Supporting Information

The roles of Asn7 and Trp67 in tuning the ferryl haem form of *Staphylococcus aureus* IsdG

Aarzoo Grover, Aiman Nabi, Matthew A. Conger, and Matthew D. Liptak*

Department of Chemistry, University of Vermont, Burlington, Vermont 05405, United States

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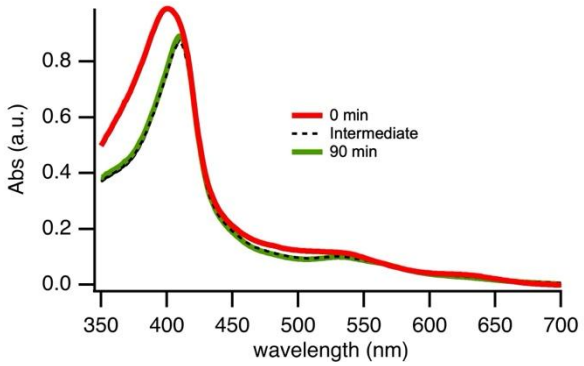

Fig. S1. UV/Vis Abs spectrum for N7A IsdG—haem (red trace), followed by its reaction with mCPBA to yield intermediates (dashed black traces) and compound Y (green trace) at 90 minutes in 125 mM KP_i pH 7.4 at room temperature.

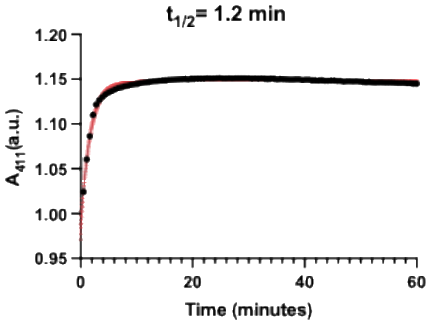

Fig. S2. Decay of N7A IsdG compound X to N7A IsdG compound Y at 5 °C. The UV/Vis absorption intensity for the growth of the N7A IsdG compound Y Soret band at 411 nm was monitored versus time for 60 min and fit to a first-order kinetic model. N7A IsdG compound X has a half-life of 1.2 ± 0.4 min and decays to compound Y at a rate of 0.60 ± 0.02 min^{-1} at 5 °C.

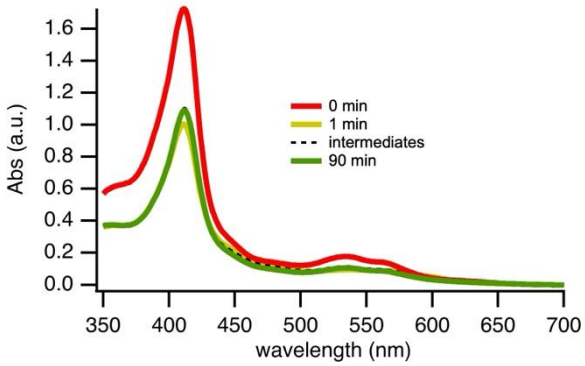

Fig. S3. UV/Vis Abs spectrum for W67F IsdG—haem (red trace), compound X (gold trace) formed after one minute of adding mCPBA, followed by the intermediates (dashed black traces), and compound Y (green trace) at 90 minutes in 125 mM KP_i pH 7.4 at room temperature.

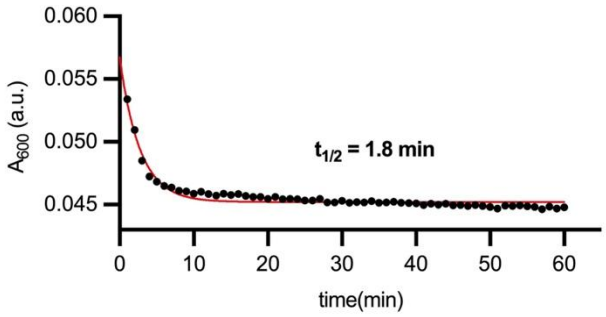

Fig. S4. Decay of W67F IsdG compound X to W67F IsdG compound Y at room temperature. The UV/Vis absorption intensity for the unique band observed at 600 nm in W67F IsdG compound X was monitored versus time for 60 min and fit to a first-order kinetic model. W67F IsdG compound X has a half-life of 1.8 ± 0.2 min and decays to compound Y at a rate of 0.39 ± 0.09 min^{-1} at room temperature.

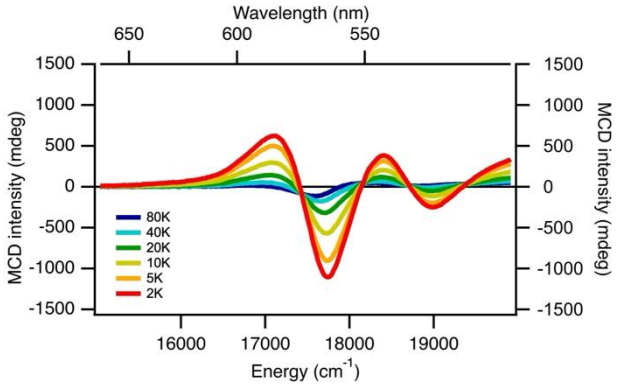

Fig. S5. 7 T MCD spectrum of the IsdG–haem reaction mixture flash frozen after 1 min. MCD spectra were acquired at temperatures ranging from 2 to 80 K.

