Supporting Information

Rationally Engineering Phototherapy Modules of Eosin-Conjugated Responsive Polymeric Nanocarriers via Intracellular Endocytic pH Gradients

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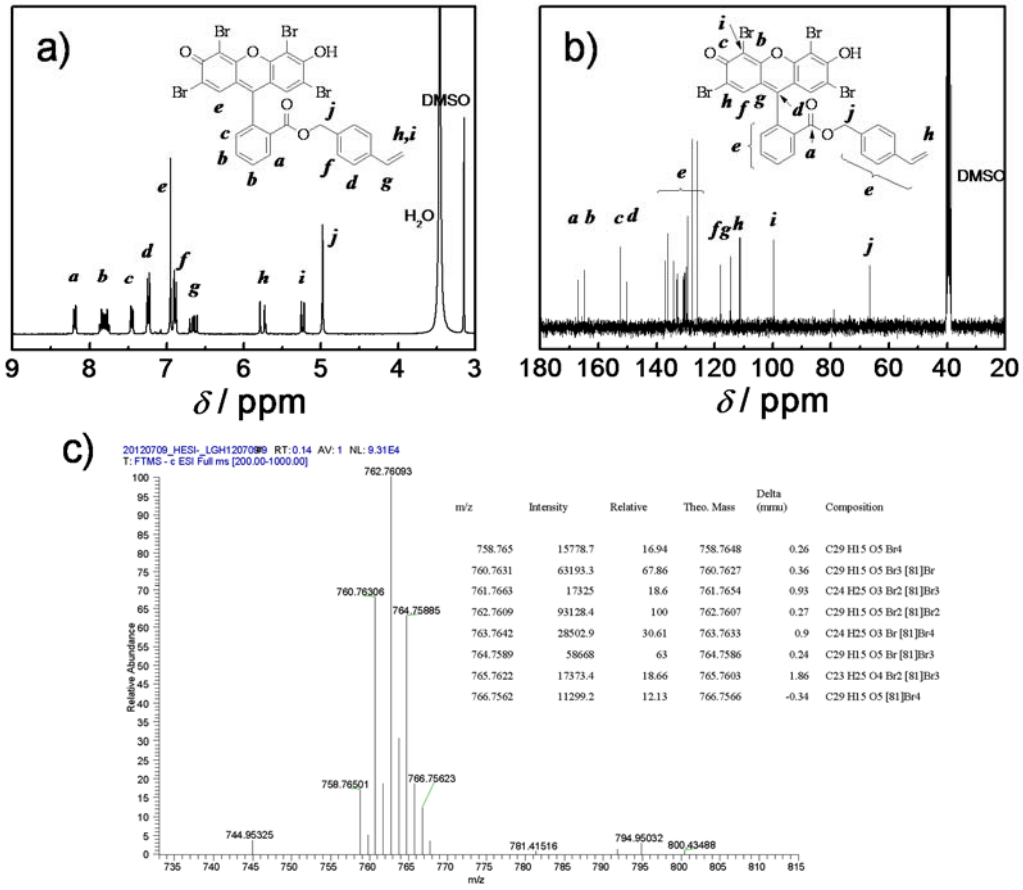
cells were treated (a) without irradiation or with (b) 30 min and (c,d) 60 min green light (520 nm) irradiation after co-incubating with **BP3** micelles for 2 h; the cells were further incubated with 6 μM acridine orange (AO) for 30 min before CLSM analysis. Green channel emission was collected at 530 ± 20 nm and red channel at 620 ± 20 nm. AO as an intracellular indicator of acid organelle integrity emits red fluorescence in lysosomes, and displays green fluorescence in the cytosol and nuclei. The addition of NAC as $^1O_2$ scavenger in (d) led to reduced lysosomal disruption.

