Electronic Supplementary Material (ESI) for Integrative Biology.

Supporting Information

Intracellular singlet oxygen photosensitizers: On the road to solving the problems of sensitizer degradation, bleaching and relocalization.

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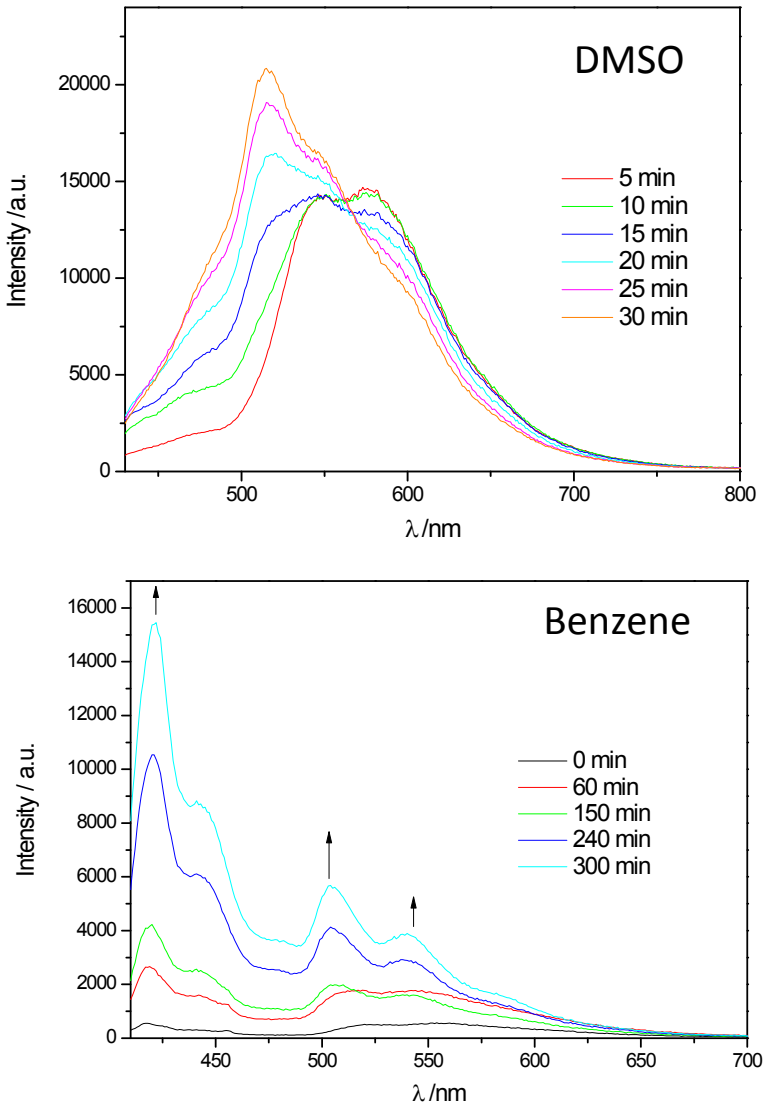

Figure S1. Emission spectra from BP in Benzene (1 kHz fs laser irradiation at 400 nm with 57 mW/cm^2) and DMSO (1 kHz fs laser irradiation at 420 nm with 43 mW/cm^2) recorded as a function of elapsed irradiation time. The data show that different products are formed upon irradiation.

