Supporting Information for

Near-infrared superoxide generator based on a biocompatible indene-bearing heptamethine cyanine dye

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S1 Experimental

S1.1 Superoxide detection with Mito-HE

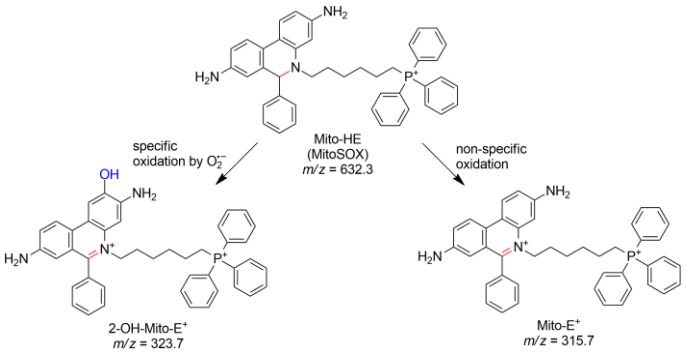

Fig. S1. The oxidation mechanism of Mito-HE.

S1.2 The demonstration of oxidative stress in vitro

Photoinduced processes in cells loaded with dye **1** were studied with H_2DCF as a fluorogenic probe (Fig. S2). Cells were incubated with the probe in the form of H_2DCF-DA that readily crosses cytoplasmic membrane. After passing to the cytoplasm, H_2DCF-DA is cleaved by esterases yielding H_2DCF. Non-fluorescent H_2DCF can be oxidized to fluorescent DCF under certain conditions. In particular, Fenton-type reactions between H_2O_2 and redox-active metal ions (primarily Fe^{2+}) result in the production of •OH that efficiently oxidizes H_2DCF to DCF. Alternatively, H_2DCF can be oxidized by cytochrome *c*. Redox-active metal ions and cytochrome *c* are mostly located within lysosomes and mitochondria, respectively. Since hydrophilic H_2DCF cannot cross membranes, cytosolic DCF-induced fluorescence can be observed only after the release of redox-active metal ions from lysosomes and/or cytochrome *c* from mitochondria[1]. These processes are a result of lysosomal and mitochondrial membrane permeabilization and can be linked to different forms of stress and cell death[2].

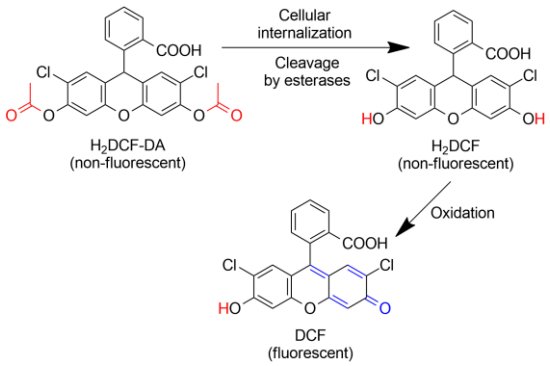

Fig. S2. The transformations of H_2DCF-DA upon cellular uptake and oxidation.

[1] ***a***. Karlsson, M.; Kurz, T.; Brunk, U. T.; Nilsson, S. E.; Frennesson, C. I. What does the commonly used DCF test for oxidative stress really show? *Biochem. J*. **2010**, 428, 183–190; ***b***. Wardman, P. Use of the dichlorofluorescein assay to measure “reactive oxygen species”. *Radiat. Res*. **2008**, 170, 406–407.

[2] ***a***. Wang, F.; Gómez-Sintes, R.; Boya, P. Lysosomal membrane permeabilization and cell death. *Traffic* **2018**, 19, 918–931; ***b***. Kroemer, G.; Galluzzi, L.; Brenner, K. Mitochondrial membrane permeabilization in cell death. *Physiol. Rev*. **2007**, 87, 99–163.

