Supporting information

Photophysical, photooxidation, and biomolecule-interaction of *meso*-tetra(thienyl)porphyrins containing peripheral Pt(II) and Pd(II) complexes. Insights for photodynamic therapy applications

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Elemental analysis and molar conductivity

The CHN% elemental analyses were performed using a Shimadzu EA112 microanalysis instrument. The molar conductance of the porphyrins ([ ] = 10^{-4} M range) in DMF solution at 25ºC was measured using an MS Tecnopon conductometry model mCA 150.1 direct-reading conductivity bridge using 0.01 M KCl to calibrate. All experiments are conducted in duplicate.

The molar conductivity in DMF solutions for porphyrins are 280 to 300 S cm^2 mol^{-1} range, indicating 1:4 electrolyte, as expected[1].

Free-base *meso*-tetra-(2-thienyl)porphyrin **H_2TTP** was synthetized and characterized according reported by Momo and co-workers[2].

Table S1. The CHN% analysis and molar conductivity of porphyrins **H_2TTP**, **PtbpyTTP**, and **PdbpyTTP**.

	Elemental analysis, exp. (calc.)
	$C_{36}H_{22}N_4S_4$
H_2TTP	%C: (67.68) 67.90; %H: (3.47) 3.49; %N: (8.77) 8.76
	$[C_{76}H_{54}Cl_4N_{12}S_4Pt_4](PF_6)_4 \cdot 8H_2O$
PtbpyTTP	%C: (32.73) 31.55; %H: (1.94) 2.40; %N: (6.11) 5.71
	$[C_{76}H_{54}Cl_4N_{12}S_4Pd_4](PF_6)_4 \cdot 9H_2O$
PdbpyTTP	%C: (37.86) 35.44; %H: (2.26) 2.79; %N: (6.97) 6.52
	Molar conductivity ($S.cm^2.mol^{-1}$)
PtbpyTTP	289.3
PdbpyTTP	296.9
DMF	1.00

Electrochemical analysis by cyclic voltammetry

Cyclic voltammograms of porphyrins were recorded with a potentiostat/galvanostat AutoLab Eco Chemie PGSTAT 128 N system at room temperature and under an argon atmosphere in a dry DMF solution. Electrochemical grade tetrabutylammonium hexafluorophosphate ($TBAPF_6$, 0.1 M) was used as a supporting electrolyte. A standard three-component system was used to carry out the CV experiments: a glassy carbon working electrode, a platinum wire auxiliary electrode, and a platinum wire *pseudo*-reference electrode. The ferrocenium/ferrocene redox couple was used as an internal reference to monitor the reference electrode ($E_{1/2}$ = 0.469 V)[3].

Table S2. Redox potentials of porphyrins **H_2TTP**, **PtbpyTTP**, and **PdbpyTTP** in dry DMF solution, at scan rate 100 mV s^{-1} (*E versus* Fc/Fc^+).

Porphyrin	$Por^{0/1-}$	$Por^{1-/2-}$	$Por^{0/1+}$	$Por^{1+/2+}$	HOMO (eV)[d]	LUMO (eV)[e]	ΔE
H_2TTP	−1.08 V[a]	-----	+0.76 V[c]	-----	−5.56	−3.72	1.84
PtbpyTTP	−1.22 V[b]	−1.46 V[a]	+0.75 V[c]	-----	−5.55	−3.58	1.97
PdbpyTTP	−1.60 V[b]	−2.13 V[b]	+0.49 V[c]	-----	−5.29	−3.20	2.09

[a]$E_{1/2} = E_{pa} + E_{pc} / 2$; [b]Cathodic peak; [c]Anodic peak; [d]$E_{HOMO} = -[4.8 + E_{oxid}$ (*versus* Fc^+/Fc)]; [e]$E_{LUMO} = -[4.8 + E_{red}$ (*versus* Fc^+/Fc)]

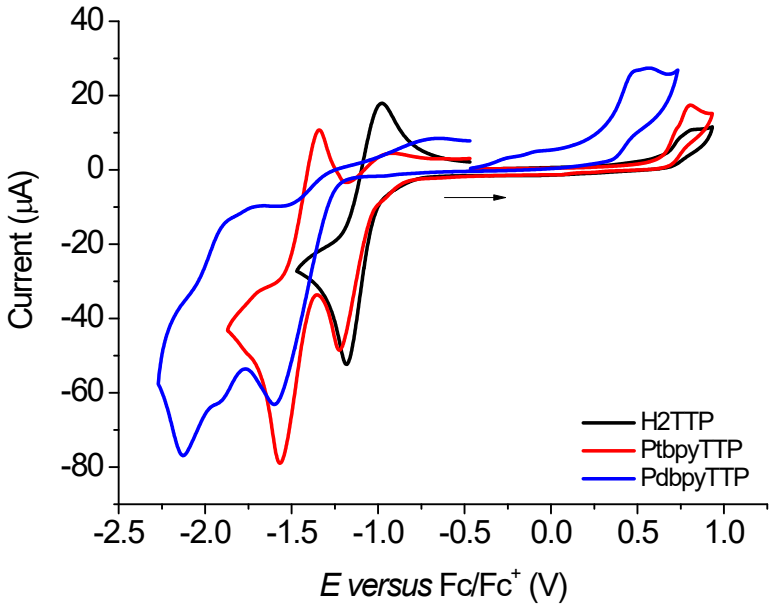

Figure S1. Cyclic voltammograms of porphyrins **H_2TTP**, **PtbpyTTP,** and **PdbpyTTP**. Electrolyte: $TBAPF_6$ 0.1 M; Solvent: DMF; Reference: Fc/Fc^+; scan rate: 100 mV s^{-1}.

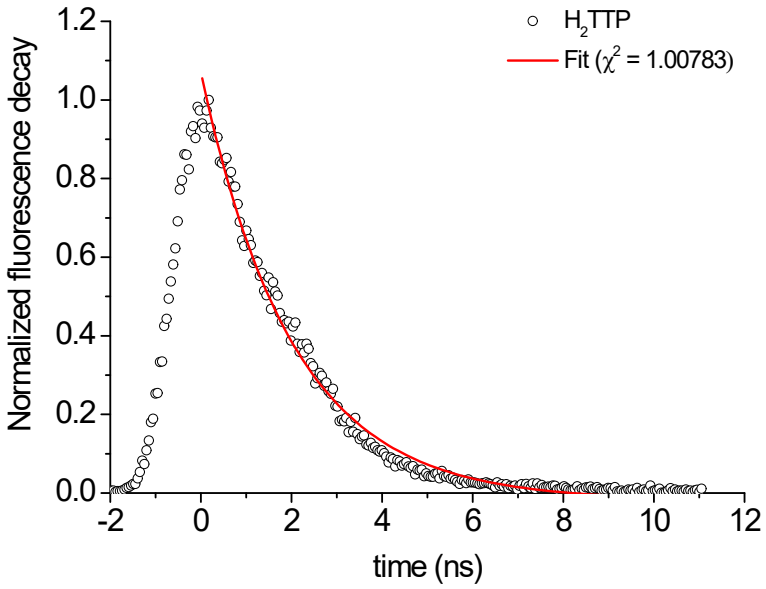

Figure S2. Normalized fluorescence emission decay of porphyrin **H_2TTP**, in DMSO solution, at λ_{exc} = 455 nm (NanoLED) and concentration of 2.0 μM.

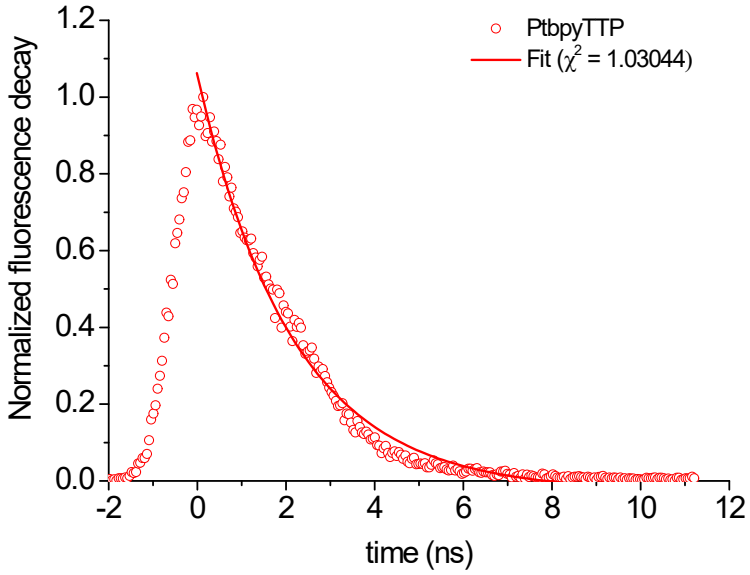

Figure S3. Normalized fluorescence emission decay of porphyrin **PtbpyTTP**, in DMSO solution, at λ_{exc} = 455 nm (NanoLED) and concentration of 2.0 μM.