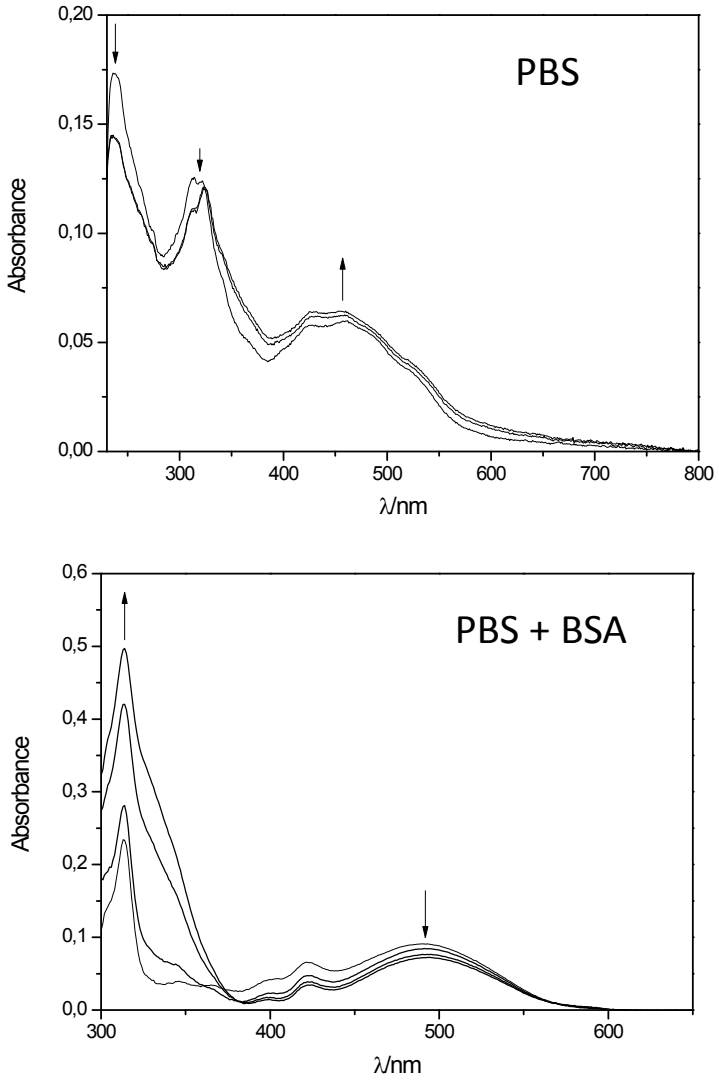

Figure S2. Absorption spectra of BP in aqueous phosphate-buffered solutions, PBS, as a function of the elapsed irradiation time with a 1 kHz fs laser at 420 nm. (top) Data were recorded over an elapsed irradiation period of 220 min with 17 mW/cm^2. (bottom) The sample also contained 0.75 mM Bovine Serum Albumin, BSA, and data were recorded over an elapsed irradiation period of 220 min with 29 mW/cm^2. The pronounced changes at ~ 310-320 nm likely reflect BSA oxidation. The differences in λ_{max} of BP absorption may reflect a combination of (a) differences in the extent of BP aggregation (see data in Figure S3), (b) BP binding to BSA, and (c) the effect of a high BSA concentration on light scattering (see discussion of Figure 5). In both experiments, DMSO (0.25% by volume) was added to facilitate BP solubility.

