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 \((E_{45^-}\text{MI})\).** \(\alpha\)-Methoxy-o-hydroxy poly(ethylene oxide) \((\text{MeO-E}_m^-\text{OH}, m=45, 15.0 \text{ g}, 7.50 \text{ mmol})\), 2-bromoisobutyryl bromide \((4.50 \text{ g}, 15.0 \text{ mmol})\) and trimethylamine \((\sim 2 \text{ mL})\) 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. \(E_{113^-}\text{MI}\) was also synthesized by following the same procedure mentioned above.

**Synthesis of the diblock copolymers, \(E_{45^-}\text{A}_{30}\).** The macroinitiator, \(E_{45^-}\text{MI}\) (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 \(^1\text{H}\) NMR spectroscopy. The peak area was integrated and compared for the methylene protons in a poly(ethylene oxide)block \((a \text{ and } b \text{ at } \sim3.6 \text{ ppm})\) and the methylene protons in a -\(CH_2\text{-SO}_3\text{Na}\) group of the AMPS block at \(\sim3.3 \text{ ppm}\).
other polymers, $E_{45}$-$A_{70}$, $E_{113}$-$A_{28}$, $E_{113}$-$A_{90}$ 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 (E113-MI) and diblock copolymers (E113-A28, E113-A90).
**Fig. S2** UV/vis and PL spectra of C1 in the presence of E₁₁₃₋ₐ₂₈ (a, b) and E₁₁₃₋ₐ₉₀ (c, d) with increasing [C₁₆]. The spectra were obtained with an aqueous solution containing [C1] = [E₁₁₃₋ₐ₂₈ or 90] = 5 μM. PL spectra were obtained by exciting at λₐ₉₃(C1) = 405 nm.

**Fig. S3** PL spectra of C1 in the presence of C₁₆ (left), and C₁₆ and poly(ethylene oxide) oligomer (MeO-E₄₅-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/E_{45-A70}/C_{16} (b) in water.

**Fig. S7** Temporal stability of TPEF signal of C1 and C1/E_{45-A70}/C_{16} in HeLa cells.
Fig. S8 PL spectra of doxorubicin-HCl (doxil) in the presence of $E_{45}-A_{70}$ with increasing $[C_{16}]$ in water. [doxil] = 5 $\mu$M and $[E_{45}-A_{70}] = 5$ $\mu$M.

Fig. S9 PL spectra of N-phenyl-1-naphthylamine (NPN) in the presence of $E_{45}-A_{70}$ with increasing $[C_{16}]$ in water. $[E_{45}-A_{70}] = 5$ $\mu$M.
**Table S1.** Spectroscopy summary of N1 and C1 in different solvents.

<table>
<thead>
<tr>
<th></th>
<th>( \lambda_{\text{max}} ) (nm)</th>
<th>Hexane</th>
<th>Toluene</th>
<th>THF</th>
<th>DCM</th>
<th>DMSO</th>
<th>Water</th>
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<tbody>
<tr>
<td><strong>N1</strong></td>
<td>Absorption</td>
<td>399</td>
<td>409</td>
<td>409</td>
<td>413</td>
<td>419</td>
<td>419</td>
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<tr>
<td></td>
<td>Emission</td>
<td>440, 472</td>
<td>458, 483</td>
<td>472</td>
<td>481</td>
<td>515</td>
<td>515</td>
</tr>
<tr>
<td><strong>C1</strong></td>
<td>Absorption</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>419</td>
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<tr>
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