Supporting Information

Highlighting Cancer Cells with Halochromic Switches

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Experimental Procedures

Materials and Methods. Chemicals were purchased from commercial sources and used as received. MeCN was distilled over CaH_2. THF was distilled over Na and benzophenone. H_2O (18.2 MΩ-cm) was purified with a Barnstead International NANOpure DIamond Analytical system. Compounds **1**, **3**, **4**, **6**, **17**, **18** and **20** were prepared according to literature procedures.[S1–6] GPC was performed with a Phenomenex Phenogel 5-μm MXM column (7.8 × 300 mm) operated with a Nexera X2 system, in THF at a flow rate of 1.0 mL min^{-1} and a detection wavelength of 250 nm for **5**, **7** and **12** and 290 nm for **8** and **13**. Monodisperse polystyrene standards (3,700–250,000) were employed to determine the M_n and polydispersity index (PDI) of the polymers from the GPC traces, following a literature protocol.[S7] NMR spectra were recorded with a Bruker Avance 400 spectrometer. DLS and SLS measurements were performed with a Malvern ZEN1600 apparatus. The values reported for the hydrodynamic diameter are averaged over ten independent experiments of ten runs of 10 s each. The values reported for the average supramolecular weight were determined from the concentration dependence of the scattering intensity, following a literature protocol.[S7] Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path length of 1.0 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Fluorescence quantum yields were determined against a methanol solution of the acetate salt of cresyl violet (ϕ = 0.54), following a literature protocol.[S8] Fluorescence images were recorded with a Leica SP5 confocal laser-scanning microscope.

5. A solution of **3** (452 mg, 2 mmol), **4** (1.0 g, 0.5 mmol) and AIBN (0.5 mg, 0.003 mmol) in degassed THF (8 mL) was heated at 75 °C for 3 days in a sealed tube. After cooling down in an ice bath, the solvent was distilled off under reduced pressure. The residue was purified by column chromatography [SiO_2, $CHCl_3$/MeOH (40:1, v/v)] to give **5** (872 mg) as a white solid. GPC: M_n = 21.0 kDa, PDI = 1.16. ^{1}H NMR ($CDCl_3$): δ = 0.84–0.93 (10H, br s), 1.22–1.39 (42H, br s), 1.69–1.77 (8H, br s), 3.37–3.41 (3H, s), 3.54–3.77 (176H, m), 3.87–4.02 (6H, br s), 4.06–4.16 (2H, br s).

7. A solution of **3** (904 mg, 4.0 mmol), **4** (2.0 g, 1.0 mmol), **6** (65 mg, 0.1 mmol) and AIBN (5 mg, 0.03 mmol) in degassed THF (20 mL) was heated at 75 °C for 3 days in a sealed tube. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure. The residue was purified by column chromatography [SiO_2, CH_2Cl_2/MeOH (20:1, v/v)] to give **7** (2.0 g) as a white solid. GPC: M_n = 16.7 kDa, PDI = 1.43; ^{1}H NMR ($CDCl_3$): δ = 0.84–0.93 (10H, br s), 1.22–1.39 (42H, br s), 1.69–1.77 (8H, br s), 2.25–2.32 (0.2H, m), 2.46–2.56 (0.2H, m), 3.37–3.41 (3H, s), 3.54–3.77 (180H, m), 3.87–4.02 (6H, br s), 4.06–4.16 (2H, br s).

8. A solution of **7** (146 mg, 0.06 mmol), folic acid (265 mg, 0.6 mmol), DCC (124 mg, 0.6 mmol) and DMAP (73 mg, 0.6 mmol) in DMSO (20 mL) was stirred at 40 °C for 3 days. The resulting precipitate was filtered off, dissolved in CH_2Cl_2 (150 mL) and washed with aqueous NaCl (1M, 6 × 100 mL). The solvent was distilled off under reduced pressure and the residue was purified by column chromatography [SiO_2, CH_2Cl_2/MeOH (10:1, v/v)] to afford **8** (42 mg) as a red solid. GPC: M_n = 19.2 kDa, PDI = 1.44; ^{1}H NMR ($CDCl_3$): δ = 0.84–0.93 (10H, br s), 1.22–1.39 (42H, br s), 1.69–1.77 (8H, br s), 3.37–3.41 (3H, s), 3.54–3.77 (176H, m), 3.87–4.02 (6H, br s), 4.06–4.16 (2H, br s).

9. A solution of 10-azidodecan-1-amine (990 mg, 5 mmol), methacrylic acid (516 mg, 6 mmol), DCC (1030 mg, 5 mmol) and DMAP (610 mg, 5 mmol) in CH_2Cl_2 (20 mL) was stirred at ambient temperature for 24 hours. The resulting precipitate was filtered off and the residue was purified by column chromatography [SiO_2, hexane/EtOAc (5:1, v/v)] to afford **9** (839 mg, 63%) as a colorless liquid. ESIMS: m/z = 267.1121 [M]+ (calcd. for $C_{14}H_{26}N_4O$ = 266.3890); ^{1}H NMR ($CDCl_3$): δ = 1.27–1.14 (14H, m), 1.40–1.59 (4H, m), 1.88 (3H, s), 3.12–3.27 (2H, m), 5.21 (1H, s), 5.60 (1H, s), 6.18 (1H, br s).

