Supporting information for:

Dynamic Molecular Recognition on the Surface of Vesicle Membranes

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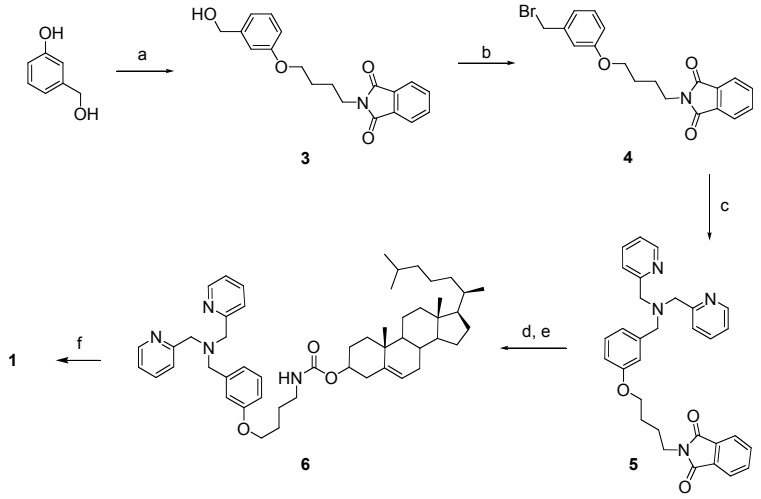
Contents

1) Preparation of receptor 1



Synthesis of 3

The solution of 3-hydroxy benzyl alcohol (1.24 g), N-4-bromobutyl phthalimide (3.1 g) and K_2CO_3 (6.9 g) was refluxed in acentonitrile (50 mL) for 12 h. The mixture was cooled and filtered. The solvent was removed. The residues were purified by silica gel column (Dichloromethane/ethyl acetate=9:1). Yield: 2.7 g. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 1.96-2.05 (m, 4H), 3.91 (t, J = 6.9 Hz, 2H), 4.15 (t, J = 5.7 Hz, 2H), 4.80 (s, 2H), 6.93-6.96 (s, 2H), 7.05- 7.07 (m, 2H), 7.36-7.41 (m, 1H), 7.85-7.89 (m, 2H), 7.96-8.01 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.4, 26.7, 37.7, 65.1, 67.2, 113.0, 113.8, 119.2, 123.3, 129.6, 132.1, 134.1, 142.8, 159.2, 168.6. MS (FAB): m/z 325 (M)

Synthesis of 4

Tetrabromomethane (2.7 g) and compound **3** (2.4 g) were dissolved in dry dichloromethane (20 mL). Triphenylphosphine (2.05 g) in dry dichloromethane (20 mL) was added over a period of 30 min. the mixture was stirred at ambient temperature overnight. The most of solvent were removed. The crude compound was purified by silica gel column (Dichloromethane/ethyl acetate=97:3). Yield: 2.73 g. ^{1}H NMR (300 MHz, $CDCl_3$, ppm): 7.86 (m, 2H), 7.73 (m, 2H), 7.22 (t, 1H), 6.96 (d, 2H), 6.92 (s, 1H), 6.81 (d, 1H), 4.46 (s, 2H), 4.01 (t, J=5.7 Hz, 2H), 3.79 (t, J=6.6 Hz, 2H), 1.88 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$, ppm): 168.69, 159.34, 139.35, 134.21,

132.35, 130.04, 123.48, 121.58, 115.29, 114.90, 67.4, 37.87, 33.80, 26.83, 25.57. MS (FAB): m/z 387(M), 388 (M+1), 389 (M+2).

Synthesis of 5

Compound **4** (2.5 g) and 2, 2'-dipicolyl amine (1.54 g) were dissolved in dry DMF (10 mL). K_2CO_3 (4.44 g) was added. The mixtures were stirred at room temperature for 20 h. DMF was removed on vacuum. The crude compound was purified by silica gel column (Ethyl acetate/0.5% Et_3N). Yield: 2.9 g. ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.86-1.91 (m, 4H), 3.67 (s, 2H), 3.75-3.79 (m, 2H), 3.83 (s, 4H), 3.97-4.01 (m, 2H), 6.74-6.77 (m, 1H), 6.97-7.00 (m, 2H), 7.12-7.28 (m, 3H), 7.59-7.34 (m, 6H), 7.83-7.87 (m, 2H), 8.51-8.53 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.6, 26.9, 37.9, 58.6, 60.0, 67.3, 113.3, 121.4, 122.3, 123.2, 123.4, 129.5, 132.3, 134.2,136.8, 149.1, 159.3, 168.7. MS (FAB): m/z 506 (M).

Synthesis of 6

Compound **5** (2.8 g) was dissolved in a mixture of dichloromethane (2 mL) and ethanol (18 mL). Hydrazine (0.5 mL) was added. The solution was refluxed for 1h. The precipitate appears during the reflux. The precipitate was removed by filtration. The filtrate was concentrated. The crude compound was dissolved in dichloromethane (5 mL), then filtered. The solvent was removed to yield deprotected amine (2.33 g) without further purification. The solution of deprotected amine (2.33 g) and triethyl amine (2.6 mL) in Dichloromethane (20 mL) was cooled to 0°C. A solution of cholestery chloroformate in dry dichloromethane was added slowly during a period of 30min, then the mixture was warmed to room temperature overnight. The solvent was removed. The residues were purified by silica gel column (Ethyl acetate/1%Et_3N). Yield: 93%. ^{1}H NMR ($CDCl_3$, ppm): 8.51(m, 2H), 7.68 (m, 4H), 7.14 (m, 3H), 6.98 (m, 2H), 6.74 (m, 1H), 5.37 (m, 1H), 4.75 (t, 1H), 4.49 (m, 1H), 3.97 (t, 2H), 3.81 (s, 4H), 3.67 (s, 2H), 3.25 (m, 2H), 2.33 (m, 4H), 0.66-2.00 (m, 42H). ^{13}C NMR ($CDCl_3$, ppm): 159.97, 159.22, 156.32, 149.05, 140.81, 140.06, 136.25, 129.27, 122.92, 122.44, 121.86, 121.36, 115.32, 113.35, 74.36, 67.611, 60.16, 58.65, 56.88, 56.43,

