Imino-Oxy Acetic Acid Dealkylation as Evidence for an Inner-Sphere Alcohol Intermediate in the Reaction Catalyzed by Peptidylglycine α-Hydroxylating Monooxygenase (PHM).

Supporting Information

Neil R. McIntyre,[†] Edward W. Lowe, Jr.[#], and David J. Merkler*

Department of Chemistry, University of South Florida, 4202 E. Fowler Ave.,

Tampa, FL 33620, USA

*Corresponding author. E-mail: merkler@cas.usf.edu

[†]Present address: Department of Molecular Medicine, College of Medicine, University of South Florida, 12901 Bruce B. Downs Blvd., Tampa, FL, 33612-4799

[#]Present address: Vanderbilt University Center for Structural Biology, 465 21st Ave. South

BIOSCI/MRBIII, Room 5144F, Nashville, TN 37232-8725

Supporting Information Available

Figure A. C_{18} -reverse phase high performance liquid chromatograph of (R/S)-α-hydroxy-*N*-benzoylglycine, *N*-benzoylglycine, benzamide, benzaldehyde imino-oxy acetic acid, and benzaldoxime using 65% 50 mM K_xPO_4(pH 6.0), 30% MeOH, and 5% CH_3CN flowing at a rate of 1mL/min, with signal detection at 248 nm.

Figure B. ^{13}C NMR analysis of PAM mediated benzaldehyde imino-oxy acetic acid catalysis. The isolated sample was analyzed with a Varian 100-MHz instrument using (d_3)-methanol as the solvent.

Figure C and Table C. Stoichiometry of the enzymatic [oxygen] versus [substrate] consumption measured below the ambient concentration of dissolved oxygen (217uM). Benzaldehyde imino-oxy acetic acid was used in this experiment as the substrate.

Figure D. Replot analysis of data represented in table B for the $^{D}(V_{MAX}/K_M)_{BIAA}$.

Figure E. Replot analysis of data represented in table A for the $^{D}(V_{MAX}/K_M)_{BIAA}$ as$[O_2]\rightarrow 0$uM and $^{D}(V_{MAX}/K_M)_{BIAA}$ as $[O_2]\rightarrow\infty$, respectively.

Scheme A. Spectrophotometric glyoxylate analysis used in tandem with C_{18} RP-HPLC for the determination and dilution benzaldoxime (BOX) concentration to 48 μM, respectively.

Scheme B. Cu_M domain for the PHM active site poses for benzaldehyde imino-oxy acetic acid Cα-radical for the reaction coordinate corresponding to figure 5, respectively. Yellow encased coordinate represents the energetic maxima. Atomic representations are carbon (grey), nitrogen (blue), sulfur (yellow), oxygen (red), and hydrogen (white). Please note, the yellow bordered box represents the energetic maxima for the reaction coordinate.

Scheme C. PHM active site poses for benzaldehyde imino-oxy acetic acid Cα-radical for the reaction coordinate corresponding to figure 6, respectively. The yellow coordinate represents the energetic maximum. Atomic representations are carbon (grey), nitrogen (blue), sulfur (yellow), oxygen (red), and hydrogen (white). Please note, the yellow bordered box represents the energetic maximum in the reaction coordinate.

Table A. Kinetic parameters for data in supporting information figures 7 and 8, respectively.

Table B. Kinetic parameters for oxygen variation with benzaldehyde imino-oxy acetic

Movie A. Cu^{II}-OH reaction coordinate for BIAA oxidation.

Movie B. Cu^{II}-O• reaction coordinate for BIAA oxidation.

