

Supporting information

Fluorescent organic ion pairs based on berberine: Counter-ion effect on the formation of particles and on the uptake by colon cancer cells

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| Ber ⁺ R ⁻ | λ_{em} (nm) | ϕ_P (solid) |
|-----------------------------------|---------------------|------------------|
| Ber ⁺ Pal ⁻ | 530 | 0.044 |
| Ber ⁺ AOT ⁻ | 546 | 0.052 |
| Ber ⁺ Tos ⁻ | 512 | 0.031 |
| Ber ⁺ TPB ⁻ | 552 | 0.006 |

Table S1. Maximum emission wavelength (λ_{em}) and photoluminescence quantum yields (ϕ_P) of the four organic salts of berberine investigated in this work. Excitation at 420 nm.

| Volume of Ber ⁺ Cl ⁻ solution (μL) | Concentration of Ber ⁺ Cl ⁻ solution (mM) | Volume of Na ⁺ X ⁻ (μL) | Concentration of Na ⁺ X ⁻ (mM) | Final concentration of Ber ⁺ X ⁻ (μM) |
|--|---|---|--|---|
| 500 | 1 | 500 | 1 | 500 |
| 750 | 0.333 | 250 | 1 | 250 |
| 975 | 0.0256 | 25 | 1 | 25 |

Table S2. Volumes and concentrations of solutions used to prepare Ber⁺X⁻ salts using Protocol 1.

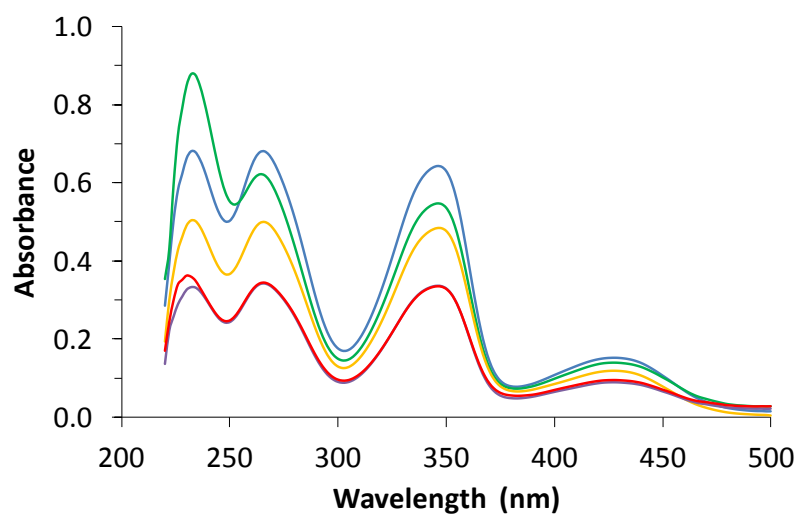


Figure S1. UV-vis absorption spectra of the berberine salts dissolved in acetonitrile. Ber^+Pal^- , orange line; Ber^+AOT^- , purple line; Ber^+Tos^- , red line; Ber^+TPB^- green line, Ber^+Cl^- , blue line. Salt concentration in the 10^{-5} M range. The deformation of the Ber^+TPB^- spectrum at low wavelengths can be attributed to absorption of TPB^- anion.

