Structural basis for potent inhibition of D-amino acid oxidase by thiophene carboxylic acids

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Supplementary Data

Supplementary Table 1

	1c-DAO	**2b-DAO**
Data collection		
X-ray source	PF-AR NW12A	PF-AR NW12A
Wavelength (Å)	1.000	1.000
Space group	$P2_12_12$	$P2_12_12$
Cell dimensions *a, b, c* (Å)	150.3, 182.6, 51.1	150.1, 182.7, 51.1
Resolution (Å)	49.23 – 2.60 (2.70 – 2.60)[a]	39.15 – 2.60 (2.70 – 2.60)[a]
Observed reflections	362,775 (37,703)[a]	355,952 (37,019)[a]
Unique reflections	44,404 (4,593)[a]	44,351 (4,577)[a]

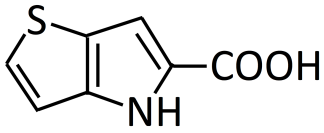
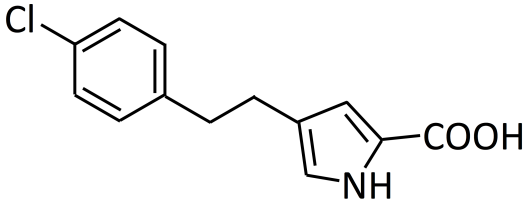
R_{meas}	0.224 (2.041)[a]	0.263 (1.542)[a]
$I/\sigma I$	9.7 (1.3)[a]	9.5 (2.2)[a]
Completeness (%)	100.0 (100.0)[a]	100.0 (100.0)[a]
Multiplicity	8.2 (8.2)[a]	8.0 (8.1)[a]
half-data set correlation (CC (1/2))	0.996 (0.517)[a]	0.993 (0.731)[a]
Refinement		
Resolution (Å)	49.23 – 2.60 (2.66 – 2.60)[a]	39.15 - 2.60 (2.66 – 2.60)[a]
No. reflections	44,280	44,233
R_{work}/R_{free}	0.2009/0.2598	0.2188/0.2550
RMSD		
Bond lengths (Å)	0.004	0.003
Bond angles (º)	0.884	0.968
No. atoms		
Protein	10,932	10,932
FAD	212	212
1c/2b	36	36
Water	106	225
Average *B*-factors (Å^2)		
Overall	50.17	38.66
Protein	50.40	38.75
FAD	42.41	28.62
1c/2b	60.44	29.56
Water	43.78	34.18
Ramachandran plot analysis[b]		
Favored region	97.5%	97.0%

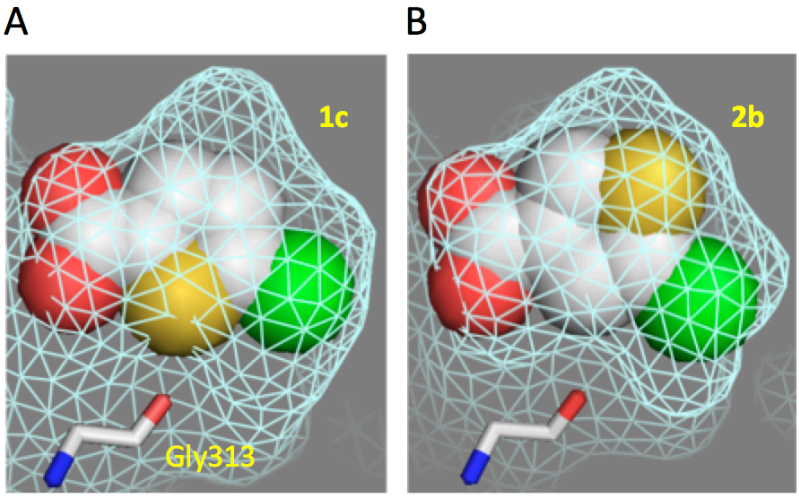
Allowed region	2.5%	3.0%
Outlier region	0.0%	0.0%
PDB code	5zja	5zj9

[a] Highest resolution shells are shown in parenthesis.

