Electronic Supplementary Information

A Hydrophilic Unimolecular Nanocapsule with Cyclodextrin Moieties in the Core: Chemically Triggered On-demand Release and pH-Response

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Instruments. The ^{1}H and ^{13}C NMR spectra were recorded using JEOL JNM-EX270 and JNM-A400II instruments with the residual proton of D_2O as a reference. The ultraviolet-visible (UV-vis) spectrum was measured using a Jasco V-550 spectrophotometer equipped with a Jasco ETC-505T temperature controller, and all measurements were carried out at 23°C. Elemental analysis was conducted using a Yanaco CHN corder MT-6 (Center for Instrumental Analysis, Hokkaido University). The fluorescence spectra were measured in a 10 mm path length cell using a Jasco FP-6300 spectrofluorometer equipped with a Jasco ETC-505T temperature controller. All measurements were carried out at 23.0 ±0.1 °C. The number-average molecular weight (M_n) and molecular weight distribution (M_w/M_n) were determined by gel permeation chromatography (GPC) using a Waters 150-C, calibrated with standard poly(styrene); eluent: DMF + 100 mM LiCl; flow rate: 1mL/min; sample concentration: 10 mg/mL; injection volume 200 μL.

Materials. α-Methyl-ω-aminopropoxy-polyoxyethylene (PEO-NH_2, M_n = 2 200) was purchased from Nippon Oil & Fats (NOF) Co., Ltd (Tokyo, Japan). Hyperbranched polyethylenimne (PEI, $M_n = 1 \times 10^4$, M_w/M_n = 2.5, degree of branch = 60%) was purchased from Aldrich. 4-(Dimethylamino) pyridine (DMAP, >99%), Rose bengal (RB), and 1-ethyl-(3,3-dimethylaminopropyl carbodiimide) hydrochloride (EDC) were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Methyl blue (MeB), methyl orange (MO), pyrene (PY) and PBS buffer (pH 7.4 and 6.4, 0.067 M phosphate) were purchased from Kanto Chemical Co., Ltd. (Tokyo, Japan). Methylene blue (MB), Nile red and sucinnic anhydride were purchased from TCI. β-Cyclodextrin (β-CD) was purchased from Junsei Chem Co., Ltd. The mono-6-(*p*-tosyl)- β-cyclodextrin was prepared based on a literature.[1] All chemicals were used without further purification unless otherwise stated.

Synthesis

CD_{24}-PEI.[2] A solution of PEI (0.34 g, 7.9 mmol equivalent NH), Mono-6-(*p*-tosyl)- β-CD (1.67 g, 1.3 mmol) in dry DMSO (5 ml) was prepared and stirred at 60°C under a nitrogen atmosphere for 66 h. The solution was diluted

with water and dialyzed against 500 ml of water (the water was changed every 12h) using a Spectro/Por dialyzing tube (MWCO 3500) for 3d. It was then freeze-dried to yield a white powder (1.26 g, 62.7%). ^{1}H NMR (D_2O, δ/ppm): 2.2-2.85 (C6-H of CD attached to PEI and CH_2CH_2 of PEI), 3.3-3.9 (C2-C6-H of CD), 4.92 (C1-H of CD). From the comparison of the signal at 4.92 ppm with that around 2.2-2.85 ppm, it could be derived that a 10.3 mol% of the amino proton was substituted by CD. Elemental analysis, C: 45.23%, H: 7.60%, N: 8.14%, which corresponded to an 11.5 mol% substitution degree. The ^{1}H NMR result was used to calculate the molecular weight of the resulting polymer: $M_n = (1135\text{-}18) \times 24 + 10000 = 3.68 \times 10^4$, where 1135 and 24 are the molecular weight and number of CD moieties attached to PEI, and 10000 was the molecular weight of PEI.

