Electronic Supplementary Material (ESI) for Nanoscale Advances.

Investigating the reactive oxygen species production of Rose Bengal and Merocyanine 540-loaded radioluminescent nanoparticles

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Supporting Information

Materials

All reagents were used without further purification. Lu_2O_3 (99.999%) and Gd_2O_3 (99.999%) were purchased from Chemicals 101 Corp. Dy_2O_3 (99.99%) was purchased from Sigma Aldrich. HCl (trace metal grade, 99.999%) was purchased from Fisher Chemical. Sodium Oleate (>97.0%) was purchased from TCI. Rose Bengal was purchased from Alfa Aesar. Merocyanine 540 (dye content 90%), Ammonium Fluoride (99.99% trace metals basis), 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) (BioReagent, suitable for fluorescence, ≥90%), 1,3-Diphenylisobenzofuran (DPBF) (97%), Tetraethyl orthosilicate (98%), Sodium azide (99.5%) and deuterium oxide (99.999%) were purchased from Sigma-Aldrich. 3,3,5,5-Tetramethyl-1-pyrroline N-Oxide (TMPO) (≥95%) and 5,5-Dimethyl-1-pyrroline-N-oxide (DMPO) (≥98%) were purchased from Santa Cruz Biotechnology.

Characterization

X-ray irradiation was performed using an Amptek MINI-X2 portable X-ray source (4 W, Au target) operating at 50 kVp, 80 μA with no collimating or beam hardening filters, leading to a dose rate of approximately 30 Gy/min at the position of the sample. Radioluminescence emissions were collected using a 600 μm diameter optical fiber from Ocean Optics Inc. coupled to a Princeton-Teledyne Instruments FERGIE BRXVR UV-NIR spectrograph for detection. The FERGIE spectrometer was equipped with a 250 grooves/mm grating blazed at 550 nm.

UV-Visible absorption spectroscopy

UV-Visible absorption spectroscopy was performed on a Cary 5000 series UV-Vis-NIR spectrophotometer from Agilent Technologies with a source changeover at 350 nm. Solutions were analyzed in a 1-cm path length quartz cuvette from Thorlabs.

Transmission electronic microscopy

Transmission electron microscopy (TEM) was performed using a Jeol-JEM-2100F microscope operating at 200 kV. Samples were prepared by dropping colloidal dispersions (~ 10 μL) in hexanes on a formvar/carbon film supported on a 300 mesh copper grid (3 mm in diameter) and subsequent evaporation of the solvent. The images obtained were analyzed in ImageJ, free software courtesy of the National Institutes of Health.

Powder X-ray diffraction

Powder X-ray diffraction (PXRD) patterns were recorded on a Scintag XDS-2000 diffractometer equipped with a Si(Li) Peltier-cooled solid state detector, Cu Kα source at a generation power of 45 kV and 40 mA. The 2θ scan range was set from 10-90° with a scanning step size of 0.01° and an integration time of 2 s.

Fourier-Transform Infrared Spectroscopy (FTIR)

To characterize the samples vibrational modes, we used a Nicolet iS50 FTIR spectrometer (Thermo Scientific). The instrument was used in its attenuated total reflectance (ATR) mode. The dried sample was placed against a diamond ATR crystal and the spectra were acquired from 500 to 4000 cm^{-1} with a resolution of 0.8 cm^{-1} and 64 scans were acquired.

Brunauer-Emmett-Teller (BET)

BET analysis was performed by first activating the sample at 150°C under vacuum for 24 hours using a Micromeritics Smart VacPrep system. BET analysis was then performed using a Micromeritics TriStar II PLUS 2.03 instrument.

Electron paramagnetic resonance (EPR) spectroscopy

The spectra were acquired on a Bruker (Karlsruhe, Germany) Elexsys E580 X band EPR instrument using a high-sensitivity resonator. The sample was analyzed in a flat cell (Suprasil, 150 µL, 0.3 mm wide) to minimize signal loss. A 1 G modulation amplitude and 20 mW power were used for the acquisition. A polynomial baseline correction was applied to each spectrum before fitting.

