Supplemental Information

S.1 Correction of Fluorescence Data for Use in Data Analysis.

The emission intensity was calculated by integrating the area under the emission peak of the spectrum. Prior to the integration the spectra are corrected for the instrument response using a data file provided by the manufacturer. The corrected data need to undergo an additional correction due to optical effects such as inner-filter and dilution using the equation [40, 80, 81]:

$$F = 10^{\left(\frac{OD_{ex} + OD_{em}}{2}\right)} F_{cor} \qquad \text{(S1)}$$

where F is the corrected fluorescence, $F_{cor}$is the fluorescence intensity obtained from the instrument-corrected spectrum, while OD_{ex} and OD_{em} are, respectively, the optical densities of the sample at the excitation wavelength and at the emission maximum of the fluorophore. Compared to the equation described in [40] (equation 2.6) we modified the nomenclature and used F for F_{cor} and F_{cor} for F_{obs}. In comparison with the nomenclature used in [80] (equation 6) for the same equation we used F_{cor} instead of F_{obs} and F instead of F_{ideal}. Finally reference [81] uses basically the same nomenclature of [40]. In comparison equation 1 in [81] we used the same change of nomenclature discussed above for 40 and assumed d_{em} and d_{ex} equal to 1 since we use 1 cm cuvettes.

In order to exemplify the protocol here is a table of values from our experiments representative of our data:

Data	**PPIX**	**ZnPPIX**
F_{raw} (i.e. before correction for the instrumental response)	28.2	20.4
F_{cor} (i.e. after correction for the instrumental response)	37.5	23.6
OD_{ex} (i.e. representative optical density of the sample at the excitation wavelength of HSA = 294 nm)	0.104	0.108
OD_{em} (i.e. representative optical density of the sample at the maximum of emission of HSA = 336 nm)	0.053	0.071
F	44.9	29.0

S.2 Calculation of f_A.

The value of f_A used in equation 9 was calculated from the absorption spectra using established values of the molar extinction coefficient of the porphyrin and HSA. Using the Beer-Lambert equation the concentration of a molecule in solution can be found as

$C = \frac{OD_\lambda}{\varepsilon_\lambda l}$ where OD_λ is the absorption at wavelength λ, ε_λ is the molar extinction coefficient of the chromophore at that wavelength and l is the thickness of the sample (in our case = 1 cm). Thus

$$f_A = \frac{C_{bPP}}{C_{HSA}} = \frac{OD_\lambda^{bPP}}{\varepsilon_\lambda^{bPP}} \cdot \frac{\varepsilon_{278}^{HSA}}{OD_{278}^{HSA}} \quad \text{(S2)}$$

Where the super and subscripts bPP refer to the bound protoporphyrin, the subscript λ for bPP sample refer to the wavelength at which we know the value of ε for the PP (405 nm for PPIX and 417 nm for ZnPPIX) and 278 is the wavelength of the tabulated value of ε for HSA. As was the case for the correct value of HSA contribution to the absorption at 294 nm (discussed in section 2.4.2) one has to consider the residual contribution of each porphyrin to the absorption at 278 nm. Using the same methods explained in section 2.4.2 we estimated the residual absorption of PPIX and ZnPPIX at 278 nm from the absorption ratio $\frac{OD_{278}}{OD_{\lambda_{max}}}$ obtained in solutions of the porphyrins in DMSO. The estimated contribution of the porphyrins was than subtracted from the total absorption at 278 nm in order to obtain the sole contribution (thus the concentration) of HSA.

In order to exemplify the protocol here is a table of values from our experiments representative of our data:

$OD_{407\,nm}^{PPIX}$	0.120	$OD_{417\,nm}^{ZnPPIX}$	0.119
$\varepsilon_{407\,nm}^{PPIX}$ **(approximated by the value of in DMSO)** $\varepsilon_{407\,nm}^{PPIX}$	1.7×10^5	$\varepsilon_{417\,nm}^{PPIX}$ **(approximated by the value of** $\varepsilon_{417\,nm}^{PPIX}$ **in DMSO)**	1.2×10^5
$[PPIX] = \frac{OD_{407\,nm}^{PPIX}}{\left(\varepsilon_{407\,nm}^{PPIX}\right)l}$	0.71 μM	$[ZnPPIX] = \frac{OD_{417\,nm}^{ZnPPIX}}{\left(\varepsilon_{417\,nm}^{ZnPPIX}\right)l}$	0.99 μM
$\varepsilon_{278\,nm}^{HSA}$	3.5×10^4	$\varepsilon_{278\,nm}^{HSA}$	3.5×10^4
$OD_{278\,nm\,\#}^{HSA}$	0.059	$OD_{278\,nm\,\#}^{HSA}$	0.11
$[HSA] = \frac{OD_{278\,nm}^{HSA}}{\left(\varepsilon_{278\,nm}^{HSA}\right)l}$	1.69 μM	$[HSA] = \frac{OD_{278\,nm}^{HSA}}{\left(\varepsilon_{278\,nm}^{HSA}\right)l}$	3.3 μM
f_A **(from equation S2)**	0.42	f_A **(from equation S2)**	0.30

[#] *This value was obtained by taking the overall OD at 278 nm and subtracting the estimated contribution of PPIX or ZnPPIX. The latter was estimated by approximating/assuming that the absorption spectrum most representative of the porphyrin bound to HSA was the one obtained in DMSO. Thus, from the spectrum of PPIX (or ZnPPIX) in DMSO we established the ratio of optical densities between the maximum of the Soret band (407 nm for PPIX; 417 nm for ZnPPIX)*

and the value at 278 nm. We assumed that this ratio would remain constant (24.0 for PPIX and 39.6 for ZnPPIX), thus, from the maximum of the Soret band of bound PPIX (or ZnPPIX) [such as the ones in Figure S2] one extrapolates the contribution of each porphyrin to the OD at 278 nm. This contribution was subtracted to the overall OD to obtain $OD_{278\,nm}^{HSA}$.