PEO-COOH. A solution of PEO-NH_2 (1.1 g, 0.5 mmol), succinic anhydride (0.1 g, 1 mmol) and DMAP (0.112 g, 1 mmol) in dry methylene chloride (8 ml) was stirred at room temperature for 36 h. The polymer was purified 3 times by dissolution/precipitation in chloroform/diethyl ether. Yield 1.15g (96%). ^{1}H NMR ($CDCl_3$, δ/ppm): 1.78 (m, 2H, $OCH_2C\mathit{H}_2CH_2N$), 2.54 (t, 2H, NC=O-$C\mathit{H}_2CH_2COOH$), 2.64 (t, 2H, NC=O-$CH_2C\mathit{H}_2COOH$), 3.40 (s, 3H, CH_3O), 3.5-3.9 (s, 194H, $C\mathit{H}_2C\mathit{H}_2O$, CH_2N), 6.98 (s, 1H, CONH). Additional signal: 3.16 (s, CH_3 of DMAP), 6.6 and 8.2 (aromatic protons of DMAP). A ~100% functionalization of PEO-NH_2 was obtained, as judged from the ^{1}H NMR integral intensity ($I_{1.78}/I_{3.40} \times 1.5 \approx 100\%$).

CD_{24}-PEI@PEO_{37} (NC2). An aqueous solution of CD_{24}-PEI (0.162 g, 1 mmol NH), PEO-COOH (0.5 g, 0.22 mmol), and EDC (0.2 g, 1 mmol) in water (8 ml) was prepared and stirred at room temperature for 3d. The mixture was dialyzed against water (Spectro/Por, MWCO 3500) at pH 5 for 12 h, then at pH 7 for 48 h (change water every 12 h). It was freeze-dried to yield 0.46 g (88.0%) of a white powder. ^{1}H NMR (D_2O, δ/ppm): 1.64 (m, 2H, $CH_2C\mathit{H}_2CH_2N$), 2.30 (t, 2H, $NHCOCH_2C\mathit{H}_2CONH$) and 2.38 (2H, $NHCOC\mathit{H}_2CH_2CONH$), 3.25 (s, 3H, CH_3O), 2.5-4.0 (m, 285H, other signal of CD, PEO and PEI), 4.92 (s, 4.47H, C1-H of CD). The molecular weight was calculated from the spectral integration by the following equation: $M_n = (I_{1.64}/2)/(I_{4.92}/7) \times 24 \times 2264 + 3.68 \times 10^4 = 12.2 \times 10^4$, corresponding to CD24-PEI@PEO37, where $I_{1.64}$ and $I_{4.92}$ are the integral intensity at 1.64 and 4.92 ppm, respectively, and 2264 is the molecular weight of the PEO moieties. FTIR (KBr, cm^{-1}): 3400 (NH, OH, CONH), 1645 (C=O), 1105 (C-O). $M_n = 6.7 \times 10^4$, $M_w/M_n = 1.97$.

PEI@PEO_{42} (NC1). An aqueous solution of PEI (0.028 g. 0.65 mmol NH), PEO-COOH (0.3 g, 0.13 mmol) and EDC (0.07 g, 0.36 mmol) in water (8 ml) was prepared and stirred at room temperature for 3d. The purification was similar to NC2 that yielded 0.295g (90.0%) of a white powder. ^{1}H NMR (D_2O, δ/ppm): 1.64 (m, 2H, $CH_2C\mathit{H}_2CH_2N$), 2.0-3.0 (t, 4H, $NHCOC\mathit{H}_2C\mathit{H}_2CONH$ + 22 H CH_2CH_2N), 3.25 (s, CH_3O), 3.0-4.0 (m, 188H, signal due to PEO and $C\mathit{H}_2NHCO$). From the comparison of the signal around 2.0-3.0 ppm with that around 1.64 ppm, it could be derived that 18.0 mol% of the amino proton was functionalized by PEO, corresponding to PEI @PEO_{42} ($M_n = 10.5 \times 10^4$). The M_n was calculated by $2I_{1.64}/(I_{2\text{-}3} - 2I_{1.64}) \times 2264 + 10000$, where $I_{1.64}$ and $I_{2\text{-}3}$ were the integral intensities at 1.64 and 2–3 ppm, respectively; 2264 is the molecular weight of the PEO moieties. FTIR (KBr, cm^{-1}): 3400 (NH, OH, CONH), 1645 (C=O), 1105 (C-O). $M_n = 6.1 \times 10^4$, $M_w/M_n = 1.99$.