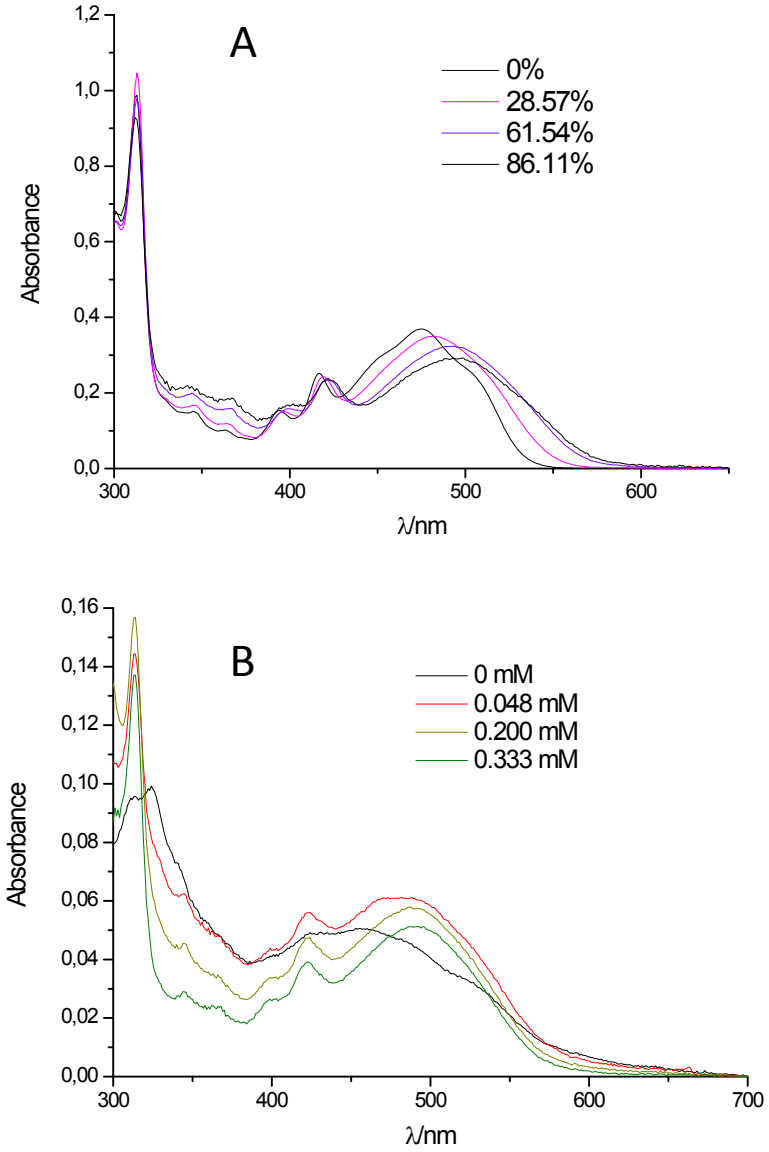

Figure S3. (A) Absorption spectra of BP in DMSO with different percentages of added aqueous PBS (% by volume). (B) Absorption spectra of BP in PBS solution containing different amounts of added BSA.

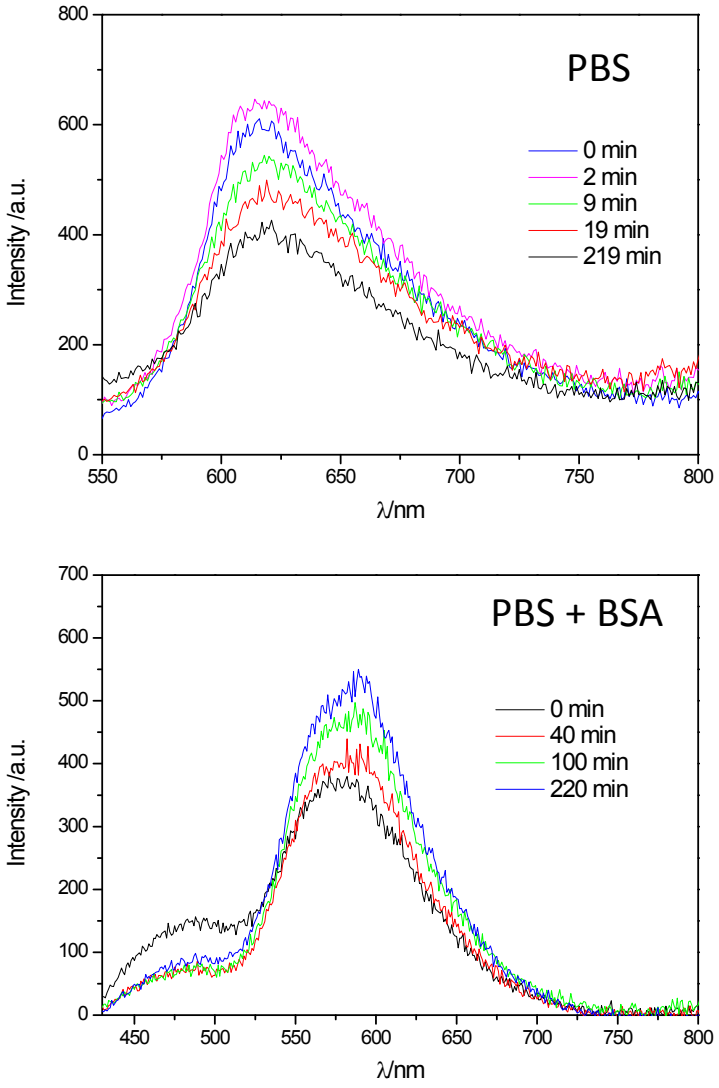

Figure S4. Emission spectra from BP in PBS and BP + BSA in PBS recorded as a function of elapsed irradiation at 400 nm (1 kHz fs laser irradiation with 57 mW/cm^2).