Methods

Synthesis of OA-capped β-$NaLuF_4$: 20% Gd^{3+}; 3% Dy^{3+} RLNPs were synthesized via a coprecipitation route adapted from literature (main text reference 30). The synthesis described below is comprised of a two-step process where the first step involves the preparation of the hexahydrate lanthanide precursors, followed by the formation of OA-coordinated nanoparticles in the second step.

All lanthanide oxides were dissolved in 20 ml of a 1:1 (v/v) mixture of H2O/ HCl (37%) and heated at 80 °C under reflux until a transparent solution of lanthanide hexahydrate $LuCl_3$, $GdCl_3$ and $DyCl_3$ was obtained. The solutions were then heated to dryness at 60 °C for several hours to evaporate the water and hydrochloric acid. In the second step of the synthetic procedure, $LuCl_3$ (0.77 mmol, 299.85 mg), $GdCl_3$ (0.2 mmol, 74.34 mg), and $DyCl_3$ (0.03 mmol, 11.31 mg) were dissolved in 20 mL of a 3:2 (v/v) mixture of ODE/OA and the mixture was heated for 30 min at 125 °C ± 5 °C under vacuum to obtain a colorless homogenous solution. Next, the solution was cooled down to 90 °C. Afterwards, sodium oleate (2.5 mmol, 761.1 mg) and ammonium fluoride (4 mmol, 148.16 mg) were added at once under argon atmosphere. A degassing step at 90 °C was then performed to generate anhydrous, oxygen-free conditions. Subsequently, the reaction temperature was increased to 320 °C (heating rate: 20 °C/min) under Ar flow and kept at this temperature for 15 min. Thereafter the mixture was allowed to cool to room temperature naturally. The nanoparticles were precipitated by the addition of ethanol and isolated via centrifugation at 4000 rpm. The resulting pellet was dispersed in a minimal amount of hexane and washed twice with ethanol, and finally dispersed in n-hexane.

Synthesis and characterization of β-NaLuF4: 20% Gd3+; 3% Dy3+ @mSiO2 nanoparticles

The mesoporous silica layer was prepared by sol-gel reaction of tetraethyl orthosilicate (TEOS) in an aqueous solution containing cetyltrimethylammonium bromide (CTAB). First, $NaLuF_4$: Gd, Dy NPs in n-hexane were added in an aqueous solution CTAB (15 mL, 0.1 M). After stirring for 12h, the solution turned clear. Then an aqueous solution of NaOH (150 µL, 2 M) was added and TEOS (0.05 mL) was added dropwise to the reaction mixture. The reaction was maintained at RT for 12 hours. The resulting silica-coated NPs were precipitated by adding 30 mL of acetone and collected via centrifugation (RCF: 1000 g; 5 minutes). The pellet was re-dispersed in 4 mL of dH_2O, again precipitated with 10 mL of ethanol and collected via centrifugation. This step was repeated once more. To extract CTAB from the nanoparticles, the as-obtained nanoparticles were redispersed in acidic ethanol solution (pH ~ 1.5) at 60 °C for 3 h. Finally, the obtained RLNPs@mSiO2 nanoparticles were isolated via centrifugation at 4000 rpm and washed with ethanol. The synthesized nanoparticles are characterized by TEM (Figure S1), and FTIR (Figure S2A).

