Supporting Information

Covalently Surface-Functionalized Porphyrins on Silica Nanoparticles for Efficient Photodynamic Therapy

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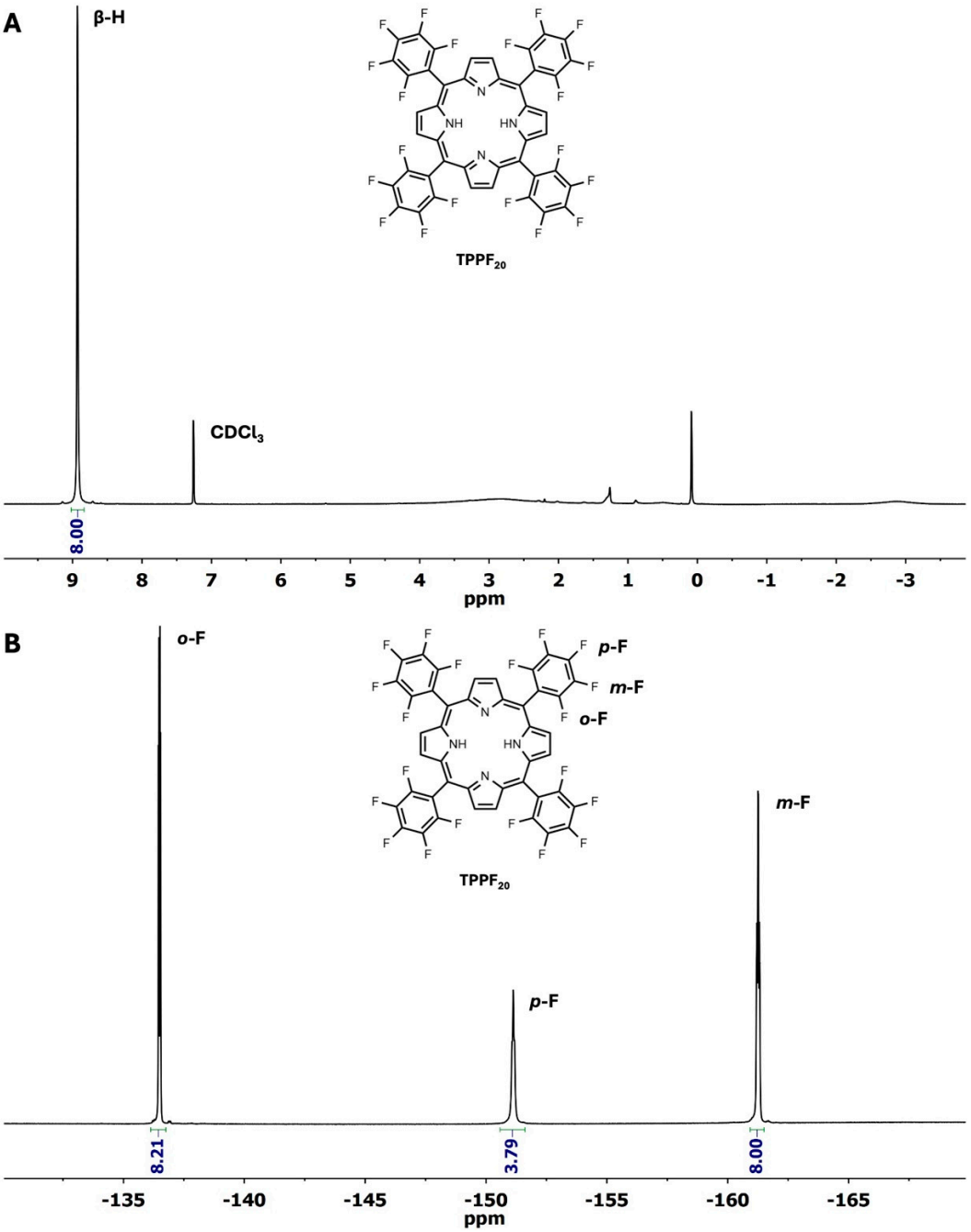

Figure S1. Characterization of $TPPF_{20}$ by NMR spectroscopy. ^{1}H (A) and ^{19}F (B) NMR spectra obtained in $CDCl_3$.

