Electronic Supplemental Information

Oxidation of an indole substrate by porphyrin iron(III) superoxide: Relevance to indoleamine and tryptophan 2,3-dioxygenases

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Materials. All chemicals were purchased from commercial sources and used without further purification unless otherwise stated. Reactions involving inert atmospheres were performed under an Ar atmosphere using standard Schlenk techniques or in an N_2-filled dry box. Toluene and THF were purified via a Pure-Solv solvent purification system from Innovative Technologies, Inc. Anhydrous *N*,*N*-dimethylformamide (DMF) was purchased from Sigma Aldrich. Deuterated solvents for NMR were purchased from Cambridge Isotopes, Inc. DMF was distilled from CaH_2, while THF and toluene were distilled from Na/benzophenone, then degassed by repeated cycles of freeze-pump-thaw and stored over 4 Å molecular sieves in an N_2-filled drybox prior to use. The synthesis of Fe^{II}(TPP) (TPP = *meso*-tetraphenylporphyrinato^{2-}) followed literature procedures,[1] and was crystallized from either pentane/toluene or THF/heptane mixtures to give Fe^{II}(TPP) as violet block crystals. A typical synthesis was performed on 170 mg of $TPPH_2$, giving 41 mg of crystalline product (22%). The compounds indole, 2-methylindole, 3-methylindole, and 2,3-dimethylindole were purchased from Sigma Aldrich, recrystallized twice in petroleum ether and dried under vacuum prior to use. The product N-(2-acetyl-phenyl)-acetamide (98% purity) was purchased from AA Blocks LLC.

Instrumentation. UV-vis spectra were collected with a Varian Cary 50 Bio Spectrophotometer coupled to a Unisoku USP-203 Cryostat or a Hewlett-Packard Agilent 8453 diode-array spectrophotometer with an air-free quartz cuvette (600 μL, path length = 0.2 cm). ^{1}H NMR spectra were recorded on a Bruker Advance 400 or 300 MHz FT-NMR spectrometer.

Generation of $Fe^{III}(O_2^{\bullet-})$(TPP) and decay to $(Fe^{III}(TPP))_2(\mu\text{-}O)$.

a) UV-vis spectroscopy. Under inert atmosphere, crystalline Fe^{II}(TPP) (0.38 μmol) was dissolved in DMF (600 μL, 0.6 mM), to give a violet stock solution. An aliquot of this solution was transferred to a custom-made Schlenk cuvette and loaded into the UV-vis cryostat. The solution was cooled to -40 °C (λ_{max} nm ($\varepsilon \times 10^4$ M^{-1} cm^{-1}): 538 (7.8), 563 (10.4), 606 (5.1)), and bubbled with excess O_2 for approximately 5 s, at which point the solution turned bright red. Disappearance of the peaks at 538, 563 and 606 nm for Fe^{II}(TPP) occurred concomitant with the appearance of a new spectrum (λ_{max} nm ($\varepsilon \times 10^4$ M^{-1} cm^{-1}): 545 (13.6), 580(sh) (5.3)) indicative of formation of

$Fe^{III}(O_2^{\bullet-})(TPP)$. Slow decay of the spectrum for $Fe^{III}(O_2^{\bullet-})(TPP)$ over 120 min led to a color change to green-brown and a new spectrum (λ_{max} nm ($\varepsilon \times 10^4$ M^{-1} cm^{-1}): 569 (9.1), 609 (4.9)) which can be assigned to the final $(Fe^{III}(TPP))_2(\mu\text{-}O)$ product.

b) ^{1}H NMR spectroscopy. Under inert atmosphere, crystalline $Fe^{II}(TPP)$ (1.9 μmol) was dissolved in DMF-d_7 (500 μL, 3.8 mM) to give a violet solution which was analyzed by ^{1}H NMR at -40 °C (400 MHz): 8.02, 8.29, 8.98, 51.1 ppm. The NMR sample was removed from the spectrometer and kept at -40 °C by immersion in a $CH_3CN/N_{2(l)}$ bath. The solution was bubbled with excess O_2 for approximately 5 s, at which point the solution turned bright red, indicative of formation of $Fe^{III}(O_2^{\bullet-})(TPP)$. The sample was returned to the spectrometer and analysis by ^{1}H NMR at -40 °C showed loss of the peaks for $Fe^{II}(TPP)$, and the appearance of a new spectrum with peaks at 9.76 and 9.06 ppm, assigned to $Fe^{III}(O_2^{\bullet-})(TPP)$. Warming of the solution to 25 °C led to a color change to green-brown, and the ^{1}H NMR spectrum for $Fe^{III}(O_2^{\bullet-})(TPP)$ was converted to a new spectrum with a peak at 13.7 ppm, assigned to $(Fe^{III}(TPP))_2(\mu\text{-}O)$.