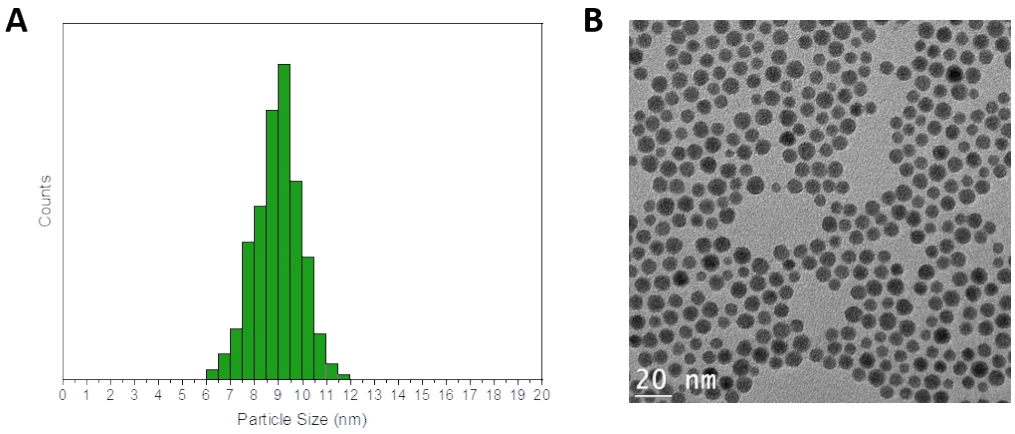


Figure S1. Histogram of oleate-coated $NaLuF_4$:Dy^{3+},Gd^{3+} nanoparticles. The average size was found to be 9 ± 1 nm. B) TEM image of the oleate coated nanoparticles.

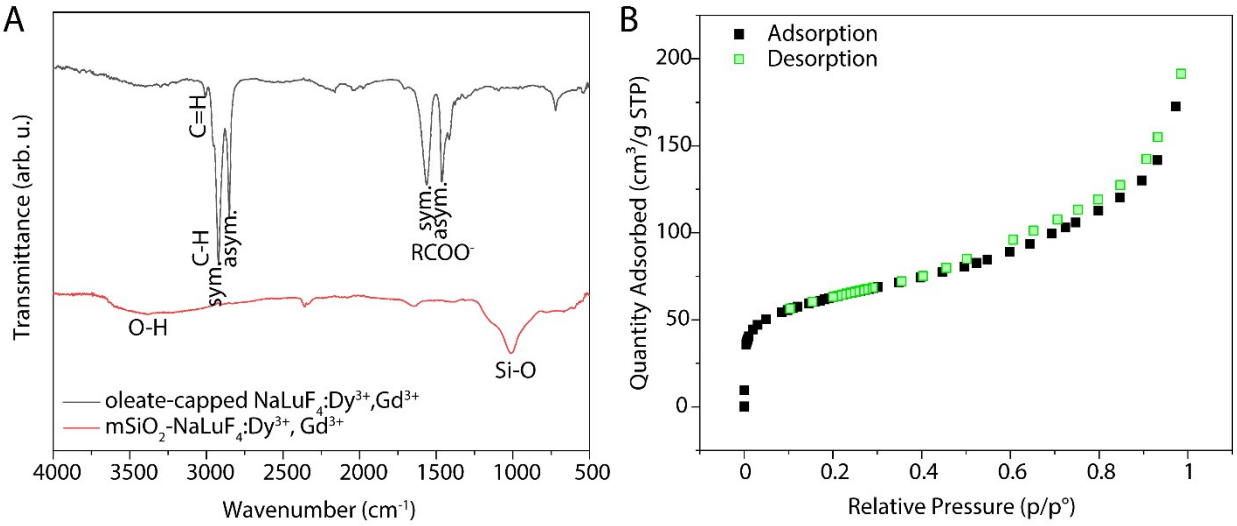


Figure S2. A) FT-IR of oleate capped (black) and mesoporous silica coated (red) $NaLuF_4$:Dy^{3+},Gd^{3+} nanoparticles. B) BET isotherm of mesoporous silica coated $NaLuF_4$:Dy^{3+},Gd^{3+} nanoparticles.

Photosensitizer loading

For this purpose, a dispersion of NPs (33 mg) in 2 mL DMSO was added to a solution containing an excess of PS molecules in DMSO (1 mL) and stirred for 12 hours protected from light. Afterwards, the particles were separated from the solution by centrifugation (13,000 g, 25 min) and washed with DMSO. The colored precipitate was finally dispersed in DMSO and stored in the dark. The supernatants were collected after the loading process for UV-vis absorption measurement and a standard RB calibration curve was used to determine the unreacted PS. The loading efficiency in wt% was calculated using equation 1:

$$\text{Photosensitizer loading} = \frac{mass\ of\ loaded\ photosensitizer}{mass\ of\ particles}$$

BET analysis was used to determine the number of photosensitizers per surface area of the RLNPs@mSiO$_2$. Equimolar starting amounts of RB and MC540 were used for loading into the nanoparticles. The results are summarized in Table S1.