Figure S10. Representative CLSM images from blue (7HC, 450 ± 20 nm), green (eosin, 560 ± 20 nm), and red (LysoTracker, 630 ± 20 nm) channels, and overlay of all three channels recorded for live A549 cells without (a) and with (b,c) 60 min green light (520 nm) irradiation at 37 ºC, the cells were incubated with **BP3** micelles for 2 h at first. The addition of (b) vitamin C and (c) *N*-acetyl-*L*-cysteine NAC) as $^1O_2$ scavengers both led to prominently reduced lysosomal disruption.

Figure S11. (a) CLSM images and (b) emission intensity ratio (I_{green}/I_{blue}) changes recorded at pH 7.4 for the aqueous mixture of **BP4** (0.35 g/L), **BP5** (0.15 g/L), and **BP2** (0.2 g/L) in the temperature range of 35-46 ºC.

Figure S12. (a) Representative CLSM images of blue and green channels and overlay of blue/green channels recorded for A549 cells upon irradiating the green box area with 543 nm laser for varying number of scans (20, 50 and 100 times). The cells were incubated for 2 h with **BP4** (0.35 g/L) and **BP5** (0.15 g/L) in the presence of **BP2** (0.2 g/L) micelles at first; after washing and replacing with fresh DMEM medium, the cell culture was further incubated for 2 h. (b,c) Laser irradiation-triggered evolution of local temperature gradient distributions (b) inside and (c) outside the irradiation square box area.



m/z	Intensity	Relative	Theo. Mass	Delta (mmu)	Composition
758.765	15778.7	16.94	758.7648	0.26	C29 H15 O5 Br4
760.7631	63193.3	67.86	760.7627	0.36	C29 H15 O5 Br3 [81]Br
761.7663	17325	18.6	761.7654	0.93	C24 H25 O3 Br2 [81]Br3
762.7609	93128.4	100	762.7607	0.27	C29 H15 O5 Br2 [81]Br2
763.7642	28502.9	30.61	763.7633	0.9	C24 H25 O3 Br [81]Br4
764.7589	58668	63	764.7586	0.24	C29 H15 O5 Br [81]Br3
765.7622	17373.4	18.66	765.7603	1.86	C23 H25 O4 Br2 [81]Br3
766.7562	11299.2	12.13	766.7566	-0.34	C29 H15 O5 [81]Br4

Figure S1. (a) ^{1}H NMR, (b) ^{13}C NMR, and (c) ESI mass spectra recorded for eosin-based monomer (EoS).

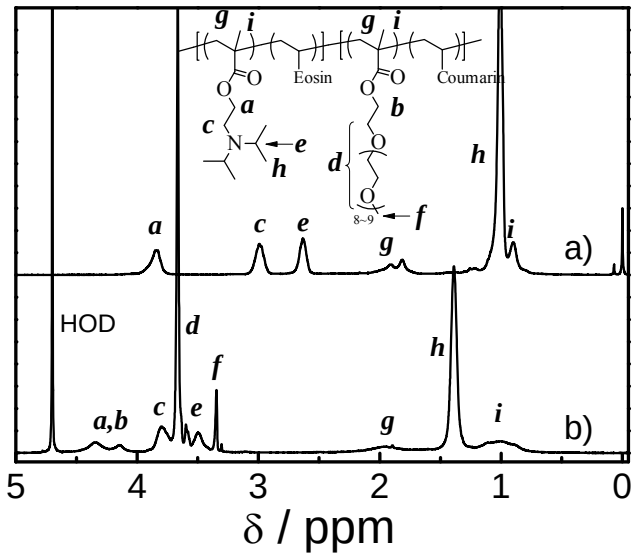


Figure S2. ^{1}H NMR spectra recorded for (a) P(DPA$_{0.95}$-*co*-EoS$_{0.05}$)$_{65}$ (**P2**) macroRAFT agent in $CDCl_3$ and (b) P(DPA$_{0.95}$-*co*-EoS$_{0.05}$)$_{65}$-*b*-P(OEGMA-*co*-7HC)$_{22}$ diblock copolymer (**BP3**) in DCl/D_2O.

