Supporting Information

The Role of Zinc in Isoform Selective Inhibitor Binding to Neuronal Nitric Oxide Synthase

Silvia L. Delker, [a] Fengtian Xue,[b,c] Huiying Li,[a] Jianguo Fang[b], Joumana Jamal, [a] Richard B. Silverman,[b*] and Thomas L. Poulos[a*]

[a]*Departments of Molecular Biology and Biochemistry, Pharmaceutical Chemistry, and Chemistry, University of California, Irvine, California 92697-3900*

[b]*Department of Chemistry, Department of Biochemistry, Molecular Biology, and Cell Biology, Center for Molecular Innovation and Drug Discovery, Chemistry of Life Processes Institute, Northwestern University, 2145 Sheridan Road, Evanston, Illinois 60208-3113*

[c]*Current address: Department of Chemistry, University of Louisiana at Lafayette, Lafayette,* Louisiana 70504

Crystallographic Statistics and Supplementary Figures

Table S1 Crystallographic data collection and refinement statistics (part 2)

Data set [1]	eNOS-**3h**	eNOS-**3k**	eNOS-**3m**
Data collection			
PDB code	3N5R	3N5S	3N5P
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	57.9, 106.7, 156.9	58.0, 106.8, 157.0	60.0, 106.1, 158.6
Resolution (Å)	2.57 (2.61-2.57)	2.18 (2.22-2.18)	2.40 (2.44-2.40)
R_{sym} or R_{merge}	0.082 (0.598)	0.067 (0.559)	0.096 (0.725)
$I / \sigma I$	14.6 (1.9)	16.8 (1.9)	12.1 (1.4)
No. unique reflections	31,470	51,666	38,918
Completeness (%)	98.8 (99.9)	99.8 (100.0)	95.4 (98.7)
Redundancy	3.6 (3.7)	3.6 (3.7)	3.6 (3.5)
Refinement			
Resolution (Å)	2.57	2.18	2.39
No. reflections used	29,883	49,032	36,918
R_{work} / R_{free}	0.181/0.244	0.177/0.220	0.206/0.260
No. atoms			
Protein	6456	6438	6461
Ligand/ion	197	199	219
Water	110	383	137
R.m.s. deviations			
Bond lengths (Å)	0.014	0.011	0.012
Bond angles (°)	1.534	1.350	1.498

Data set [1]	eNOS N368D-**3j**	eNOS N368D-**3k**	eNOS N368D-**3n**
Data collection			
PDB code	3N6F	3N6G	3N6E
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	58.0, 106.7, 157.2	57.7, 106.4, 156.9	58.0, 106.9, 157.1
Resolution (Å)	2.18 (2.22-2.18)	2.22 (2.26-2.22)	2.20 (2.24-2.20)
R_{sym} or R_{merge}	0.062 (0.551)	0.065 (0.705)	0.068 (0.640)
$I / \sigma I$	20.4 (2.1)	20.6 (1.9)	19.0 (1.9)
No. unique reflections	51,400	49,005	50,214
Completeness (%)	99.0 (96.5)	99.4 (98.3)	99.7 (98.6)
Redundancy	4.0 (3.9)	4.0 (3.9)	3.9 (3.8)
Refinement			
Resolution (Å)	2.18	2.21	2.20
No. reflections used	48,775	46,518	47,647
R_{work} / R_{free}	0.178/0.218	0.187/0.238	0.179/0.215
No. atoms			
Protein	6422	6448	6438
Ligand/ion	199	199	201
Water	289	234	235
R.m.s. deviations			
Bond lengths (Å)	0.011	0.013	0.010
Bond angles (°)	1.350	1.470	1.339

Data set [1]	eNOS N368D/V106M-**3h**	eNOS N368D/V106M-**3j**	eNOS N368D/V106M-**3k**	eNOS N368D/V106M-**3n**
Data collection				
PDB code	3N68	3N69	3N6A	3N67
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	57.8, 106.6, 157.1	58.2, 106.6, 157.0	58.1, 106.6, 156.9	58.0, 106.6, 156.8
Resolution (Å)	2.54 (2.58-2.54)	2.10 (2.14-2.10)	2.50 (2.54-2.50)	2.10 (2.14-2.10)
R_{sym} or R_{merge}	0.079 (0.463)	0.074 (0.576)	0.103 (0.600)	0.061 (0.431)
$I / \sigma I$	13.7 (1.8)	17.3 (2.2)	12.2 (1.6)	20.0 (2.5)
No. unique reflections	32,342	57,493	34,483	54,937
Completeness (%)	97.8 (96.7)	99.4 (99.9)	99.5 (95.3)	94.5 (88.3)
Redundancy	3.5 (3.4)	4.0 (4.1)	3.6 (3.3)	3.8 (4.0)
Refinement				
Resolution (Å)	2.53	2.10	2.49	2.09
No. reflections used	30,718	54,552	32,727	52,119
R_{work} / R_{free}	0.190/0.254	0.167/0.215	0.184/0.243	0.173/0.219
No. atoms				
Protein	6428	6515	6432	6473
Ligand/ion	191	187	199	201
Water	96	440	154	364
R.m.s. deviations				
Bond lengths (Å)	0.014	0.016	0.013	0.016
Bond angles (°)	1.530	1.512	1.470	1.509

