Supplementary Information (SI) for Physical Chemistry Chemical Physics.

Unusual Mechanism of Aziridine Biosynthesis Catalysed by an αKG-dependent Non-heme Enzyme TqaL

Warispreet Singh[1,2]* and Meilan Huang[1]*

[1] Department of Chemistry & Chemical Engineering, Queen's University, Belfast, BT9 5AG, United Kingdom

[2] Department of Applied Sciences, Northumbria University, Newcastle upon Tyne, NE1 8ST, United Kingdom, Hub for Biotechnology in Build Environment, Newcastle upon Tyne, United Kingdom

Corresponding Authors: m.huang@qub.ac.uk, w.singh@northumbria.ac.uk

Table of Contents

Table S1 The clustering analysis of runs for L-Val in complex with TqaL enzyme

Cluster* rank	Lowest binding energy (kcal/mol)	Run	Mean binding energy (kcal/mol)	Number in cluster
1	-5.81	112	-4.67	37
2	-5.62	86	-4.41	101
3	-4.2	43	-3.08	38
4	-3.56	260	-3.2	2
5	-3.42	162	-2.78	62
6	-3.4	208	-3.25	3
7	-3.25	270	-2.67	4
8	-3.09	258	-3.09	1
9	-3.09	132	-2.69	9
10	-3.03	125	-2.43	9
11	-2.84	131	-2.41	4
12	-2.48	121	-2.39	3
13	-2.34	200	-2.05	12
14	-2.21	294	-2.16	5
15	-2.16	119	-2.16	2
16	-2.05	170	-1.87	3
17	-1.91	197	-1.91	1
18	-1.89	218	-1.89	1
19	-1.75	96	-1.75	1
20	-1.4	120	-1.4	1
21	-1.25	195	-1.25	1

*The table shows the flexible docking poses of L-Val grouped into 21 distinct clusters out of 300 runs based on a RMSD tolerance of 2.0 Å. The average energies associated with each structure from these clusters are shown. The reaction catalyzed by the TqaL enzyme is initiated by the hydrogen atom abstraction from the C-beta hydrogen of L-Val.[1] Therefore the distance of the oxygen atom of the Fe(IV)=O to the Cβ of the L-Val was examined for the selection of a docked complex for the subsequent MD simulations.

Table S2. The energies of the QM/MM optimized structures of the Fe(IV)=O in complex with L-Val at different spin states of iron.

	Energy (a.u)	**Relative Energy (kcal/mol)**
Quintet (S=2)	-3116.81887	0
Singlet (S=0)	-3116.790988	17.4966097
Triplet (S=1)	-3116.805975	8.091785225
Septet (S=3)	-3116.79425	15.44946165