[b] Analyzed with Rampage [1]

Supplementary Table 2

Cmpd	Structure	IC_{50} (μM)
TPC		0.0054 [2]
CPC		0.0115 [2]

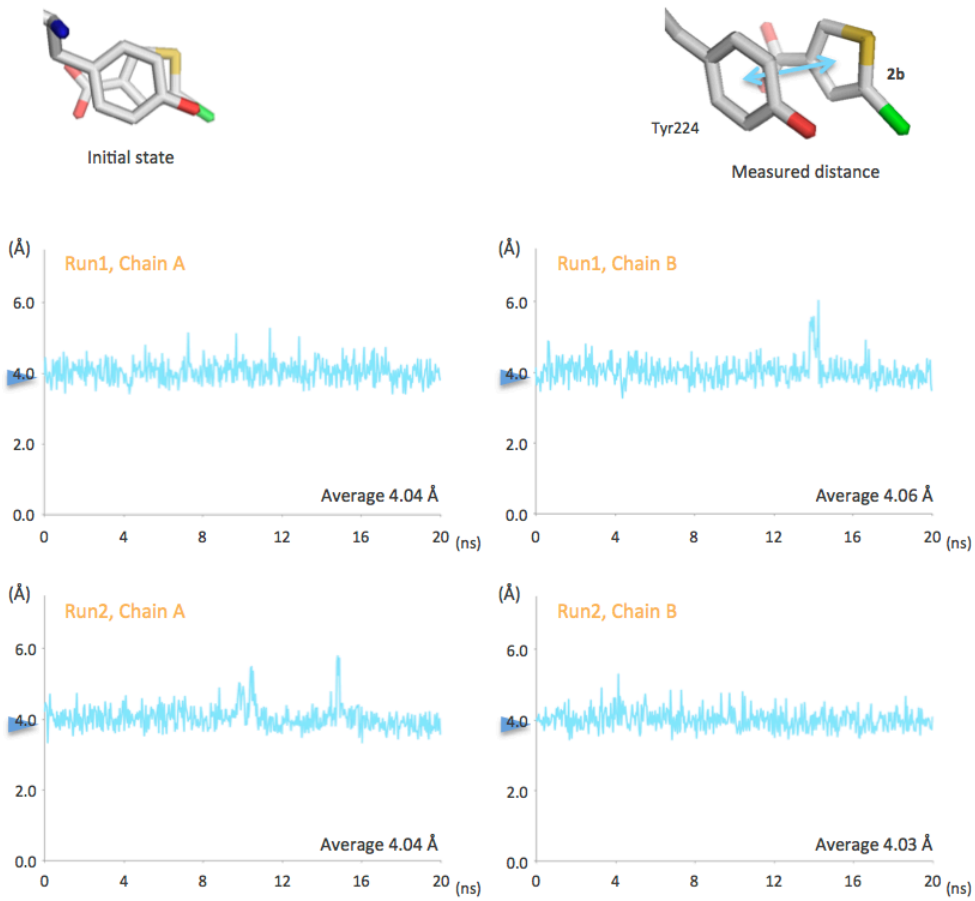


crystal structure of DAO in complex with **1c**

crystal structure of DAO in complex with **2b**

Supplementary Fig. 1

A lone pair of the sulfur atom of **1c** may repulse that of the oxygen atom of Gly313 of DAO (**A**). The sulfur atom of **2b** is far from the oxygen atom of Gly313 (**B**). In addition, a gap between **1c** and the wall of the substrate-binding pocket appeared wider than that of the **2b**-DAO complex. Accordingly, the substrate-binding pocket of **2b**-DAO complex was smaller than that of the **1c**-DAO complex (**Table 5**). These may collectively cause a decrease in the stability of the **1c**-DAO complex relative to the **2b**-DAO complex.