Reaction of $Fe^{III}(O_2^{\bullet-})(TPP)$ with 2,3-dimethylindole (2,3-DMI) at -40 °C.

a) UV-Vis spectroscopy. A solution of $Fe^{II}(TPP)$ (0.6 mM) in DMF in a Schlenk cuvette, prepared under inert atmosphere, was cooled to -40 °C in the UV-vis cryostat. An amount of O_2 (~2 mL) was added via gas tight syringe, generating $Fe^{III}(O_2^{\bullet-})(TPP)$ as seen by UV-vis. An aliquot of a stock solution of 2,3-DMI (73 equiv, 50 μL) was added, and the reaction was monitored by UV-vis spectroscopy every 30 s, until no significant changes in the spectra were observed.

b) ^{1}H NMR spectroscopy. A solution of $Fe^{II}(TPP)$ (1.9 μmol) and 2,3-DMI (10.0 mg, 69 μmol) in DMF-d_7 (500 μL) was cooled to -40 °C in a $CH_3CN/N_{2(l)}$ bath. An excess amount of O_2 was bubbled through the solution and a color change of violet to bright red was observed. The solution was transferred quickly to the NMR spectrometer, which was precooled to -40 °C, and the reaction was monitored by ^{1}H NMR over time for 2 h. A peak at 8.80 ppm appeared and was assigned to the C3-H phenyl proton of *N*-(2-acetyl-phenyl)-acetamide by comparison with an independent sample. An internal standard, 1,3,5-trimethoxybenzene (6.0 μmol), was added for quantitation of the product by integration of the peak at 8.80 ppm and comparison to the peak at 6.34 ppm for the standard. The yield of *N*-(2-acetyl-phenyl)-acetamide was 49% ± 8% (average of 3 trials). **Control**

reactions. A control reaction of 2,3-DMI (10 mg) plus excess O_2 in DMF-d_7 at -40 °C showed only peaks for the starting material 2,3-DMI after 2 h. Another control reaction was prepared by mixing $(Fe^{III}(TPP))_2(\mu\text{-}O)$ (1.9 μmol) and 2,3-DMI (10.0 mg, 69 μmol) in the presence of excess O_2 in DMF-d_7 at -40 °C for 2 h, and the resulting ^1H NMR spectrum did not show any formation of *N*-(2-acetyl-phenyl)-acetamide.