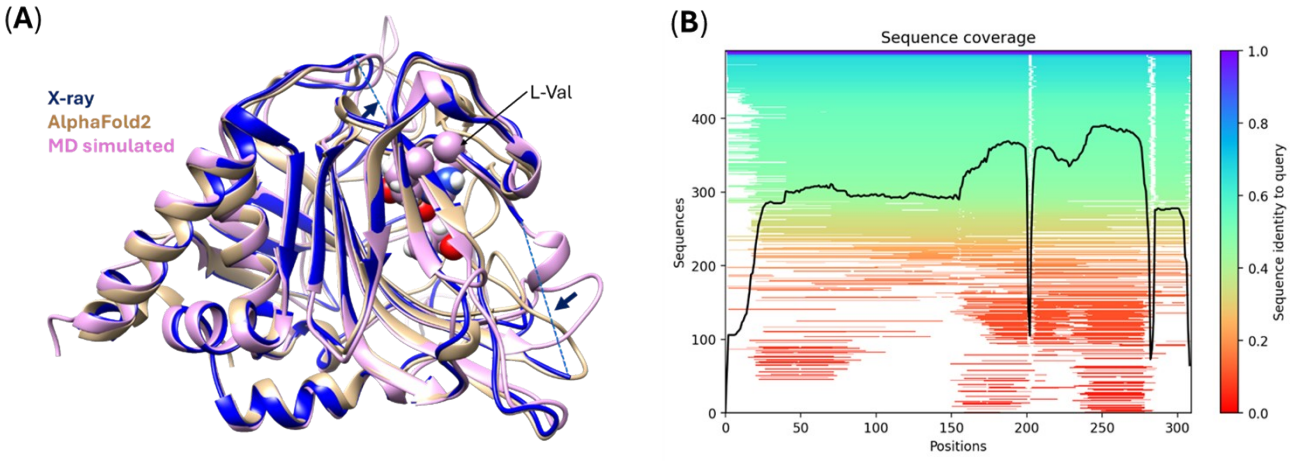


Figure S1 Comparison of the crystal structure and AlphaFold2 modelled structure of TqaL (**A**). The superposition of the crystal, MD simulated crystal structure and AlphaFold2 of TqaL shown by blue, pink and gold ribbons, respectively. The active site residues are shown by sphere representation. The missing loop residues in the crystal structure (shown by dotted dark blue lines) were modelled using modeller and the entire crystal structure was refined by MD simulations. (**B**). The coverage plot of multiple sequence alignment (MSA) used by AlphaFold2 in predicting the 3D structure of TqaL. Regions with high coverage which indicate better sequence alignment, are associated with higher confidence in structure prediction. Whereas regions with low coverage which reflect fewer sequence alignment, are associated with lower confidence in structure prediction.

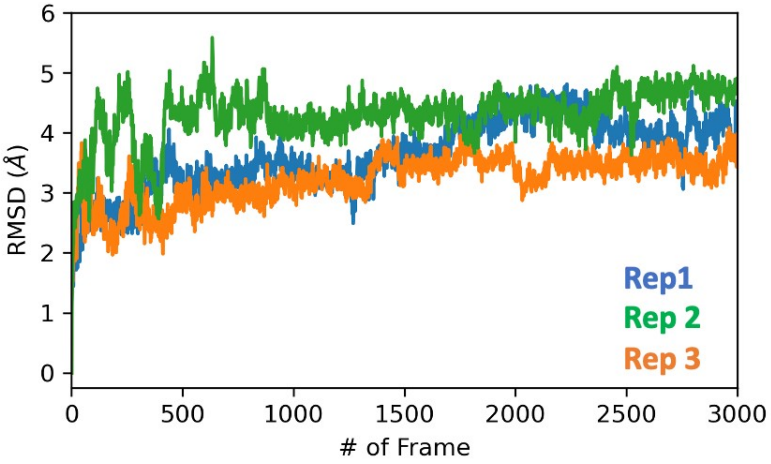


Figure S2 The RMSD analysis of the heavy atom of the TqaL enzyme obtained for the Fe(IV)=O species in complex with the L-Val substrate from 300-ns MD (Three replicas were run).