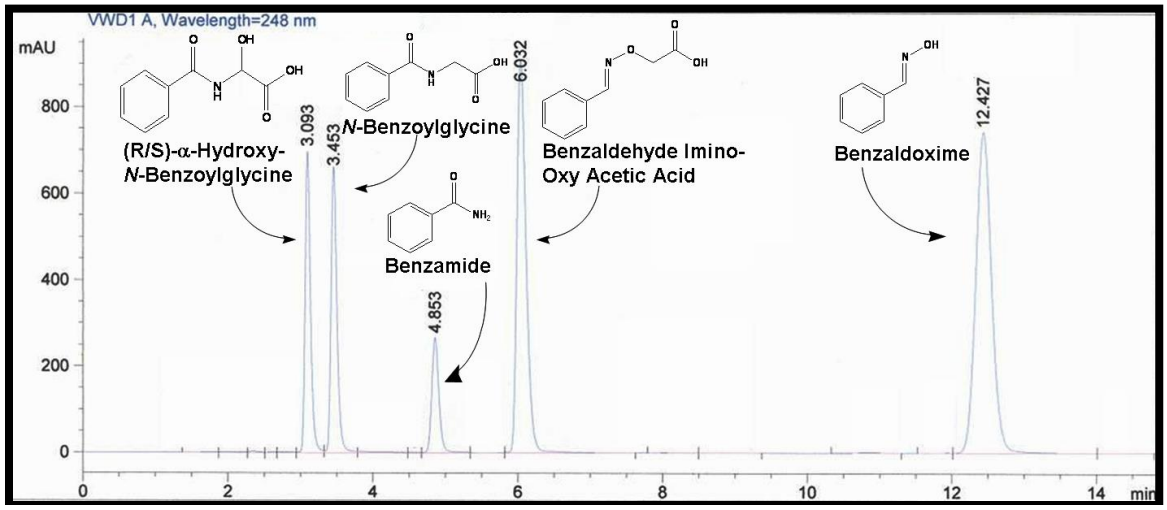


Figure A.

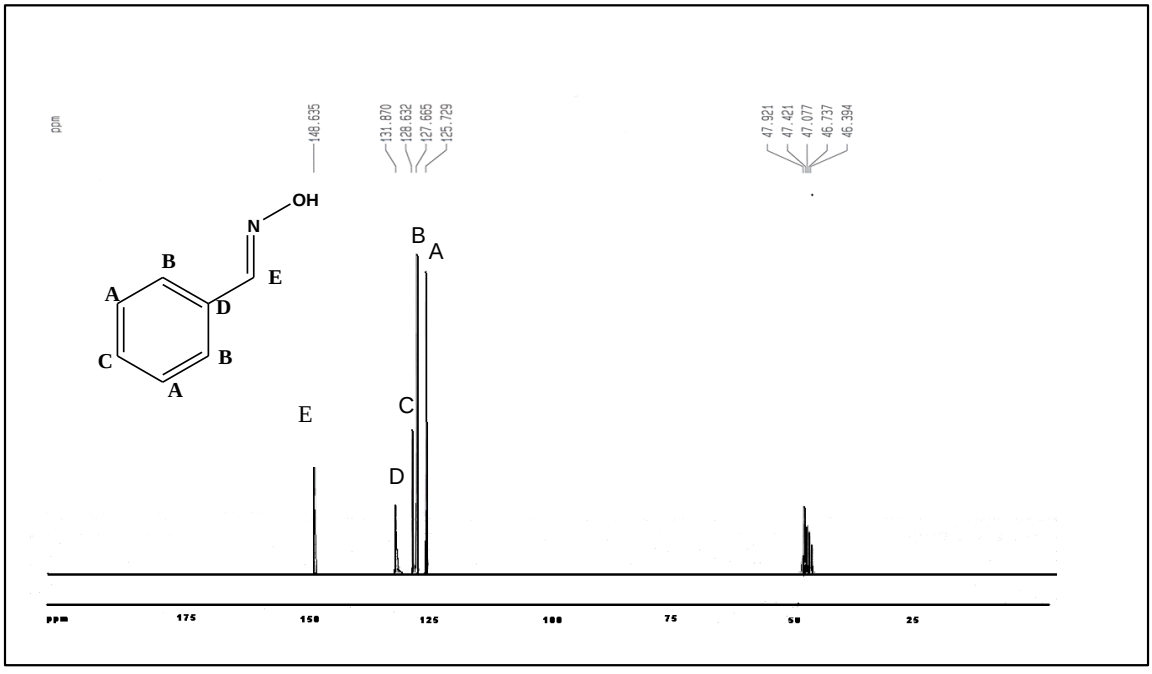


Figure B.