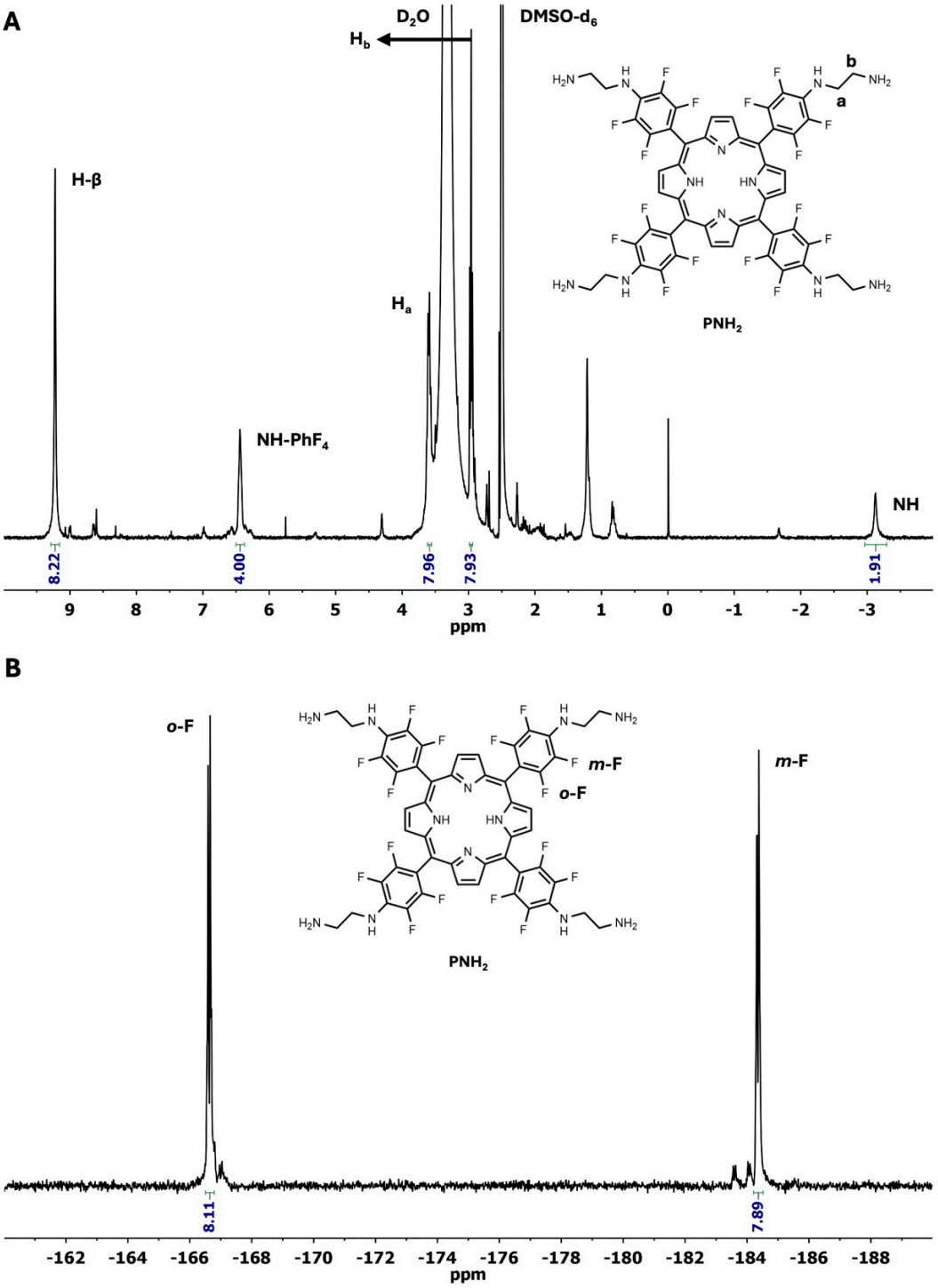

Figure S2. Characterization of PNH_2 by NMR spectroscopy. ^{1}H (A) and ^{19}F (B) NMR spectra obtained in DMSO-d_6.

