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[a]	Space Group	Resolution (Å)	Muta-tion	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	$I4_122$	1.90	WT	ox	in
PvdA	3S61	FAD, NDP, ORN	$I4_122$	3.03	WT	red	in
KtzI	4TLX	FDA, K, NAP, ORN	$P2_12_12_1$	2.23	WT	red	in
KtzI	4TLZ	FAD, K, NAP, ORN	$P2_12_12_1$	2.41	WT	ox	**out**
KtzI	4TM0	FAD, K, NAP, ORN	$P2_12_12_1$	2.74	WT	reoxidised	**out**
KtzI	4TM1	FDA, BR, NAP	$P2_12_12_1$	2.39	WT	red	in
KtzI	4TM3	FAD, BR	$P2_12_12_1$	2.09	WT	ox	**out**
KtzI	4TM4	FDA, BR, NAP	$P2_12_12_1$	2.63	WT	red	in

[a]Lists the PDB ligand IDs stated in the entry.

Table S2
X-ray diffraction and data collection statistics

	FAD_{ox}	**FAD_{ox} – $NADP^+$**	**FAD_{red} – $NADP^+$ – L-Orn**	**FAD_{red} – L-Orn**
Beamline	APS (24-ID-C)	ALS (4.2.2)	APS (24-ID-C)	APS (24-ID-E)
Space group	$P2_1$	$P2_1$	$P2_1$	$P2_1$
Unit cell parameters (Å,°)	a = 76.8, b = 156.9, c = 88.6, β = 110.4	a = 80.5, b = 154.9, c = 90.5, β = 109.2	a = 85.2, b = 153.0, c = 91.1, β = 110.9	a = 105.9 b =155.0 , c =146.85, 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 (2.12 – 2.09)	63.2 – 1.95 (1.98 – 1.95)	153.0 – 2.34 (2.38 – 2.34)	155.04 - 2.23 (2.26-2.23)
Observations[a]	265666 (10926)	544409 (24487)	374864 (9559)	1006051 (37428)
Unique reflections[a]	114542 (5054)	151085 (7458)	90888 (3292)	225621 (8596)
$R_{merge}(I)$[a]	0.110 (0.858)	0.092 (0.790)	0.198 (1.372)	0.209 (1.400)
$R_{meas}(I)$[a]	0.142 (1.113)	0.108 (0.947)	0.227 (1.637)	0.239 (1.583)
$R_{pim}(I)$[a]	0.088 (0.702)	0.056 (0.516)	0.111 (0.874)	0.113 (0.727)
Mean I/σ[a]	6.0 (1.0)	12.1 (1.5)	8.9 (0.8)	7.8 (1.0)
$CC_{1/2}$	0.991 (0.416)	0.997 (0.523)	0.984 (0.8)	0.986 (0.399)
Completeness (%)[a]	98.4 (87.4)	99.5 (99.0)	98.5 (71.9)	97.6 (75.2)
Multiplicity[a]	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
$NADP^+$	N/A	192	192	N/A
Water	526	1069	398	383
R_{cryst}[a]	0.1823 (0.2856)	0.1682 (0.2588)	0.1835 (0.2987)	0.2307 (0.3103)
R_{free}[a,b]	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[c]				
Favored (%)	96.72	97.14	96.59	97.39
Outliers (%)	0.11	0.00	0.11	0.03
Clashscore (PR)[c]	2.13 (99)	1.78 (100)	2.81 (100)	3.73 (99)
MolProbity score (PR)[c]	1.34 (99)	1.09 (100)	1.49 (99)	1.48 (99)
Average B ($Å^2$)				
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
$NADP^+$	N/A	27.6	30.5	N/A
Water	33.9	31.3	30.0	43.0
Coord. error (Å)[d]	0.29	0.21	0.36	0.39
PDB code	6X0H	6X0I	6X0J	6X0K

[a]Values for the outer resolution shell of data are given in parenthesis. [b]5% test set. [c]From MolProbity. The percentile ranks (PR) for Clashscore and MolProbity score are given in parentheses. [d]Maximum likelihood-based coordinate error estimate from PHENIX.

