Electronic Supplementary Information

Chemicals. Doxorubicin hydrochloride was obtained from Selleck Chemicals (>99% purity, cat. no. S1208). Ampliflu™ Red (>98% purity, cat. no. 90101), horseradish peroxidase (type VI-A, cat. no. P6782) and dimethyl sulfoxide (>99.5% purity, cat. no. 41647) were obtained from Sigma-Aldrich. Sodium borohydride was obtained from Spectrum Chemicals (>98% purity, cat. no. S1187). All chemicals were used as received. Milli-Q water was used for aqueous solutions preparation. $N_2(g)$ used was provided in-house as boil-off from $N_2(l)$.

The concentrations of doxorubicin, horseradish peroxidase, and resorufin solutions were determined spectrophotometrically using ε_{480}=1.15 x 10^4 $M^{-1}cm^{-1}$ (doxorubicin),[1] ε_{403}=1.02 x 10^5 $M^{-1}cm^{-1}$ (HRP),[2] and ε_{571}=6.97 x 10^4 $M^{-1}cm^{-1}$ (resorufin).[3] The Ampliflu™ Red solution was prepared by dissolving excess Ampliflu™ Red in a 6 μM horseradish peroxidase solution that is 2:1 water:DMSO.

Chemical reduction and recovery of doxorubicin. In order to investigate chemical reduction and recovery of doxorubicin, $NaBH_4$ was added to a 60μM doxorubicin solution ($NaBH_4$ is the limiting reagent). The reaction mixture was placed in a 1 cm x 1 cm quartz cuvette equipped with a magnetic stirrer. The progress of the reaction was monitored using a SPEX Fluorolog 3 fluorescence spectrometer with excitation at 530 nm (2 nm bandwidth) and emission collected at 590 nm (2 nm bandwidth).

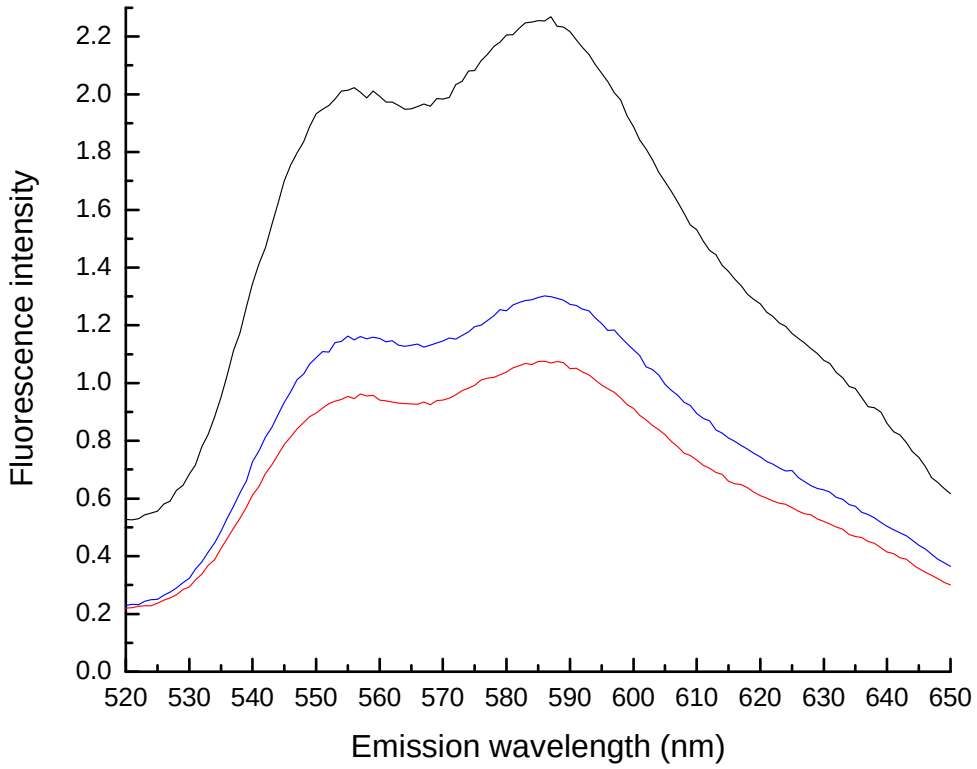
Photochemical degradation and recovery. The photochemical degradation of doxorubicin was carried out in the SPEX Fluorolog 3 fluorescence spectrometer. This instrument is equipped with a 450 W Xe arc lamp. A 40 μM doxorubicin solution was placed in a 1 cm x 1 cm quartz cuvette equipped with a magnetic stirrer. Photoreduction was performed using 320 nm light (29 nm bandwidth) and emission was collected at 590 nm (2 nm bandwidth). The measurement was

carried out for both open cuvette (for exposure to atmospheric O_2) and closed cuvette (equipped with a septum, for anaerobic measurements). To reduce the concentration of dissolved oxygen, the solution was purged with $N_2(g)$ for 15 minutes before measurement. Once measurements were started, the fluorescence intensity of solutions was measured with the sample under a continuous flow of N_2 over the solution. For purged samples, the recovery of doxorubicin was observed only after removal of nitrogen protection, and with excitation at 530 nm (2 nm bandwidth) and emission monitored at 590 nm (4 nm bandwidth).

