Supporting Information

Effects of Interdomain-Tether Length and Flexibility on the Kinetics of Intramolecular Electron Transfer in Human Sulfite Oxidase†

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Table S1. Primer Design for Site-Directed Mutagenesis of Human Sulfite Oxidase.

Mutant	Template	Primer	Primer sequence
P105A	*wt* HSO	forward	GAGCTGAATGCTGAAGACAAGGTA
		reverse	TACCTTGTCTTCAGCATTCAGCTC
P111A	*wt* HSO	forward	AAGGTAGCCGCCACCGTGGA
		reverse	TCCACGGTGGCGGCTACCTT
P118A	*wt* HSO	forward	AGACCTCTGACGCTTATGCTGAT
		reverse	ATCAGCATAAGCGTCAGAGGTCT
P105A/P111A	P105A	forward	AAGGTAGCCGCCACCGTGGA
		reverse	TCCACGGTGGCGGCTACCTT
P105A/P111A/P118A	P105A/P111A	forward	AGACCTCTGACGCTTATGCTGAT
		reverse	ATCAGCATAAGCGTCAGAGGTCT
ΔK108V109A110	*wt* HSO	forward	CCTGAAGACCCCACCGTGGAGACCTCTGACCCTTAT
		reverse	ATAAGGGTCAGAGGTCTCCACGGTGGGGTCTTCAGG
ΔK108V109A110T112	ΔKVA	forward	CCTGAAGACCCCGTGGAGACC
		reverse	GGTCTCCACGGGGTCTTCAGG
ΔK108V109A110T112V113	ΔKVAT	forward	GAAGACCCCGAGACCTCT
		reverse	AGAGGTCTCGGGGTCTTC
ΔK108V109A110T112V113T115	ΔKVATV	forward	ATCCTGAAGACCCCGAGTCTGACCCTTA
		reverse	TAAGGGTCAGACTCGGGGTCTTCAGGAT

Table S2 Iron to Molybdenum Ratios Determined using Inductively Coupled Plasma

HSO Mutant	Iron to Molybdenum Ratio
P111A	**0.70 ± 0.03**
P105A/P111A	**1.0 ± 0.03**
ΔKVA	**1.0 ± 0.03**
ΔKVAT	**0.64 ± 0.02**
ΔKVATV	**0.70 ± 0.02**

Table S3. Laser Flash Photolysis Results for Proline to Alanine Mutants

		pH 6.8	pH 7.0	pH 7.4	pH 7.61
wt HSO	k_{et} (s^{-1})	427 ± 36	465 ± 24	467 ± 19	338 ± 16
	k_f (s^{-1})	88 ± 7	121 ± 6	147 ± 6	105 ± 5
	k_r (s^{-1})	339 ± 29	344 ± 18	320 ± 13	233 ± 11
	K_{eq}	0.26 ± 0.01	0.35 ± 0.01	0.46 ± 0.02	0.45 ± 0.02
P105A	k_{et} (s^{-1})	136 ± 18	236 ± 11	146 ± 23	118 ± 6
	k_f (s^{-1})	25 ± 3	65 ± 3	23 ± 4	36 ± 2
	k_r (s^{-1})	111 ± 19	171 ± 12	123 ± 23	81 ± 6
	K_{eq}	0.22 ± 0.01	0.38 ± 0.02	0.19 ± 0.02	0.44 ± 0.03
P111A	k_{et} (s^{-1})	435 ± 26	403 ± 28	359 ± 19	283 ± 12
	k_f (s^{-1})	95 ± 6	107 ± 7	108 ± 6	86 ± 4
	k_r (s^{-1})	340 ± 20	296 ± 21	251 ± 13	197 ± 8
	K_{eq}	0.28 ± 0.02	0.36 ± 0.01	0.43 ± 0.01	0.44 ± 0.02
P105A/P111A	k_{et} (s^{-1})	*ND	136 ± 18	93 ± 11	95 ± 6
	k_f (s^{-1})	*ND	22 ± 3	23 ± 3	24 ± 2
	k_r (s^{-1})	*ND	114 ± 18	70 ± 11	70 ± 7
	K_{eq}	*ND	0.19 ± 0.01	0.33 ± 0.02	0.34 ± 0.01

*no heme reoxidation was observed.

Table S4. Laser Flash Photolysis Results for Tether Deletion Mutants of HSO

		pH 6.8	pH 7.0	pH 7.4	pH 7.61
wt HSO	k_{et} (s^{-1})	427 ± 36	465 ± 24	467 ± 19	338 ± 16
	k_f (s^{-1})	88 ± 7	121 ± 6	147 ± 6	105 ± 5
	k_r (s^{-1})	339 ± 29	344 ± 18	320 ± 13	233 ± 11
	K_{eq}	0.26 ± 0.01	0.35 ± 0.01	0.46 ± 0.02	0.45 ± 0.02
ΔKVA	k_{et} (s^{-1})	406 ± 15	393 ± 15	294 ± 14	241 ± 10
	k_f (s^{-1})	107 ± 4	102 ± 4	98 ± 5	85 ± 4
	k_r (s^{-1})	299 ± 11	291 ± 11	196 ± 9	156 ± 6
	K_{eq}	0.36 ± 0.01	0.35 ± 0.01	0.5 ± 0.02	0.54 ± 0.02
ΔKVAT	k_{et} (s^{-1})	188 ± 12	165 ± 3	165 ± 3	143 ± 3
	k_f (s^{-1})	55 ± 5	43 ± 4	59 ± 2	50 ± 3
	k_r (s^{-1})	133 ± 13	122 ± 5	106 ± 4	94 ± 4
	K_{eq}	0.41±0.02	0.35 ± 0.03	0.56 ± 0.01	0.53 ± 0.03
ΔKVATV	k_{et} (s^{-1})	7.6 ± 0.2	7.0 ± 0.1	6.4 ± 0.1	5.9 ± 0.1
	k_f (s^{-1})	2.8 ± 0.1	2.6 ± 0.1	2.8 ± 0.1	2.4 ± 0.1
	k_r (s^{-1})	4.8 ± 0.1	4.4 ± 0.1	3.6 ± 0.1	3.4 ± 0.1
	K_{eq}	0.59 ± 0.01	0.63 ± 0.02	0.91 ± 0.03	0.87 ± 0.03