Supporting Information

The ferric-superoxo intermediate of the TxtE nitration pathway resists reduction, facilitating its reaction with nitric oxide

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KEYWORDS

Nitric oxide, metalloenzyme, nitration, natural product, Streptomyces.

Supplementary information

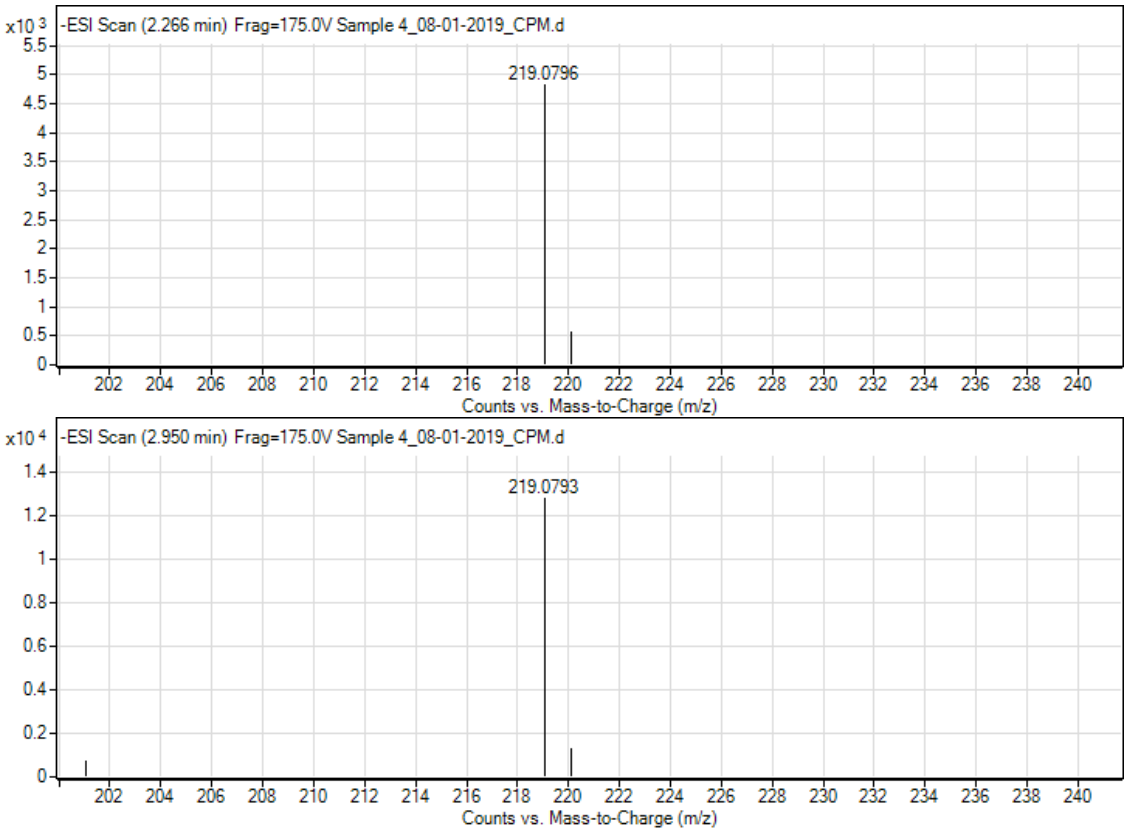

Figure S1. MS spectra of peaks eluted at 2.3 and 3.0 minutes resulting from LC separation of TB14 peroxide shunt sample shown in cyan trace of **Fig. 2** in the main text.