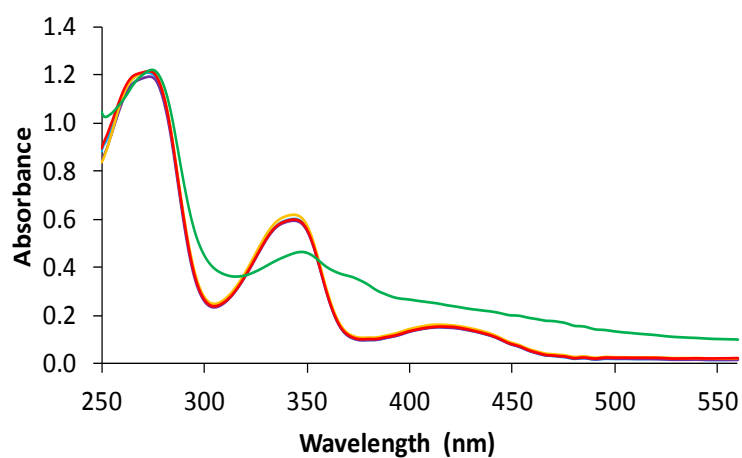


Figure S2. UV-vis absorption spectra of the berberine salts prepared in Opti-MEM according to Protocol 1. Ber^+Pal^- , orange line; Ber^+AOT^- , purple line; Ber^+Tos^- , red line; Ber^+TPB^- green line, Ber^+Cl^- , blue line. Salt concentration: 25 μM .

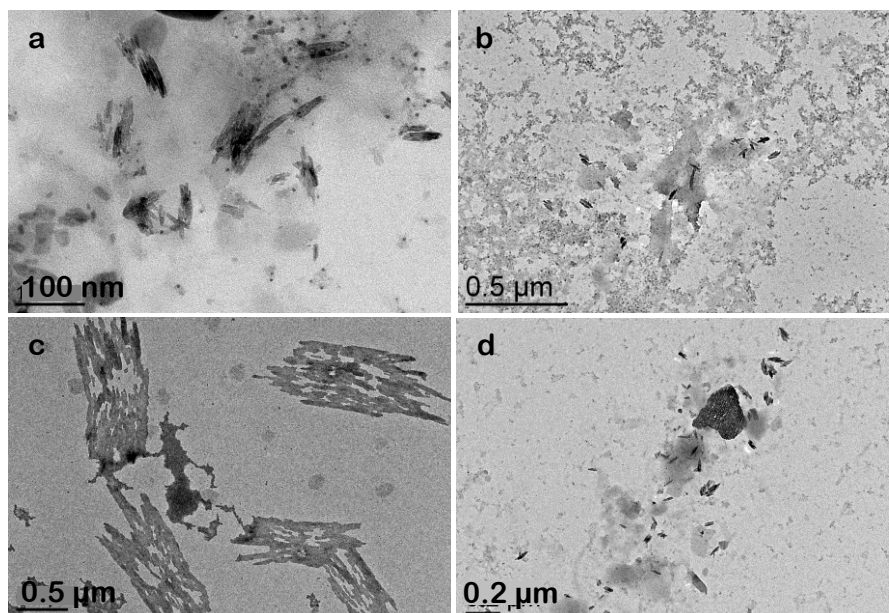


Figure S3. Transmission electron microscopy images of berberine salts in water at 50 μM . a) Ber^+Tos^- , b) Ber^+Pal^- , and c) Ber^+AOT^- prepared according to Protocol 1. d) Ber^+TPB^- prepared according to Protocol 2.