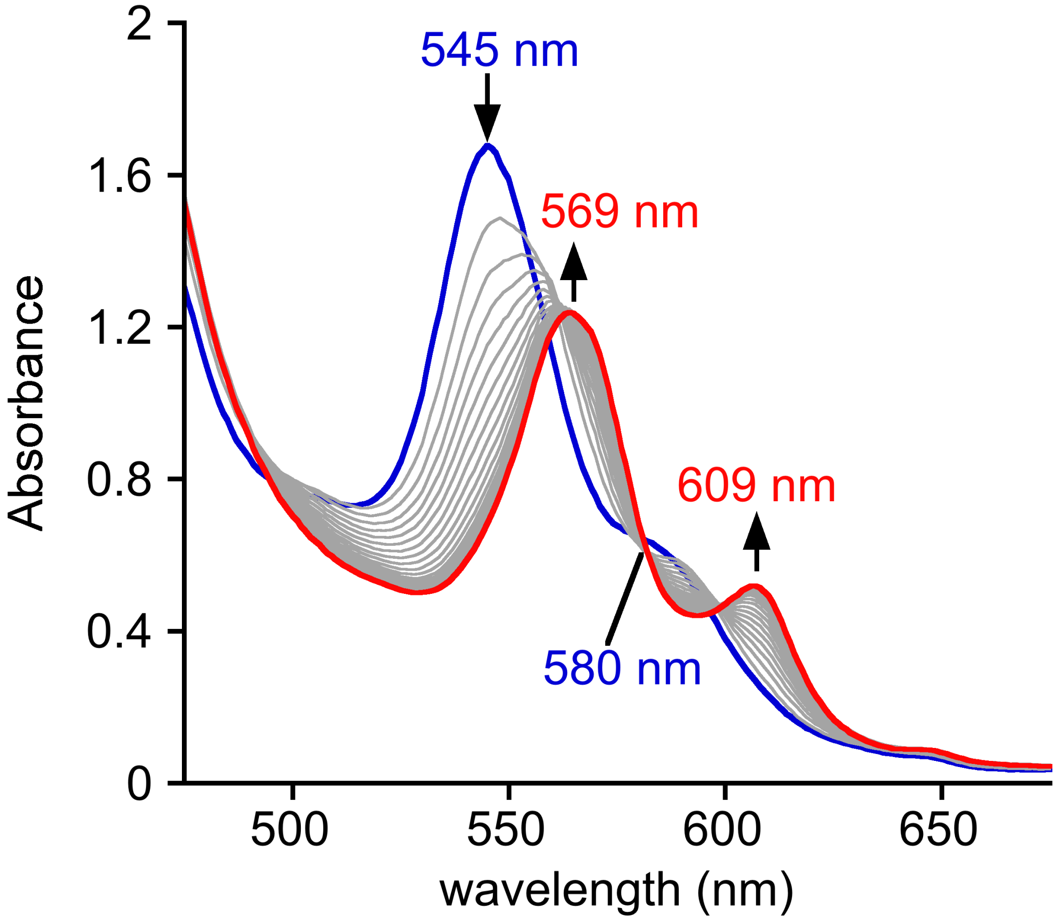

Figure S1. Time-resolved UV-vis spectral changes (0 – 120 min) observed for the self-decay of $Fe^{III}(O_2^{\bullet -})(TPP)$ (0.6 mM) to form $(Fe^{III}(TPP))_2(\mu\text{-}O)$ in DMF at -40 °C.

