Electronic Supplementary Material (ESI)

A Cationic Copolymer as a Cocatalyst for a Peroxidase-Mimicking Heme-DNAzyme

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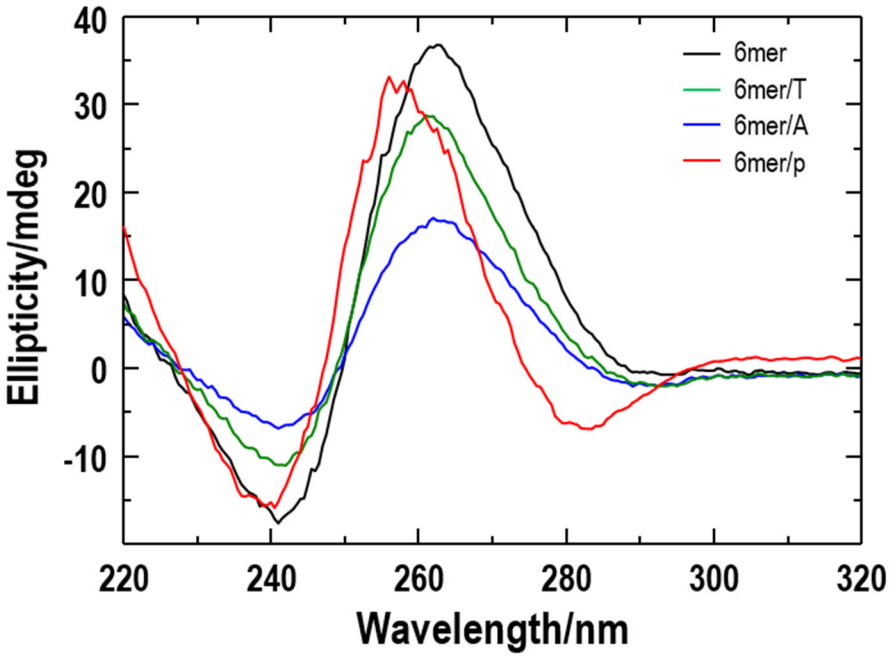

Fig. S1. CD spectra of parallel-stranded tetrameric G-quadruplex DNAs, 6mer (black), 6mer/T (green), and 6mer/A (blue), formed from 5.0 μM d(TTAGGG), d(TTAGGG/T), and d(TTAGGGA), respectively, and that, 6mer/p (red), formed from 10 μM d(TTAGGGp) in 50 mM potassium phosphate buffer, pH 6.80, and 50 mM KCl at 25 °C. The negative and positive Cotton effects at ~240 and ~260 nm, respectively, are CD spectral signatures for formation of parallel-stranded G-quadruplex DNA (R. Del Villar-Guerra, R. D. Gray, J. B. Chaires *Curr. Protoc. Nucleic Acid Chem.*, 2017, **2**, 1-17).