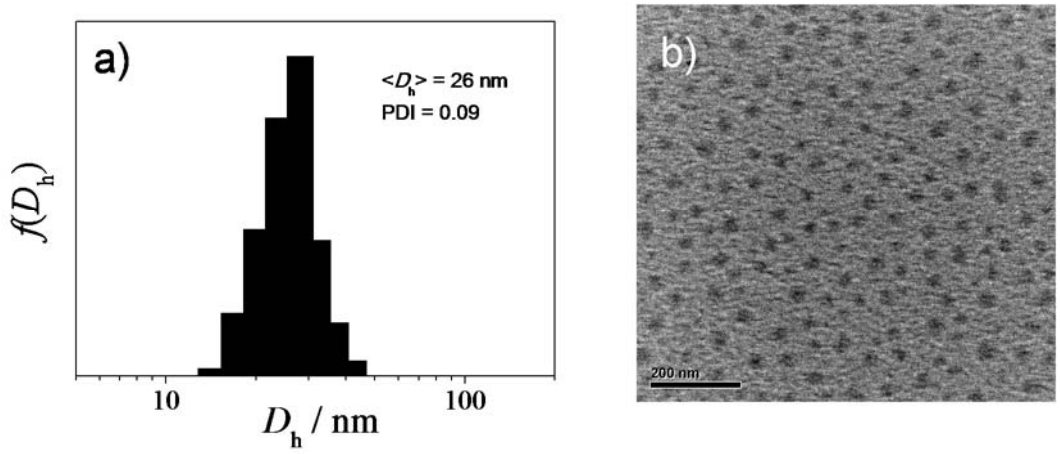


Figure S3. (a) Hydrodynamic diameter distribution, $f(D_h)$, and (b) typical TEM image recorded for **BP2** micelles (pH 7.4).

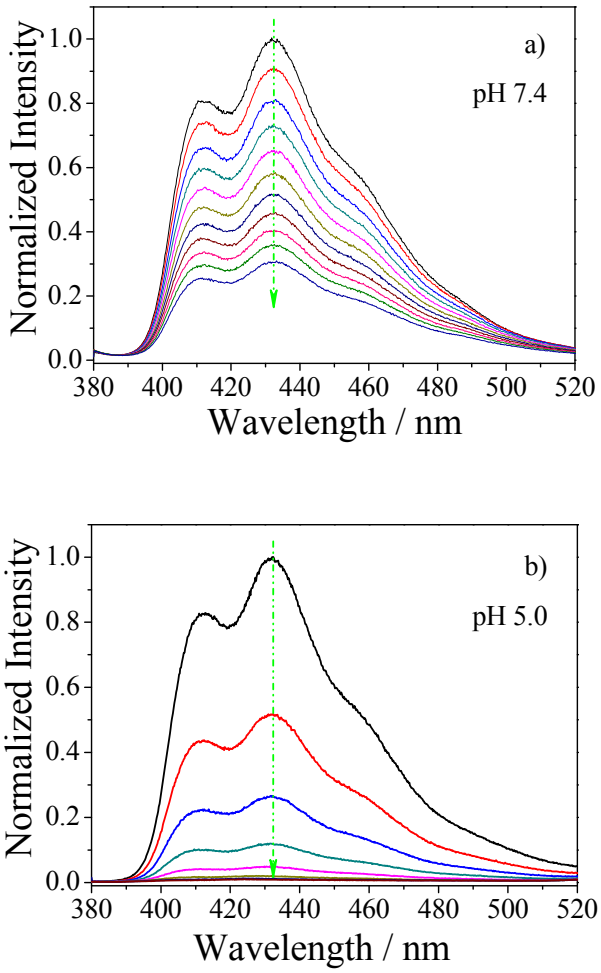


Figure S4. Fluorescence emission spectrum (λ_{ex} = 370 nm) recorded at 25 ºC for oxygen-saturated aqueous **BP2** solution (0.5 g/L, 22 μM eosin moieties) at (a) pH 7.4 and (b) pH 5.0 in the presence of *An*-2NH_2 (180 μM, λ_{ex} = 370 nm) after being subjected to green light irradiation (520 nm) for varying time periods.