50.28, 42.49, 40.83, 39.95, 39.65, 38.77, 37.19, 36.71, 36.36, 35.85, 32.08, 32.02, 28.36, 28.28, 28.04, 27.00, 26.71, 24.38, 23.98, 22.84, 22.62, 21.10, 19.40, 18.87, 11.98. MS (FAB): m/z 789 (M+1).

Synthesis of 1

To a solution of compound **6** (0.41 g) in methanol (5 mL), zinc nitrate hydrate (0.17 g) was added. The solvent was removed after 30 min. The solid was used without further purification.

2) General procedure for vesicle preparation

A chloroform solution containing 2 mol % DOPE-PEG-2000, 20-35 mol % **1** and various amounts of POPC and/or DOTAP was evaporated using a rotary evaporator (<30°C). The lipid film was dried under high vacuum for 1h, and then soaked by buffer (10 mM TES, 145 mM NaCl, pH 7.4). Pyrex glass beads were added before vortexing to facilitate the removal of lipid film from the side of the flask. The resulting mixture was extruded at ambient temperature 29 times through a 19mm polycarbonate filter with 200 nm diameter pores using a hand held Basic Liposo Fast device.

3) Fluorescence and UV-Vis titration of CS with vesicles

All fluorescent experiments were carried out in aqueous buffer (pH 7.4, 10mM and 145mM NaCl) at 25 °C. A spectral scan (λ_{ex}=400 nm) was recorded after each addition of vesicles from a 5 mM (total lipid) stock solution. The initial concentration of **CS** is 10 μM in all cases.

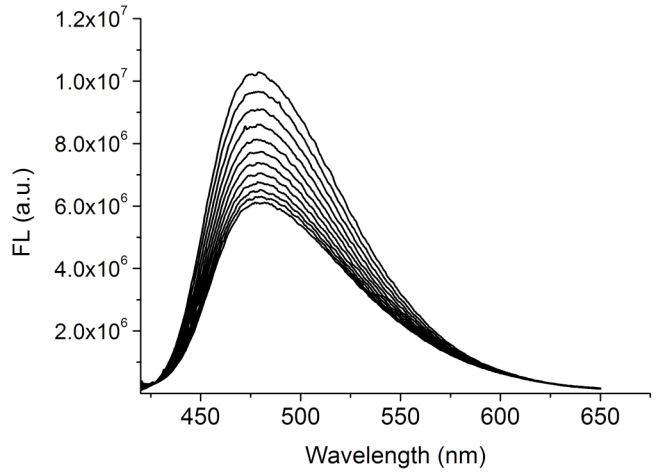


Fig. S1 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon the titration with vesicles at 25°C. Vesicle components: 10 mol % receptor **1**, 88 mol % POPC and 2 mol % DPPE-PEG-2000.

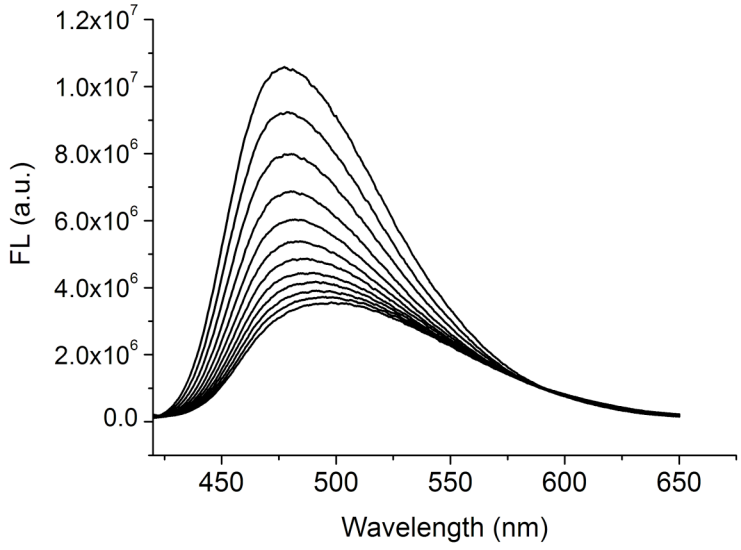


Fig. S2 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon the titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

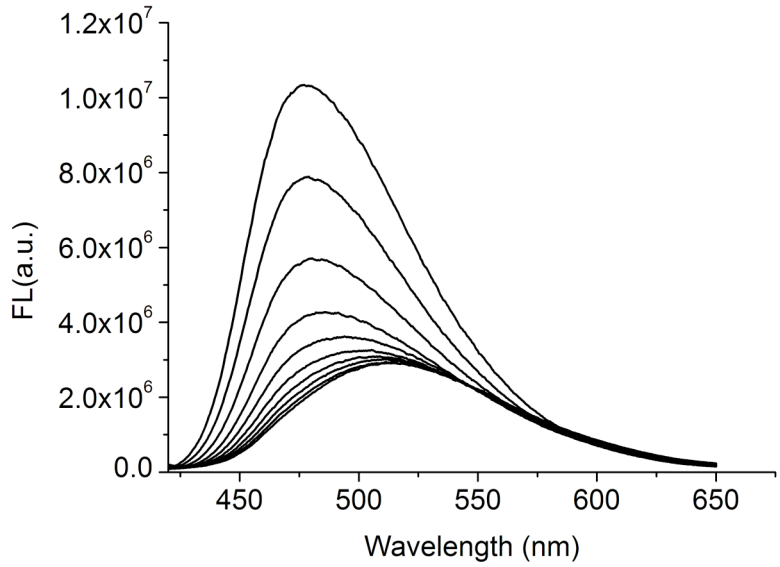


Fig. S3 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon the titration with vesicles at 25°C. Vesicle components: 30 mol % receptor **1**, 68 mol % POPC and 2 mol % DPPE-PEG-2000.

