SUPPORTING INFORMATION

Trapping conformational states of a flavin-dependent N-monooxygenase in crystallo reveals protein and flavin dynamics
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Table S1
FAD conformations in SidA, PvdA, and KtzI ornithine hydroxylase structures

|       | PDB ID | Active Site Ligands<sup>a</sup> | Space Group | Resolution (Å) | Mutation | Flavin Redox State | Flavin Conformation |
|-------|--------|-------------------------------|-------------|----------------|----------|---------------------|---------------------|
| SidA  | 4B63   | FAD, NAP, ORN                 | I222        | 1.90           | WT       | ox                  | in                  |
| SidA  | 4B64   | FAD, NAP, LYS                 | I222        | 2.28           | WT       | ox                  | in                  |
| SidA  | 4B65   | FAD, NDP                      | I222        | 2.32           | WT       | red                 | in                  |
| SidA  | 4B66   | FAD, NAP, ARG                 | I222        | 2.90           | WT       | red                 | in                  |
| SidA  | 4B67   | FAD, NAP, ORN                 | I222        | 2.75           | WT       | reoxidised          | in                  |
| SidA  | 4B68   | FAD, NAP, ARG                 | I222        | 2.29           | WT       | reoxidised          | in                  |
| SidA  | 4B69   | FAD, ORN                      | I222        | 2.30           | WT       | ox                  | in                  |
| SidA  | 5CKU   | FAD, NAP, ORN                 | I222        | 2.10           | N323A    | ox                  | in                  |
| PvdA  | 3S5W   | FAD, NAP, ONH                 | I4122       | 1.90           | WT       | ox                  | in                  |
| PvdA  | 3S61   | FAD, NDP, ORN                 | I4122       | 3.03           | WT       | red                 | in                  |
| KtzI  | 4TLX   | FDA, K, NAP, ORN              | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.23 | WT | red | in |
| KtzI  | 4TLZ   | FDA, K, NAP, ORN              | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.41 | WT | ox | <sup>out</sup> |
| KtzI  | 4TM0   | FAD, K, NAP, ORN              | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.74 | WT | reoxidised | <sup>out</sup> |
| KtzI  | 4TM1   | FDA, BR, NAP                  | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.39 | WT | red | in |
| KtzI  | 4TM3   | FAD, BR                       | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.09 | WT | ox | <sup>out</sup> |
| KtzI  | 4TM4   | FDA, BR, NAP                  | P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub> | 2.63 | WT | red | in |

<sup>a</sup>Lists the PDB ligand IDs stated in the entry.
## Table S2
### X-ray diffraction and data collection statistics

