Electronic Supplementary Information

Supramolecular nanoreactors for intracellular singlet-oxygen sensitization†

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Experimental Procedures

Materials and methods

Chemicals were purchased from commercial sources and used as received with the exception of THF, which was distilled over sodium and benzophenone. Compounds **1**, **3** and **4** were prepared according to literature procedures.[S1–S3,] Absorption spectra were recorded with a Varian Cary 100 Bio spectrometer, using quartz cells with a path length of 1.0 cm. Emission spectra were recorded with a Varian Cary Eclipse spectrometer in aerated solutions. Time-resolved absorption spectra were recorded with a Luzchem Research mLFP–111 spectrometer by illuminating orthogonally the sample with a Continuum Surelite II–10 Nd:YAG pulsed laser (355 nm, 6 ns at FWHM), using quartz cells with a path length of 1.0 cm. Steady-state emission of $^1O_2$ was recorded with a Fluorolog-2 Mod- 111 spectrometer, equipped with a InGaAs detector maintained at –196 °C, by illuminating orthogonally the sample at 405 nm with a continuous-wave laser. The luminescence decay of $^1O_2$ at 1.27 μm was probed orthogonally to the exciting pulsed-laser beam (355 nm, 6 ns at FWHM) with a pre-amplified (low impedance) Hamamatsu EI-P Ge-photodiode (300 ns resolution) maintained at –196 °C and coupled to a long-pass silicon filter (>1.1 μm) and an interference filter (1.27 μm). Plate readings were performed with a Flex station at 470 nm, using an excitation wavelength of 435 nm. Fluorescence images were recorded with a Leica SP5 confocal laser-scanning multiphoton microscope, using an excitation wavelength of 459 nm and a detection window of 470–520 nm.

Crystallographic analysis

The data crystal of **4** was glued onto the end of a thin glass fiber. X-ray intensity data were measured with a Bruker SMART APEX2 CCD-based diffractometer, using Mo Kα radiation (λ = 0.71073 Å).[S4] The raw data frames were integrated with the SAINT+ program by using a narrow-frame integration algorithm. Corrections for Lorentz and polarization effects were also applied with SAINT+. An empirical absorption correction based on the multiple measurement of equivalent reflections was applied using the program SADABS. The structure was solved by a combination of direct methods and difference Fourier syntheses and refined by full-matrix least-squares on F^2 with the SHELXTL software package.[S5] Crystal data, data collection parameters and results of the analyses are listed in Table S1. Crystallographic data were deposited with the Cambridge Crystallographic Data Centre (CCDC No. 1056448). Copies of this information may be obtained free of charge from: The Director, CCDC12 Union Road, Cambridge CB2 1EZ, UK [fax: +44(1223) 336−033; e−mail: deposit@ccdc.cam.ac.uk; www.http://www.ccdc.cam.ac.uk].

Red single crystals of **4**, suitable for X-ray diffraction analysis, were obtained from a MeCN solution after the slow evaporation of the solvent. Compound **4** crystallized in the monoclinic crystal system and the space group $P2_1/n$ was chosen based on the systematic absences in the intensity data. All non-hydrogen atoms were refined with anisotropic displacement parameters. Hydrogen atoms were placed in geometrically idealized positions and included as standard riding atoms during the least-squares refinements.

Polymer nanoparticles

Aliquots (200 μL) of a $CHCl_3$ solution of **1** (2.5 mg mL^{-1}) were mixed with aliquots (50 μL) of $CHCl_3$ solutions of **2** (0.1 mg mL^{-1}), **4** (0.1 mg mL^{-1}) or both (0.1 mg mL^{-1} each). The solvent of each mixture was distilled off under reduced pressure. The residues were dried under vacuum for 30 min and dispersed in PBS (1.0 mL, pH = 7.0). After sonication for 5 min, the dispersions were filtered and the filtrates were used for spectroscopic experiments without further purification. Alternatively, aliquots (100 μL) of the $CHCl_3$ solution of **1** were mixed with aliquots (50 μL) of the $CHCl_3$ solutions of **2** and **3**. The solvent of each mixture was distilled off under reduced pressure. The residues were dried under vacuum for 30 min and dispersed in PBS (0.5 mL, pH = 7.0). After sonication for 5 min, the dispersions were filtered, equal volumes of the two filtrates were mixed and the resulting solution was used for spectroscopic experiments without further purification.

