Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Water-Mediated Network in the Resistance Mechanism of Fosfomycin

James McClory, [†] Jun-Tang Lin [‡], David Timson[§], Jian Zhang[//] Meilan Huang[†,*]

[†] School of Chemistry and Chemical Engineering, Queen's University Belfast, David Keir Building, Stranmillis Road, Belfast, BT9 5AG, Northern Ireland, United Kingdom

[‡] Stem Cells and Biotheraphy Engineering Research Centre of Henan, College of Biomedical Engineering, Xinxiang Medical University, Xinxiang 453003, China

§ School of Pharmacy and Biomolecular Sciences, The University of Brighton, Huxley Building, Lewes Road, Brighton, BN2 4GJ, United Kingdom

[//] Shanghai Jiaotong University, 280 Chongqing Road, Shanghai 200025, China

*Corresponding Author: m.huang@qub.ac.uk

Table S1. RESP charges of atoms of Fosfomycin

No.	atom Name	atom Type	RESP Charge
1	O1	OS	-0.39
2	O2	O2	-0.87
3	O3	O2	-0.87
4	O4	OH	-0.49
5	C1	CT	-0.18
6	C2	CT	0.14
7	C3	CT	0.02
8	P1	P	1.16
9	H1	H1	0.08
10	H2	H1	-0.01
11	H3	HC	0.01
12	H4	HC	0.01
13	H5	HC	0.01
14	H6	HO	0.38

Table S2. QM/MM Benchmark calculations on FomA

	Reactant	**TS**	**Product**
6-31g(d) Opt	0	17.31	-14.13
6-31g(d,p) SP	0	17.64	-14.48
6-31+g(d) SP	0	19.01	-11.83
6-311++g(d,p) SP	0	19.44	-12.22

Table S3. Summary of key interactions in FomA from crystal, MD and QM/MM structures

	Lys18-ATP	**dLys18-α-PO_4 (Å)**	**dLys18-β-,γ–bridging oxygen (Å)**	**dHis58-** γ – PO_4 **(Å)**	**dHis58-** -Sub-PO_4 **(Å)**	**Ser13-Water**
Crystal FomA-FM (pdb: 3D41)	γ-PO_4	4.40	5.35	3.45	3.88	β-,γ-bridging oxygen
Representative structure (MD Cluster)	α -,γ-PO_4	3.82	3.76	3.28	2.74	Four-Water mediated H-bond with three water tethering ATP
Reactant (QM/MM)	α -,γ-PO_4	2.92	3.84	3.28	3.71	Four-Water mediated H-bond with three water tethering ATP
TS (QM/MM)	α -,γ-PO_4	2.87	3.78	3.56	2.70	Four-Water mediated H-bond with three water tethering ATP
Product (QM/MM)	α -,γ-PO_4	2.86	3.58	3.15	2.95	Four-Water mediated H-bond with three water tethering ATP
Crystal FomA-FM (pdb: 3QUO)	γ-PO_4	4.20	4.75	3.64	3.58	β-PO_4