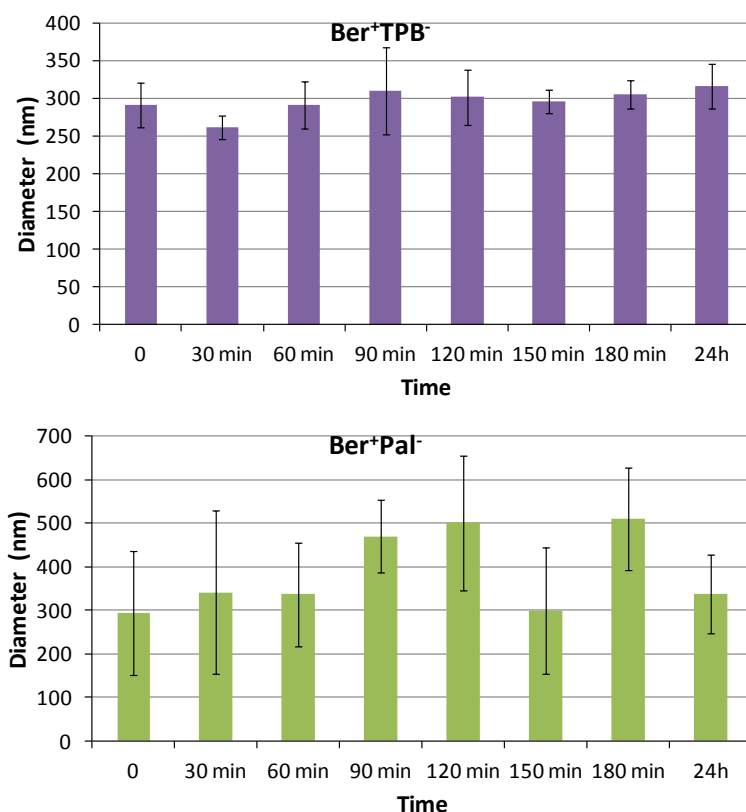


Figure S4. Evolution over time of the hydrodynamic diameter (in intensity) measured by DLS. NPs of Ber^+TPB^- and Ber^+Pal^- in water were prepared using Protocol 1 and incubated at 25°C. Salt concentration: 50 μM .

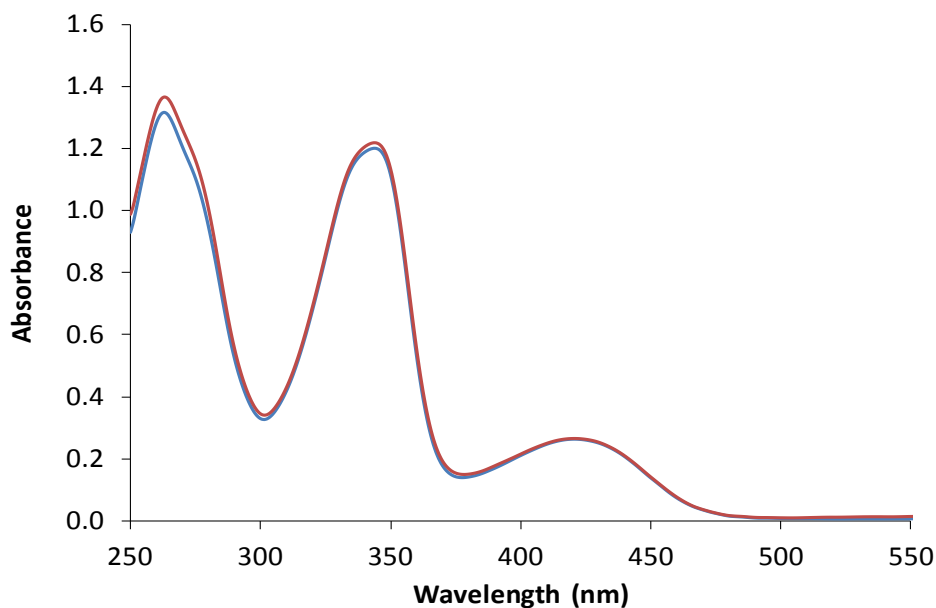


Figure S5. UV-vis absorption spectra of Ber^+Cl^- in water (blue line) and in Opti-MEM (red line) at $50\ \mu\text{M}$. Each spectrum is the average of 5 independent measurements.