10. A solution of **4** (904 mg, 4.0 mmol), **6** (65 mg, 0.1 mmol), **9** (2.0 g, 1.0 mmol) and AIBN (5 mg, 0.03 mmol) in degassed THF (20 mL) was heated at 75 °C for 3 days in a sealed tube. After cooling down to ambient temperature, the

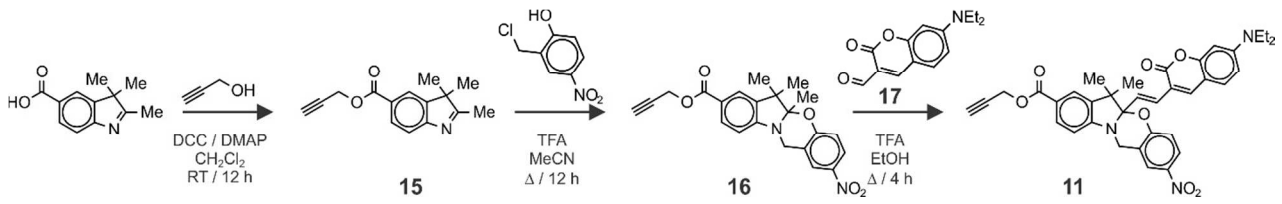

Figure S1. Synthesis of **11**.

solvent was distilled off under reduced pressure. The residue was purified column chromatography (Sephadex LH-20, MeOH) to give **10** (1.89 g) as a white solid. GPC: M_n = 17.2 kDa, PDI = 1.15; ^{1}H NMR ($CDCl_3$): δ = 0.95–1.08 (2H, br s), 1.21–1.42 (37H, br s), 1.89–2.09 (5H, br s), 2.23–2.30 (3H, br s), 3.23–3.30 (2H, br s), 3.38 (3H, s), 3.45–3.83 (176H, m), 3.87–4.01 (2H, br s), 4.03–4.20 (2H, br s).

11. A solution of **15** (204 mg, 0.5 mmol), **17** (147 mg, 0.6 mmol) and TFA (250 μL) in EtOH (20 mL) was heated under reflux for 4 hours. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was purified by column chromatography [SiO_2, hexane/EtOAc (3:1, v/v)] to afford **11** (241 mg, 76%) as a green solid. ESIMS: *m/z* = 634.2562 $[M]^+$ (calcd. for $C_{37}H_{35}N_3O_7$ = 633.7010); ^{1}H NMR ($CDCl_3$): δ = 0.87 (6H, t), 1.22 (6H, s), 2.03 (1H, s), 2.61–2.70 (2H, m), 3.43 (4H, q), 4.40 (2H, t), 4.66 (2H, d, 3 Hz), 6.49 (1H, s), 6.57–6.66 (3H, m), 6.88–6.99 (2H, m), 7.25 (1H, d, 8 Hz), 7.52 (1H, s), 7.82 (1H, s), 7.90 (1H, d, 8 Hz), 7.98–8.06 (2H, m).

12. CuI (4 mg, 0.02 mmol) was added to a solution of **10** (64 mg, 0.1 mmol) and **11** (1.0 g) in degassed MeCN. The mixture was stirred at ambient temperature for 12 hours under Ar. The solvent was distilled off under reduced pressure and the residue was purified by column chromatography (Sephadex LH-20, MeOH) to give **12** (890 mg) as a green solid. GPC: M_n = 17.8 kDa, PDI = 1.21; ^{1}H NMR ($CDCl_3$): δ = 1.03–1.17 (3H, br s), 1.22–1.47 (45H, br s), 2.05–2.52 (8H, br s), 3.21–3.33 (2H, br s), 3.40 (3H, s), 3.52–3.83 (190H, m), 3.85–3.99 (3H, br s), 4.03–4.20 (2H, br s).

13. A solution of **12** (400 mg), folic acid (22 mg, 0.5 mmol), DCC (10 mg, 0.05 mmol) and DMAP (6 mg, 0.05 mmol) in DMSO (2 mL) was stirred at 40 °C for 4 days in the dark. After cooling down to ambient temperature, the resulting precipitate was filtered off and the filtrate diluted in CH_2Cl_2 (50 mL) and washed with aqueous NaCl (1M, 3 × 50 mL). The solvent was distilled off under reduced pressure and the residue was purified by column chromatography (Sephadex LH-20, MeOH) to give **13** (322 mg) as a green solid. GPC: M_n = 18.9 kDa, PDI = 1.45; ^{1}H NMR ($CDCl_3$): δ = 1.02–1.18 (3H, br s), 1.21–1.48 (46H, br s), 2.01–2.55 (8H, br s), 3.23–3.30 (2H, br s), 3.38 (3H, s), 3.50–3.83 (180H, m), 3.87–4.01 (3H, br s), 4.03–4.20 (2H, br s).