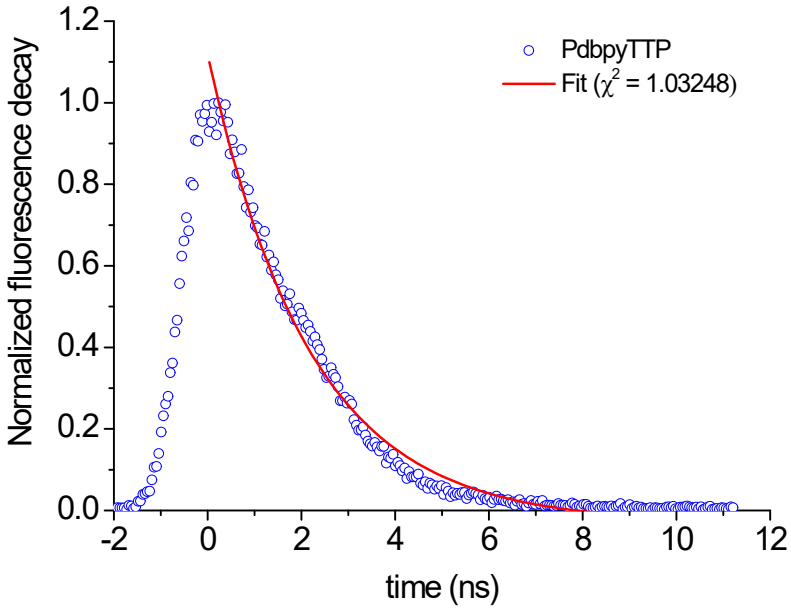

Figure S4. Normalized fluorescence emission decay of porphyrin **PdbpyTTP**, in DMSO solution, at λ_{exc} = 455 nm (NanoLED) and concentration of 2.0 μM.

Aggregation study

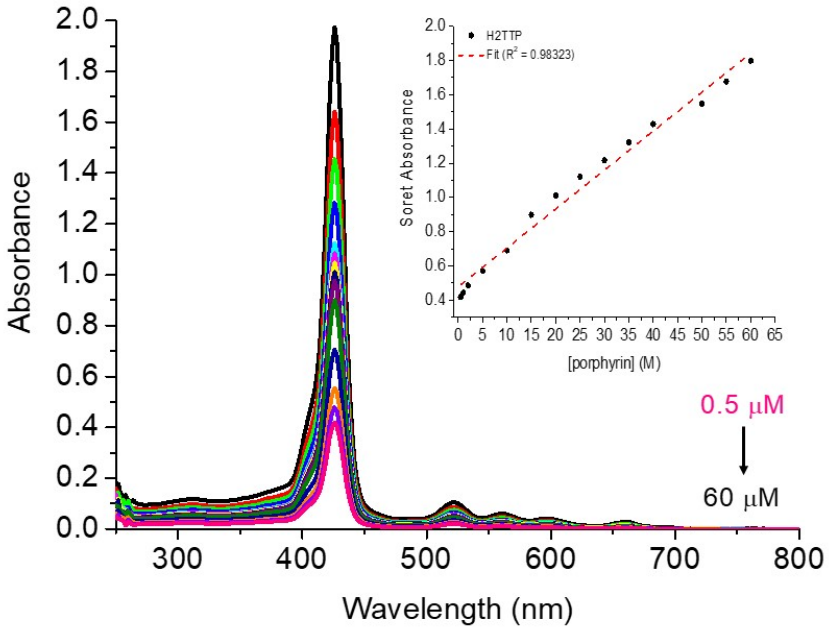

Figure S5. Aggregation study for **H_2TTP**, using dimethyl sulfoxide (DMSO) as solvent. The inset shows the linear behavior of the absorbance at Soret band as a function of the concentration.

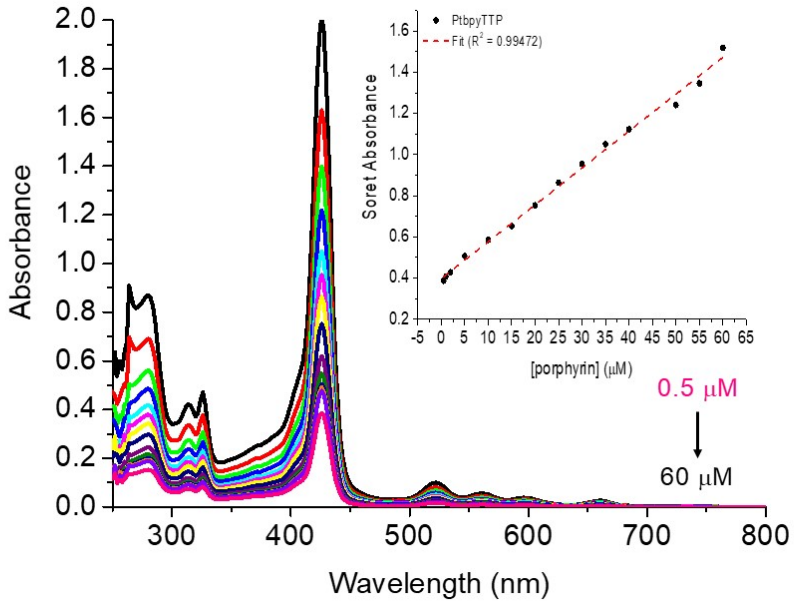

Figure S6. Aggregation study for **PtbpyTTP**, using dimethyl sulfoxide (DMSO) as solvent. The inset shows the linear behavior of the absorbance at Soret band as a function of the concentration.

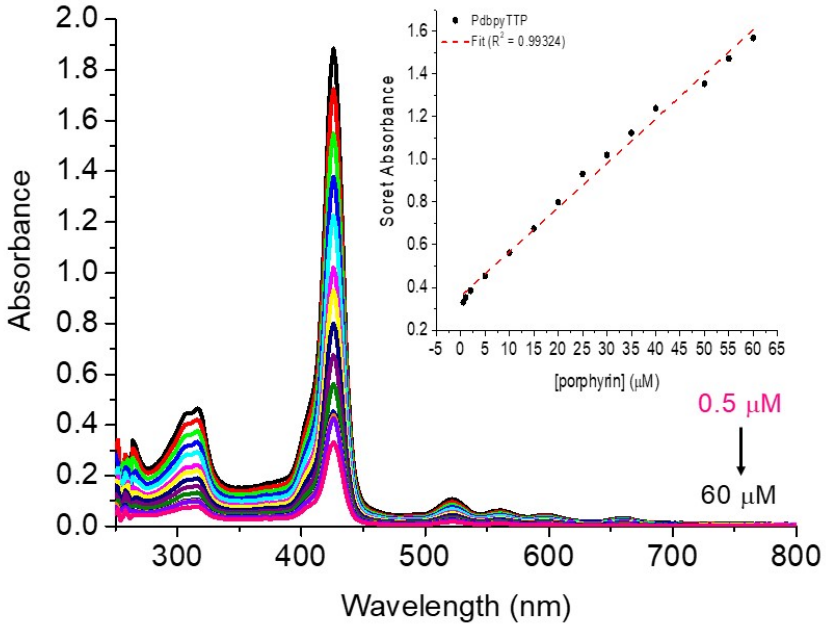

Figure S7. Aggregation study for **PdbpyTTP**, using dimethyl sulfoxide (DMSO) as solvent. The inset shows the linear behavior of the absorbance at Soret band as a function of the concentration.

Photostability assays

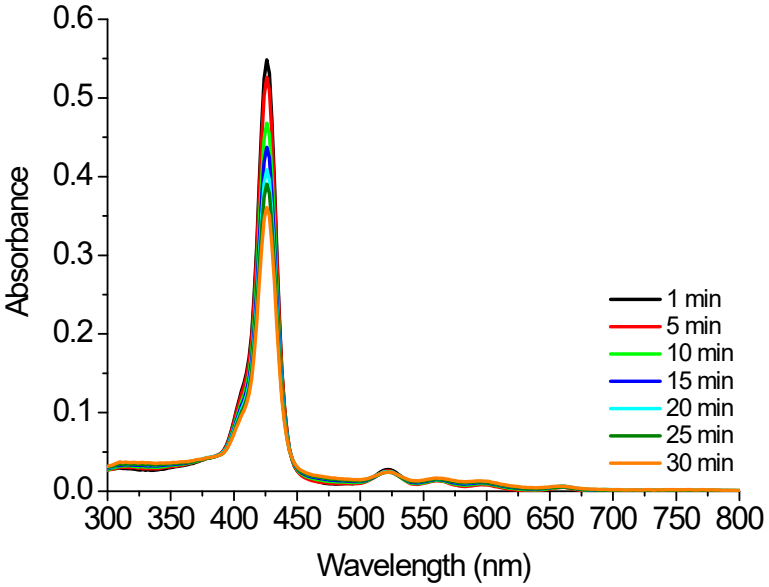

Figure S8. Photostability assay by UV-Vis of the porphyrin **H_2TTP** in DMSO solution after irradiation with a white-light source (400-800 nm) at a fluence rate of 50 mW cm^{-2} and a total light dosage of 90 J cm^{-2} for different periods (0 to 30 min) and initial concentration of 1.0 μM (black line spectrum).

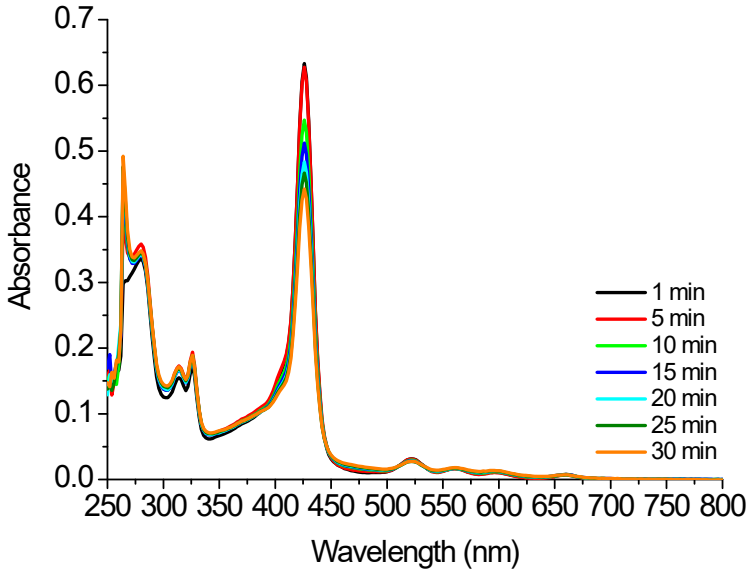

Figure S9. Photostability assay by UV-Vis of the porphyrin **PtbpyTTP** in DMSO solution after irradiation with a white-light source (400-800 nm) at a fluence rate of 50 mW cm^{-2} and a total light dosage of 90 J cm^{-2} for different periods (0 to 30 min) and initial concentration of 1.0 μM (black line spectrum).