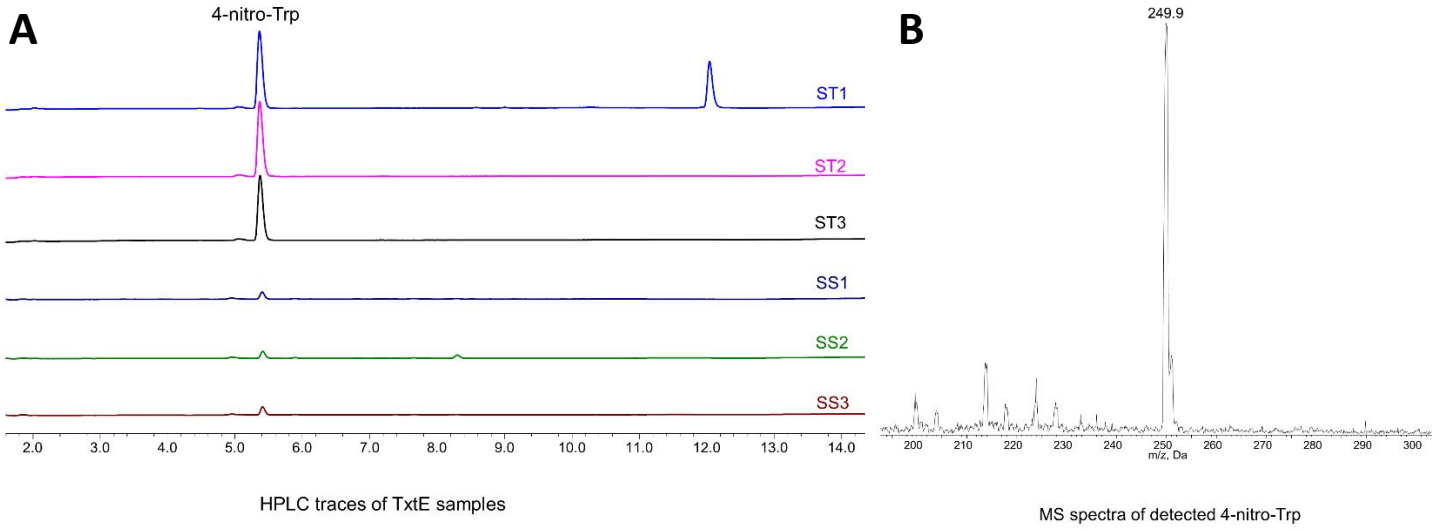

Figure S2. A) HPLC traces of single turnover TxtE (ST) and steady state TB14 (SS) replicates monitoring 380-nm absorbance of 4-NO_2- Trp. **B)** MS spectrum of 5.5 min elution peak. Reaction conditions: 100 µM Fe^{II} TxtE, 500 µM Trp, 0.86 mM PROLI-NONOate in pH 8.0 buffer (ST); 0.5 µM TB14, 500 µM Trp, 2 mM NADPH, and 1.33 mM DEA-NONOate (~2 mM NO) in pH 8.0 buffer (SS).

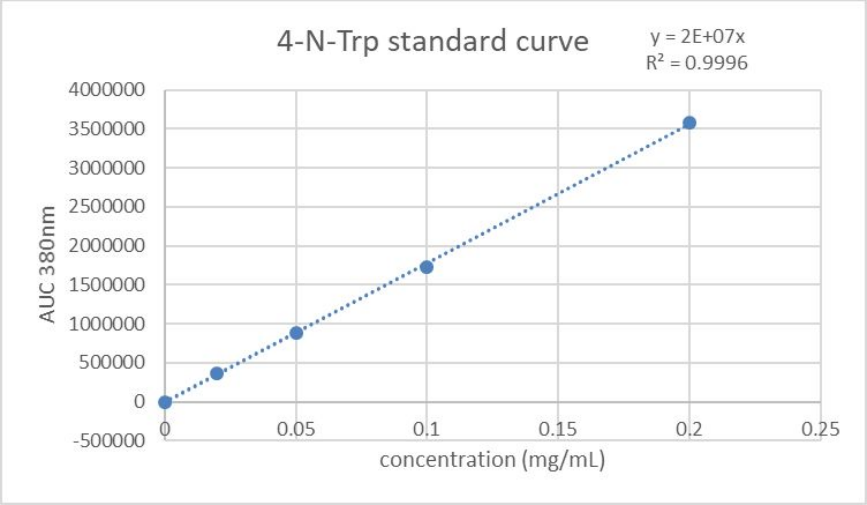

Figure S3. Calibration curve for 4-NO_2-Trp standard. AUC = area-under-curve.