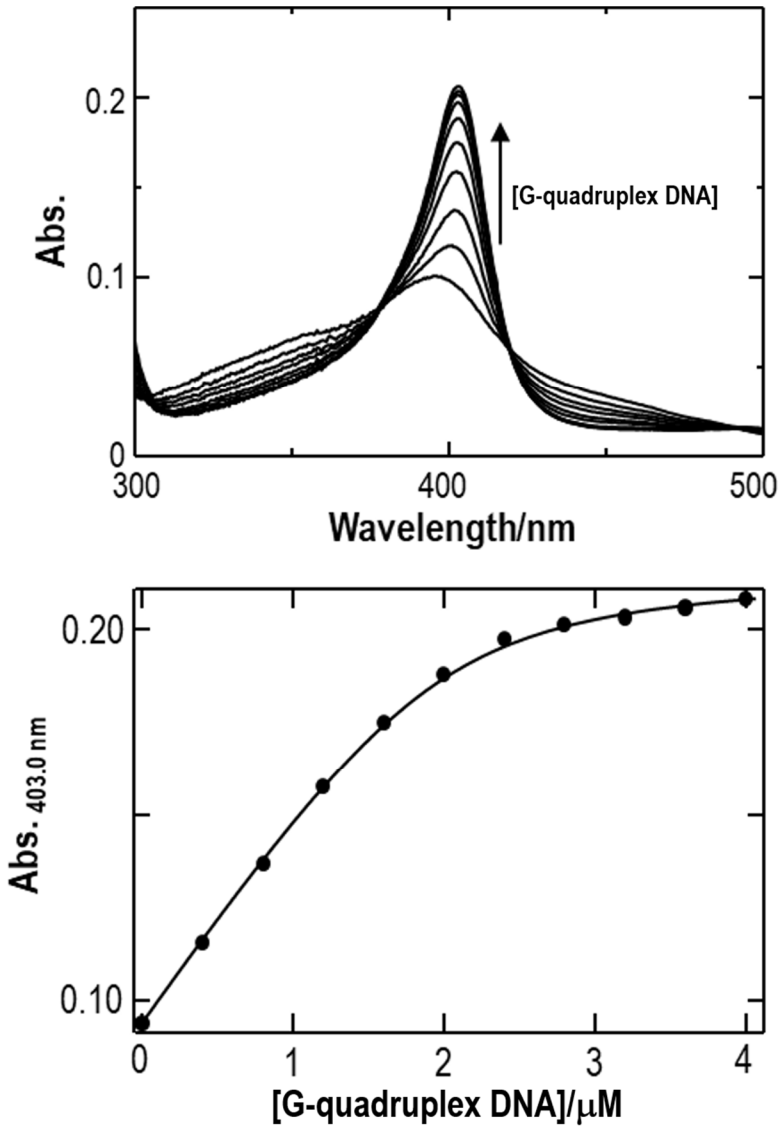

Fig. S2 Soret absorption, 300-500 nm, of a parallel-stranded tetrameric G-quadruplex DNA (6mer), 0 – 6 μM, titrated against 2.0 μM heme(Fe^{3+}) in 300 mM KCl and 50 mM potassium phosphate buffer, pH 6.80, together with 0.08 w/v% Triton X-100 and 0.5 v/v% dimethyl sulfoxide, at 25 °C (top). A heme binding constant (K_a) of 6.5 ± 0.7 μM^{-1} was obtained for the heme(Fe^{3+})-6mer complex (bottom).