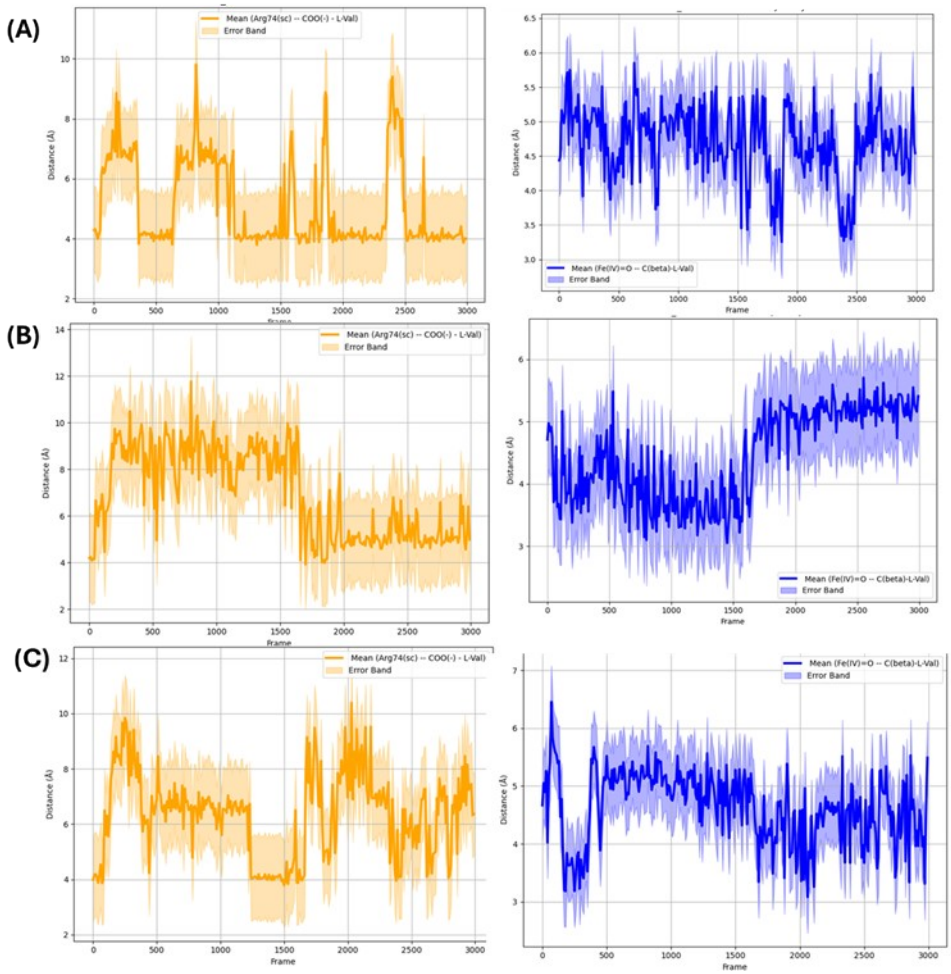


Figure S3 The important distances of TqaL enzyme for three replica runs of 300-ns MD simulations. The distance between the guanidino nitrogen of Arg74 to the carboxylate oxygen of L-Val substrate is shown in orange; the distance between Fe(IV)=O to Cβ of L-Val substrate is shown in blue. (A) Replica 1, (B) Replica 2, (C) Replica 3. The distance plots include a standard error of the mean (SEM) band, calculated over a 10-frame window.

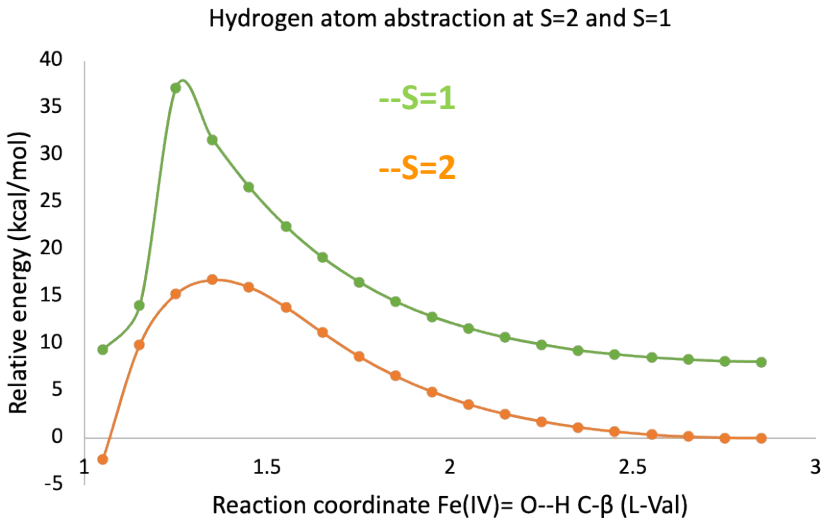


Figure S4 The potential energy surface for the hydrogen atom abstraction of the L-Val substrate by the Fe(IV)=O species at S=1 (triplet) and S=2 (quintet) spin states of iron.