14. A solution of DCC (103 mg, 0.5 mmol), folic acid (220 mg, 0.5 mmol), **19** (120 mg, 0.05 mmol) and DMAP (61 mg, 0.5 mmol) in DMSO (20 mL) was stirred at 40 °C for 3 days. The resulting precipitate was filtered off and the mixture was dissolved in CH_2Cl_2 (150 mL) and washed with aqueous NaCl (1M, 6 × 100 mL). The solvent was distilled off under reduced pressure and the residue was purified by column chromatography [SiO_2, CH_2Cl_2/MeOH (10:1, v/v)] to afford **14** (24 mg, 17%) as a red solid. ^{1}H NMR [$(CD_3)_2SO$]: δ = 1.13 (6H, t, 8 Hz), 1.27 (6H, s),

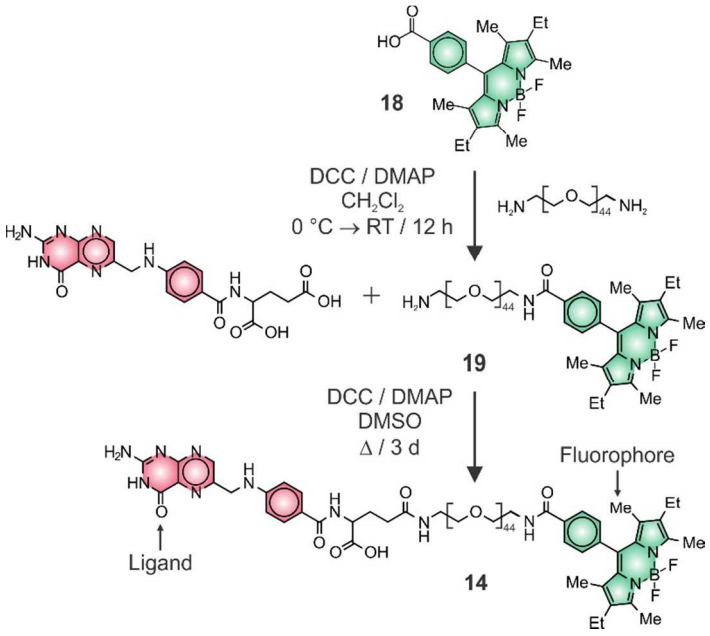

Figure S2. Synthesis of **14**.

2.29 (4H, m), 2.44 (4H, q, 8 Hz), 2.56 (6H, s), 3.27–3.48 (176H, m), 4.33 (1H, br), 4.46 (2H, br), 6.63 (2H, br), 6.91 (1H, br), 7.58 (2H, d, 8 Hz), 7.78 (1H, s), 8.09 (2H, d, 6 Hz), 8.64 (1H, s).

15. A solution of 2,3,3-trimethyl-5-carboxy-3*H*-indole (1015 mg, 5 mmol), propargyl alcohol (420 mg, 6 mmol), DCC (1030 mg, 5 mmol) and DMAP (610 mg, 5 mmol) in CH_2Cl_2 (20 mL) was stirred at ambient temperature for 12 hours. The resulting precipitate was filtered off and the residue was purified by column chromatography [SiO_2, hexane/EtOAc (3:1, v/v)] to afford **15** (800 mg, 62%) as a white solid. ESIMS: *m/z* = 256.1347 $[M]^+$ (calcd. for $C_{16}H_{17}NO_2$ = 255.3170); ^1H NMR ($CDCl_3$): δ = 1.32 (6H, s), 2.01 (1H, t, 3 Hz), 2.28 (3H, s), 2.53–2.58 (2H, m), 4.37 (2H, t, 9 Hz), 7.48 (1H, d, 8 Hz), 7.90 (1H, s), 7.99 (1H, d, 8 Hz).

16. A solution of **15** (400 mg, 1.6 mmol) and 2-chloromethyl-4-nitrophenol (375 mg, 2 mmol) in MeCN (30 mL) was heated under reflux for 12 hours. After cooling down to ambient temperature, the solvent was distilled off under reduced pressure and the residue was purified by column chromatography [SiO_2: hexane/EtOAc (4:1, v/v)] to afford **16** (204 mg, 32%) as a yellow solid. ESIMS: *m/z* = 407.1603 $[M]^+$ (calcd. for $C_{23}H_{22}N_2O_5$ = 406.4380); ^1H NMR ($CDCl_3$): δ = 1.19 (3H, s), 1.58 (6H, d, 8 Hz), 2.01 (1H, t, 3 Hz), 2.60–2.65 (2H, m), 4.37 (2H, t, 7 Hz), 4.67 (2H, d, 4 Hz), 6.60 (1H, d, 9 Hz), 6.73 (1H, d, 9 Hz), 7.79 (1H, d, 2 Hz), 7.87 (1H, dd, 2 and 2 Hz), 7.96 (1H, dd, 3 and 3 Hz), 8.01 (1H, d, 3 Hz).

19. A solution of DCC (41 mg, 0.2 mmol) in CH_2Cl_2 (5 mL) was added dropwise over 10 min to a solution of **18** (42 mg, 0.1 mmol), poly(ethylene glycol) bis(amine) (M_n = 2.0 kDa, 400 mg, 0.2 mmol) and DMAP (25 mg, 0.2 mmol) in CH_2Cl_2 (15 mL) maintained at 0 °C. The reaction was allowed to warm up to ambient temperature and then stirred for 12 hours under these conditions. The resulting precipitate was filtered off and the solvent of the filtrate was distilled off under reduced pressure. The residue was purified by column chromatography [SiO_2, CH_2Cl_2/MeOH (10:1, v/v)] to afford **19** (140 mg, 63%) as a red solid. ^1H NMR ($CDCl_3$): δ = 1.03 (6H, t, 8 Hz), 1.31 (6H, s), 2.35 (4H, q, 8 Hz), 2.57 (6H, s), 3.78–3.62 (176H, m), 7.38 (2H, d, 8 Hz), 8.00 (2H, d, 8 Hz).