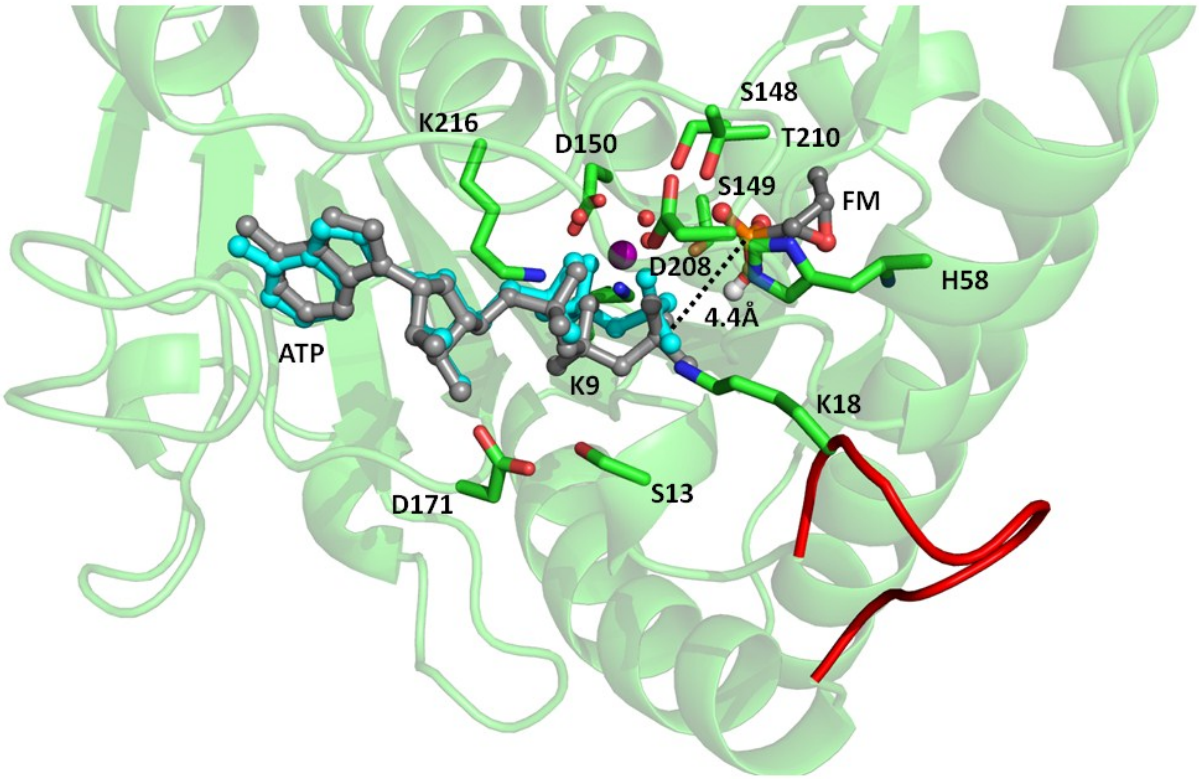


Figure S1. ATP in the built reactant model (grey) and representative cluster structure of ATP-FM-Mg^{2+}-FomA complex (cyan) shows significant conformational change. The built reactant model is shown in green ribbon representation, with the flexible glycine loop shown in red.