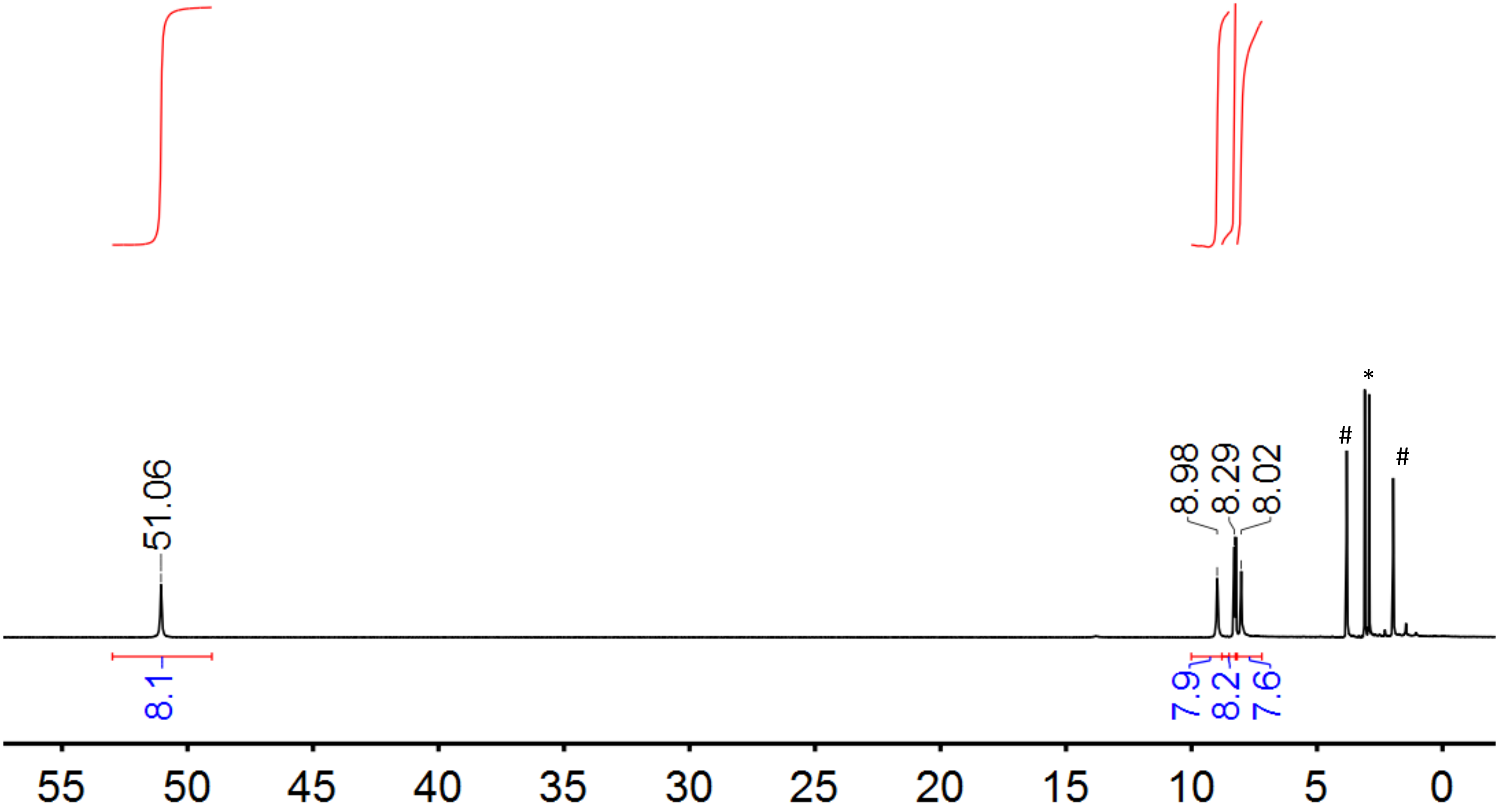

Figure S2. ^{1}H NMR spectrum (400 MHz) of Fe^{II}(TPP) (4 mM) in DMF-d_7 at 25 °C; * = DMF, # = THF.

