Supporting Information

Spatiotemporal Monitoring Endocytic and Cytosolic pH Gradients with Endosomal Escaping pH-Responsive Micellar Nanocarriers

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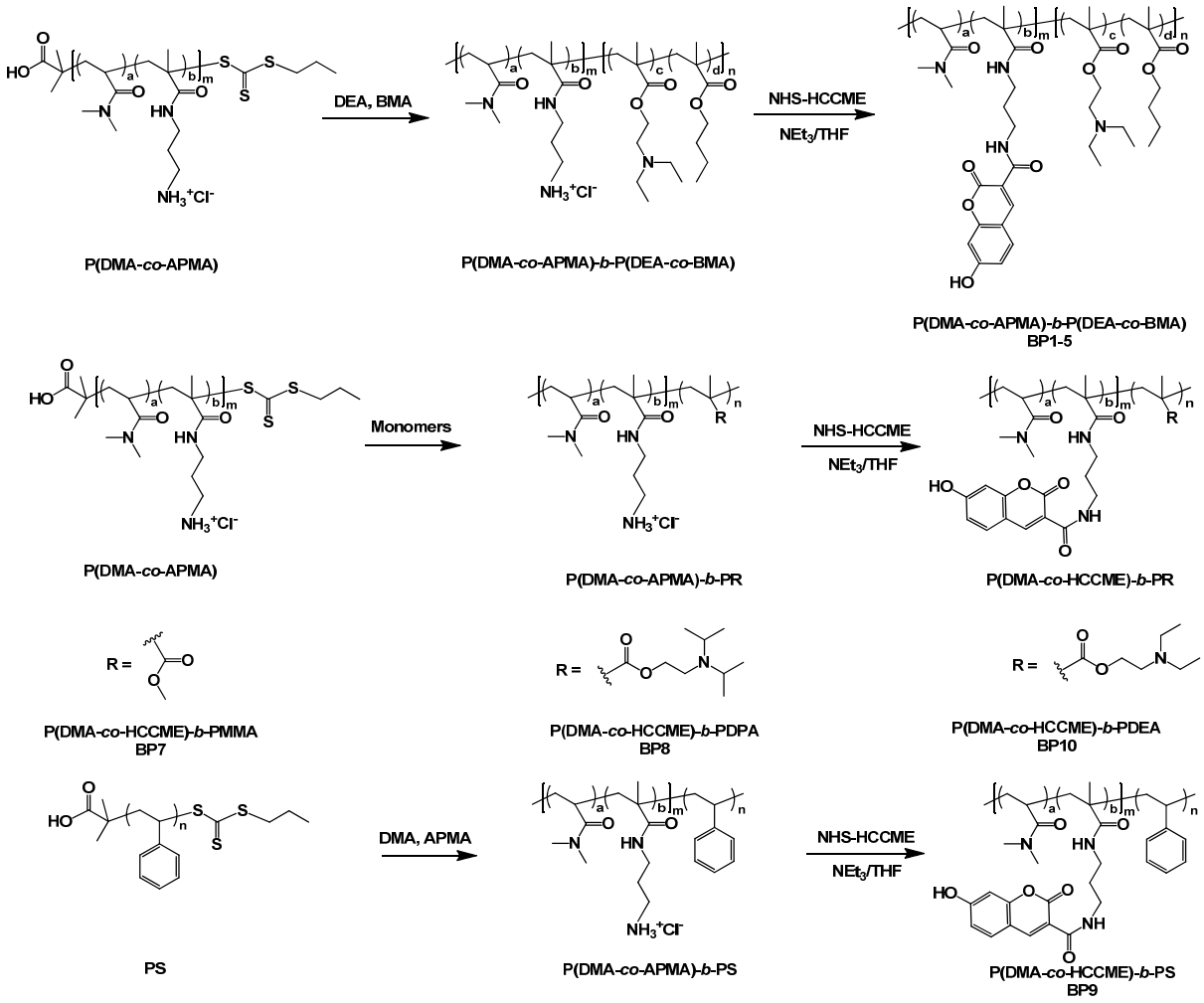

Scheme S1. Synthetic routes employed for the preparation of HCCME-labeled diblock copolymers, P(DMA-*co*-HCCME)-*b*-P(DEA-*co*-BMA) with varying BMA molar fractions (**BP1**-**BP5**), P(DMA-*co*-HCCME)-*b*-PMMA (**BP7**), P(DMA-*co*-HCCME)-*b*-PDPA (**BP8**), P(DMA-*co*-HCCME)-*b*-PS (**BP9**), and P(DMA-*co*-HCCME)-*b*-PDEA (**BP10**) by sequential RAFT polymerizations.

Table S1. Intensity-Average Hydrodynamic Diameter, <D_h>, Polydispersity Index, pK_a, and Zeta Potentials (ξ) of Micelles Self-Assembled from Diblock Copolymers.

Entry	<D_h> (nm) [a]	PDI [a]	pK_a [b]	ξ (mV) [c]
BP1	34.8	0.054	6.73	-2.7
BP2	30.4	0.054	6.51	-3.7
BP3	41.6	0.099	6.12	-4.5
BP4	37.6	0.10	5.92	-6.2
BP5	35.6	0.20	5.57	-5.6
BP6	24.2	0.11	6.12	-5.8
BP7	108.0	0.21	/	-24.0
BP8	32.8	0.13	6.30	-6.7
BP9	31.2	0.23	/	-21.4
BP10	35.4	0.044	7.07	5.8

[a] Determined by DLS at a copolymer concentration of 1.0 g/L and pH 7.4; [b] Determined by pH titration experiments; [c] Zeta potentials were determined by Malvern Zetasizer Nano ZS at copolymer concentrations of 0.5 g/L in PBS buffer (pH 7.4). Note: all data were averaged over three measurements.

