation were 560 nm and 490 nm respectively.

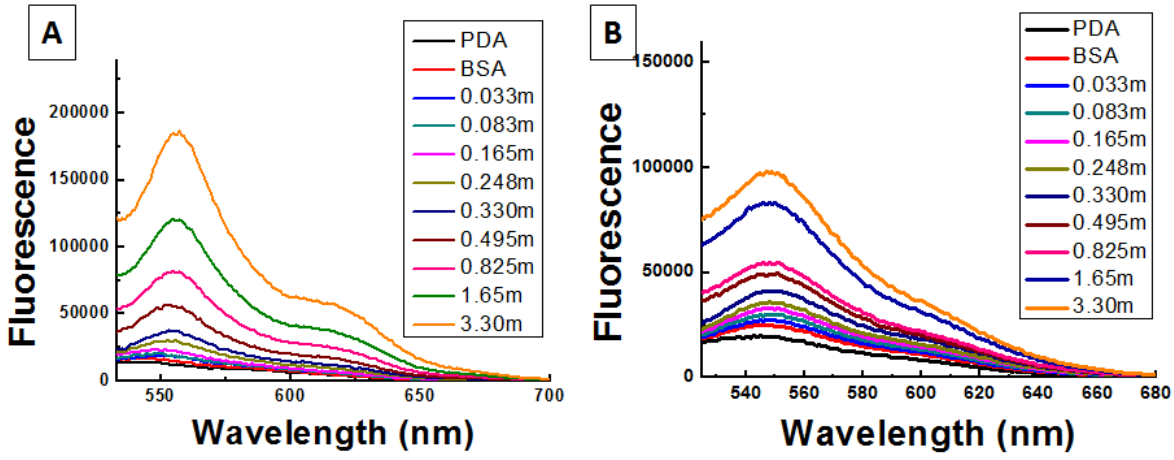


Figure 6S. Direct emission response of PDA liposomes *N* (A) and liposomes *C*(B) at different concentration of *E. Coli* in the solution. The excitation wavelength for all the spectra was 490 nm.

Estimation of liposomes interacted with E. Coli and the number of glucose molecules that interacted with one E. Coli. We assume that the liposomes are monodispersed and have an average diameter of 250 nm, and cross section area of one molecule was assumed to be ~ 0.27 nm^2. The total concentration of all the monomers is 1 mM or in 2 mL of solution, there are 0.2 μmoles ~ 1.2 x 10^{17} of molecules present in the solution. The number of glucose molecules in the solution/mL ~ 0.05 x 0.001 x 2 x 10^{-6} x 6.023 * 10^{23} ~ 6 * 10^{13}

Surface area of one liposome is 4 x π x r^2 = 7.85 * 10^5 nm^2

Average number of molecules in one liposome = 2.9 * 10^6

Number of liposomes in the solution = 1.2 x 10^{17}/2.9 x 10^6 = 4.19 x 10^{10}

Average number of glucose molecules/liposome = 0.05 * 2.9 * 10^6 ~ 1.45 x 10^5

Only half of the glucose molecules per liposome interacted with *E. Coli* = 7.2 x 10^4. Other half of the glucose molecules are inside of the bilayer membrane of the liposomes.

Half of the total glucose molecules present on the outer surface interacted with *E. Coli* ~ 3 x 10^{13}. Other half of the glucose molecules cannot interact with *E. Coli* because they are away from the *E. Coli* surface. Under high *E. Coli* concentration where aggregation is possible, there is possibility of all the glucose molecules on liposome surface to interact with *E. Coli*. We do not consider this possibility for simplicity of the calculations.

Average number of glucose molecules interacted per *E. Coli* ~ 3 x 10^{13}/10^7 ~ 3 x 10^6

The average number of liposomes interact with *E. Coli*, ~ 3 x 10^6/1.45 x 10^5 ~ 20 liposomes per *E. Coli*. However, for glucose concentration of 10 mol % and 15 mol%, we estimate the average number of liposomes per *E. Coli* ~40 and 60 respectively.

We have taken 10^7 *E. Coli* for these calculations because the signal get saturated at ~ 10^7 *E. Coli* particles under over the experimental conditions. Thus, only a fraction of the *E. Coli* surface is decorated with liposomes.

References:

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