Supporting Information

Interaction between heme and tau-derived R1 peptides: Binding and oxidative reactivity

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1. Synthesis of R1τ and AcR1τ peptides

The peptides R1τ and AcR1τ were synthesized using the standard fluorenyl methoxycarbonyl solid-phase method (Fmoc) in dimethylformamide (DMF). Rink-amide resin was used as solid support, which yielded the peptide amidated at the C-terminus. After deprotection of the resin with 20 mL of 20 % (v:v) piperidine in DMF, the first amino acid (3 mol equiv. *vs.* resin sites, estimated to be 0.64 mmol/g of resin), was added in the presence of 3 equiv. of *N*-hydroxybenzotriazole, 3 equiv. of benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate and 6 equiv. of *N*,*N*-diisopropylethylamine. After 45 min, the same coupling procedure was repeated.
After recoupling of each amino acid, a capping step was performed by using 20 mL of 4.7 % acetic anhydride and 4 % of pyridine in DMF. Deprotection of the Fmoc group was performed by treating twice the resin, for 3 min and 7 min, respectively, with 15 mL of 20 % piperidine in DMF.
To obtain AcR1τ, acetylation of Val^{256} was made through the capping step procedure.
At the end of the synthesis, the protections of the amino acid side chains were removed with a solution of 95 % trifluoroacetic acid (TFA, 25 mL for 1 g of resin), triisopropyl silane (2.5 %) and water (2.5 %), which serves also to release the peptide from the resin.
After stirring for 3 h, the solution was concentrated under vacuum and cold diethyl ether was added to precipitate the peptide. The mixture was filtered and the precipitate washed with cold diethyl ether; then, it was dissolved in water and purified by HPLC, using a 0-100 % linear gradient of 0.1 % TFA in water to 0.1 % TFA in CH_3CN over 30 min (flow rate of 3 mL/min, loop 2 mL), as eluent. The products were then lyophilized (yield ~80%) and characterized by ESI-MS.

ESI-MS data (direct injection, MeOH, positive-ion mode, capillary temperature 200 °C): *m/z* 1836 ($R1\tau H^+$), 919 ($R1\tau H_2^{2+}$), 613 ($R1\tau H_3^{3+}$) a.m.u.; 1879 ($AcR1\tau H^+$), 940 ($AcR1\tau H_2^{2+}$), 627 ($AcR1\tau H_3^{3+}$) a.m.u.

2. Binding experiments

Following recommended procedures,[1S] the optical cells used for solutions of hemin, hemin-H, and hemin-GH prepared for spectral titrations were cleaned by washing them with 1 M NaOH and then extensively with water; the cells were subsequently washed with 1 M HNO_3 and with water, and then they were air-dried. The titration experiments were performed as described in the Experimental section, using thermostated cells at 25 °C. All the spectra were corrected for dilution effects.

(a) Titration of hemin with AcR1τ. Figure 1S shows the decrease in intensity of the Soret band upon ligand binding of hemin (4 μM) with AcR1τ.