Cells

Cells were cultured in Dulbecco's modified Eagle's media, supplemented with foetal bovine serum (10%, v/v), penicillin (200 U mL^{-1}), streptomycin (200 μg mL^{-1}), non-essential amino acids (0.1 mM) and incubated at 37 °C in O_2/CO_2/air (20:5:75, v/v/v). For the intracellular energy-transfer experiments, cells were seeded in 96-well glass-bottom plates at a density of 5×10^4 cells mL^{-1} and incubated overnight at 37 °C in O_2/CO_2/air (20:5:75, v/v/v). The cultured cells were incubated with a PBS solution of nanoparticles of **1** (125 μg mL^{-1}), containing either **2** (2.5 μg mL^{-1}) or **4** (2.5 μg mL^{-1}), or with a mixture (1:1, v/v) of the two solutions for 3 hours and washed three times with PBS (80 μL) and the intensity of each well was measured using a plate reader. For the one-photon viability experiments, HeLA, HUVECs or Hs 27 cells, seeded in 96-well plates as described above, were incubated without or with a PBS solution of nanoparticles of **1** (125 μg mL^{-1}), containing either **2** (0–5 μg mL^{-1}) or **4** (0–5 μg mL^{-1}), or with a mixture (1:1, v/v) of the two solutions for 3 hours. Selected wells were irradiated at 435 nm (5 mW) for 0–60 s with a continuous-wave laser. After replacing PBS with fresh media (100 μL), the illuminated cells were maintained in the dark at 37 °C under O_2/CO_2/air (20:5:75, v/v/v) for a further 24 hours. A PBS solution (5 mg mL^{-1}, 20 μL) of (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added to each well and the plates were maintained under the same conditions for a further 4 hours. After removing a portion (85 μL) of the medium from each well and adding dimethylsulfoxide (100 μL), the absorbance was recorded at 595 nm with a plate reader. For the two-photon viability experiments, HeLA cells, seeded in 386-well plates as described above, were incubated with a PBS solution of nanoparticles of **1** (125 μg mL^{-1}), containing either **2** (2.5 μg mL^{-1}) or **4** (2.5 μg mL^{-1}), or a mixture (1:1, v/v) of the two solutions for 3 hours. Selected wells were irradiated at 900 nm for 30 s with a mode-locked Ti:sapphire laser, generating pulses of 100 fs at a rate of 80 MHz and an average power of 80 mW. The illuminated cells were maintained in the dark at 37 °C under O_2/CO_2/air (20:5:75, v/v/v) for a further 24 hours. Then, the number of viable cells per well was determined using a microscope fitted with a counting grid.

S1 Y. Gabe, Y. Urano, K. Kikuchi, H. Kojima and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 3357.

S2 I. Yildiz, S. Impellizzeri, E. Deniz, B. McCaughan, J. F. Callan, and F. M. Raymo, *J. Am. Chem. Soc.*, 2011, **133**, 871.

S3 W. Wu, H. Guo, W. Wu, S. Ji and J. Zhao, *J. Org. Chem.*, 2011, **76**, 7056.

S4 Apex2 Version 2.2-0 and SAINT+ Version 7.46A; Bruker Analytical X-ray System, Inc., Madison, Wisconsin, USA, 2007.

S5 (a) G. M. Sheldrick, SHELXTL Version 6.1; Bruker Analytical X-ray Systems, Inc., Madison, Wisconsin, USA, 2000. (b) G. M. Sheldrick, *Acta Cryst.*, 2008, **A64**, 112.

Table S1. Crystallographic Data for **4**.