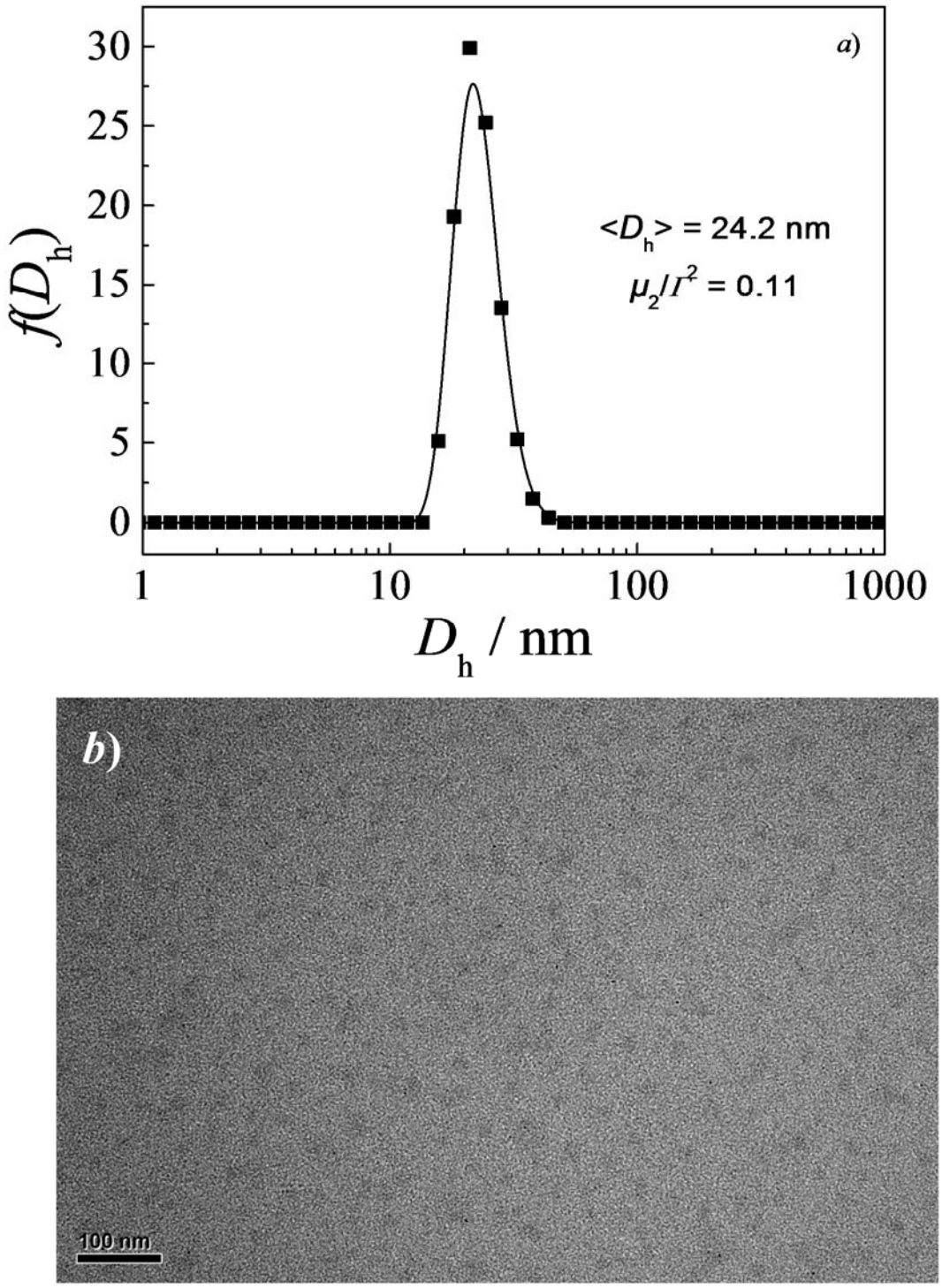

Figure S1. Typical (a) hydrodynamic diameter distribution, $f(D_h)$, and (b) TEM image recorded for **BP6** micelles (0.2 g/L, pH 7.4).

