Electronic Supplementary Material (ESI) for Photochemical & Photobiological Sciences

Evaluation of phototoxicity of dendritic phosphorescent oxygen probes: an *in vitro* study

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Supporting Information

Molecular dynamics simulations

Simulations were performed on a porphyrin **G0** and dendrimers **G1**-**G3**. The peripheral groups on the dendrimers consisted of monomethoxy-polyethyleneglycol ester residues as in PdTBP-AG^n-(CO_2PEG), where PEG=-$(CH_2)_7$-OMe. The simulations included molecular dynamics (CHARMM force field, Hyperchem 7.0) executed on a PC platform. The dynamics simulations were allowed to sample 50-200 ps at 500 K in order to represent sufficient conformational space. All simulations were performed in the medium with distance-dependent dielectric (scale factor=4) to represent water.

Ten conformations were arbitrary selected for each probe, and the "molecular diameters" were determined as averages of five arbitrary selected distances measured across the molecular skeleton – from end to end across the whole molecule.

Electrochemical measurements

Electrochemical measurements were carried out using argon-purged CH_3CN (Romil Hi-Dry™) solutions at room temperature with an EcoChemie Autolab 30 multipurpose instrument interfaced to a PC. The working electrode was a glassy carbon electrode (0.08 cm^2, Amel) and the counter electrode was a Pt wire. A silver wire

was employed as a quasi-reference electrode (QRE). Tetraethylammonium hexafluorophosphate ($TEAPF_6$) (0.1 M) was used as a supporting electrolyte. The potentials are reported relative to SCE. These were measured with AgQRE (quasi-reference electrode) using ferrocene as an internal standard (+0.395 V *vs* SCE). The concentrations of the compounds examined were ca 10^{-3} M. Cyclic voltammograms were obtained at scan rates in the range 0.2–5 V s^{-1}. The experimental error for the potential values was estimated to be ±10 mV. The peak reduction and oxidation potentials for compound **G1** are shown in Table 1.

Table S1. Peak potentials (V, *vs* SCE) in acetonitrile/$TEAPF_6$ solution at 298 K. Scan rate: 1 V/s.

Compound	I_{red}	II_{red}	I_{ox}
G1	-1.47	-1.95	1.46

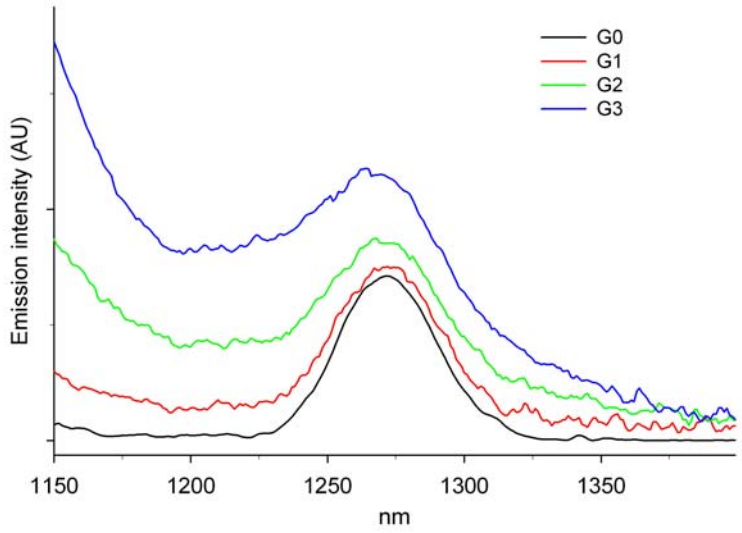


Figure S1. Phosphorescence of singlet oxygen in air-equilibrated C_2H_5OH / CH_3OH 4:1 (v/v) solutions (λ_{max}=1270 nm) as sensitized by compounds **G0-G3**. The emission intensities are directly comparable, since the absorbances of the sensitizer solutions at the excitation wavelength (635 nm) were kept equal for all the compounds.

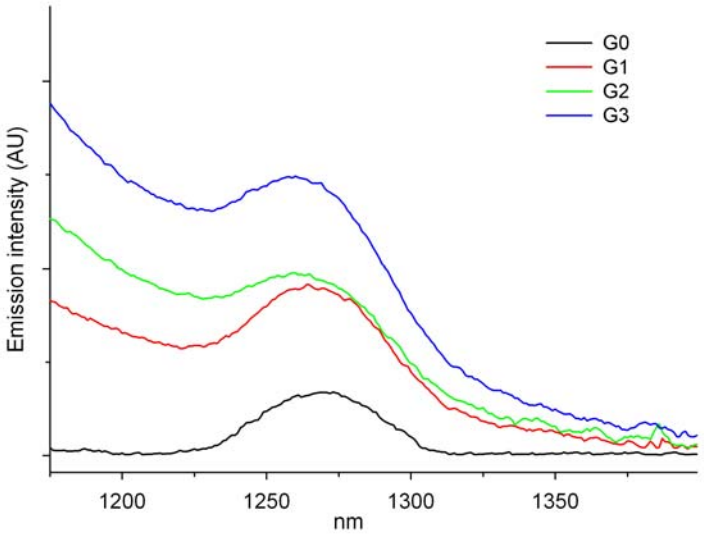


Figure S2. Phosphorescence of singlet oxygen in air-equilibrated D_2O solutions (λ_{max}=1270 nm) as sensitized by compounds **G0-G3**. The emission intensities are directly comparable, since the absorbances of the sensitizer solutions at the excitation wavelength (627 nm) were kept equal for all the compounds.