Table S3
SMILES of the top 10 compounds from docking

Site 1	
1	O=C1c2ccccc2c2cc([N+](=O)[O-])cc3cccc1c23
2	Oc1cc(c2ccccc2)c2cc3c(cc2n1)OCCO3
3	CC(=O)NCCc1c(c2ccccc2)[nH]c2ccccc12
4	Oc1nc2cc(C(F)(F)F)cc(n3cccc3)c2nc1O
5	CCCc1nc2c3ccc(F)cc3c3c(=O)[nH]ccc3c2[nH]1
6	Cc1ccc(C(O)(c2ccc(C)cc2)c2ccccn2)cc1
7	COc1cccc(c2cccc3c2CC(=O)C(N)CC3)c1
8	O=C(/C=C/c1cccc(/C=C/C(=O)c2ccccc2)c1)NO
9	Cc1ccc(O)c2[nH]c(Cc3cccc4ccccc34)nc12
10[a]	O=c1c2ccccc2c2nc3[nH]c(=O)[nH]c(=O)c3cc12
Site 2	
1	O=c1[nH]c(c2ccccc2)cc2onc(c3ccccc3)c12
2	CC(=O)Oc1cccc(c2cc3cc(C)ccc3oc2=O)c1
3	O=C(Nc1ccc(F)cc1)C(=O)C1C(=O)Nc2ccccc12
4	Cc1ccc2cccc(NC3OC(=O)c4ccccc34)c2n1
5[a]	O=c1c2ccccc2c2nc3[nH]c(=O)[nH]c(=O)c3cc12
6	O=C1/C(=C/c2ccc(O)c([N+](=O)[O-])c2)/COc2ccccc12
7	O=C(Nc1nnc(c2ccccc2)s1)c1ccccc1F
8	CC(=O)N1CC2(NC(=O)c3ccccc3N2)c2ccccc12
9	O=C(O)/C(=C/C(=O)c1cccc(c2nnn[nH]2)c1)/O
10	N=c1oc2ccccc2cc1C(=O)Nc1cccc(O)c1

[a]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 sp^3	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

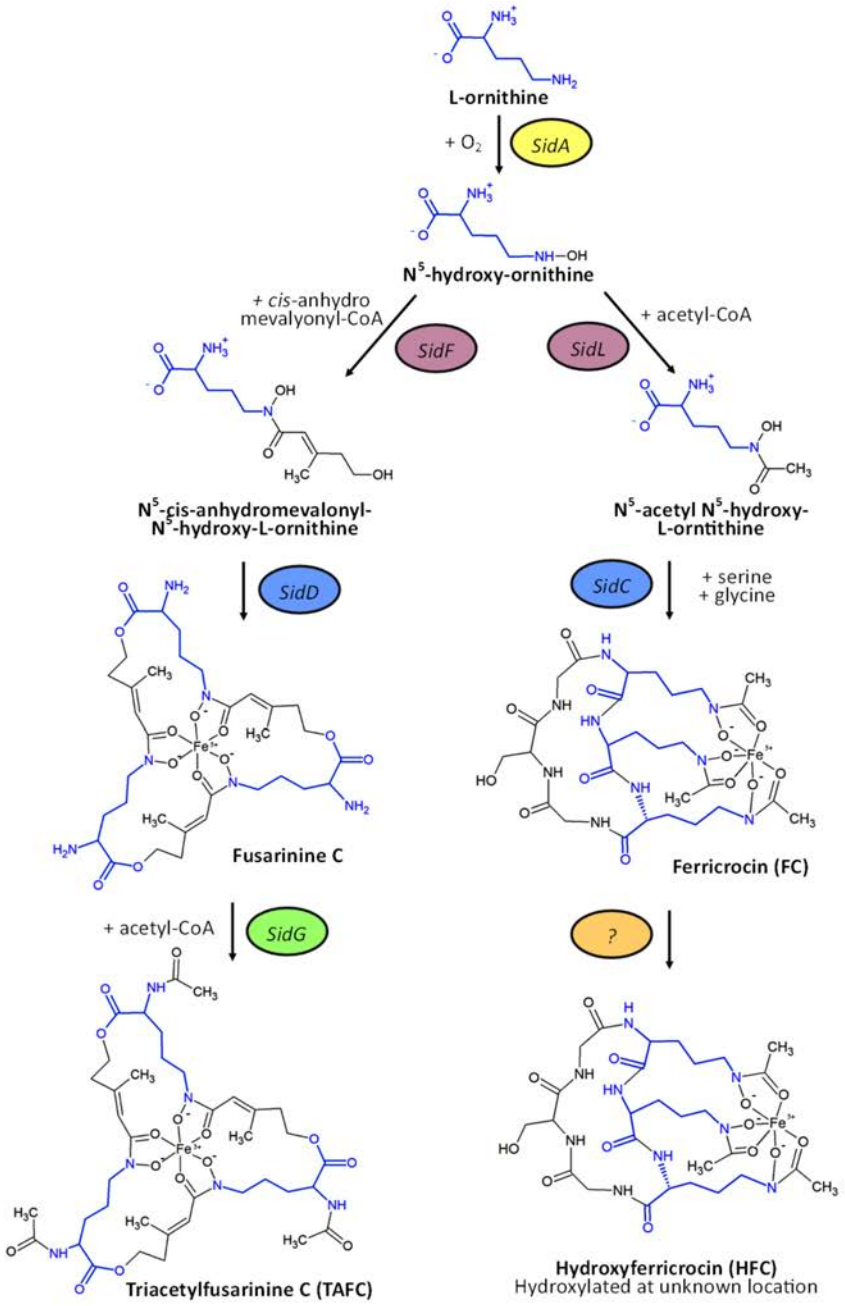

Figure S1. Biosynthetic pathway for hydroxamate-containing siderophores. SidA (yellow) catalyzes the first step in the production of hydroxamate-containing siderophores in *Aspergillus fumigatus.* The components of each molecule which originate from L-Orn are shown in blue. SidF and SidL (purple) are N^5-acetylases. SidD and SidC (blue) are non-ribosomal peptide synthetases. SidG (green) is an N^2-acetlyase. An unidentified enzyme (orange) is a hydroxylase.