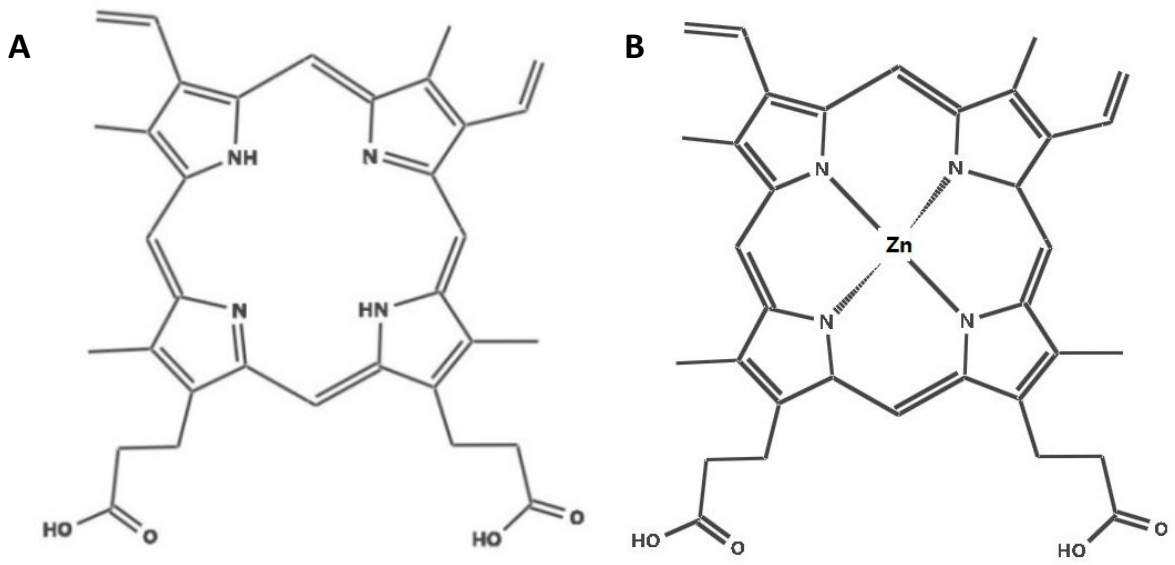

Figure S1. *Molecular structures of PPIX (A) and ZnPPIX (B).*

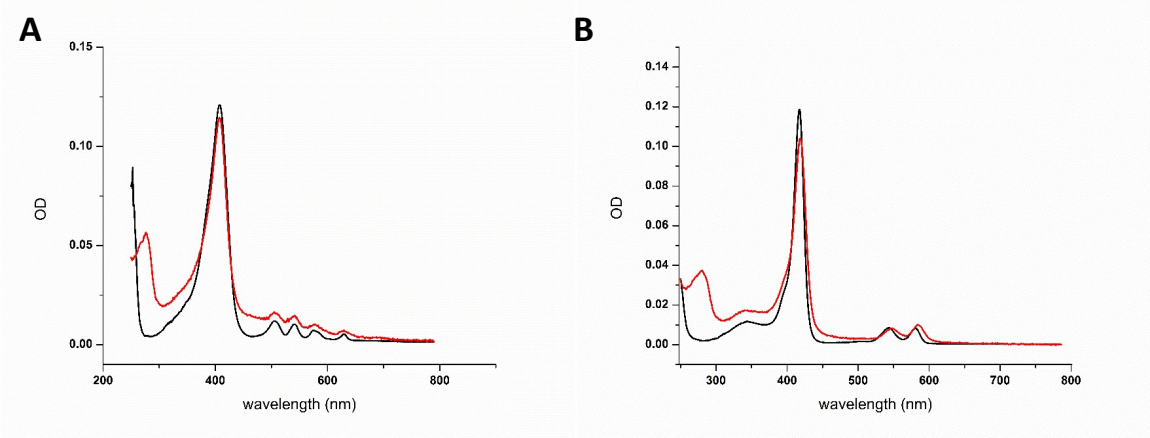

Figure S2. *(A) Spectral overlap of the absorption spectra of PPIX in DMSO (black) and a solution of PPIX bound to HSA (red) in aqueous solution. The concentration of the second one can only be inferred by similarities as discussed in the manuscripts since the procedure to eliminate the aggregated porphyrins renders the final concentration of bound PPIX unpredictable and it can only be measured after the procedure using the method described in the manuscript. From the spectrum in DMSO we established that the optical density of PPIX in DMSO at the maximum of the Soret band (407 nm) is 24-times larger than the OD at 294 nm where Trp214 was excited in our experiments. This ratio was then assumed to extrapolate the contribution to the OD of monomeric PPIX at 294 nm in the experiments where PPIX was bound*

*to HSA. This assumption enabled us to extrapolate from the total OD at 294 nm the contribution of Trp214 in the quenching experiments where one has both bound PPIX and HSA in solution. (**B**) Spectral overlap of the absorption spectra of 0.8μM solution of ZnPPIX in DMSO (black) and a solution of ZnPPIX bound to HSA in aqueous solution. The concentration of the second one can only be inferred by similarities as discussed in the manuscripts since the procedure to eliminate the aggregated porphyrins renders the final concentration of bound ZnPPIX unpredictable and it can only be measured after the procedure using the method described in the manuscript. From the spectrum in DMSO we established that the optical density of ZnPPIX in DMSO at the maximum of the Soret band (407 nm) is 39.6-times larger than the OD at 294 nm where Trp214 was excited in our experiments. This ratio was then assumed to extrapolate the contribution to the OD of monomeric ZnPPIX at 294 nm in the experiments where ZnPPIX was bound to HSA. This assumption enabled us to extrapolate from the total OD at 294 nm the contribution of Trp214 in the quenching experiments where one has both bound ZnPPIX and HSA in solution. The black spectra (PPIX or ZnPPIX in DMSO) were recorded from a dilution of the sample shown below (blue spectrum in Figure S3A and S3b). The dilution was carried out in order to bring the intensity of the peak near the same value as that obtained from the complex of HSA with each protoporphyrin after elimination of the aggregates. Also, the overall increase in the spectrum is due to the presence of the protein which contributes in the region of the absorption of the aromatics (<310 nm) but also at longer wavelengths due to the increased scattering contribution produced by HSA.*

