Supplementary Information for

pH-responsive water soluble smart vesicles containing a bis(styryl)benzene derivative for two-photon microscopy imaging

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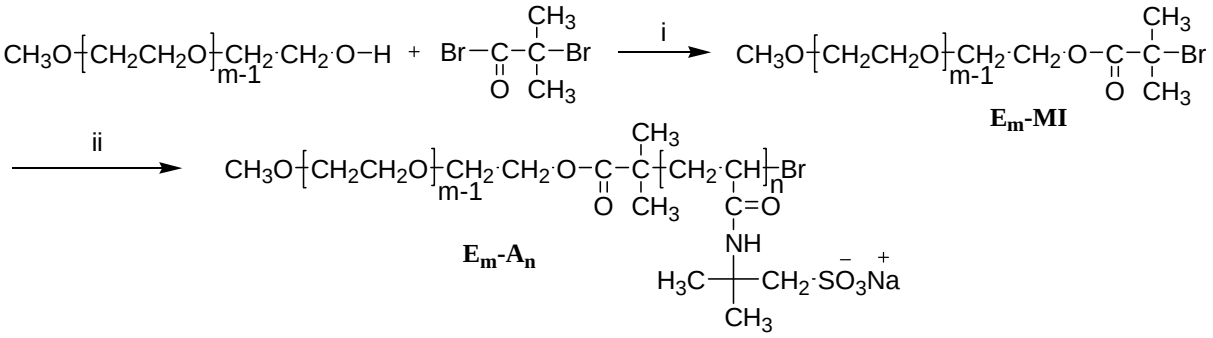
Synthesis of poly[(ethylene oxide)-*b*-(sodium 2-acrylamido-2-methyl-1-propane sulfonate)] diblock copolymers, (**E_m-A_n**).

Poly(ethylene oxide) macroinitiator (E_{45}-MI). α-Methoxy-o-hydroxy poly(ethylene oxide) (MeO-E_m-OH, m=45, 15.0 g, 7.50 mmol), 2-bromoisobutyryl bromide (4.50 g, 15.0 mmol) and trimethylamine (~2 mL) were mixed in 60 mL of dry THF. The reaction mixture was stirred overnight at room temperature. The precipitate from the reaction solution was filtered out and the supernatant was concentrated under reduced pressure. To remove the excess 2-bromoisobutyryl bromide, the concentrated solution was precipitated several times into cold *n*-hexane, filtered and dried to afford 15.0 g (yield: 93.0%) of the macroinitiator. **E_{113}-MI** was also synthesized by following the same procedure mentioned above.

Synthesis of the diblock copolymers, E_{45}-A_{30}. The macroinitiator, **E_{45}-MI** (0.54 g, 0.25 mmol) and 2-acrylamido-2-methyl-1-propanesulfonic acid, sodium salt (AMPS) (2.87 g, 12.5 mmol) were dissolved in a 40 mL water/methanol mixture (3:1,v/v). After the reaction solution was degassed, 2,2′ -bipyridine (78 mg, 0.50 mmol) and Cu(I)Cl (0.46 g, 0.25 mmol) were added. The resulting dark-brown solution was stirred at room temperature for 3 hrs. To remove the catalyst, silica gel was added to the polymer solution in water with stirring for 2 hrs, resulting in a colorless polymer solution. Finally the polymer was purified by dialysis (cellulose tubing, M_w cut-off: 3,500 g/mol) against water for 3 days and freeze-dried (1.58 g, yield 70.0%). The number average molecular weight was estimated by ^{1}H NMR spectroscopy. The peak area was integrated and compared for the methylene protons in a poly(ethylene oxide)block (a and b at ~3.6 ppm) and the methylene protons in a -CH_2-SO_3Na group of the AMPS block at ~3.3 ppm. The

other polymers, $\mathbf{E_{45}\text{-}A_{70}}$, $\mathbf{E_{113}\text{-}A_{28}}$, $\mathbf{E_{113}\text{-}A_{90}}$ were also synthesized by following the same procedure.



Scheme S1. Reagents and conditions: (i) THF, triethylamine, room temperature, 12 hrs; (ii) AMPS, 2,2′-bipyridine, Cu(I)Cl, in water/ methanol (3:1,v/v), 3hrs.