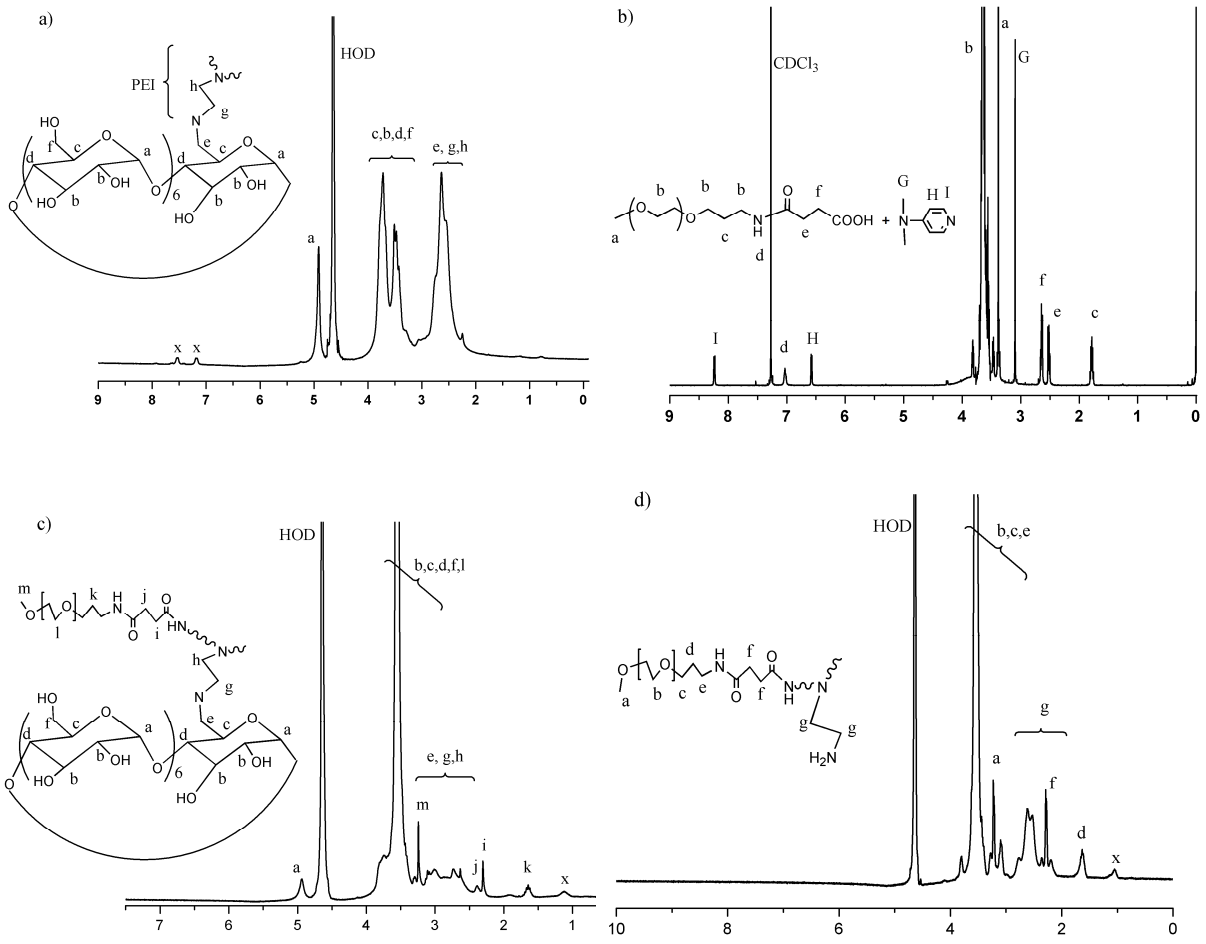

Fig. S1 ^{1}H NMR of (a) CD_{24}-PEI in D_2O, (b) PEO-COOH in $CDCl_3$, (c) NC2 in D_2O, and (d) NC1 in D_2O. (The signals marked with x represent impurities).