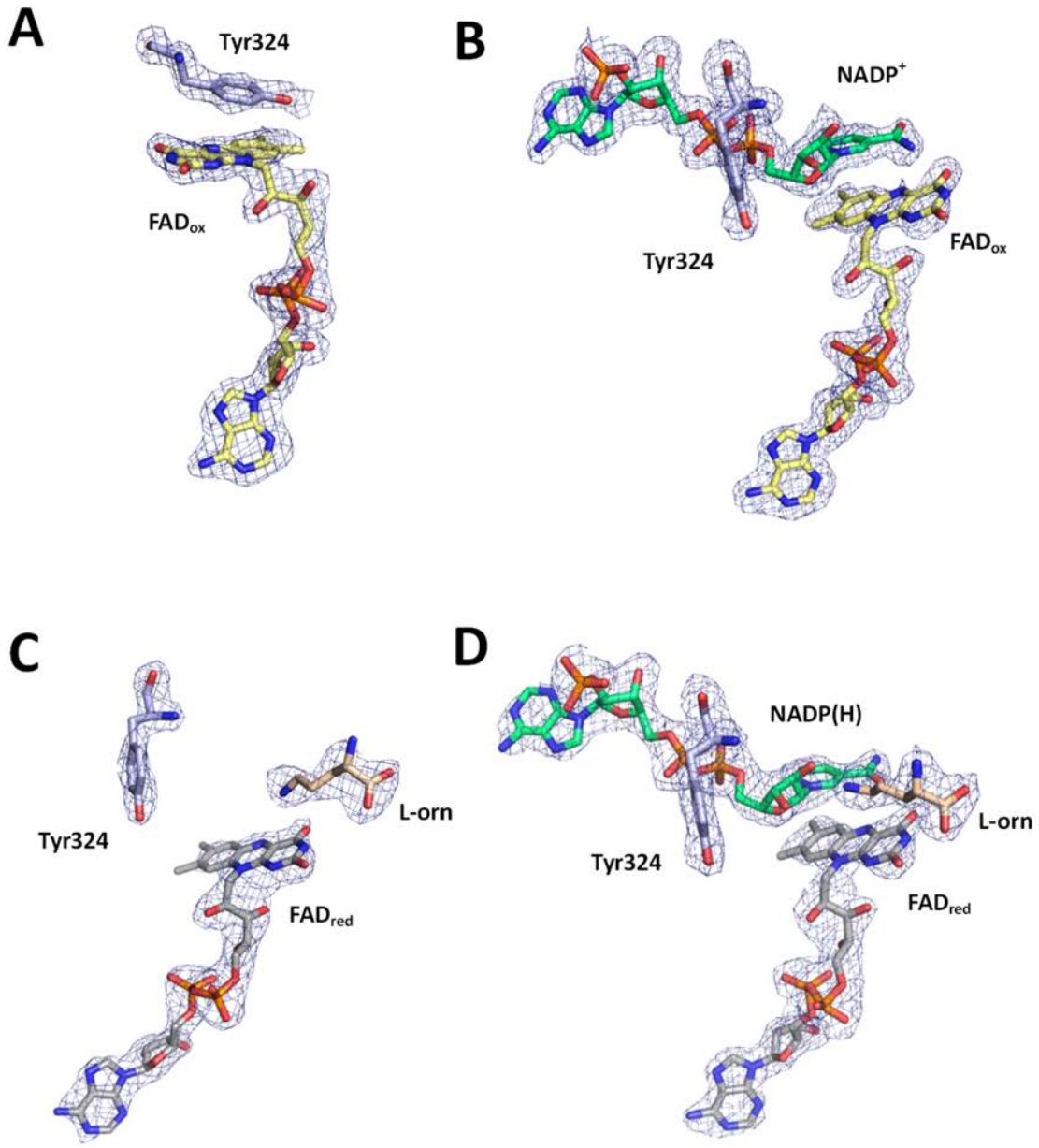

Figure S2. Electron density maps for the FAD, Tyr324, $NADP^+$ and L-Orn in SidA structures (polder omit maps contoured at 3.5σ). (A) Oxidized enzyme without ligands bound. (B) Oxidized enzyme complexed with $NADP^+$. (C) Dithionite-reduced enzyme complexed with L-Orn. (D) Reduced enzyme complexed with $NADP^+$ and L-Orn.

```
CLUSTAL O(1.2.4) multiple sequence alignment

KtzI                ------------------------------MTVAHAGESPTHDVVGVGFGPANLSLAVALE         31
WP_011603723.1      ---------------------------------MSAREFDIYDVVGIGFGPSNLSLAVALD         28
WP_109279069.1      ---------------------------------MDVNAADIYDVVGVGFGPSNLAFAIALE         28
WP_026218888.1      ---------------------------------MGMFGHEIHDVVGIGFGPSNLSLAIALE         28
PvdA                ------------------------------MTQATATAVVHDLIGVGFGPSNIALAIALQ         30
OKP15176.1          MEPIPRKSELPFHSEMA---PRTQAPTNRTLLRSVPADEMHDLLCVGFGPASLAIAIALH         57
SidA                MESVERKSESSYLGMRNMQ-PEQRLSLDPPRLRSTPQDELHDLLCVGFGPASLAIAIALH         59
XP_026607835.1      MEPLQRKSE-DFQSYRKMPLAQQRA---PQRLRPTPPDELHDLLCVGFGPASLSIAVALH         56
KAB8068769.1        MEPVERKLEIGSRSYPKMPLTQQRSSGESPRLKASPKDELHDLLCVGFGPASLAIAIALN         60
                                                                :*:: :****:.:::*:**.