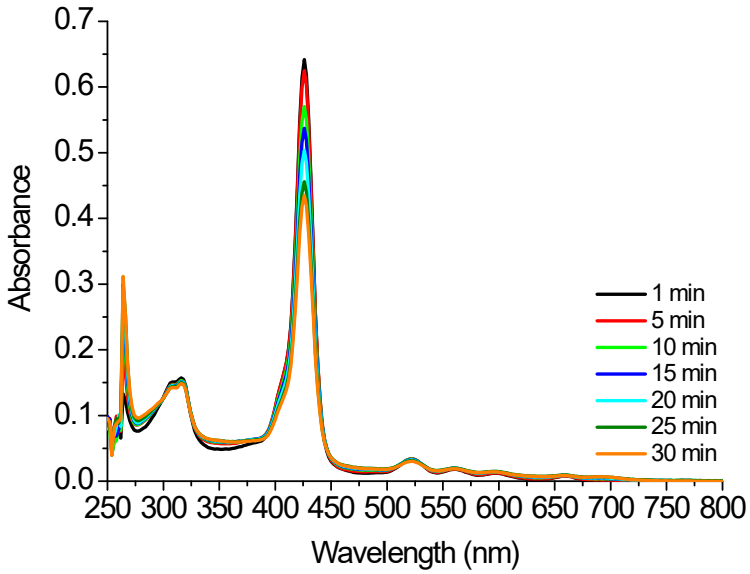

Figure S10. Photostability assay by UV-Vis of the porphyrin **PdbpyTTP** in DMSO solution after irradiation with a white-light source (400-800 nm) at a fluence rate of 50 mW cm^{-2} and a total light dosage of 90 J cm^{-2} for different periods (0 to 30 min) and initial concentration of 1.0 μM (black line spectrum).

Stability in solution assays

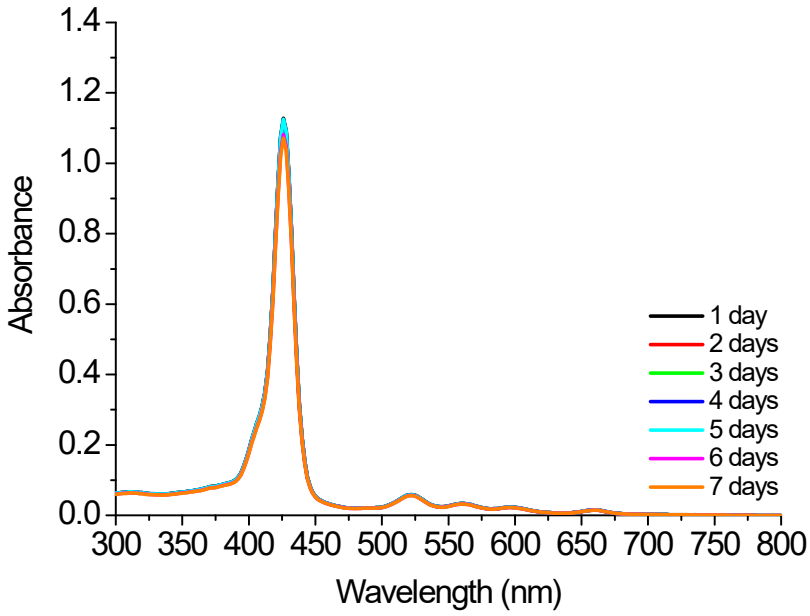

Figure S11. Solution stability by UV–visible spectra for porphyrin **H_2TTP** in DMSO solution and initial concentration of 1.5 μM (black line spectrum).

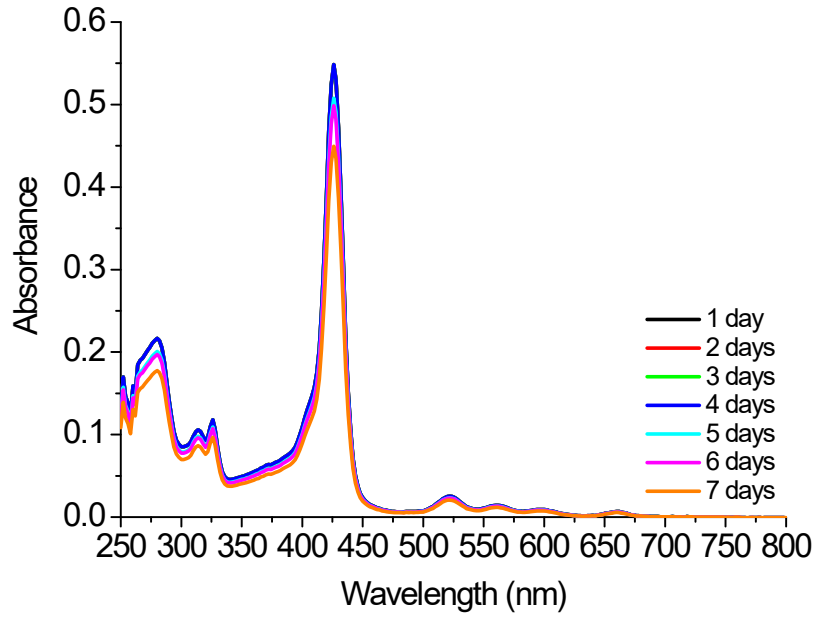

Figure S12. Solution stability by UV–visible spectra for porphyrin **PtbpyTTP** in DMSO solution and initial concentration of 1.5 μM (black line spectrum).

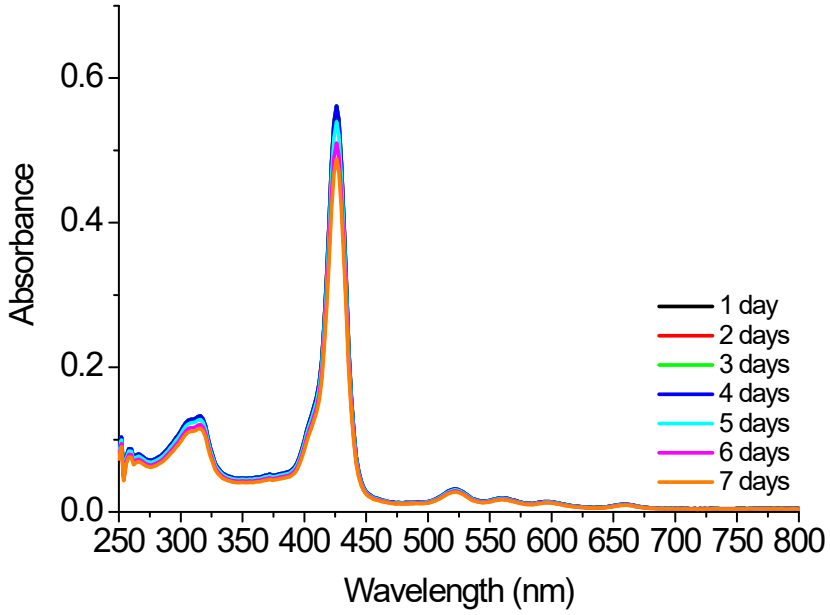

Figure S13. Solution stability by UV–visible spectra for porphyrin **PdbpyTTP** in DMSO solution and initial concentration of 1.5 μM (black line spectrum).

Singlet oxygen quantum yield (Φ_Δ) determination

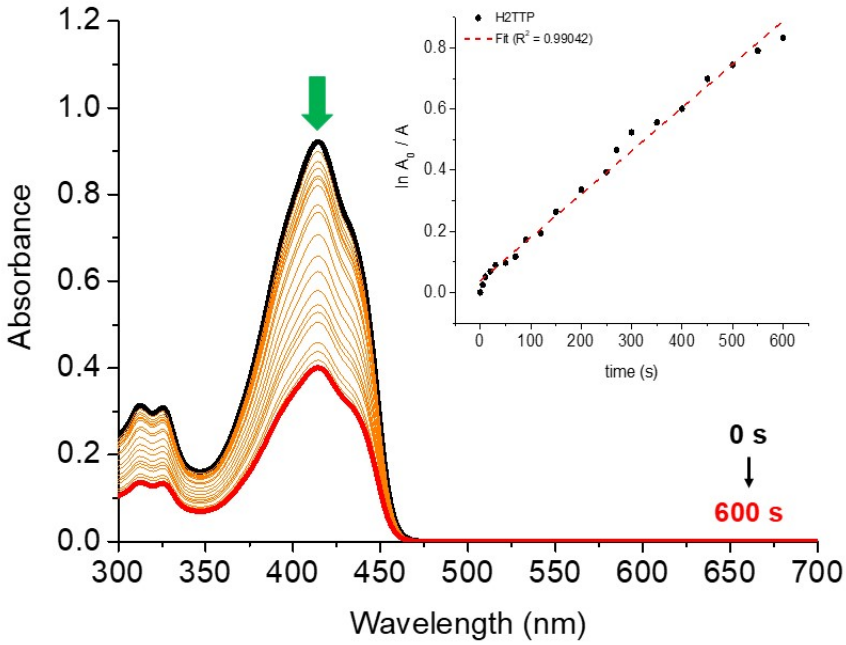

Figure S14. Photo-oxidation of DPBF by irradiation with diode laser (660 nm) in the presence of **H_2TTP**. The *inset* shows the first-order kinetic profile.