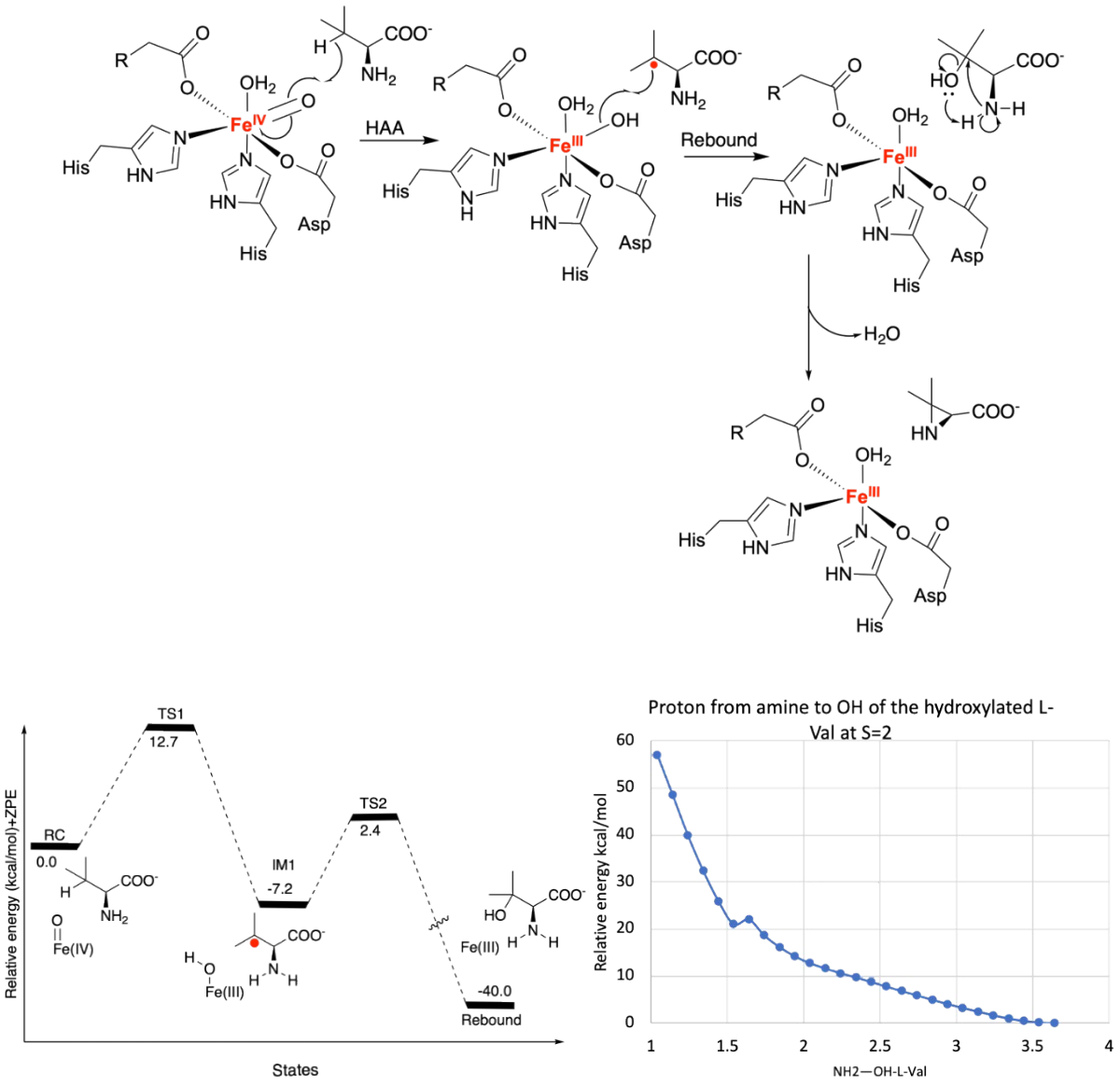


Figure S5 The rebound step and the postulated proton transfer from the amine to the OH of the hydroxylated L-Val that would lead to the aziridine product. The calculations were conducted at S=2 (quintet) spin state of iron.