Data set [1]	eNOS H373S-**3j**	eNOS H373S-**3m**	eNOS H373S-**3n**
Data collection			
PDB code	3N6B	3N6C	3N6D
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	57.7, 106.6, 157.8	58.1, 105.7, 159.2	57.2, 106.7, 156.8
Resolution (Å)	3.10 (3.15-3.10)	3.05 (3.10-3.05)	3.05 (3.10-3.05)
R_{sym} or R_{merge}	0.097 (0.611)	0.152 (0.682)	0.118 (0.702)
$I / \sigma I$	13.5 (1.8)	9.4 (1.7)	11.9 (2.0)
No. unique reflections	18,319	18,687	18,759
Completeness (%)	99.2 (100.0)	97.8 (98.4)	99.2 (100.0)
Redundancy	3.9 (3.9)	4.0 (3.7)	3.7 (3.8)
Refinement			
Resolution (Å)	3.10	3.06	3.05
No. reflections used	17,380	17,732	17,785
R_{work} / R_{free}	0.187/0.274	0.233/0.332	0.199/0.289
No. atoms			
Protein	6401	6398	6411
Ligand/ion	219	197	181
Water	10	26	22
R.m.s. deviations			
Bond lengths (Å)	0.016	0.014	0.016
Bond angles (°)	1.704	1.534	1.704

Data set[1]	nNOS-**3h**	nNOS-**3k**	nNOS-**3m**
Data collection			
PDB code	3N5V	3N5X	3N5Z
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions			
a, *b*, *c* (Å)	52.1, 112.1,165.5	52.3, 111.1,164.8	51.8, 111.1,164.6
Resolution (Å)	2.30 (2.34-2.30)	1.80 (1.83-1.80)	2.18 (2.22-2.18)
R_{sym} or R_{merge}	0.071 (0.48)	0.058 (0.501)	0.065 (0.486)
$I / \sigma I$	9.3 (2.1)	25.2 (2.6)	21.6 (2.1)
No. unique reflections	43,487	89,351	49,860
Completeness (%)	98.9 (98.2)	99.6 (100.0)	99.2 (94.2)
Redundancy	5.4 (3.8)	4.1 (4.0)	4.0 (3.8)
Refinement			
Resolution (Å)	2.30	1.80	2.18
No. reflections used	41,255	84,829	47,303
R_{work} / R_{free}[2]	0.185/0.238	0.173/0.198	0.194/0.243
No. atoms			
Protein	6682	6713	6668
Ligand/ion	182	190	182
Water	191	635	195
R.m.s. deviations			
Bond lengths (Å)	0.014	0.012	0.014
Bond angles (°)	1.427	1.236	1.482

	nNOS D597N-**3n**	nNOS D597N/M336V-**3n**	nNOS S602H-**3j**	nNOS S602H-**3n**
Data collection				
PDB code	3N64	3N63	3N65	3N66
Space group	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions				
a, *b*, *c* (Å)	51.8, 110.2, 164.2	51.8, 111.0, 164.0	52.0,110.6,164.2	51.9, 110.6, 164.0
Resolution (Å)	1.95 (1.98-1.95)	2.00 (2.03-2.00)	1.80 (1.83-1.80)	1.78 (1.81-1.78)
R_{sym} or R_{merge}	0.065 (0.543)	0.065 (0.464)	0.060 (0.593)	0.051 (0.651)
$I / \sigma I$	22.5 (2.5)	20.9 (2.0)	28.1 (2.5)	29.1 (2.2)
No. unique reflections	69,474	62,815	88,164	91,167
Completeness (%)	99.8 (100.0)	97.0 (82.4)	99.4 (99.0)	99.6 (99.7)
Redundancy	4.0 (4.0)	3.9 (3.2)	5.1 (5.0)	4.0 (3.9)
Refinement				
Resolution (Å)	1.95	2.00	1.80	1.78
No. reflections used	65,979	59,663	83,704	86,523
R_{work} / R_{free}	0.183/0.217	0.195/0.236	0.167/0.194	0.182/0.216
No. atoms				
Protein	6662	6566	6746	6682
Ligand/ion	158	157	249	174
Water	371	267	679	449
R.m.s. deviations				
Bond lengths (Å)	0.013	0.013	0.013	0.013
Bond angles (°)	1.404	1.424	1.470	1.361

[1] See Table 1 for the inhibitor codes and formula.