Doped Polymer Nanoparticles for Spectroscopy. CH_2Cl_2 solutions of **1** (0.2 mM, 50 μL) and either **5** or **8** (2.5 mg mL^{-1}, 200 μL) were mixed and the solvent was distilled off under reduced pressure. The residue was dispersed in phosphate buffer (1.0 mL) with pH ranging from 3.0 to 10.0. The resulting dispersions were sonicated for 5 min, stored for 10 min at ambient temperature and used for the spectroscopic experiments without further purification. The concentration of **1** in the final solutions was estimated from the absorbance for the coumarin chromophore and the ε measured in acetonitrile, which is 17.6 mM^{-1} cm^{-1}. The concentration of the nanoparticles was determined from the mass of **5** and **8** and the corresponding average supramolecular weight, which is 588 and 435 kDa respectively according to SLS measurements. The ratio between the concentrations of molecular guest and supramolecular host is 23 in both instances. This value is the average number of pre-fluorophores per nanosized container.

Doped Polymer Nanoparticles for Imaging. CH_2Cl_2 solutions of **1** (0.5 mM, 40 μL) and either **5** or **8** (10 mg mL^{-1}, 100 μL) were mixed and the solvent was evaporated under reduced pressure. The residue was dispersed in Dulbecco's PBS (400 μL). The resulting dispersions were sonicated for 5 min, stored for 10 min at ambient temperature and passed through a syringe filter with a pore size of 200 nm. The filtrate was used for the imaging experiments without further purification.

Cell Culture. HEK-293 cells were cultured in Dulbecco's modified Eagle's media supplemented with fetal bovine serum (10% v/v), penicillin (100 U mL^{-1}) and streptomycin (0.01% v/v). MCF-7 cells were cultured in Roswell Park Memorial Institute medium supplemented with fetal bovine serum (10% v/v), penicillin (100 U mL^{-1}) and streptomycin (0.01% v/v). Cells were maintained in a humidified CO_2 (5% v/v) atmosphere at 37 °C, seeded in 96-well glass-bottom plates at a density of 10^5 cells cm^{-2} and incubated overnight. The culture medium was switched to L-15 and then a PBS solution (10 μL) of *(i)* nanoparticles of **5** containing **1**, (*ii*) nanoparticles of **8** containing **1**, (*iii*) **12** (5.0 mg mL^{-1}), *(iv)* **13** (5.0 mg mL^{-1}) or *(v)* a

mixture of **13** (5.0 mg mL^{-1}) and **14** (0.2 mM) was added to the cells (20 μL). For the inhibition experiments, a PBS solution of folic acid (0.02–0.50 M) was added to the cells and, after 0.5 hours, a PBS solution of **13** (5.0 mg mL^{-1}) was also added to the sample. All cells were incubated at 25 °C for 2–24 hours and imaged without any washing.

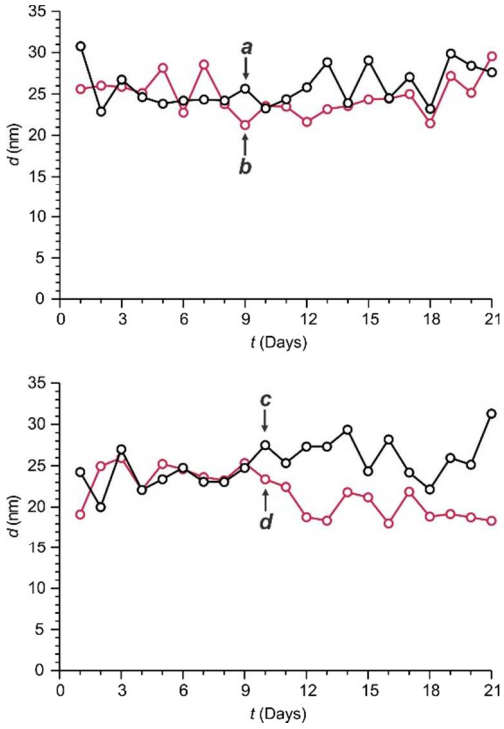

Figure S3. Temporal evolutions of the average hydrodynamic dynamic diameter of **5** (***a***), **8** (***b***), **12** (***c***) and **13** (***d***) in buffer at a pH of 7.15.

