Electronic Supplementary Material (ESI) for RSC Advances

Electronic Supplementary Information

A self-assembled nanostructured material with photosensitising properties

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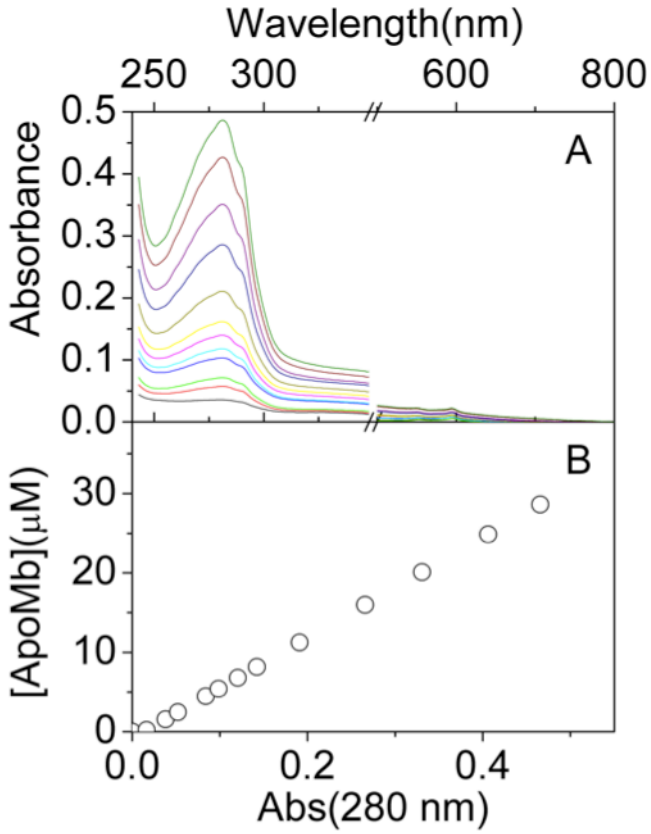

Figure S1. A. Absorption spectra of 0.6 μM Hyp-ApoMb mixtures at increasing ApoMb concentrations (between 0 and 30 μM). The concentration of ApoMb, calculated from the absorbance at 280 nm using the known molar extinction coefficient 15800 $M^{-1}cm^{-1}$ [31], is reported in **B**.

Derivation of eqn (1) for binding of Hyp to ApoMb:

$$\mathrm{Hyp} + \mathrm{ApoMb} \underset{k_{-b}}{\overset{k_b}{\rightleftarrows}} \mathrm{Hyp-ApoMb}$$

the equilibrium binding constant is given by:

$$K_a = \frac{[\mathrm{Hyp-ApoMb}]}{[\mathrm{Hyp}][\mathrm{ApoMb}]}$$

From this expression, the concentrations of free and protein bound Hyp can be derived:

$$[\mathrm{Hyp-ApoMb}] = \frac{K_a[\mathrm{ApoMb}][\mathrm{Hyp}]_{\mathrm{tot}}}{(1 + K_a[\mathrm{ApoMb}])}$$

$$[\mathrm{Hyp}] = [\mathrm{Hyp}]_{\mathrm{tot}}\left(1 - \frac{K_a[\mathrm{ApoMb}]}{(1 + K_a[\mathrm{ApoMb}])}\right)$$

The total emitted fluorescence is the sum of the fluorescence emission from free and from protein bound Hyp which are in turn proportional to the concentrations of these two species:

$$F = F^{\mathrm{Hyp}}[\mathrm{Hyp}]_{\mathrm{tot}}(1 - K_a[\mathrm{ApoMb}]/(1 + K_a[\mathrm{ApoMb}])\,) + F^{\mathrm{Hyp-ApoMb}}(K_a[\mathrm{ApoMb}][\mathrm{Hyp}]_{\mathrm{tot}}/(1 + K_a[\mathrm{ApoMb}])\,)$$

where F^{Hyp} and $F^{\mathrm{Hyp\text{-}ApoMb}}$ are scaling factors proportional to fluorescence quantum yields of the two species.

Under the simplifying assumption that the total concentration of ApoMb, $[\mathrm{ApoMb}]_{\mathrm{tot}}$, is larger than the concentration of bound Hyp, [Hyp-ApoMb], the concentration of free protein, [ApoMb], can be well approximated by $[\mathrm{ApoMb}]_{\mathrm{tot}}$. Hence, the above expression can be written as:

$$F \sim F^{\mathrm{Hyp}}[\mathrm{Hyp}]_{\mathrm{tot}}/(1 + K_a[\mathrm{ApoMb}]_{\mathrm{tot}}) + F^{\mathrm{Hyp-ApoMb}}(K_a[\mathrm{ApoMb}]_{\mathrm{tot}}[\mathrm{Hyp}]_{\mathrm{tot}}/(1 + K_a[\mathrm{ApoMb}]_{\mathrm{tot}})) \quad (1)$$

Cumulative irradiation of the Hyp-ApoMb complex

ApoMb contains aminoacids that may quench $^1O_2$ before it can escape off the protein, e.g., histidines in the heme pocket. It was thus of interest to ascertain whether photooxidation of these residues upon prolonged irradiation of the ApoMb-Hyp complex would result in the enhancement of its Φ_Δ value, as recently observed for a related flavin-binding protein.[1] Fig. S2A shows that the intensity of $^1O_2$ phosphorescence actually decreases upon irradiation, concomitant with changes in the absorption spectrum of the protein (Fig. S2B). However, a transient increase is observed at 60 minutes.