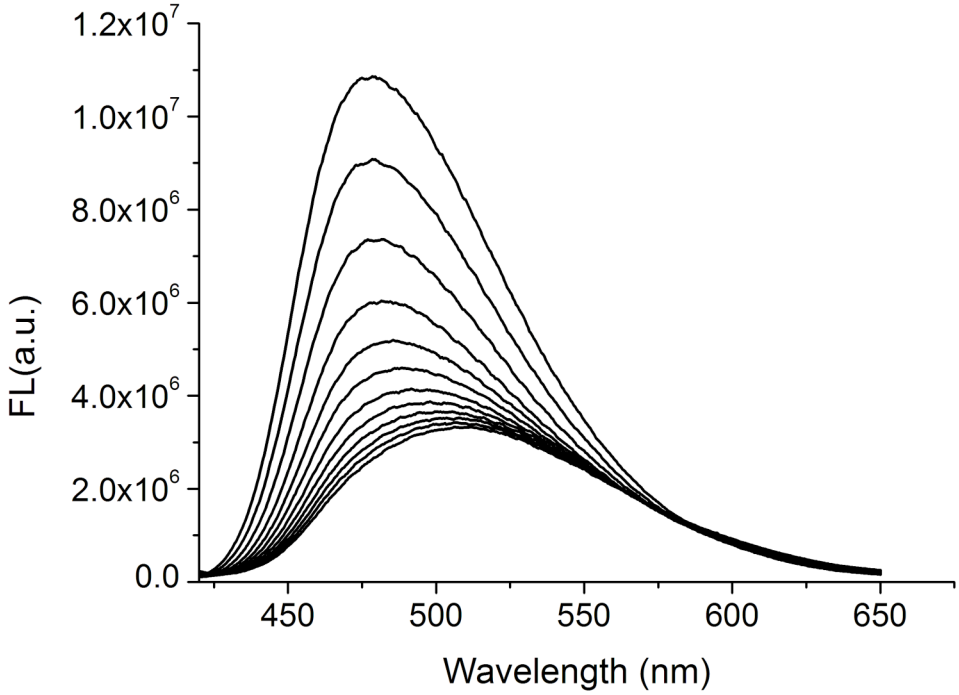


Fig. S4 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 73 mol % POPC, 5 mol % DOTAP and 2 mol % DPPE-PEG-2000.

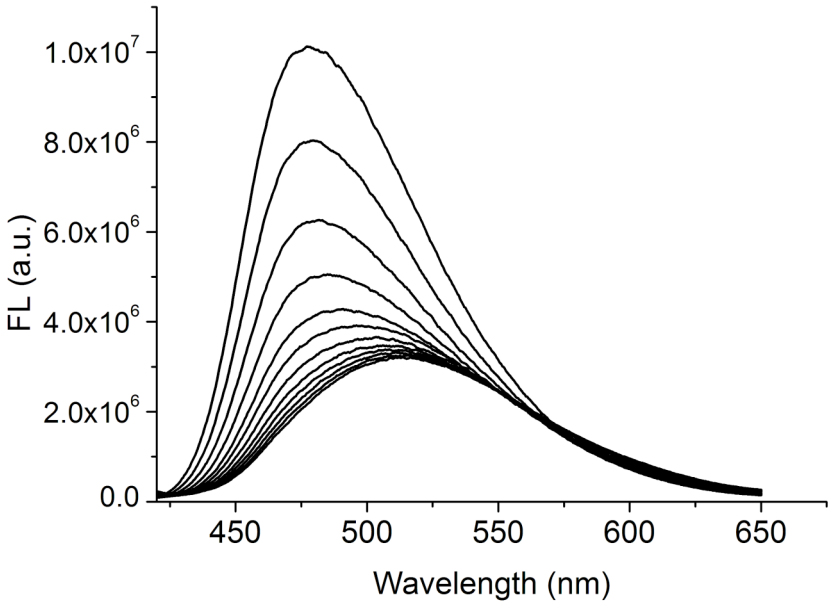


Fig. S5 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 68 mol % POPC, 10 mol % DOTAP and 2 mol % DPPE-PEG-2000.