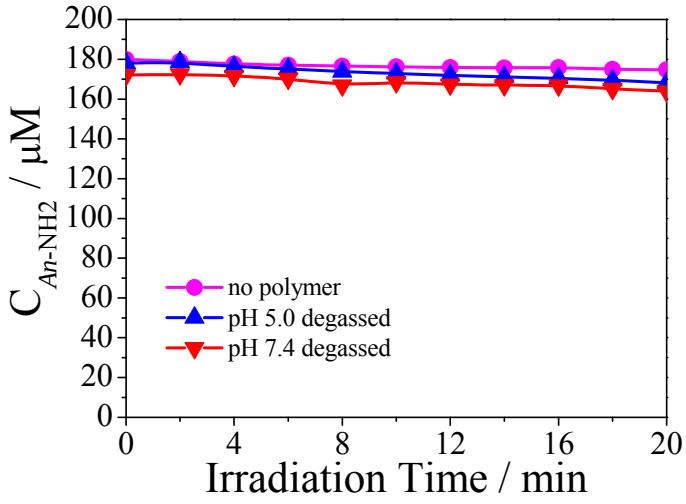


Figure S5. Time-dependent consumption of *An*-2NH_2 ($^1O_2$ generation) recorded at 25 °C for aqueous **BP2** solution (0.5 g/L, 22 μM eosin moieties) in the presence of *An*-2NH_2 (180 μM, λ_{ex} = 370 nm) after being subjected to green light irradiation (520 nm) for varying time periods.

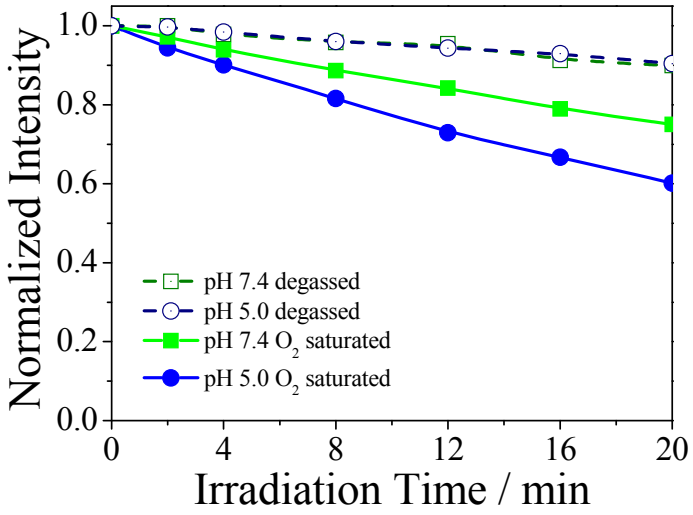


Figure S6. Emission intensities (λ_{ex} = 500 nm, λ_{em} = 555 nm) recorded at 25 ºC for the aqueous solution of **BP2** (0.5 g/L, 22 μM eosin moieties) after being subjected to green light irradiation (520 nm) for varying time periods.