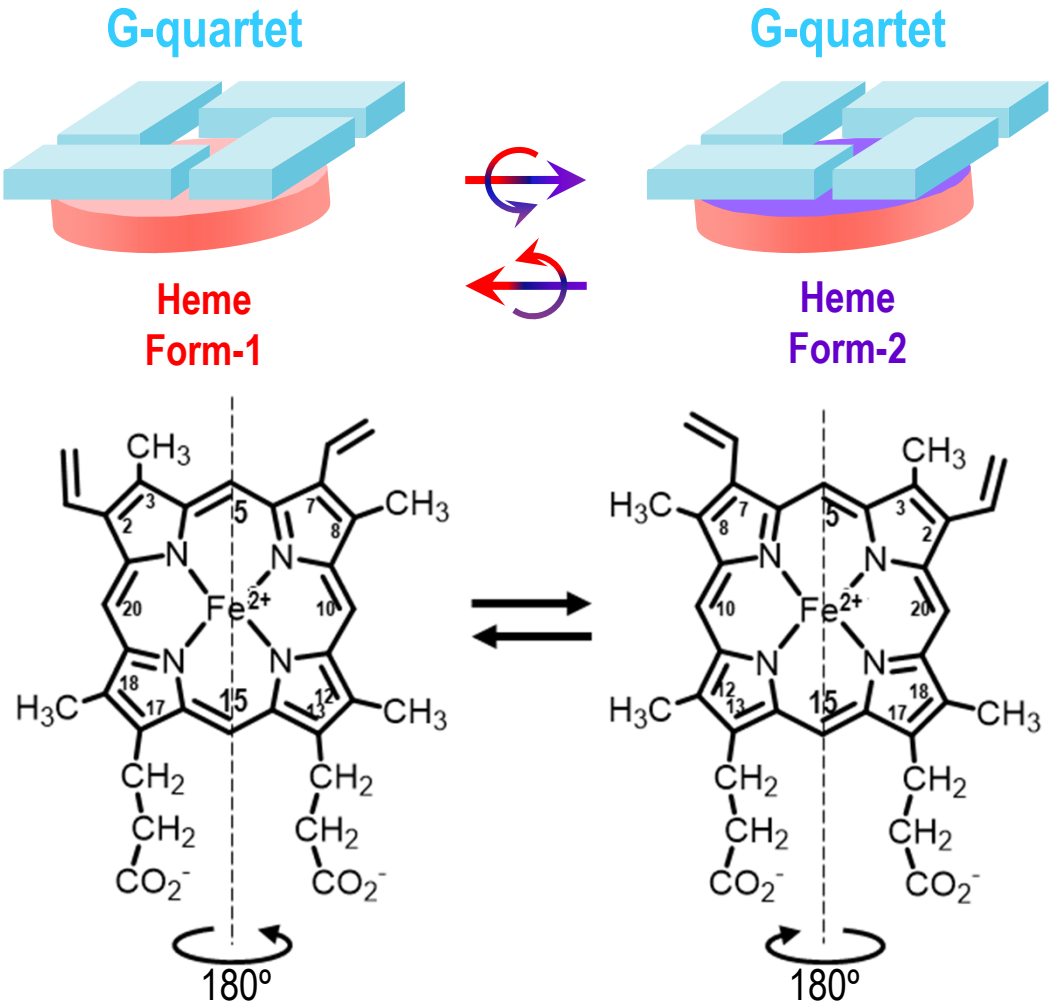

Figure S3. Two different orientations of heme(Fe^{2+}) with respect to a G-quartet. These orientations can be interconverted through 180º rotation of heme(Fe^{2+}) around the *pseudo*-C_2 axis passing through the *meso* 5- and 15-H hydrogens.

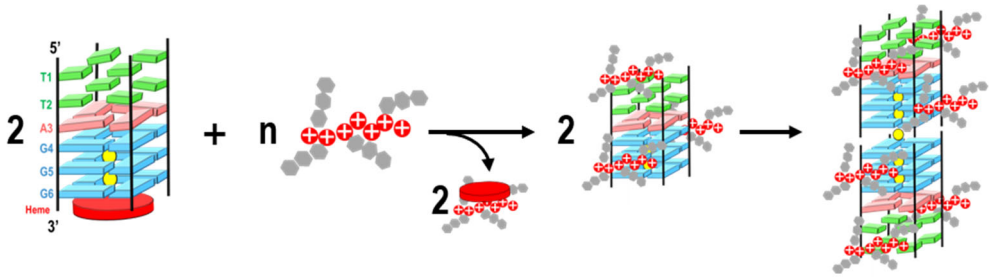

Fig. S4 Schematic representation of dimerization of the 6mer produced through the detachment of heme from the heme-6mer complex due to the polymer. The dimerization occurs through end-to-end stacking of the 3'-terminal G-quartets (Y. Kato, T. Ohyama, H. Mita, Y. Yamamoto, *J. Am. Chem. Soc.* 2005, **127**, 9980−9981).

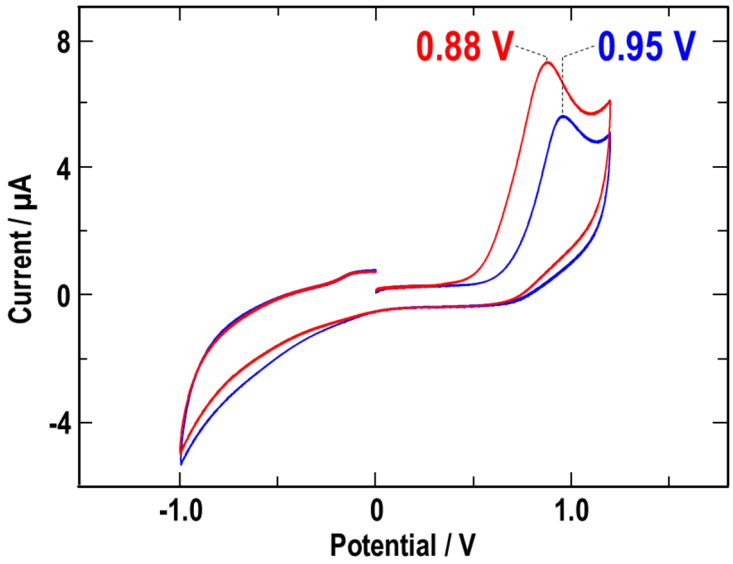

Fig. S5. Cyclic voltammograms of 50 mM Amplex Red aqueous solutions containing 50 mM potassium phosphate buffer, pH 6.80, in the absence (bule) and presence of PLL-*g*-Dex at *N/P* = 3.6 (red), at room temperature. The oxidation potentials of 0.95 and 0.88 V (vs. SCE) were obtained for Amplex Red in the absence and presence of the polymer, respectively. The measurements were made using a BAS ALS-619EZ electrochemical analyzer with a boron-doped diamond electrode as a working electrode, a platinum wire as a counter electrode, and saturated calomel electrode (SCE) as a reference electrode.