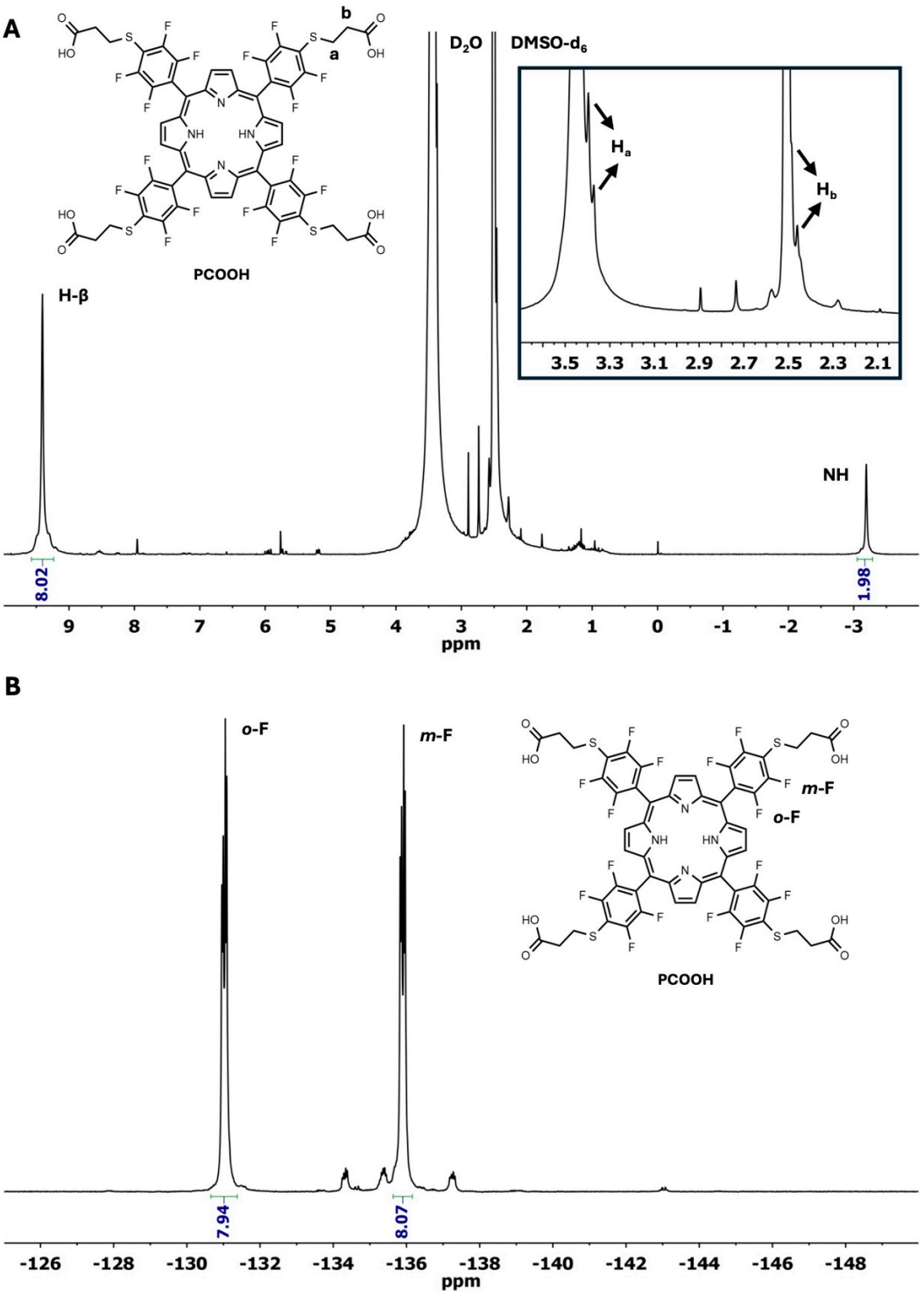

Figure S3. Characterization of PCOOH by NMR spectroscopy. ^{1}H (A) and ^{19}F (B) NMR spectra obtained in DMSO-d_6.

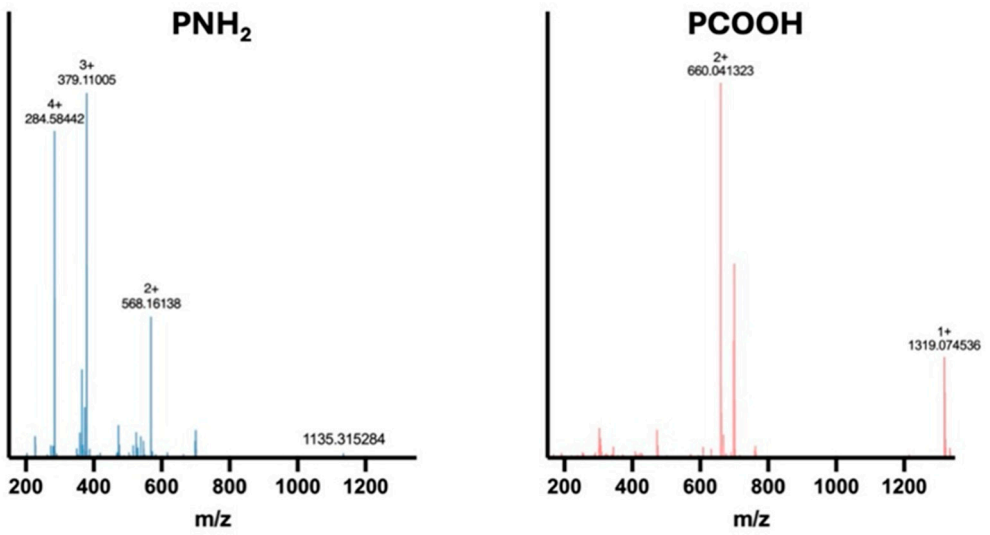

Figure S4. High-resolution ESI mass spectra of PNH_2 (m/z $[C_{52}H_{39}F_{16}N_{12} + H]^+$: observed 1135.315284, calculated 1135.315966), and PCOOH (m/z $[C_{56}H_{31}F_{16}N_4O_8S_4 + H]^+$: observed 1319.074536, calculated 1319.076376).