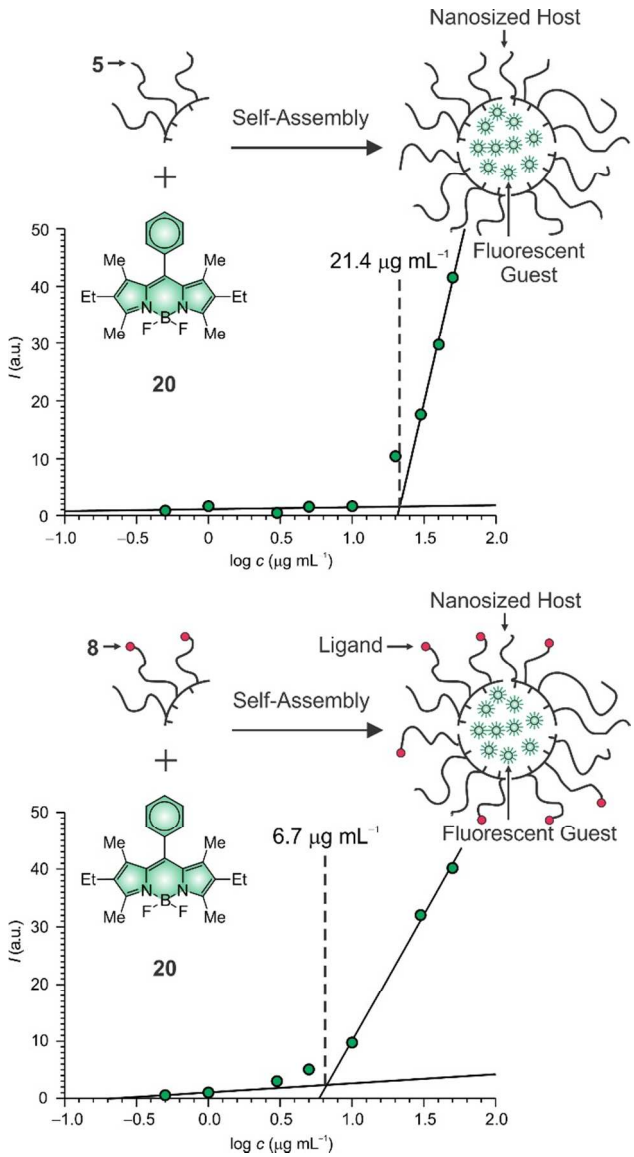

Figure S4. Plots of the emission intensity (λ_{Ex} = 500 nm, λ_{Em} = 540 nm), recorded at 25 °C after combining CH_2Cl_2 solutions of **20** (0.1 mM, 10 μL) and either **5** or **8** (50 μg mL^{-1}, 20–100 μl or 500 μg mL^{-1}, 100–1000 μL), distilling the solvent off under reduced pressure, dispersing the residue in buffer (1.0 mL) with pH of 7.15 and passing the resulting dispersion through a nanoporous membrane (200 nm), against the polymer concentration.

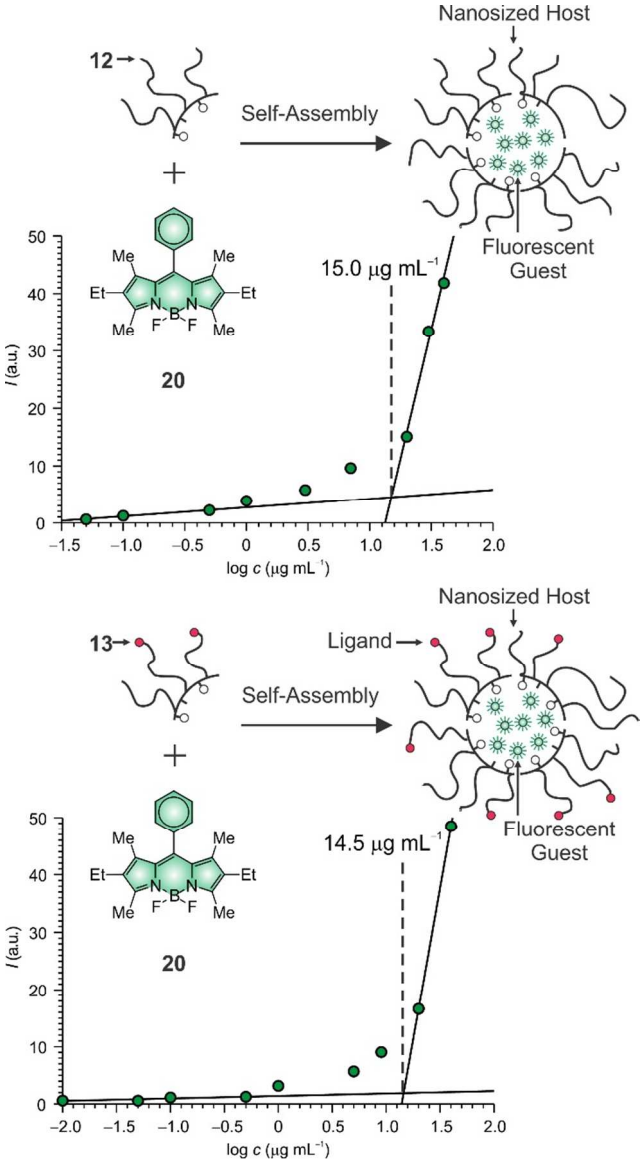

Figure S5. Plots of the emission intensity (λ_{Ex} = 500 nm, λ_{Em} = 540 nm), recorded at 25 °C after combining CH_2Cl_2 solutions of **20** (0.1 mM, 10 μL) and either **12** or **13** (50 μg mL^{-1}, 20–100 μl or 500 μg mL^{-1}, 100–1000 μL), distilling the solvent off under reduced pressure, dispersing the residue in buffer (1.0 mL) with pH of 7.15 and passing the resulting dispersion through a nanoporous membrane (200 nm), against the polymer concentration.