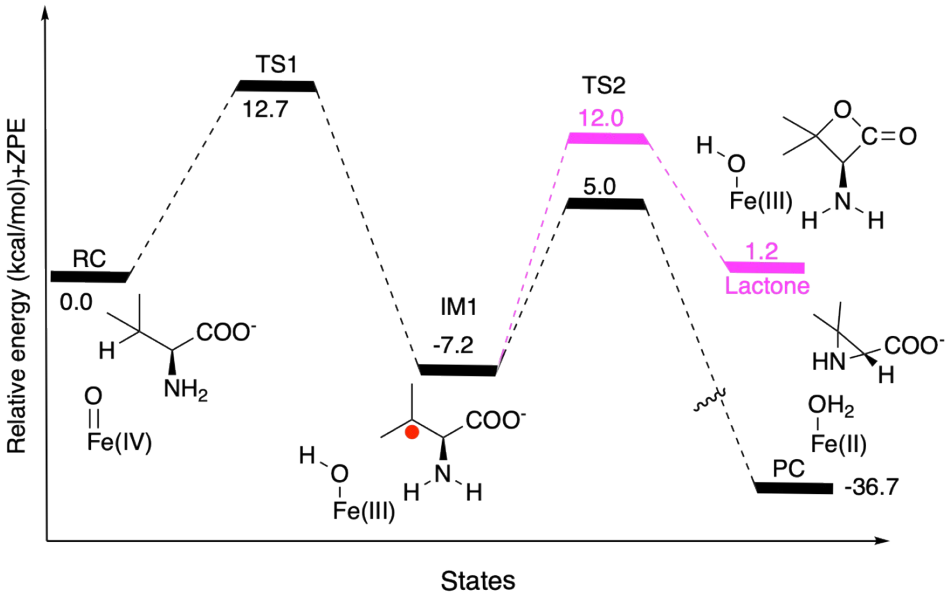
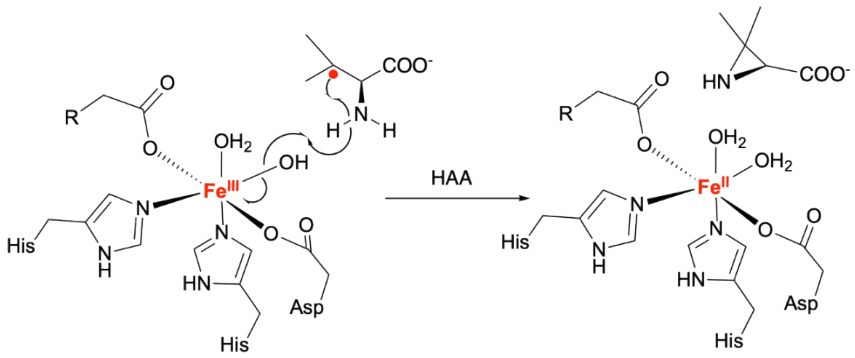


Figure S6 The QM/MM reaction profile of the lactone formation in comparison to the aziridine formation catalysed by the TqaL enzyme at quintet state (S=2).



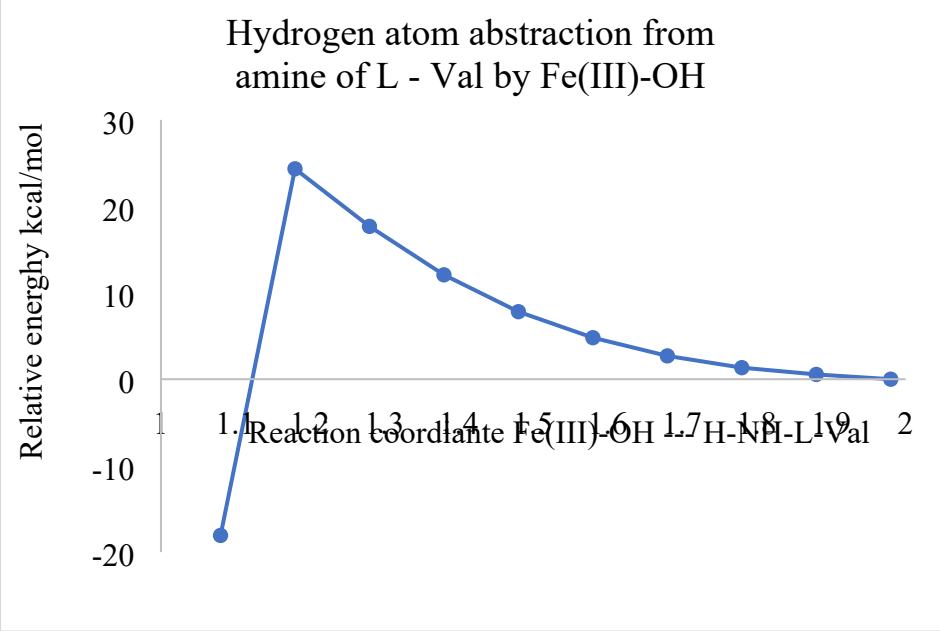


Figure S7 The potential energy scan of the hydrogen atom abstraction by Fe(III)-OH from the amine of L-Val. The calculations were conducted at S=2 (quintet) spin state of iron.

Reference

1. Cha, L.; Paris, J. C.; Zanella, B.; Spletzer, M.; Yao, A.; Guo, Y.; Chang, W.-c., Mechanistic Studies of Aziridine Formation Catalyzed by Mononuclear Non-Heme Iron Enzymes. *J. Am. Chem. Soc.* **2023,** *145* (11), 6240-6246.