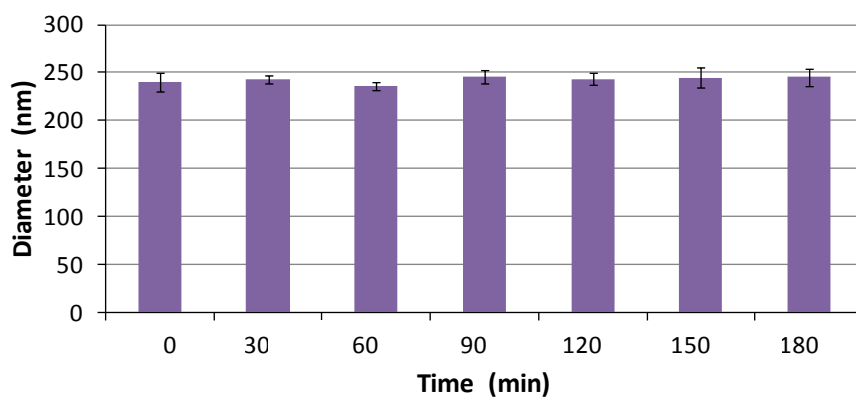


Figure S6. Evolution over time of the hydrodynamic diameter (in intensity) measured by DLS. NPs of Ber^+TPB^- in Opti-MEM containing 5% fetal calf serum were prepared using Protocol 1 and incubated at 37°C . Salt concentration: $50\ \mu\text{M}$.

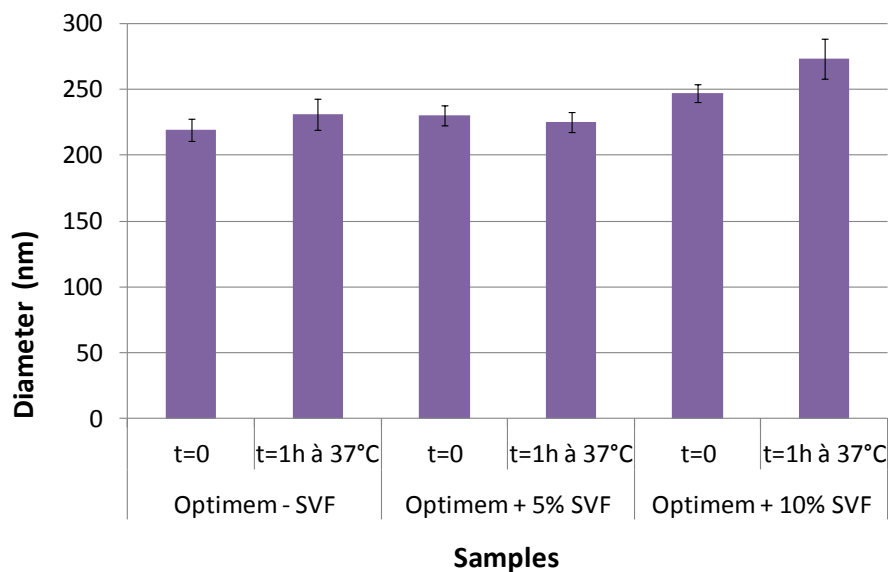


Figure S7. Hydrodynamic diameter (in intensity) measured by DLS of Ber^+TPB^- nanoparticles in Opti-MEM containing 0%, 5% and 10% fetal calf serum, one measurement immediately after dilution ($t = 0$), and after 1h incubation at 37°C ($t = 1\text{h}$). NPs were prepared using Protocol 1, salt concentration: $50\ \mu\text{M}$.

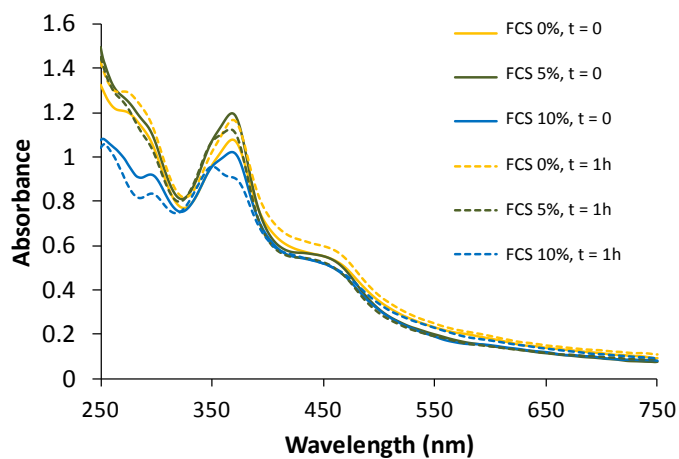


Figure S8. UV-vis absorption spectra of Ber^+TPB^- in Opti-MEM containing 0%, 5% and 10% fetal calf serum. Samples were prepared using Protocol 1 and absorbance was measured immediately after dilution (solid lines) and after 1h incubation at 37°C (dashed lines). Salt concentration: $50\ \mu\text{M}$.