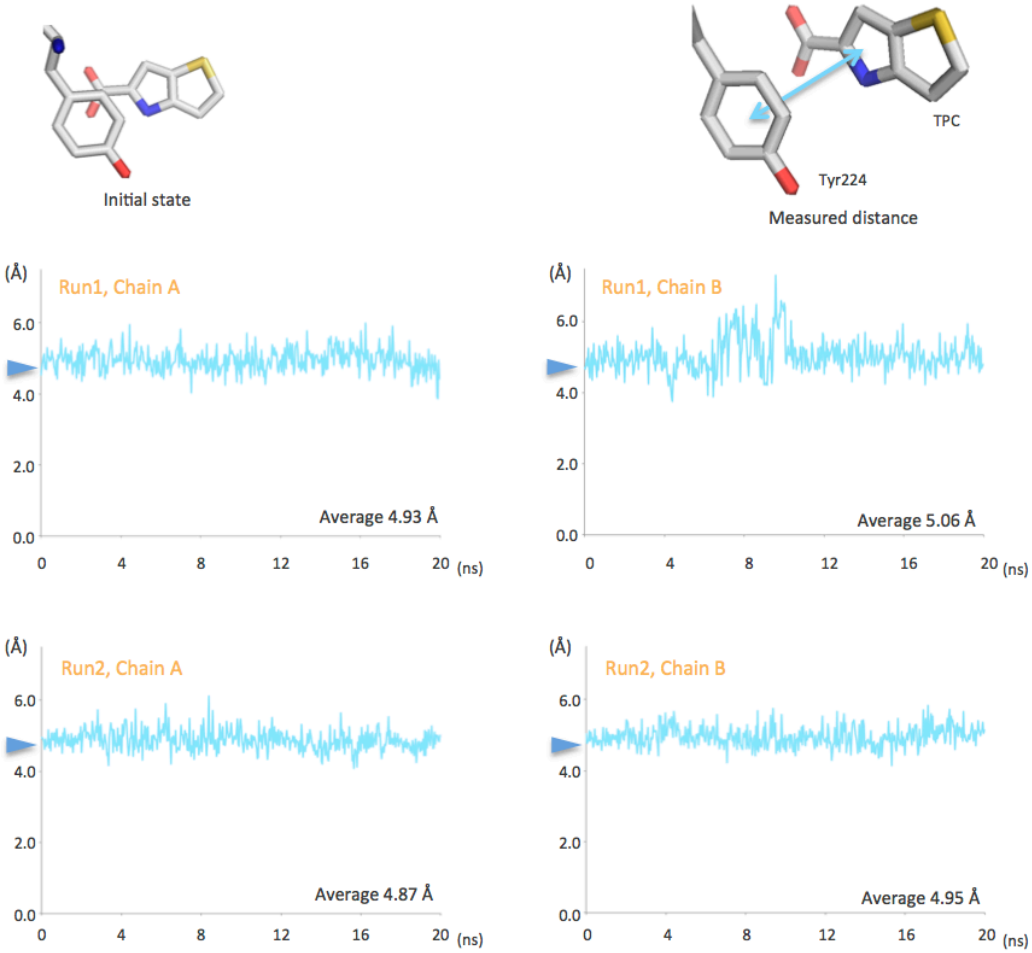


Supplementary Fig. 2

Distance between the centroids of the thiophene ring of 2b and benzene ring of Tyr224

Initial structure: S state with 2b
(crystal structure of DAO in complex with 2b)