KtzI                ESP------------AALTSAFFERRASISWHQGMLLPAAKMQVSFLKDLATFRNPASRF         79
WP_011603723.1      EFRVN-------GMGNVFSNIFFERRSSFAWHPSMLLPSATMQISFLKDLVTFRNPTSSF         81
WP_109279069.1      ENGAD-------SPAGPVTSAFFERQSSFGWHRNMLLPSATMQVSFLKDLATLRNPVSRF         81
WP_026218888.1      EHQAN-------ESARPVTAAFFERQPAFGWHRNMLLPSTTMQISFLKDLATFRNPVSRF         81
PvdA                ERAQ---------AQGALEVLFLDKQGDYRWHGNTLVSQSELQISFLKDLVSLRNPTSPY         81
OKP15176.1          DAMDPALSRAAS-GSFKPKVCFLERQKQFAWHSGMLVPGSRMQISFIKDLATLRDPRSSF        116
SidA                DALDPRLNKSASNIHAQPKICFLERQKQFAWHSGMLVPGSKMQISFIKDLATLRDPRSSF        119
XP_026607835.1      DALDPCLNKCAPTSGWQPKVAFLERQKQFAWHSGMQIPGAKMQISFIKDLATLRDPRSSF        116
KAB8068769.1        DALDPCLNKSAPNPHWQPKVCFLERQKQFAWHSGMLVPGSKMQISFIKDLATMRNPRSSF        120
                    :                   *::::    ** .  :  : :*:**:***.::*:* * :

KtzI                SFVSFLHERGRLVRFANNHDFFPTRREFHDYLEWAESKLAHEVSYDSEVTAIRPGPGRPV        139
WP_011603723.1      SFVAYLHESGRLPRFVNNQDFFPTREEFHQYLEWAQARVAHRVAYGSEARSLRLPAGVGP        141
WP_109279069.1      GFISFLHASGRLEQFINTQTFFPTREEFHQYLDWAQGLFSDRVSYGSEVTGIQLPTETTS        141
WP_026218888.1      GFISYLHASGRLPQFVNAQDFFPTRQEFHQYLEWAESSVTDRVSYGSDVTSIRPPQGIAA        141
PvdA                SFVNYLHKHDRLVDFINLGTFYPCRMEFNDYLRWVASHFQEQSRYGEEVLRIEPMLS---        138
OKP15176.1          TFLNYLHNKNRLIHFTNLGTFLPARMEFEDYMRWCAQPFENVVSYGEEVVDVVPGSK--T        174
SidA                TFLNYLHQKGRLIHFTNLSTFLPARLEFEDYMRWCAQQFSDVVAYGEEVVEVIPGKSDPS        179
XP_026607835.1      TFLNYLHQKDRLIHFTNLSTFLPARMEFEDYMRWCANQFSDVVSYGEEVIEVLPGKSSPD        176
KAB8068769.1        TFLNYLHQKDRLIHFTNLSTFLPARLEFEDYMRWCAQKFAHVVTYGEEVIEVIPGKTNPS        180
                     *: :**  .**  * *   * * * **.:*: *    . .   *..:.  :

KtzI                -DS-----VLVDVSTPEATRTVEARNIVISTGLVPRMPAG---VQSDEFVWHSSRFLDHF        190
WP_011603723.1      -ERADRLCLQVADAASGTSRMVEARNVVISTGLVPTMPTG---VERGERVWHSSEFLERF        197
WP_109279069.1      -ATAQYLQVNVRDCGTGDTRTVAARNIVISTGLVPQVPDG---VASDERVWHSSEFLERF        197
WP_026218888.1      -RDAKHLEIEVEDLVSGATRLVKARNVTVSTGLVPRLPQG---IERDERVWHSSEFLEKF        197
PvdA                AGQVEALRVISRNAD-GEELVRTTRALVVSPGGTPRIPQVFRALKGDGRVFHHSQYLEHM        197
OKP15176.1          DGVVDYFVVRSRNAETGEISSRRARKVVVALGGKAKMPPG---FPQDARIMHSSKYCTGL        231
SidA                SSVVDFFTVRSRNVETGEISARRTRKVVIAIGGTAKMPSG---LPQDPRIIHSSKYCTTL        236
XP_026607835.1      SHVVDYFTVLSRNVETGEISSRSARKVVLALGGSAKLPAE---LPQDPRIMHSSKYCAVL        233
KAB8068769.1        STIVDFFTVKSRNIETGEISARSARKVVVALGGTAKLPKE---LPQDPRIMHSSKYCTTL        237
                            :             :* :.:: *    :*     .  .  : * *.:   :

KtzI                RDRD--PRSLRRVAVAGGGQSAAEIVRFLHDNRPDTVVHAIMPSYGYVVADNTPFANQIF        248
WP_011603723.1      RRTS--PARIRRVAVVGAGQSAAEITRFLYDELPHAEVSAIIPSYGYCVADDTPFANEVF        255
WP_109279069.1      KQAS--PDTLKKVAVVGAGQSGAEIARFLYDSLPQAQIYAIIPSYGYSIADDTPFANRVF        255
WP_026218888.1      GRMD--AAGLGSVAVVGAGQSAAEITRFLYDTLPHARVSAILPAYGYSVADDTPFANQVF        255
PvdA                AKQPCSSGKPMKIAIIGGGQSAAEAFIDLNDSYPSVQADMILRASALKPADDSPFVNEVF        257