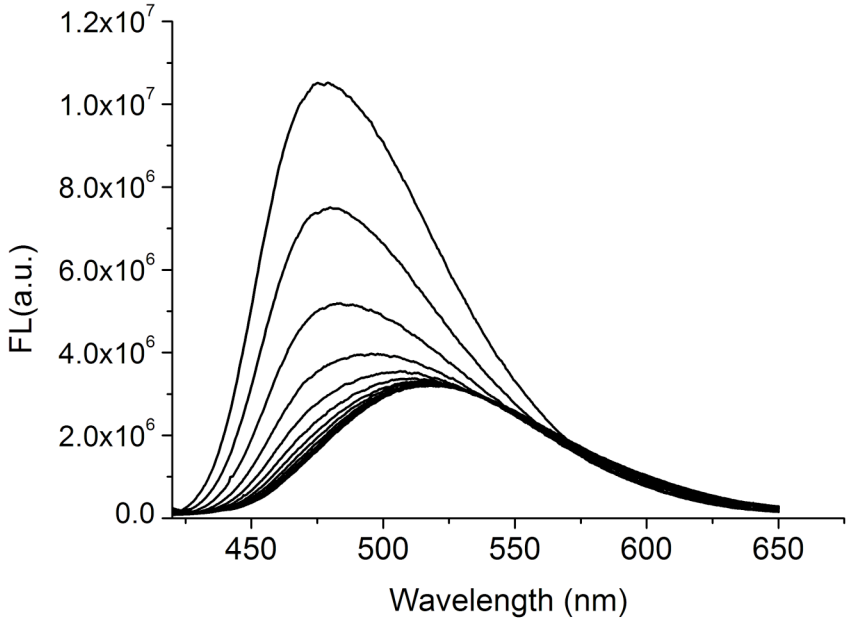


Fig. S6 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 58 mol % POPC, 20 mol % DOTAP and 2 mol % DPPE-PEG-2000.

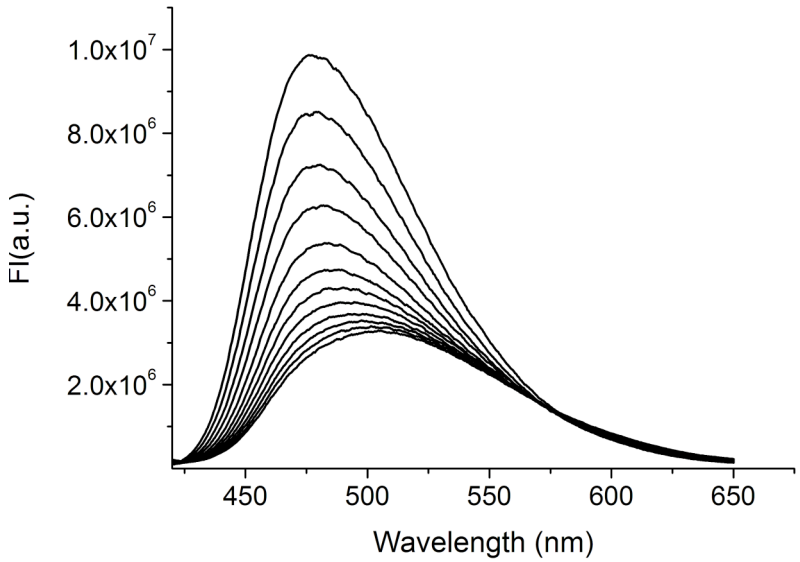


Fig. S7 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon the titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 63 mol % POPC, 15 mol % cholesterol and 2 mol % DPPE-PEG-2000.

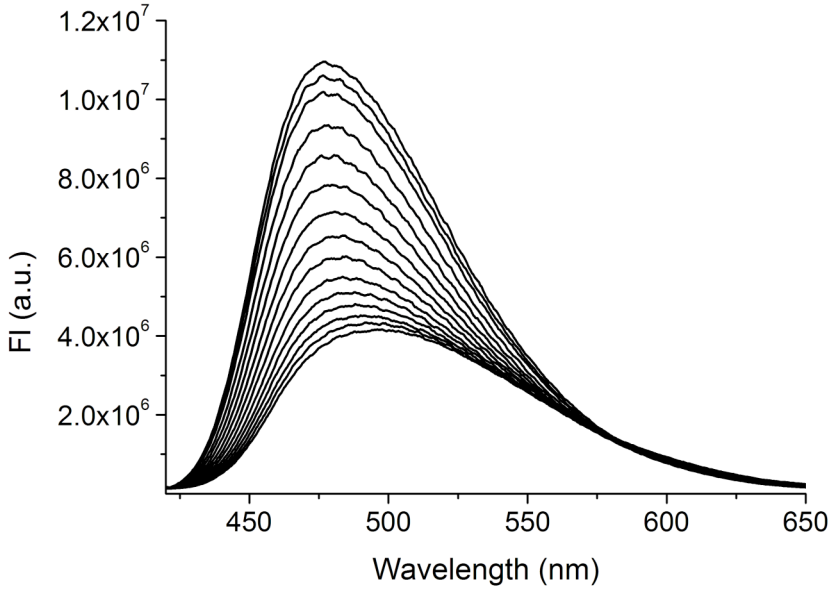


Fig. S8 Fluorescence spectra of **CS** (10 μM) in buffer (pH 7.4, 10 mM TES and 145 mM NaCl) upon the titration with vesicles at 25°C. Vesicle components: 20 mol % receptor **1**, 38 mol % POPC, 40 mol % sphingomyelin and 2 mol % DPPE-PEG-2000.

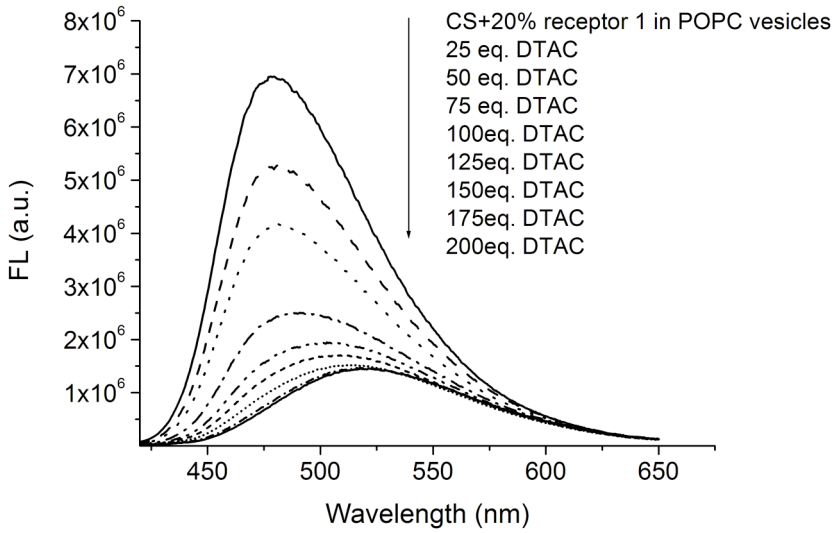


Fig. S9 Fluorescence spectra of a solution of **CS** ($1x10^{-5}$ M) and vesicles (20% receptor **1** in POPC, **1** is $2x10^{-5}$ M) in buffer (2 mL) upon titration with DTAC (from 0.5 M stock solution). Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