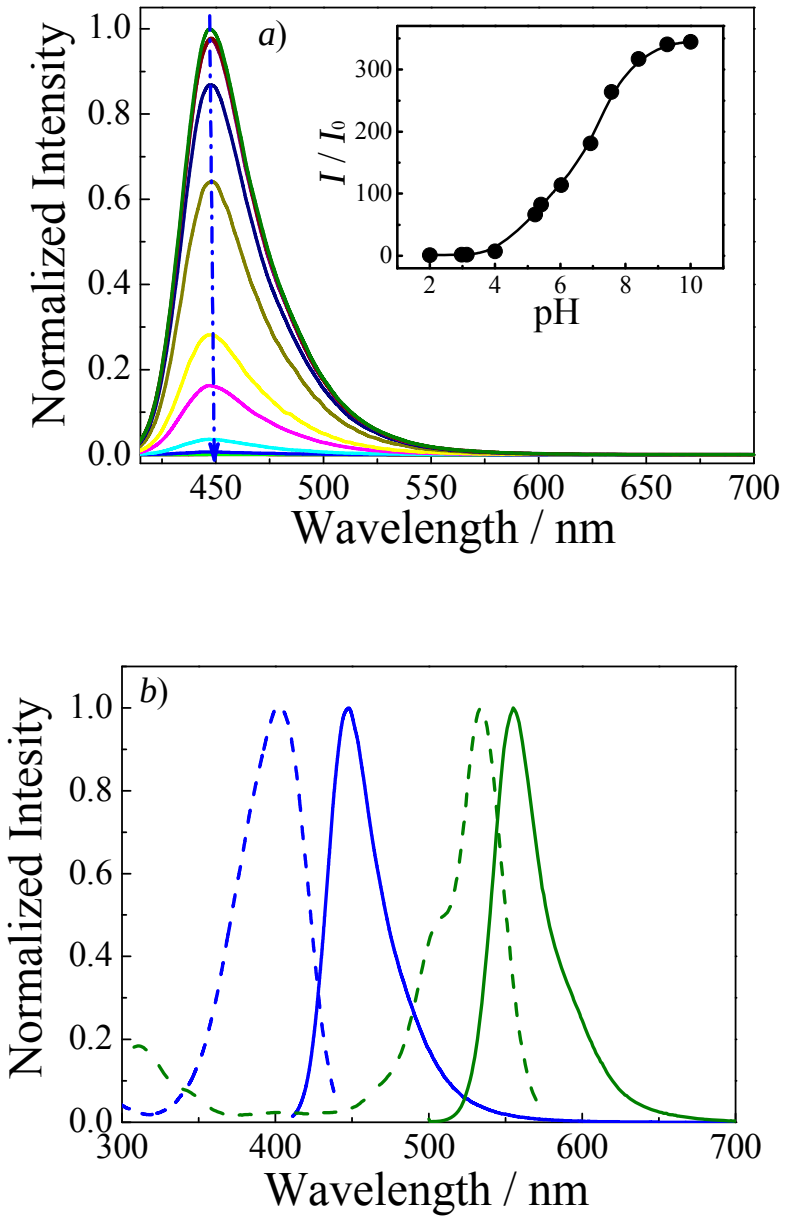


Figure S7. (a) pH-dependent fluorescence emission spectra (λ_{ex} = 405 nm) recorded at 25 °C for aqueous **BP1** solution (0.5 g/L) in the pH range of 2-10; the inset in (a) shows pH-dependent emission intensity changes (λ_{em} = 448 nm). (b) Fluorescence emission and excitation spectra recorded for 0.5 g/L aqueous **BP1** solution (blue lines) at pH 7.4 and aqueous **BP2** solution (green lines) at pH 5.0.