S.3 Calculating the contribution of each porphyrin to the optical density at the excitation wavelength of Trp214 (i.e., 294 nm). The data in the two spectra above can be taken to explain the protocol for obtaining the contribution of Trp214 to the OD at 294 nm without the contribution of the porphyrins. Assuming that the OD ratio $\left(\frac{OD_{Soret\ \lambda_{max}}}{OD_{294\,nm}}\right)$ described in the manuscript remains constant, one can estimate the contribution of each porphyrin to the $OD_{294\,nm}$. Thus, from spectra such as the ones in Figures S5A or S6A the OD at the maximum of the Soret band can be used to calculate the residual absorption of the porphyrin at 294 nm by assuming that the ratio between the OD of the Soret band and the OD at 294 nm is maintained for the porphyrins as it is in the DMSO samples (see legend of Figure S3). This contribution is then subtracted from the overall $OD_{294\,nm}$ to estimate the sole contribution of Trp214 to the absorption at this wavelength. This value was then used as the OD_{ex} value in equation S1 used to correct the emission spectra of the protein.

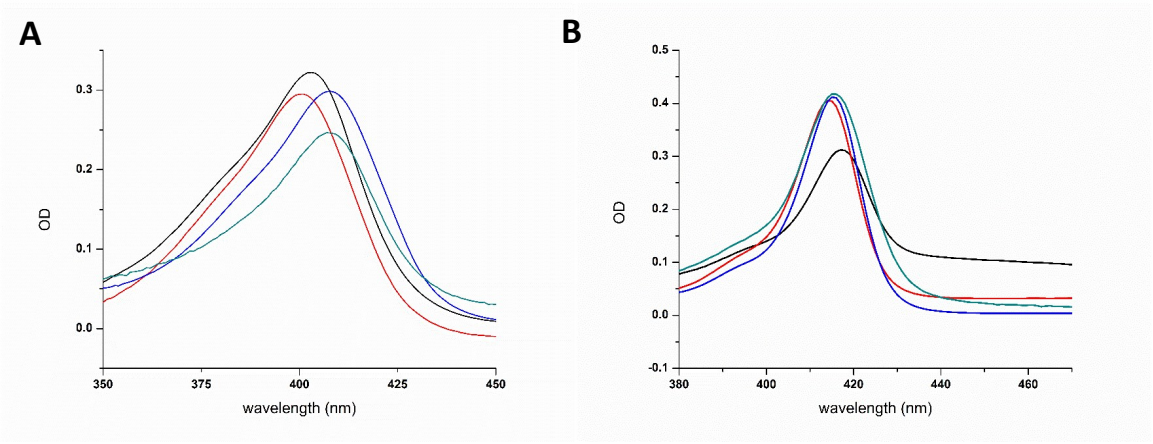

Figure S3. *(**A**) Spectral overlap of the absorption spectra of 1.5μM solution of PPIX in methanol (red), ethanol (black), DMSO (blue) and PPIX bound to HSA (teal). The spectra only show the region of the Soret band. The similarity between the peaks of PPIX in DMSO and PPIX bound to HSA was the reason for choosing the spectra of PPIX in DMSO as representative (even in terms of molar extinction coefficient) of the porphyrin bound to HSA. (**B**) Spectral overlap of the absorption spectra of 1.7 μM solution of ZnPPIX in methanol (red), ethanol (black), DMSO (blue) and PPIX bound to HSA (teal). The spectra only show the region of the Soret band. The similarity between the peaks of ZnPPIX in DMSO and ZnPPIX bound to HSA was the reason for choosing the spectra of ZnPPIX in DMSO as representative (even in terms of molar extinction coefficient) of the porphyrin bound to HSA.*