Detection of H_2O_2. The measurement of resorufin fluorescence was performed in a 1 mm pathlength cuvette. A 40 μM doxorubicin solution (0.3 mL) was irradiated with 320 nm light (29 nm bandwidth) for 40 minutes, while an identical sample was kept in the dark. After that time both samples were mixed with Ampliflu™ Red solution (50 μL) and 10 minutes later the emission spectrum of resorufin produced by this reaction was recorded with excitation at 575 nm (2 nm bandwidth) and emission collection from 585 nm to 700 nm (2 nm bandwidth).

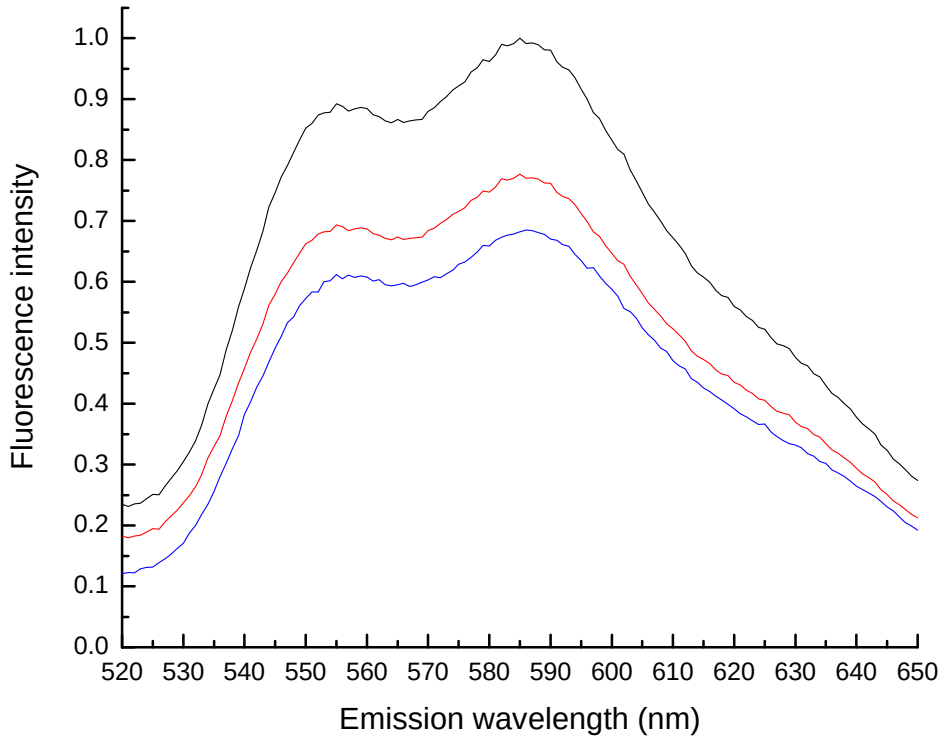
Spectroscopic measurements. Absorbance measurements were made using a Cary model 300 double beam UV-visible absorption spectrometer, with a spectral resolution of 1 nm. The 3mL of 50μM doxorubicin solution was placed in 1 cm pathlength cuvette equipped with a magnetic stirrer. The sample was irradiated with 320 nm light (29 nm bandwidth) under nitrogen-purged conditions for 120 minutes in the fluorescence spectrometer sample chamber. After photoreduction, the absorbance spectrum of doxorubicin was recorded. The sample was exposed to O_2 and 100 μL of Ampliflu™ Red solution was introduced, then the absorbance spectrum was measured (Fig. 3b). The resorufin absorbance band intensity corresponds to a H_2O_2 concentration of 1 μM.

Fluorescence spectra associated with Figure 1.



Black line – doxorubicin emission spectrum registered at the beginning of chemical reduction.
Red line - doxorubicin emission spectrum after ~650 sec.
Blue line – doxorubicin emission spectrum after ~2000 sec.

Fluorescence spectra associated with Figure 2a.



Black line – doxorubicin emission spectrum before exposure to UV.
Blue line- doxorubicin emission spectrum after exposure to UV in a nitrogen atmosphere
Red line – doxorubicin emission spectrum after exposure to UV in an air atmosphere

References

1. M. Fiallo, A. Laigle, A. Garnier-Suillerot, C. Amirand, J.-P. Ballini, L. Chinsky, M. Duquesne, B. Jolles, F. Sureau, P. Y. Turpin and P. Vigny, *Biochimica et Biophysica Acta*, 1993, **1177**, 236-244.
2. P. I. Ohlsson and K. G. Paul, *Acta Chemica Scand. B*, 1976, **30**, 373-375.
3. T. M. Kitson and K. E. Kitson, *Biochemical Journal*, 1997, **322**, 701-708.