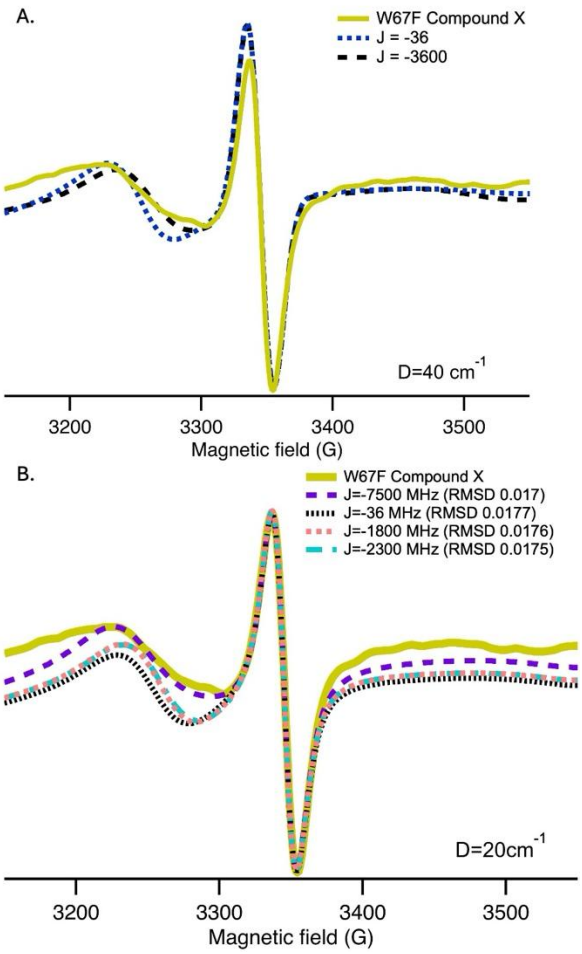

Fig. S6. Fig. 8 W67F IsdG compound X is best simulated with a significantly larger exchange (*J*) coupling between the *S* = 1 ferryl center and the *S* = ½ organic radical compared to WT IsdG compound X. If the MCD-derived zero-field splitting (*D*) value of +40 cm^{-1} for WT IsdG compound X is used to fit the W67F IsdG compound X radical signal, a *J* value of -3600 MHz yields the best fit of the radical signal (A). If a *D* value of +20 cm^{-1} is used instead, a *J* value of -7500 MHz yields the best fit of the radical signal (B). Regardless, the exchange coupling between the ferryl center and the organic radical is at least 100x larger in W67F IsdG compound X compared to WT enzyme. This suggests that the organic radical may be localized on the porphyrin moiety, and W67F IsdG compound X may have a compound I-like electronic structure.

Table S1. EPR Simulations of N7A IsdG

Ferric	S	g	Nucs	lwpp	weight	A
S1	0.5	[2.97 2.24 1.73]	56Fe	3, 2	0.5	
S2	0.5	[3.15 2.29 1.55]	56Fe	1.2, 3.3	0.45	
S3	2.5	[5.925, 1.9]	56Fe	2, 5	0.12	
S4	1.5	4.28	56Fe	2, 4	0.001	
S5	1.5	2.01	63 Cu	6	0.00001	305, 289
Compound Y	**S**	**g**	**Nucs**	**lwpp**	**weight**	**A**
S1	0.5	[2.925 2.27 1.55]	56Fe	2, 5	1	
S3	2.5	[5.915, 1.9]	56Fe	0, 4	0.005	
S4	1.5	4.28	56Fe	0, 4	0.0038	
S5	1.5	2.01	63 Cu	6	0.00001	305, 289

Table S2. EPR Simulations of W67F IsdG

Ferric	S	g	Nucs	lwpp	weight	A		
S1	0.5	[2.91 2.07 1.56]	56Fe	4, 2	0.2			
S2	0.5	[3.11 2.26 0.98]	56Fe	8, 1	1			
S3	2.5	[5.925 2]	56Fe	4, 3	0.004			
S4	1.5	4.27	56Fe	3, 1	0.0002			
S5	1.5	2.01	63Cu	10	0.00009	305, 289		
Compound X	**S**	**g**	**Nucs**	**lwpp**	**weight**	**A**	**J**	**D**
S1	0.5	[2.928 2.27 1.55]	56Fe	4, 4	1			
S3	2.5	[5.925, 2]	56Fe	4, 3	0.0017			
S4	1.5	4.27	56Fe	3, 1	0.0012			
S5	1.5	2.01	63 Cu	10	0.00009	305, 289		
S6	0.5, 1	[2.019, 2]	12C, 56Fe	2, 4.5	0.0135		-7500	125, 600000
S7	0.5	2.0039	12C	0.9, 1.1	0.00275			
S8	0.5	[2.285 2.285 1.885]	56Fe	4, 3	0.036			
Compound Y	**S**	**g**	**Nucs**	**lwpp**	**weight**	**A**		
S1	0.5	[2.928 2.27 1.55]	56Fe	4.2, 4	1			
S3	2.5	[5.925, 1.99]	56Fe	3, 4	0.002			
S4	1.5	4.27	56Fe	2, 3	0.00025			
S5	1.5	2	63 Cu	10	0.00001	305, 289		
S8	0.5	[2.285 2.285 1.885]	56Fe	3, 4	0.033			