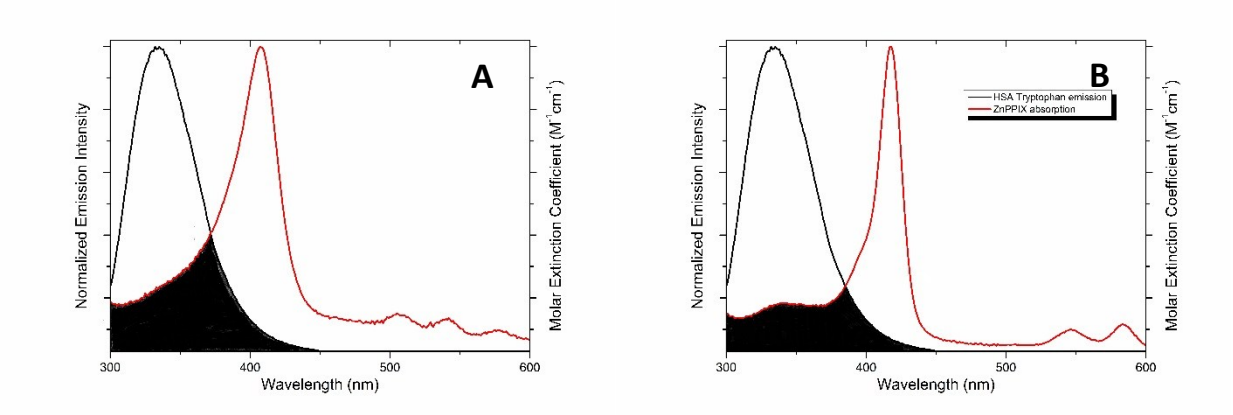

Figure S4. *(**A**) Spectral overlap between the fluorescence spectrum of Trp214 (black) and the absorption spectrum of PPIX in DMSO (red). The shaded area represented the overlap between the emission of the Trp residue and the absorption of the porphyrin. The value of the overlap integral $J(\lambda)$ [equation 6] is proportional to this area. (**B**) Spectral overlap between the fluorescence spectrum of Trp214 (black) and the absorption spectrum of ZnPPIX in DMSO (red). The concentration of HSA was 3.0 μM (A) and 3.3 μM (B). The concentration of PPIX was 4.1 μM while the concentration of ZnPPIX was 3.5 μM. Tha values of the concentrations are not important in the calculation of the Forster distance, however we reported them to convey the*

concept that the concentrations were sufficiently low to enable us to neglect aggregation of the single species. Moreover as explained in the manuscript and earlier in the Supplemental Information, the absorption spectrum of the porphyrins was selected as the one obtained in DMSO since one cannot obtain a separate absorption spectrum of bound porphyrin, and the spectral characteristics in DMSO are the ones that most closely resemble the spectra of the porphyrins bound to HSA. The shaded area represented the overlap between the emission of the Trp residue and the absorption of the porphyrin. The value of the overlap integral $J(\lambda)$ [equation 6] is proportional to this area.