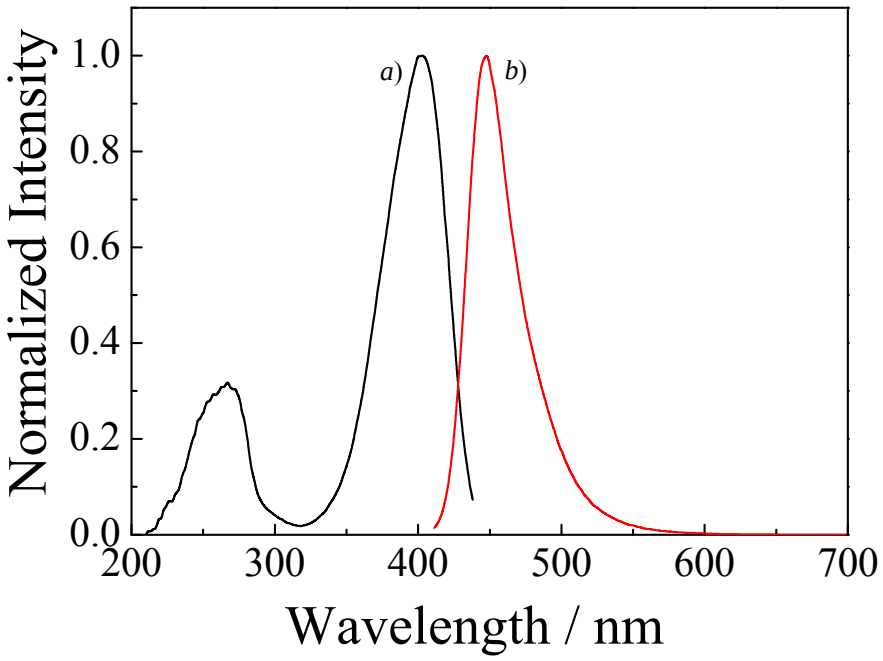

Figure S2. Normalized fluorescence (a) excitation and (b) emission spectra recorded for the aqueous solution of **P1** (pH 9.0; 0.018 g/L, [HCCME] = 1.0×10^{-6} M; slit widths: Ex. 2.5 nm, Em. 2.5 nm).

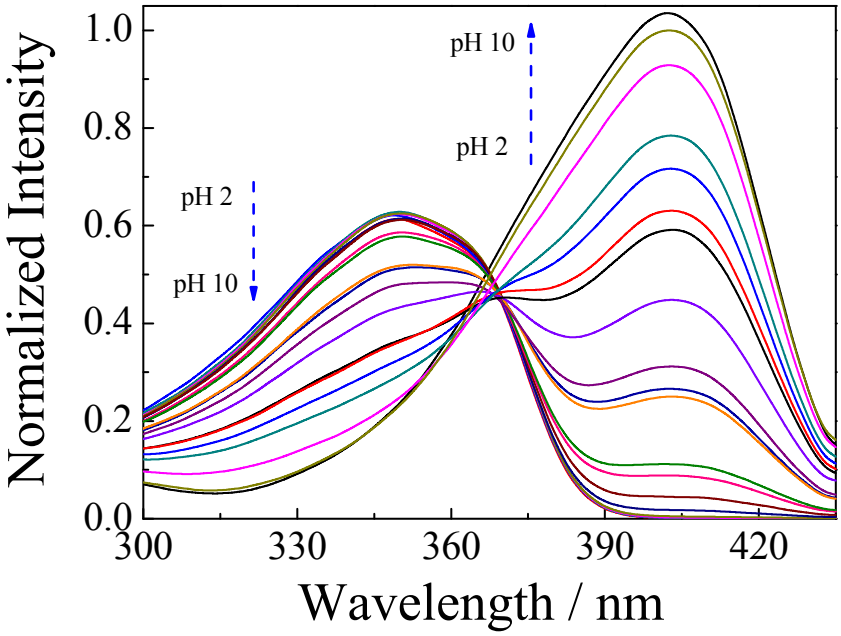

Figure S3. Normalized fluorescence excitation spectra (λ_{em} = 447 nm, slit widths: Ex. 2.5 nm, Em. 2.5 nm) recorded for the aqueous solution of **P1** (0.018 g/L, [HCCME] = 1.0×10^{-6} M) in the pH range of 2-10.