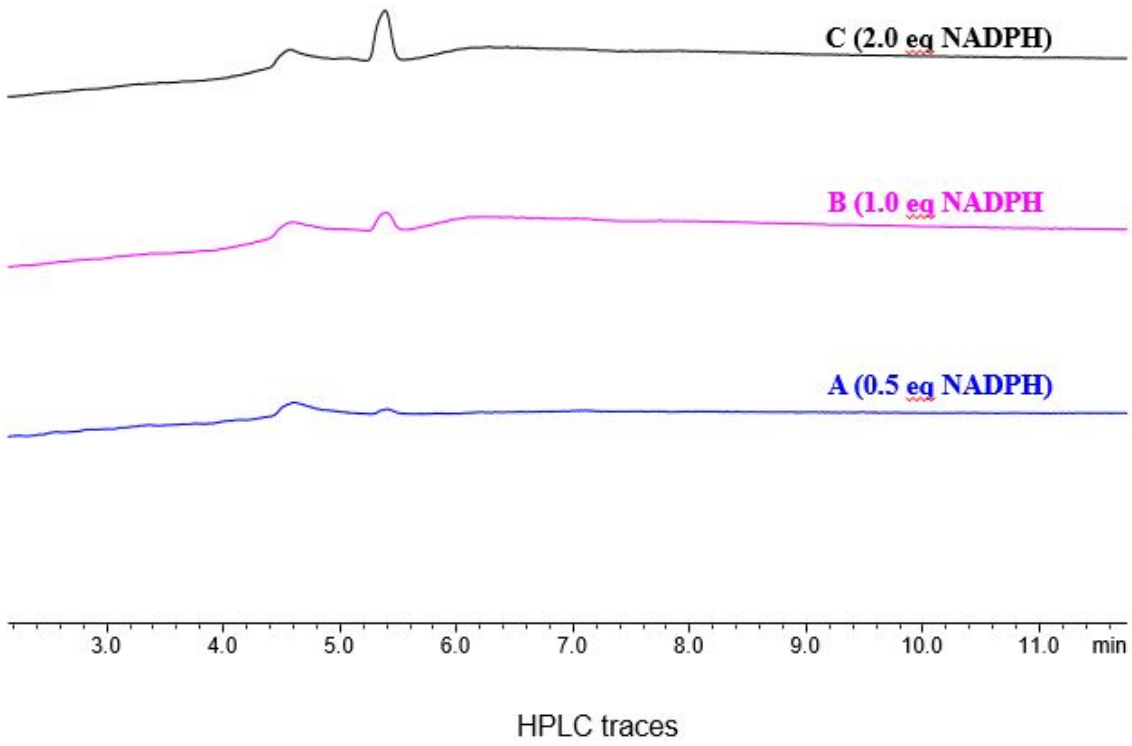

Figure S4. Representative HPLC traces of single nitration turnover TB14 samples containing 0.5 eq (A), 1.0 eq (B), or 2.0 eq NADPH monitoring 380-nm absorbance of 4-NO_2-Trp. Reaction conditions: 20 µM TB14, 0.5 mM Trp, and 1 mM PROLI-NONOate (~2 mM NO equivalent) with 10 µM (A), 20 µM (B), or 40 µM (C) NADPH.