[2] A 5% of reflections has been set aside during the entire course of refinement for the free R calculation only. For each NOS isoform the choice of free R flags have been kept the same as those used in the starting model data set.

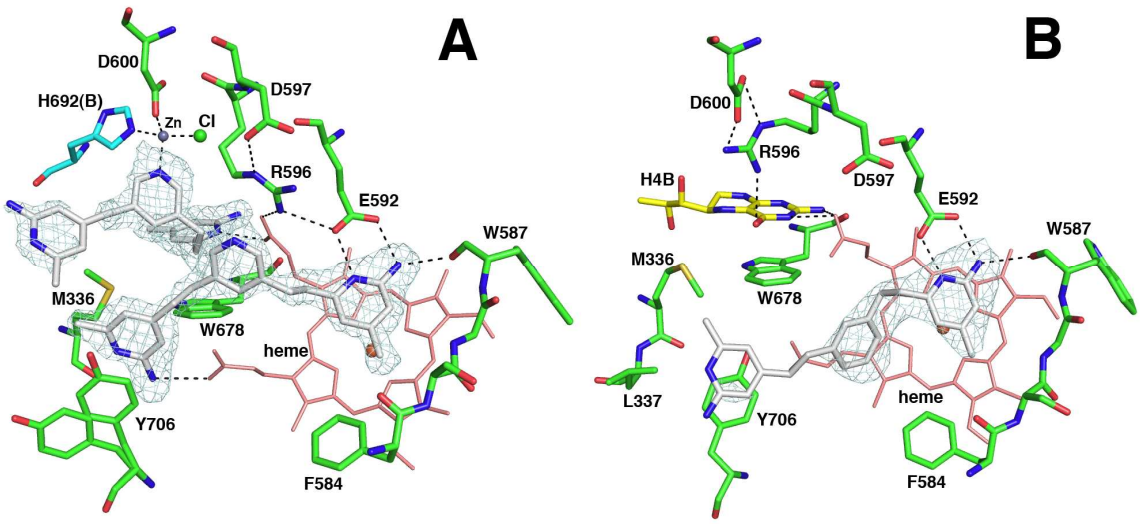

Figure S1 The active site of (A) nNOS with two molecules of **3k** bound or (B) nNOS complexed with one molecule of **3h**. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 3 σ contour level. Major hydrogen bonds and the Zn coordination sphere are depicted with dashed lines. Note the differences in the Tyr706 side chain position: in panel A, Tyr706 swings out (two alternate conformations) allowing one of the aminopyridine rings of **3k** to make hydrogen bond with the heme propionate D; in panel B, Tyr706 is in its original rotamer position that hydrogen bonds with the heme propionate D. The second aminopyridine of **3h** (B) is less well defined but likely stacks with Tyr706.