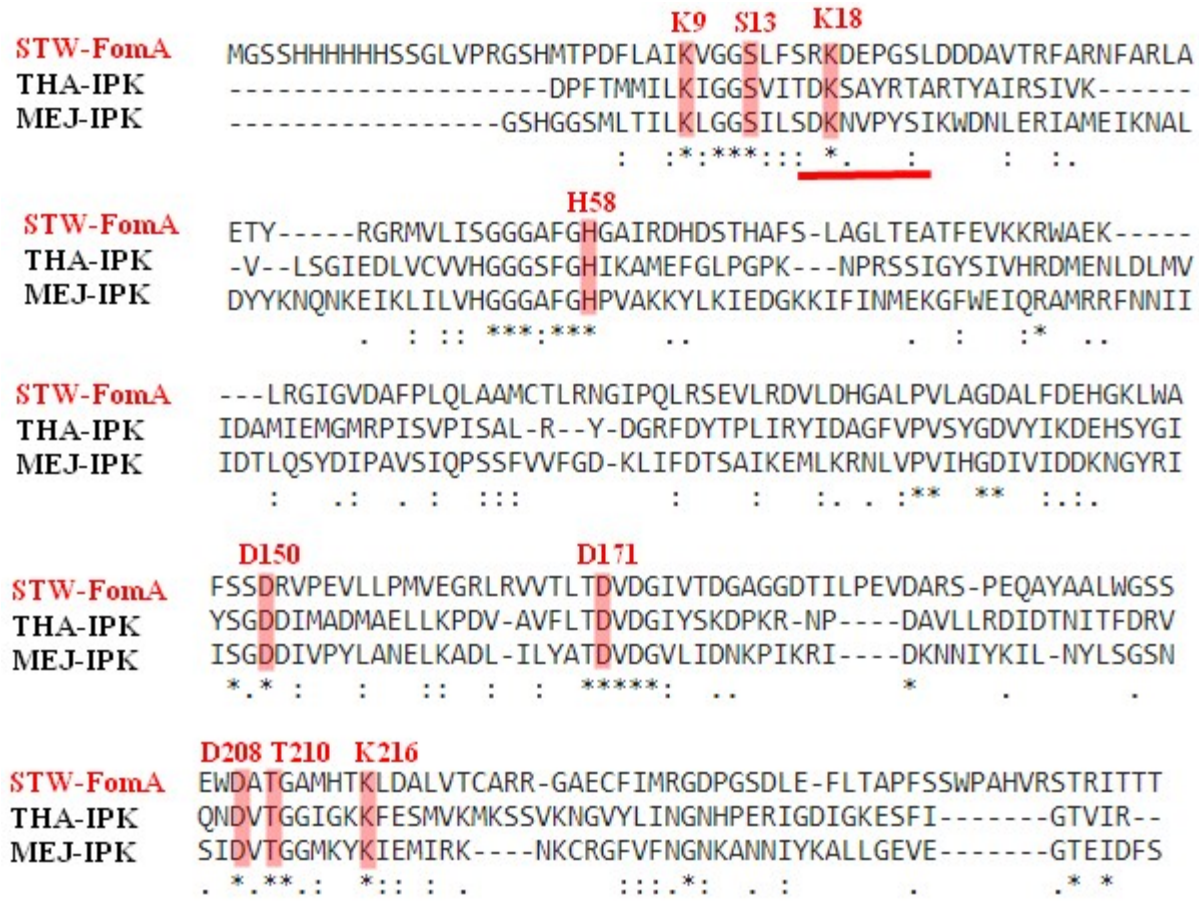
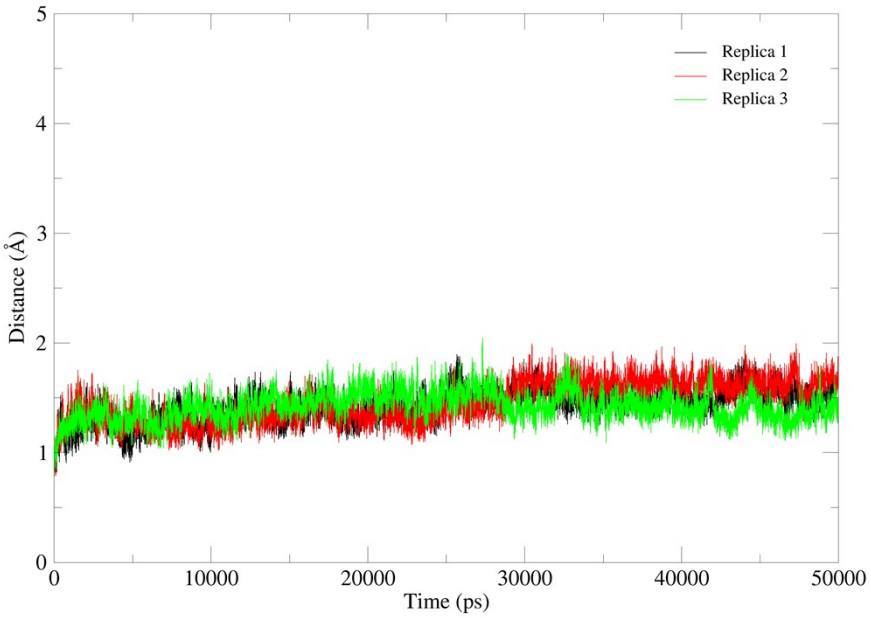
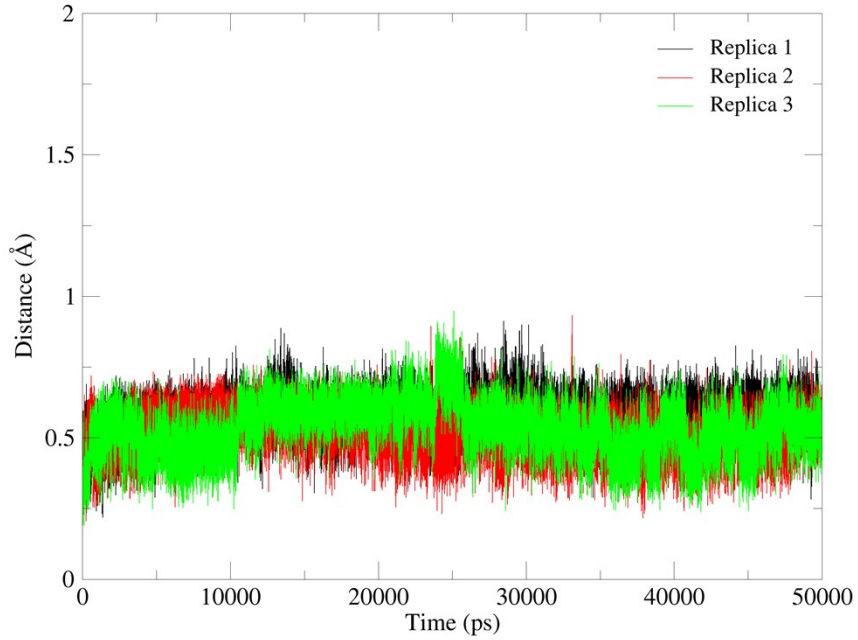


Figure S2. Sequence alignment of AAK enzymes FomA and IPK using the Clustal Omega web server. Key residues in FomA and their corresponding residues in IPK are highlighted in red. Species names are abbreviated as follows: STW, *Streptomyces wedmorensis* (STW-FomA: BAA32493.1); THA, *Thermoplasma acidophilum* (THA-IPK: CAC11251.1); MEJ, *Methanocaldococcus jannaschii* (MEJ-IPK: AAB98024.1). The glycine-rich loop consisting residues 16-24 is underlined by red line.