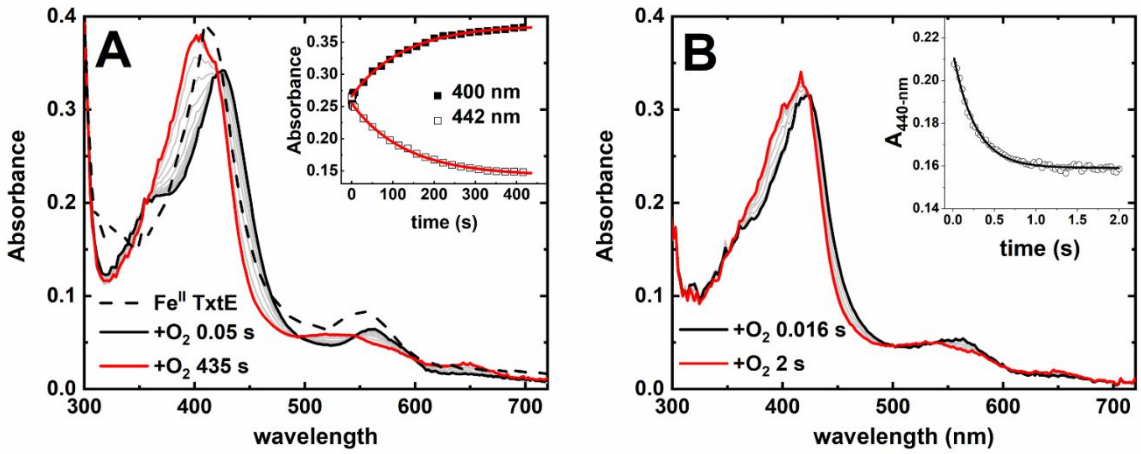

Figure S5. Stopped-flow spectral time courses of anaerobic Fe^{II} TxtE mixed with O_2 in the presence (A) and absence (B) of Trp at pH 8.0. Dashed trace obtained by mixing Fe^{II} TxtE solution against deoxygenated buffer. Solid black and red traces are the first and last collected spectra in the time courses, respectively. Gray traces

were collected at intermediate times. Inset: single-exponent fits to representative A) A_{400} and $A_{442\text{-}nm}$ or B) $A_{440\text{-}nm}$ traces. Final conditions: A) 5 μM Fe^{II} TxtE, 250 μM Trp, 130 μM O_2 and B) 5 μM Fe^{II} TxtE, 130 μM O_2. All solutions were in 100 mM Tris, pH 8.0 and performed at 20 ˚C in a 1 cm path length cuvette.

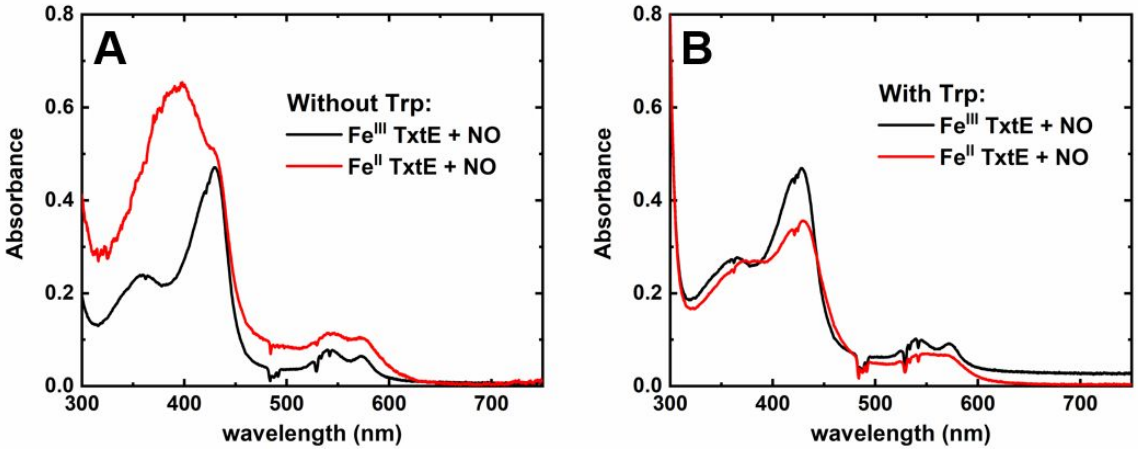

Figure S6. UV-vis absorption spectra of TxtE $\{FeNO\}^6$ (black) and $\{FeNO\}^7$ (red) species in the absence (Panel A) or presence (Panel B) of Trp. The red trace in Panel A was obtained by reducing TxtE with dithionite and then addition of NO (*g*) whereas all other spectra were obtained by introduction of 1 mM PROLI NONOate (~2 mM NO equivalents). Where relevant, tryptophan was present at 1 mM concentration and all spectra were recorded in 100 mM Tris (pH 8.0). TxtE concentration for all spectra except the Panel A red trace (20 μM) was 5 μM.