Table 1. Summary of RB and MC540 nanoparticle loading.

	Rose Bengal	Merocyanine 540
Concentration (M)	2.64×10^{-15}	2.48×10^{-6}
Concentration (molecules PS/mL)	1.59×10^{16}	2.98×10^{-15}
Surface Loading (molecules/cm^2)	5.66×10^{12}	1.06×10^{15}

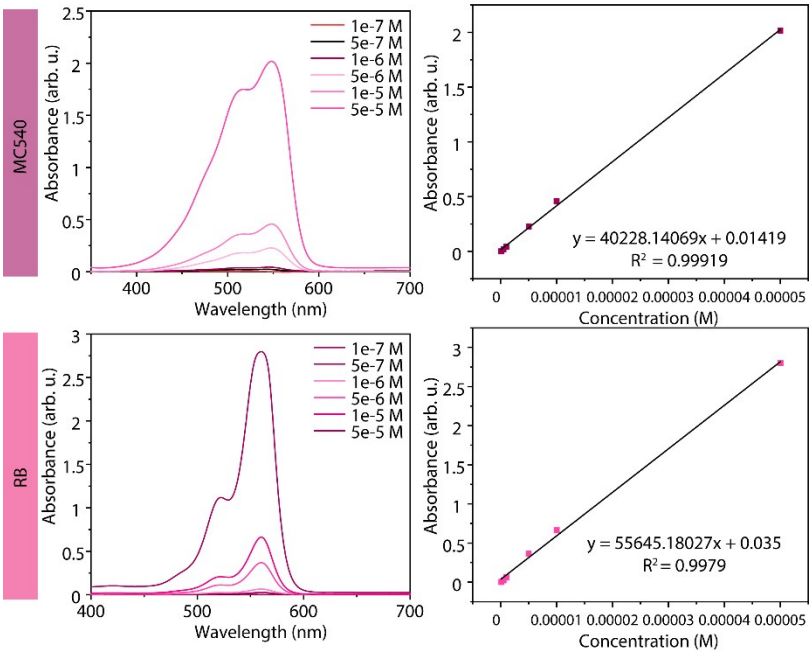


Figure S3. UV-Vis absorption spectra and corresponding calibration curves for MC540 (top) and RB (bottom).

Figure S4 shows the loaded amount of photosensitizer, which exhibit similar trends: the loaded amount of photosensitizer molecules increases with increased added amount until a plateau is reached.

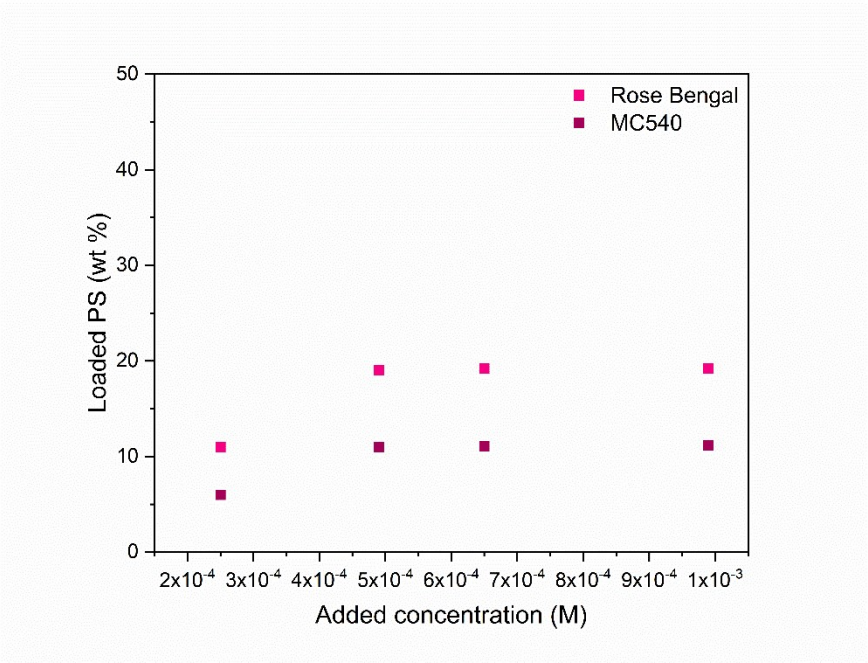
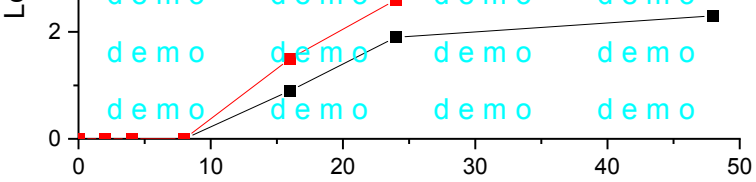