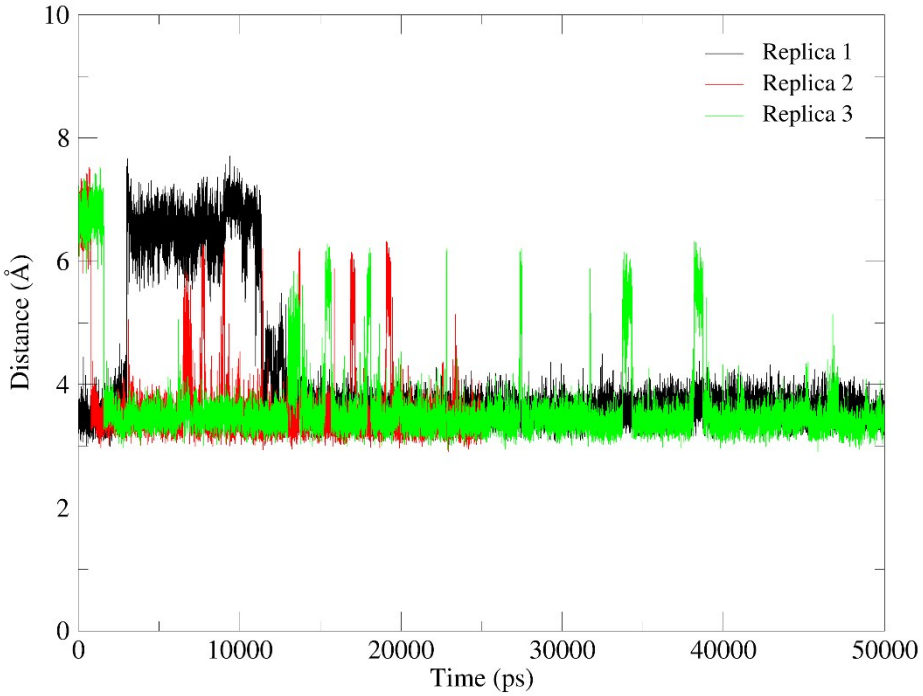


(A)



(B)

Figure S3. (A) RMSD values for Cα carbon of the backbone of ATP-FM-Mg^{2+}-FomA complex (B) RMSD values for heavy atoms of ATP in the ATP-FM-Mg^{2+}-FomA complex (plotted based on the last 50 ns trajectory).


Replica 1
Replica 2
Replica 3
Distance (Å)
Time (ps)
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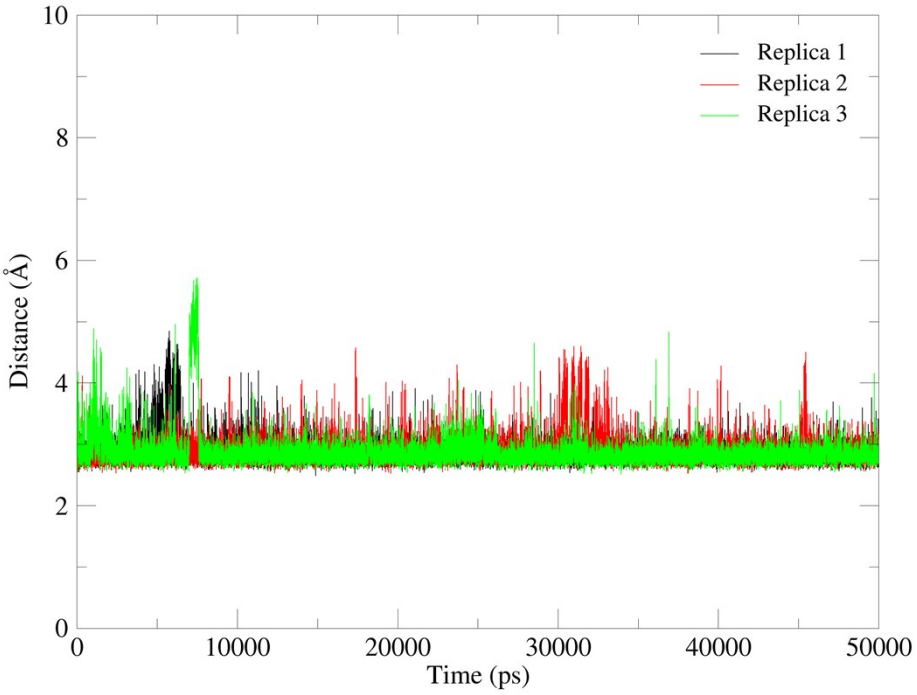
2
4
6
8
10
0
10000
20000
30000
40000
50000