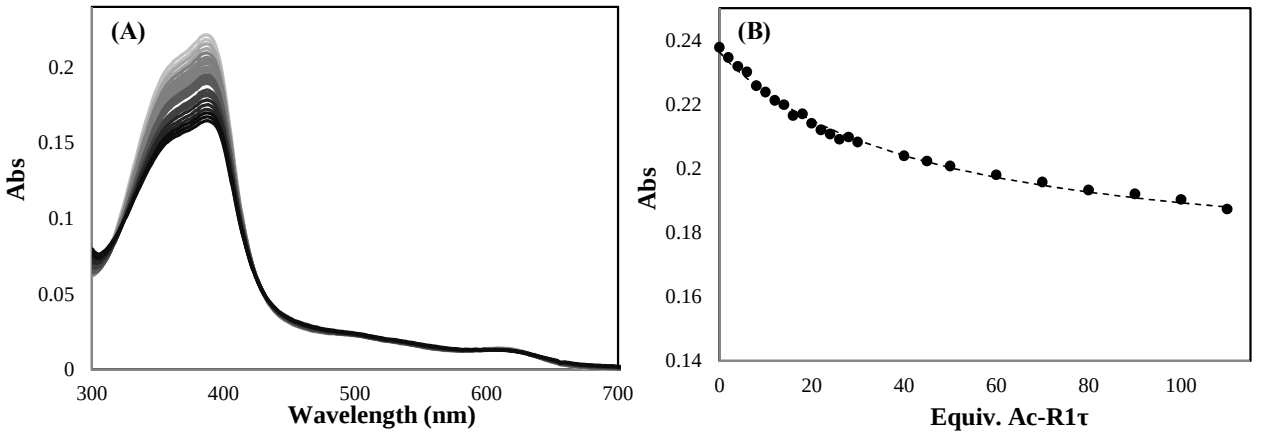

Figure 1S. (A) UV-Vis spectrophotometric titration in 20 mM phosphate buffer solution, pH 7.4, of hemin (4 μM) with AcR1τ (from 0 to 110 equiv.) using an optical cell of 1-cm path length; (B) Fitting of the absorbance maximum at 390 nm *vs.* peptide equiv. added to determine the binding constants. Spectra were corrected for dilution.

(b) Stoichiometry of hemin-R1τ complexes. Figure 1B, in the text, shows the fitting of the titration data of hemin with R1τ, processed with the Hyperquad package.[2S] The data fitted equally well assuming the formation of a 1:1 complex between R1τ and monomeric or dimeric hemin, yielding complexes [hemin(R1τ)] and [$hemin_2$(R1τ)], respectively; the two traces displayed in Figure 1S are actually superimposed. In contrast, when formation of the 2:2 species [$hemin_2(R1\tau)_2$] was assumed, the fitting of the observed data was poor, as shown in the same figure.

(c) Titration of hemin-GH with imidazole. Figure 2S shows the optical changes occurring upon titration of hemin-GH with imidazole.

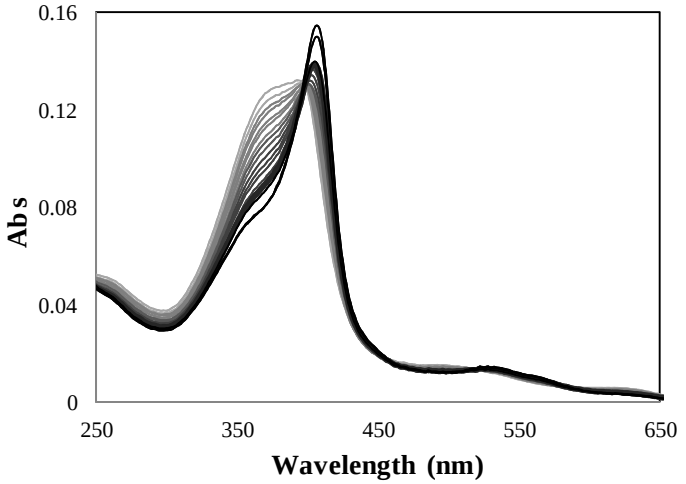

Figure 2S. UV-Vis spectrophotometric titration of hemin-GH (4 μM) with imidazole, from 0 to 200 equiv., in an optical cell of 1 cm path length, in 20 mM phosphate buffer solution, pH 7.4.

(d) Titration of [hemin(R1τ)] with imidazole. Figure 3S shows the optical changes occurring upon titration of [hemin(R1τ)] with imidazole.

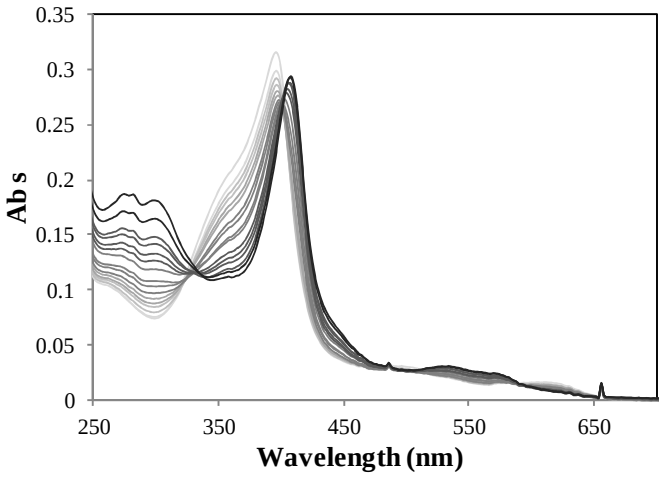

Figure 3S. UV-Vis spectrophotometric titration of [hemin(R1τ)] complex (obtained by mixing 0.4 μM hemin and 20 μM R1τ) with imidazole, from 0 to 4×10^4 equiv. (16 mM), in 20 mM phosphate buffer solution, pH 7.4, using an optical cell of 10 cm path length.