OKP15176.1          PNVLKNESAPYNIAVVGSGQSAAEIFHDLQRRYPNSRTTLILRDTALRPSDDSPFVNEIF        291
SidA                PALLKDKSKPYNIAVLGSGQSAAEIFHDLQKRYPNSRTTLIMRDSAMRPSDDSPFVNEIF        296
XP_026607835.1      PSLLKDNDEPYNIAVLGSGQSAAEIFQDLHRRYPNSRTSLIMRDSAMRPSDDSPFVNEVF        293
KAB8068769.1        PTMLKDNQEAYNIAVLGSGQSAAEIFHDLQKRYPNSKTTLIMRDTAMRPSDDSPFVNELF        297
                                :*: *.***.**    *    *      *:   .   :*::**.*.:*

KtzI                DPAAVDDYFDGSKQAKDAFWRYHRNTNYSVVDDEVIRDLYRRGYDDEVAGAPRLNFVNLA        308
WP_011603723.1      DPEAIDDYYYATERTREALWRYHSNTNYSVVDDSVIRDLYRRSYEDDLRDVGRLRFLRLT        315
WP_109279069.1      DSAAVDDYYFGTERGREAFWRYHKNTNYSVADDEVIRDLYQRMYMEELRGTKRLHLLNLA        315
WP_026218888.1      DPGAVDEYYFGSDRTREAFWRYHKNTNYSVVDDEVIRDLYRRSYEEEVRGVRRLNFLNLT        315
PvdA                APKFTDLIYSREHAERERLLREYHNTNYSVVDTDLIERIYGVFYRQKVSGIPRHAFRCMT        317
OKP15176.1          NPERVEKFYQLPQEERHARIAKEKATNYSVVRLELIEEIYNTMYLQRVQNPDESTWQHRI        351
SidA                NPERVDKFYSQSAAERQRSLLADKATNYSVVRLELIEEIYNDMYLQRVKNPDETQWQHRI        356
XP_026607835.1      NPERTDKFFNLSASERQRSLKQDKATNYSVVRLELIEEIYHEMYLQRVKNPDETQWQHRV        353
KAB8068769.1        NPERVDKFFNLSPAERQRSLAADKATNYSVVRLELIEEIFHDMYVQRIKNPDETQWQHRI        357
                         :  :      :.        *****.  .:*. ::   * : : .  .

KtzI                HV----VGAKRIADD--TRVTVYS-------MAREESYDLDVDVLVCATGYDPMDPGDLL        355
WP_011603723.1      RV----AGVRSVGAQ--TRVSLRA-------GIDGDLRDLDVDVLVCATGYAAMEPTGLL        362
WP_109279069.1      RV----TNVKRMGNE--SRVSMES-------LLDGGSQDLDVDALVFATGYDSMDPSGVL        362
WP_026218888.1      RV----DQVKRSGDE--TRVSLRS-------LLDDRVRELDVDALVFATGYDSPEPSGLL        362
PvdA                TVERATAT------AQGIELALRDA-------GSGELSVETYDAVILATGYERQLHRQLL        364
OKP15176.1          LPERKVARIEHHNPAHRMRIHVKSVNDD----ASDGKEVLEVDALMVATGYQRDAHEQLL        407
SidA                LPERKITRVEHHGPQSRMRIHLKSSKPESEGAANDVKETLEVDALMVATGYNRNAHERLL        416
XP_026607835.1      LPGRKITRVEHYGPNKRMRVHVKAVKDGKDSLVGDGKEVLEVDALMVATGYNRNGHEQLL        413
KAB8068769.1        LPGRKITRVEHYGPHRRMRLHVRALKDGKDSIVGDGKEILEIDALMVATGYNRNAHEQLL        417
                                      .: :                      *.:: ****       :*

KtzI                GELAEHCVQDAEGRWQVDRDYRMVTTP---DLRCGIYLQGGTEHTHGLSSSLLSNLATRS        412
WP_011603723.1      GDLDQYCLRDEAGRYRIERDYRIVTAP---EMQCGIYLQGGTEHTHGLSSSLLSNIAVRS        419
WP_109279069.1      GVLDDYCLRDGAGRHRVGRDYRLVTTP---DVSCGIYLQGGTEHTHGLTSSLLSNIAVRG        419
WP_026218888.1      GDLDRYCLRDEAGRHRVGRDYRLVTSP---ELSCGIYLQGGTEHTHGLTSSLLSNIAIRS        419
PvdA                EPLAEYL-G----DHEIGRDYRLQTDE---RCKVAIYAQGFSQASHGLSDTLLSVLPVRA        416
OKP15176.1          EQVCGLR-PVGQTAWAPGRDYRVELDGGKVSAGAGIWLQGCNEKTHGLSDSLLSILAVRG        466
SidA                SKVQHLR-PTGQDQWKPHRDYRVEMDPSKVSSEAGIWLQGCNERTHGLSDSLLSVLAVRG        475
XP_026607835.1      SKVQQLR-PATQDRWTPSRDYRVDLDRSKVSADAGIWLQGSNEQTHGLSDSLLSVLAARG        472
KAB8068769.1        KNVQHLR-PAGQERWTPNREYRAELDPSKVSAQAGIWLQGCNEQTHGLSDSLLSILASRG        476
                      :                 *:**            .*: ** .: :***:.:*** :  *.