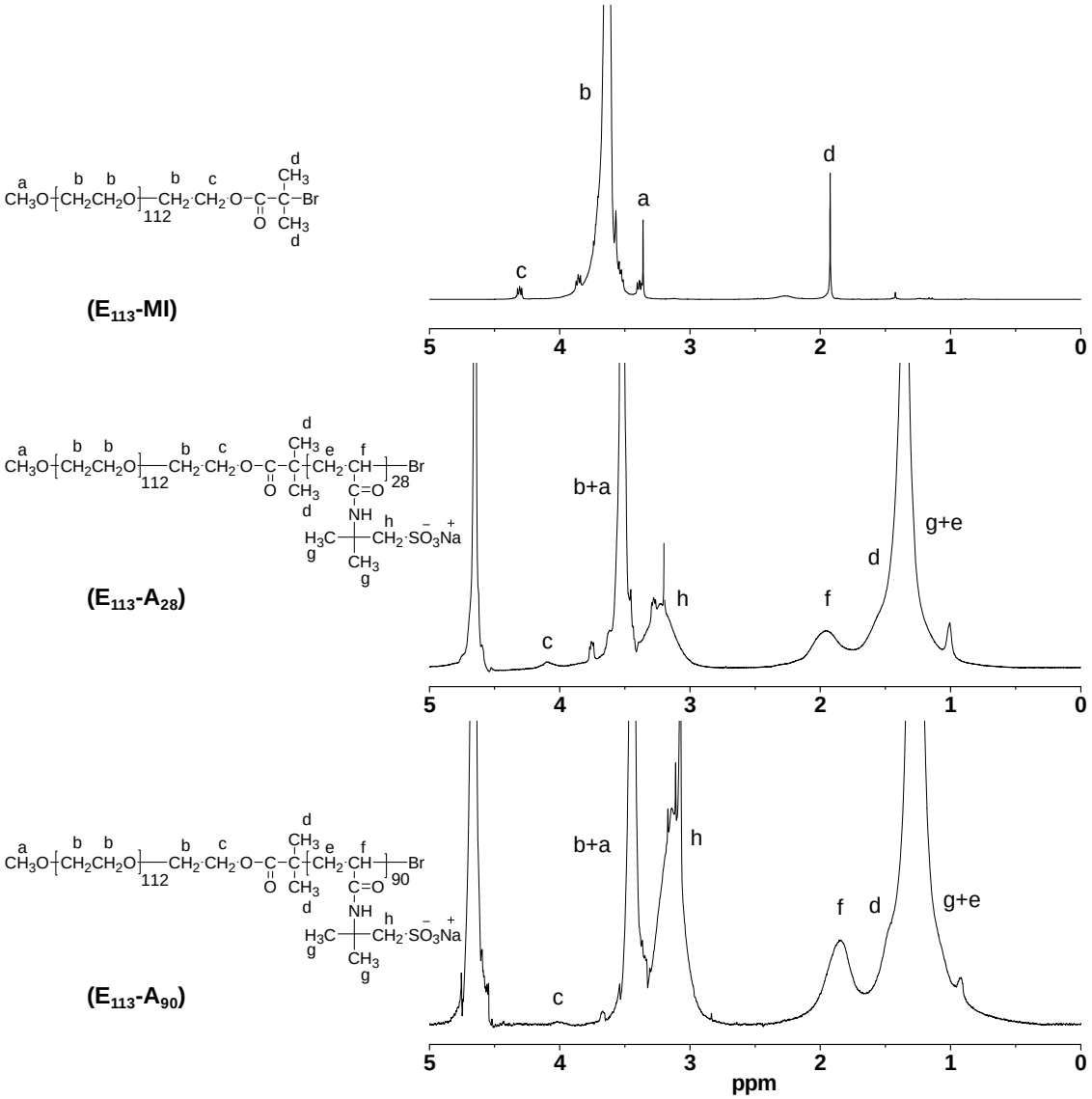


Fig. S1 ^{1}H NMR spectra of macroinitiator (**E_{113}-MI**) and diblock copolymers (**E_{113}-A_{28}**, **E_{113}-A_{90}**).