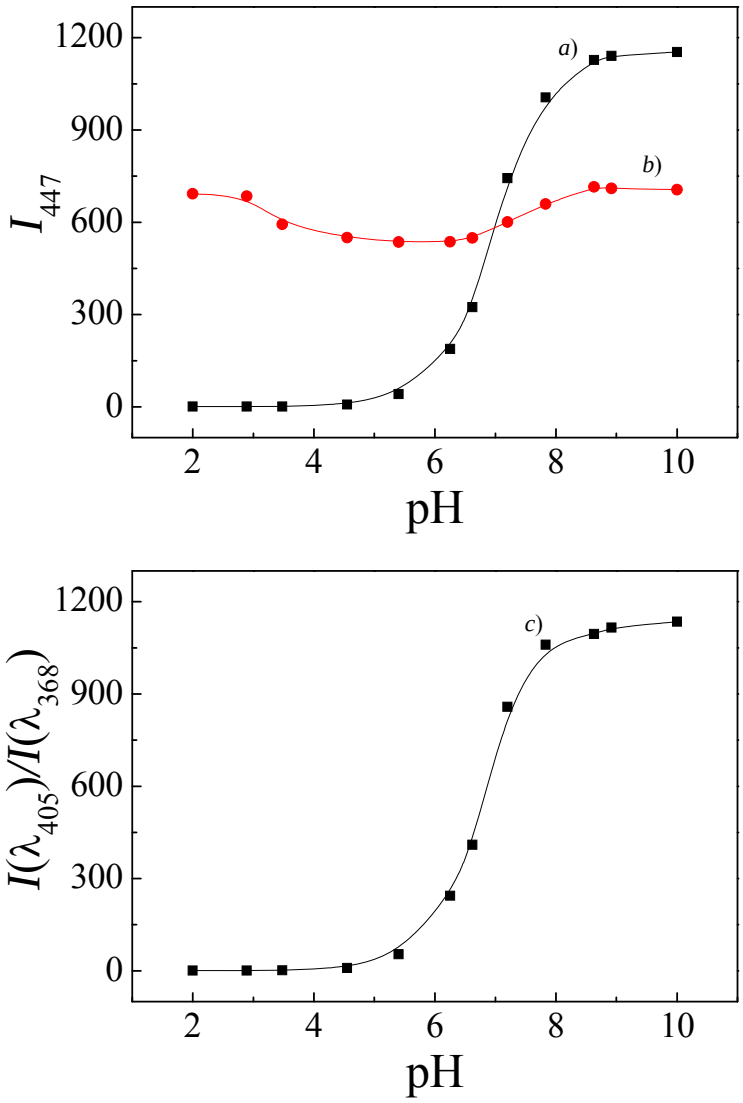

Figure S4. Relative fluorescence emission intensity changes excited at (a) 405 nm (λ_{em} = 447 nm) and (b) 368 nm (λ_{em} = 447 nm) recorded for the aqueous solution of **P1** (0.018 g/L, [HCCME] = 1.0 × 10^{-6} M) in the pH range of 2-10. (c) Normalized fluorescence intensity ratio changes, $I(\lambda_{405})/I(\lambda_{368})$, recorded for the aqueous solution of **P1** in the pH range of 2-10. $I(\lambda_{405})$ and $I(\lambda_{368})$ refer to the fluorescence intensities at 447 nm when excited at 405 nm and 368 nm, respectively.

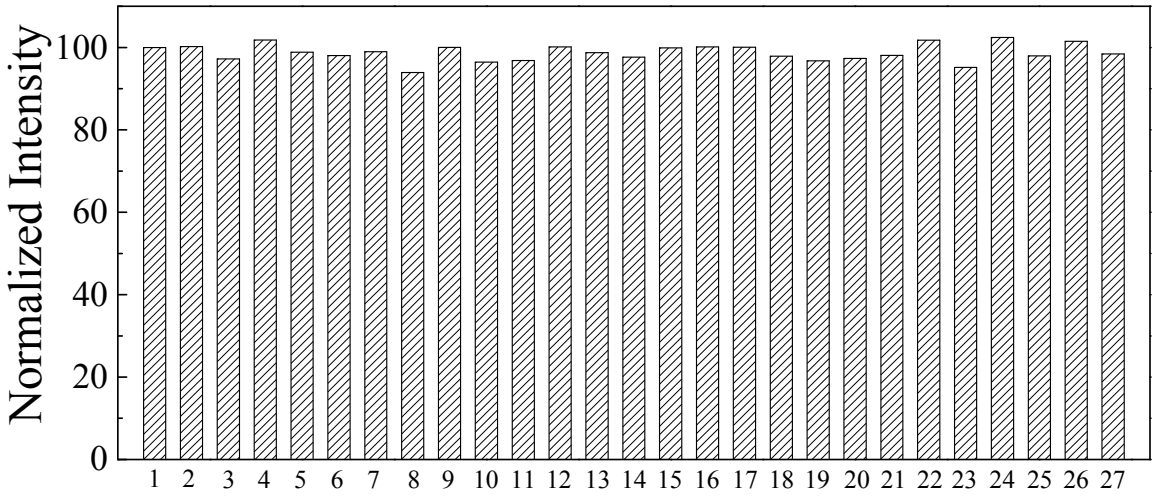

Figure S5. Fluorescent emission intensities recorded at 447 nm for aqueous solutions of **P1** (0.018 g/L, [HCCME] = 1.0×10^{-6} M) in the presence of 1 mM (2) Ag^{+}, (3) Al^{3+}, (4) Ca^{2+}, (5) Cu^{2+}, (6) Mg^{2+}, (7) Pb^{2+}, (8) Zn^{2+}, (9) H_2O_2, (10) *L*-cysteine, (11) *L*-phenylalanine, (12) *L*-alanine, (13) glycine, (14) *L*-lysine, (15) *L*-tyrosine, (16) *L*-tryptophan, (17) *L*-aspartic acid, (18) *L*-histidine, (19) glucose, and (20) mannose, respectively, or in the presence of 0.01 g/L (21) lipase, (22) glucose oxidase, (23) acid phosphatase, (24) amylase, (25) alkaline phosphase, (26) lysozyme, and (27) trypsin. The mixture was incubated at 37 ^{o}C for 1 h prior to fluorescence measurement. Column 1 was taken as the control.