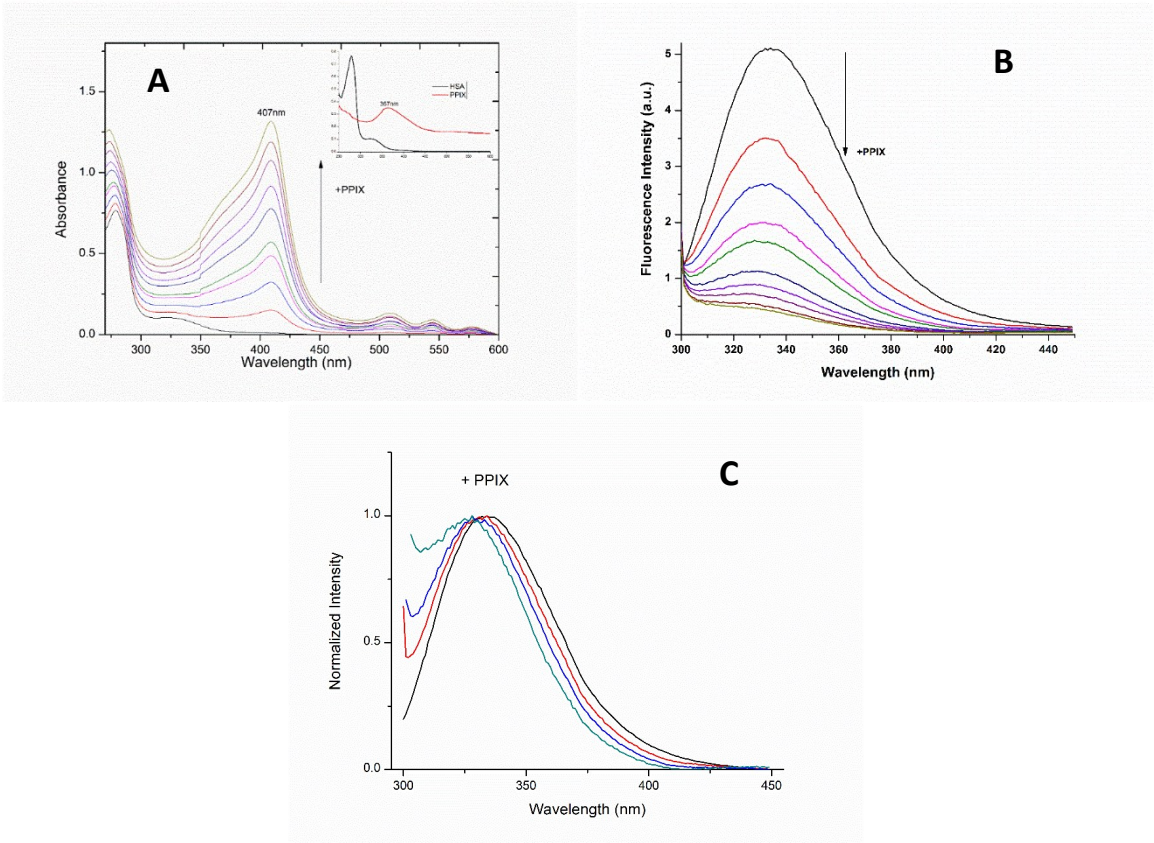

Figure S5. *(**A**) Absorption spectra of HSA solution with PPIX added from 0 - 6 μM. The arrow indicates the direction of the change in absorption as PPIX is added to the solution. The inset shows the absorption spectra of free HSA and free PPIX in aqueous solution at concentrations of ~ 17 μM and ~ 6 μM respectively (the spectrum of PPIX was offset to allow a better visualization of the absorption peak). (**B**) Emission spectra of HSA solution with PPIX added from 0 - 8 μM upon excitation at 294 nm. The arrow indicates the direction of fluorescence intensity change as the concentration of PPIX increases. (**C**) Same emission spectra as in (**B**), normalized for the maximum intensity.*