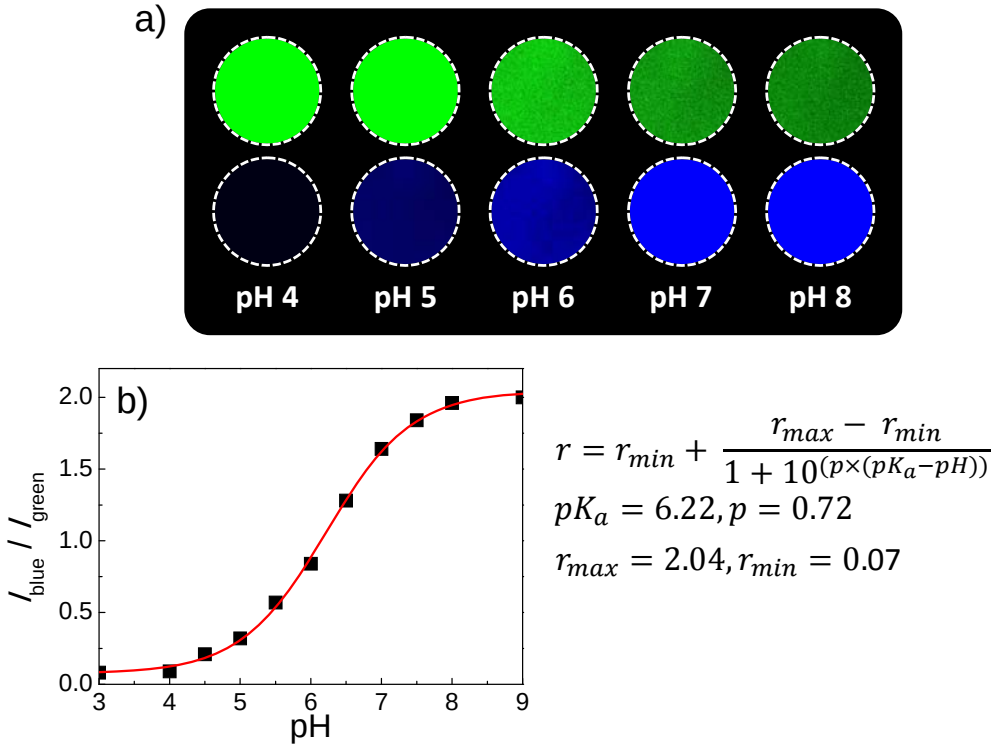


Figure S8. (a) CLSM images and (b) emission intensity ratio (I_{blue}/I_{green}) changes recorded for 0.5 g/L aqueous solution of **BP3** at varying pH.

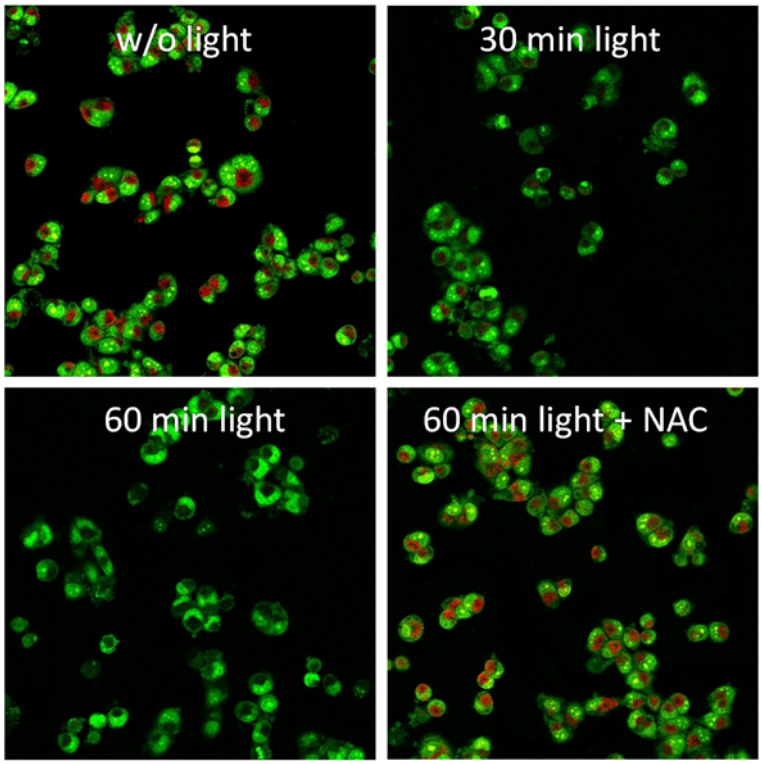
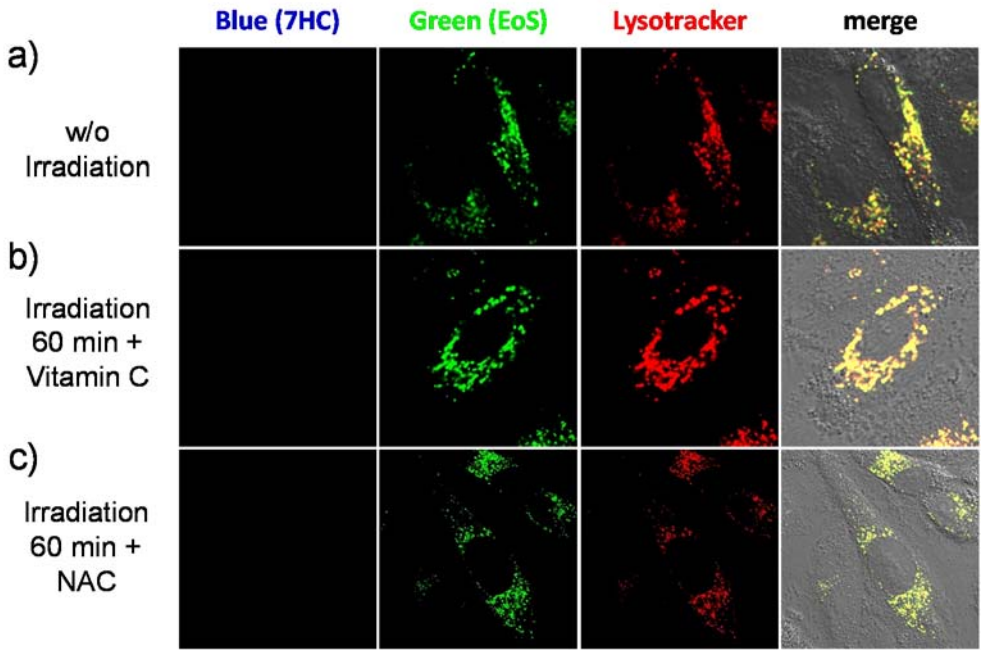