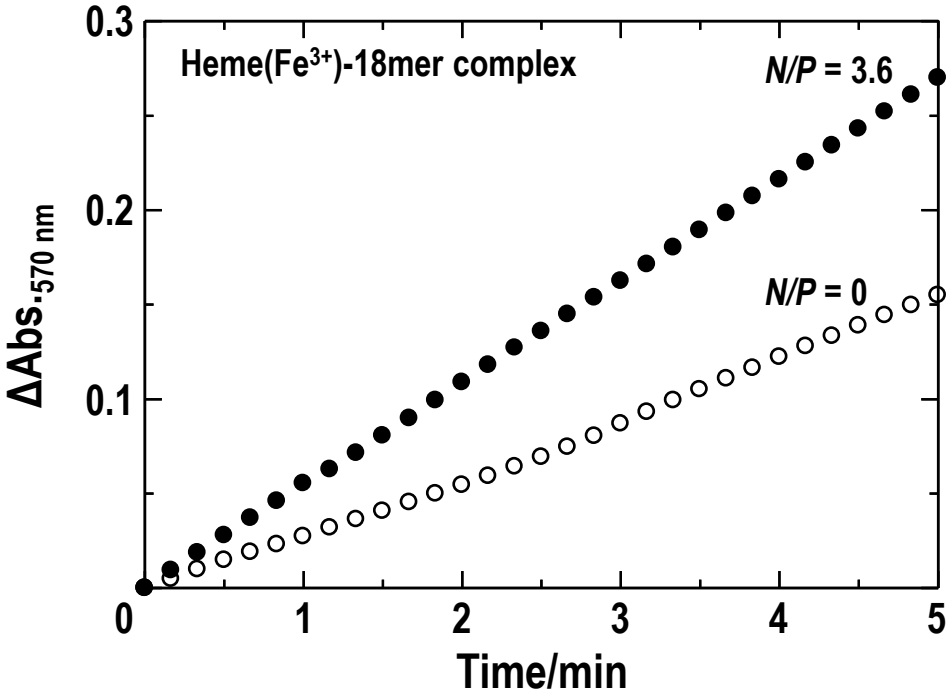

Fig. S6. Time-evolution of 570-nm absorbance due to Resorufin produced on the oxidation of Amplex Red by a complex between heme(Fe^{3+}) and an all parallel-stranded monomeric G-quadruplex DNA of non-standard base inosine(I)-containing sequence d(TAGGGTGGGTTGGGTGIG) (18mer), i.e., the heme(Fe^{3+})-18mer complex in the absence (○) and presence of PLL-*g*-Dex at *N*/*P* = 3.6 (●). All samples contained 0.5 μM heme(Fe^{3+}), 20 μM DNA, 50 μM Amplex Red, and 200 μM H_2O_2 in 50 mM K^+ phosphate buffer, pH 6.80, at 25 ºC. Initial slopes (R_0) of 0.57 ± 0.06 and 1.00 ± 0.10 μM/min were obtained for the complex in the absence and presence of the polymer, respectively.