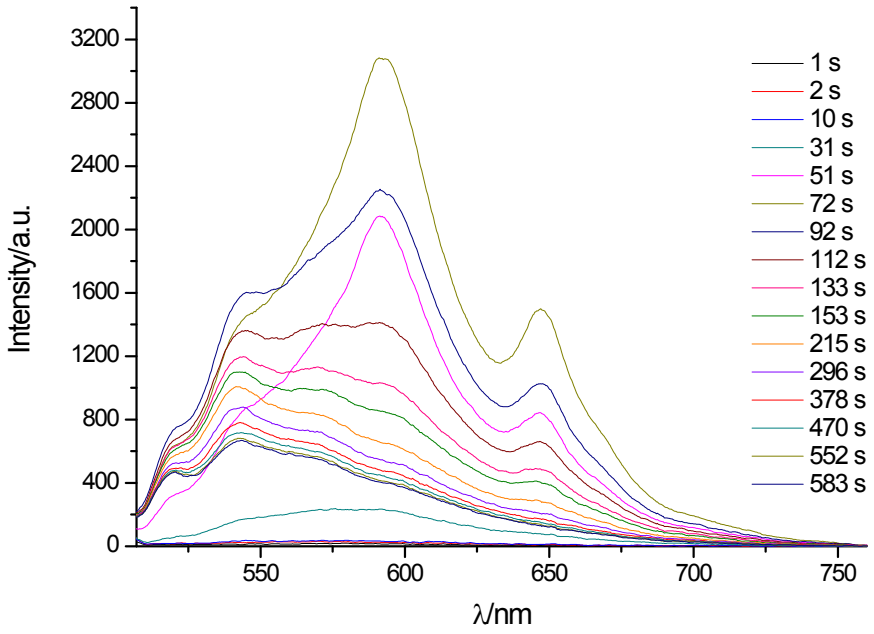

Figure S5. BP emission from HeLa cells. Emission detected at wavelengths longer than 500 nm upon excitation at 480 nm (8 mW/cm^2 from a *cw* metal halide lamp). Images were recorded as a function of the elapsed irradiation time at 480 nm.

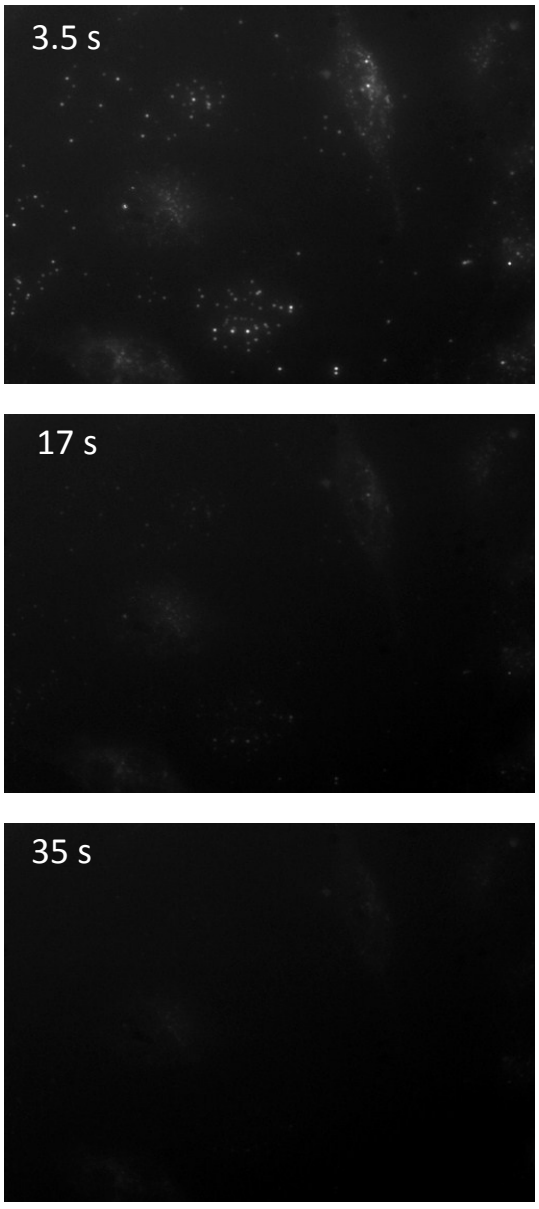

Figure S6. Emission from BBB-containing Hela cells. Emission detected at wavelengths longer than 500 nm upon excitation at 425 nm (6.2 mW/cm^2 from a *cw* metal halide lamp). Images were recorded as a function of the elapsed irradiation time at 425 nm.