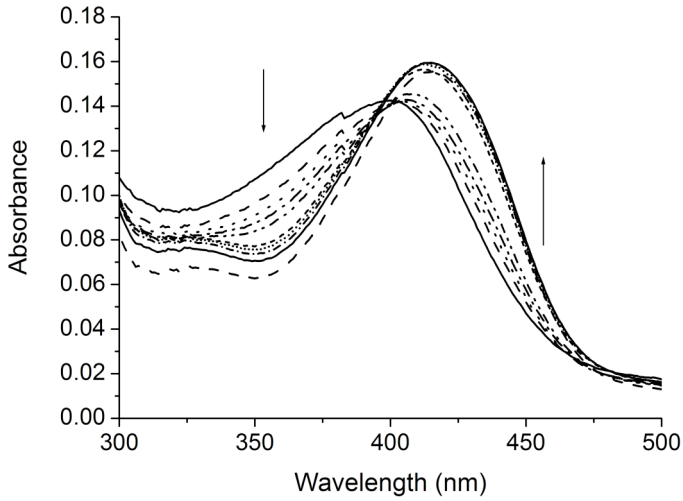


Fig. S10 UV spectra of a solution of **CS** ($1x10^{-5}$ M) and vesicles (20% receptor **1** in POPC, **1** is $2x10^{-5}$ M) in buffer (2 mL) upon titration with DTAC (from 0.5 M stock solution). Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

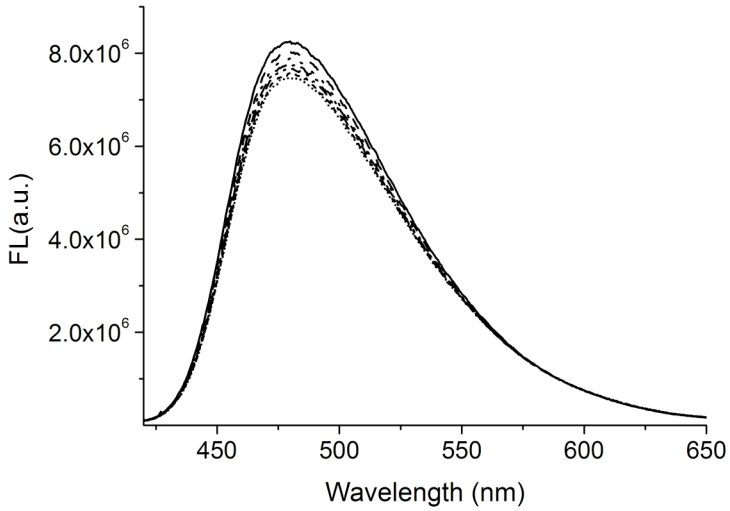


Fig. S11 Fluorescence spectra of a solution of **CS** ($1x10^{-5}$ M) and vesicles (20% receptor **1** in POPC, **1** is $2x10^{-5}$ M) in buffer (2 mL) upon titration with TEGME (0-200eq.). Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

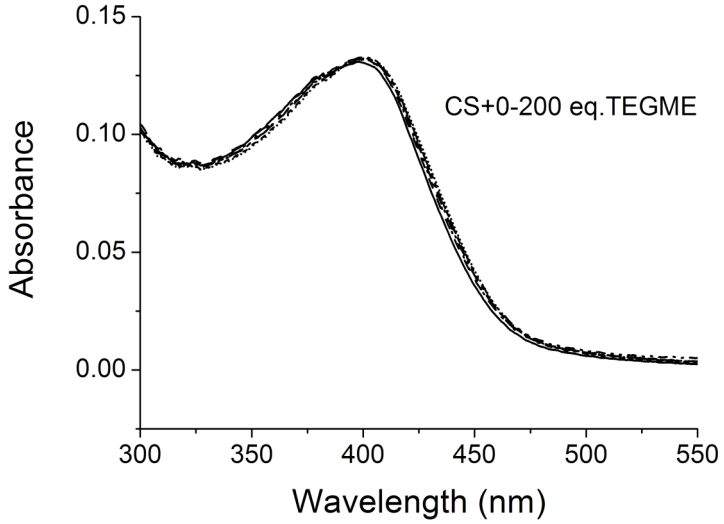


Fig. S12 UV spectra of a solution of **CS** ($1x10^{-5}$ M) and vesicles (20% receptor **1** in POPC, **1** is $2x10^{-5}$ M) in buffer (2 mL) upon titration with TEGME (0-200eq.). Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