Figure S9. Observation of lysosomal disruption of A549 cells induced by **BP3** micelles. The cells were treated (a) without irradiation or with (b) 30 min and (c,d) 60 min green light (520 nm) irradiation after co-incubating with **BP3** micelles for 2 h; the cells were further incubated with 6 μM acridine orange (AO) for 30 min before CLSM analysis. Green channel emission was collected at 530 ± 20 nm and red channel at 620 ± 20 nm. AO as an intracellular indicator of acid organelle integrity emits red fluorescence in lysosomes, and displays green fluorescence in the cytosol and nuclei. The addition of NAC as $^1O_2$ scavenger in (d) led to reduced lysosomal disruption.



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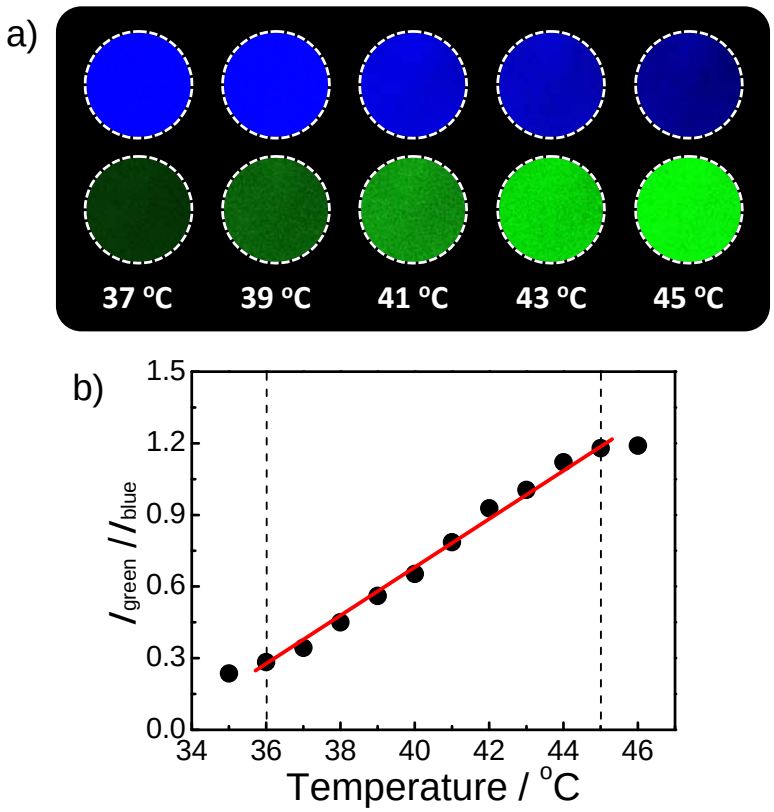