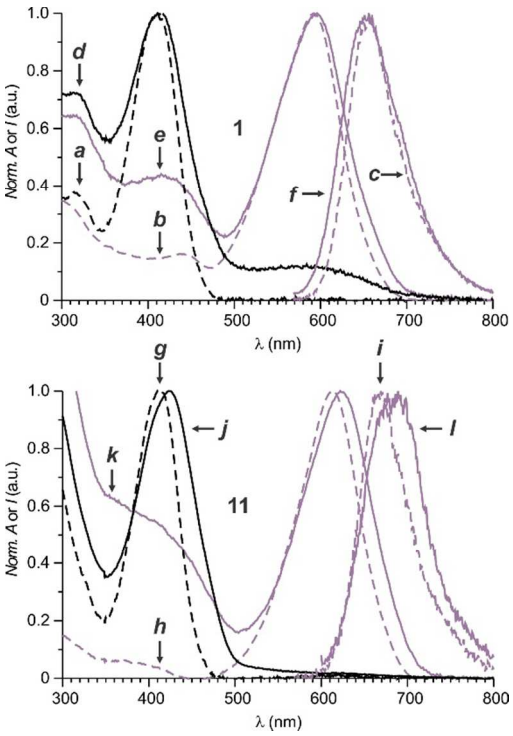

Figure S6. Normalized absorption and emission (λ_{Ex} = 560 nm) spectra of **1** (10 μM), before (***a***) and after (***b*** and ***c***) the addition of CF_3CO_2H (1 eq.) in MeCN, of **1** and **5** in buffer with pH of 7.15 (***d***) or 4.01 (***e*** and ***f***), of **11** (10 μM), before (***g***) and after (***h*** and ***i***) the addition of CF_3CO_2H (10 eq.) in MeCN, and of **12** (500 μg mL^{-1}) in buffer at a pH of 6.88 (***j***) or 3.02 (***k*** and ***l***).

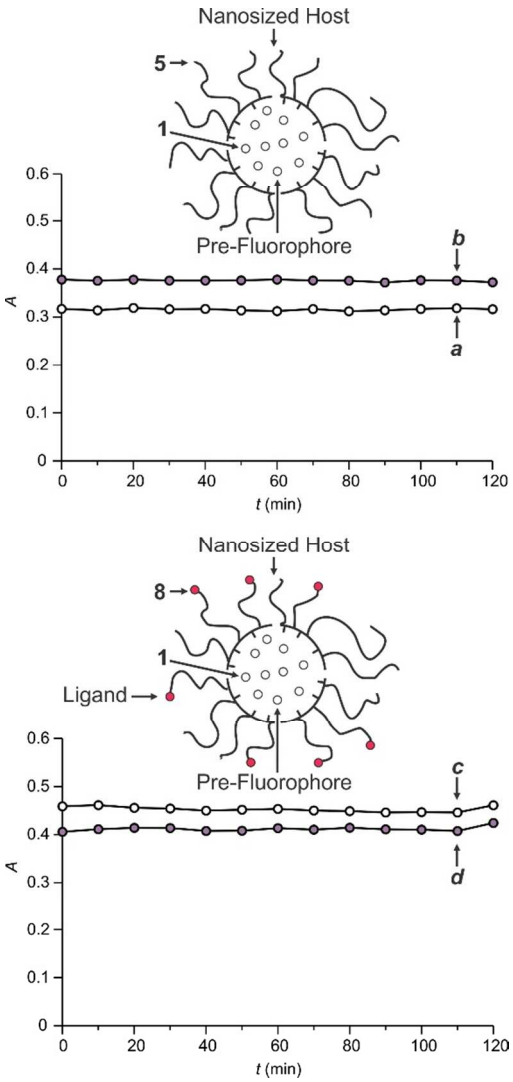

Figure S7. Temporal evolution of the absorbance detected at 414 (***a*** and ***c***) and 596 nm (***b*** and ***d***) for either **1** and **5** or **1** and **8** in buffer with pH of either 7.15 (***a*** and ***c***) or 4.01 (***b*** and ***d***).

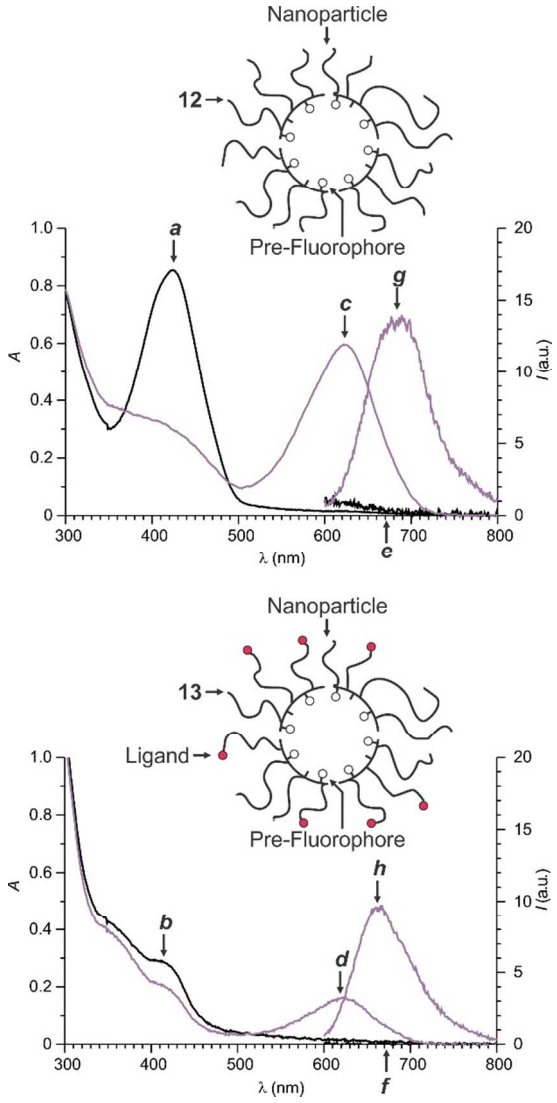

Figure S8. Absorption and emission (λ_{Ex} = 560 nm) spectra of **12** in buffer at a pH of 6.88 (***a*** and ***e***) or 3.02 (***c*** and ***g***) and of **13** in buffer at a pH of 6.88 (***b*** and ***f***) or 3.02 (***d*** and ***h***).

