Supporting Information

Characterizing Y224 conformational flexibility in FtmOx1-catalysis using ^{19}F NMR spectroscopy

Xinye Wang, [‡a] Lingyun Yang,[‡b] Shenlin Wang,[a] Jun Wang,[c] Kelin Li,[d] Nathchar Naowarojna,[e] Yi Ju,[a] Ke Ye,[a] Yuchen Han,[a] Wupeng Yan,[c*] Xueting Liu,[a*] Lixin Zhang,[a*] Pinghua Liu[d*]

[a.] *State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, Shanghai 200237, China. E-mail: liuxueting@ecust.edu.cn; lizhang@ecust.edu.cn*
[b.] *iHuman Institute, ShanghaiTech University, Shanghai 201210, China.*
[c.] *School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China. E-mail: yanwupeng@sjtu.edu.cn*
[d.] *Department of Chemistry, Boston University, Boston, Massachusetts 02215, United States. E-mail: pinghua@bu.edu*
[e.] *Program of Chemistry, Faculty of Science and Technology, Sakon Nakhon Rajabhat University, Sakon Nakhon 47000, Thailand*

Table of Contents

Supplementary Figures

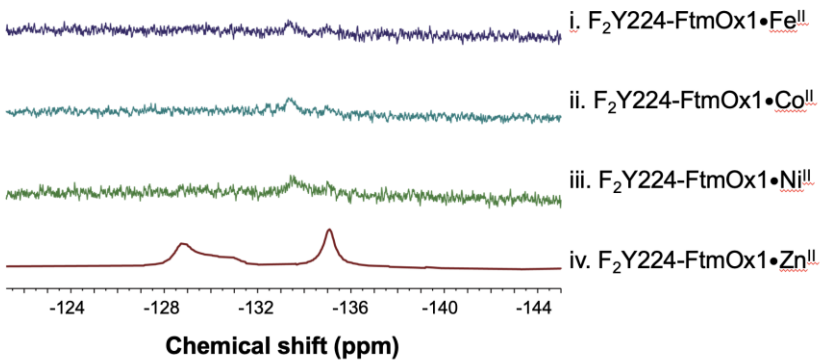

Figure S1. ^{19}F NMR studies on F_2Y224-FtmOx1 complexed with different metal ions.

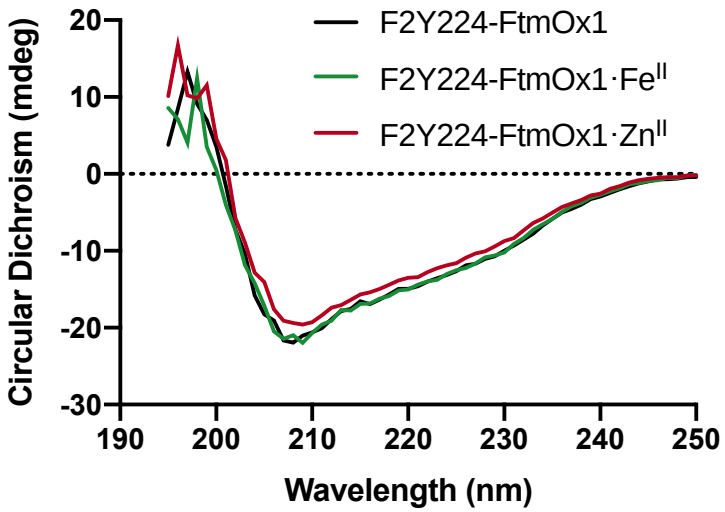

Figure S2. CD spectrum of F2Y224-FtmOx1 apo protein (black), F2Y224-FtmOx1·Fe(II) (red), and F2Y224-FtmOx1·Zn(II) (green).