|                      | FAD<sub>ox</sub> | FAD<sub>ox</sub> – NADP<sup>+</sup> | FAD<sub>red</sub> – NADP<sup>+</sup> | FAD<sub>red</sub> – L-Orn |
|----------------------|-------------------|---------------------------------|-------------------------------|------------------------|
| **Beamline**         | APS (24-ID-C)     | ALS (4.2.2)                     | APS (24-ID-C)                 | APS (24-ID-E)          |
| **Space group**      | P<sub>2</sub><sub>1</sub> | P<sub>2</sub><sub>1</sub>     | P<sub>2</sub><sub>1</sub> | P<sub>2</sub><sub>1</sub> |
| **Unit cell**        | a = 76.8,         | a = 80.5,                       | a = 85.2,                     | a = 105.9              |
| **parameters (Å,°)** | b = 156.9,        | b = 154.9,                      | b = 153.0,                    | b = 155.0              |
|                      | c = 88.6,         | c = 90.5,                       | c = 91.1,                     | c = 146.85,            |
|                      | β = 110.4         | β = 109.2                       | β = 110.9,                    | β = 91.01              |
| **Mols. in asu.**    | 4                 | 4                               | 4                             | 8                      |
| **Wavelength (Å)**   | 0.97918           | 1.00000                         | 0.97918                       | 0.97918                |
| **Resolution (Å)**   | 156.9 – 2.09      | 63.2 – 1.95                     | 153.0 – 2.34                  | 155.04 - 2.23          |
| **Observations**     | 265666 (10926)    | 544409 (24487)                  | 374864 (9559)                 | 1006051 (37428)        |
| **Unique reflections** | 114542 (5054)   | 151085 (7458)                   | 90888 (3292)                  | 225621 (8596)          |
| **R<sub>merge</sub>** | 0.110 (0.858)    | 0.092 (0.790)                   | 0.198 (1.372)                 | 0.209 (1.400)          |
| **R<sub>meas</sub>** | 0.142 (1.113)    | 0.108 (0.947)                   | 0.227 (1.637)                 | 0.239 (1.583)          |
| **R<sub>pim</sub>**  | 0.088 (0.702)    | 0.056 (0.516)                   | 0.111 (0.874)                 | 0.113 (0.727)          |
| **Mean I/σ**         | 6.0 (1.0)         | 12.1 (1.5)                      | 8.9 (0.8)                     | 7.8 (1.0)              |
| **CC<sub>1/2</sub>** | 0.991 (0.416)    | 0.997 (0.523)                   | 0.984 (0.8)                   | 0.968 (0.399)          |
| **Completeness (%)** | 98.4 (87.4)       | 99.5 (99.0)                     | 98.5 (71.9)                   | 97.6 (75.2)            |
| **Multiplicity**     | 2.3 (2.2)         | 3.6 (3.3)                       | 4.1 (2.9)                     | 4.5 (4.4)              |
| **No. of protein residues** | 1788          | 1771                            | 1807                          | 3510                   |
| **No. of atoms**     |                   |                                 |                               |                        |
| **Protein**          | 13867             | 14033                           | 13964                         | 27310                  |
| **FAD**              | 212               | 265                             | 212                           | 424                    |
| **L-Orn**            | N/A               | N/A                             | 36                            | 54                     |
| **NADP<sup>+</sup>** | N/A               | 192                             | 192                           | N/A                    |
| **Water**            | 526               | 1069                            | 398                           | 383                    |
| **R<sub>cryst</sub>** | 0.1823 (0.2856)  | 0.1682 (0.2588)                 | 0.1835 (0.2987)               | 0.2307 (0.3103)        |
| **R<sub>free</sub><sup>a,b</sup>** | 0.2322 (0.3321) | 0.2110 (0.2990)                | 0.2491 (0.3375)               | 0.2807 (0.3580)        |
| **rmsd bonds (Å)**   | 0.007             | 0.006                           | 0.007                         | 0.008                  |
| **rmsd angles (°)**  | 0.919             | 0.869                           | 1.020                         | 1.029                  |
| **Ramachandran plot** |                   |                                 |                               |                        |
| **Favored (%)**      | 96.72             | 97.14                           | 96.59                         | 97.39                  |
| **Outliers (%)**     | 0.11              | 0.00                            | 0.11                          | 0.03                   |
| **Clashscore (PR)<sup>c</sup>** | 2.13 (99)   | 1.78 (100)                      | 2.81 (100)                    | 3.73 (99)              |
| **MolProbity score (PR)<sup>c</sup>** | 1.34 (99) | 1.09 (100)                      | 1.49 (99)                     | 1.48 (99)              |
| **Average B (Å<sup>2</sup>)** |               |                                 |                               |                        |
| **Protein**          | 37.7              | 30.2                            | 35.0                          | 57.8                   |
| **FAD**              | 32.6              | 23.6                            | 28.6                          | 49.4                   |
| **L-Orn**            | N/A               | N/A                             | 26.1                          | 41.5                   |
| **NADP<sup>+</sup>** | N/A               | 27.6                            | 30.5                          | N/A                    |
| **Water**            | 33.9              | 31.3                            | 30.0                          | 43.0                   |
| **Coord. error (Å)<sup>d</sup>** | 0.29               | 0.21                           | 0.36                          | 0.39                   |
| **PDB code**         | 6X0H              | 6X0I                           | 6X0J                          | 6X0K                   |

<sup>a</sup>Values for the outer resolution shell of data are given in parenthesis. <sup>b</sup>5% test set. <sup>c</sup>From MolProbity. The percentile ranks (PR) for Clashscore and MolProbity score are given in parentheses. <sup>d</sup>Maximum likelihood-based coordinate error estimate from PHENIX.
## Table S3
SMILES of the top 10 compounds from docking

| Site 1                      | SMILES                                                                 |
|-----------------------------|------------------------------------------------------------------------|
| 1                           | O=C1c2cccccc2c2cc([N+])(=O)(O=)cc3cccc1c23                             |
| 2                           | Oc1ccc(c2cccccc2)c2cc3c(cc2n1)OCCO3                                   |
| 3                           | CC(=O)NCCc1c(c2cccccc2)[nH]c2cccccc12                                  |
| 4                           | Oc1nc2cc(C(F)(F)F)cc(n3cccccc3)c2nc10                                 |
| 5                           | CCCc1nc2c3ccc(F)cc3c3c(=O)[nH]cc3c3c2[nH]1                              |
| 6                           | Cc1ccc(C(O)(c2ccc(C)cc2)c2cccn2)cc1                                  |
| 7                           | C0c1cccc(c2cccccc3c2CC(=O)C(N)CC3)c1                                  |
| 8                           | O=C(/C=C/c1cccc(/C=C/C(=O)c2cccccc2)c1)NO                              |
| 9                           | Ce1ccc(O)c2[nH]e(Cc3cccc4cccccc34)nc12                                |
| 10*                         | O=c1c2cccccc2c2nc3[nH]e(=O)[nH]c(=O)c3cc12                            |