Theoretical (μM)	Experimental (μM)	Ratio
100	95.6	1.05
75	76.6	0.98
35	38.8	0.90
20	20.8	0.96

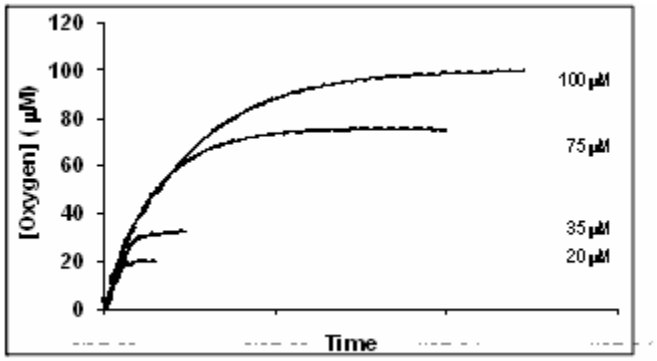


Figure C and Table C.

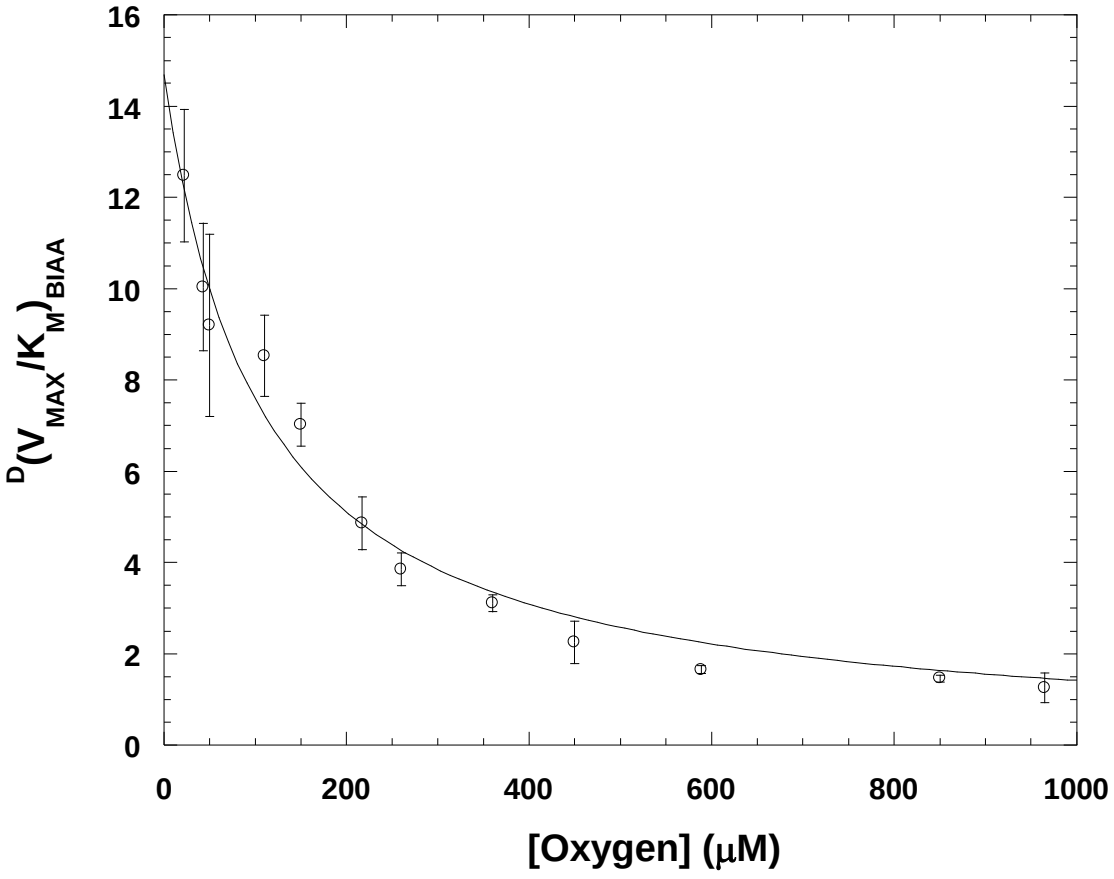


Figure D.

