Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

Supporting Information

A genetically-encoded photosensitiser demonstrates killing of bacteria by purely endogenous singlet oxygen

Rubén Ruiz-González[1], John H. White[2], Montserrat Agut[1], Santi Nonell[1,*] and Cristina Flors[2,3]*

[1] IQS School of Engineering, Universitat Ramon Llull, Vía Augusta 390, E-08017, Barcelona, Spain

[2] EastChem School of Chemistry, University of Edinburgh, Joseph Black Building, The King's Buildings, West Mains Rd, EH9 3JJ, Edinburgh, UK

[3]IMDEA Nanociencia, C/ Faraday 9, Ciudad Universitaria de Cantoblanco, 28049 Madrid, Spain

Bacterial expression and culture conditions

pET20bTagRFPHis[1] or pET20b plasmids were transformed into competent *E. coli* strain BL21 (DE3). *E. coli* were aerobically grown overnight at 37 ºC in an orbital shaking incubator (250 rpm) in luria-bertani (LB) broth (Fischer Scientific) in the presence of the appropriate amount of antibiotic (100 μg/mL carbenicillin; Sigma) to stationary phase. A reinoculum was then grown in fresh LB medium at 37 ºC to an OD600 = 0.2 (start of log phase). TagRFPHis expression was induced with 50 μM solution of isopropyl β-D-1-thiogalactopyranoside (IPTG; Sigma) for 1 hour at 37°C to an OD600 ≈ 0.70 (logarithmic phase) corresponding to *ca.* 10^8 colony forming units per milliliter (CFU)/mL. The suspensions were then centrifuged (10 min, 5000 rpm) and resuspended with sterile PBS or D-PBS at pH 7.4 at the same concentration for aPDT experiments.

Photodynamic inactivation studies

Cell suspension aliquots (400 μL) were placed in 8-well optical non-treated glass plates (Lab-Tek, Nalgene NUNC international, Rochester, New York). The wells were illuminated from the bottom of the plates by means of an expanded CW 532 nm laser beam (Cobolt Samba, Sweden) at fluences ranging from 0 to 3500 $J \cdot cm^{-2}$. Irradiation was carried out at low power density (40 $mW \cdot cm^{-2}$) and during long periods of time (up to 24 hours), and no significant temperature increase was observed during the experiments. At different time intervals during the illumination, when the desired fluences had been delivered, aliquots were taken from the well (the suspensions were thoroughly mixed before sampling to avoid cell settlement). For determination of cell viability after treatments, aliquots were serially diluted (1:10 dilutions until single colonies could be observed), streaked on nutrient agar, and incubated in the dark for 20 h at 37 ºC. Experiments were carried out in triplicate for each experimental condition.

Electronic Supplementary Material (ESI) for Photochemical & Photobiological Science

Supporting Information

Spectroscopic measurements and microscopy

Emission and excitation spectra were recorded in a Spex Fluoromax-4 spectrofluorometer (Horiba Jobin-Ybon, Edison, NJ). Fluorescence images of living cells expressing TagRFP, immobilized on polylysine-coated glass plates, were captured using a Nikon Eclipse TE2000 inverted microscope, equipped with an oil-immersion objective (Apochromat, 60x, NA 1.49, Nikon). Excitation was provided by a 532 nm DPPS CW laser (Cobolt Samba, Sweden). Wide-field illumination was achieved by focusing the expanded and collimated laser beam onto the back-focal plane of the objective. Emission was collected by the same objective and imaged by an Andor Luca(S) EMCCD camera after passing through a T565lpxr dichroic mirror (Chroma Technology) and a ET605/70 bandpass filter (Chroma Technology).

In Figure S1 small spectroscopic differences –due to differences in microenvironment- can be observed between purified (D-PBS) and cell-expressed TagRFP both in the excitation spectrum (2-nm hypsochromic shift in cells) and in the fluorescence spectrum (7-nm hypsochromic shift in cells).

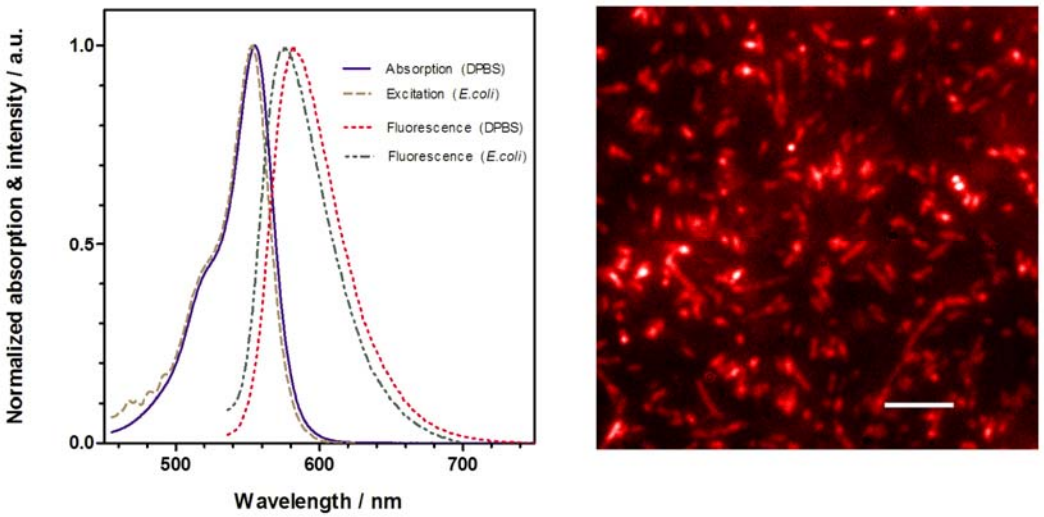

Fig. S1. Spectroscopic measurements of TagRFP. Left panel: Excitation (λ_{obs} = 650 nm) and emission (λ_{exc} = 500 nm) spectra were recorded both in D-PBS and in *E. coli*. Right panel: fluorescence image of TagRFP-expressing bacteria upon laser illumination at 532 nm (scale bar 5 μM).