KtzI                GEIVSSIERRKS--------------------             424
WP_011603723.1      GEIIDSIVARSAERTAPCAVLAEA-------              443
WP_109279069.1      GEIADSILQRRSVDGPAASGLTAPA------              444
WP_026218888.1      GEIADSVIRRRVEHELELERNAALEVARETR              450
PvdA                EEISGSLYQHLKPGTAARALHE-HALAS---              443
OKP15176.1          GEIVDSVFGDELAGKTVQDTR-LRAML----              492
SidA                GEMVQSIFGEQLERAAVQGHQ-LRAML----              501
XP_026607835.1      GEMVESIFGEQLESAAVPDTRF-RAML----              498
KAB8068769.1        GEMVNSIFGGEIAGAAVPDTTQIRAML----              503
                            *:  *:
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Figure S3. Multiple sequence alignment of ornithine hydroxylases. The Tyr-loop is highlighted in yellow.

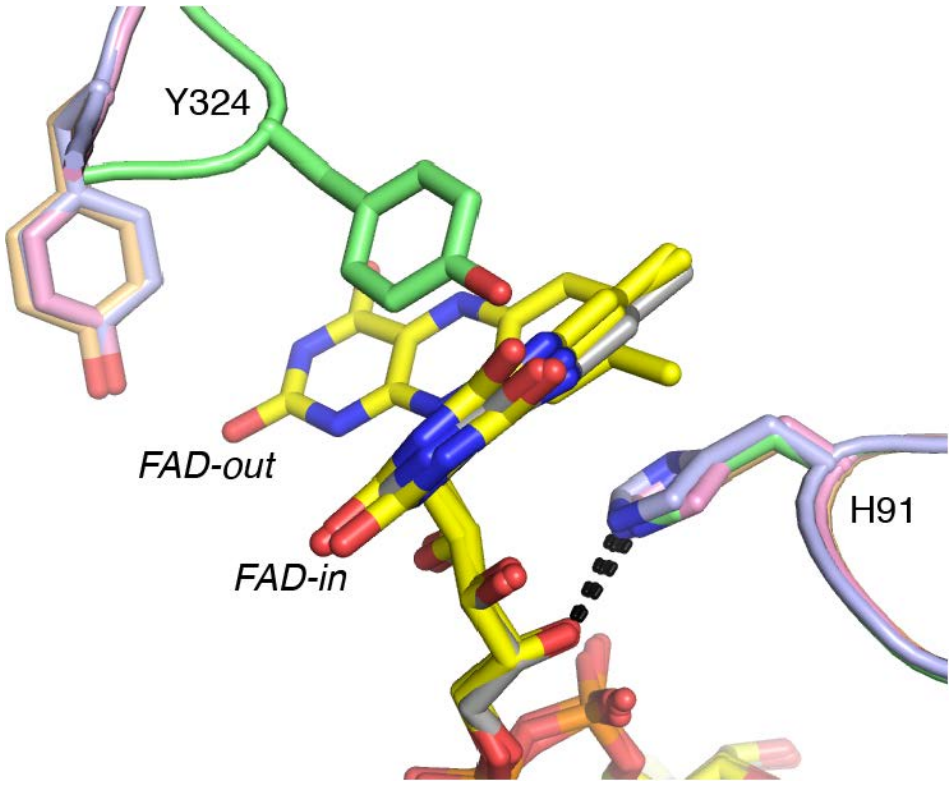

Figure S4. Hydrogen bonding between His91 and the ribityl 4′-OH of SidA in the resting state (green), dithionite-reduced enzyme with L-Orn bound (light orange), oxidized enzyme with $NADP^+$ (pale blue), and reduced enzyme with $NADP^+$ (pink). Oxidized and reduced FADs are colored yellow and gray, respectively.