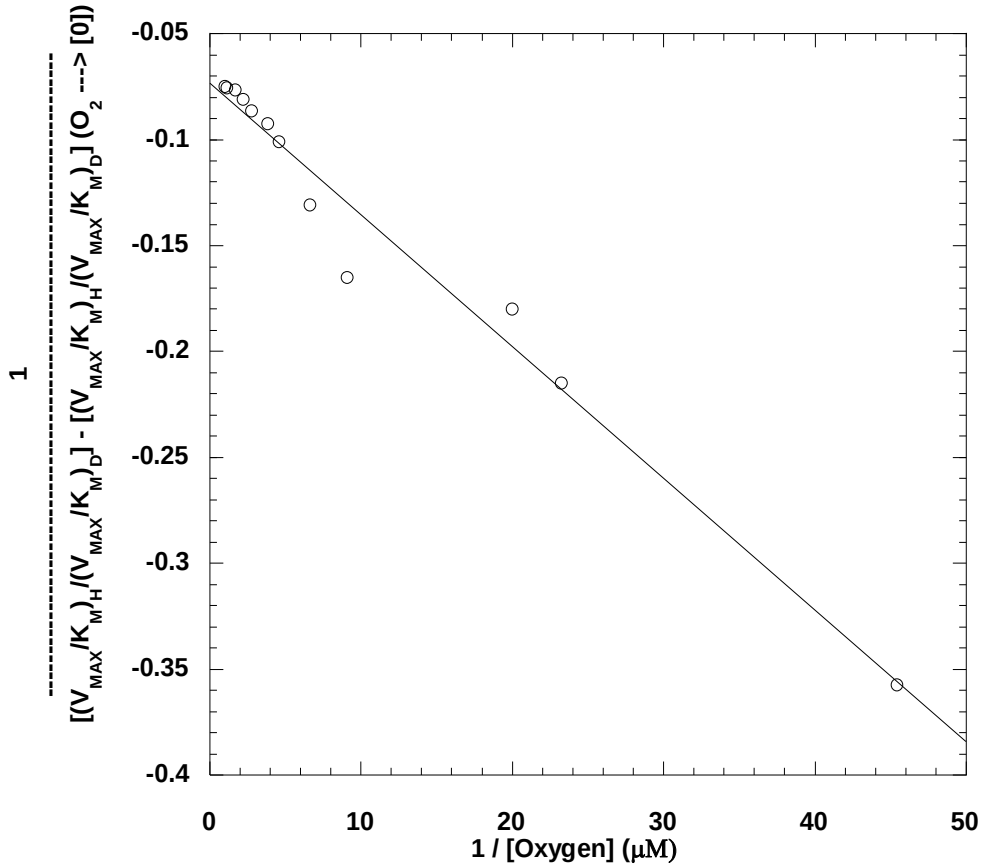
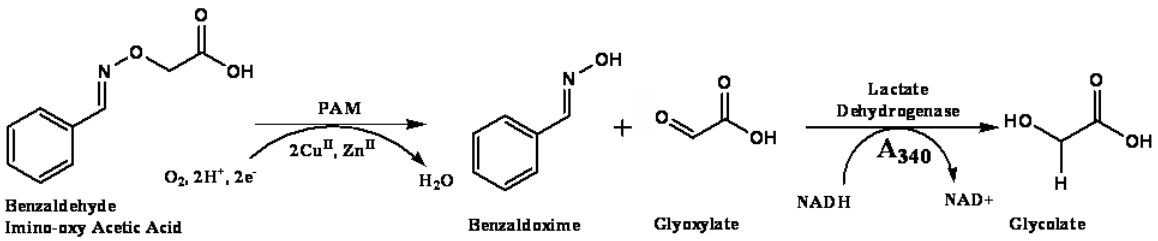
