

**Supplemental materials.**

**Table S1.** The sensitivity analyses were based on varying one specific parameter while keeping all others unchanged and observing the effect on  $Q_{NO}$  at  $t = 10$  s at steady state. The NOS1 concentration was  $0.9 \mu\text{M}$ , the  $\text{O}_2$  concentration was  $100 \mu\text{M}$ , and the arginine concentration was  $100 \mu\text{M}$ .

	k <sub>1</sub>		k <sub>1</sub>		k <sub>2</sub>		k <sub>3</sub>		k <sub>4</sub>		k <sub>4</sub>	
	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )
Original	6.6	1.16	6.6	1.16	20.8	1.16	20.8	1.16	6.6	1.16	6.6	1.16
Test 1	0.66	1.16	0.66	1.16	2.08	1.15	2.08	0.66	0.66	1.16	0.66	1.16
Test 2	33.0	1.16	33.0	1.16	104.0	1.17	104.0	1.24	33.0	1.16	33.0	1.16
Test 3	66.0	1.16	66.0	1.16	208.0	1.19	208.0	1.26	66.0	1.16	66.0	1.16
	k <sub>5</sub>		k <sub>5</sub>		k <sub>6</sub>		k <sub>7</sub>		k <sub>8</sub>		k <sub>8</sub> '	
	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )
Original	8.5	1.16	215.6	1.16	175.6	1.16	20.8	1.16	13.2	1.16	13.2	1.16
Test 1	0.85	1.10	2.16	1.16	17.6	1.01	2.08	0.58	1.32	1.16	1.32	1.18
Test 2	42.5	1.16	21.6	1.16	878.0	1.17	104.0	1.27	66.0	1.16	66.0	1.10
Test 3	85.0	1.16	2156.0	1.12	1756.0	1.17	208.0	1.28	132.0	1.17	132.0	1.04
	k <sub>9</sub>		k <sub>9</sub> '		k <sub>10</sub>		k <sub>11</sub>		k <sub>12</sub>		k <sub>13</sub>	
	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\mu\text{M}^{-1} \cdot \text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )	parameter ( $\text{s}^{-1}$ )	Q <sub>NO</sub> ( $\mu\text{M}/\text{s}$ )
Original	8.6	1.16	399.2	1.16	39.1	1.16	20.8	1.16	0.01	1.16	39.9	1.16
Test 1	0.86	0.86	3.99	1.20	3.91	0.65	1.04	4.36	0.001	0.16	3.99	0.14
Test 2	43.0	1.20	39.9	1.20	195.5	1.25	2.08	3.80	0.1	2.93	199.5	3.25
Test 3	86.0	1.20	3992.0	0.88	391.0	1.26	208.0	0.15	1.0	3.46	399.0	4.21

**Figure S1.** NO production by NOS1 when the binding rates of L-arginine to the ferric ( $k_1$ ) and ferrous ( $k_4$ ) forms of NOS1 and the dissociation rates of L-arginine from the ferric ( $k_1'$ ) and ferrous ( $k_4'$ ) forms of NOS1 varied. Total NOS1 concentration was 0.9  $\mu\text{M}$ , L-arginine concentration was 100  $\mu\text{M}$ , and  $\text{O}_2$  concentration was 100  $\mu\text{M}$ . The apparent binding rate ( $k_1 + k_4$ ) of L-arginine to NOS1 heme was 2.5  $\mu\text{M}^{-1}\cdot\text{s}^{-1}$  and the apparent dissociation rate ( $k_1' + k_4'$ ) was 2.5  $\text{s}^{-1}$  at 15  $^\circ\text{C}$ . In (A), Case 1:  $k_1$  was taken as 10% and  $k_4$  was taken as 90% of the apparent binding rate; Case 2:  $k_1$  and  $k_4$  were each taken as 50% of the apparent binding rate; Case 3:  $k_1$  was taken as 90% and  $k_4$  was taken as 10% of the apparent binding rate. In (B), Case 1:  $k_1'$  was taken as 10% and  $k_4'$  was taken as 90% of the apparent dissociation rate; Case 2:  $k_1'$  and  $k_4'$  were each taken as 50% of the apparent dissociation rate; Case 3:  $k_1'$  was taken as 90% and  $k_4'$  was taken as 10% of the apparent dissociation rate. All other kinetic parameters were listed in Table 1. In all cases, the NO production was virtually the same.