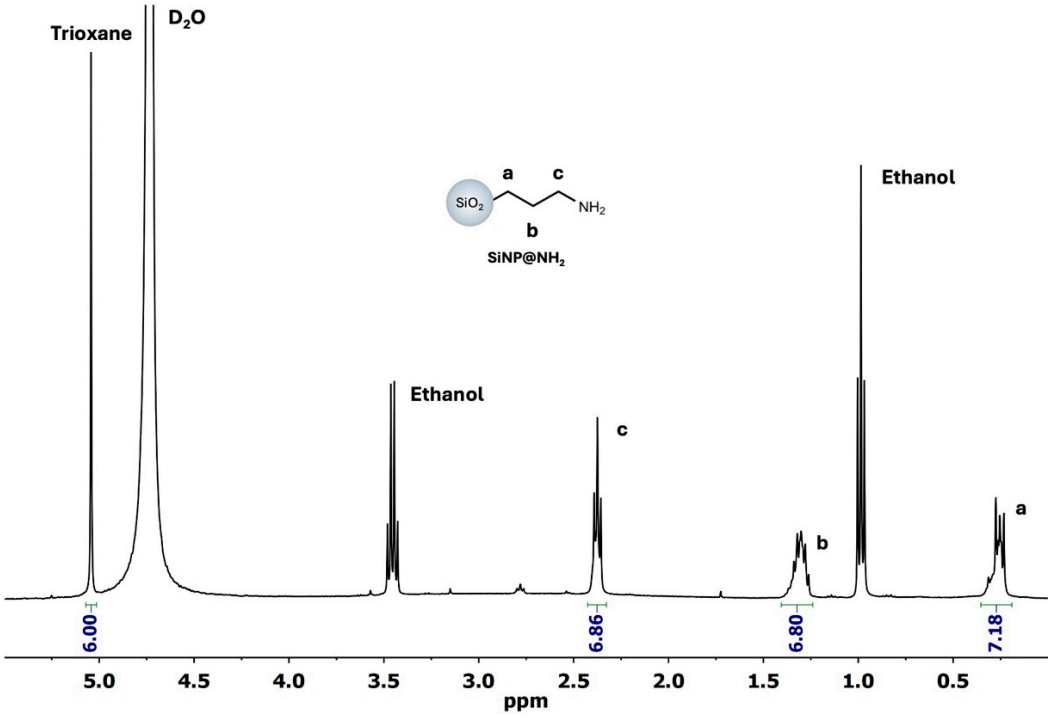

Figure S5. Characterization of $SiNP@NH_2$ by NMR spectroscopy. ^1H NMR spectra obtained in $NaOH/D_2O$.

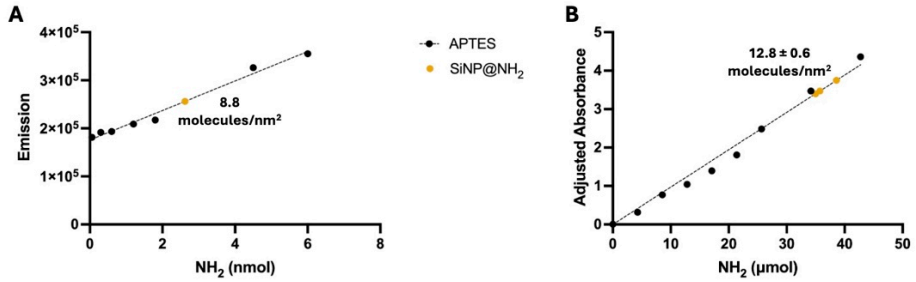

Figure S6. Evaluation of accessible amine groups using colorimetric assays. Fluorescamine assay (A) estimated a coverage of 8.8 amines/nm^2, while ninhydrin assay (B) estimated a coverage of 12.8±0.6 amines/nm^2.