Figure S4: The amount of loaded photosensitizer versus the added photosensitizer molecules



Formulation stability

To estimate the stability of the loaded RB and MC540 onto the ScNPs, a photosensitizer release test was performed at different time points. After incubation in DI H_2O at room temperature, the nanocomposites were centrifuged, and the supernatants were quantified by UV-vis absorption measurement.

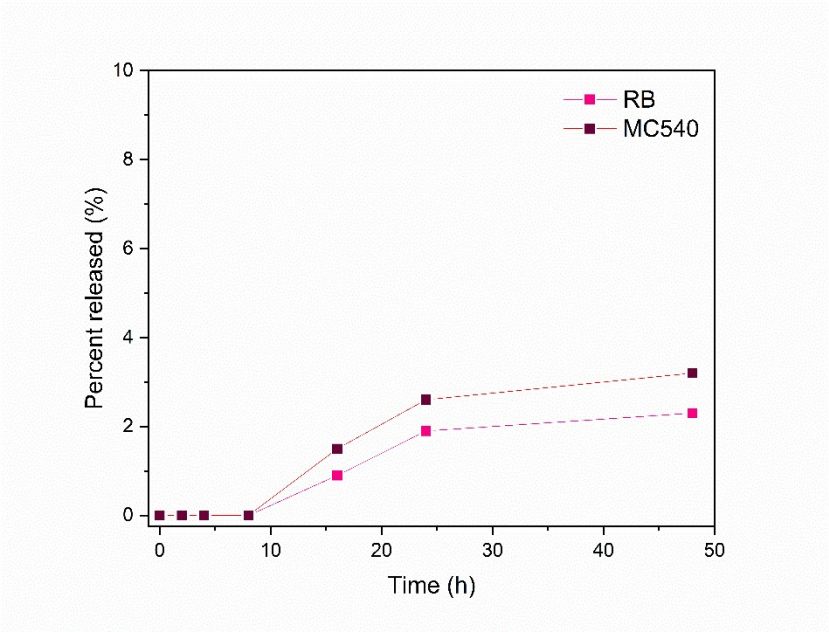


Figure S5: The stability test of the loaded PSs after 48 h showed less than 3% of RB and less than 5% of MC540 was released to the supernatant.

Singlet oxygen and ROS detection

ABDA assays were performed using 2 mg/mL solutions of PS-RLNP dispersions and 0.6 mM ABDA in either 50/50 v/v% DMSO/H_2O or DMSO/D_2O. DPBF assays were performed using 2 mg/mL PS-RLNP dispersions and 0.3 mM DPBF ABDA in either 50/50 v/v% DMSO/H_2O or DMSO/D_2O. All assays were performed in triplicate and results were averaged. Solutions were irradiated with X-rays at room temperature and stirred continuously during irradiation. UV-Vis absorption spectra of the solutions were taken every 30 seconds for an irradiation duration of 5 minutes. The sodium azide assay was performed using a 2 mg/mL dispersion of PS-RLNPs and 20 mM of sodium azide in DMSO/H_2O and DMSO/D_2O. EPR spectroscopy was performed using a Bruker D2 Phaser XRD as an X-ray source (30 kVp, 10 mA, Cu anode, unfiltered) and solutions of PS-RLNPs (2 mg/mL) and TMPO (50 mM) or DMPO (50 mM) were irradiated for 5 minutes and then transferred into a flat cell for EPR measurement.