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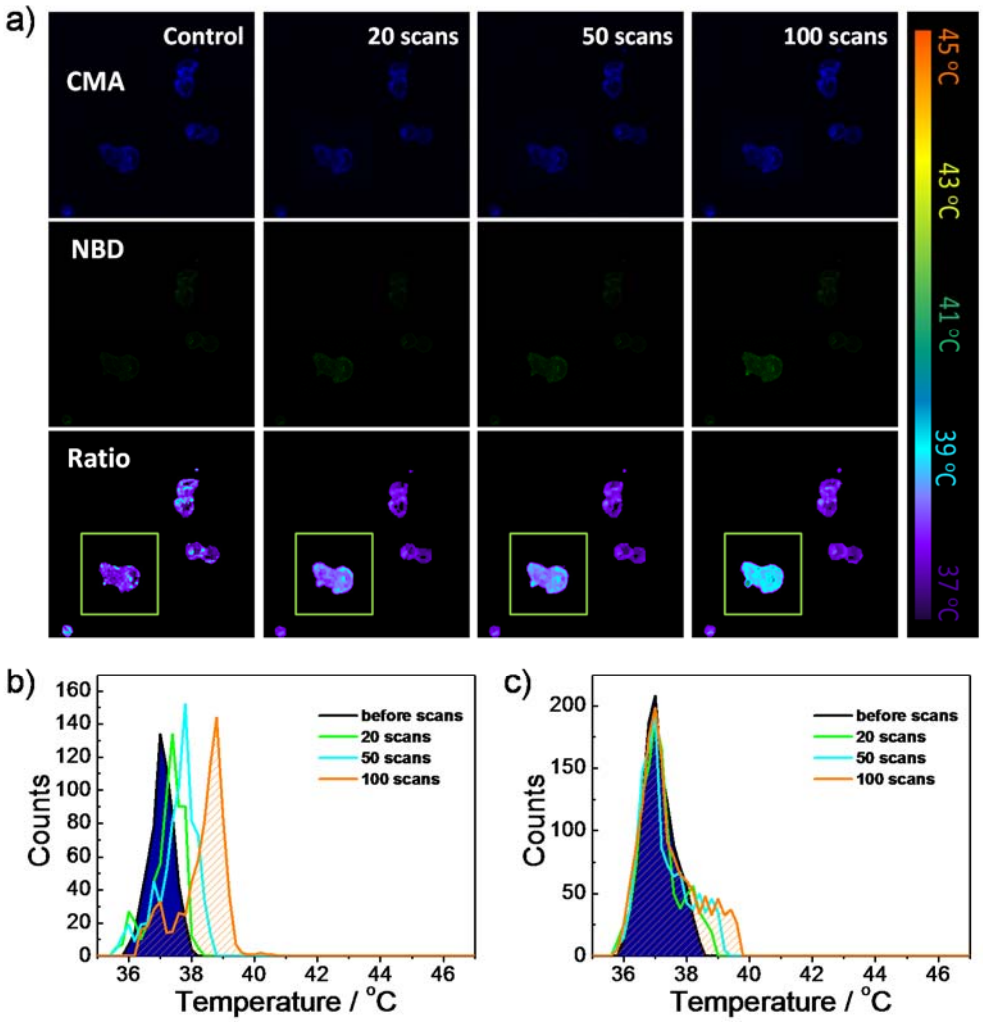

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