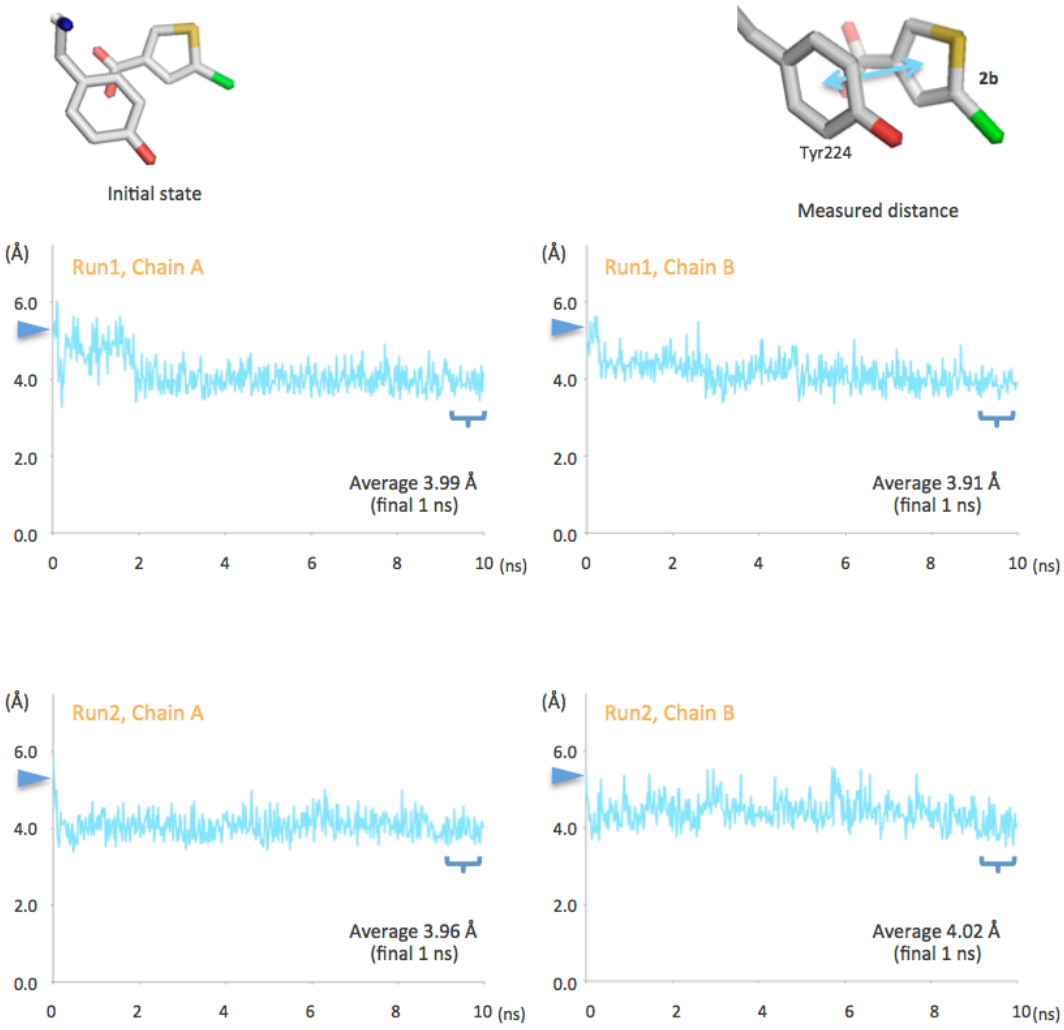
: Distance in the crystal structure (3.86 Å and 3.81 Å for A and B chains, respectively)



Supplementary Fig. 3

Distance between the centroids of the pyrrole ring of TPC and benzene ring of Tyr224
Initial structure: D state with TPC
(crystal structure of DAO in complex with TPC)

: Distance in the crystal structure (4.67 Å and 4.73 Å for A and B chains, respectively)