(A)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Replica 2
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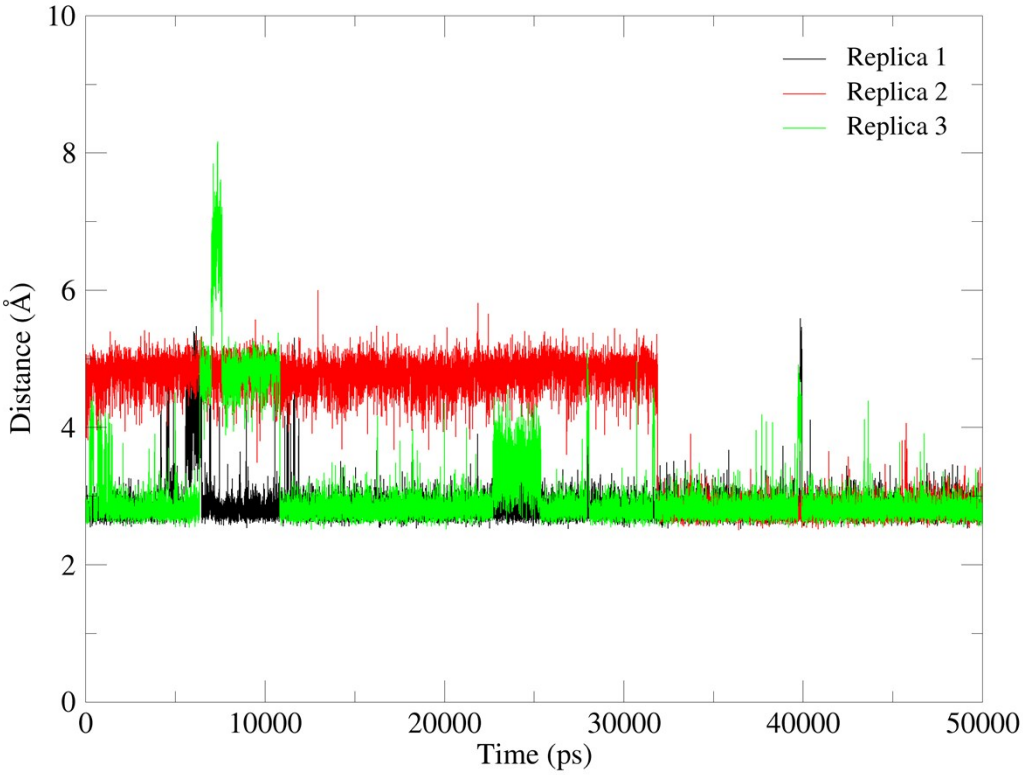
(B)



(C)

Figure S4. (A) Distance between the γ-phosphate of ATP and the phosphate group of fosfomycin from 3 replicas of 100-ns MD simulations of the ATP-FM-Mg^{2+}-FomA complex (plotted based on the last 50 ns trajectory) (B). Distance between the α-phosphate of ATP and Lys18from 3 replicas of 100-ns MD simulations of the ATP-FM-Mg^{2+}-FomA complex (plotted based on the last 50 ns trajectory) (C). Distance between Lys18 and Asp208 from 3 replicas of 100-ns MD simulations of the ATP-FM-Mg^{2+}-FomA complex (plotted based on the last 50 ns trajectory).