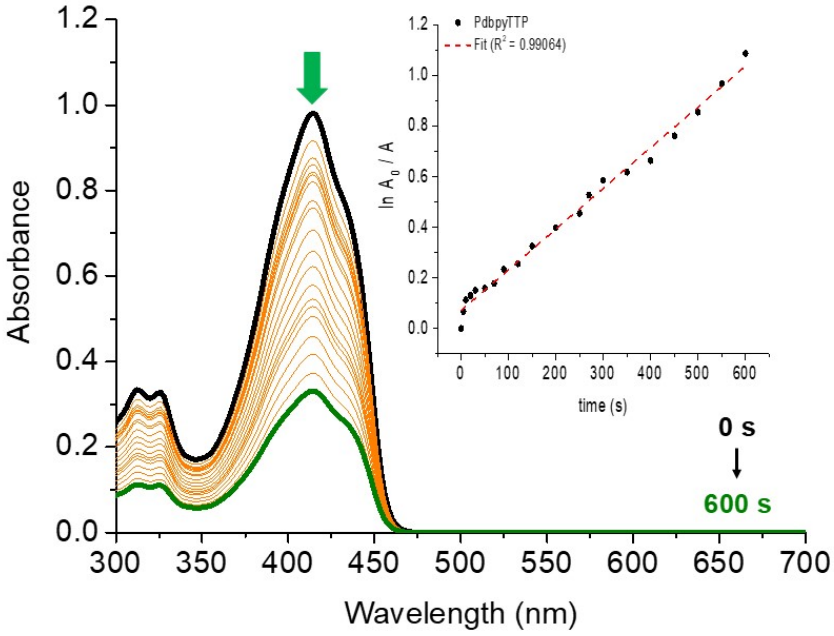

Figure S15. Photo-oxidation of DPBF by irradiation with diode laser (660 nm) in the presence of **PdbpyTTP**. The *inset* shows the first-order kinetic profile.

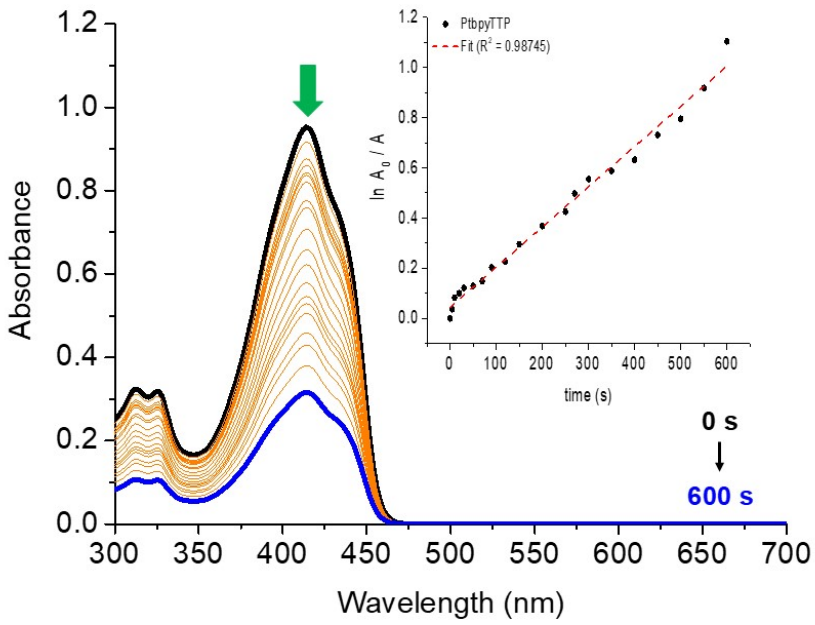

Figure S16. Photo-oxidation of DPBF by irradiation with diode laser (660 nm) in the presence of **PtbpyTTP**. The *inset* shows the first-order kinetic profile.

HSA-binding assays by UV-Vis absorption analysis

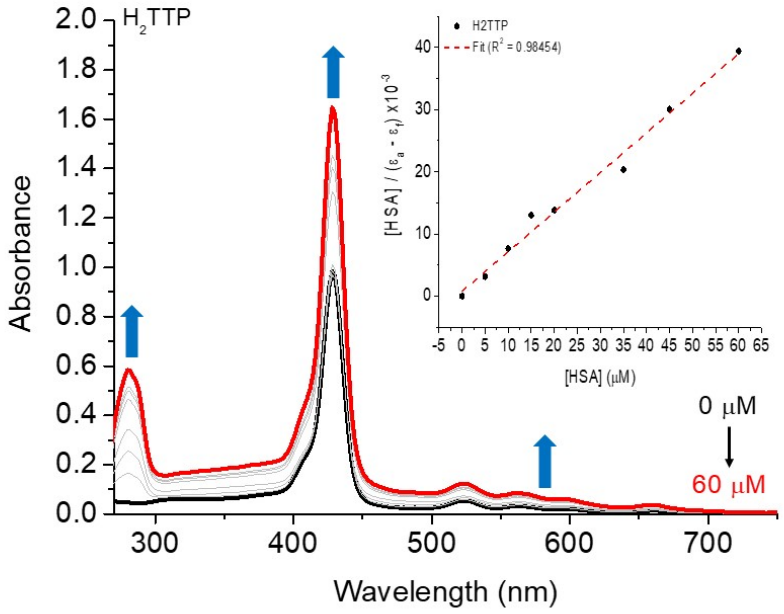

Figure S17. UV–Vis absorption spectra of porphyrin **H_2TTP** with increase HSA concentrations, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution. The insert shows the plot of [HSA]/(ε_a – ε_f) *versus* [HSA].

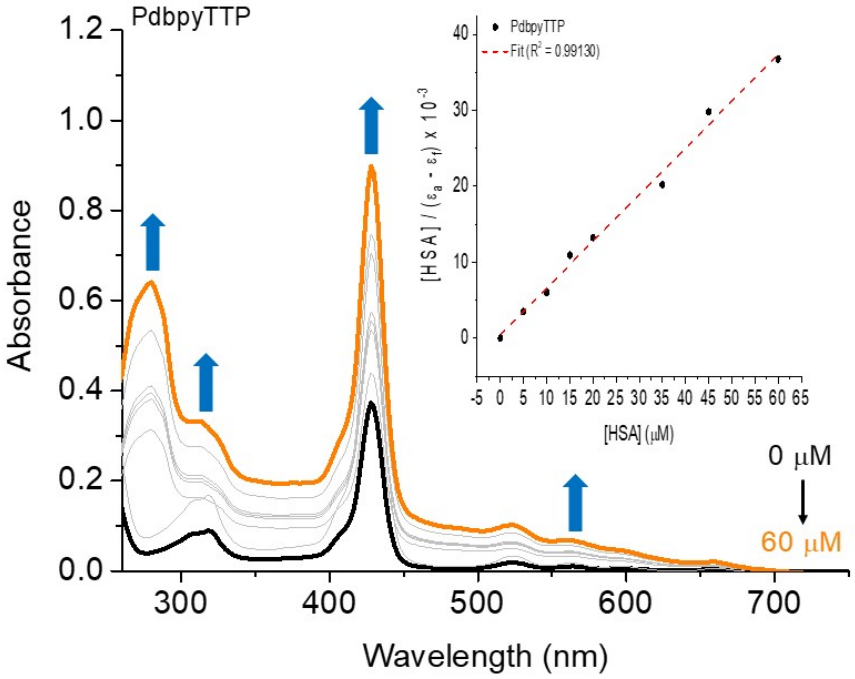

Figure S18. UV–Vis absorption spectra of porphyrin **PdbpyTTP** with increase HSA concentrations, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution. The insert shows the plot of [HSA]/(ε_a – ε_f) *versus* [HSA].

HSA-binding assays by steady-state fluorescence emission analysis

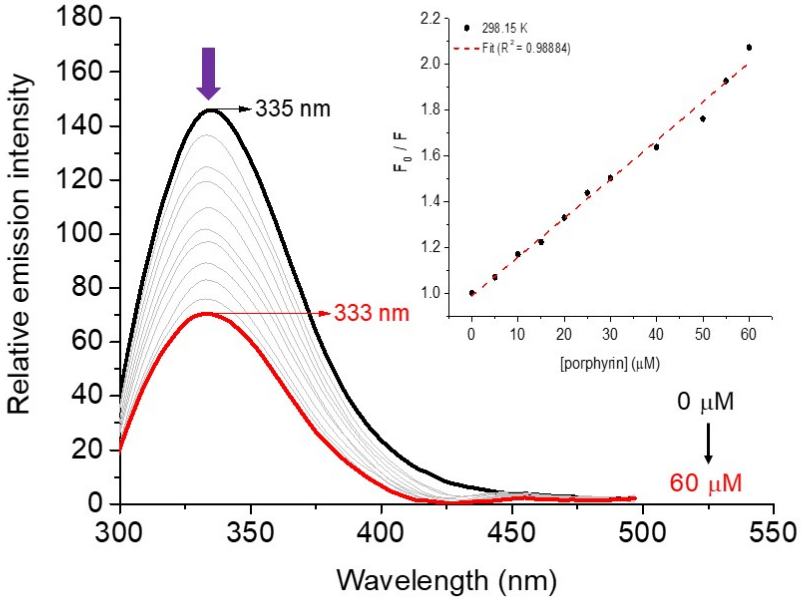

Figure S19. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **H_2TTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 298.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