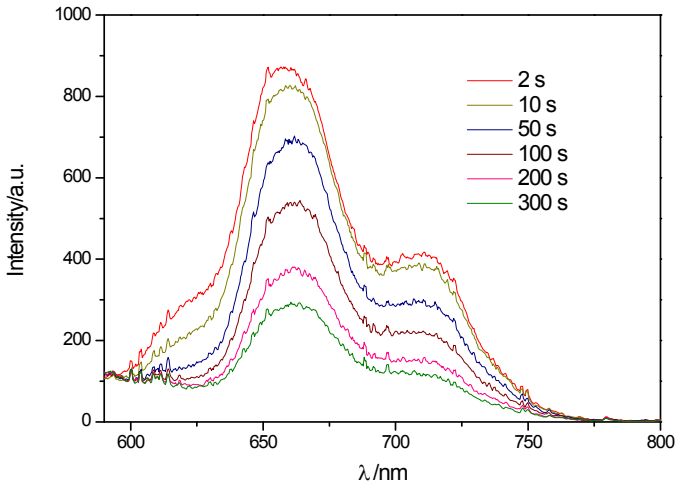

Figure S7. TMPyP fluorescence emission from Hela cells. Emission detected at wavelengths longer than 500 nm upon excitation at 425 nm (2.6 mW/cm^2 from a *cw* metal halide lamp). Images were recorded as a function of the elapsed irradiation time at 425 nm. The absence of pronounced changes in the spectrum, combined with corresponding fluorescence-based images of cells (see reference 26 in the main paper), lead us to infer that re-localization of TMPyP is the principal factor causing the decrease in fluorescence intensity.

Further Details about the Photobleaching Quantum Yields:

The photobleaching quantum yield can be expressed as:

$$\Phi_{pd} = \frac{initial\ rate\ of\ disappearance\ of\ photosensitizer\ molecule\ (v_m)}{initial\ rate\ of\ absorption\ of\ photons\ (v_p)} \quad (1)$$

The initial disappearance rate of the photosensitizer can be approximated by

$$v_m = \frac{\Delta n}{\Delta t} \quad (2)$$

where the number of photosensitizer molecules is given by $n = CVN_A$, and C is the concentration in mol/dm^3, V the volume in dm^3 and N_A Avogadro's number. The change in the concentration of photosensitizer molecules, ΔC, can be approximated by the change in absorbance, ΔA, per unit time

$$\Delta C = \frac{\Delta A}{\varepsilon l} \quad (3)$$

where it is assumed that ΔA only quantifies sensitizer disappearance and does not reflect the appearance of degradation products. Thus,

$$\Delta n = \frac{VN_A \Delta A}{\varepsilon l} \quad (4)$$

It is important to emphasize that the quantity $(\Delta A)/(\varepsilon l \Delta t)$ only approximates the initial rate of disappearance of the photosensitizer and can become unreliable for large Δt values. For a more accurate treatment, one should ideally determine the order of the reaction rate for sensitizer disappearance.

The initial rate of absorption of the photons is given by the difference between the number of incident (I_0) and transmitted (I_t) photons, by unit time

$$v_p = I_0 - I_t = I_0\left(1 - 10^{-A_0}\right) \quad (5)$$

where A_o is the initial absorbance of the medium at the wavelength λ of the (monochromatic) incident light. The number of incident photons per unit time (the photon flux) is determined by the radiant power (P) of the incident light taking into consideration the energy of each incident photon

$$I_0 = \frac{P}{hc/\lambda} \tag{6}$$

Thus,

$$v_p = \frac{\lambda P(1 - 10^{-A_0})}{hc} \tag{7}$$

From these two initial rates, we obtain

$$\Phi_{pb} = \frac{VN_A hc}{\varepsilon l \lambda P(1 - 10^{-A_0})} \frac{\Delta A}{\Delta t} \tag{8}$$

The value of Φ_{pd} accounts for the photon flux of the light source, the volume irradiated and the photodecomposition rate constant.