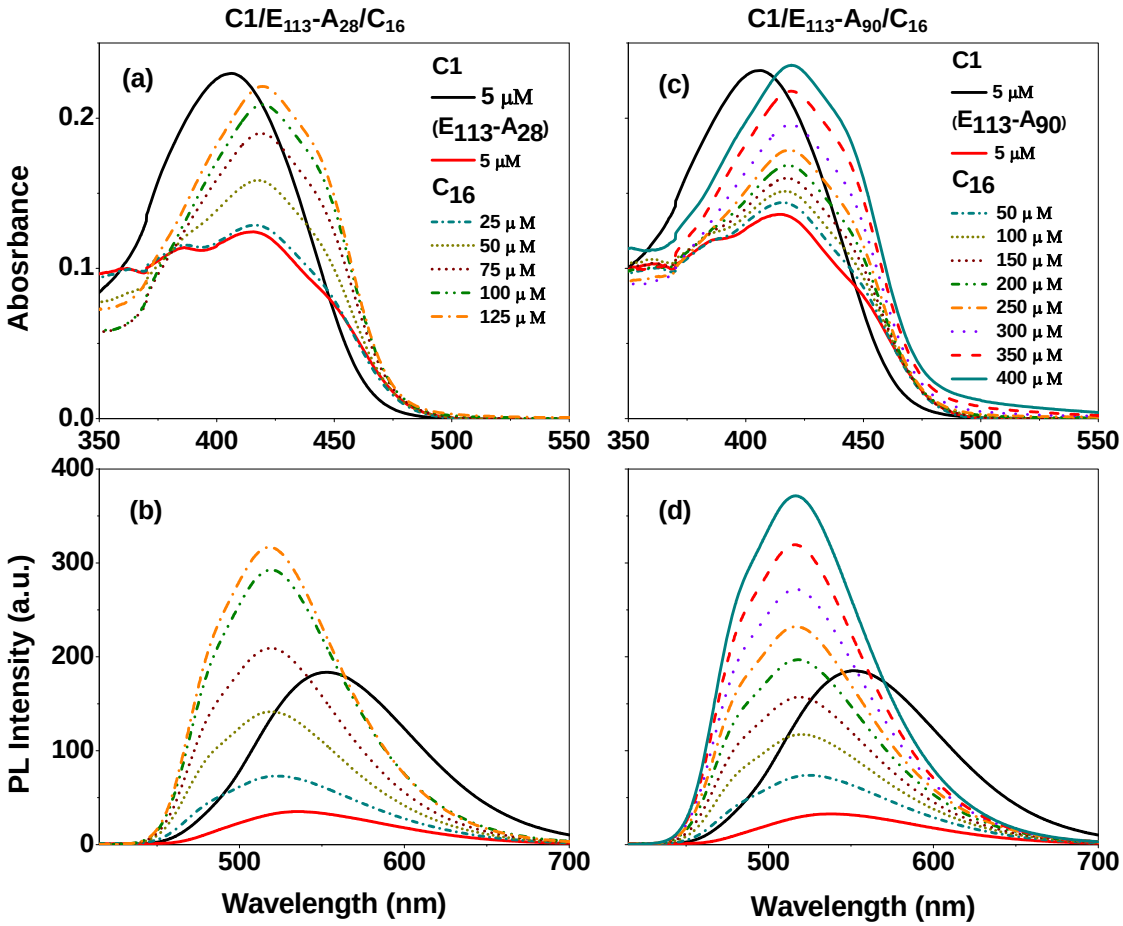


Fig. S2 UV/vis and PL spectra of **C1** in the presence of $\mathbf{E_{113}\text{-}A_{28}}$ (a, b) and $\mathbf{E_{113}\text{-}A_{90}}$ (c, d) with increasing [$\mathbf{C_{16}}$].The spectra were obtained with an aqueous solution containing [**C1**] = [$\mathbf{E_{113}\text{-}A_{28\ or\ 90}}$] = 5 μM. PL spectra were obtained by exciting at λ_{abs} (**C1**) = 405 nm.