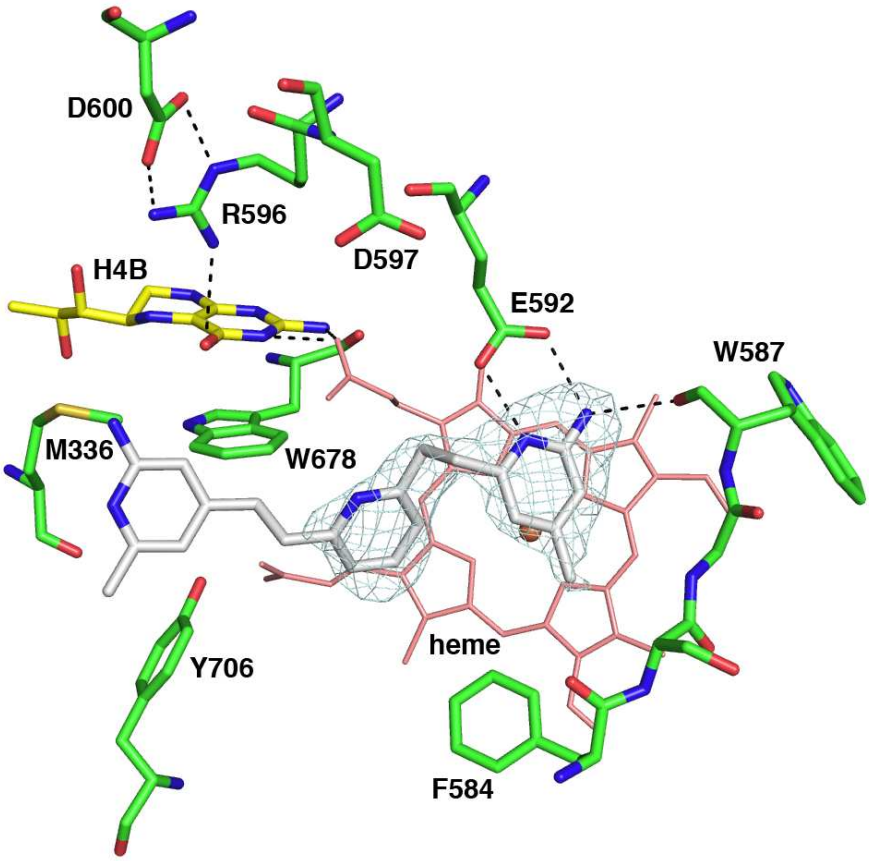

Figure S2 The active site of nNOS with one molecule of **3m** bound. Shown also the sigma-A weighted Fo-Fc omit density map for the inhibitor contoured at 3 σ. Major hydrogen bonds are depicted with dash lines. The second aminopyridine of **3m** is disordered indicated by poor density in the region.