General procedure for UV/vis measurement (Fig. 1A)

All the aqueous solutions of NC1, NC2, PY, and dye were prepared using the PBS buffered water. For the PY saturated solution, excess crystalline PY was added to the corresponding solution and stirred overnight, and the mixture was then filtered to remove the solid. When a precise amount of PY was needed, an acetone solution of PY with the precise content of PY was prepared, and certain volume of the solution was subjected to rotary evaporation to obtain the precise weight of the pure PY.

Fluorescent titration for the encapsulation of Pyrene by NC2 (Fig. 1B and Fig. 4)

A 3 ml PY-saturated water (pH 7.4, buffer) was titrated with aqueous NC2 ($1 \times 10^{-4} \sim 6.1 \times 10^{-9}$M (equivalent [CD] = $2.4 \times 10^{-3} \sim 1.46 \times 10^{-7}$ M) in water (pH 7.4 buffer). The titration was monitored by the fluorescent spectra of PY, whose I_1/I_3 ratio was recorded and plotted as a function of the [CD]/[PY]. The excitation wavelength was set at 335 nm, and emission was recorded within 350-550 nm.

Critical aggregation concentration (CAC) of NC1 and NC2 (Fig. S2)[2-6]

The CAC of NC1 was measured by employing either PY or Nile red as a fluorescent probe, while that of NC2 was measured only by Nile red. Typically, the NC2 (saturated with PY) samples at the diverse concentrations of 3×10^{-8} ~ 2×10^{-5} M in water were prepared; each sample (4 ml) was mixed with a 4 μl acetone solution of Nile red (0.4 mg·ml^{-1}). Each sample was subjected to a fluorescence measurement. The excitation wavelength was set at 555 nm, and emission was recorded within 560-740 nm. The fluorescent intensity was plotted as a function of the concentration of NC2. When PY was used as the fluorescent probe, the measurement was similar to the fluorescent titration.