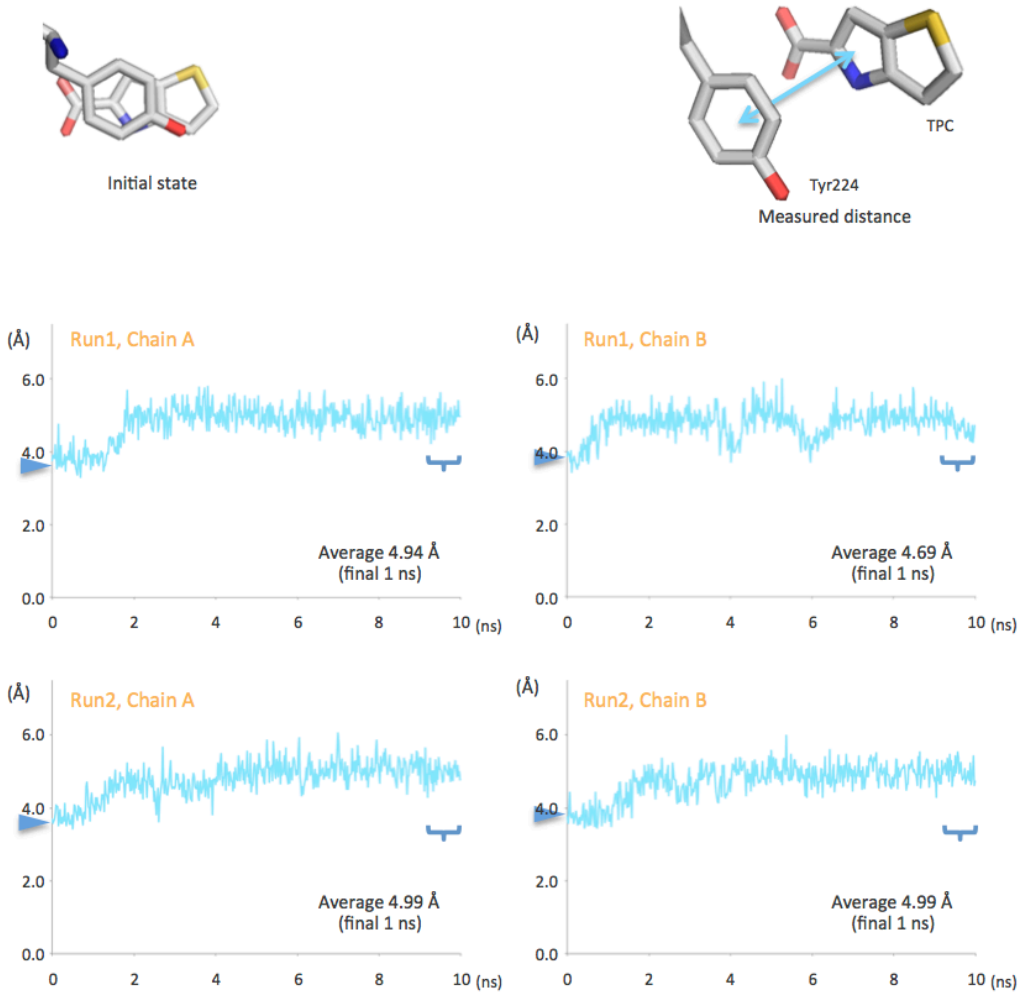


Supplementary Fig. 4

Distance between the centroids of the rings of 2b and Tyr224

Initial structure: D state with 2b

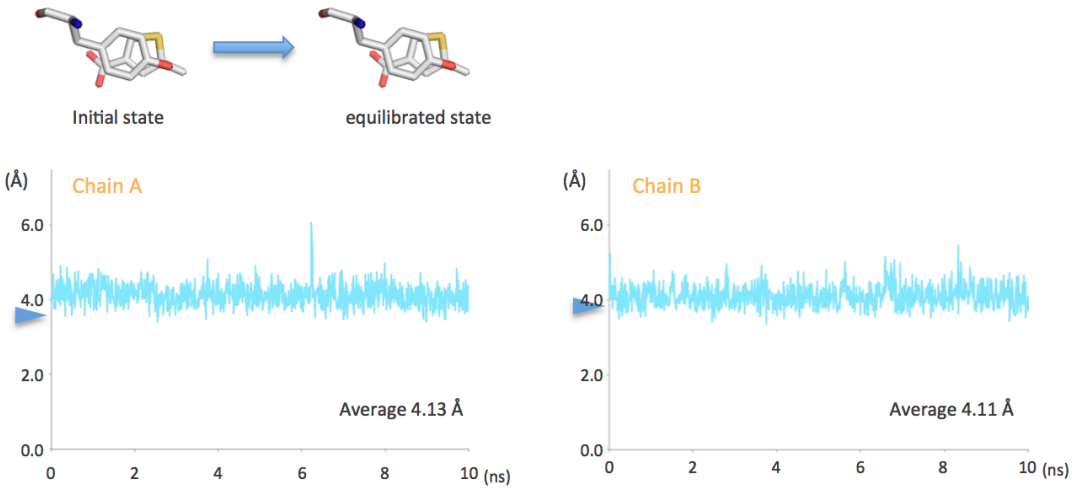
: Distance in the initial structure (5.28 Å and 5.34 Å for A and B chains, respectively)



Supplementary Fig. 5

Distance between the centroids of the pyrrole ring of TPC and benzene ring of Tyr224
Initial structure : S state with TPC

: Distance in the initial structure (3.60 Å and 3.84 Å for A and B chains, respectively)