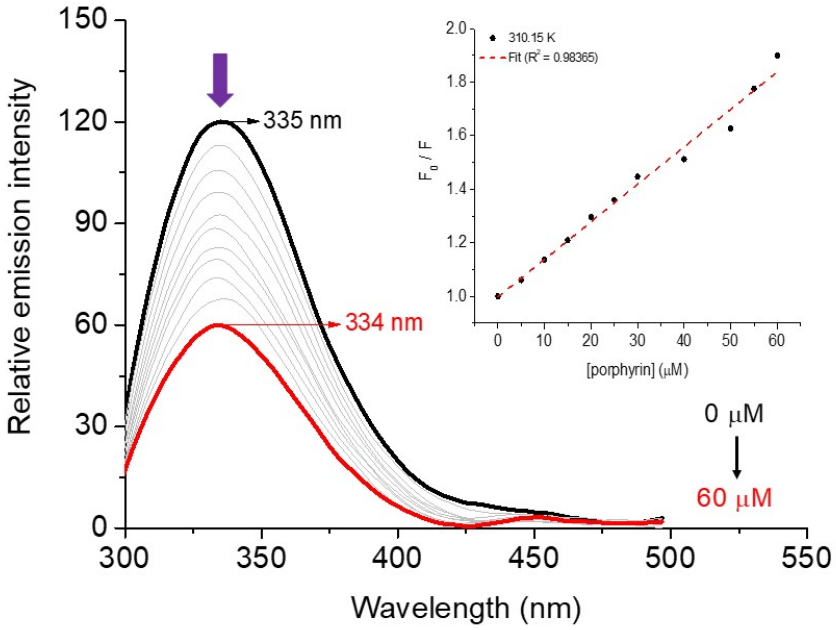

Figure S20. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **H_2TTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 310.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

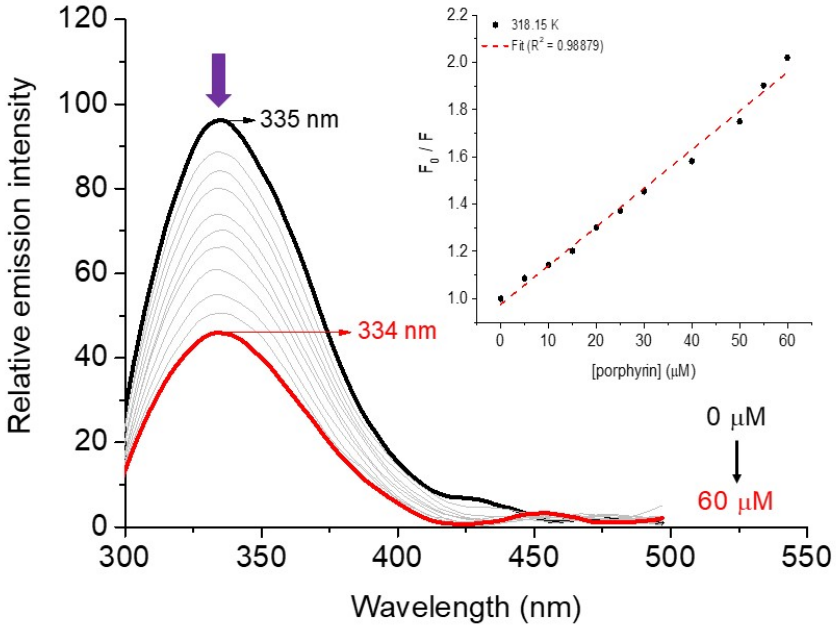

Figure S21. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **H_2TTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 318.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

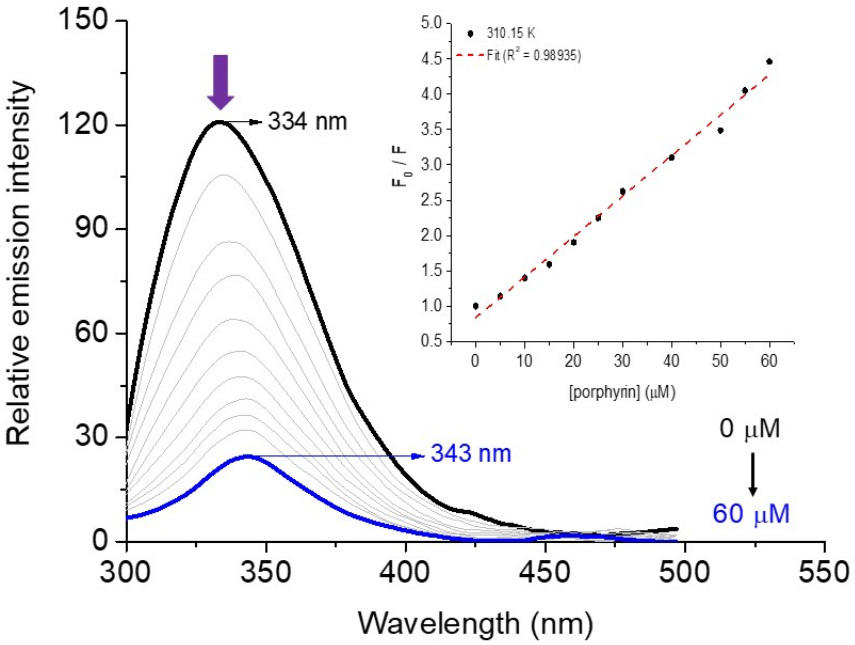

Figure S22. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **PtbpyTTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 310.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

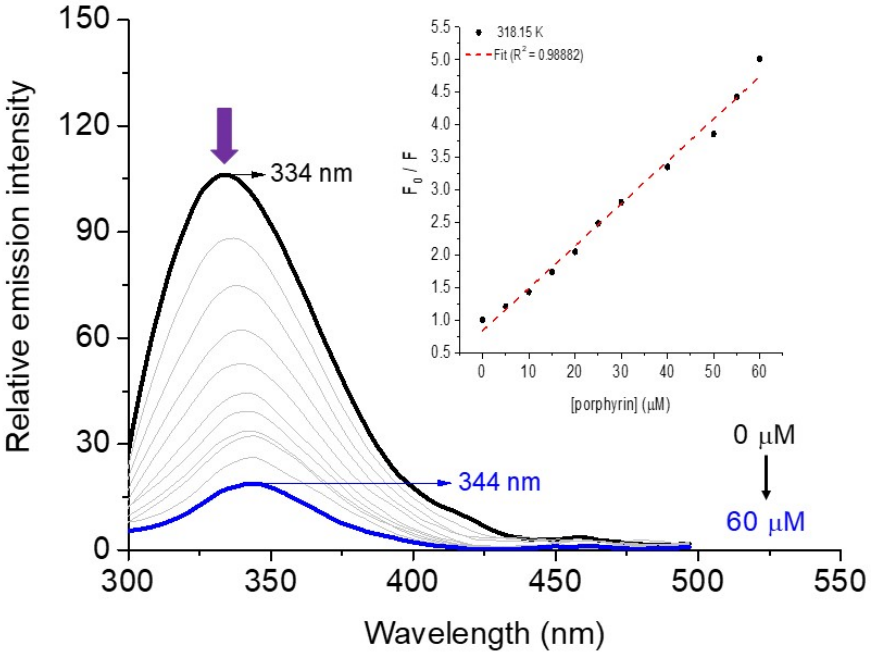

Figure S23. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **PtbpyTTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 318.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

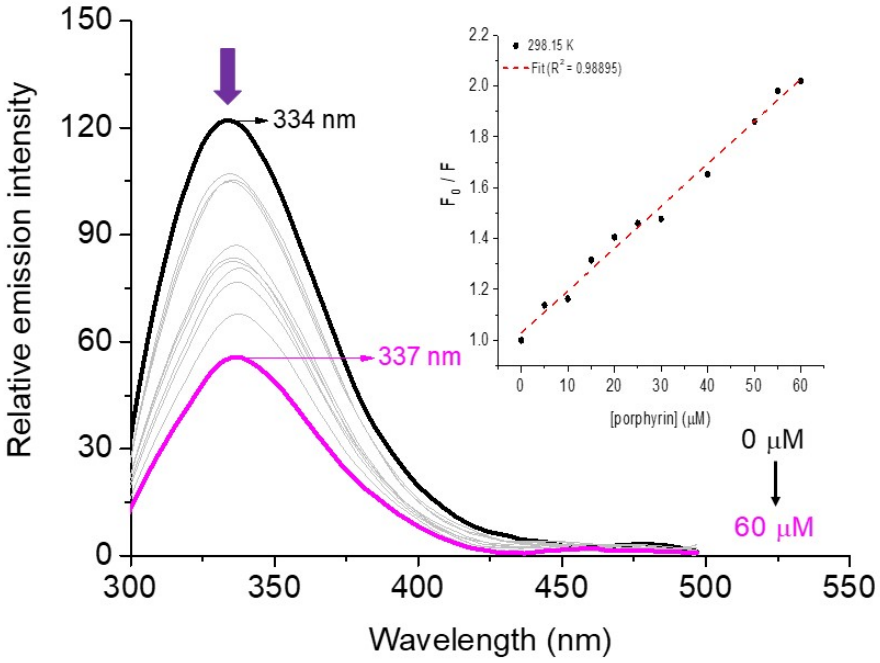

Figure S24. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **PdbpyTTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 298.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

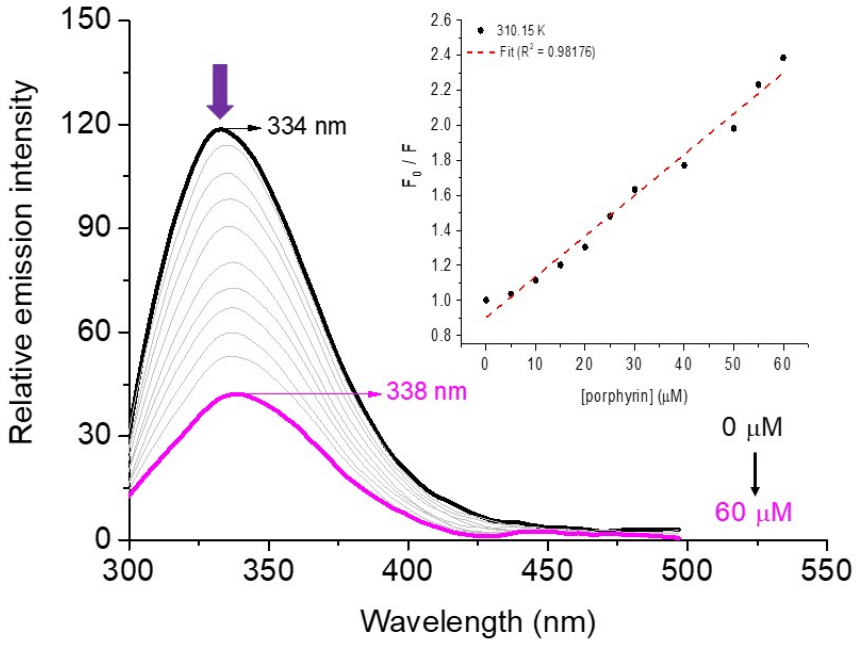

Figure S25. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **PdbpyTTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 310.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