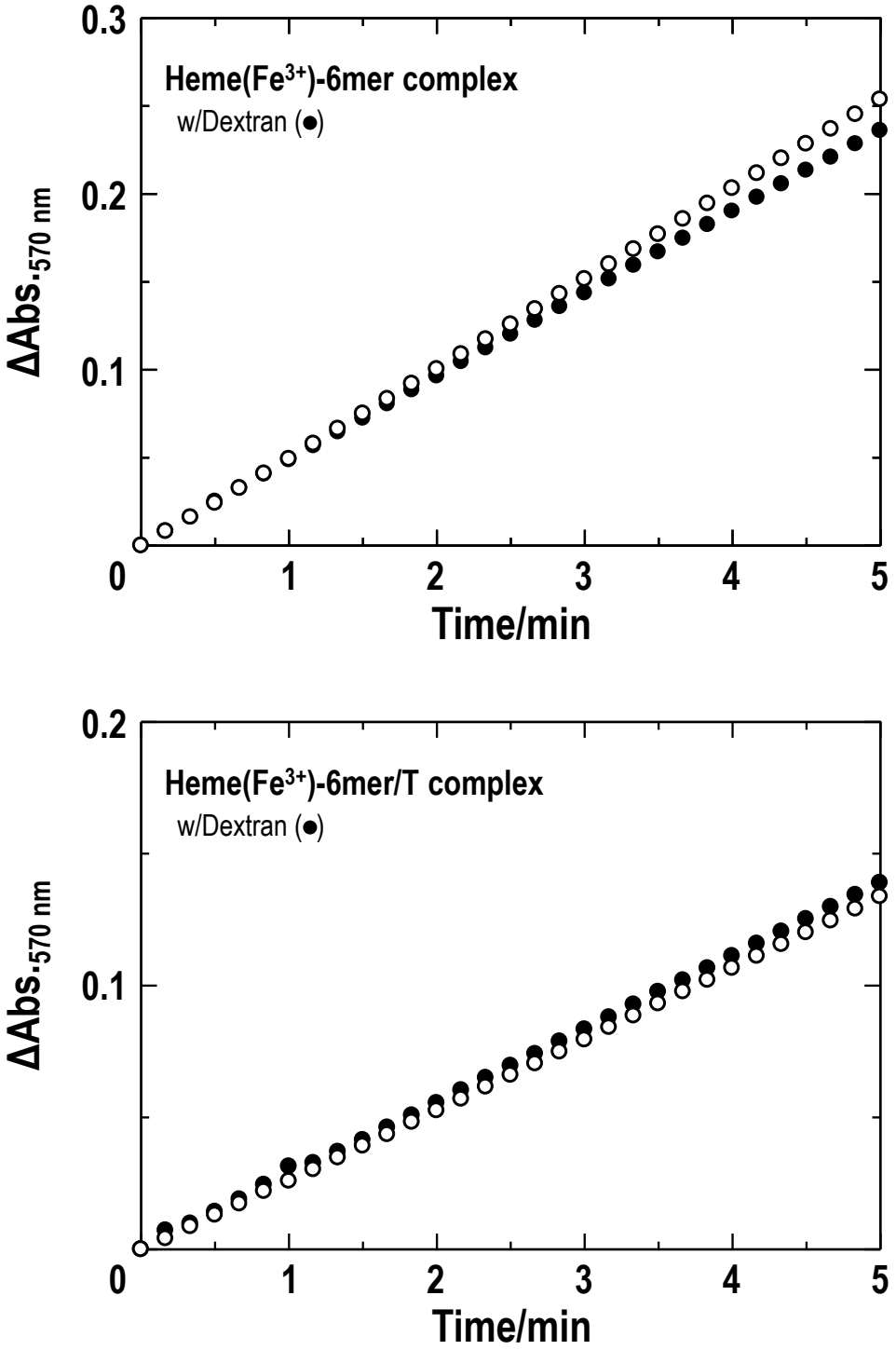

Fig. S7. Time-evolution of 570-nm absorbance due to Resorufin produced on the oxidation of Amplex Red by the heme(Fe^{3+})-6mer (top) and heme(Fe^{3+})-6mer/T complexes (bottom) in the absence (○) and presence of the dextran used to prepare PLL-*g*-Dex (●). All samples contained 0.5 μM heme(Fe^{3+}), 20 μM DNA, 50 μM Amplex Red, and 200 μM H_2O_2 in 50 mM K^+ phosphate buffer, pH 6.80, at 25 ºC. The dextran in amount equal to the weight of the dextran moiety of PLL-g-Dex at N/P = 3.6 was added to the reaction mixture. Initial slopes (R_0) of 0.94 ± 0.09 and 0.88 ± 0.09 μM/min were obtained for the heme(Fe^{3+})-6mer complex in the absence and presence of the dextran, respectively, and those of 0.50 ± 0.05 and 0.51 ± 0.05 μM/min for the heme(Fe^{3+})-6mer/T one in the absence and presence of the dextran, respectively.