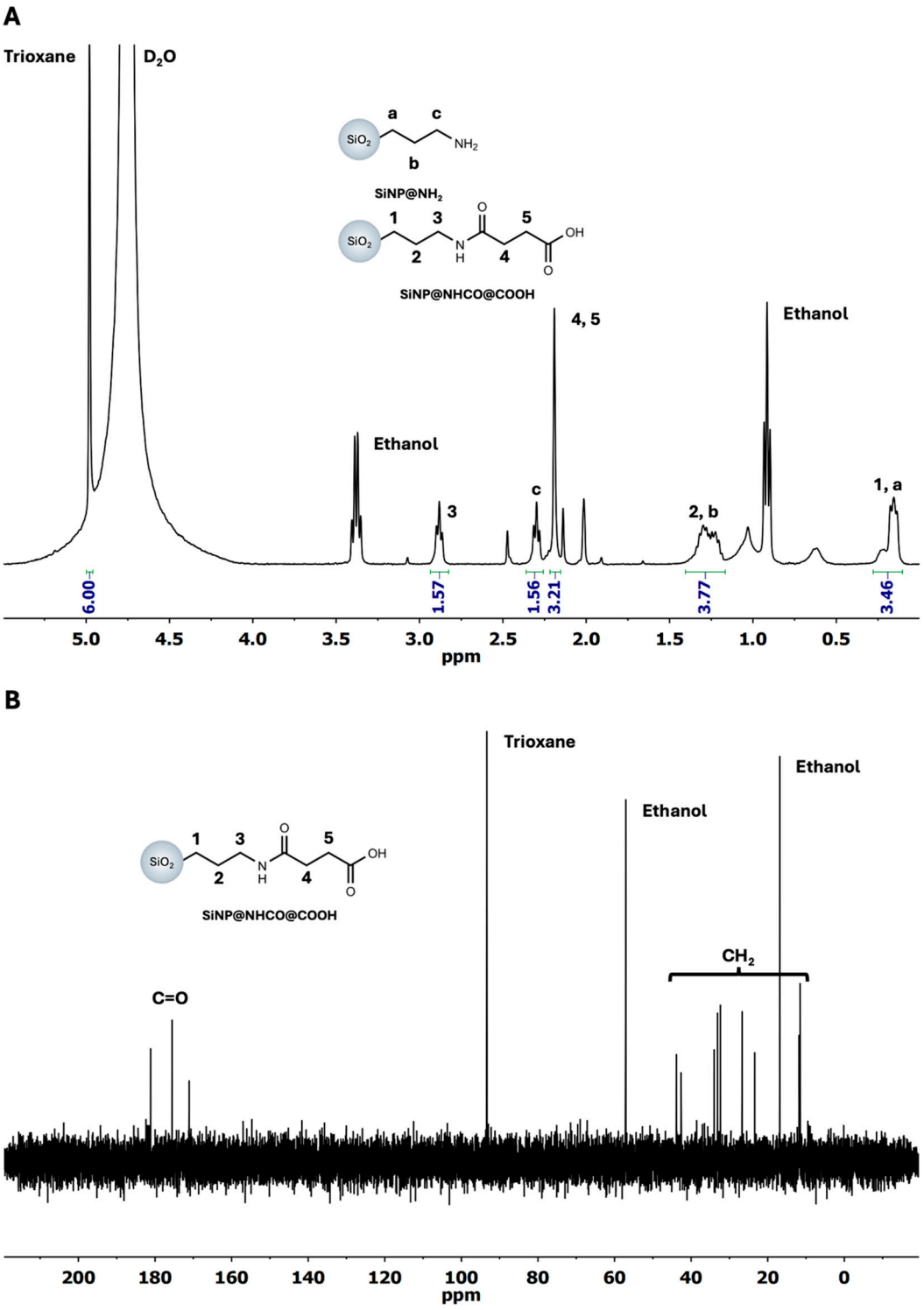

Figure S7. Characterization of SiNP@NHCO@COOH by NMR spectroscopy. ^{1}H (A) and ^{13}C (B) NMR spectra obtained in NaOH/D_2O.

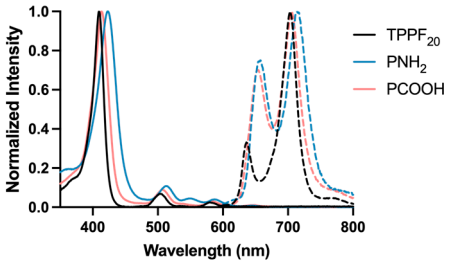

Figure S8. Normalized absorption (solid lines) and emission (dashed lines) spectra of $TPPF_{20}$, PNH_2, and PCOOH in DMF.

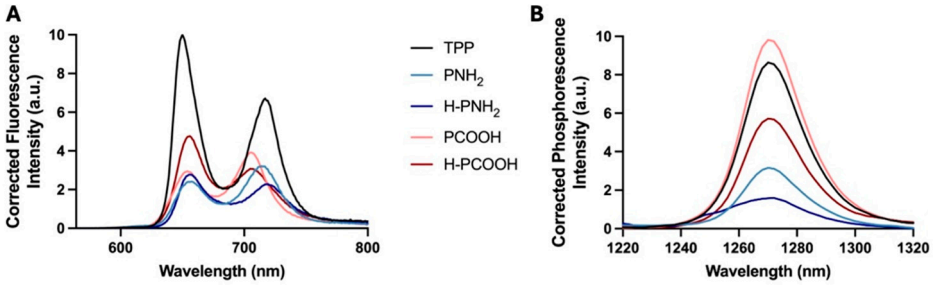

Figure S9. (A) Fluorescence emission spectra, corrected for absorption differences at excitation wavelength (λ_{ex} = 420 nm). (B) Corrected singlet oxygen phosphorescence spectra (λ_{ex} = 405 nm). Spectra were used to determine fluorescence (Φ_F) and singlet oxygen (Φ_Δ) quantum yields. Measurements were performed in DMF with absorbance values below 0.2 at excitation wavelengths, using TPP as a reference compound.