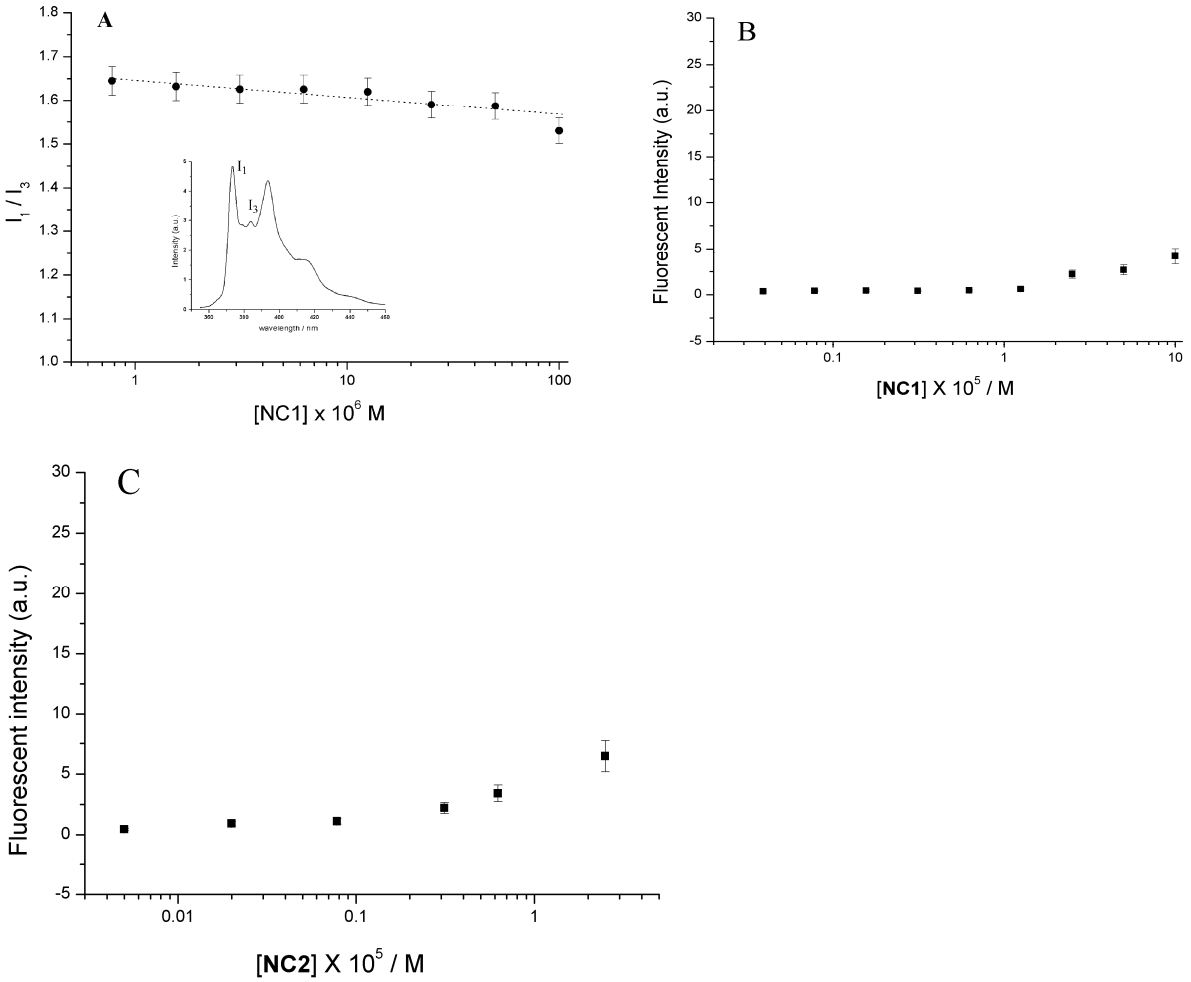

Fig. S2 PY (A) and Nile red (B, C) as Fluorescent probe for the detection of CAC of NC1 (A, B) and NC2 saturated with pyrene (C). The insert in A shows a typical fluorescent spectrum of PY, where I_1 and I_3 are shown. Conditions: pH 7.4 (PBS buffer); for PY, [PY] = 6.9×10^{-7} M, λ_{ex} = 335 nm and emission within 350~460 nm was recorded; for Nile red, [Nile red] = 1.26×10^{-6} M, λ_{ex} = 555 nm and emission within 560~720 nm was recorded.

PY release triggered by water-soluble dyes (Fig. 3)

Typically, a stock solution of NC2 (2.3×10^{-7} M) saturated with PY in buffered water (PBS, pH 7.4) was prepared. Three ml of the stock solution was titrated with aqueous RB (1×10^{-4} M in buffered water, pH 7.4). Initially, 14 μl was added each time, and the fluorescent spectrum of PY was monitored by the I_1/I_3 ratio. When I_1/I_3 ratio was no

longer sensitive to the addition of RB, a larger volume (e.g. 70 μl) of RB could be added each time. The excitation wavelength was λ_{ex} = 335 nm, and emission within 350-550 nm was recorded. In a control experiment, the β-CDs were used in place of NC2 (with an equivalent amount of CD), and the titration was similarly carried out. The titration with MO, MeB and MB was also similarly carried out.

Biphasic extraction of dyes from water to chloroform by NC2 (Fig. 2)

An arbitrary aqueous solution of MO, RB, MeB and MB was prepared and mixed with NC2 in chloroform (5×10^{-6} M), the mixture was subjected to vigorous shaking and then long time standing (>12h). MO and RB can be transferred to the organic phase but MeB and MB can't be.