(e) Formation of [hemin-GH(R1τ)] and [hemin-GH(Aβ16)] complexes. Figure 4S and 5S show the optical changes occurring upon titration of hemin-GH with R1τ and Aβ16, respectively.

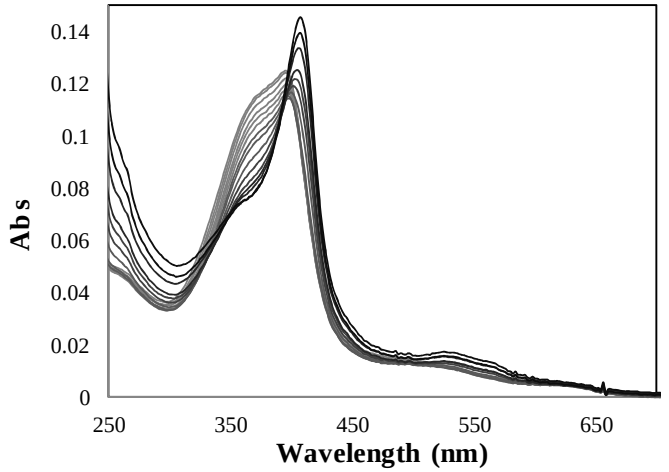

Figure 4S. UV-Vis spectrophotometric titration of hemin-GH (4 μM) with R1τ, from 0 to 220 equiv., in an optical cell of 1 cm path length, performed in 20 mM phosphate buffer solution, pH 7.4.

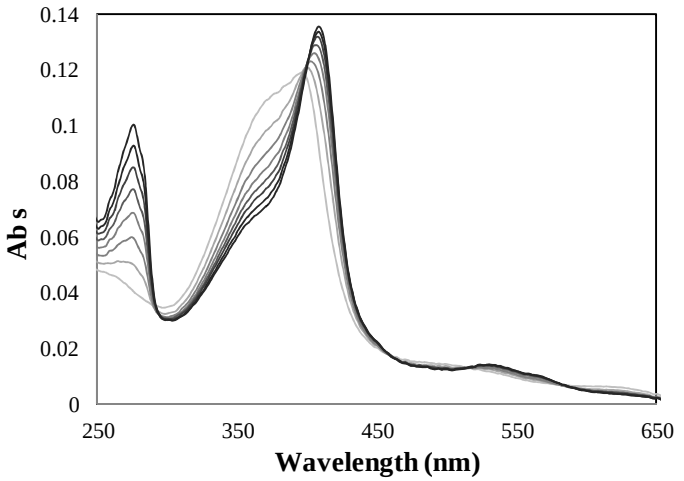

Figure 5S. UV-Vis spectrophotometric titration of hemin-GH (4 µM) with Aβ16, from 0 to 14 equiv. (56 µM), in an optical cell of 1 cm path length, performed in 20 mM phosphate buffer solution, pH 7.4.

(f) Titration of hemin with R1τ*. Figure 6S shows the decrease in intensity of the Soret band at 390 nm upon addition of R1τ* to hemin (4 µM). In spite of the amino acid substitution, the formation of the six-coordinated bis(imidazole) adduct of hemin requires a large excess of peptide (1.3×10^3 equiv.).