Empirical Formula	$C_{19}H_{17}BN_2F_2I_2$
Formula Weight	575.96
Crystal System	Monoclinic
Lattice Parameters:	
a (Å)	11.5103(4)
b (Å)	13.0569(5)
c (Å)	13.4292(5)
β (°)	90.900(1)
V (Å^3)	2018.01(13)
Space Group	$P2_1/n$ (# 14)
Z Value	4
ρ_{calc} (g cm^{-3})	1.896
μ (Mo Kα) (mm^{-1})	3.139
T (K)	296
$2\Theta_{max}$ (°)	54.0
No. Obs. ($I > 2\sigma(I)$)	3925
No. Parameters	239
Goodness of Fit	1.070
Max. Shift in Cycle	0.001
*Residuals**: R1; wR2	0.0398; 0.0918
Absorption Correction, Max/min	Multi-Scan 0.7442 / 0.3523
Largest Peak in Final Diff. Map (e$^-$ Å^{-3})	1.197

* $R = \Sigma_{hkl}(\,|\,|F_{obs}|-|F_{calc}|\,|)/\Sigma_{hkl}|F_{obs}|$; $R_w = [\Sigma_{hkl}w(|F_{obs}|-|F_{calc}|)^2/\Sigma_{hkl}wF_{obs}^2]^{1/2}$, $w = 1/\sigma^2(F_{obs})$; $GOF = [\Sigma_{hkl}w(|F_{obs}|-|F_{calc}|)^2/(n_{data}-n_{vari})]^{1/2}$.

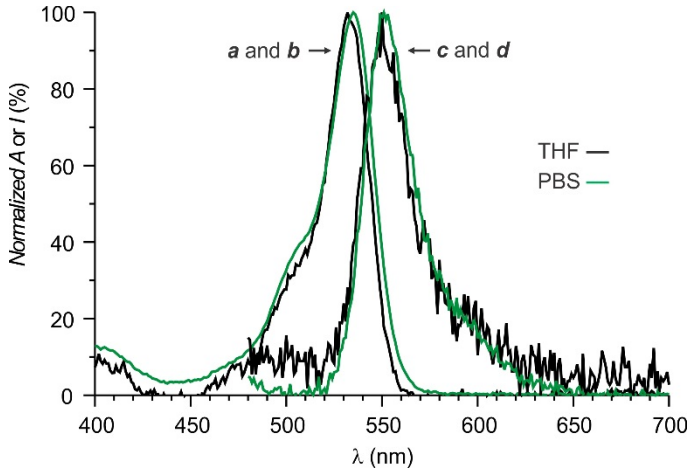

Fig. S1. Normalized absorption (***a*** and ***b***) and emission (***c*** and ***d***, λ_{Ex} = 470 nm) spectra of a THF (1.0 μM, 25 °C) solution of **4** and of a PBS solution (pH = 7.0, 25 °C) of nanoparticles of **1** (500 μg mL^{-1}), containing **3** (5 μg mL^{-1}).

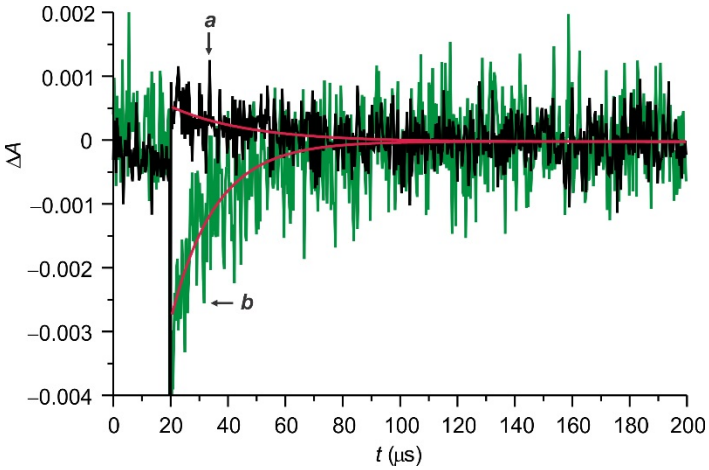

Fig. S2. Absorbance evolution at 420 (***a***) and 530 nm (***b***) of degassed PBS solutions (pH = 7.0, 25 °C) of nanoparticles of **1** (500 μg mL^{-1}), containing **4** (0.2 μg mL^{-1}), upon pulsed-laser excitation (355 nm, 6 ns, 12 mJ per pulse) and corresponding mono-exponential fittings.