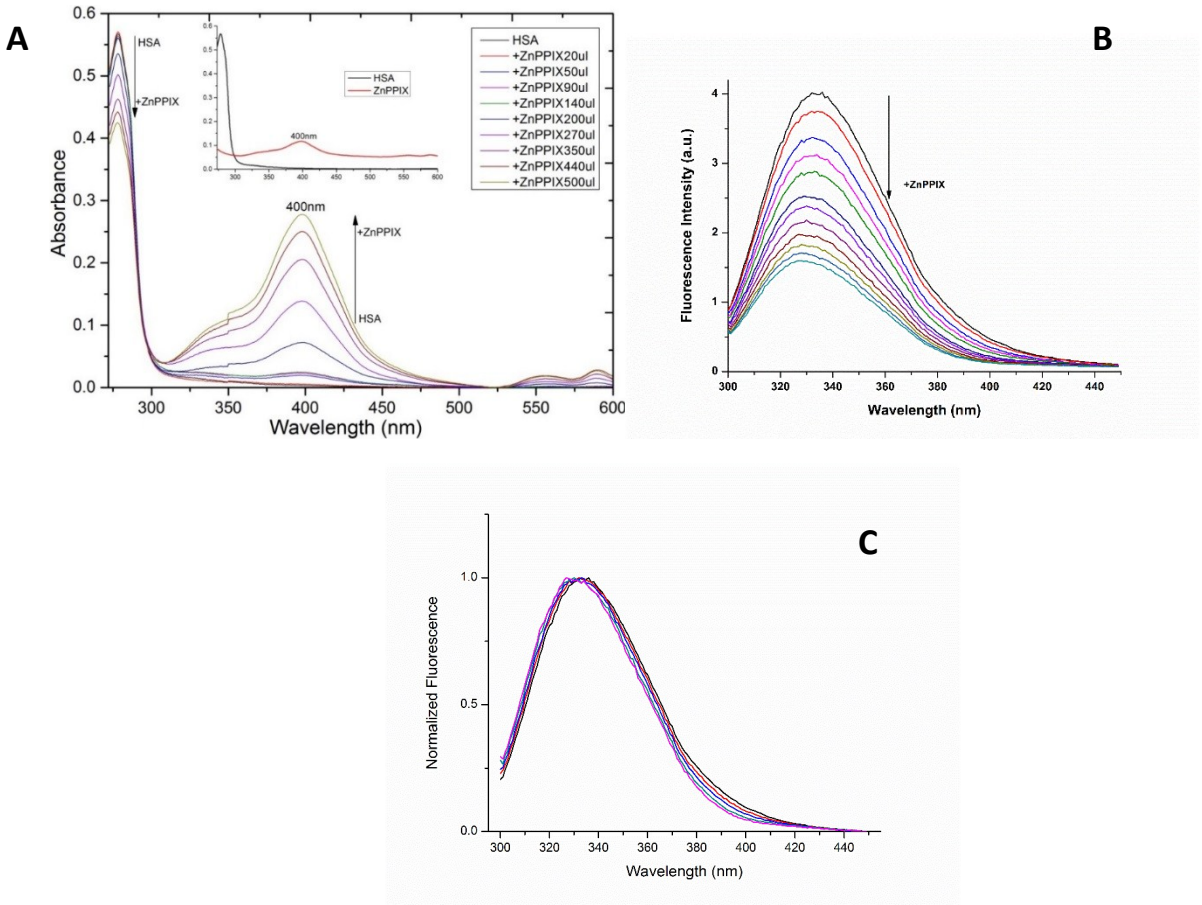

Figure S6. *(A) Absorption spectra of HSA solution with ZnPPIX added from 0 – 6 μM The arrow indicates the direction of the change in absorption as ZnPPIX is added to the solution. The inset shows the absorption spectra of free HSA and free ZnPPIX in aqueous solution at concentrations of ~ 16 μM and ~ 0.7 μM respectively (the spectrum of ZnPPIX was offset to allow a better visualization of the absorption peak).* ***(B)*** *Emission spectra of HSA solution with ZnPPIX added from 0 - 6 μM upon excitation at 294nm. The arrow indicates the direction of fluorescence intensity change as the concentration of ZnPPIX increases. (****C****) Same emission spectra as in (****B****), normalized for the maximum intensity.*