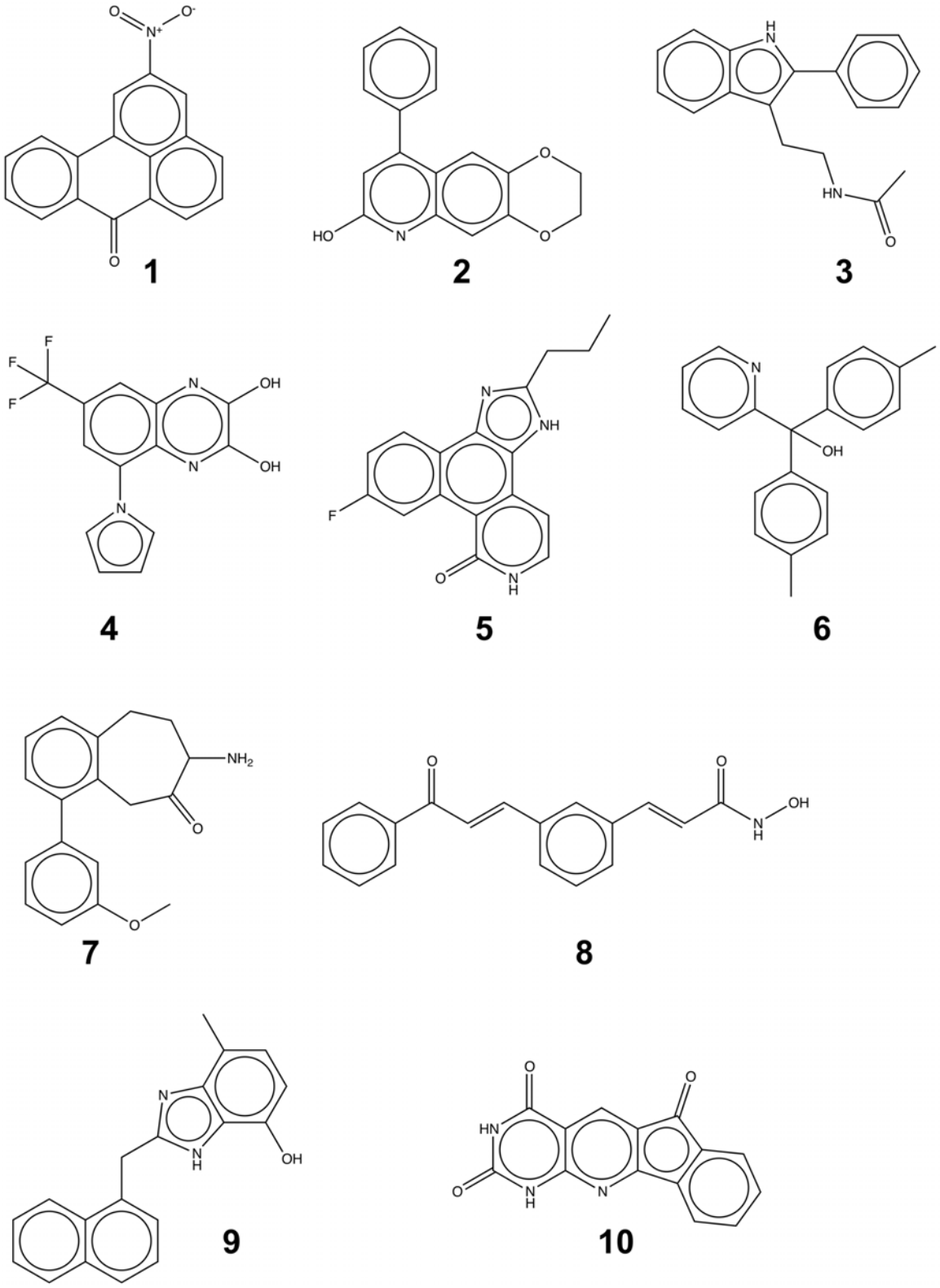

Figure S5. Chemical structures of the top 10 compounds from docking to site 1.

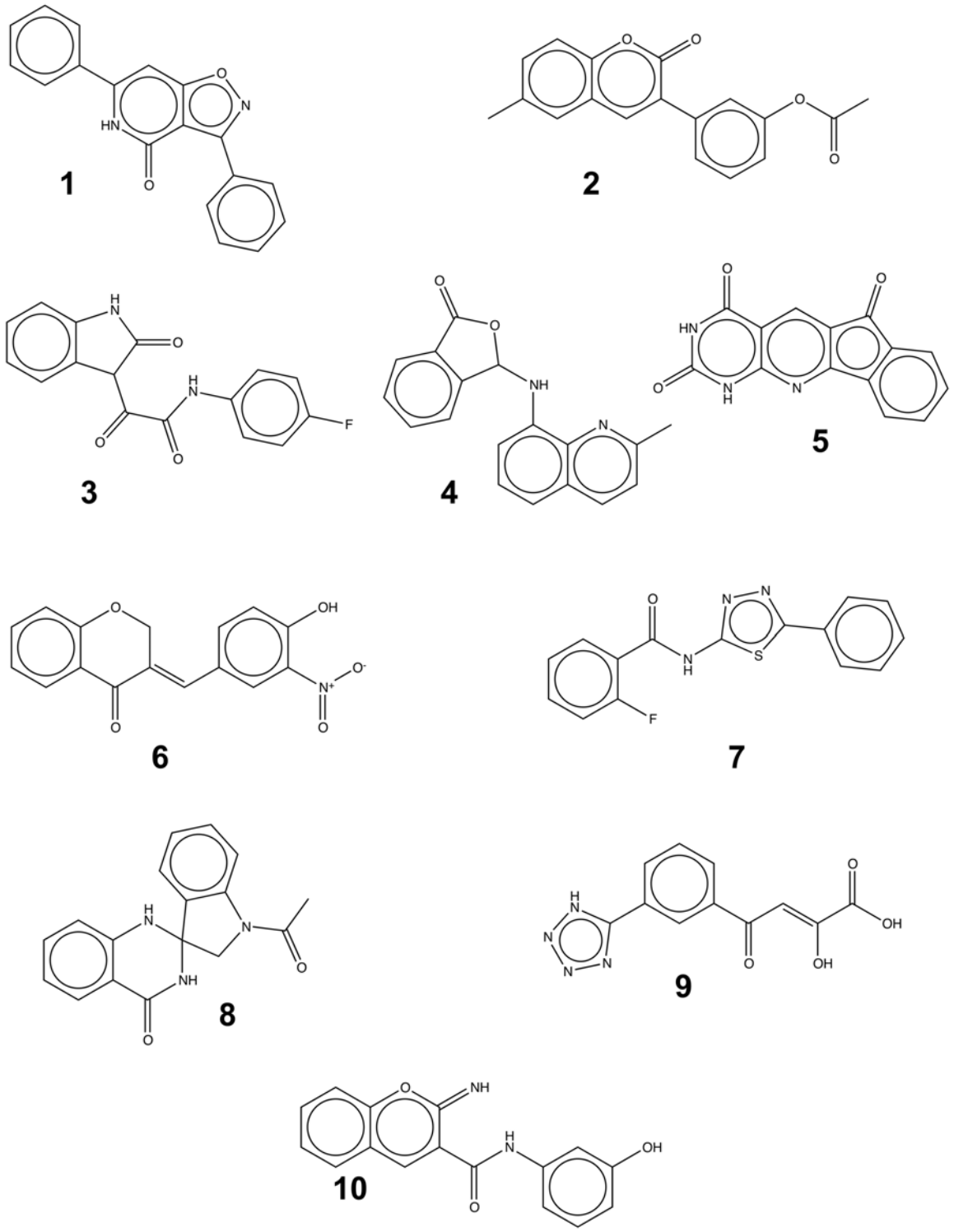