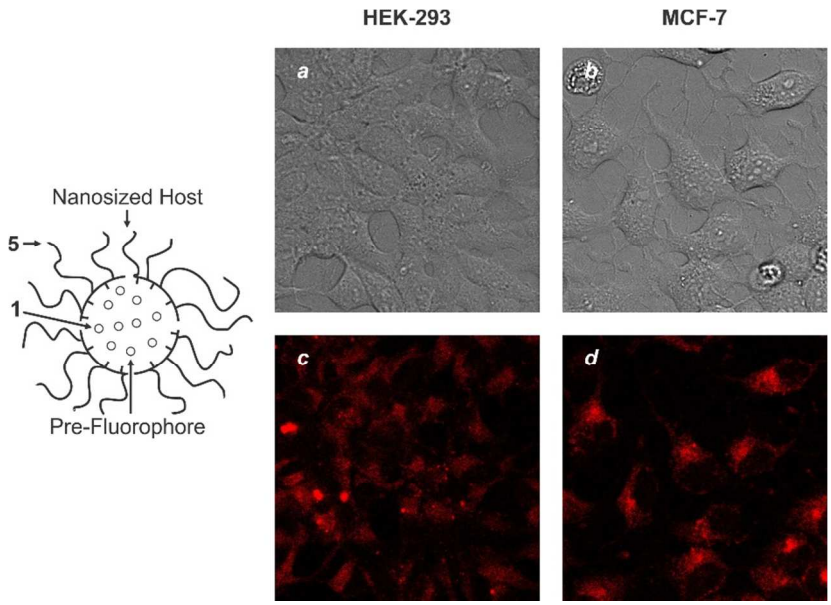

Figure S9. Transmittance (***a*** and ***c***) and fluorescence (***b*** and ***d***) images (λ_{Ex} = 561 nm, λ_{Em} = 575–750 nm, scale bar = 25 μm) recorded after incubation of HEK-293 and MCF-7 cells with PBS solutions of **1** and **5** for 1 hour.

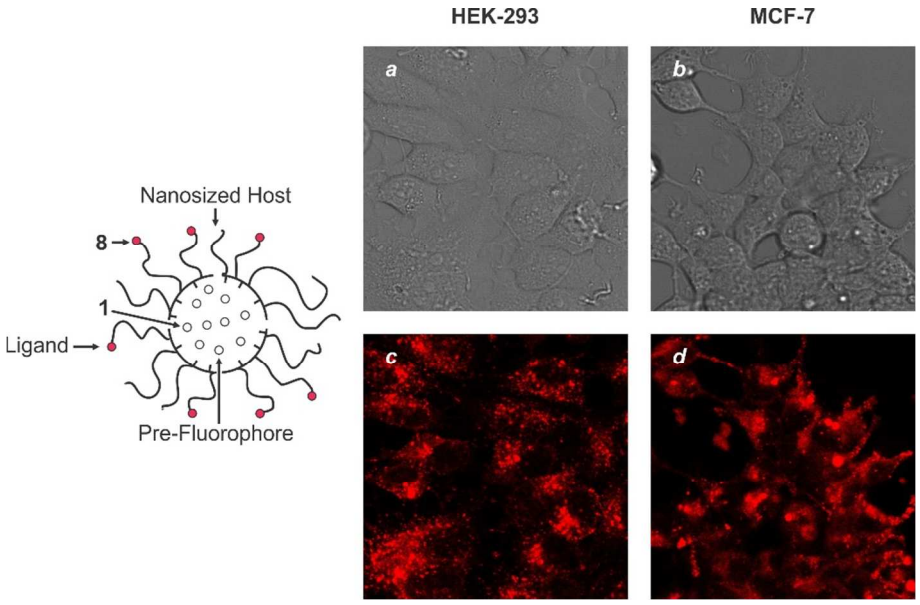

Figure S10. Transmittance (***a*** and ***c***) and fluorescence (***b*** and ***d***) images (λ_{Ex} = 561 nm, λ_{Em} = 575–750 nm, scale bar = 25 μm) recorded after incubation of HEK-293 and MCF-7 cells with PBS solutions of **1** and **8** for 1 hour.