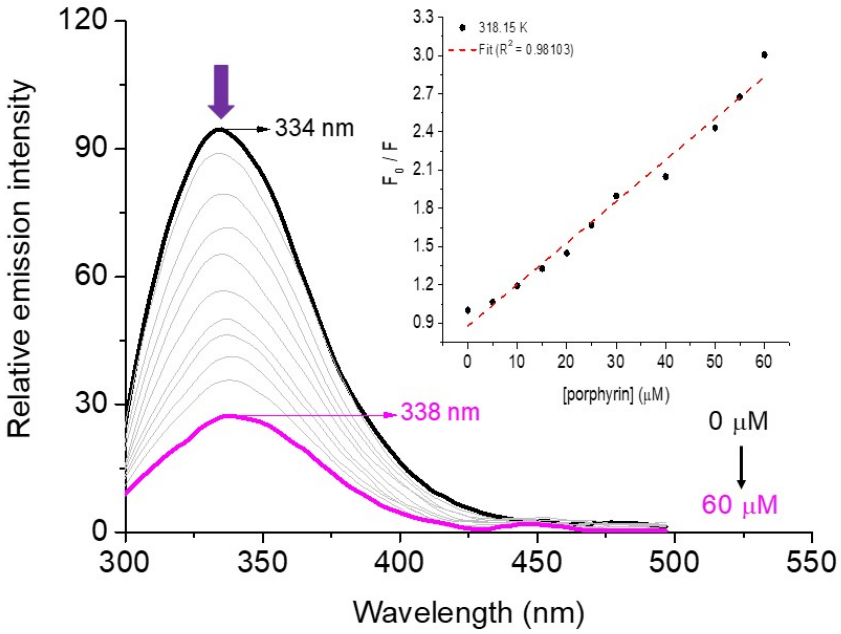

Figure S26. Steady-state fluorescence emission spectra for HSA without and upon successive additions of **PdbpyTTP**, in a DMSO(5%)/Tris-HCl buffer (pH 7.4) solution at 318.15 K. The concentration of compounds ranged from 0 to 60 μM. *Insert graph* shows the plot of F_0/F *versus* [porphyrin].

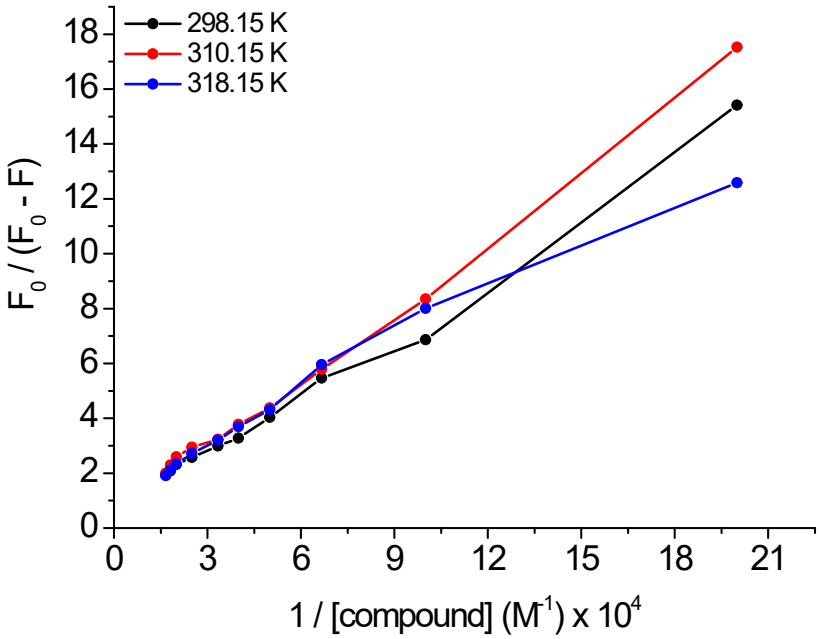

Figure S27. Modified Stern–Volmer plots for HSA:**H_2TTP** at three different temperatures.

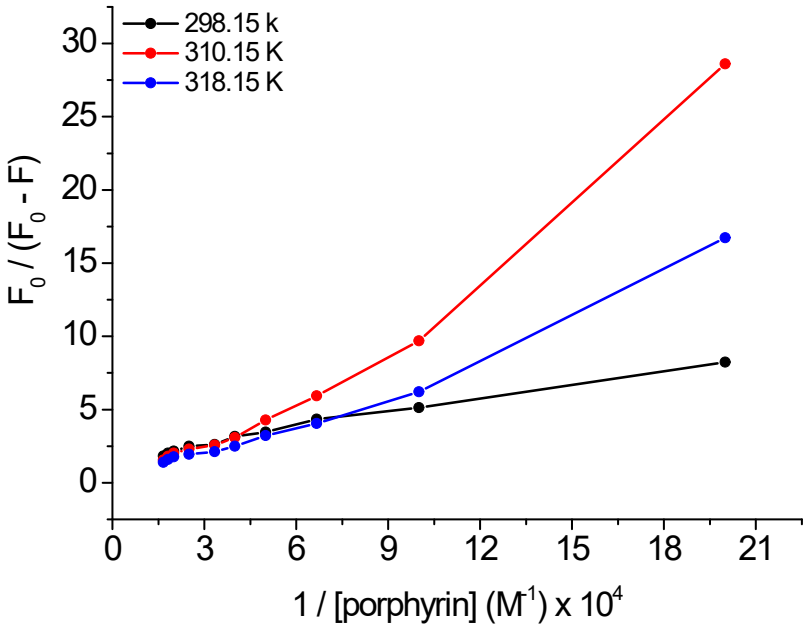

Figure S28. Modified Stern–Volmer plots for HSA:**PdbpyTTP** at three different temperatures.

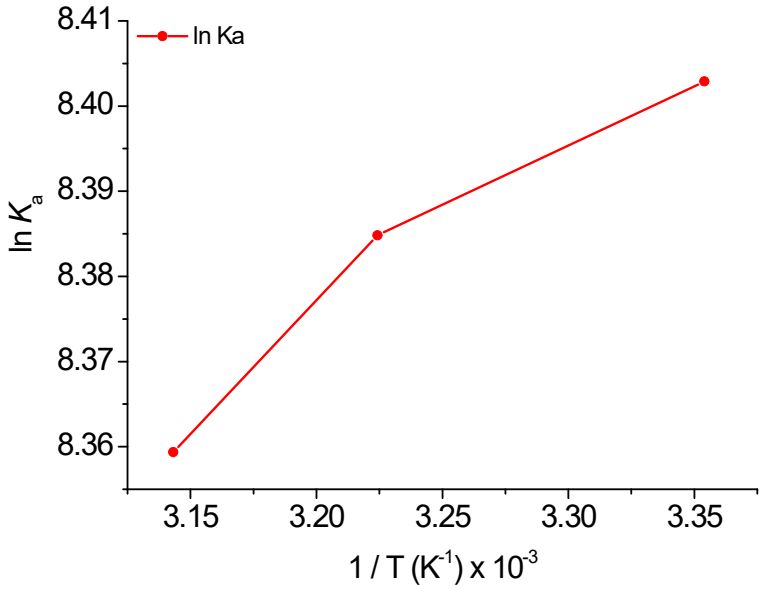

Figure S29. Van´t Hoff plot for HSA:**H_2TTP**.

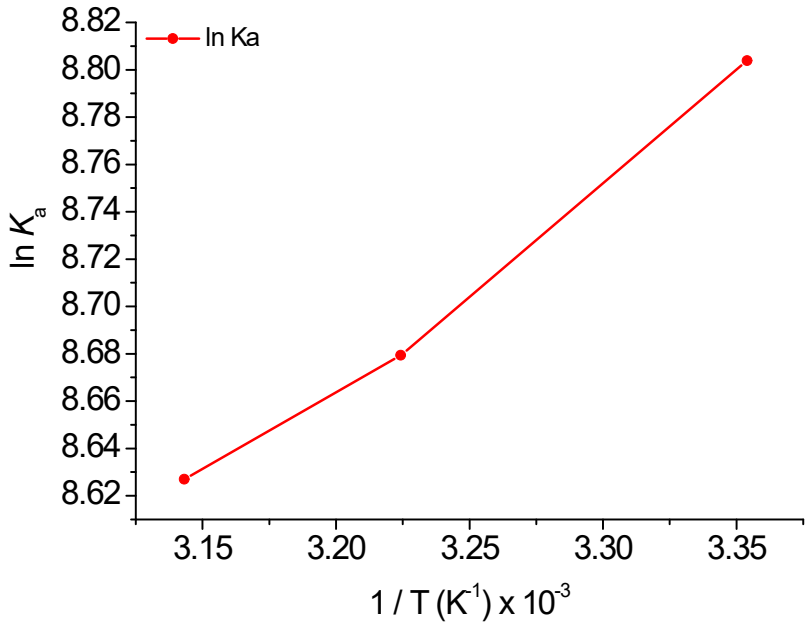

Figure S30. Van´t Hoff plot for HSA:**PtbpyTTP**.