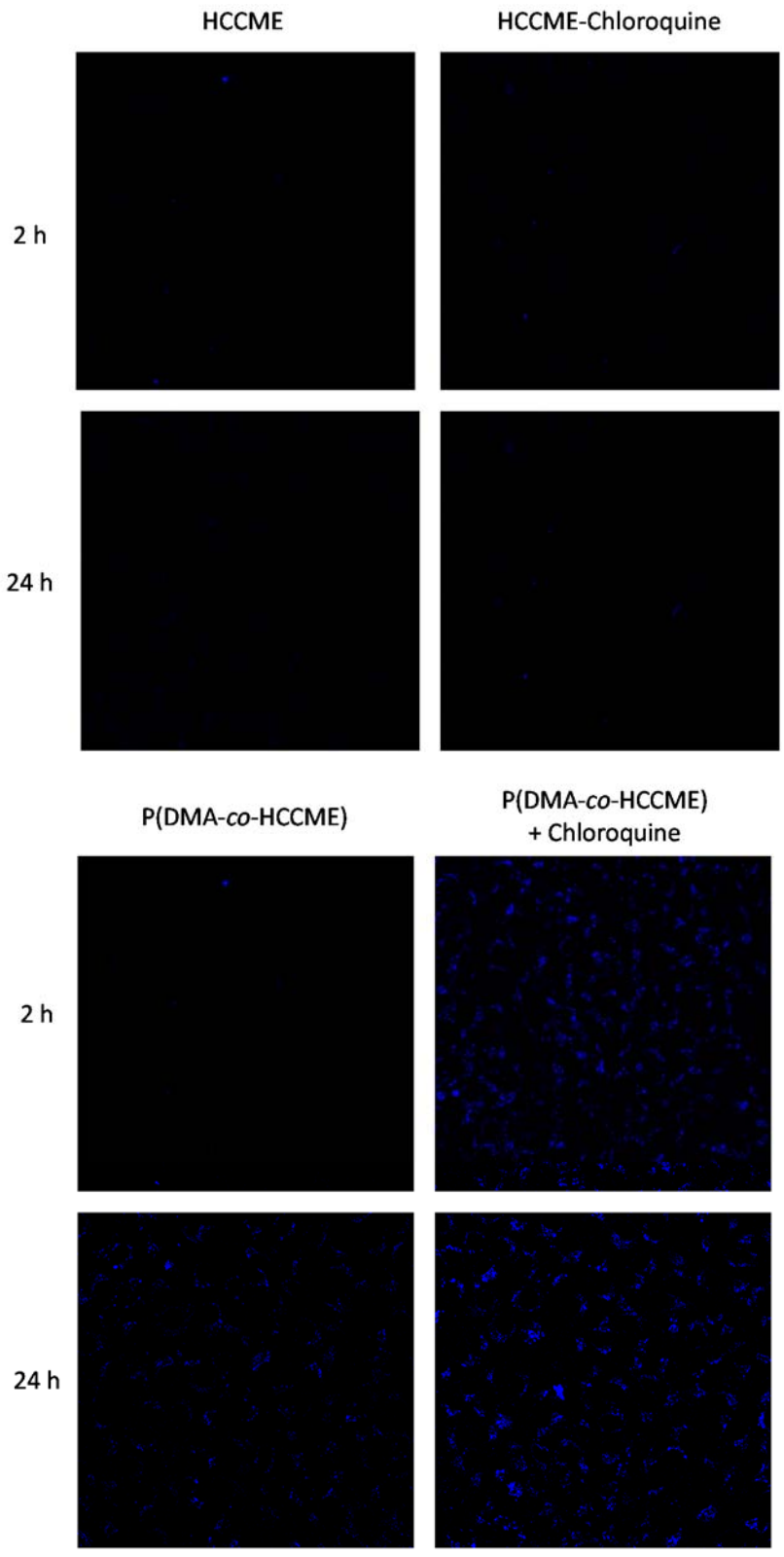

Figure S6. CLSM images recorded for live HepG2 cells after incubating with (top) HCCME and (bottom) **P1** (0.018 g/L, [HCCME] = 1.0×10^{-6} M) in the (left) absence and (right) presence of chloroquine (100 μM) at 37 ºC for 2 h and 24 h, respectively.