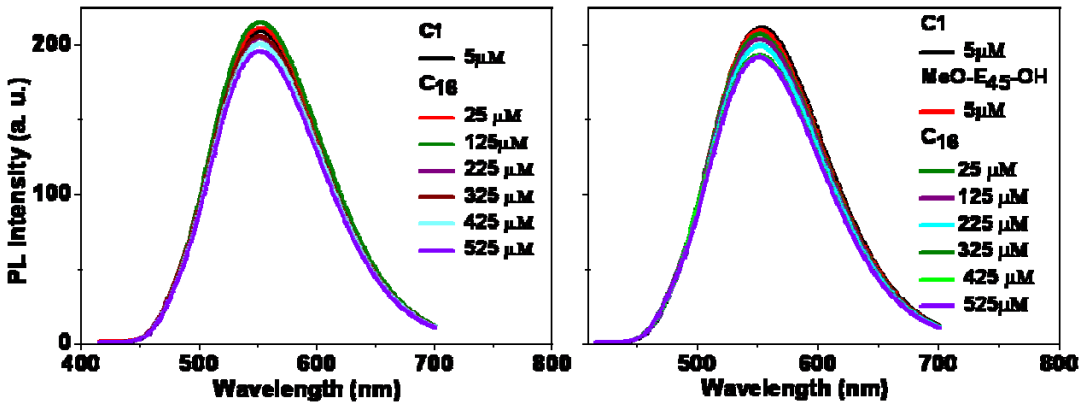


Fig. S3 PL spectra of **C1** in the presence of $\mathbf{C_{16}}$ (left), and $\mathbf{C_{16}}$ and poly(ethylene oxide) oligomer (MeO-E_{45}-OH) (right).

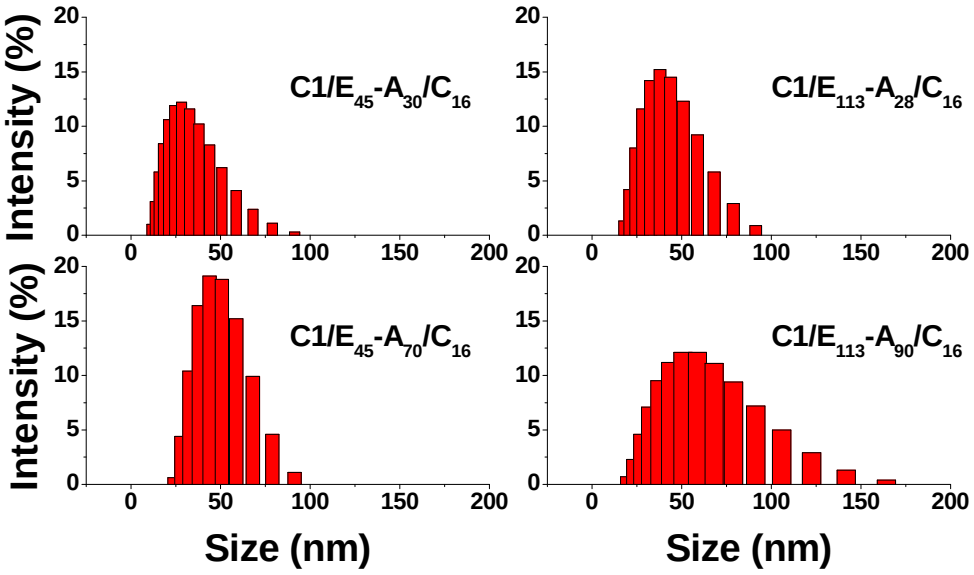


Fig. S4 Particle size distribution of vesicular complexes measured by dynamic light scattering.

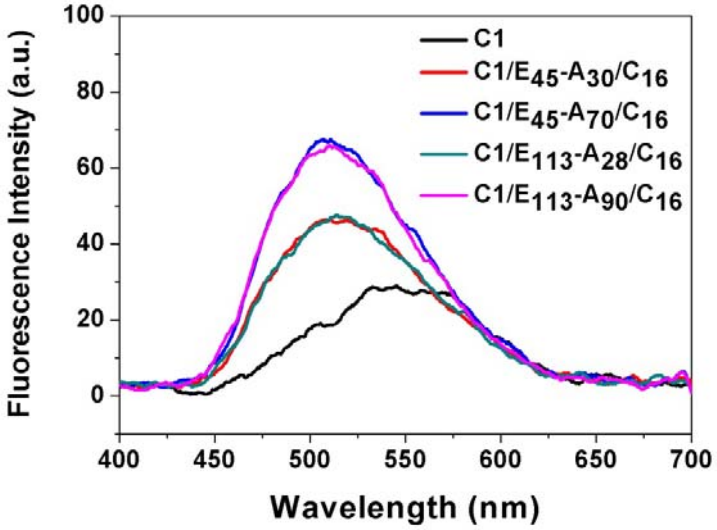


Fig. S5 Two-photon excited fluorescence spectra of **C1** and vesicular complexes in water.