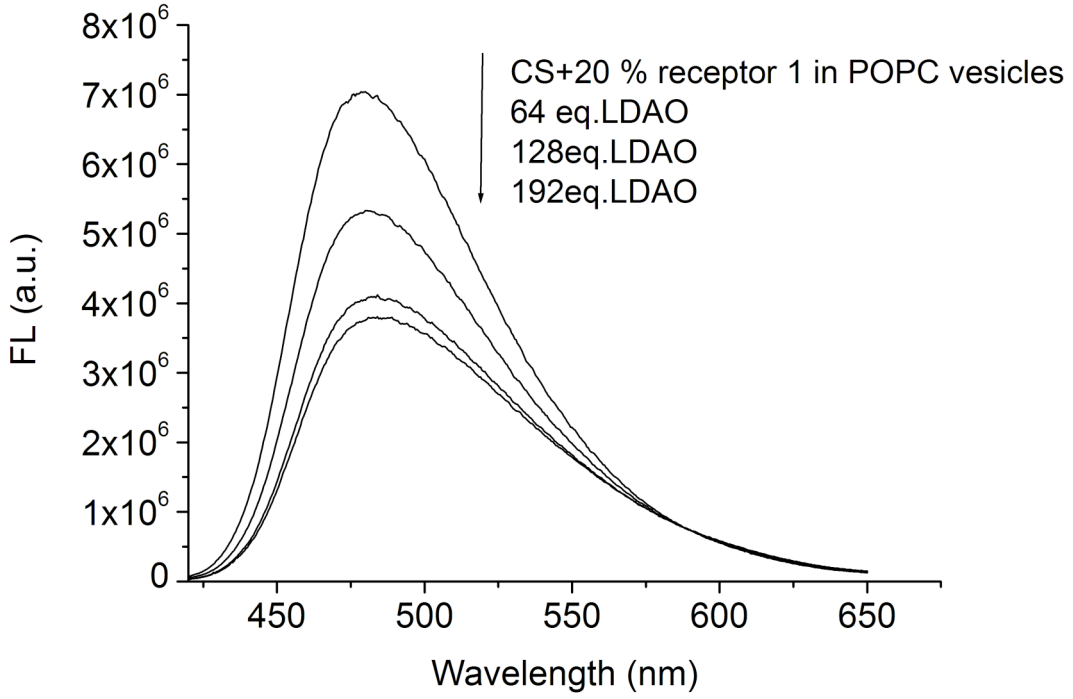
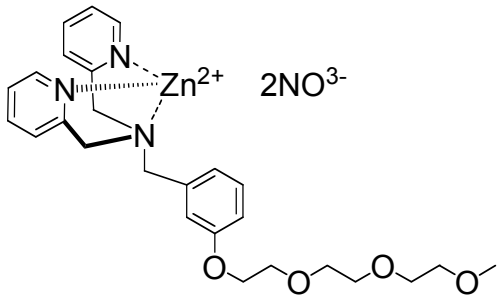


Fig. S13 Fluorescence spectra of a solution of **CS** ($1x10^{-5}$ M) and vesicles (20% receptor **1** in POPC, **1** is $2x10^{-5}$ M) in buffer (2 mL) upon titration with LDAO. Vesicle components: 20 mol % receptor **1**, 78 mol % POPC and 2 mol % DPPE-PEG-2000.

4) Fluorescence properties of model receptor 2 in buffer

Due to the poor solubility of receptor **1** in water, a hydrophilic model **2** was used to determine the binding constant. A typical quenching titration and Job plot with **2** are as follows:



Structure of model compound **2**

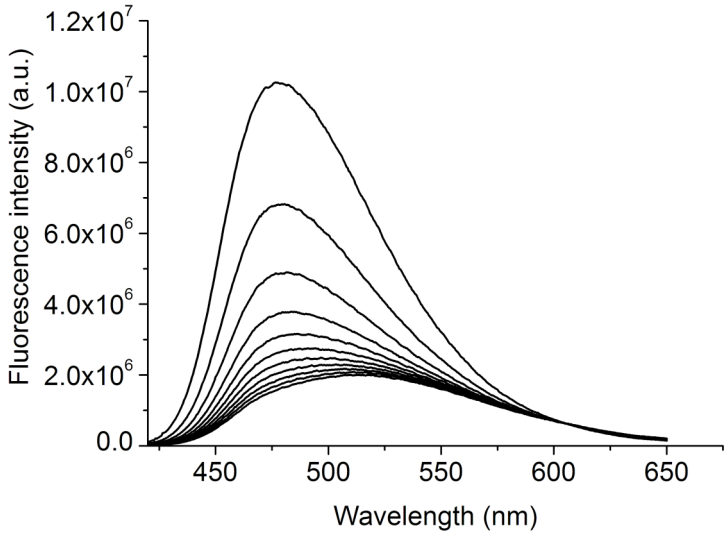


Fig. S14 Fluorescence quenching of **CS** upon the titration with **2** in buffer.

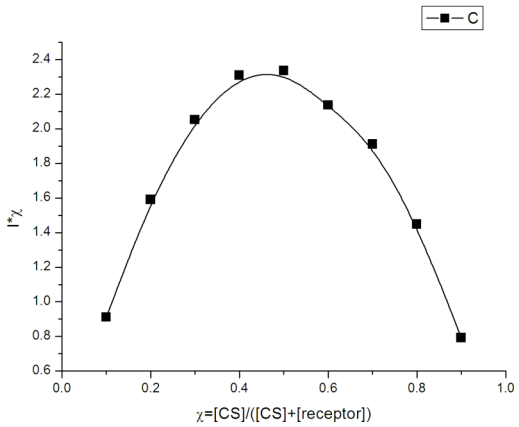


Fig. S15 Job plot of **CS** and **2** in buffer showing 1:1 complex is dominant.

5) Determination of binding constants.