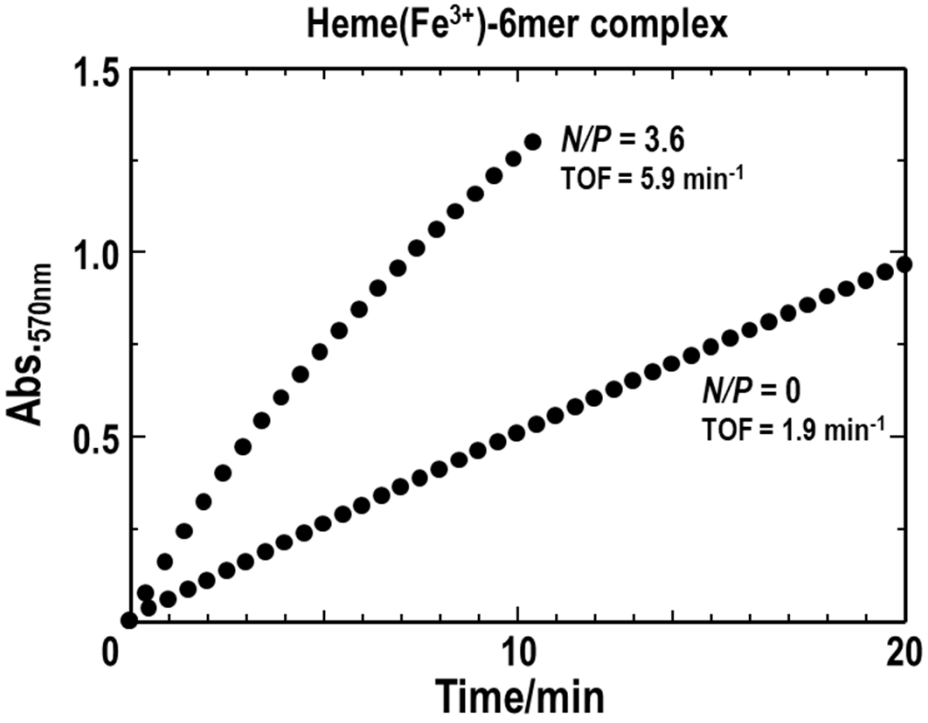

Fig. S8. Time-evolution of 570-nm absorbance due to Resorufin (ε_{570} = 5.4 × 10^4 M^{-1} cm^{-1}) produced on the oxidation of Amplex Red by the heme(Fe^{3+})-6mer complex in the absence (top) and presence of PLL-g-Dex at *N/P* = 3.6 (bottom). All samples contained 0.5 μM heme(Fe^{3+}), 20 μM DNA, 50 μM Amplex Red, and 200 μM H_2O_2 in 50 mM K^+ phosphate buffer, pH 6.80, at 25 ºC. The turnover frequencies (TOFs) of 1.9 and 5.9 min^{-1} were obtained for the complex in the absence and presence of the polymer, respectively.

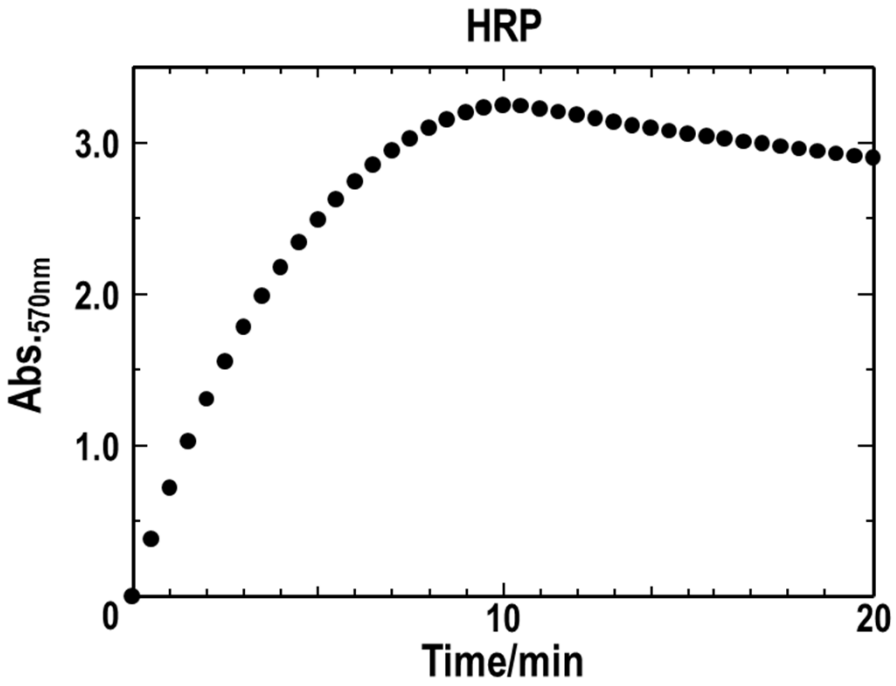

Fig. S9. Time-evolution of 570-nm absorbance due to Resorufin (ε_{570} = 5.4 × 10^4 M^{-1} cm^{-1}) produced on the oxidation of Amplex Red by HRP. The reaction mixture contained 1 nM HRP, 50 μM Amplex Red, and 200 μM H_2O_2 in 50 mM K^+ phosphate buffer, pH 6.80, at 25 ºC. The turnover frequency (TOF) of 1.1 × 10^4 min^{-1} was obtained.