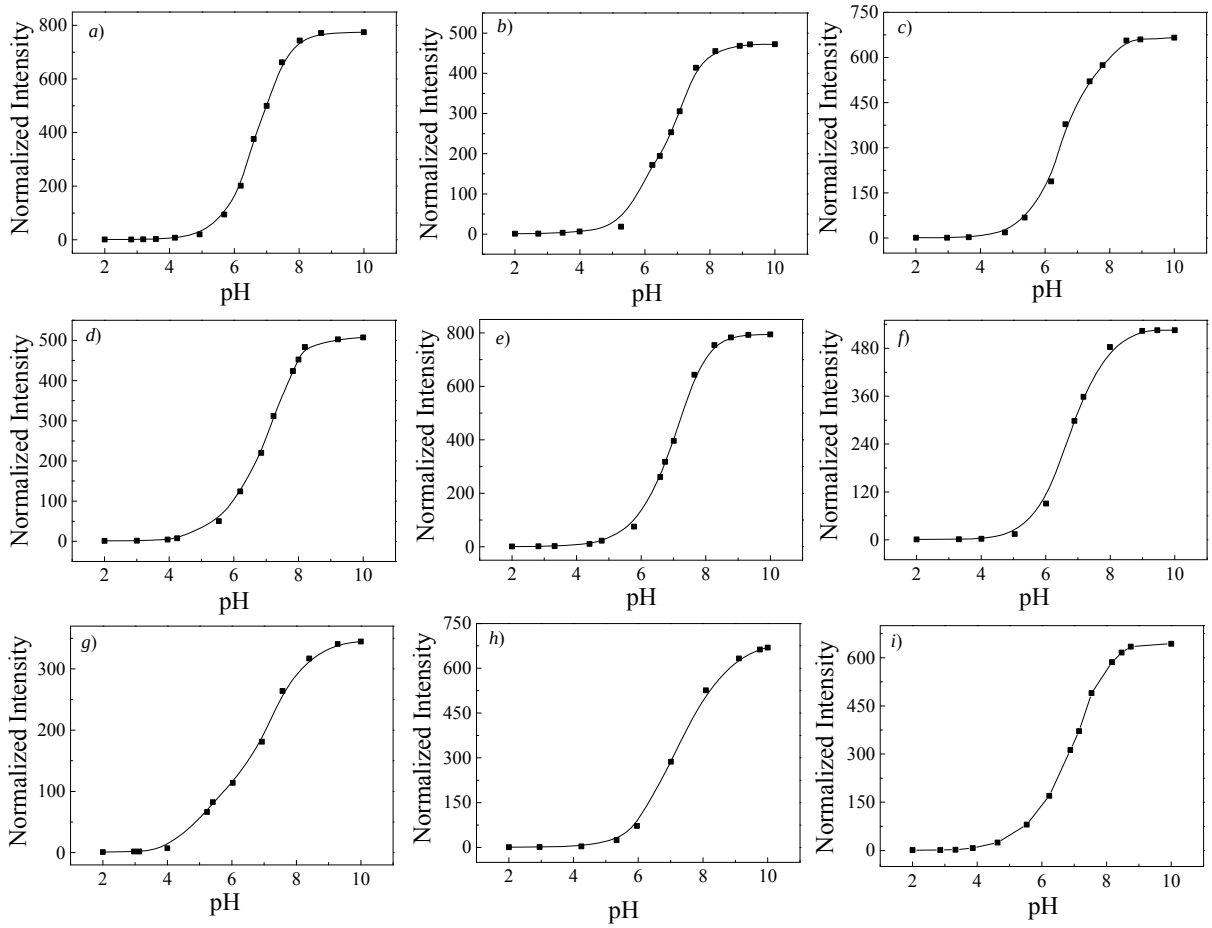

Figure S7. Normalized fluorescence emission intensity changes (λ_{ex} = 405 nm, λ_{em} = 447 nm) recorded for aqueous solutions of (a) **BP1**, (b) **BP2**, (c) **BP3**, (d) **BP4**, (e) **BP5**, (f) **BP7**, (g) **BP8**, (h) **BP9**, and (i) **BP10** in the pH range of 2-10, respectively. In all cases, the concentration of HCCME moieties was fixed at 1.0×10^{-6} M.

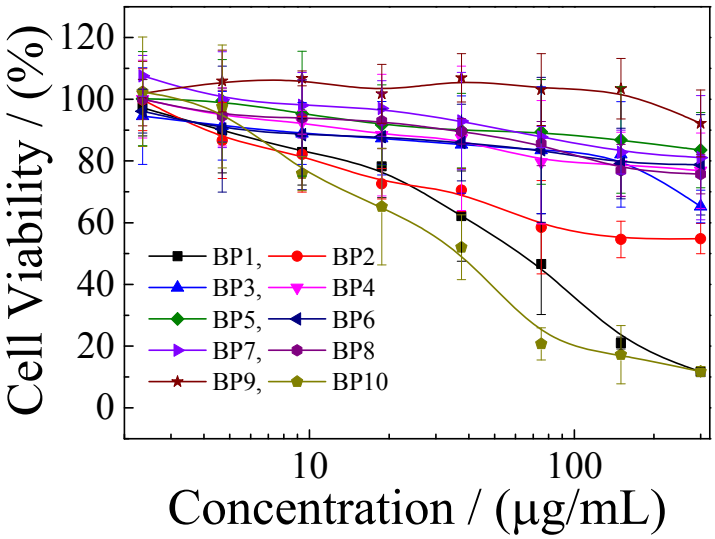

Figure S8. *In vitro* cytotoxicity of **BP1-10** micelles determined by MTT assay against HepG2 cells. The data represent a single experiment performed in quadruple with the error bars denoting the standard deviation.

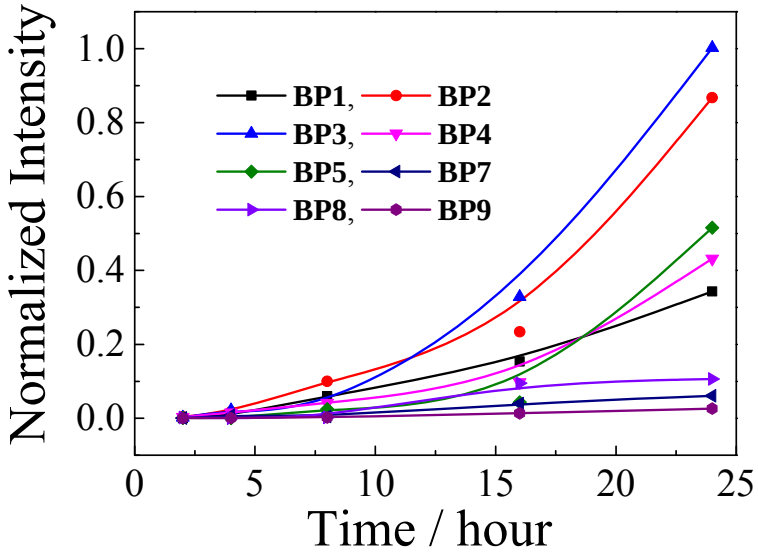

Figure S9. Time-dependent relative changes in intracellular fluorescence intensities recorded for **BP1-5** and **BP7-9** micellar dispersions, respectively. In all cases, the concentration of HCCME moieties was fixed at 1.0×10^{-6} M.