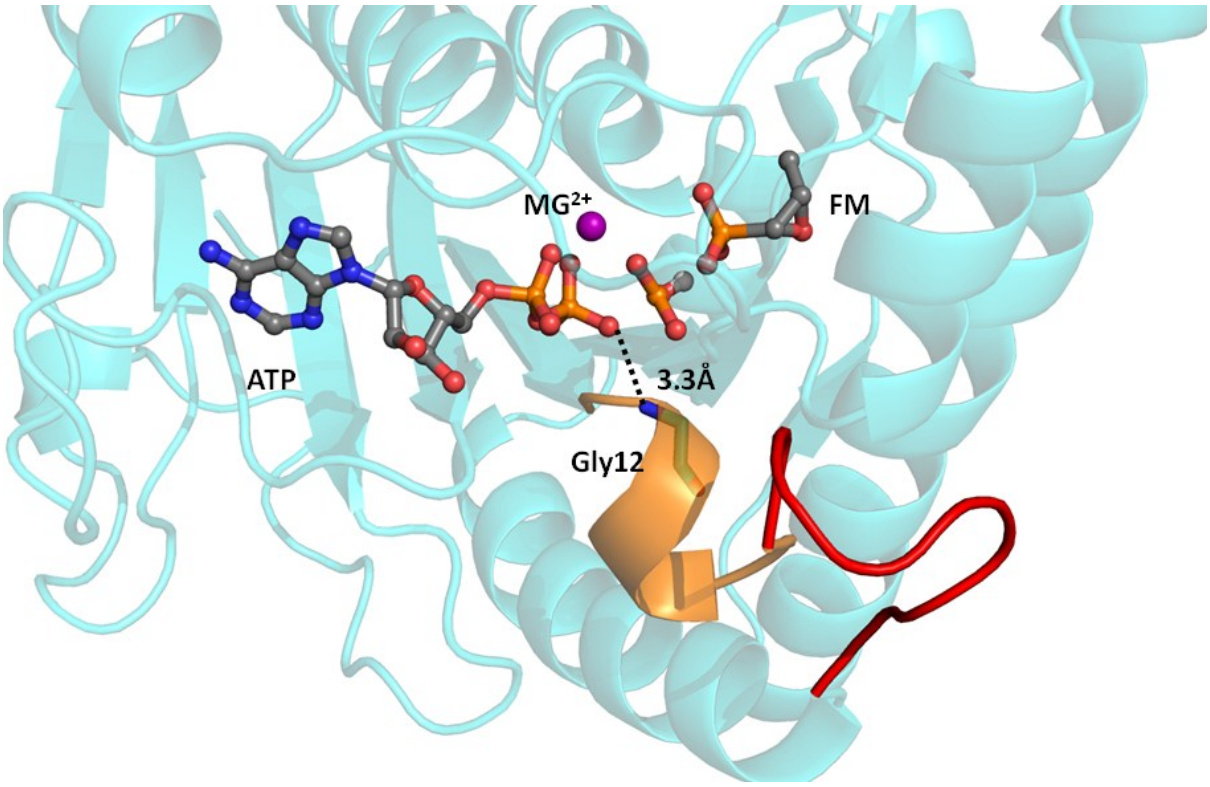


Figure S5. The QM/MM optimized structure of the transition state shows that Gly12 on the γ-turn (orange) connecting to the flexible glycine rich loop (red) forms a H-bond with the β-, γ-bridging oxygen.