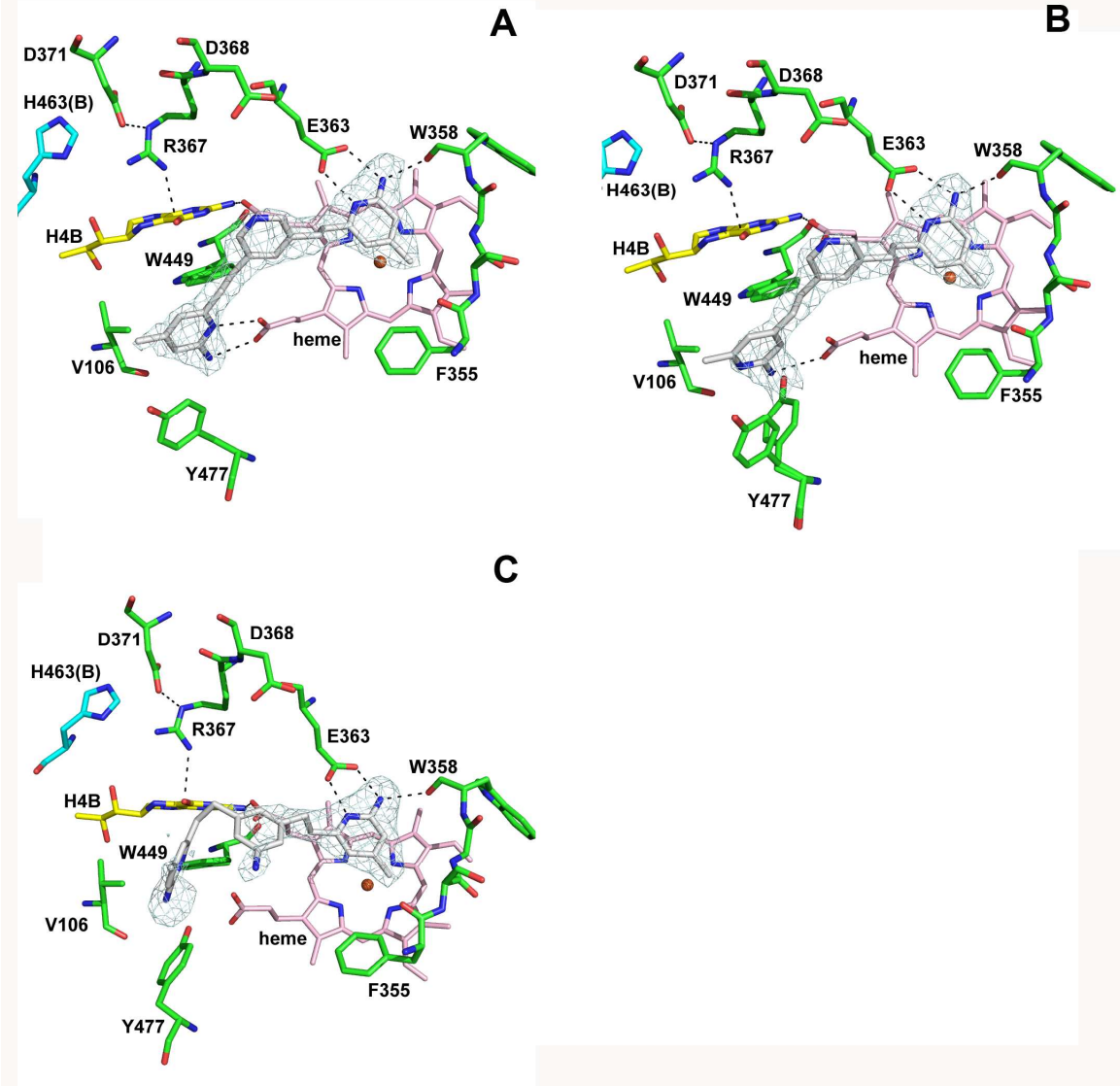

Figure S3 The active site of eNOS N368D mutant with (A) one molecule of **3j** bound or (B) with one molecule of **3k** bound or (C) with inhibitor **3n** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 3.0 σ (**3j**, **3k**) or 2.5 σ (**3n**) contour level. Major hydrogen bonds are depicted with dashed lines. Note the differences in the Y477 side chain position: in both panels A and B, Y477 swings out allowing one of the aminopyridine rings of **3j** or **3k** to make hydrogen bond with the heme propionate D; in panel C, Y477 is in its original rotamer position that hydrogen bonds with the heme propionate D. The second aminopyridine of **3n** is partially disordered.