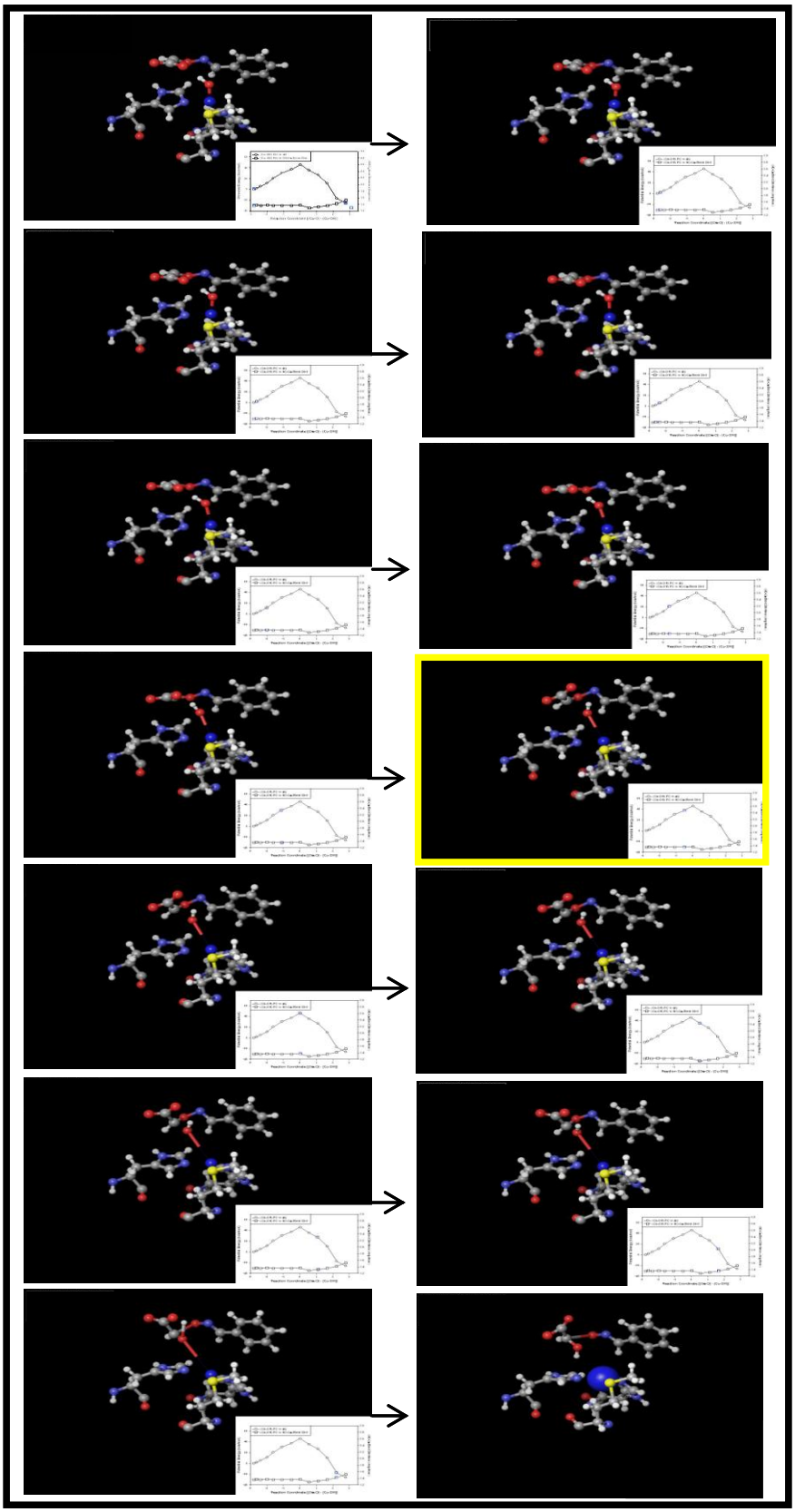