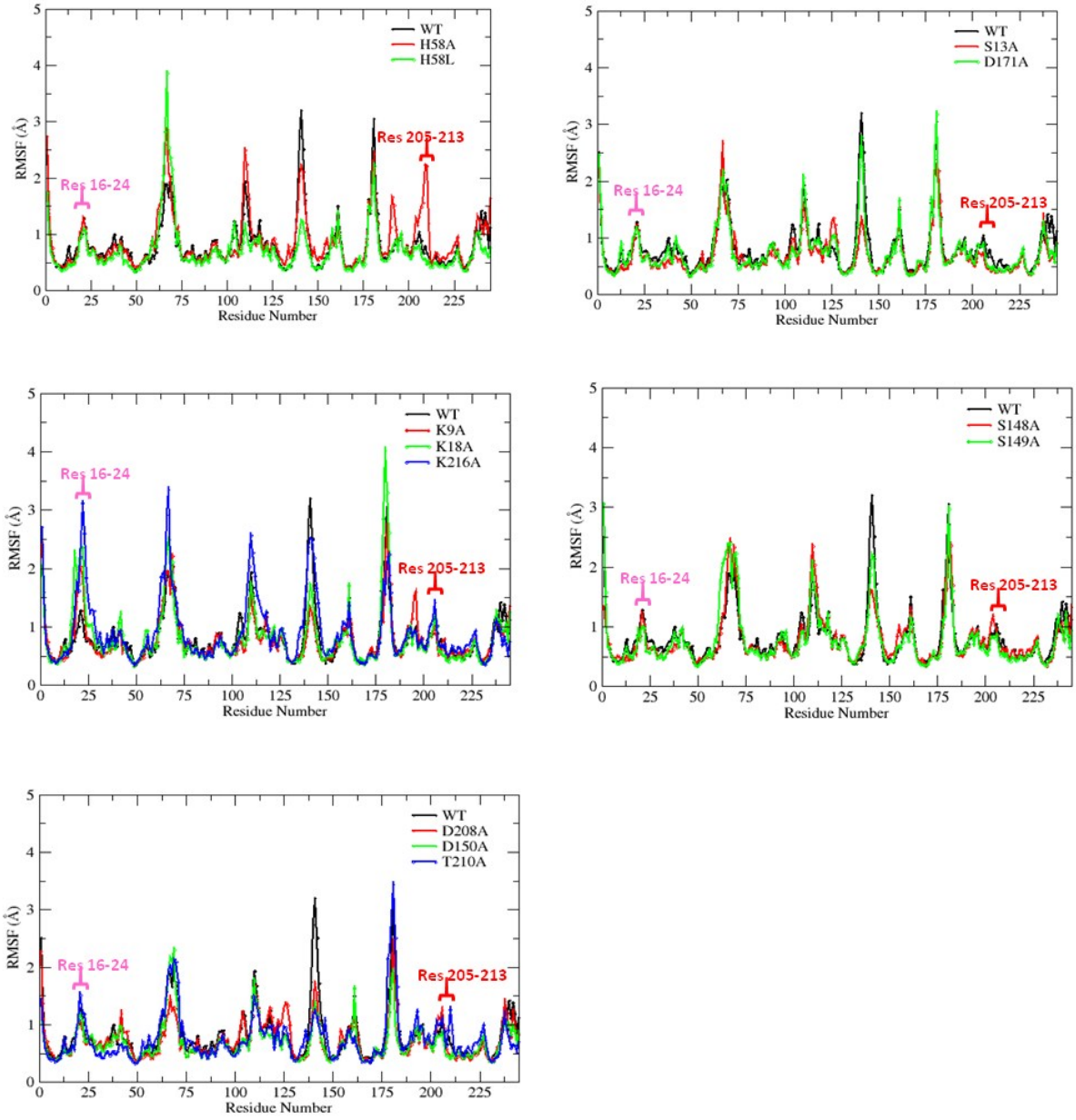
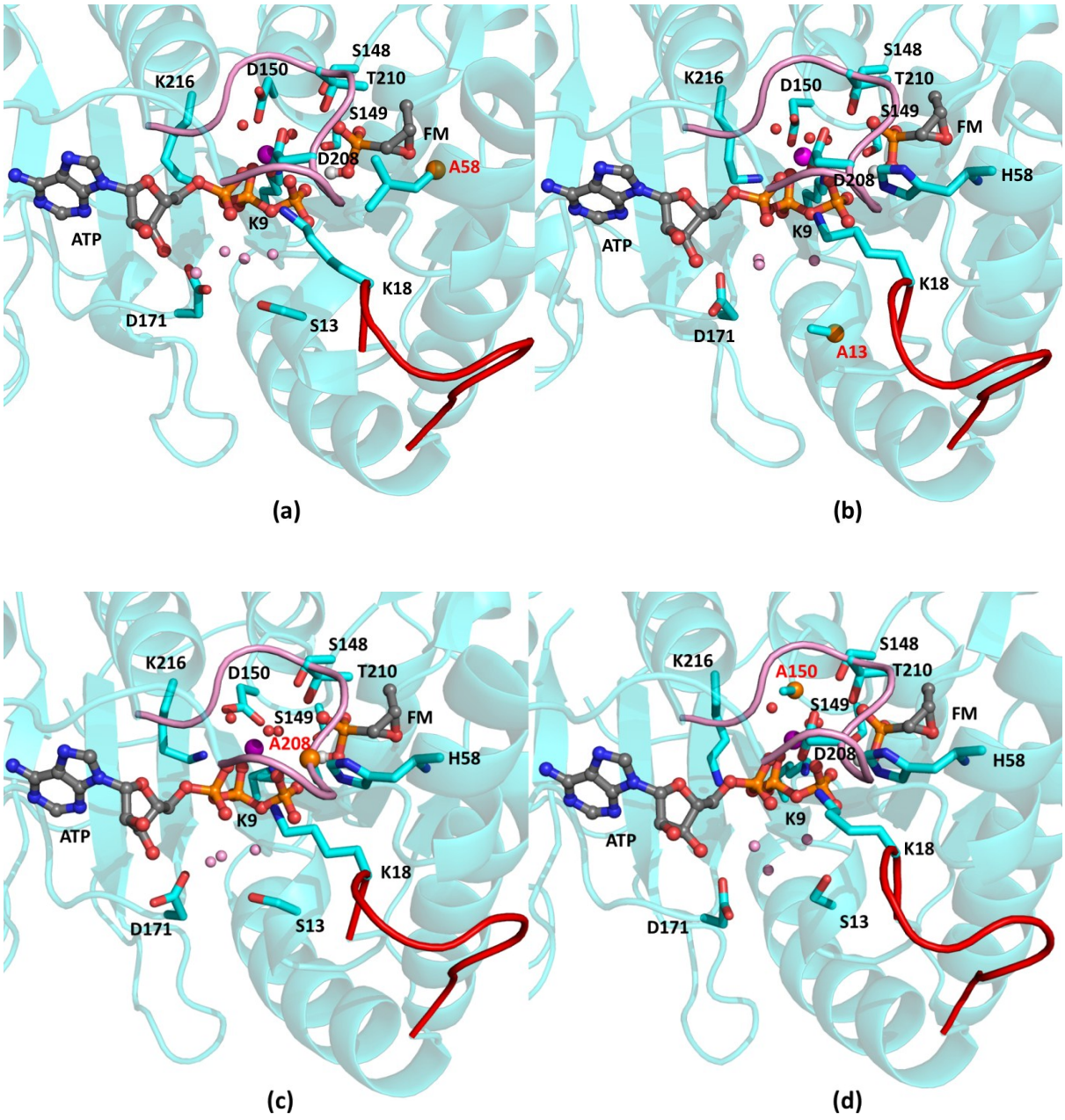