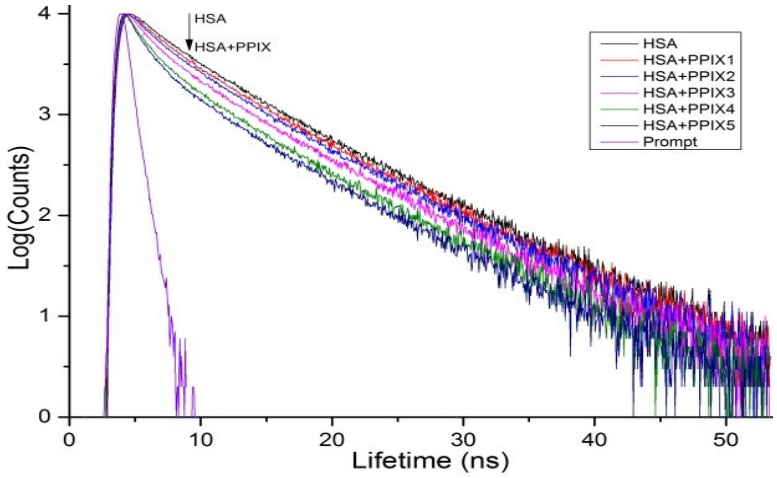

Figure S7*. Fluorescence lifetime decay of HSA upon addition of PPIX.*

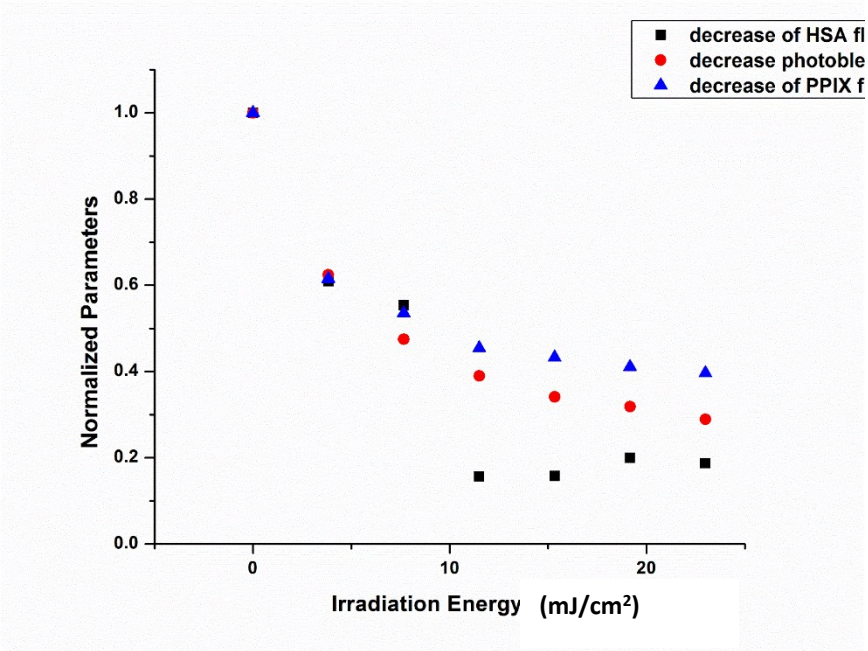

Figure S8. *Comparison of the photobleaching of the Soret band of PPIX (▱) with the decrease in fluorescence intensity of PPIX(●) and the decrease in Trp214 emission intensity (■), along with the total fluence from 0 – 23 mJ/cm².*

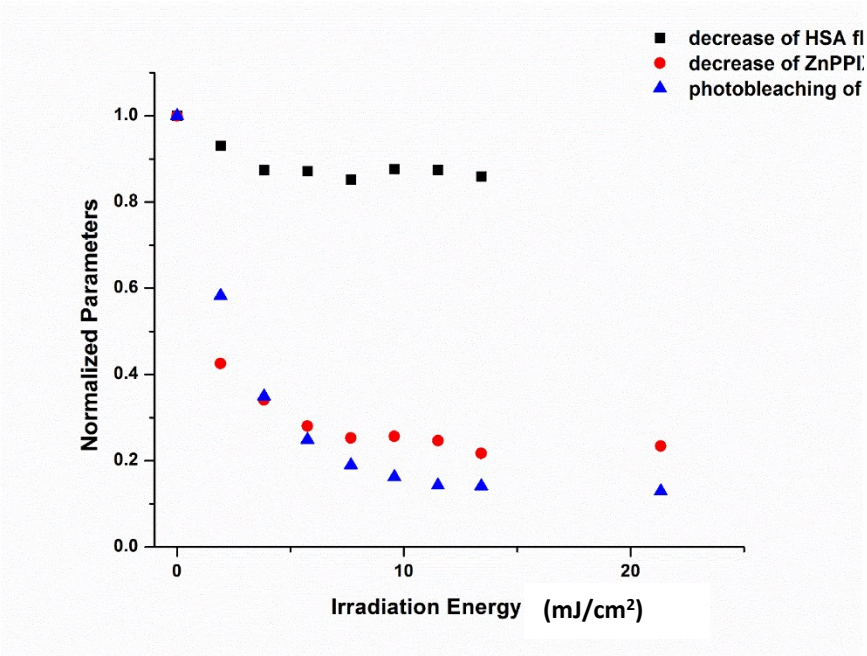

Figure S9. *Comparison of the photobleaching of the Soret band of ZnPPIX PPIX (□) with the decrease in fluorescence intensity of PPIX(●) and the decrease in Trp214 emission intensity (■), along with the total fluence from 0 – 23 mJ/cm².*