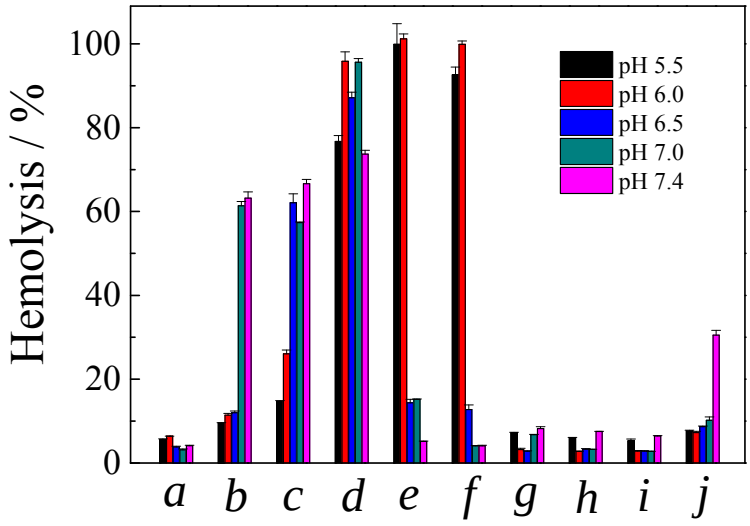

Figure S10. Hemolysis dose-response curves recorded for aqueous solutions (20 μg/mL) of (a) **P1,** (b) **BP1** (c) **BP2**, (d) **BP3**, (e) **BP4**, (f) **BP5**, (g) **BP7**, (h) **BP8**, (i) **BP9**, and (j) **BP10** at varying pH conditions. PBS (10 mM phosphate, 150 mM NaCl, pH 7.4) and Triton X-100 (1% v/v) were used as negative and positive controls, respectively. The data represent a single experiment performed in triplicate with the error bars denoting the standard deviation.

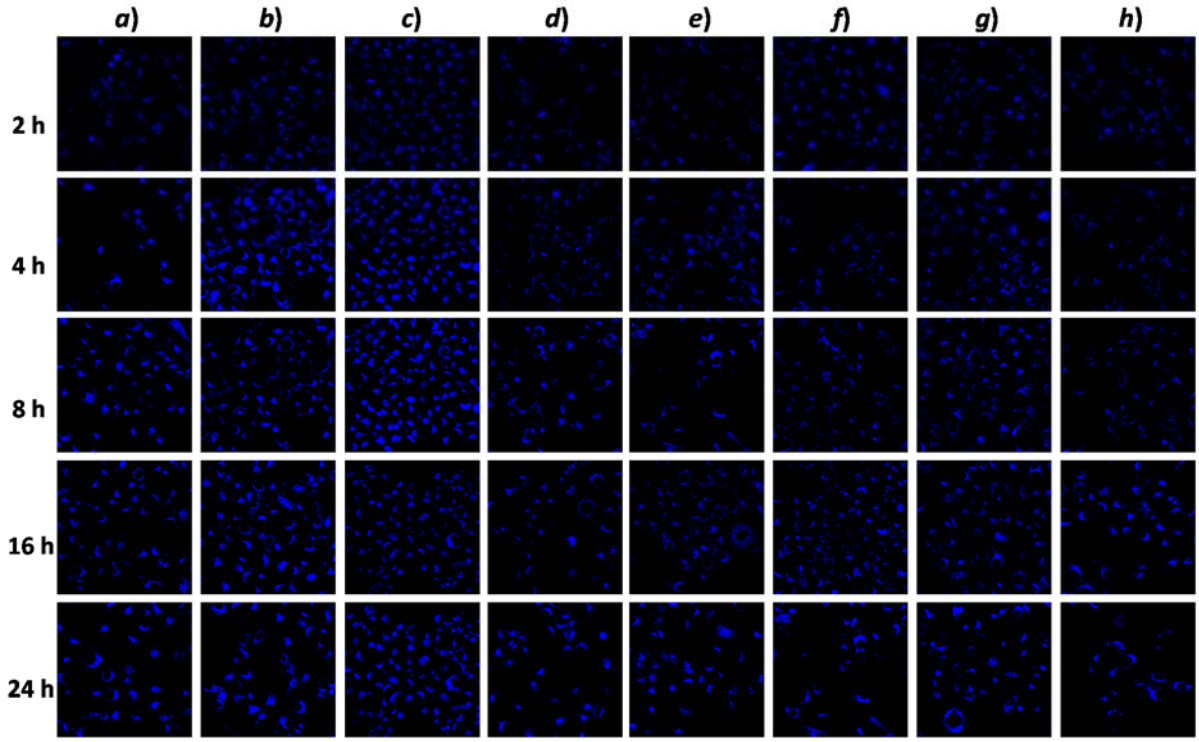

Figure S11. CLSM images recorded for live HepG2 cells incubated at 37 ºC for varying times with (a) **BP1**, (b) **BP2**, (c) **BP3**, (d) **BP4**, (e) **BP5**, (f) **BP7**, (g) **BP8**, and (h) **BP9**, respectively. The cells were further treated with chloroquine (100 μM) and incubated at 37 ºC for 1 h before recording fluorescence images. In all cases, the concentration of HCCME moieties was fixed at 1.0×10^{-6} M.