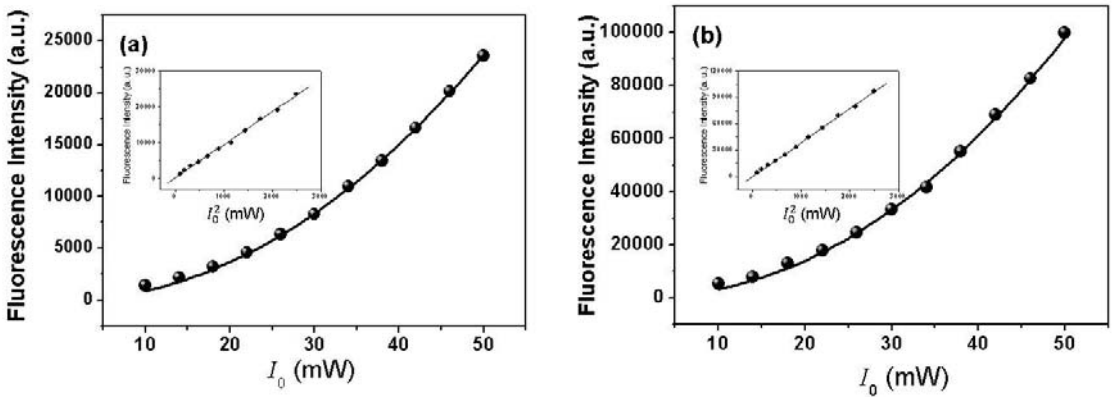


Fig. S6 Quadratic power dependence of TPEF signal for **C1** (a) and **C1**/$\mathbf{E_{45}}$-$\mathbf{A_{70}}$/$\mathbf{C_{16}}$ (b) in water.

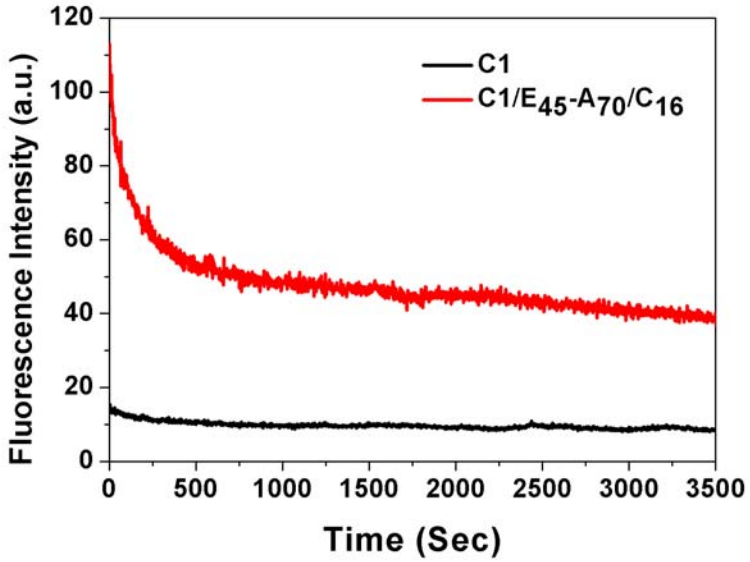


Fig. S7 Temporal stability of TPEF signal of **C1** and **C1**/$\mathbf{E_{45}}$-$\mathbf{A_{70}}$/$\mathbf{C_{16}}$ in HeLa cells.