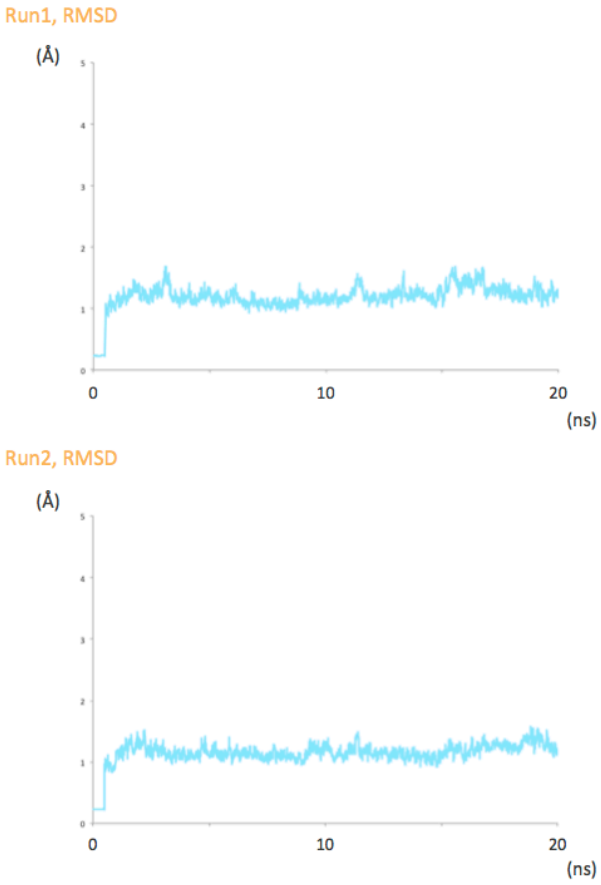


Supplementary Fig. 6

Distance between the centroids of the rings of **2c** and Tyr224

Initial structure: S state with **2c**

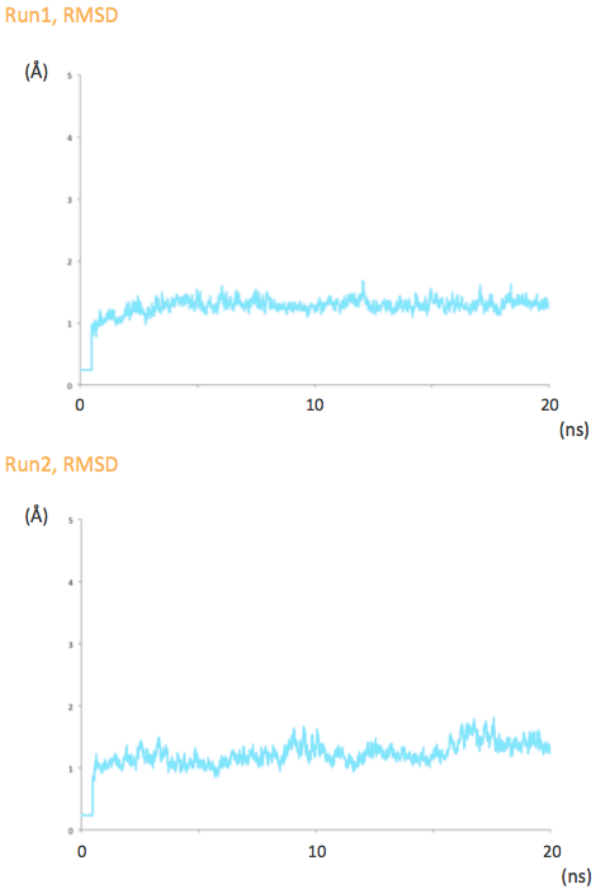
: Distance in the initial structure (3.65 Å and 3.86 Å for A and B chains, respectively)



Supplementary Fig. 7

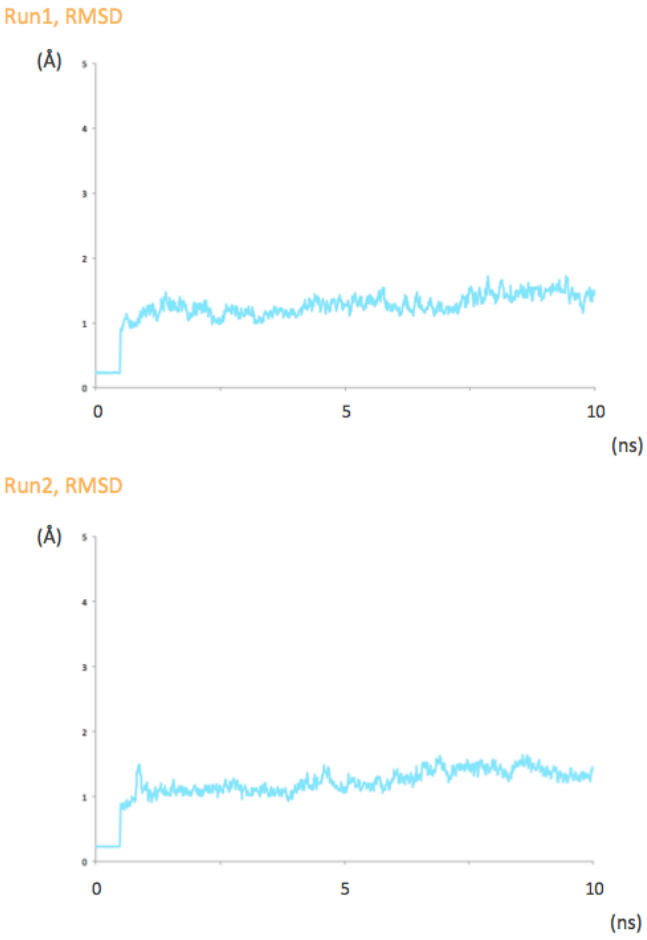
Backbone RMSD of DAO trajectories with respect to the initial structure.