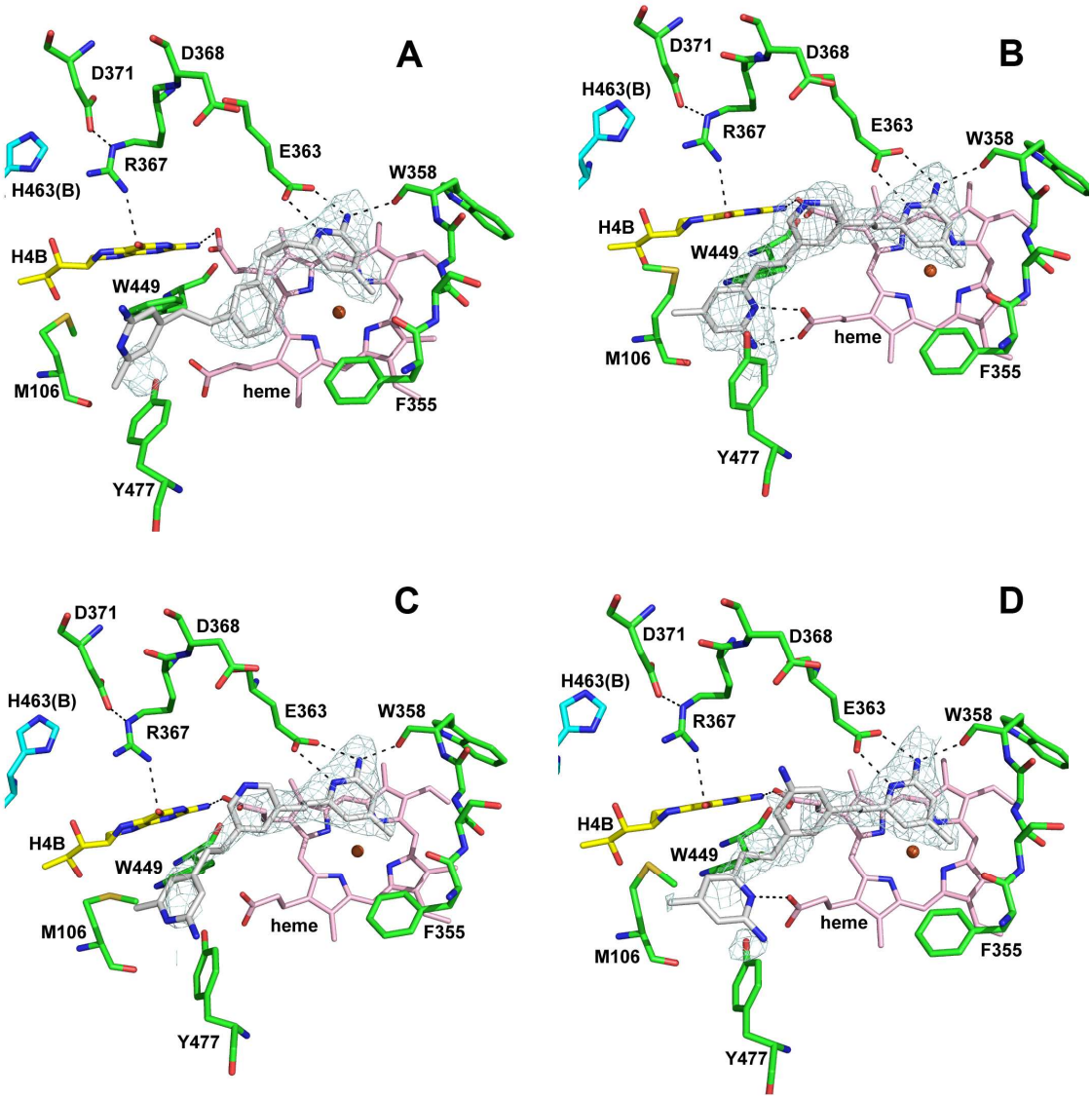

Figure S4 The active site of eNOS N368D/V106M double mutant with (A) one molecule of **3h** or (B) one molecule of **3j** or (C) one molecule of **3k** or (D) inhibitor **3n** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 3.0 σ (**3k**) or 2.5 σ (**3h, 3k, 3n**) contour level. Major hydrogen bonds are depicted with dashed lines. Note the differences in the Y477 side chain position: in panel B, it swings out allowing one of the aminopyridine rings of **3k** to make hydrogen bond with the heme propionate D; in panels A and C, Y477 is in its original rotamer position that hydrogen bonds with the heme propionate D; in panel D, Y477 is in its original rotamer position making H-bond with the heme propionate D, but the second aminopyridine ring of **3n** contorts to also make a weak hydrogen bond with the other oxygen from the same propionate. In panels A, C, and D, weak density for the second aminopyridine of inhibitor indicates disordering.

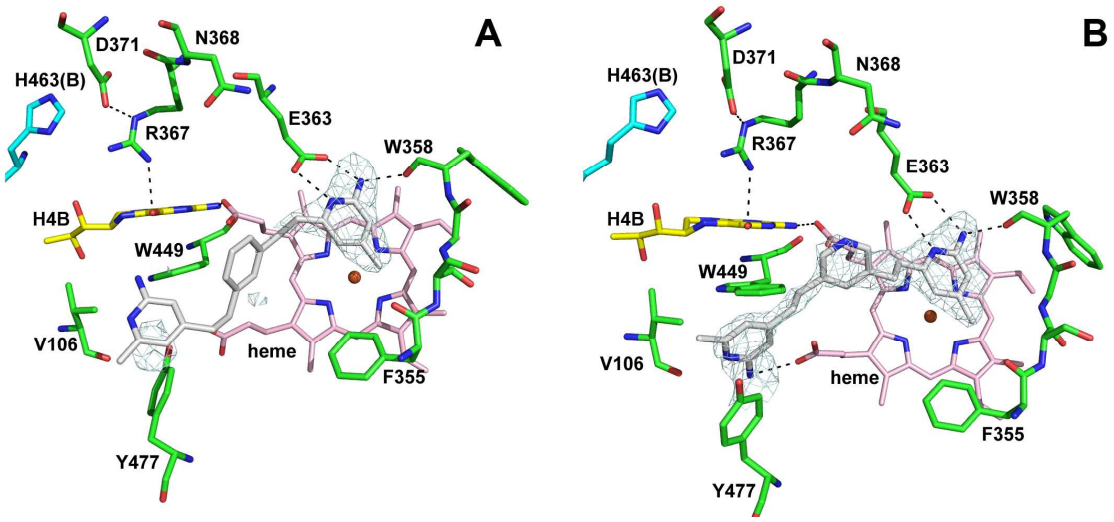

Figure S5 The active site of eNOS (A) with one molecule of **3h** or (B) with one molecule of **3k** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 2.5 σ (**3h**) or 3.0 σ (**3k**) contour level. Major hydrogen bonds are depicted with dashed lines. Note the differences in the Y477 side chain position: in panel B, Y477 swings out allowing one of the aminopyridine rings of **3k** to make hydrogen bond with the heme propionate D; in panel A, Y477 is in its original rotamer position that hydrogen bonds with the heme propionate D. The second aminopyridine of **3h** in panel A is disordered.