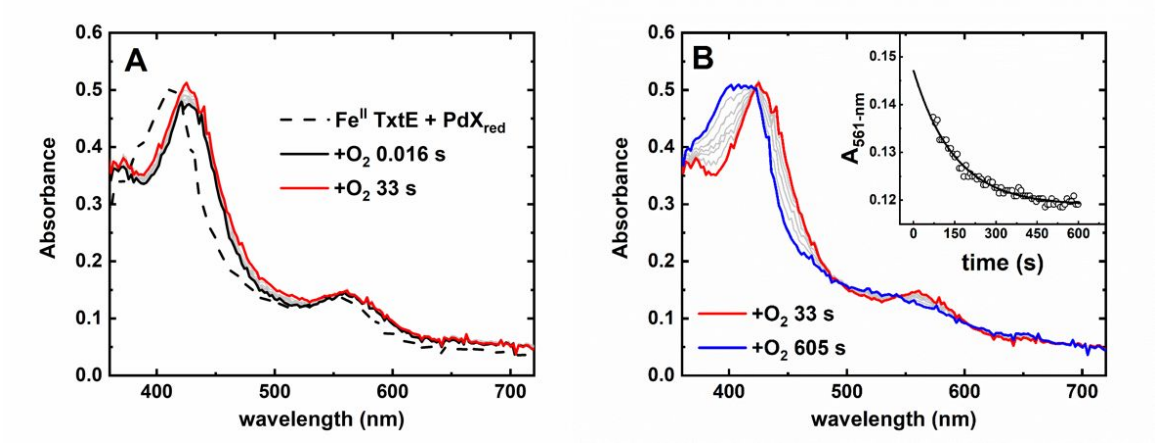

Figure S7. Stopped-flow spectral time courses of anaerobic Fe^{II} TxtE and PdX_{red} mixed with O_2 in the presence of excess Trp at pH 8.0 and 21 ˚C. Dashed trace obtained by mixing the anaerobic solution against deoxygenated buffer. Solid black, red, or blue traces were collected at times indicated in the figure legends. Gray traces were collected at intermediate times. Inset: single-exponent fits to representative $A_{561\text{-nm}}$ traces. Conditions after mixing: 15 μM Fe^{II} TxtE, 15 μM PdX_{red}, 250 μM Trp, 130 μM O_2. All solutions were in 100 mM Tris, pH 8.0 and measured at 20 ˚C in a 1 cm path length cuvette.

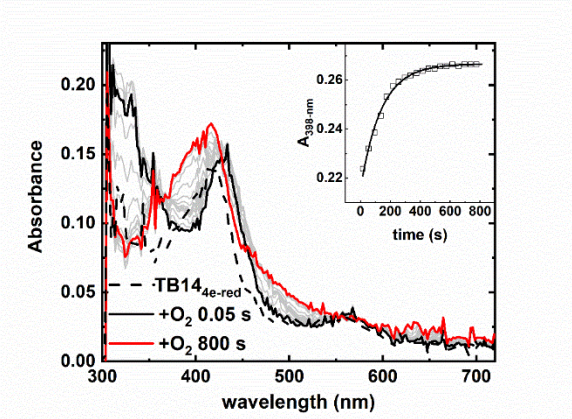

Figure S8. Stopped-flow spectral time courses of anaerobic $Tb14_{4e\text{-red}}$ mixed with O_2 at pH 8.0. Dashed trace obtained by mixing Fe^{II} TxtE solution against deoxygenated buffer. Solid black and red traces are the first and

last collected spectra in the time courses, respectively. Gray traces were collected at intermediate times. Inset: Representative single-wavelength traces extracted low light intensity time courses and fit with single-exponent functions. Final conditions: A) 5 μM TB14, 250 μM Trp, 10 μM NADPH, and 130 μM O_2 All solutions were in 100 mM Tris, pH 8.0 and performed at 20 ˚C in a 1 cm path length cuvette.

Table S1. AUC of 380-nm elution peaks for all samples HPLC samples in **Fig. S4** of ST and SS samples and calculated [4-NO_2- Trp] based on standard curve for each sample.

Sample	**AUC (380nm)**	**Calculated Concentration (mg/mL)**	**Calculated Concentration (μM)**
ST1	335557	0.0189	75.6896
ST2	323518	0.0182	72.9741
ST3	271855	0.0153	61.3208
SS1	26407	0.0015	5.9565
SS2	26337	0.0015	5.9407
SS3	30424	0.0017	6.8626

Table S2. AUC of 380-nm elution peaks for all samples HPLC samples in **Fig. S6** of TB14 single-turnover samples with varying NADPH concentration.

	AUC (380 nm)					
Sample	**Trial 1**	**Trial 2**	**Trial 3**	**average**	**[4-NO_2-Trp] (mg/mL)**	**[4-NO_2-Trp] (μM)**
X	2557	2531	2693	2593 (87)	0.000145803	0.58 (0.02)
Y	8921	8679	14711	10770 (3414)	0.000605455	2.4 (0.8)
Z	25084	19003	18965	21017 (3521	0.001181492	4.7 (0.8)