S2 The measurement of the photodegradation quantum yields

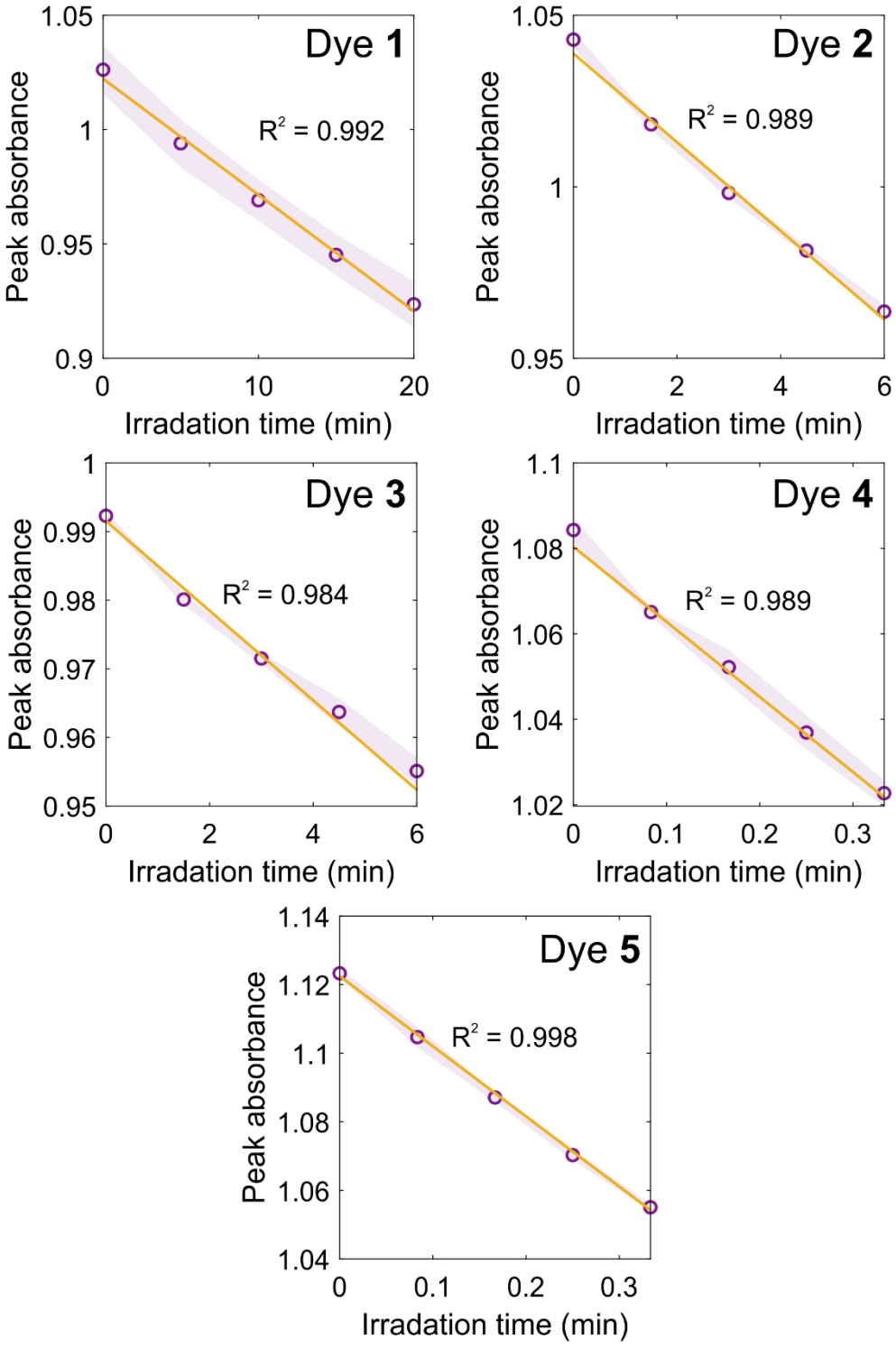

Fig. S3. Absorbance in the peak of the absorption spectrum as a function of irradiation time for 5 μM solutions of dyes **1**–**5** in MeCN/H_2O ($v/v = 1:1$). The samples were exposed to 750 nm laser radiation with an irradiance of 130 mW cm^{-2}. The measurements for each dye were repeated four times.