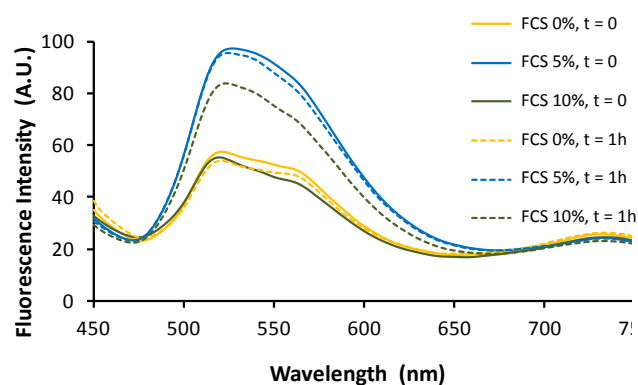


Figure S9. Fluorescence spectra of Ber^+TPB^- in Opti-MEM containing 0% (yellow line), 5% (green line) and 10% (blue line) fetal calf serum. Samples were prepared using Protocol 1 and fluorescence was measured immediately after dilution (solid lines), and after 1h incubation at 37 °C (dashed lines). Salt concentration: 50 μM . $\lambda_{\text{ex}} = 420 \text{ nm}$.

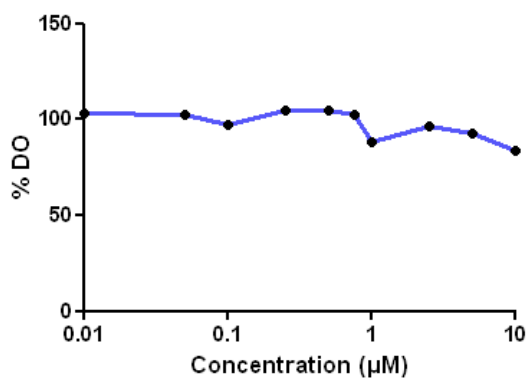


Figure S10. Absorbance of HCT-116 cells incubated with various concentrations of Ber^+Pal^- after reaction with reagent WST-1. Absorbance measured at 450 nm.