Initial structure: S state with **2b**
(crystal structure of DAO in complex with **2b**)



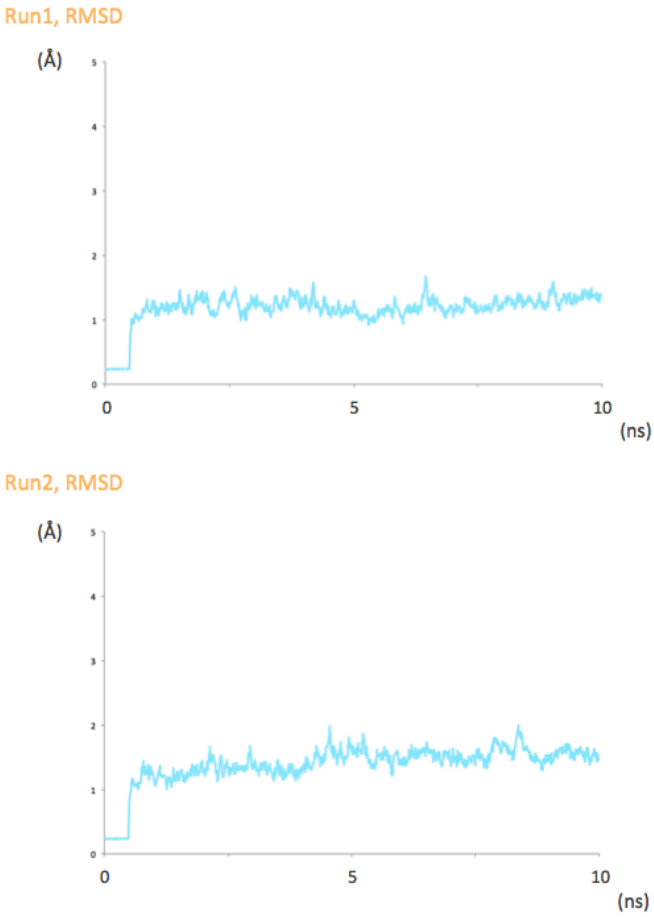
Supplementary Fig. 8

Backbone RMSD of DAO trajectories with respect to the initial structure.
Initial structure: D state with TPC



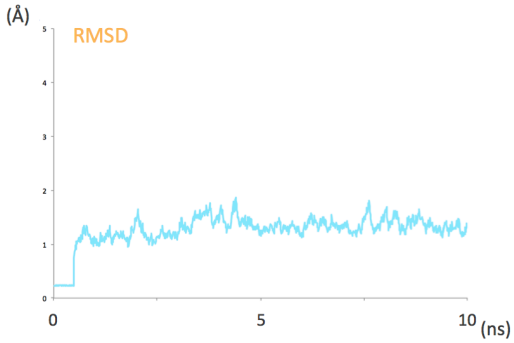
Supplementary Fig. 9

Backbone RMSD of DAO trajectories with respect to the initial structure.
Initial structure: D state with 2b



Supplementary Fig. 10

Backbone RMSD of DAO trajectories with respect to the initial structure.
Initial structure : S state with TPC



Supplementary Fig. 11

Backbone RMSD of DAO trajectories with respect to the initial structure.
Initial structure : S state with **2c**

Reference

[1] Lovell SC, Davis IW, Arendall WB, 3rd, de Bakker PI, Word JM, Prisant MG, et al. Structure validation by Calpha geometry: phi,psi and Cbeta deviation. Proteins. 2003;50:437-50.

[2] Hopkins SC, Heffernan ML, Saraswat LD, Bowen CA, Melnick L, Hardy LW, et al. Structural, kinetic, and pharmacodynamic mechanisms of D-amino acid oxidase inhibition by small molecules. Journal of medicinal chemistry. 2013;56:3710-24.