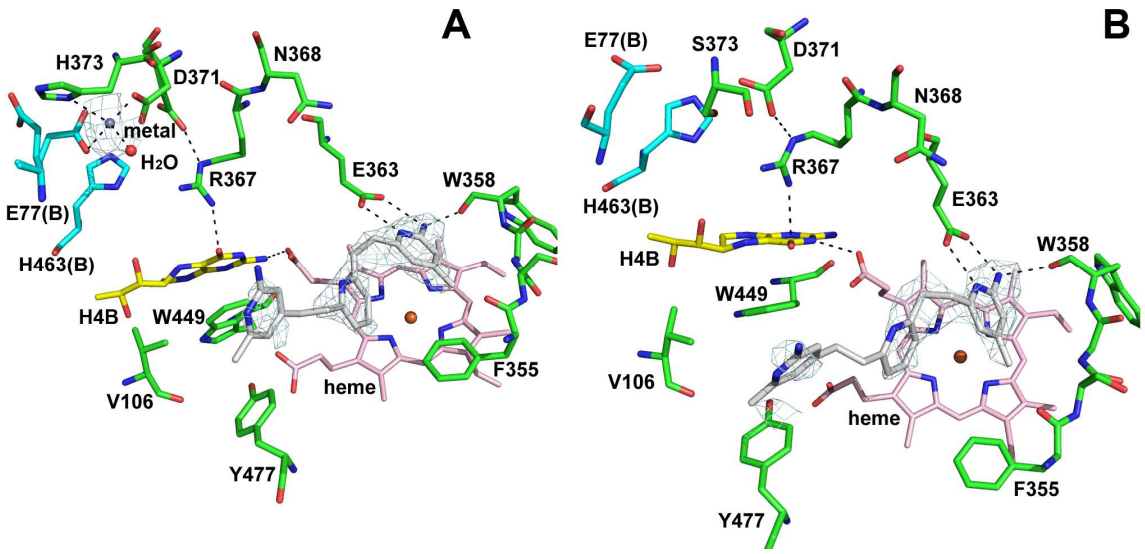

Figure S6 The active site of (A) eNOS with one molecule of **3m** bound or (B) eNOS H373S mutant complexed with one molecule of **3m**. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 2.5 σ contour level. Major hydrogen bonds and the unknown metal coordination sphere are depicted with dashed lines. In panel A, R367 does not swing out and the Zn site seen in nNOS was not observed, however a partially occupied metal site was discovered nearby. The metal site is ligated with the eNOS unique His373, one out of two alternate rotamers of the conserved Asp371 and Glu77 from B subunit, and a water molecule. The compound **3m** binds in the same manner in both eNOS and eNOS H373S mutant, but we did not observe the unknown metal or alternate conformations of residues Asp371 and Glu77 (B subunit) in the mutant.

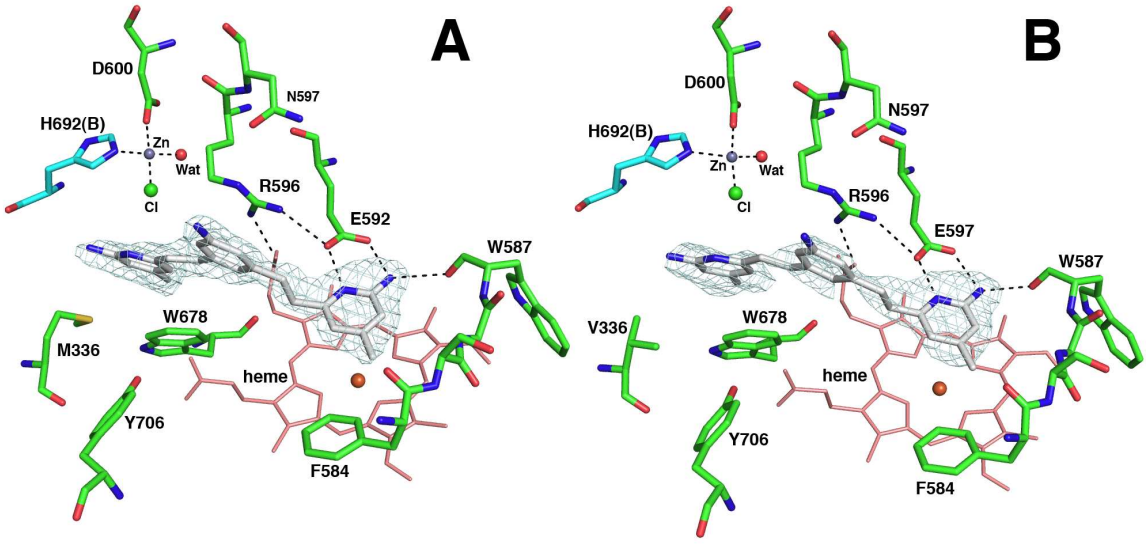

Figure S7 The active site of (A) nNOS D597N or (B) nNOS D597N/M336V mutant with inhibitor **3n** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 3 σ contour level. Major hydrogen bonds and the Zn coordination sphere are depicted with dashed lines.