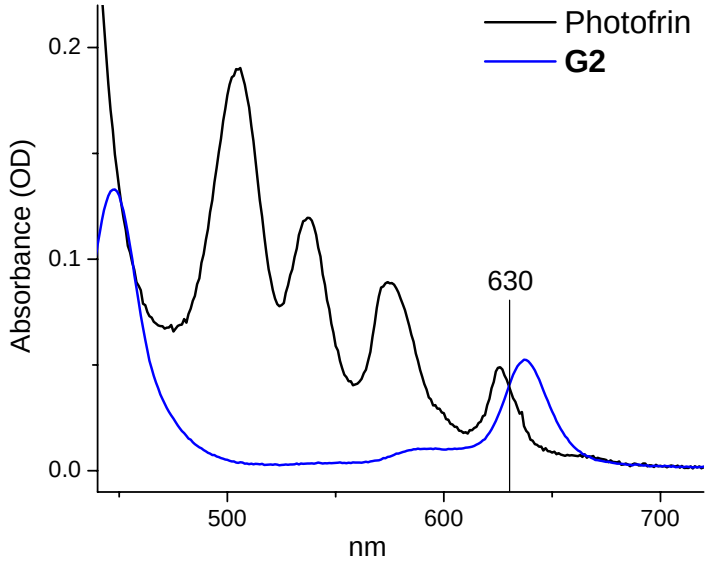


Figure S3. Absorption spectra of Photofrin and **G2** in aqueous solutions. The solutions are isoabsorbing at 630 nm.

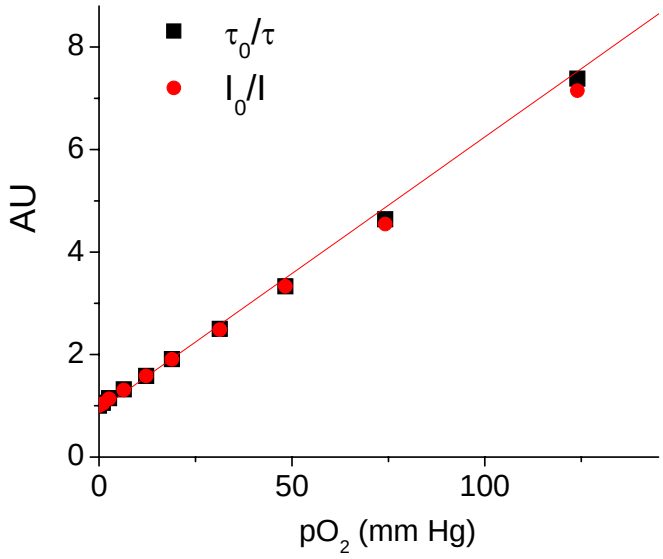


Figure S4. Stern-Volmer plots of the phosphorescence lifetime (black) and phosphorescence intensity (red) of **G2** as a function of partial pressure of oxygen (pO_2). The phosphorescence lifetimes (τ_{av}) were obtained as intensity-weighted averages, determined from double-exponential fits: $\tau_{av}=(I_1\tau_1+I_2\tau_2)/(I_1+I_2)$, where I_1 and I_2 are initial intensities of the decays with lifetimes τ_1 and τ_2, respectively. Lifetime τ_0 was measured a using solution completely deoxygenated by Ar flow. The emisson intensities were obtained from the integrated phosphorescence spectra (λ_{ex}=630 nm). The solution was placed in a fluorimetric cell, equipped with a rubber septa for needle-inlet and outlet of Ar and a stirring bar. Ar flow was slowely passed through the soluiton under continuous stirring, while the phosphorescence lifetime was continuously monitored. When a value of τ close to the desirable was reached, the stirring and the bubbling were stopped, the needles were removed from the cell and the emisson spectrum was recorded, after which the cell was connected to the Ar flow again, and the whole measurement procedure was repeated etc. The final lifetime was measured right before and right after recording the spectrum, and the average value was used to construct the graph. The drift due to the leak of air into the cuvette during the spectral registration was found to be very small, typically no more than 5-7 μs. The values of pO_2 were obtained from the calibration plots consutructed by independent oxygen titration experiments (see main text for details).

