Mobility of a conserved tyrosine residue controls isoform-dependent enzyme-inhibitor interactions in nitric oxide synthases

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Supporting Information

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## Table S1. Crystallographic data collection and refinement statistics

| Data set | eNOS-2 | eNOS-3 | eNOS-4 | eNOS-5 |
|----------|--------|--------|--------|--------|

### Data collection

| PDB code | 4CWV | 4CWW | 4CWX | 4CWY |
|----------|------|------|------|------|
| Space group | P2₁,2,₁ | P2₁,2,₁ | P2₁,2,₁ | P2₁,2,₁ |
| Cell dimensions (Å) | 58.3 106.7 156.9 | 58.0 106.8 156.8 | 57.9 106.5 157.0 | 57.9 106.5 157.0 |
| a, b, c (Å) | 58.3 (2.38-2.34) | 58.0 (2.20-2.16) | 57.9 (2.19-2.15) | 57.9 (2.19-2.15) |
| Resolution (Å) | 2.34 | 2.16 | 2.15 | 2.15 |
| Rmerge | 0.067 (0.654) | 0.064 (0.460) | 0.073 (0.666) | 0.071 (0.619) |
| I / σ(I) | 23.0 (2.1) | 24.7 (2.7) | 31.8 (2.3) | 32.7 (2.3) |
| No. unique reflections | 41,977 | 52,912 | 53,868 | 53,726 |
| Completeness (%) | 99.7 (100.0) | 99.5 (99.8) | 99.9 (100.0) | 99.8 (100.0) |
| Redundancy | 4.0 (4.1) | 4.0 (3.9) | 5.7 (4.1) | 5.7 (4.1) |

### Refinement

| Resolution (Å) | 2.34 | 2.16 | 2.15 | 2.15 |
| No. reflections used | 39,696 | 50,039 | 51038 | 50,915 |
| R<sub>work</sub> / R<sub>free</sub> | 0.177/0.224 | 0.171/0.212 | 0.171/0.210 | 0.167/0.204 |
| No. atoms |
| Protein | 6407 | 6474 | 6418 | 6407 |
| Ligand/ion | 187 | 197 | 197 | 202 |
| Water | 196 | 328 | 299 | 290 |
| R.m.s. deviations |
| Bond lengths (Å) | 0.015 | 0.015 | 0.010 | 0.010 |
| Bond angles (deg) | 1.54 | 1.48 | 1.93 | 1.94 |
|                             | Y477A-3 | Y477A-6 | L111A-3 | L111A-5 |
|-----------------------------|---------|---------|---------|---------|
| **Data collection**         |         |         |         |         |
| PDB code                    | 4CWZ    | 4CX0    | 4CX1    | 4CX2    |
| Space group                 | P2₁2₁2₁ | P2₁2₁2₁ | P2₁2₁2₁ | P2₁2₁2₁ |
| Cell dimensions             | 57.8 106.4 156.7 | 57.7 106.5 156.2 | 57.7 106.2 156.1 | 57.9 106.6 156.8 |
| a, b, c (Å)                 |         |         |         |         |
| Resolution (Å)              | 2.08 (2.12-2.08) | 2.20 (2.24-2.20) | 2.13 (2.17-2.13) | 2.05 (2.09-2.05) |
| Rmerge                      | 0.071 (0.798) | 0.082 (0.614) | 0.083 (0.534) | 0.070 (0.556) |
| I / σI                      | 20.5 (2.0) | 16.9 (2.1) | 16.1 (1.7) | 21.0 (1.9) |
| No. unique reflections      | 59,073  | 50,206  | 54,328  | 59,766  |
| Completeness (%)            | 99.8 (100.0) | 99.4 (97.9) | 99.0 (100.0) | 95.7 (92.4) |
| Redundancy                  | 3.7 (3.7) | 3.6 (3.5) | 3.6 (3.6) | 3.5 (3.5) |
| **Refinement**              |         |         |         |         |
| Resolution (Å)              | 2.08    | 2.20    | 2.13    | 2.05    |
| No. reflections used        | 55,954  | 47,001  | 51,281  | 56,742  |
| R<sub>work</sub> / R<sub>free</sub>² | 0.154/0.197 | 0.155/0.203 | 0.172/0.214 | 0.163/0.201 |
| No. atoms                   |         |         |         |         |
| Protein                     | 6447    | 6437    | 6439    | 6435    |
| Ligand/ion                  | 197     | 207     | 196     | 199     |
| Water                       | 537     | 427     | 362     | 483     |
| R.m.s. deviations           |         |         |         |         |
| Bond lengths (Å)            | 0.011   | 0.012   | 0.011   | 0.009   |
| Bond angles (deg)           | 1.54    | 1.56    | 2.03    | 1.92    |
| Data set | nNOS- M336V/D597N-2 | nNOS- M336V/D597N-3 | nNOS- H341L-2 | nNOS- H341L-6 | Human iNOS-7 |
|----------|---------------------|---------------------|----------------|----------------|-------------|