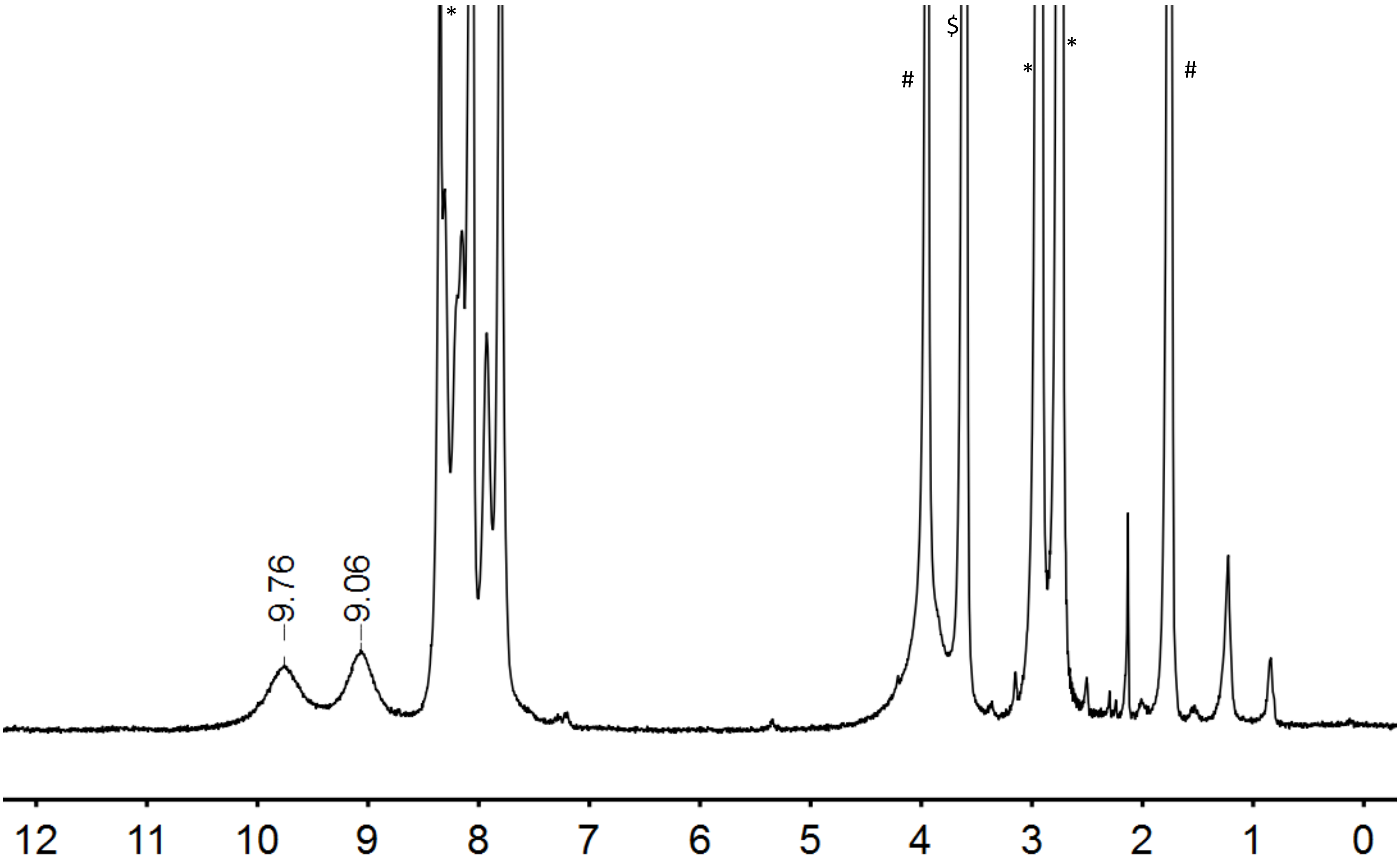

Figure S3. ^{1}H NMR spectrum (400 MHz) of $Fe^{III}(O_2^{\bullet-})(TPP)$ (4 mM) in DMF-d_7 at -40 °C; * = DMF, # = THF, $ = H_2O.

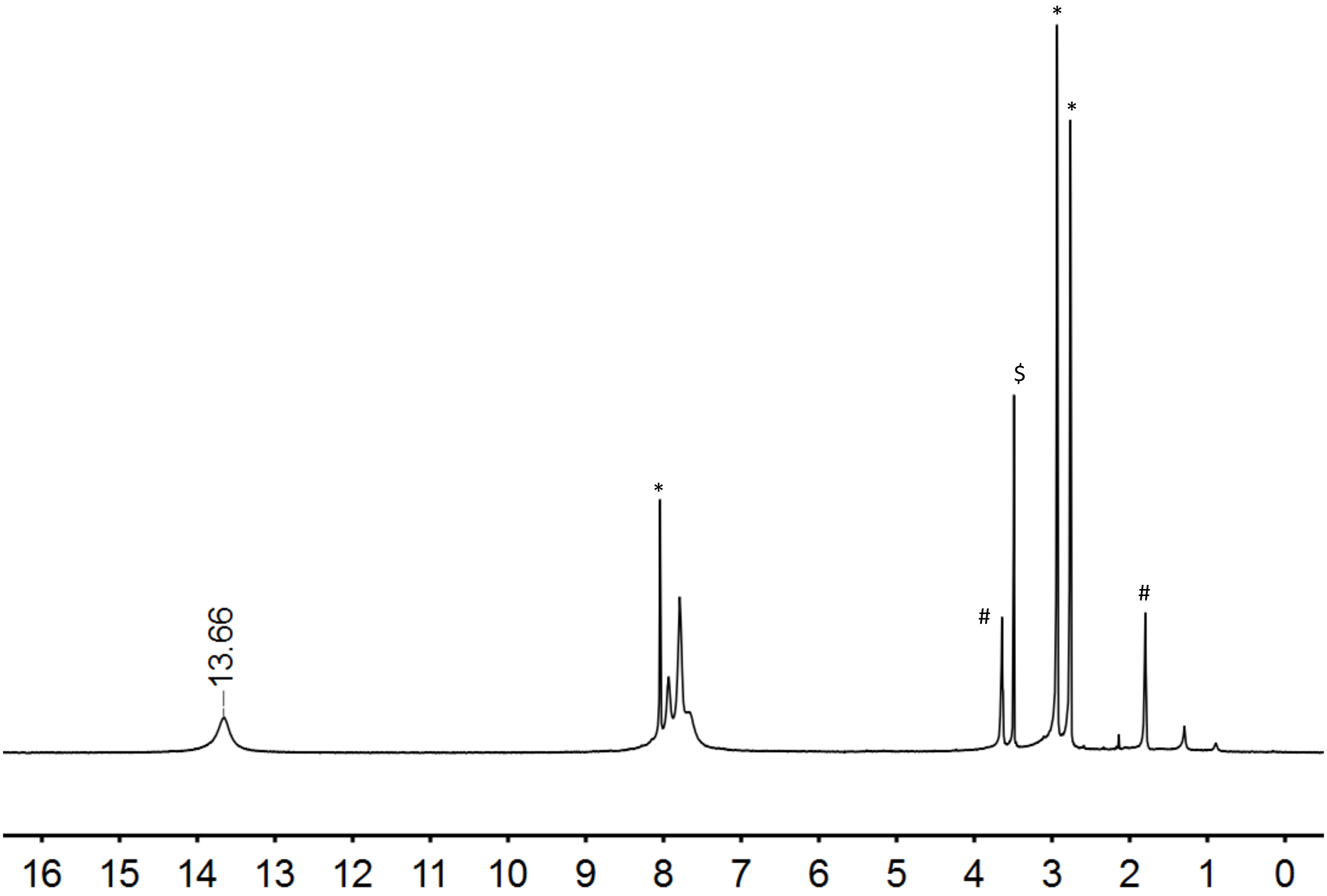

Figure S4. ^{1}H NMR spectrum (400 MHz) of $(Fe^{III}(TPP))_2(\mu\text{-}O)$ (4 mM) in DMF-d_7 at 25 °C; * = DMF, # = THF, $ = H_2O.