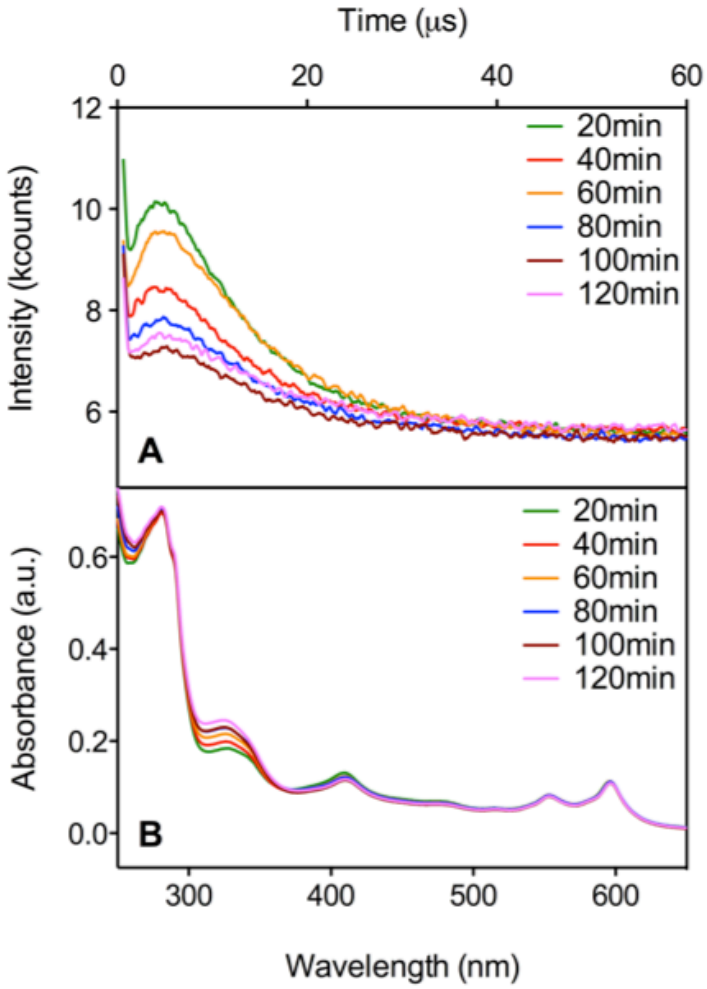

Figure S2. Effect of cumulative irradiation of the Hyp-ApoMb complex on its $^1O_2$ photosensitisation ability in PBS (λ_{exc} = 532 nm, λ_{obs} = 1275 nm; **A**) and on its absorption spectrum (**B**).

Time-resolved anisotropy measurements

Additional evidence for Hyp binding to ApoMb was obtained from time-resolved anisotropy measurements. Fig. S3 shows the anisotropy decay for Hyp-ApoMb in PBS. The best fit is obtained with a single exponential relaxation, with a pre-exponential factor of 0.32 and a time constant of 7.1±0.2 ns. The anisotropy at time zero is consistent with literature data for fluorescent dyes bound to the hydrophobic pocket of ApoMb.[2]

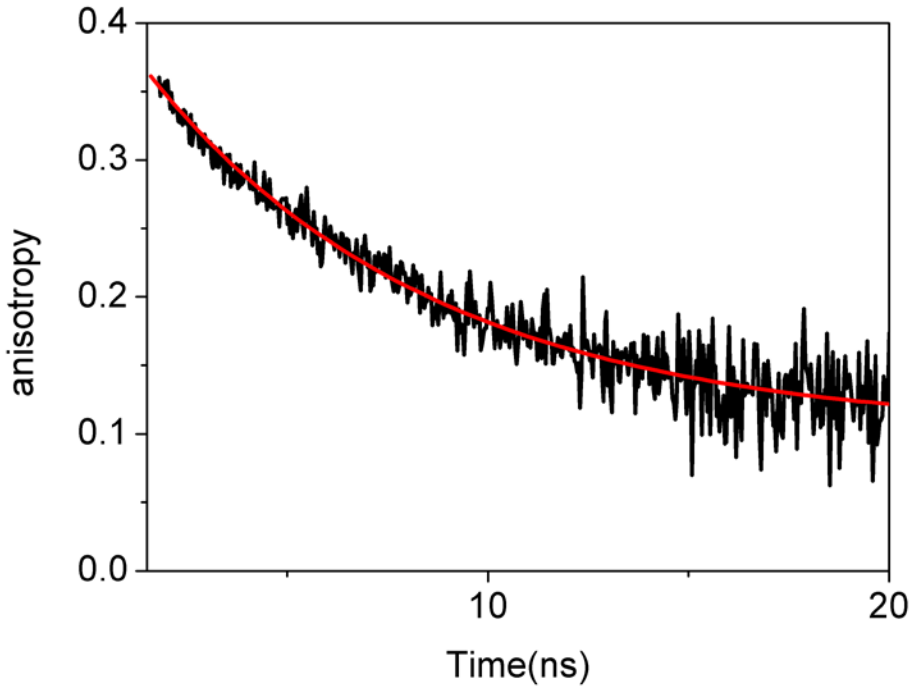

Figure S3. Fluorescence anisotropy decay (black) of Hyp-ApoMb in PBS using a 596 nm LED as the excitation light source. The red line is the best fit with a single exponential relaxation.

Notes and references

1. Ruiz-González, R.; Cortajarena, A.L.; Mejias, S.H.; Agut, M.; Nonell, S.; Flors, C., *J. Am. Chem. Soc.* 2013, 135, 9564-9567.
2. J. R. Lakowicz, E. Gratton, H. Cherek, B. P. Maliwal and G. Laczko, *J. Biol. Chem.*, 1984, 259, 10967-10972.