The equations used for analyzing the fluorescent titration data[1] are given below. Equation 1 and 2 are for 1:2 (CS:receptor) and 1:1 binding model, respectively. Where I is the experimental fluorescent intensity, I_i is the initial fluorescent intensity, I_f is the final fluorescent intensity. c_t is the total concentration of receptor. It is

[1] R. P. Bonar-Law and J. K. M. Sanders. *J. Am. Chem. Soc.,* 1995, **117**, 259-271.

assumed that only the receptors on the outer surface of the vesicles are available for binding. Previous work has shown that the outer monolayer of a 200 nm unilamellar vesicle membrane has more surface area and contains 66% of receptor. Thus, the concentration of available receptor was the total receptor concentration multiplied by 0.66. K_1 and K_2 are the first and second binding constants, respectively. All binding constants were obtained by non-linear curve fitting.

$$I = I_i + (I_f - I_i)K_1c_t(1 + K_2c_t)/(1 + K_1c_t(1 + K_2c_t)) \quad (1)$$

$$I = I_0 + (I_f - I_i)K_1c_t/(1 + K_1c_t) \quad (2)$$

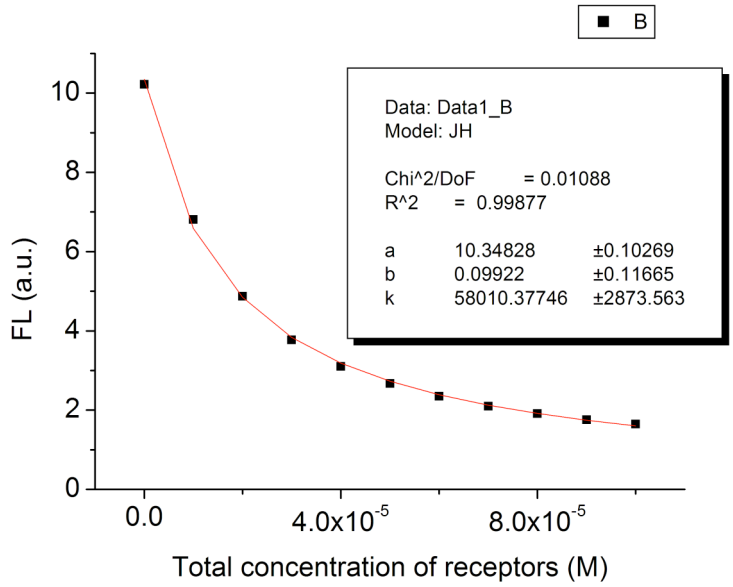


Fig. S16 The non-linear curve fitting of model receptor **2** with eq.2.

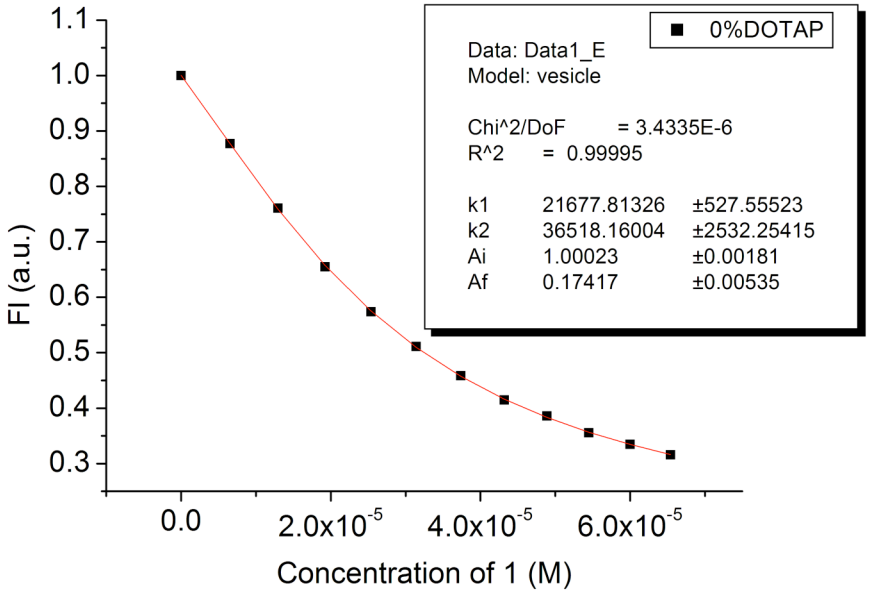


Fig. S17 A representative non-linear curve fitting of 20% receptor **1** in POPC vesicles with eq. (1).

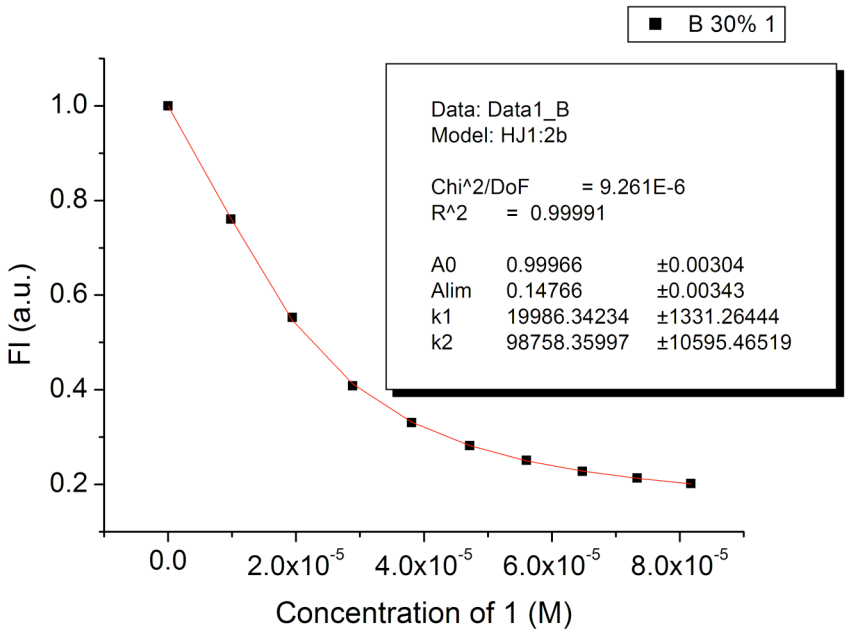


Fig. S18 A representative non-linear curve fitting of 30% receptor **1** in POPC vesicles with eq. (1).