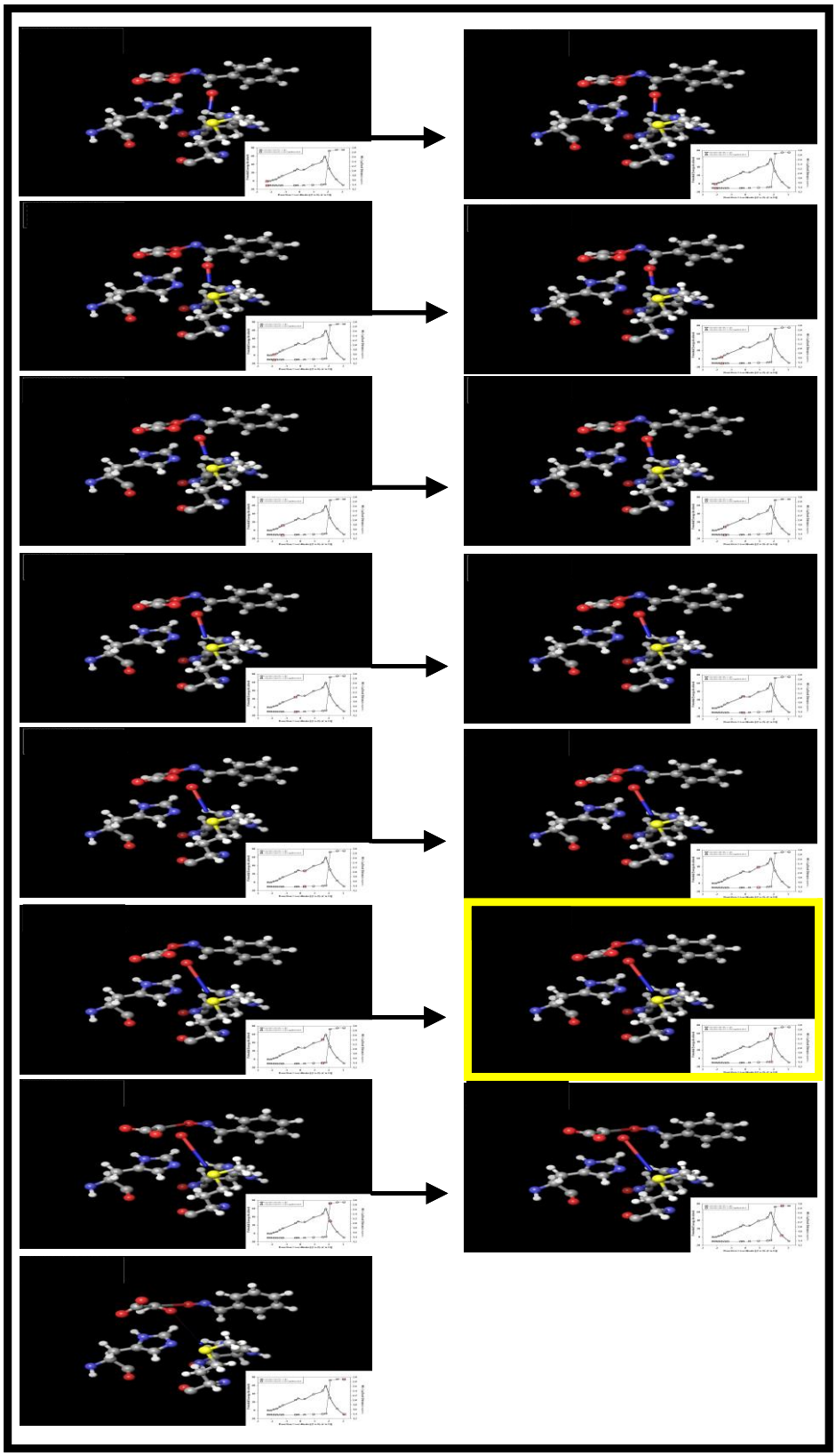
Figure E.