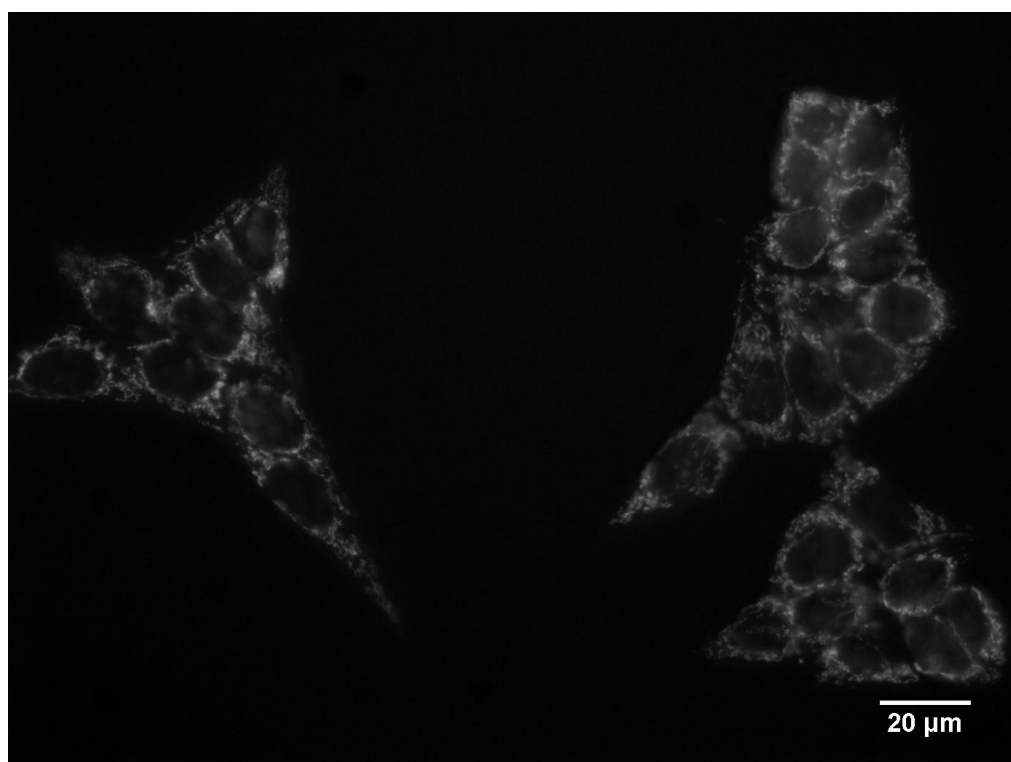
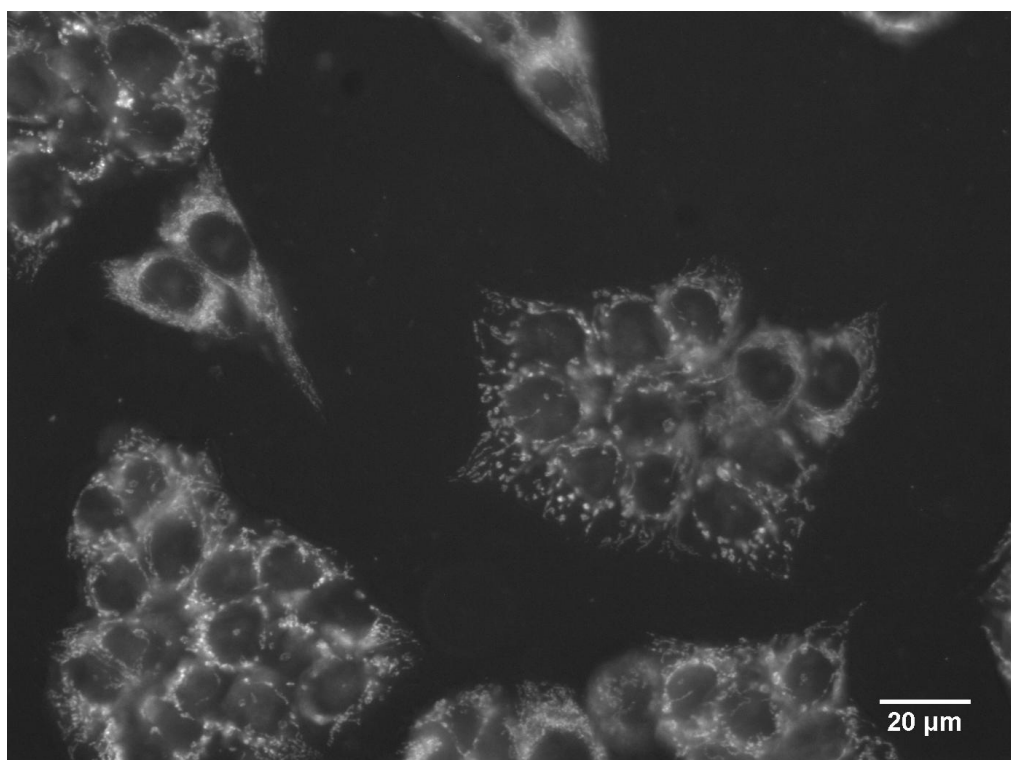


Figure S11. Microscopy images of HCT 116 wt cells incubated during 1h at 37 °C in the presence of Ber^+TPB^- at 50 μM . $\lambda_{\text{ex}} = 470 \text{ nm}$.