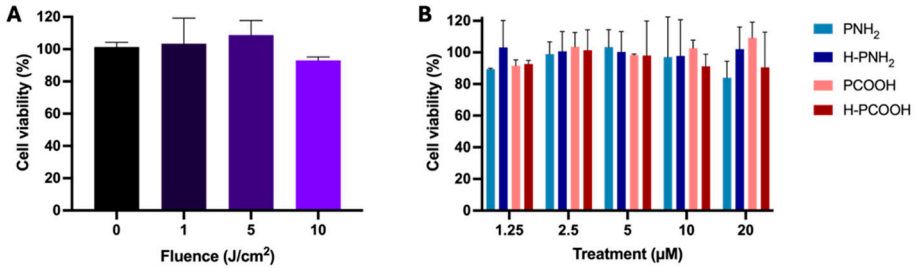

Figure S10. Toxicity control assays. Cell viability following irradiation in the absence of compounds (A) and in the presence of compounds under dark conditions (B).

Table S1. Cell viability results for HeLa cells incubated with the compounds: IC_{50} values (μM), curve fitting r^2, and lowe limit of the phototoxicity index (PI) for compounds in HeLa cells.

Compound	Fluence (J/cm²)	$IC_{50,\,light}$ (μM)[a]	r^2	PI[b]
PNH_2	1	0.90 ± 0.02	1.00	>22
	5	0.32 ± 0.02	1.00	>63
	10	<0.0625	1.00	>320
H-PNH_2	1	>5	0.73	n.d.
	5	0.7 ± 0.7	0.88	>27
	10	0.5 ± 0.3	0.92	>42
PCOOH	1	>1	0.97	n.d.
	5	0.34 (n.d.)	0.96	>59
	10	0.08± 0.04	0.96	>250
H-PCOOH	1	< 0.3	n.d.	>64
	5	< 0.3	n.d.	>64
	10	< 0.3	n.d.	>64

[a] IC_{50} values are reported with 95% confidence intervals**.** Values preceded by ">" or "<" indicate that 50% viability was not reached within the tested concentration range. [b] Lower limit for the PI calculated as the ratio of IC_{50} under irradiation over IC_{50} in the dark. Since all the compounds show IC_{50} values in the dark above the tested concentration range, the highest tested concentration (20 μM) was used to estimate a lower limit for the PI.