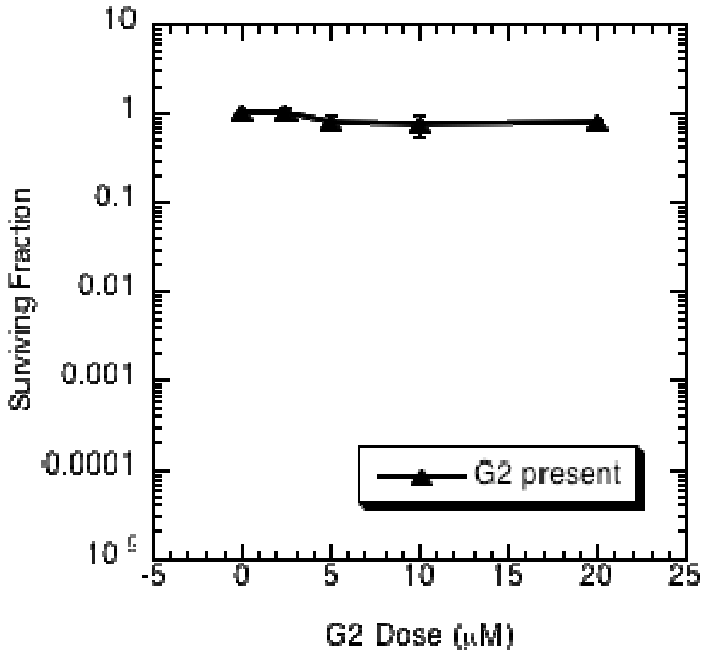
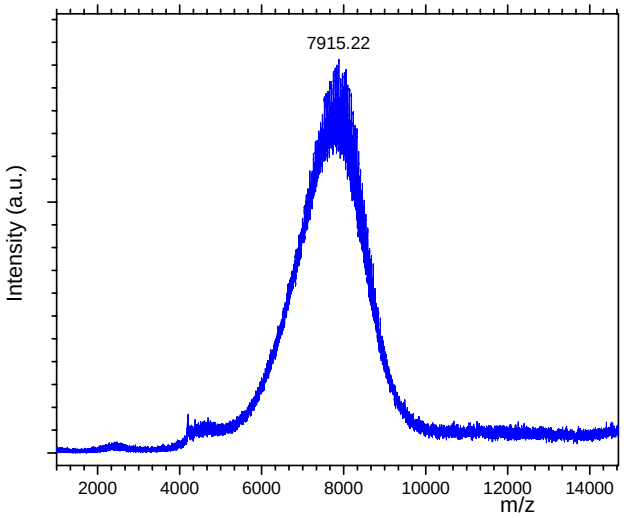
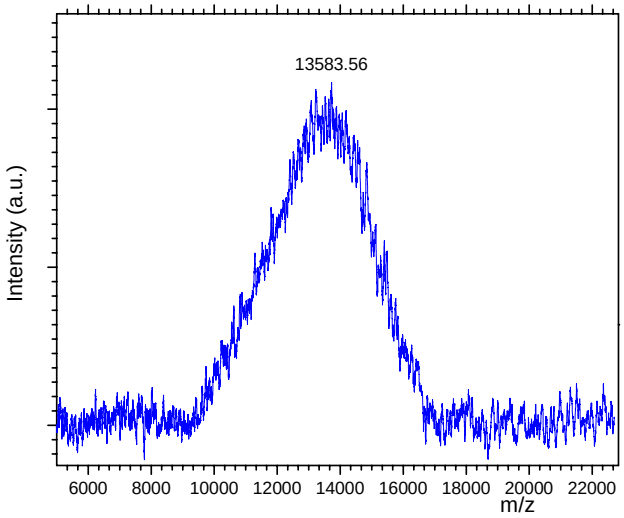


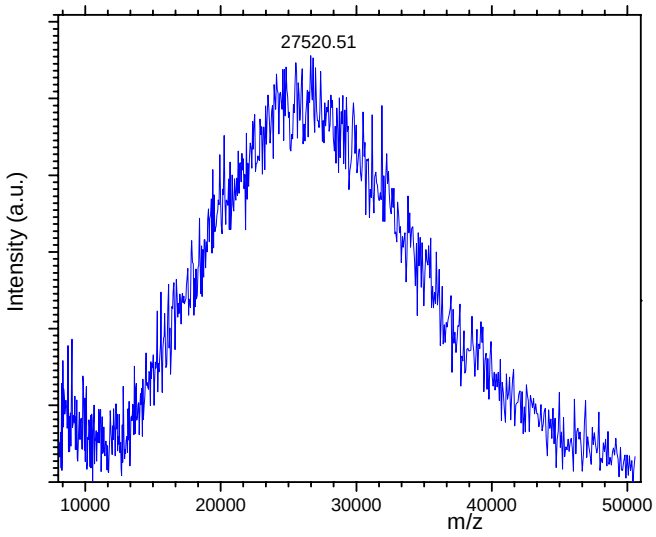
Figure S5. Clonogenic survival data for RIF cells incubated for 18 h in the presence of increasing doses of**G2**and irradiated with 630 nm light at 2 J/cm^2. Surviving fractions are calculated as fractions of the colony-forming cells in the treated samples compared to controls that received neither photosensitizer nor light. The data are shown as average ± SE from 2 independent studies.



G1



G2



G3

Figure S6. MALDI-TOF spectra of PEGylated dendritic probes **G1-G3**.