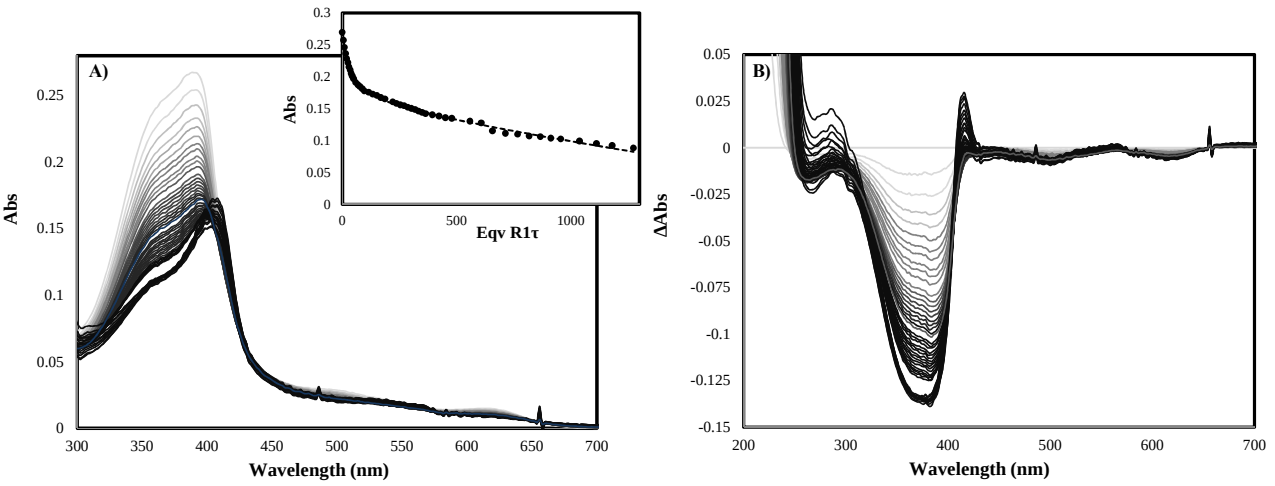

Figure 6S. UV-Vis spectrophotometric titrations in 20 mM phosphate buffer solution, pH 7.4, of hemin (4 µM) with (A) R1τ* (from 0 to 1300 equiv.) in a cell of 1 cm path length, with plot of absorbance maxima at 390 nm *vs.* equiv. of R1τ* added; (B) difference spectra for the previous experiment. Spectra were corrected for dilution.

3. Kinetic experiments

Plots corresponding to the determination of the rate dependence on the concentration of hydrogen peroxide (Figure 7S), at fixed substrate (ABTS) concentration, and the rate dependence on substrate concentration (Figure 8S), at saturating H_2O_2 concentration, for the oxidation of ABTS by hydrogen peroxide catalyzed by the hemin and hemin-peptide complexes.