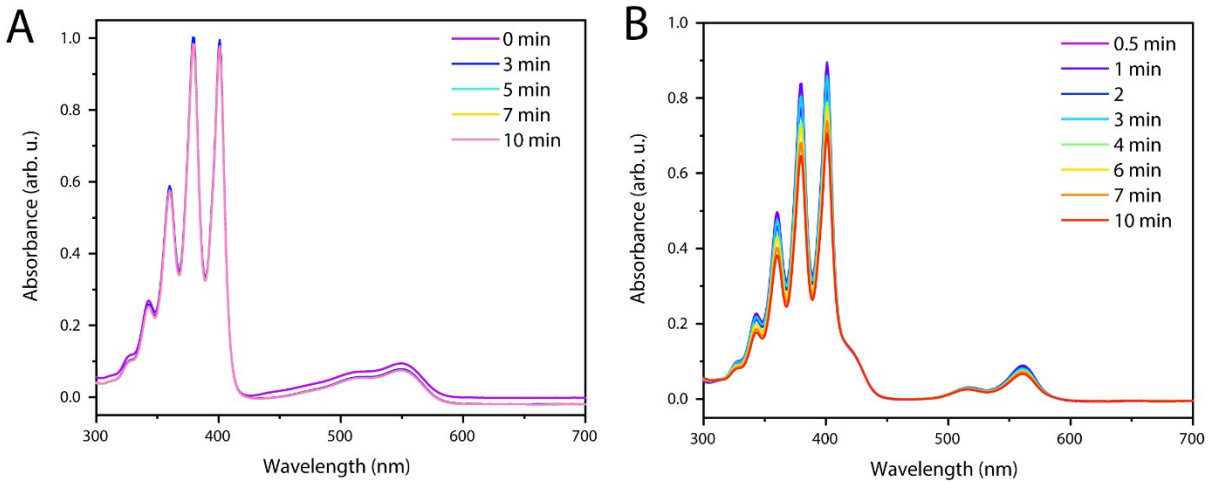


Figure S6. A) UV-Vis absorption spectra of MC540 + ABDA under 532 nm excitation as a function of irradiation time. B) UV-vis absorption spectra of ABDA + RB under 532 nm excitation as a function of irradiation time.

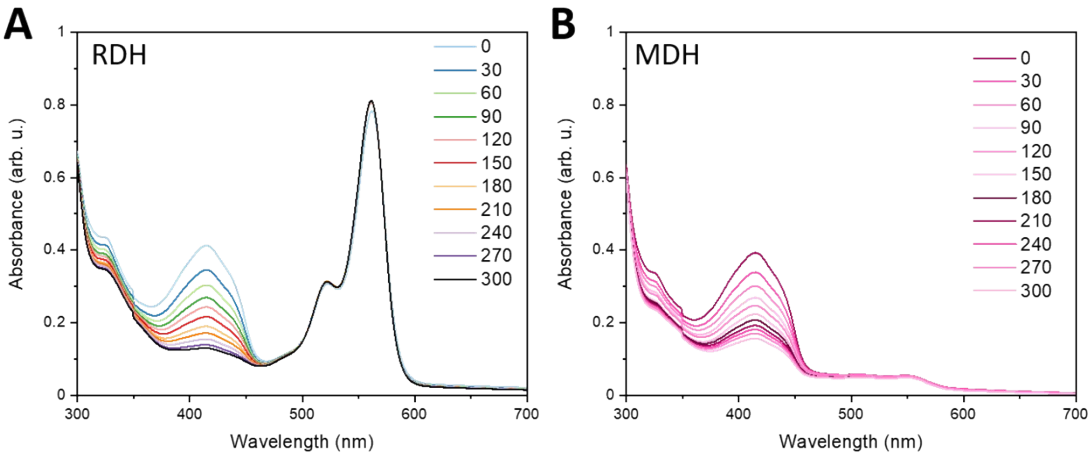


Figure S7. A) UV-vis absorption spectra of sample RDH (RB-RLNPs with DPBF, in DMSO/H2O) as a function of irradiation time in seconds. B) UV-Vis absorption spectra of sample MDH (MC540-RLNPs with DPBF, in DMSO/H2O) as a function of irradiation time in seconds.