### Data collection

| PDB code | 4CX3 | 4CX4 | 4CX5 | 4CX6 | 4CX7 |
|----------|------|------|------|------|------|
| Space group | P2₁,2,2₁ | P2₁,2,2₁ | P2₁,2,2₁ | P2₁,2,2₁ | P4₂,2₂ |
| Cell dimensions | 51.9 110.7 164.3 | 51.8 110.6 164.4 | 52.1 110.7 164.2 | 52.0 110.7 164.4 | 189.2 189.2 223.8 |
| a, b, c (Å) | | | | | |
| Resolution (Å) | 1.97 (2.00-1.97) | 1.98 (2.01-1.98) | 1.80 (1.83-1.80) | 1.90 (1.93-1.90) | 3.16 (3.33-3.16) |
| Rmerge | 0.041 (0.313) | 0.051 (0.419) | 0.055 (0.642) | 0.057 (0.714) | 0.232 (1.863) |
| I / σI | 34.6 (4.7) | 33.0 (3.6) | 27.1 (2.1) | 25.9 (2.4) | 12.6 (1.5) |
| No. unique reflections | 67,100 | 66,365 | 88,511 | 75,577 | 72,855 |
| Completeness (%) | 98.5 (91.9) | 99.1 (99.9) | 99.7 (100.0) | 99.6 (99.9) | 99.8 (98.9) |
| Redundancy | 4.0 (3.1) | 4.0 (4.0) | 4.0 (4.0) | 4.0 (4.0) | 11.9 (10.9) |

### Refinement

| Resolution (Å) | 1.97 | 1.98 | 1.80 | 1.90 | 3.16 |
| No. reflections used | 63,545 | 62,884 | 84,013 | 71,736 | 68,962 |
| Rwork / Rfree | 0.175/0.209 | 0.175/0.212 | 0.191/0.226 | 0.185/0.217 | 0.173/0.216 |
| No. atoms | | | | | |
| Protein | 6689 | 6691 | 6685 | 6674 | 13,468 |
| Ligand/ion | 194 | 227 | 181 | 181 | 421 |
| Water | 428 | 413 | 358 | 382 | 0 |
| R.m.s. deviations | | | | | |
| Bond lengths (Å) | 0.014 | 0.015 | 0.011 | 0.010 | 0.014 |
| Bond angles (deg) | 1.39 | 1.50 | 1.43 | 1.33 | 1.95 |
1 See Table 1 for the inhibitor chemical formula.

2 \( R_{\text{free}} \) was calculated with the 5\% of reflections set aside throughout the refinement. The set of reflections for the \( R_{\text{free}} \) calculation were kept the same for all data sets of each isoform according to those used in the data of the starting model.
Fig. S1 Comparison of the hyperbolic and quadratic curve fittings using SigmaPlot. (A) An apparent $K_s$ of 1.97 µM was given by fitting ($R = 0.996$) the titration data of (2R, 4S)-6 to the wild type nNOS with a hyperbolic equation, \[ \Delta A = B_{\text{max}} \frac{[L]}{(K_s + [L])}, \] where $\Delta A$ is the absorbance difference, $B_{\text{max}}$ is the maximum absorbance change to infinite ligand concentration, and $[L]$ the ligand concentration. (B) An apparent $K_s$ of 0.33 µM was given by a better fitting ($R = 0.999$) to the same titration data as in (A) but with the
quadratic equation,\textsuperscript{1} \( \Delta A = A_0 + (B_{\text{max}}/2[E])(K_s + [E] + [L]) - ((K_s + [E] + [L])^2 - 4[E][L])^{1/2} \), where [E] is the total enzyme concentration and \( A_0 \) a constant. (C) Titration of (3R, 4R)-2 to the nNOS M336V/D597N mutant can be fit vary well (R = 0.999?) with the hyperbolic equation to give an apparent \( K_s \) of 14.6 m M.
Fig. S2  The rat nNOS active site bound with (A) (3R, 4R)-4 (3UFW, ^2^) and (B) (3R, 4R)-5 (4EUX, ^3^). The omit Fo – Fc density map for each inhibitor is shown at the 2.5 σ contour level. Major hydrogen bonds are depicted with dashed lines.
Fig. S3 The eNOS active site bound with (A) (3R, 4R)-4 and (B) (3R, 4R)-5. The omit Fo – Fc density map for each inhibitor is shown at the 3.0 σ contour level. Major hydrogen bonds are depicted with dashed lines.
Fig. S4  Compound (2R, 4S)-6 bound to the active site of (A) nNOS (4C39, 1) and (B) eNOS (4C3A, 1). The omit Fo – Fc density map for each inhibitor is shown at the 3.0 σ contour level. Major hydrogen bonds are depicted with dashed lines.
**Fig. S5** The eNOS L111A active site bound with (A) $(3R, 4R)$-$3$ and (B) $(3R, 4R)$-$5$. The omit Fo – Fc density map for each inhibitor is shown at the $3.0 \sigma$ contour level. Major hydrogen bonds are depicted with dashed lines. The binding modes of $(3R, 4R)$-$3$ and $(3R, 4R)$-$5$ are the same as that observed in wild-type eNOS (Fig. 3B and Fig. S2B, respectively).
**Fig. S6** The nNOS H341L active site bound with (A) (3R, 4R)-2 and (B) (2R, 4S)-6. The omit Fo – Fc density map for each inhibitor is shown at the 3.0 σ contour level. Major hydrogen bonds are depicted with dashed lines. The binding modes of 2 and 6 are the same as that seen in wild-type nNOS (Fig. 2A and Fig. S3A, respectively).
Fig. S7  Compound 7 bound to the active site of (A) nNOS (4CTW \textsuperscript{4}) and (B) eNOS (4CU0 \textsuperscript{4}). The omit Fo – Fc density map for each inhibitor is shown at the 3.0 σ contour level. Major hydrogen bonds are depicted with dashed lines.
References

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