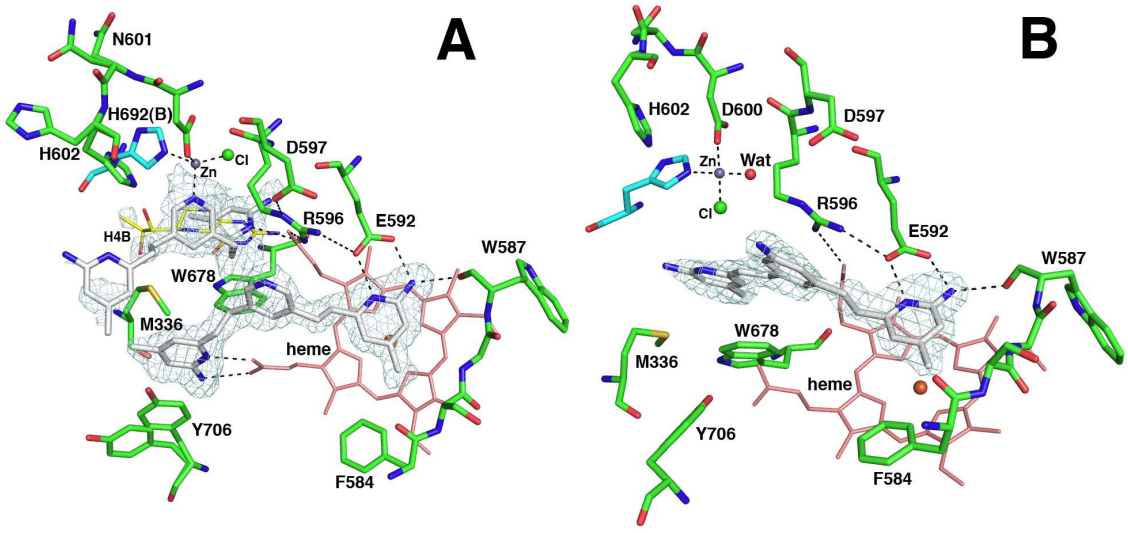

Figure S8 The active site of nNOS S602H mutant with (A) two molecules of **3j** or (B) one molecule of **3n** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 2.5 σ (**3j**) or 3 σ (**3n**) contour level. Major hydrogen bonds and the Zn coordination sphere are depicted with dashed lines. In panel A the second molecule of **3j** is only partially occupied (occupancy ~ 0.7) therefore H_4B is sharing the same space (occupancy ~ 0.3); also the side chains of Asn601, His602, and Tyr706 show alternate conformations.

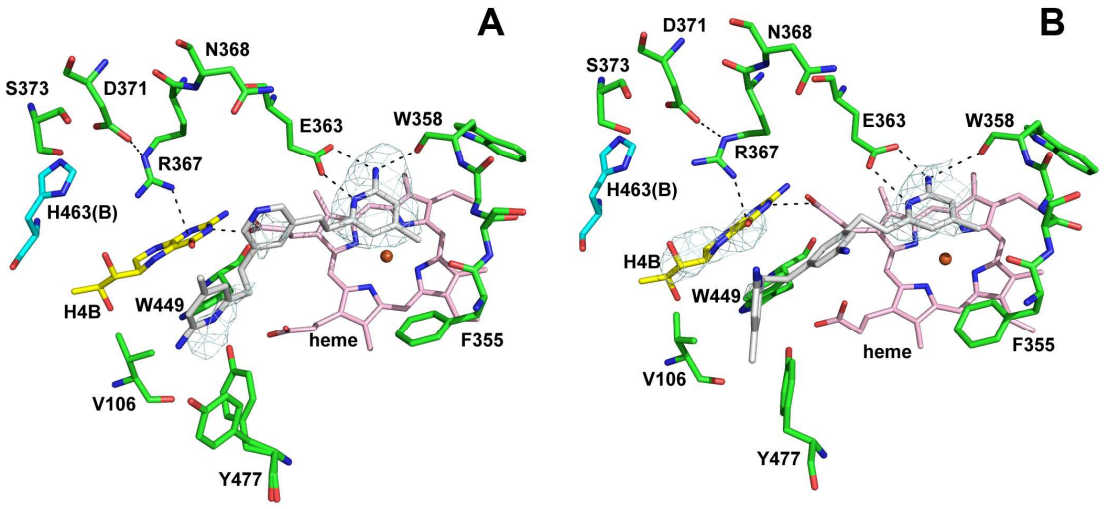

Figure S9 The active site of eNOS H373S mutant (A) bound with inhibitor **3j** or (B) with ligand **3n** bound. The sigma-A weighted Fo-Fc omit density maps for the inhibitors are shown at 2.5 σ contour level. Major hydrogen bonds are depicted with dashed lines. In panel B, the second aminopyridine of **3n** is partially disordered. The H_4B (yellow) is not disturbed by ligand binding illustrated by the omit electron density for the pterin at 2.5 σ.