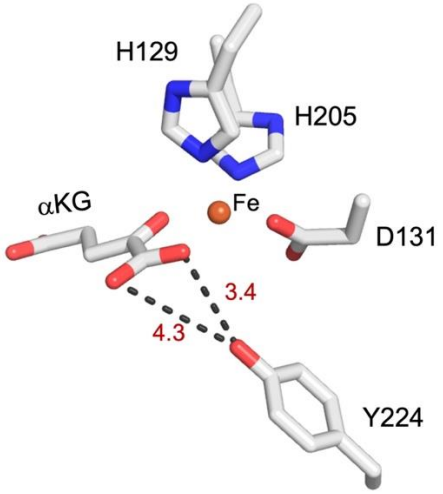

Figure S3. Binary structure of FtmOx1•Fe^{II}•*α*KG (pdb entry 4Y5S) complex. The distances between Y224 and two oxygen atoms of 1-carboxyl group of αKG are presented on the black dashed lines.

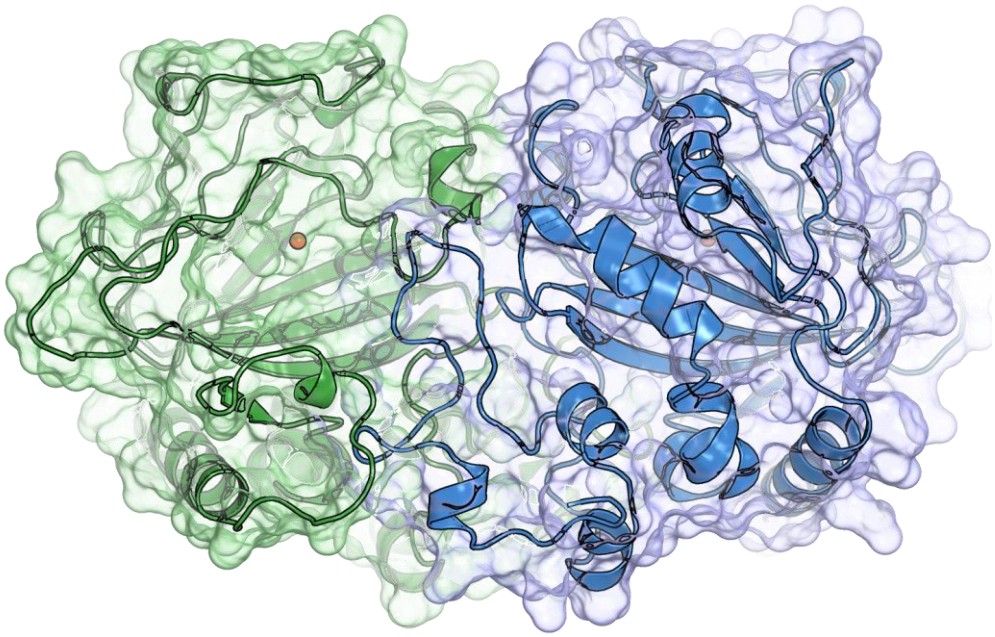

Figure S4. Overall architecture of F_2Y224-FtmOx1•Fe^{II} (pdb entry 9J1I) shown as a functional dimer with one monomer color-coded based on secondary and surface structures (shown as stereo images). The iron centers are labelled as deep olive spheres.

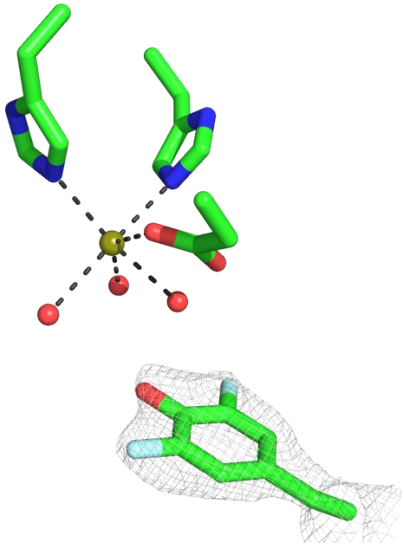

Figure S5. The composite omit map of F_2Y224-FtmOx1•Fe^{II} metallo-center (pdb entry 9J1I) (2m*F*o − D*F*c) at 1σ contour. The electron density around F2Y224 is shown in grey mesh.