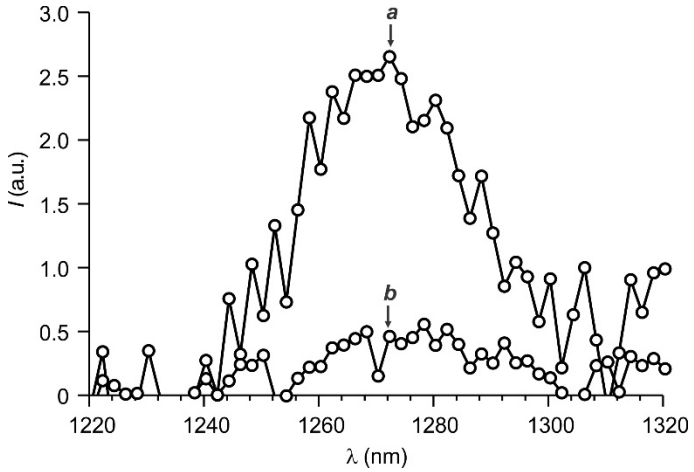

Fig. S3. Emission spectra (λ_{Ex} = 405 nm) of aerated (***a***) and degassed (***b***) deuterated PBS solutions (pH = 7.0, 25 °C) of nanoparticles of **1** (500 μg mL^{-1}), containing **2** (1.2 μg mL^{-1}) and **4** (0.2 μg mL^{-1}).

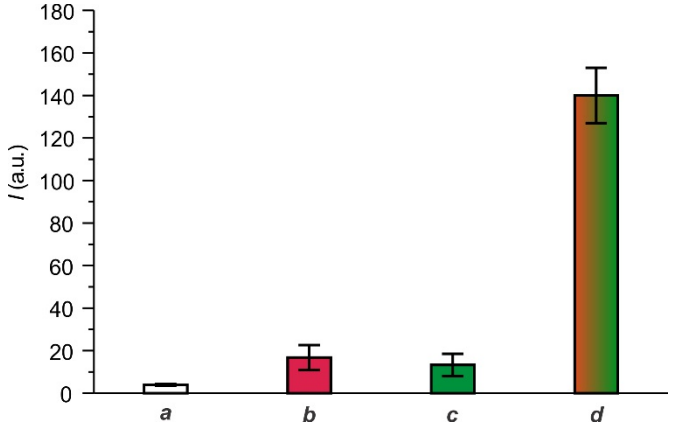

Fig. S4 Emission intensities (λ_{Ex} = 504 nm, λ_{Em} = 535 nm) of PBS solutions of SOSG (5 μM) without (***a***) and with nanoparticles of **1** (500 μg mL^{-1}), containing **2** (***b***, 5 μg mL^{-1}), **4** (***c***, 5 μg mL^{-1}) or both (***d***, 5 μg mL^{-1} each), recorded with a plate reader after irradiation with a continuous-wave laser (435 nm, 5 mW) for 30 s.

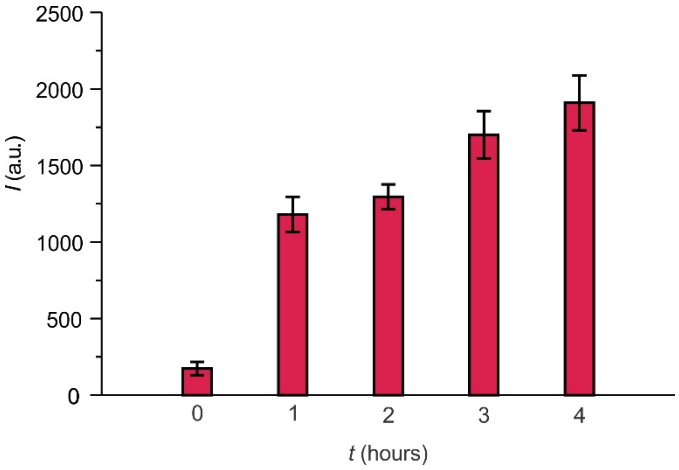

Fig. S5. Emission intensity (λ_{Ex} = 435 nm, λ_{Em} = 470 nm) recorded after incubation of HeLA cells with PBS solutions of nanoparticles of **1** (125 μg mL^{-1}), containing **2** (2.50 μg mL^{-1}) for the times indicated on the horizontal axis, washing and storage in the dark for 24 hours.