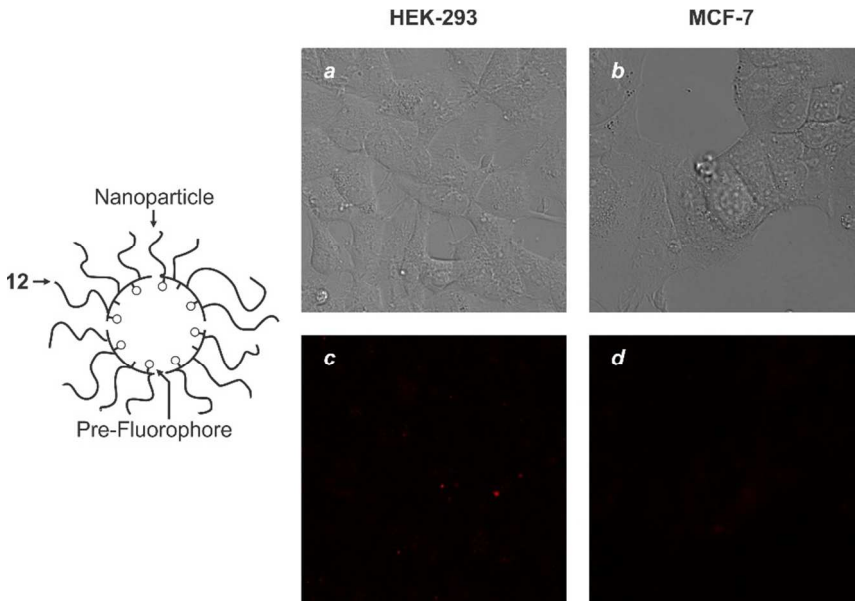

Figure S11. Transmittance (***a*** and ***c***) and fluorescence (***b*** and ***d***) images (λ_{Ex} = 561 nm, λ_{Em} = 575–750 nm, scale bar = 25 μm) recorded after incubation of HEK-293 and MCF-7 cells with PBS solutions of **12** for 2 hours.

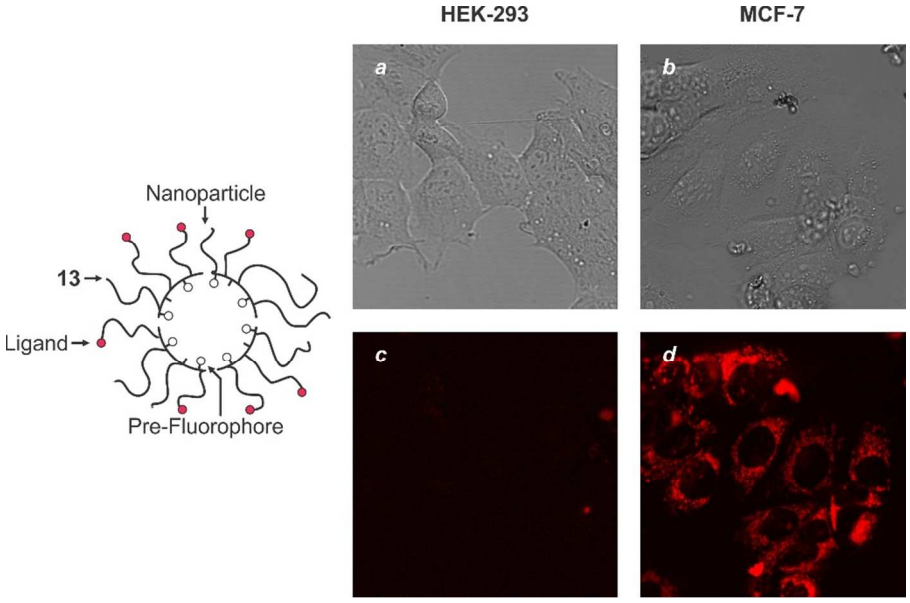

Figure S12. Transmittance (***a*** and ***c***) and fluorescence (***b*** and ***d***) images (λ_{Ex} = 561 nm, λ_{Em} = 575–750 nm, scale bar = 25 μm) recorded after incubation of HEK-293 and MCF-7 cells with PBS solutions of **13** for 2 hours.

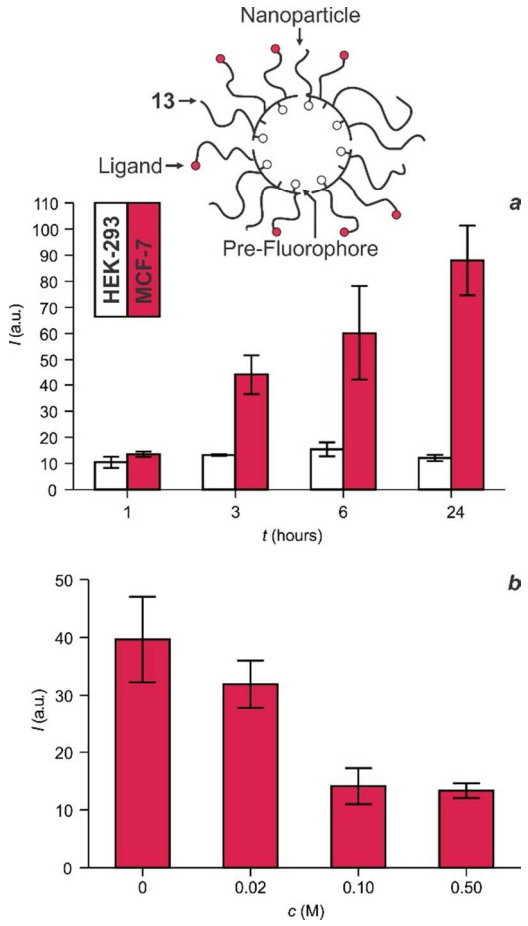

Figure S13. Average intracellular emission intensities (λ_{Ex} = 561 nm, λ_{Em} = 575–750 nm) recorded after incubation of (***a***) HEK-293 and MCF-7 cells with PBS solutions of **13** for the time indicated on the horizontal axis or (***b***) MCF-7 cells with first a PBS solution of folic acid, at the concentration indicated on the horizontal axis, for 0.5 hours and then a PBS solution of **13** for 2 hours.

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