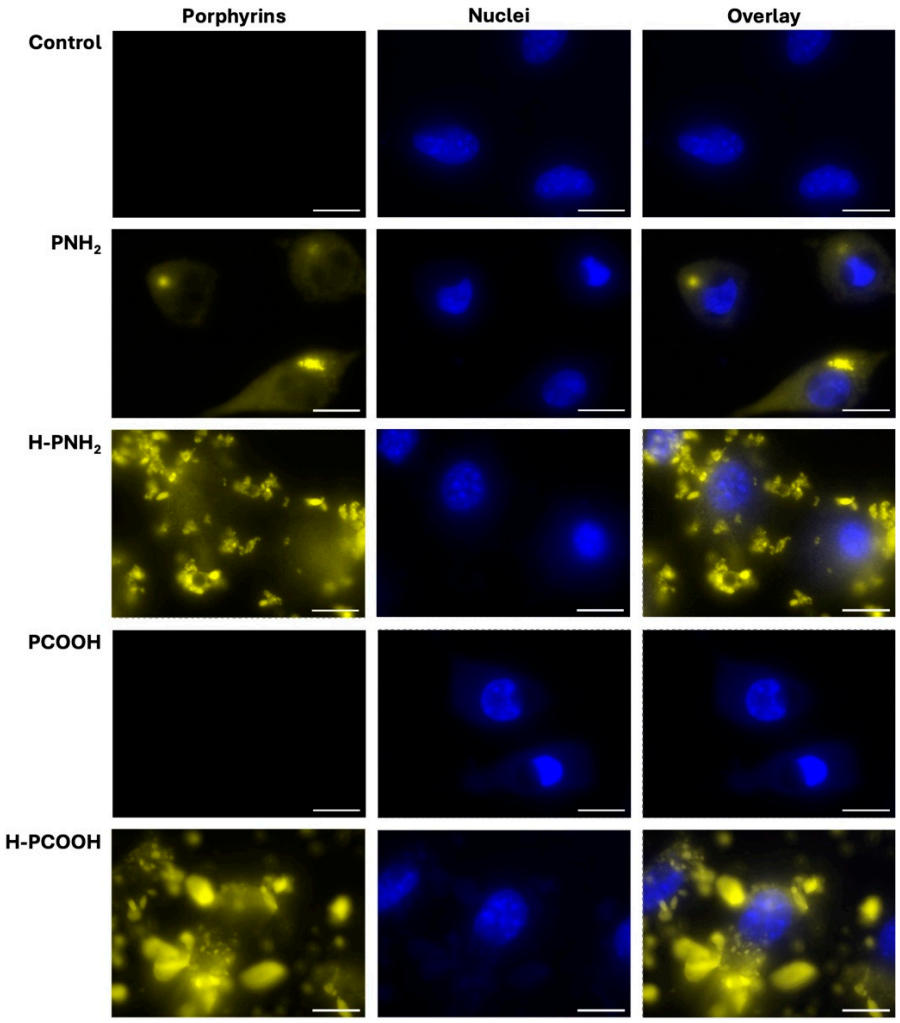

Figure S11. Widefield fluorescence microscopy images of cellular internalization of compounds in HeLa cancer cells. Yellow channel: emission from porphyrins (λ_{ex} = 515–560 nm, λ_{em} > 590 nm). Blue channel: emission from Hoechst 33342 (λ_{ex} = 340–380 nm, λ_{em} > 425 nm). Scale bar is 25 μm.

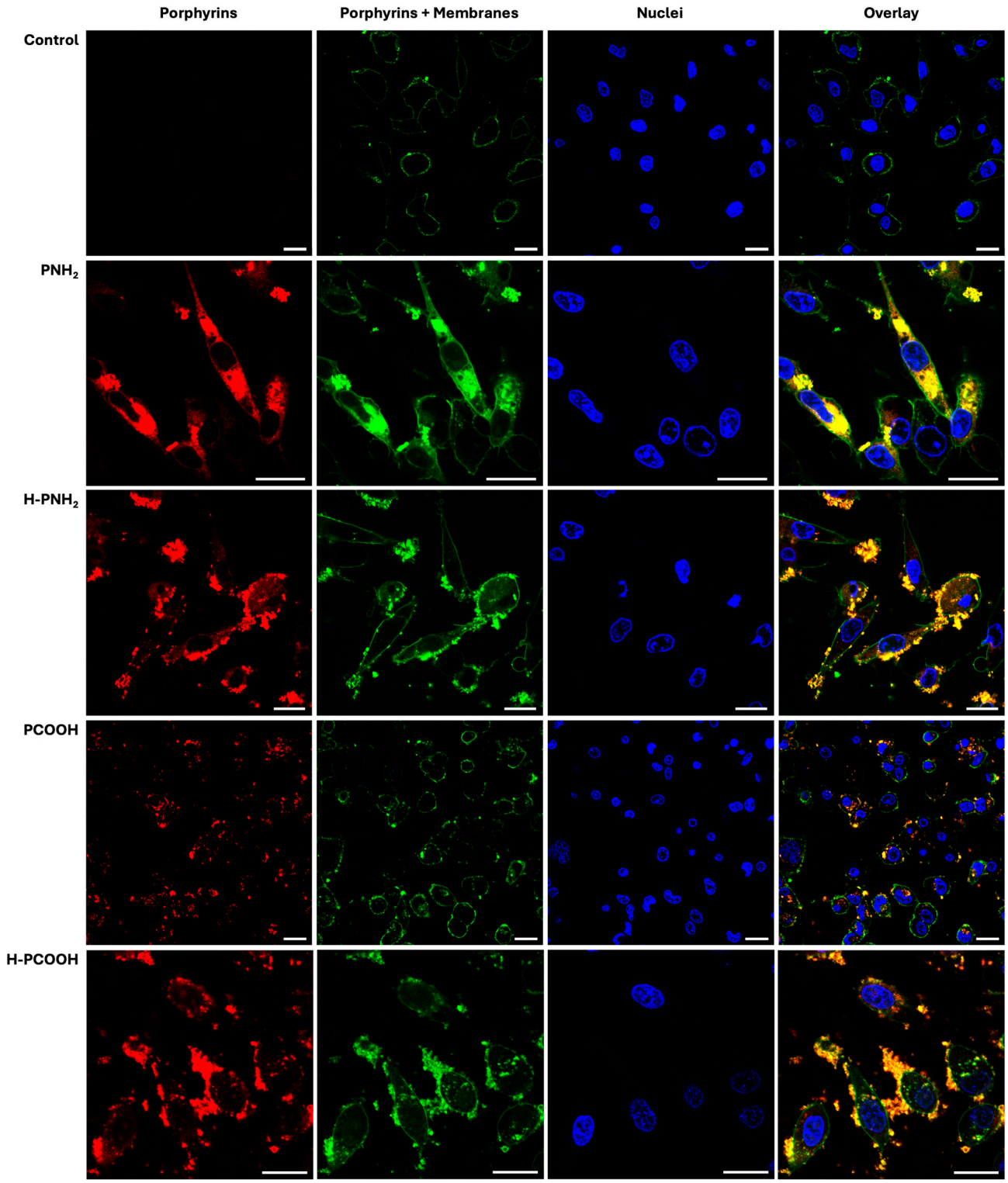

Figure S12. Confocal fluorescence microscopy images of cellular internalization of compounds in HeLa cancer cells. Red channel: emission from porphyrins (λ_{ex} = 514 nm, λ_{em} = 600–720 nm). Green channel: emission from porphyrins and Alexa Fluor 633 (λ_{ex} = 633 nm, λ_{em} = 650–700 nm). Blue channel: emission from Hoechst 33342 (λ_{ex} = 800 nm, λ_{em} = 390–500 nm). Scale bar is 25 μm.