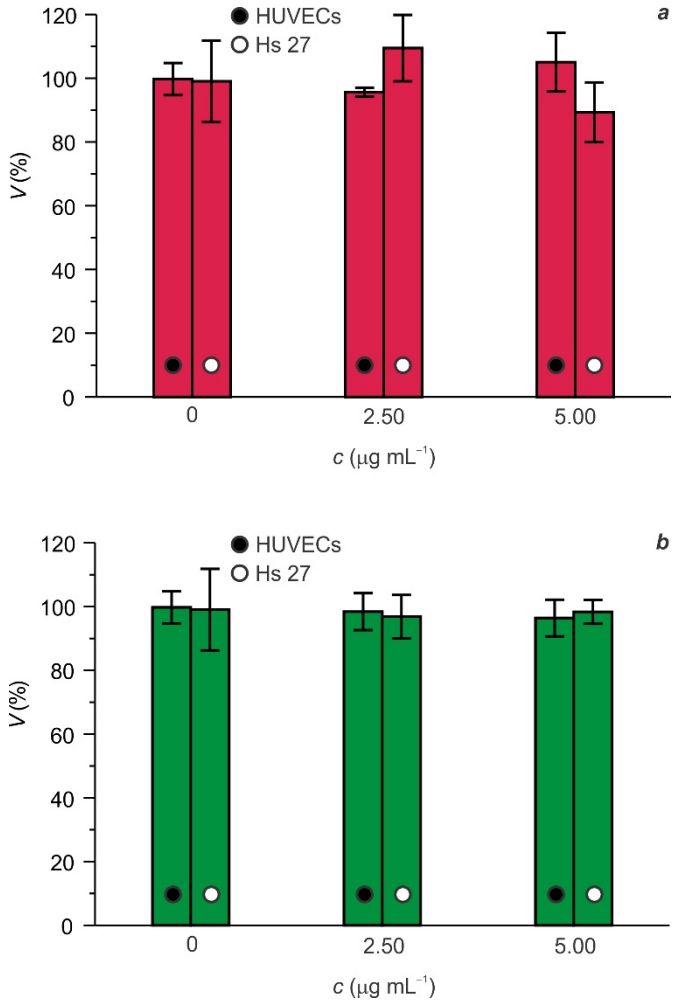

Fig. S6 Viability of HUVECs and Hs 27 cells recorded after incubation with PBS solutions of nanoparticles of **1** (125 μg mL^{-1}), containing increasing amounts of either **2** (***a***) or **4** (***b***), for 3 hours, washing and storage in the dark for 24 hours.

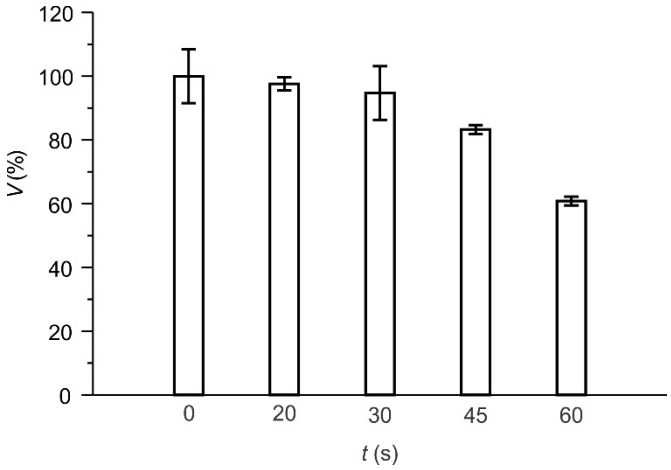

Fig. S7. Viability of HeLA cells recorded after irradiation with a continuous-wave laser (435 nm, 5 mW) for the times indicated on the horizontal axis and subsequent storage in the dark for 24 hours.