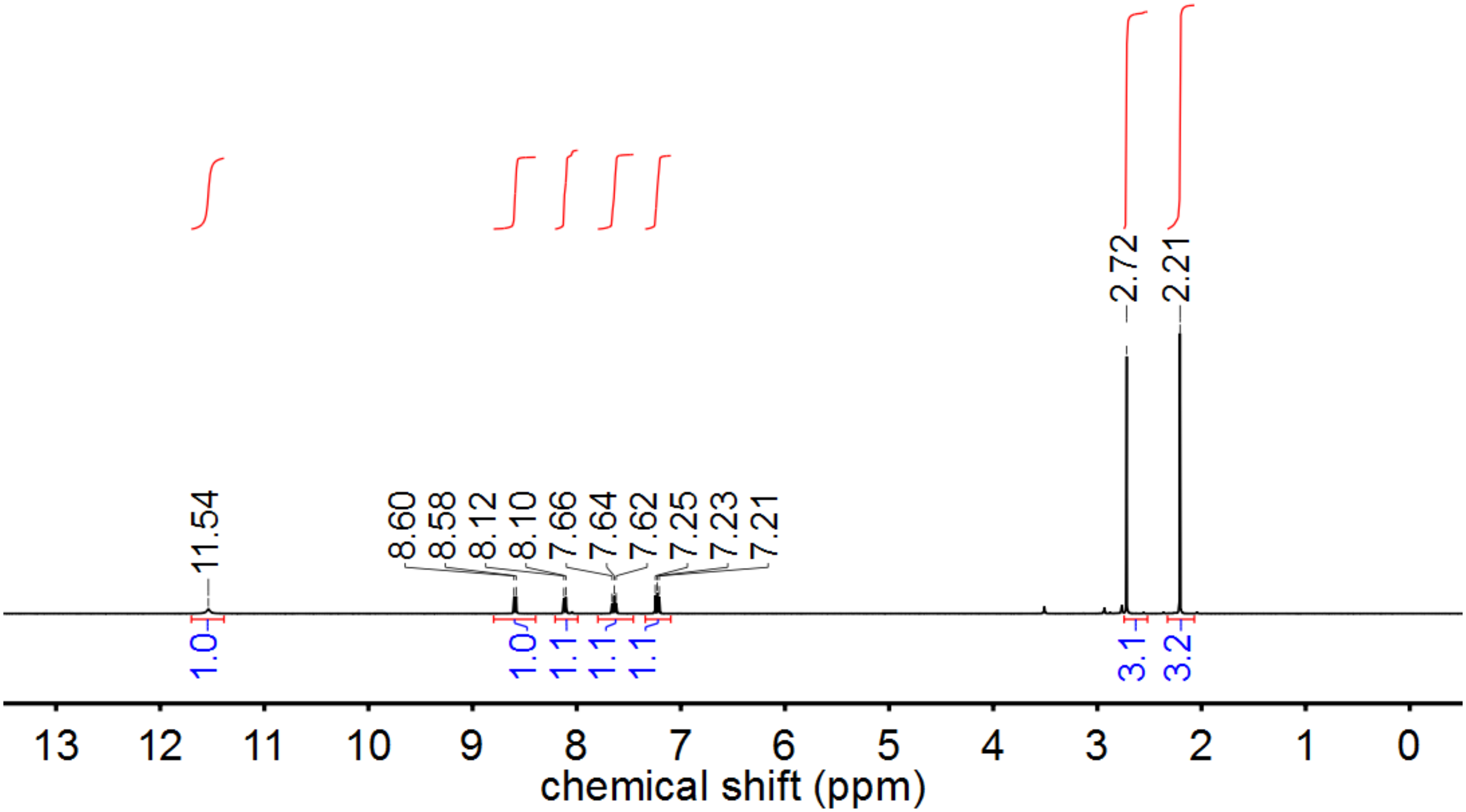

Figure S5. ^{1}H NMR spectrum (400 MHz) of *N*-(2-acetyl-phenyl)-acetamide (4 mM) in DMF-d_7 at 25 °C: δ (ppm) 2.72 (s, 3H), 2.21 (s, 3H), 7.23 (t, 1H), 7.64 (t, 1H), 8.11 (d, 1H), 8.59 (d, 1H), 11.54 (s, 1H).