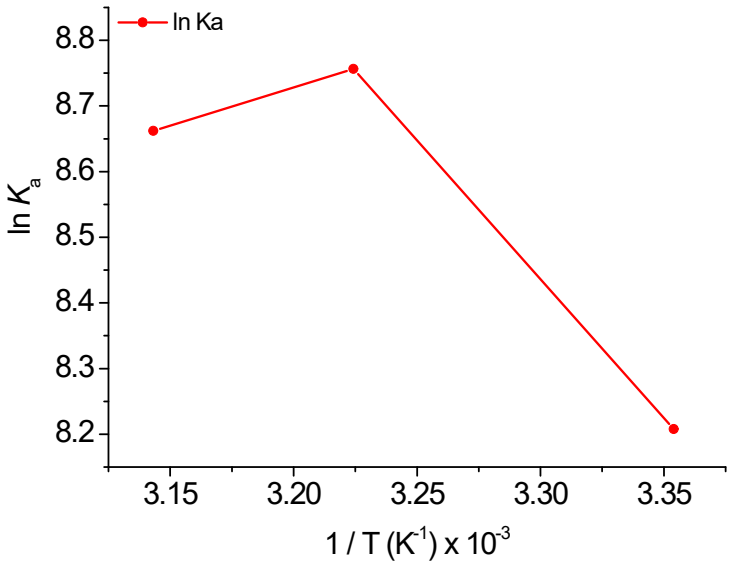

Figure S31. Van´t Hoff plot for HSA:**PdbpyTTP**.

Time-resolved fluorescence decay with HSA

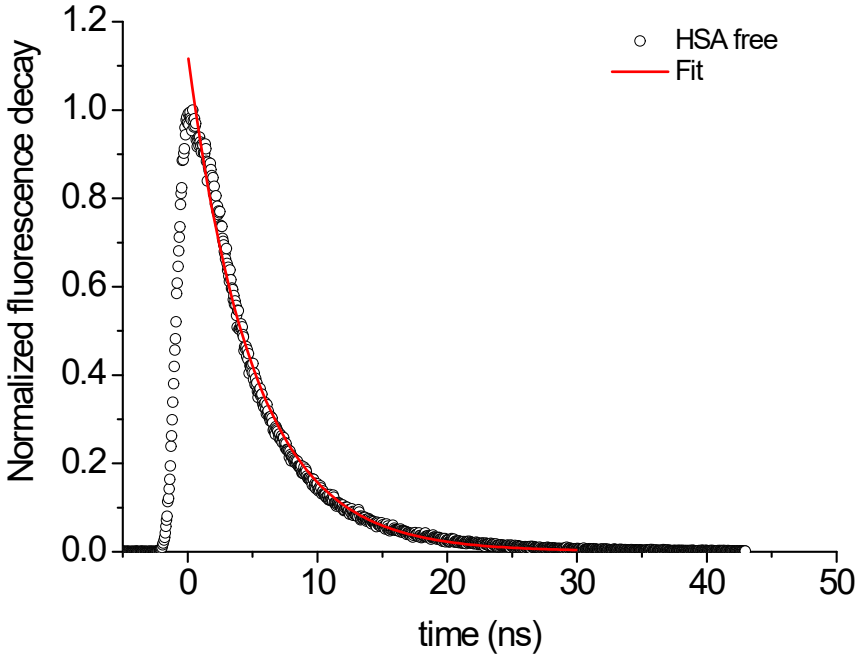

Figure S32. Normalized fluorescence emission decay for free **HSA**, in DMSO(5%)/Tris-HCl buffer (pH 7.4) solution, at λ_{exc} = 284 nm (NanoLED).

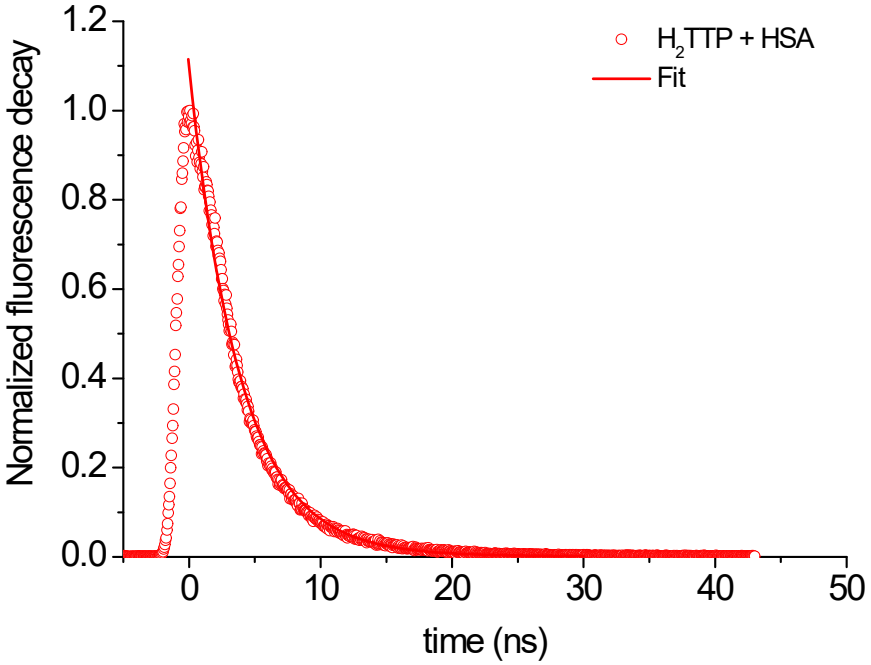

Figure S33. Normalized fluorescence emission decay for **HSA:H_2TTP**, in DMSO(5%)/Tris-HCl buffer (pH 7.4) solution, at λ_{exc} = 284 nm (NanoLED).

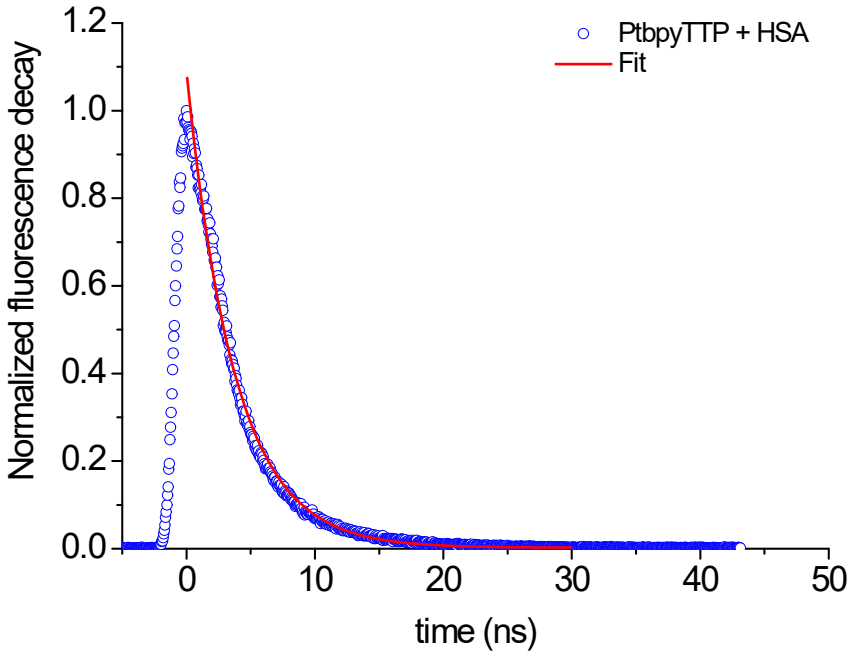

Figure S34. Normalized fluorescence emission decay for **HSA:PtbpyTTP**, in DMSO(5%)/Tris-HCl buffer (pH 7.4) solution, at λ_{exc} = 284 nm (NanoLED).

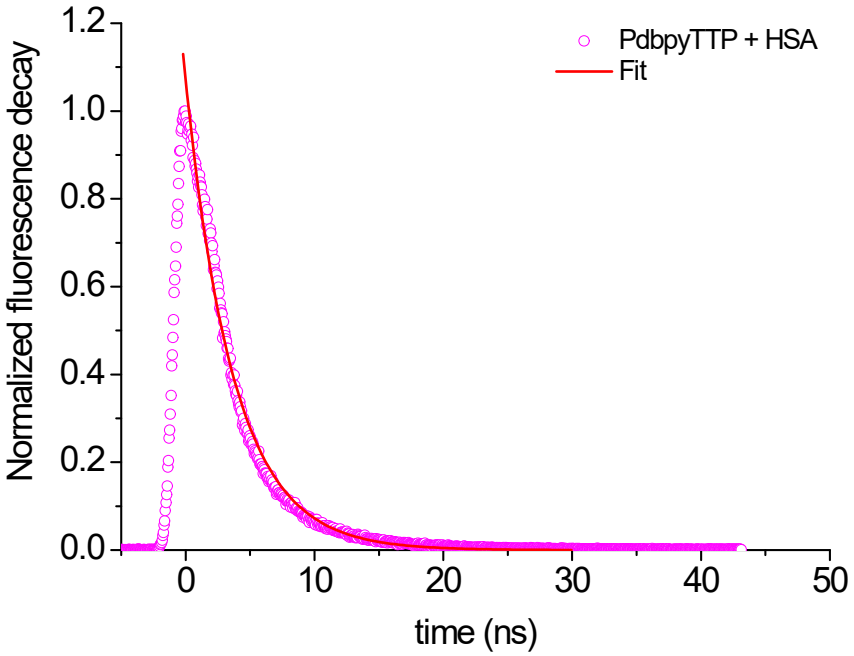

Figure S35. Normalized fluorescence emission decay for **HSA:PdbpyTTP**, in DMSO(5%)/Tris-HCl buffer (pH 7.4) solution, at λ_{exc} = 284 nm (NanoLED).