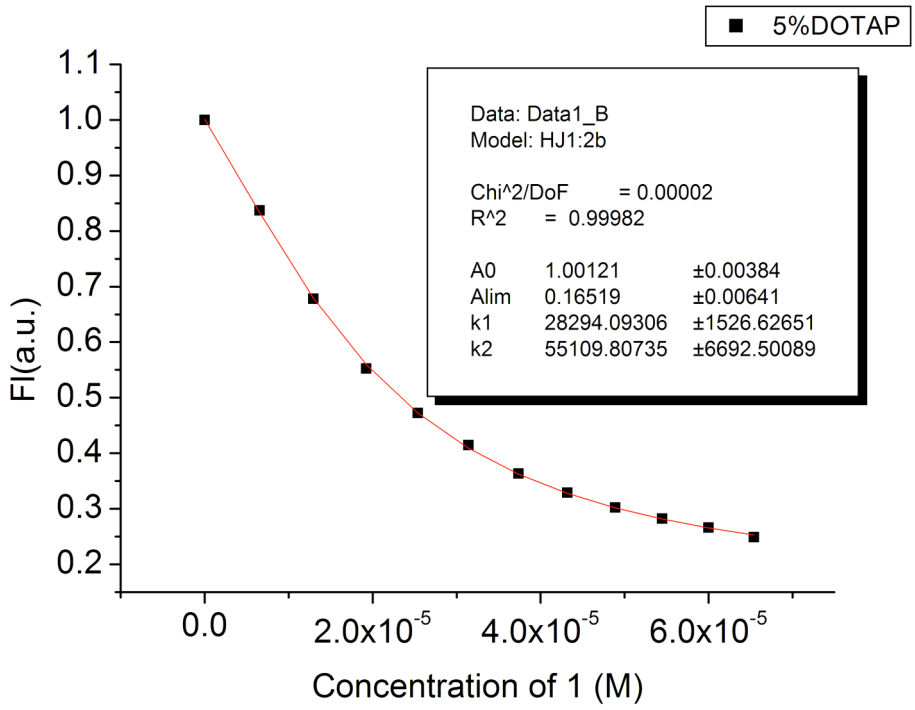


Fig. S19 Non-linear curve fitting of 20% receptor **1** in POPC vesicles also containing 5% DOTAP with eq. (1).

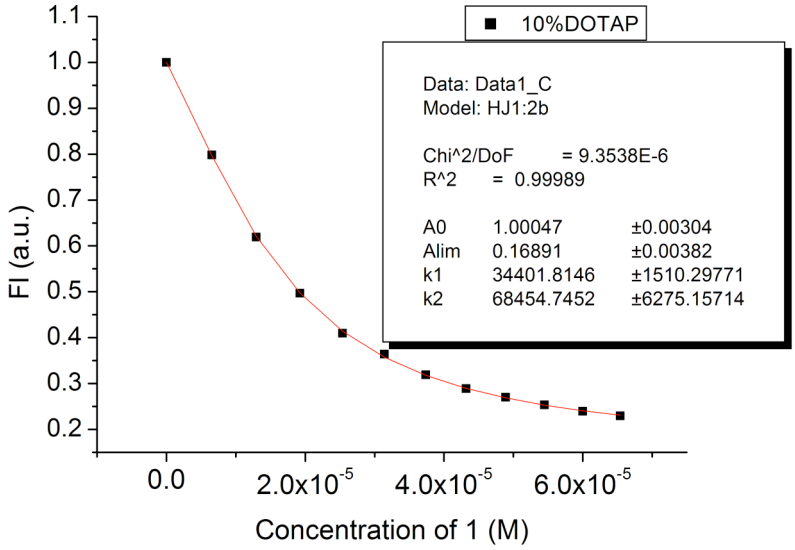


Fig. S20 Non-linear curve fitting of 20% receptor **1** in POPC vesicles also containing 10% DOTAP with eq. (1).

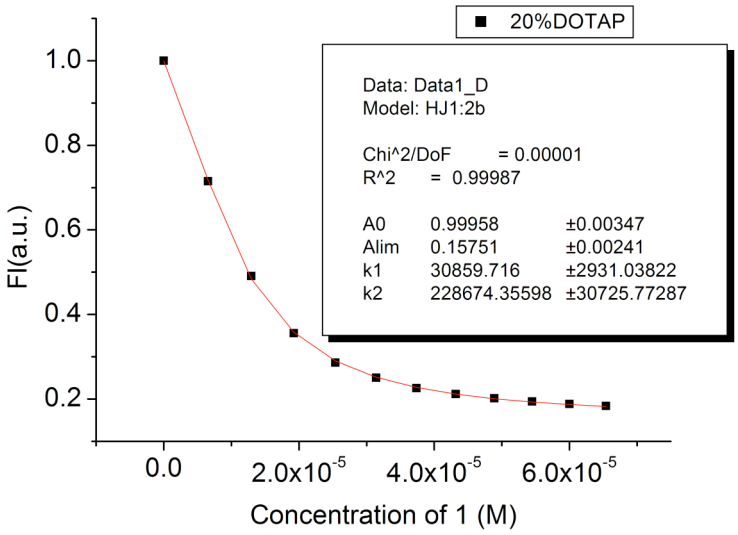


Fig. S21 Non-linear curve fitting of 20% receptor **1** in POPC vesicles also containing 20 % DOTAP with eq. (1).