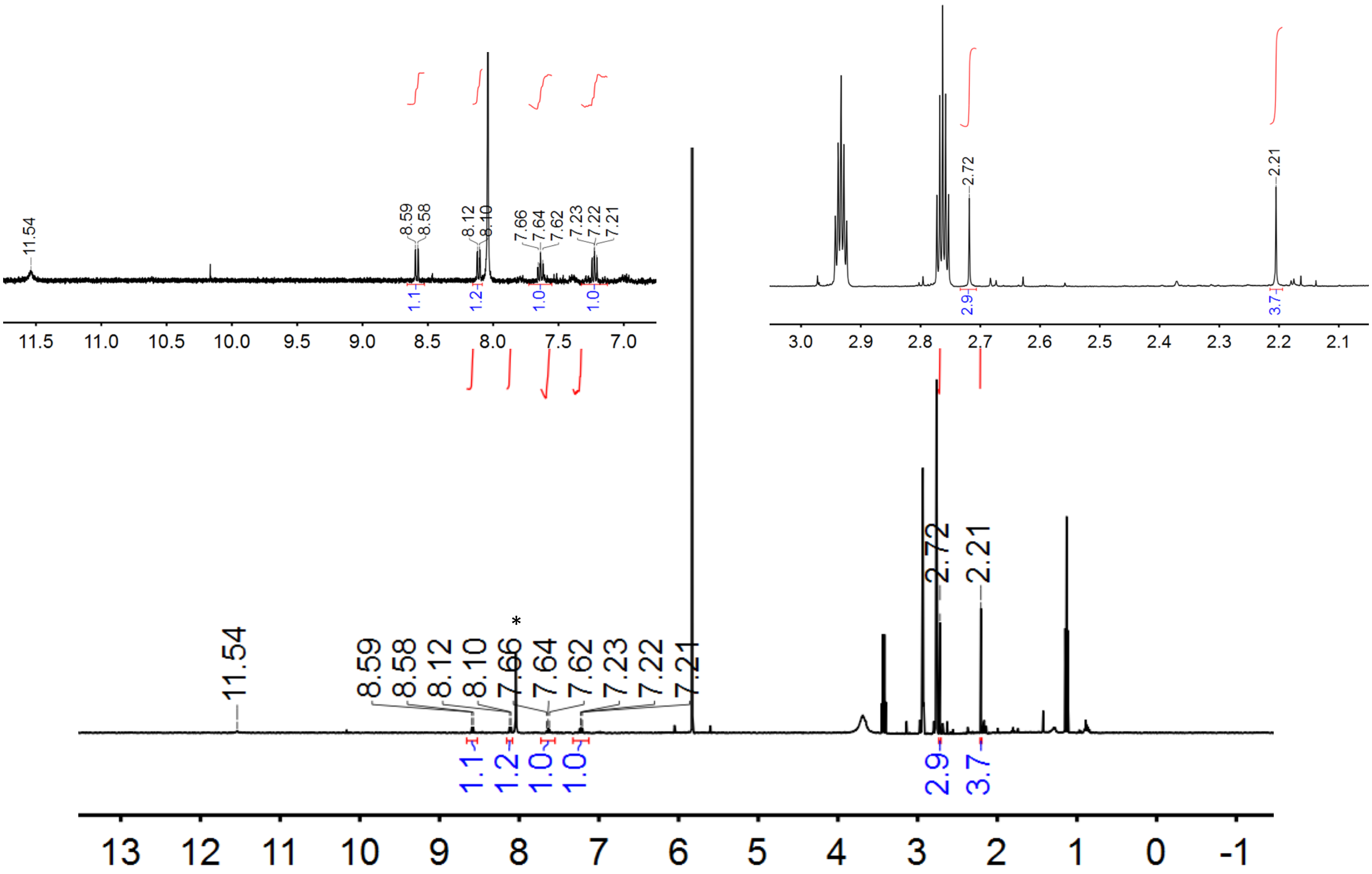

Figure S6. ^{1}H NMR spectrum (400 MHz) of the product *N*-(2-acetyl-phenyl)-acetamide, isolated from a reaction mixture of Fe^{III}(O$_2^{\bullet-}$)(TPP) and 2,3-DMI in DMF-d_7.