EPR analysis

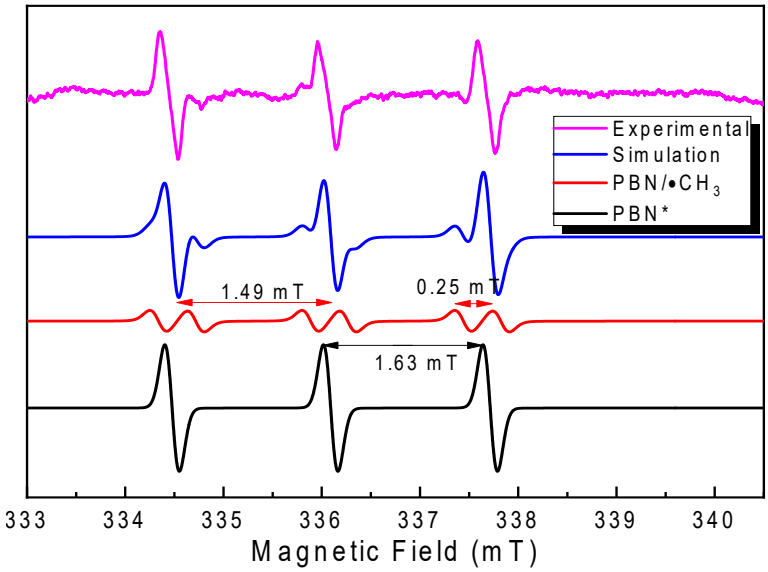

Figure S36. Experimental EPR spectrum of the PBN spin adduct (pink line) and calculated spectrum (blue line) after illumination with white-light. The same EPR spectrum is observed for the porphyrins **H_2TTP**, **PtbpyTTP** and **PdbpyTTP** in DMSO solution.

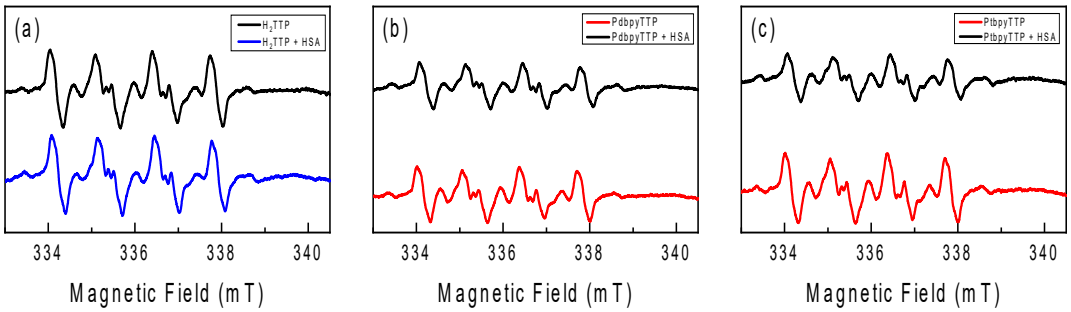

Figure S37. Experimental EPR spectra of spin adducts produced by DMPO under white-light illumination in a DMSO solution containing the porphyrins (a) **H_2TTP**, (b) **PtbpyTTP** and (c) **PdbpyTTP** with the spin trap DMPO (0.3 M) as a function of the presence of HSA (red lines) protein.

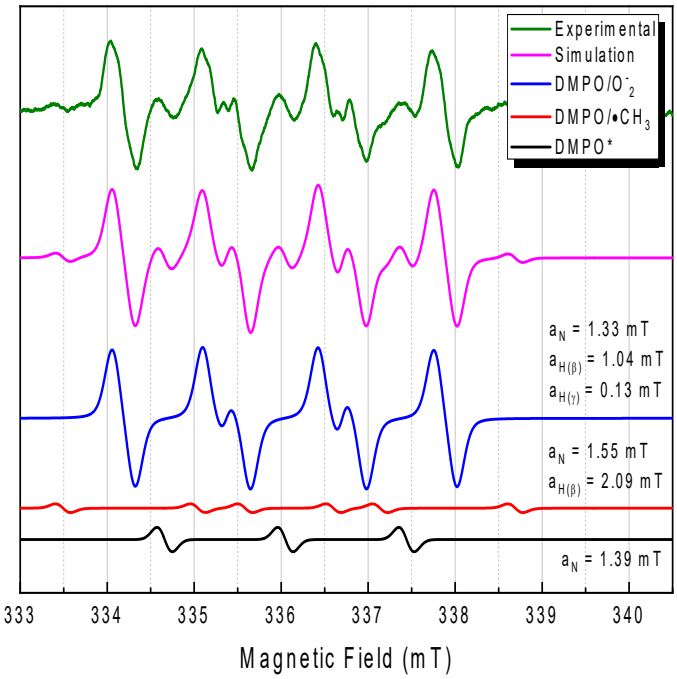

Figure S38. Simulated EPR spectra of the DMPO* (black line), DMPO/•CH_3 (red line) and DMPO/O_2^- (blue line) spin adducts which together constitute the common experimental EPR spectrum (green line) for the porphyrins **H_2TTP**, **PtbpyTTP** and **PdbpyTTP**, with and without HSA proteins, after exposure to white-light. In pink, the sum of the simulated spectra of the spin adducts is shown, reproducing the experimental data with good precision.

HSA photooxidation analysis by steady-state fluorescence emission

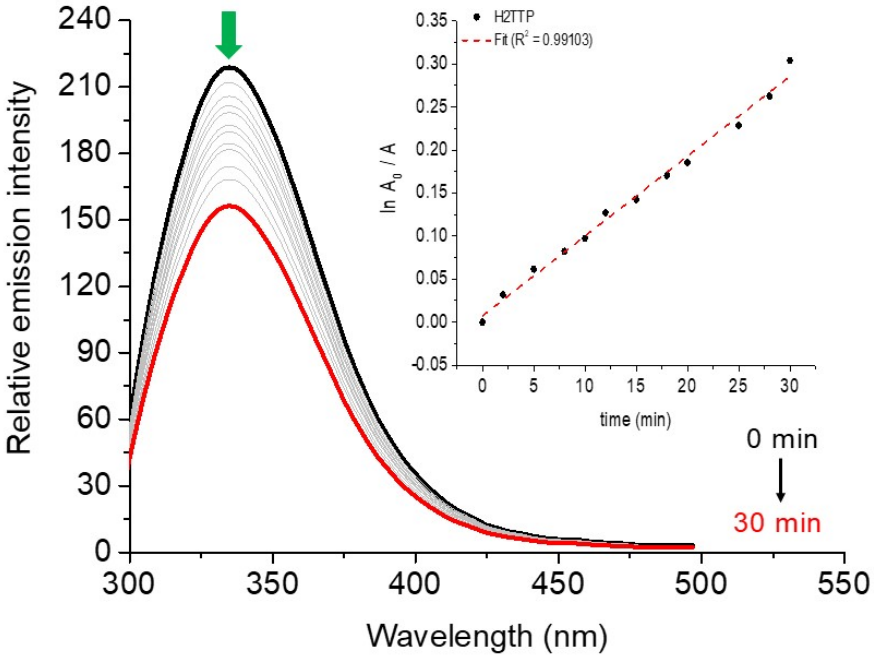

Figure S39. The HSA photooxidation assays in the presence of porphyrin **H_2TTP** upon excitation excitation of 290 nm, in the white-light irradiation conditions at period 0 to 30 min. *Inset plot:* first-order kinetic graphical profile.

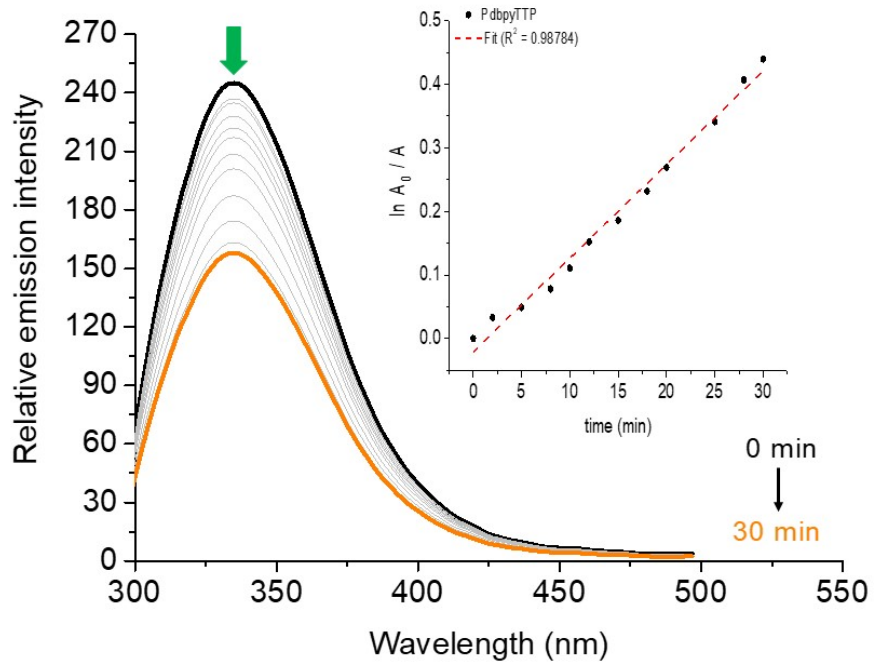

Figure S40. The HSA photooxidation assays in the presence of porphyrin **PdbpyTTP** upon excitation excitation of 290 nm, in the white-light irradiation conditions at period 0 to 30 min. *Inset plot:* first-order kinetic graphical profile.

References

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