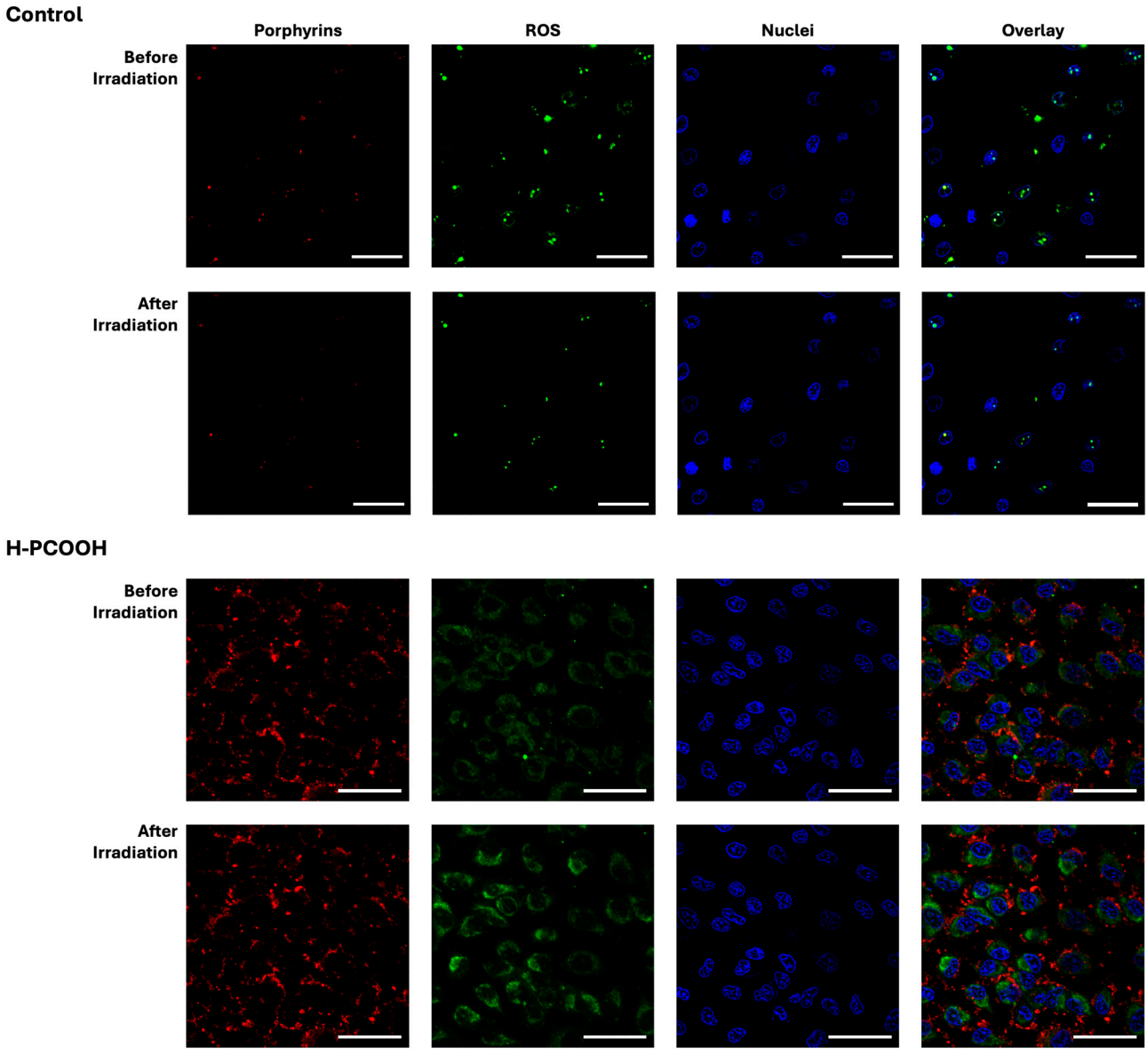

Figure S13. Confocal fluorescence microscopy images of HeLa cells showing ROS-associated fluorescence under control conditions and in the presence of H-PCOOH, before and after 60 s of blue light irradiation. Red channel: emission from the nanohybrid (λ_{ex} = 514 nm, λ_{em} = 620–720 nm). Green channel: emission from the ROS-responsive probe MitoSOX (λ_{ex} = 514 nm, λ_{em} = 570–620 nm). Blue channel: emission from Hoechst 33342, staining cell nuclei (λ_{ex} = 405 nm, λ_{em} = 400–560 nm). Scale bar: 50 μm.