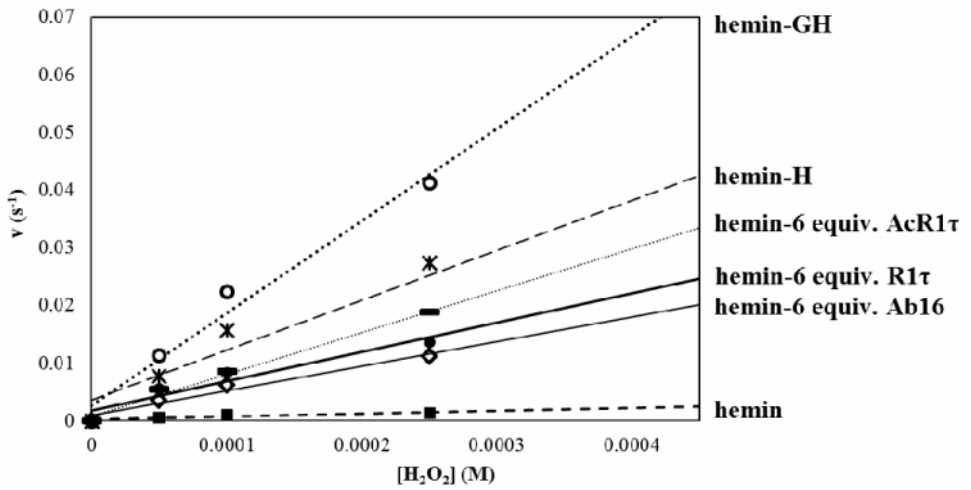

Figure 7S. Determination of the H_2O_2 concentration dependence of the reaction rate. Concentration of the hemin catalysts was 3 μM in all cases (hemin, hemin-H, hemin-GH, hemin-R1τ, hemin-AcR1τ and hemin-Aβ16). The hemin-peptide complexes were generated by adding 18 μM of R1τ, AcR1τ or Aβ16 to the hemin solution prior to starting the experiments. The concentration of ABTS was maintained fixed at 1 mM. The experiments were carried out in phosphate buffer 20 mM at pH 7.4 at 25 °C. The reaction rates (in s^{-1}) were obtained by dividing the initial rates for the catalyst concentration, the optical path (1 cm), the extinction coefficient of the product $ABTS^{\cdot+}$ (ε_{660} = 14700 M^{-1} cm^{-1}) and a factor 2 because one cycle corresponds to two $ABTS^{\cdot+}$ formed.[3S]

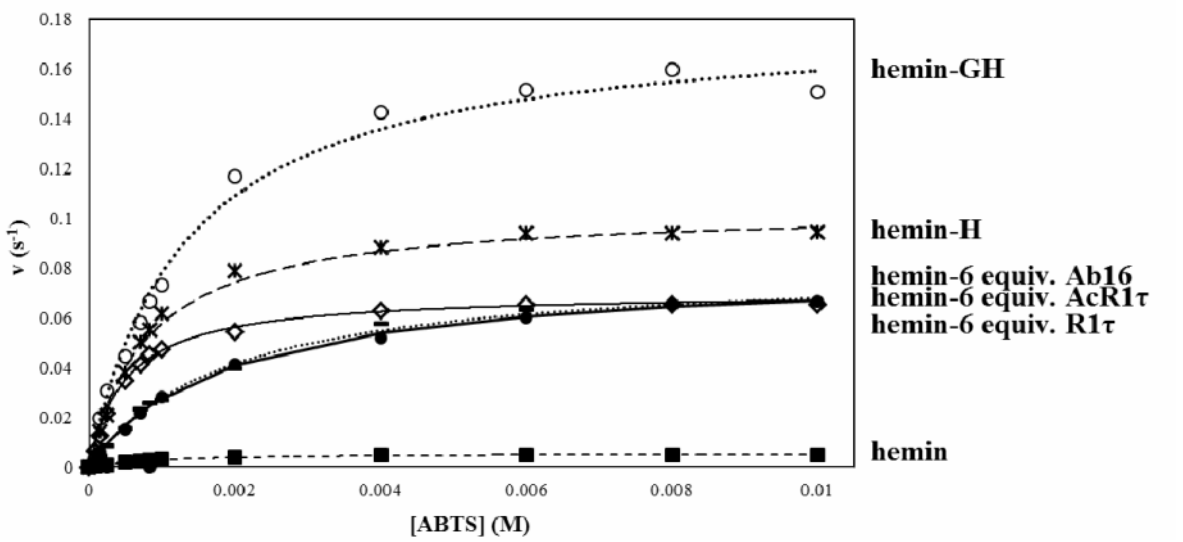

Figure 8S. Determination of the substrate concentration dependence of the reaction rate. Plots of the initial rates *vs.* ABTS concentration for $ABTS^{\cdot+}$ formation catalyzed by the various hemin complexes (3 μM). The catalysts are: hemin-GH, hemin-H, hemin-Aβ16, hemin-AcR1τ, hemin-

R1τ, and free hemin. The hemin-peptide complexes were obtained by adding six equiv. of Aβ16, AcR1τ or R1τ to the hemin solution as above. The concentration of H_2O_2 was saturating in all cases (4 mM for free hemin, 1 mM for hemin-H and hemin-GH, and 14 mM for hemin-peptide complexes). The experiments were carried out at 25 °C in 20 mM phosphate buffer, pH 7.4. The reaction rates were determined as above.

References

1S. C. Asher, K. A. De Villiers, T. J.; Egan, *Inorg. Chem.* **2009**, *48*, 7994-8003.
2S. P. Gans, A. Sabatini, A. Vacca, *Talanta* **1996**, *43*, 1739-1753.
3S. P. A. Adams, *J. Chem. Soc. Perkin Trans* **1990**, *2*, 1407-1414.