Figure S6. RMSF plots of the variants in comparison with the WT FomA. The glycine-rich loop comprising residues 16-24 is marked in pink and the loop connecting α-helix 6 and α-helix 7 is marked in red.


S148
K216
D150
T210
S149
FM
D208
A58
ATP
K9
K18
D171
S13
(a)
S148
K216
D150
T210
S149
FM
D208
H58
ATP
K9
K18
D171
A13
(b)
S148
K216
D150
T210
S149
FM
A208
H58
ATP
K9
K18
D171
S13
(c)
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S149
FM
D208
H58
ATP
K9
K18
D171
S13
(d)

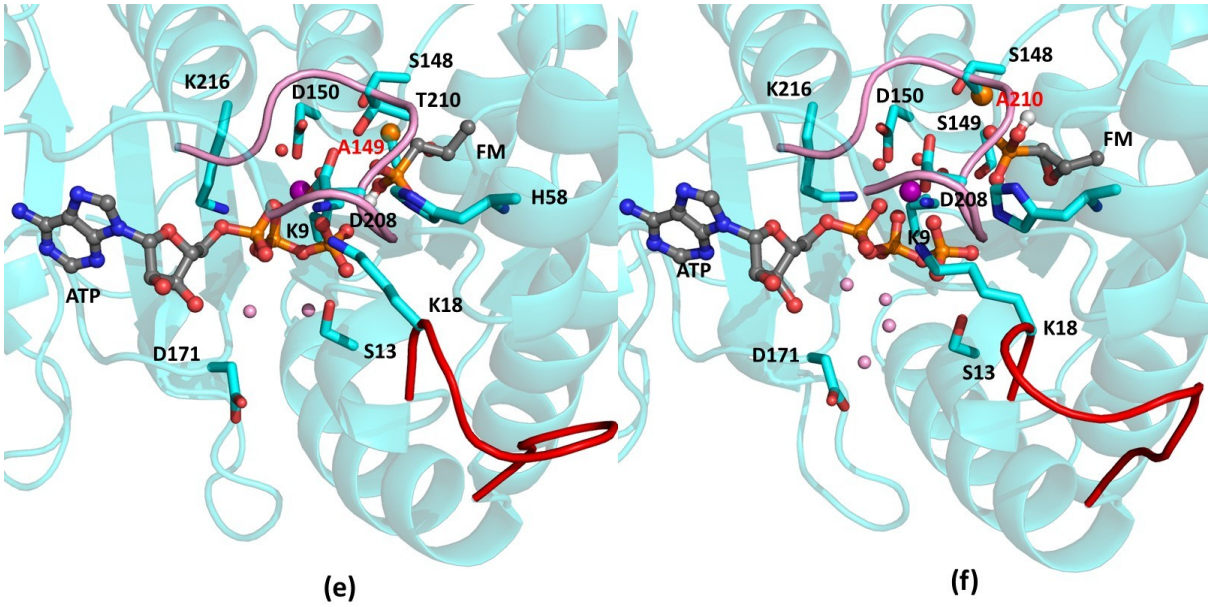

Figure S7. Representative structures of FomA mutants from MD simulations (a) H58L (b) S13A (c) D208A (d) D150A (e) S149A (f) T210A.