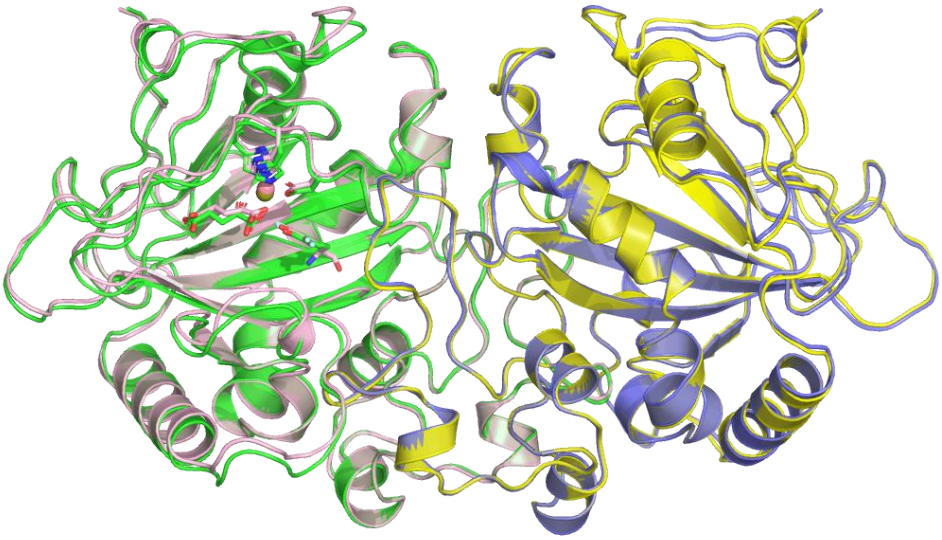

Figure S6. Superimposition of F_2Y224-FtmOx1•Co^{II}•αKG (pdb entry 9J1H) complex with F_2Y224-FtmOx1•Fe^{II}•αKG (pdb entry 4Y5S) complex.

The F_2Y224-FtmOx1•Co^{II}•αKG overall architecture and metallo-center are shown in green and purple cartoon and green sticks, respectively. The F_2Y224-FtmOx1•Fe^{II}•αKG overall architecture and metallo-center are shown in pink and yellow cartoon and sticks, respectively.

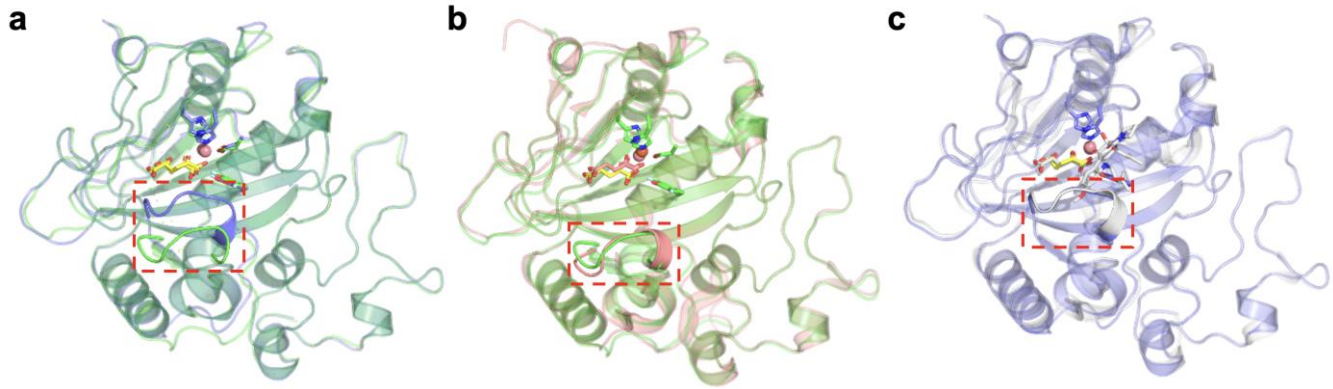

Figure S7. Structural comparions of the loops (^{65}ADKYPPHF^{72}) in different FtmOx1 crystal structures.