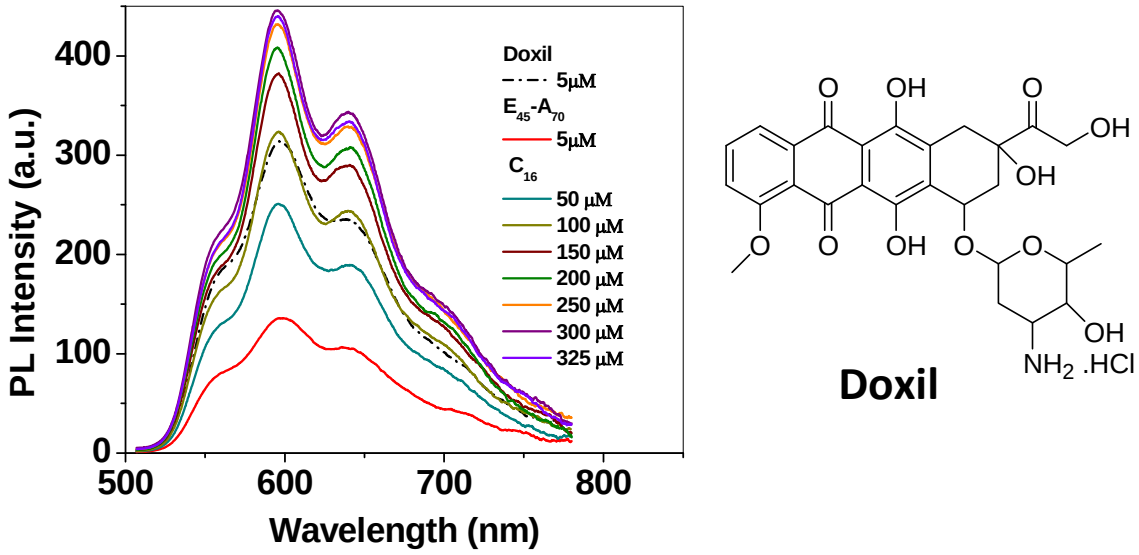


Fig. S8 PL spectra of doxorubicin-HCl (doxil) in the presence of $\mathbf{E_{45}\text{-}A_{70}}$ with increasing [$\mathbf{C_{16}}$] in water. [doxil] = 5 μM and [$\mathbf{E_{45}\text{-}A_{70}}$] = 5 μM.

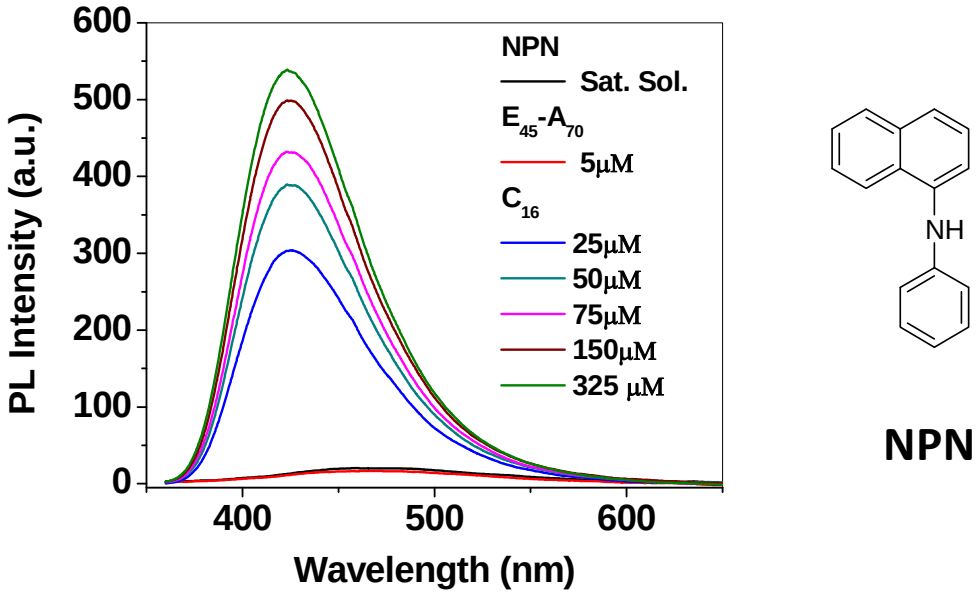


Fig. S9 PL spectra of *N*-phenyl-1-naphthylamine (NPN) in the presence of $\mathbf{E_{45}\text{-}A_{70}}$ with increasing [$\mathbf{C_{16}}$] in water. [$\mathbf{E_{45}\text{-}A_{70}}$] = 5 μM.

Table S1. Spectroscopy summary of **N1** and **C1** in different solvents.

	λ_{max}(nm)	Hexane	Toluene	THF	DCM	DMSO	Water
N1	Absorption	399	409	409	413	419	
	Emission	440, 472	458, 483	472	481	515	
C1	Absorption					419	405
	Emission					515	556