Integrity of cell membrane

After photodynamic treatments, samples were centrifuged (13000 rpm, 10 min) and the supernatant was monitored by UV—vis spectroscopy (Cary, Varian, Palo Alto) in order to measure the absorbance values at 260 nm, which indicates leakage of small cellular components due to inner membrane damage.[2]

To test the integrity of the outer membrane, the pellet was resuspended and 1-N-phenylnaphthylamine (NPN; sigma) was added to a final concentration of 15 μM. [3,4] Fluorescence spectra was readily measured after NPN addition upon excitation at 350 nm (Figure S2). Cell lysate (3 x 1 minute sonication) was used as a positive control.

Supporting Information

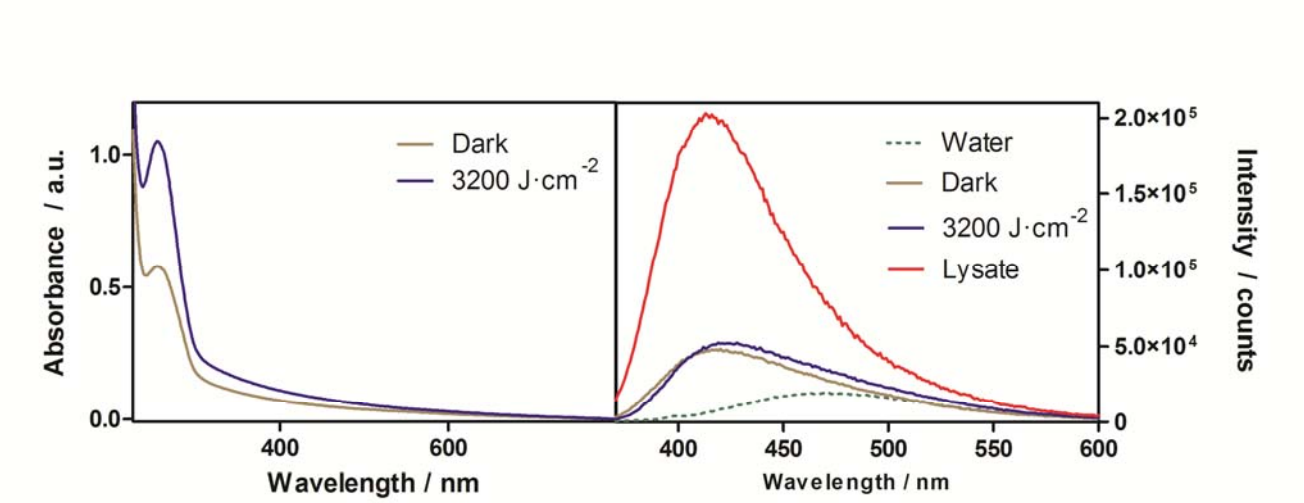

Fig. S2. Cell membrane integrity assays. Left panel: Absorption spectra of supernatants of non irradiated samples (solid brown line) and samples irradiated at 3200 J·cm^{-2} (solid blue line), consistent with cytoplasmic membrane damage. Right panel: Fluorescence spectra of NPN in water (dotted green line), in non irradiated samples (solid brown line), in treated samples (solid blue line) and in cell lysate suspensions (solid red line), consistent with no outer membrane damage in irradiated cells.

DNA purification and electrophoresis

Bacterial cultures of *E. coli* cells were grown and photodynamic treatments were performed as described above. Genomic DNA was immediately extracted from the cells after irradiation by means of a Wizard Genomic DNA Purification Kit (Promega). DNA samples were gently mixed with 3 μL of Loading Buffer 6x and analyzed by agarose gel electrophoresis (0.6 % agarose in TBE buffer). The nucleic acid stain Sybr Green (1 mg/mL; Invitrogen) was incorporated during preparation of agarose gel. The Lamda DNA/EcoRI + Hind III Marker (BioRad) was used as molecular weight marker (MK) (0.5 mg/mL) with DNA fragments between 564 and 21,226 bp.

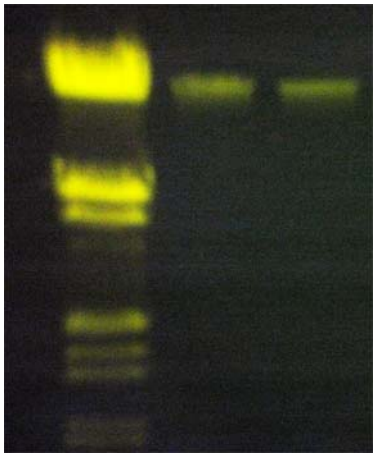

Fig S3. Agarose electrophoresis (0.6% agarose) of extracted genomic DNA samples. Line 1: Lambda DNA/EcoRI+HindIII Marker; Line 2: Dark control; Line 3: Irradiated sample (3200 J·cm^{-2})

References

(1) X. Ragàs, L. P. Cooper, J. H. White, S. Nonell and C. Flors, *Chemphyschem*, 2011, **12**, 161
(2) C. Z. Chen and S. L. Cooper, *Biomaterials*, 2002, **23**, 3359.
(3) B. Loh, C. Grant and R. E. Hancock, *Antimicrob.Agents Chemother.*, 1984, **26**, 546.
(4) M. B. Spesia, D. A. Caminos, P. Pons and E. N. Durantini, *Photodiagnosis Photodyn Ther.*, 2009, **6**, 52.