(a) Superimposition of chain A (green) onto chain B (purple) of F_2Y224-FtmOx1•Co^{II}•αKG (pdb entry 9J1H) complex. (b) Superimposition of chain A (green) of F_2Y224-FtmOx1•Co^{II}•αKG with FtmOx1•Fe^{II}•αKG (pdb entry 4Y5S) complex. (c) Superimposition of chain B (purple) of F_2Y224-FtmOx1•Co^{II}•αKG with FtmOx1•Co^{II}•αKG•13-oxo-fumitremorgin B (**6**) (pdb entry 7WSB) complex. The structures were shown in cartoon mode with 75% transparency while structurally different loops (^{65}ADKYPPHF^{72}) are highlighted by red-dotted squares with no transparency.

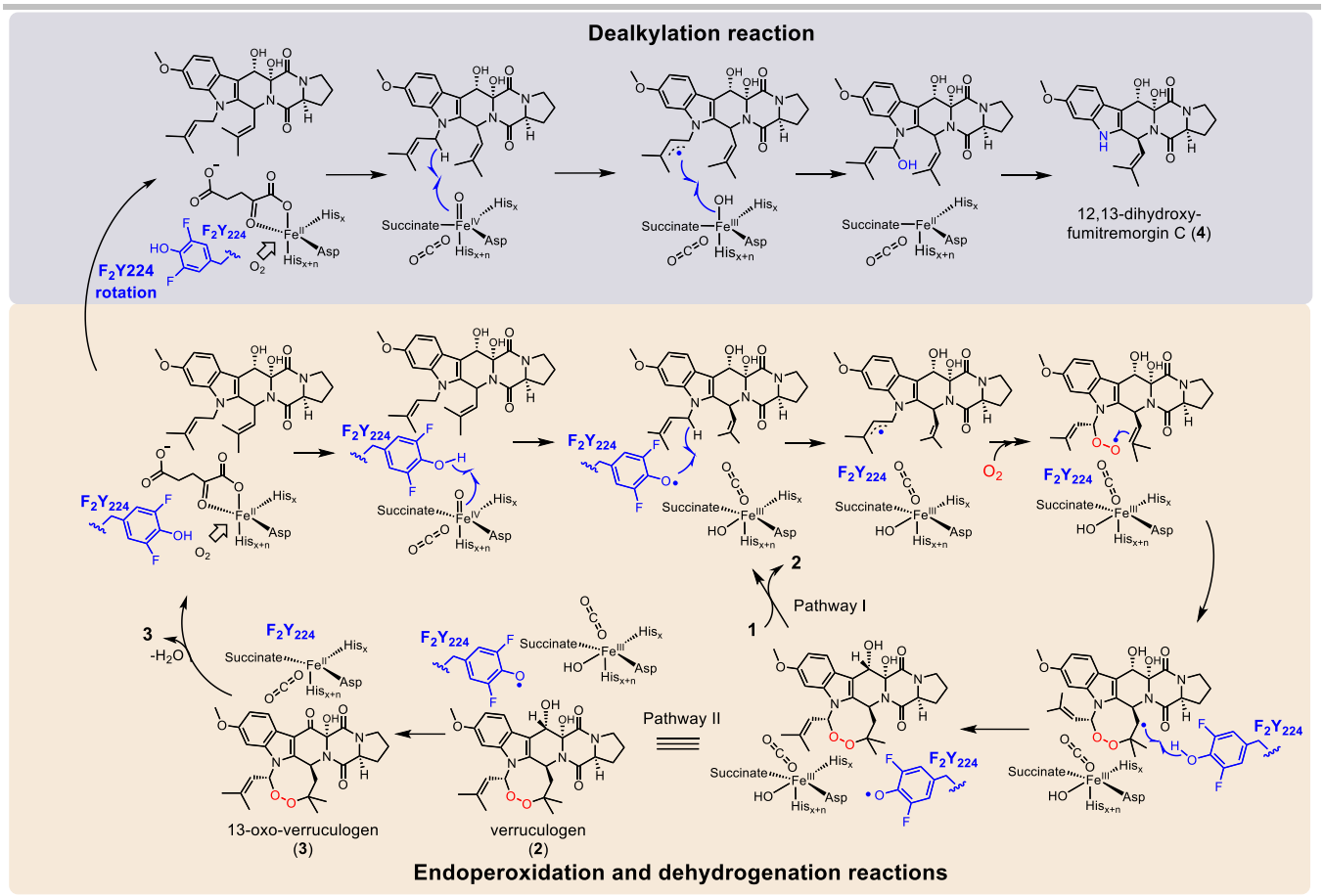

Figure S8. The proposed mechanistic models for F_2Y224-FtmOx1- catalysis.

Supplementary Tables

Table S1. Crystallographic data collection and refinement statistics for the FtmOx1•Fe^{II} structure.

Data Collection	
Wavelength (Å)	0.9785
Resolution range (Å)	36.08 - 2.11 (2.19 – 2.11)
Space group	P 1 2_1 1
Unit cell a, b, c (Å) α, β, γ (°)	60.44 45.61 105.15 90 100.02 90
Unique reflections	32539 (3155)
Multiplicity	3.2 (2.6)
Completeness (%)	99.16 (97.23)
Mean I/sigma(I)	11.18 (1.79)
$CC_{1/2}$	0.997 (0.686)
R_{sym}	0.069 (0.536)
Refinement	
R_{work}	0.1746 (0.2312)
R_{free}	0.2146 (0.2739)
Number of non-hydrogen atoms	4726
macromolecules	4501
ligands	3
water	222