| Site 2                      | SMILES                                                                 |
|-----------------------------|------------------------------------------------------------------------|
| 1                           | O=c1[nH]c(c2cccccc2)cc2onc(c3cccccc3)c12                               |
| 2                           | CC(=O)Oc1cccc(c2cc3cc(C)cccc3oc2=O)c1                                  |
| 3                           | O=C(Nc1ccc(F)cc1)C(=O)C1C(=O)Nc2cccccc12                               |
| 4                           | Cc1ccc2ccce(NC30C(=O)c4cccccc34)c2n1                                   |
| 5*                          | O=c1c2cccccc2c2nc3[nH]e(=O)[nH]c(=O)c3cc12                            |
| 6                           | O=C/C(=C/c2ccc(O)c((N+)/)(=O)[O-])c2)/COc2cccccc12                     |
| 7                           | O=C(Nc1nnc(c2ccccc2)s1)c1cccccc1F                                      |
| 8                           | CC(=O)N1CC2(NC(=O)c3cccccc3N2)c2cccccc12                               |
| 9                           | O=C(O)/C(=C/C(=O)c1cccc(c2nnn[nH]2)c1)/O                               |
| 10                          | N=c1oc2cccccc2cc1C(=O)Nc1cccc(O)c1                                    |

*Compound 10 of site 1 and compound 5 of site 2 are the same.*
### Table S4
**Physicochemical properties of the top 10 compounds from docking**

|                         | Site 1       | Site 2       |
|-------------------------|--------------|--------------|
| Molecular weight (g/mol)| 284 ± 10     | 287 ± 14     |
| No. heavy atoms         | 21.3 ± 0.7   | 21.3 ± 1.1   |
| No. aromatic atoms      | 15.7 ± 2.3   | 15 ± 3.2     |
| Fraction of C atoms in sp³ | 0.109 ± 0.09 | 0.052 ± 0.06 |
| No. rotatable bonds     | 2.4 ± 1.8    | 2.5 ± 1.4    |
| No. H-bond acceptors    | 3.2 ± 1.6    | 4.0 ± 1.3    |
| No. H-bond donors       | 1.5 ± 0.7    | 1.6 ± 1.0    |
Figure S1. Biosynthetic pathway for hydroxamate-containing siderophores. SidA (yellow) catalyzes the first step in the production of hydroxamate-containing siderophores in *Aspergillus fumigatus*. The components of each molecule which originate from L-Orn are shown in blue. SidF and SidL (purple) are N\textsuperscript{5}-acetylases. SidD and SidC (blue) are non-ribosomal peptide synthetases. SidG (green) is an N\textsuperscript{2}-acetylase. An unidentified enzyme (orange) is a hydroxylase.
Figure S2. Electron density maps for the FAD, Tyr324, NADP\(^+\) and L-Orn in SidA structures (polder omit maps contoured at 3.5\(\sigma\)). (A) Oxidized enzyme without ligands bound. (B) Oxidized enzyme complexed with NADP\(^+\). (C) Dithionite-reduced enzyme complexed with L-Orn. (D) Reduced enzyme complexed with NADP\(^+\) and L-Orn.
Multiple sequence alignment of ornithine hydroxylases. The Tyr-loop is highlighted.

| Accession | Sequence |
|-----------|----------|
| KAB8068769.1 | KAB8068769.1 |
| XP_026607835.1 | XP_026607835.1 |
| SidA | SidA |
| OKP15176.1 | OKP15176.1 |
| PvdA | PvdA |
| WP_026218888.1 | WP_026218888.1 |
| WP_011603723.1 | WP_011603723.1 |
| KtzI | KtzI |

Figure S3. Multiple sequence alignment of ornithine hydroxylases. The Tyr-loop is highlighted in yellow.
Figure S4. Hydrogen bonding between His91 and the ribityl 4’-OH of SidA in the resting state (green), dithionite-reduced enzyme with L-Orn bound (light orange), oxidized enzyme with NADP⁺ (pale blue), and reduced enzyme with NADP⁺ (pink). Oxidized and reduced FADs are colored yellow and gray, respectively.
Figure S5. Chemical structures of the top 10 compounds from docking to site 1.
Figure S6. Chemical structures of the top 10 compounds from docking to site 2.
Figure S7. The top-ranked compound docked to site 2. The FAD is colored yellow.