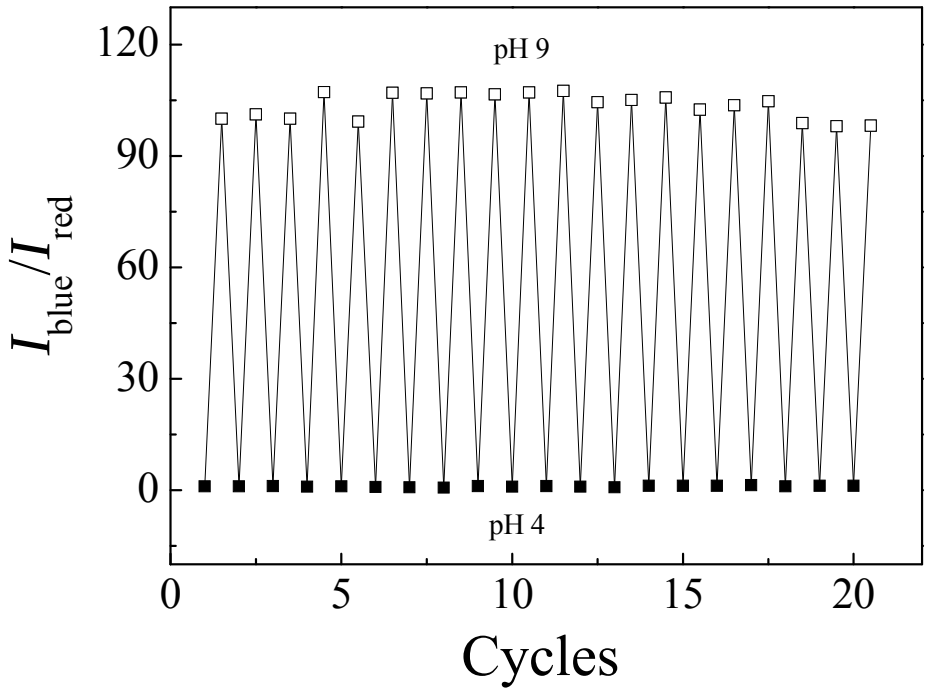

Figure S12. Emission intensity ratio changes (I_{blue}/I_{red}) recorded for the aqueous solution (0.049 g/L) of **BP6** (λ_{ex} = 405 nm for HCCME and λ_{ex} = 543 nm for TMR; slit widths: Ex. 2.5 nm, Em. 2.5 nm; [HCCME] = 1.0×10^{-6} M, [TMR] = 1.6×10^{-6} M) when the solution pH was cycled between 4 and 9.

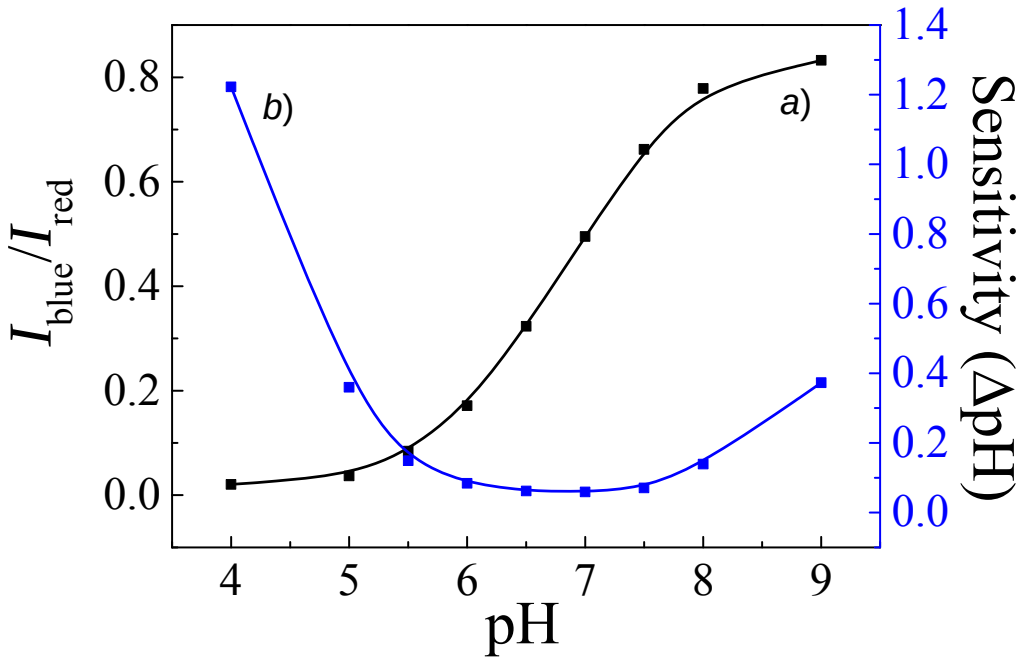

Figure S13. (a) Changes in fluorescence intensity ratios (I_{blue}/I_{red}) and (b) detection sensitivity for **BP6** aqueous solution (0.049 g/L, [HCCME] = 1.0×10^{-6} M, [TMR] = 1.6×10^{-6} M) with excitation at 405 nm and collected between 420-470 nm for the blue channel and excitation at 543 nm and collected between 555-595 nm for the red channel.