S3 Mass spectrometry data

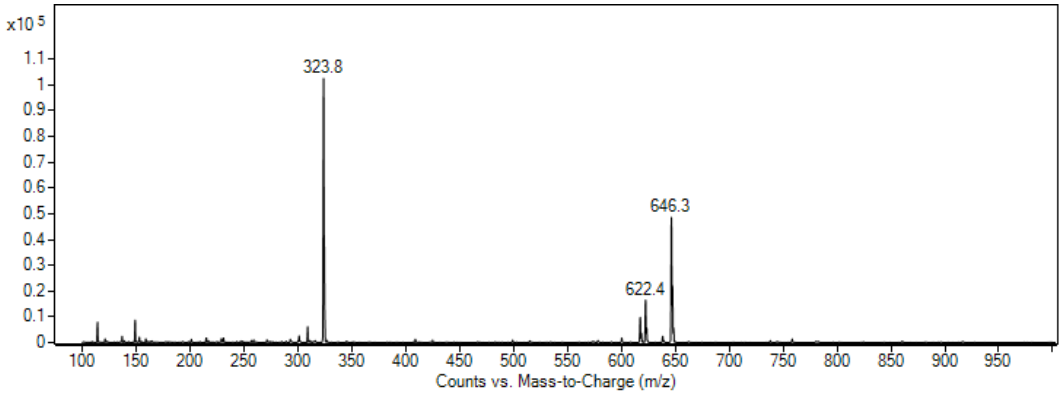

Fig. S4. Mass spectrum for 2-OH-Mito-E$^+$ (m/z calculated 323.7, found 323.8) photogenerated in a 5 µM solution of dye **1** in the presence of 5 µM Mito-HE in phosphate-buffered saline (pH 7.0, 50 mM) under 750 nm irradiation for 4 min (130 mW cm^{-2} irradiance, 31 mW cm^{-2} light dose). Mass spectrometry was performed after HPLC separation as described in the main text (Section 2.5). The presented mass spectrum corresponds to a retention time of 3.35 min in HPLC. The additional peaks in the spectrum can be assigned to the photooxidation products of dye **1** and impurities from the HPLC column.

S4 Absorption and fluorescence spectra for dye 1 inside Vero E6 cells

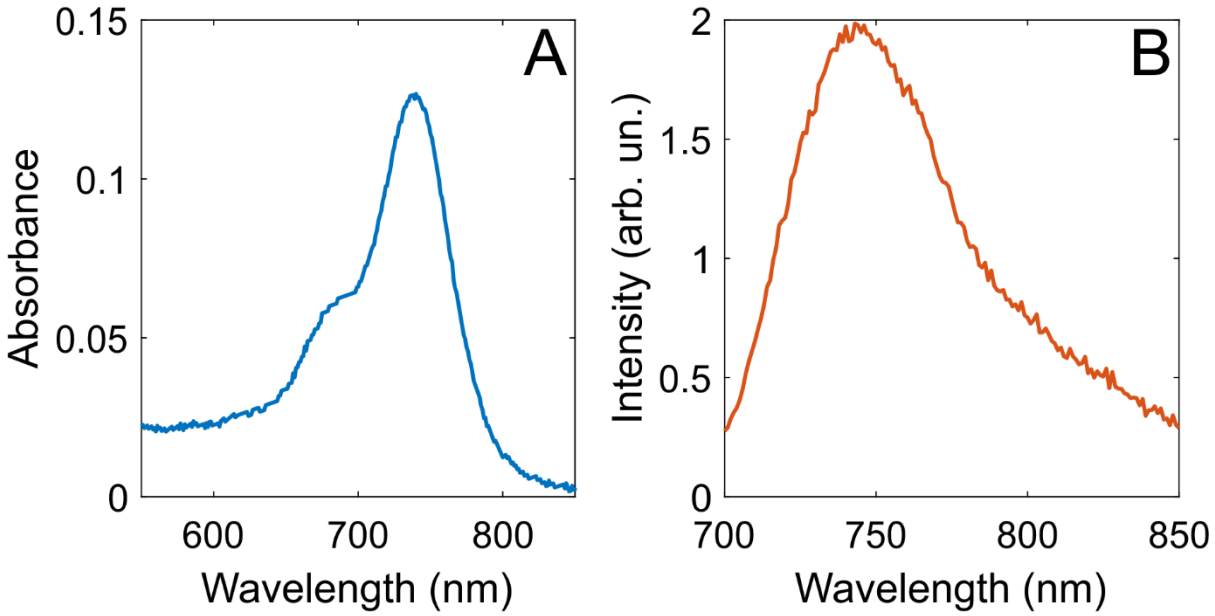

Fig. S5. The absorption spectrum (A) and fluorescence spectrum excited at 650 nm (B) for Vero E6 cells incubated with 10 µM dye **1** for 30 min, washed, and resuspended in HEPES buffer. This excitation wavelength was chosen to minimize the impact of excitation light scattering on the shape of the fluorescence spectrum.