UV/vis titration for the encapsulation of RB (Fig. S3)[7]

A stock solution of RB (6×10^{-6} M, 3ml) in buffered water (pH 7.4) was titrated with aqueous NC2 (1.8~ 18 μl each time) in buffered water (pH 7.4), and the UV/vis absorbance within 500-650 nm was recorded. The ratio of A_{563}/A_{547} (after correction of dilution) was plotted as a function of the [RB]/[NC2] ratio. For [RB]/[NC2] = 0.001:1~ 0.009: 1, NC2 at 10^{-5} M was used; for [RB]/[NC2] = 0.01:1~ 0.09: 1, NC2 at 10^{-4} M was used; for [RB]/[NC2] = 0.1:1~ 11: 1, NC2 at 10^{-3} M was used. All buffered solutions were prepared with PBS.

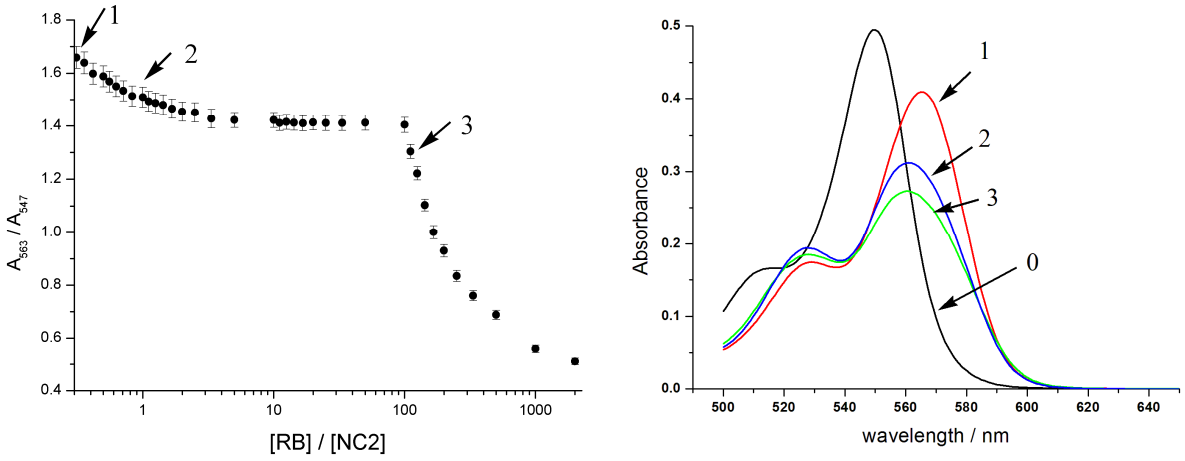

Fig. S3 (Left) UV/vis titration of RB (6×10^{-6} M) with NC2, in which the ratio of absorbance at 563 nm and 547 nm (A_{563}/A_{547}) was plotted as a function of [RB]/[NC2], (Right) several typical UV/vis spectra marked **0**, **1**, **2**, and **3**, which correspond to [RB]/[NC2] = 0 (**0**), 0.04 (**1**); 1 (**2**) and 111 (**3**). Conditions: pH 7.4 (PBS buffer) in water.

Detection of the released PY by fluorescence (Fig. S4)

To an aqueous solution of NC2 (8.2×10^{-6} M, 3 ml, saturated with PY) was added aqueous RB (1×10^{-3} M, 0.74 ml) and the fluorescent spectrum was recorded, in which the excitation wavelength was 335 nm. For comparison, the RB solution was replaced by an equivalent volume of fresh water and subjected to a similar measurement.