Protein residues	569
RMS bonds (Å)	0.008
RMS angles (°)	0.88
Ramachandran favored (%)	98.39
Ramachandran outliers (%)	0
Average B-factor ($Å^2$)	41.12
macromolecules	41.17
ligands	39.80
solvent	40.28
[1]Statistics for the highest-resolution shell are shown in parentheses.	
[2] R_{free} is calculated with 5% of the data randomly omitted from refinement.	

Table S2. Crystallographic data collection and refinement statistics for the FtmOx1•Co^{II}•αKG binary complex.

Data Collection	
Wavelength (Å)	0.9785
Resolution range (Å)	47.93 - 2.00 (2.07 – 2.00)
Space group	P 2_1 2_1 2_1
Unit cell a, b, c (Å) α, β, γ (°)	62.032 62.077 151.02 90 90 90
Unique reflections	39836 (3641)
Multiplicity	5.9 (3.7)
Completeness (%)	99.04 (91.92)
Mean I/sigma(I)	9.28 (1.99)
$CC_{1/2}$	0.992 (0.846)
R_{sym}	0.118 (0.470)
Refinement	
R_{work}	0.1726 (0.2505)
R_{free}	0.1939 (0.2932)
Number of non-hydrogen atoms	4919
macromolecules	4466
ligands	33
water	420
Protein residues	565
RMS bonds (Å)	0.007
RMS angles (°)	0.98
Ramachandran favored (%)	95.66
Ramachandran outliers (%)	0.36
Average B-factor ($Å^2$)	31.68
macromolecules	31.53
ligands	31.71
solvent	33.24
[1]Statistics for the highest-resolution shell are shown in parentheses.	
[2] R_{free} is calculated with 5% of the data randomly omitted from refinement.	