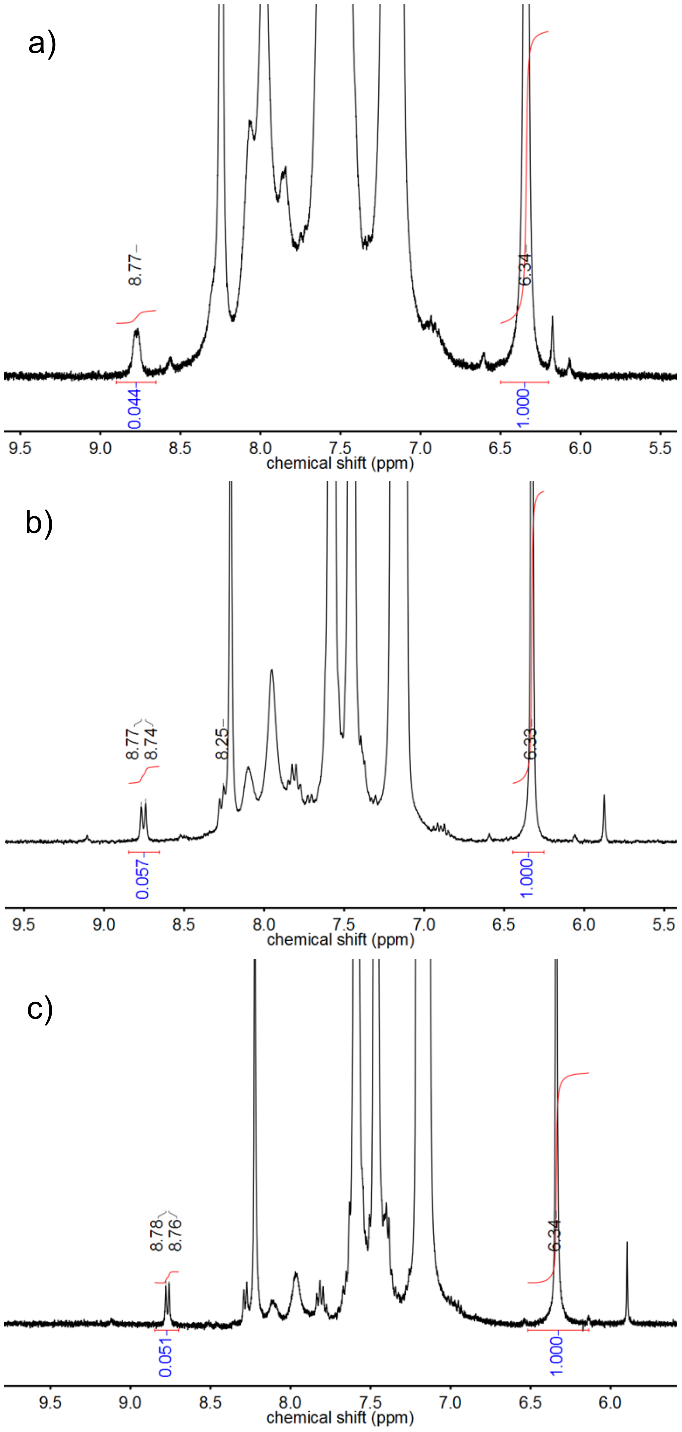

Figure S7. ^{1}H NMR spectra for the reaction of $Fe^{III}(O_2^{\bullet -})(TPP)$ (3.8 mM in 500 μL) with 2,3-DMI (36 equiv) with the internal standard 1,3,5-trimethoxybenzene (6.0 μmol) a) run at -40 °C, b) upon warm-up of the reaction mixture from (a) to 25 °C, and c) run at 25 °C. The peak corresponding to the phenyl proton of *N*-(2-acetyl-phenyl)-acetamide, at 8.80 ppm (d, 1H) was integrated versus the phenyl proton of 1,3,5-trimethoxybenzene at 6.34 ppm (s, 3H). Average yield for *N*-(2-acetyl-phenyl)-acetamide was 49 ± 8% (3 trials at -40 °C) and 61 ± 9% (3 trials following thermal equilibration to 25 °C), as computed using the formula below.

$$\textbf{\% yield } \textbf{\textit{N}-(2-acetyl-phenyl)-acetamide} = \frac{n_{std} \times \frac{I_{pdt}}{I_{std}} \times \frac{3}{1}}{n_{Fe^{II}}} \times 100\%$$

1. M. L. Pegis, D. J. Martin, C. F. Wise, A. C. Brezny, S. I. Johnson, L. E. Johnson, N. Kumar, S. Raugei and J. M. Mayer, *J. Am. Chem. Soc.*, 2019, **141**, 8315-8326.