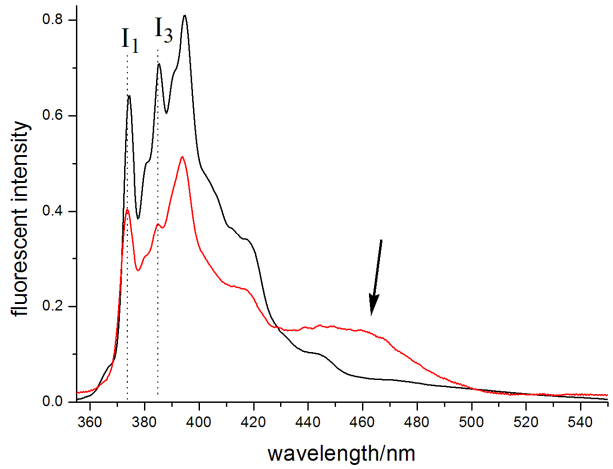

Fig. S4 Fluorescent spectra of aqueous NC2 (8.2×10^{-6} M, 3 ml, saturated with PY) before (black line) and after (red line) the addition of aqueous RB (1×10^{-3} M, 0.74 ml). Conditions: [RB]/[NC2] = 30; pH 7.4 (PBS buffer); λ_{ex} = 335 nm; the intensity of the black line was reduced 300-fold. The signal marked with an arrow corresponds to tiny crystals of PY.

Detection of the released PY by UV/vis (Fig. S5)

To an aqueous NC2 saturated with PY (1×10^{-6} M, 3.45 ml) was added aqueous RB (1×10^{-3} M, 56 µl), and the solution was subjected to a UV/vis measurement. For comparison, 56 µl of water was used in place of the aqueous RB and the measurement was similarly carried out. NC2 without PY was also prepared with identical concentrations for similar measurements.

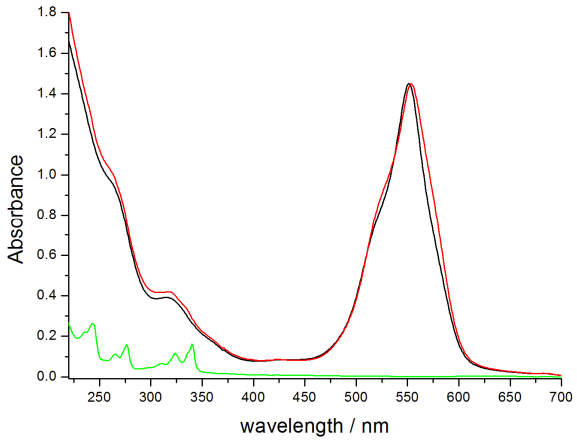

Fig. S5 UV/vis spectra of NC2 saturated with PY before (green line) and after (red line) the addition of the aqueous RB. For comparison, UV/vis spectrum of NC2/RB without PY is also presented (black line). Conditions: [NC2] = 0.98×10^{-6} M; [RB] = 1.6×10^{-5} M; pH 7.4 (PBS buffer); the red line and black line are normalized at λ = 547 nm.

Detection of the RB–β-CD interaction (Fig. S6)

To learn if RB could replace the PY trapped in the small β-CD, the following experiment was designed: to an aqueous solution of β-CD (2.4×10^{-7} M, 3ml) saturated with PY, aqueous RB (1×10^{-4} M) was added (3.6 μl each time) and the fluorescent spectra (λ_{ex} = 335 nm) were recorded, in which the corresponding I_1/I_3 ratio was plotted as a function of the [RB]/[β-CD] ratio. The UV/vis technique was also employed for the RB-CD interaction: to a 3−ml aqueous solution of RB (1.54×10^{-5} M), solid β-CD (0.08 mg) was added, and the UV/vis spectra before and after the addition were recorded.