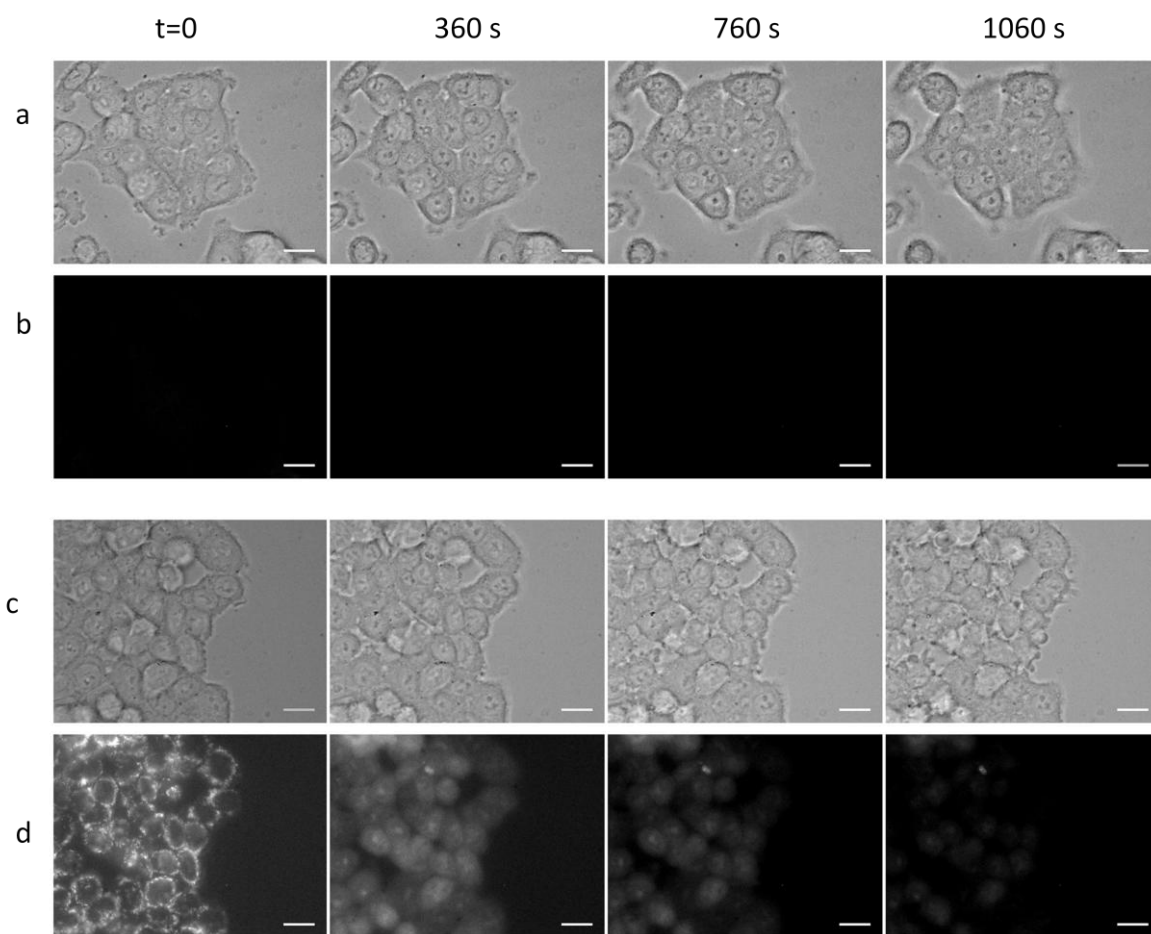


Figure S12. Videomicroscopy images of HCT-116 cells incubated at 37°C for 1h without berberine salt (a, b) and with Ber⁺TPB⁻ (c, d) at the concentration of 50 μM in Opti-MEM, and then washed with Opti-MEM, recorded in transmitted light (a, c) and fluorescence light (b, d). One image was taken every 40 s but only selected images are shown here. $\lambda_{\text{ex}} = 470 \text{ nm}$ and $\lambda_{\text{em}} = 525 \text{ nm}$. Scale bar: 25 μm.