Figure S6. Chemical structures of the top 10 compounds from docking to site 2.

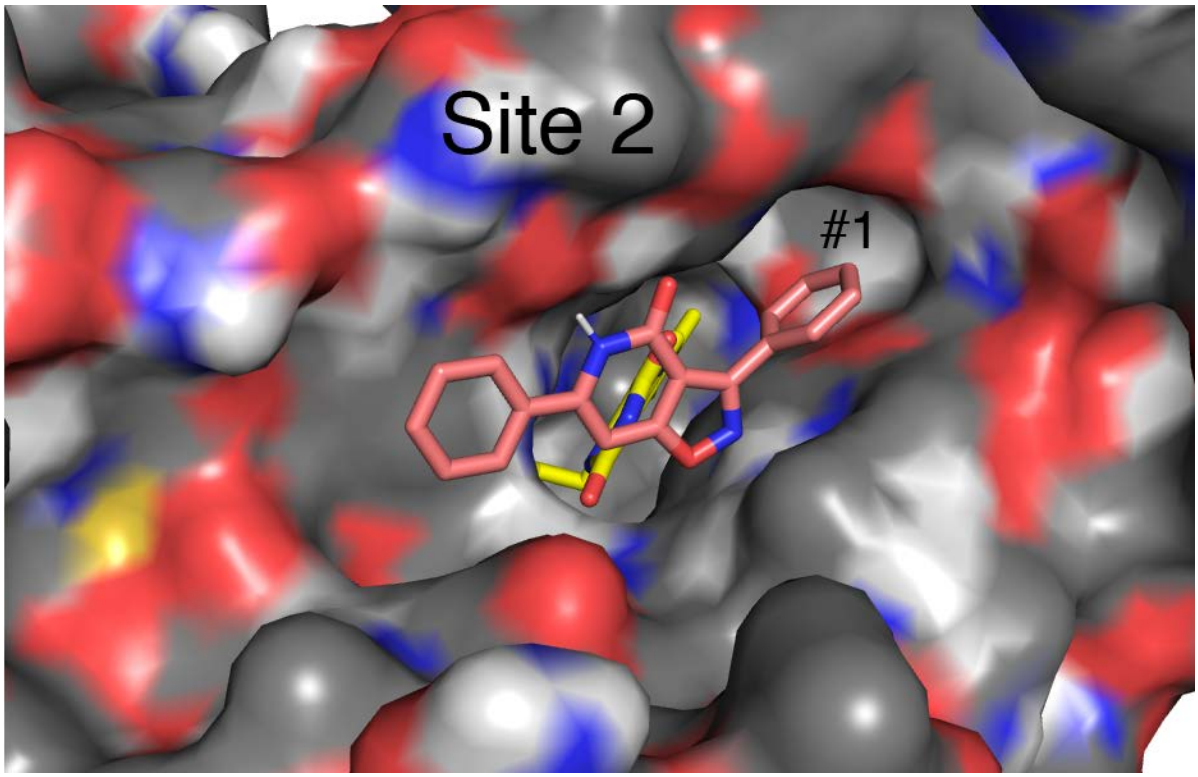

Figure S7. The top-ranked compound docked to site 2. The FAD is colored yellow.