Scheme A.



Scheme B.



Scheme C.

	Protiated						Deuterated							
$[O_2]$ (μM)	(V_{MAX}) (s^{-1})	error	(K_M) (μM)	error	(V_{MAX}/K_M) $(M^{-1}s^{-1})$	error	(V_{MAX}) (s^{-1})	error	(K_M) (μM)	error	(V_{MAX}/K_M) $(M^{-1}s^{-1})$	error	$^{D}(V_{MAX}/K_M)$	error
22	1.16	0.05	2500	214	4.62E+02	4.37E+01	0.12	0.00	3349	211	3.70E+01	2.48E+00	12.5	1.4
43	1.56	0.05	1200	92	1.30E+03	1.10E+02	0.42	0.02	3211	329	1.29E+02	1.43E+01	10.0	1.4
50	1.94	0.10	789	100	2.46E+03	3.34E+02	0.53	0.03	1989	323	2.68E+02	4.53E+01	9.2	2.0
110	2.95	0.06	666	38	4.42E+03	2.69E+02	0.88	0.02	1690	135	5.18E+02	4.36E+01	8.5	0.9
217	3.27	0.10	141	14	2.33E+04	2.35E+03	1.94	0.04	405	23	4.79E+03	2.98E+02	4.9	0.6
450	3.82	0.14	107	13	3.59E+04	4.54E+03	2.37	0.08	149	23	1.59E+04	2.57E+03	2.3	0.5
965	4.25	0.24	77	19	5.50E+04	1.37E+04	3.25	0.05	74	5	4.37E+04	2.94E+03	1.3	0.3

Single Point (V_{MAX}/K_M)

	Protiated					Deuterated						
$[O_2]$ (μM)	Rate (s^{-1})	SE	[S] (μM)	(V_{MAX}/K_M) $(M^{-1}s^{-1})$	error	Rate (s^{-1})	error	[S] (μM)	(V_{MAX}/K_M) $(M^{-1}s^{-1})$	error	$^{D}(V_{MAX}/K_M)$	error
160	0.205	0.001	20	1.03E+04	5.66E+01	0.0977478	0.016	75	1.46E+03	2.19E+02	7.0	1.1
260	0.456	0.003	20	2.27E+04	1.73E+02	0.2378863	0.045	35	5.90E+03	1.28E+03	3.8	0.8
360	0.632	0.002	20	3.15E+04	1.02E+02	0.3873916	0.046	35	1.01E+04	1.31E+03	3.1	0.4
595	0.858	0.044	15	5.93E+04	2.91E+03	0.5401725	0.018	15	3.69E+04	1.19E+03	1.6	0.1
865	1.364	0.042	15	9.29E+04	2.79E+03	0.9763579	0.002	15	6.50E+04	1.15E+02	1.4	0.0

Table A.

	Protiated							Deuterated						Isotope Effect	
[Substrate] (μM)	V_{MAX} (s^{-1})	SE	K_M (mM)	SE	$(V_{MAX}/K_M)_H$	SE	V_{MAX} (s^{-1})	SE	K_M (mM)	SE	$(V_{MAX}/K_M)_D$	SE	$^{D}(V_{MAX}/K_M)$	SE
600	3.58	0.16	204.89	24.62	1.75E+04	2.24E+03	5.88	0.89	1659.80	330.69	3.54E+03	8.87E+02	4.94	1.39
800	4.15	0.15	212.40	20.29	1.95E+04	2.00E+03	4.71	0.86	1015.20	270.93	4.64E+03	1.50E+03	4.21	1.43
1900	4.03	0.14	122.47	11.26	3.29E+04	3.23E+03	3.93	0.25	653.02	73.72	6.02E+03	7.84E+02	5.47	0.89
3000	4.12	0.14	140.42	11.91	2.93E+04	2.68E+03	3.90	0.23	547.84	60.01	7.11E+03	8.87E+02	4.13	0.64

Table B.