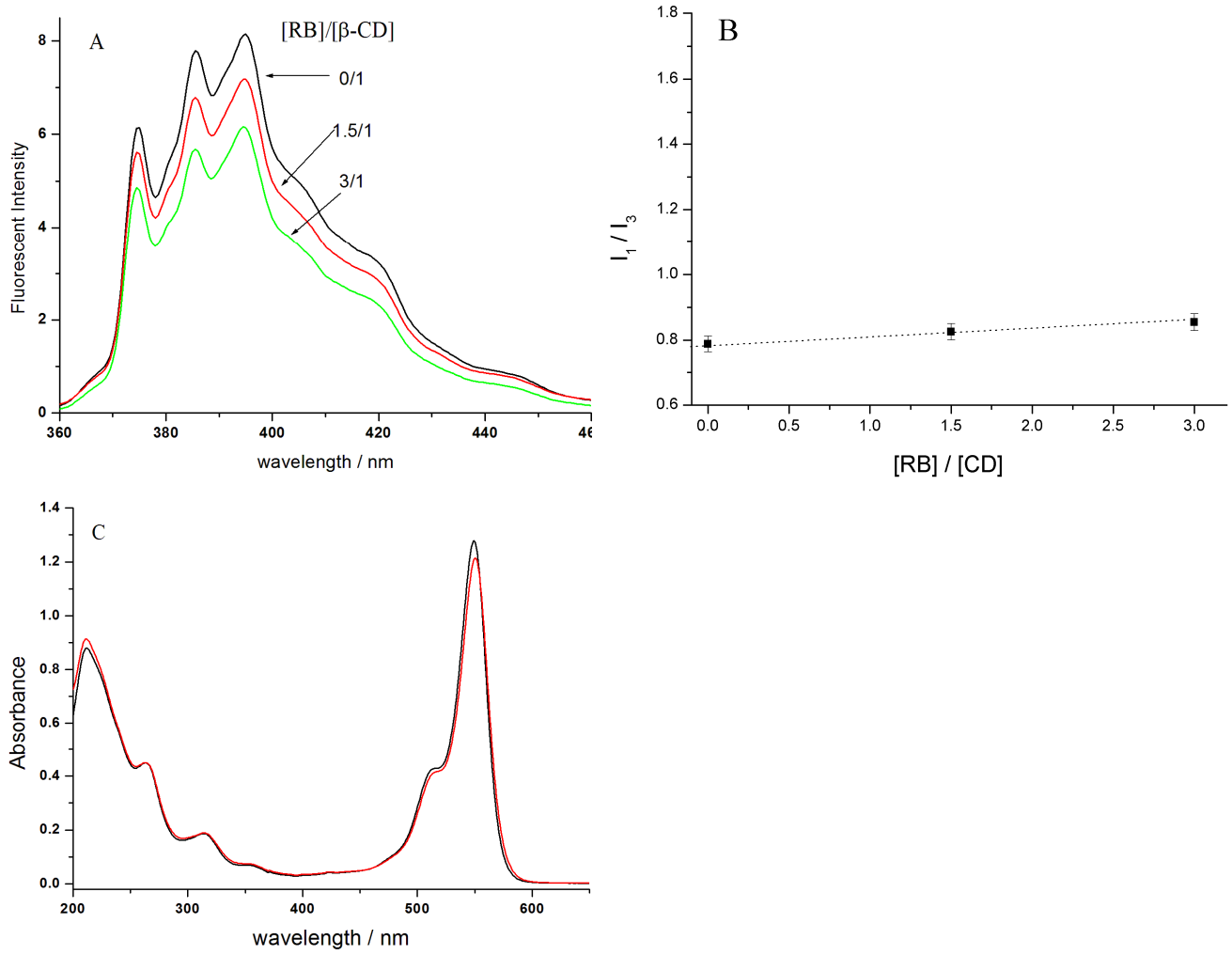

Fig. S6 (A) small β-CD, after being saturated with PY, was titrated with RB and the fluorescent spectra were recorded, (B) the corresponding I_1/I_3 ratio was plotted as a function of the [RB]/[β-CD] ratio. Conditions: [RB] = 1×10^{-4} M; [CD] = 2.4×10^{-7} M; water as solvent; λ_{ex} = 335 nm. (C) The UV/vis spectral of RB before (black line) and after (red line) the addition of small β-CD. [RB] = 1.54×10^{-5} M; [β-CD] = 2.4×10^{-5} M; solvent: water.

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