Supplementary Figures and Tables

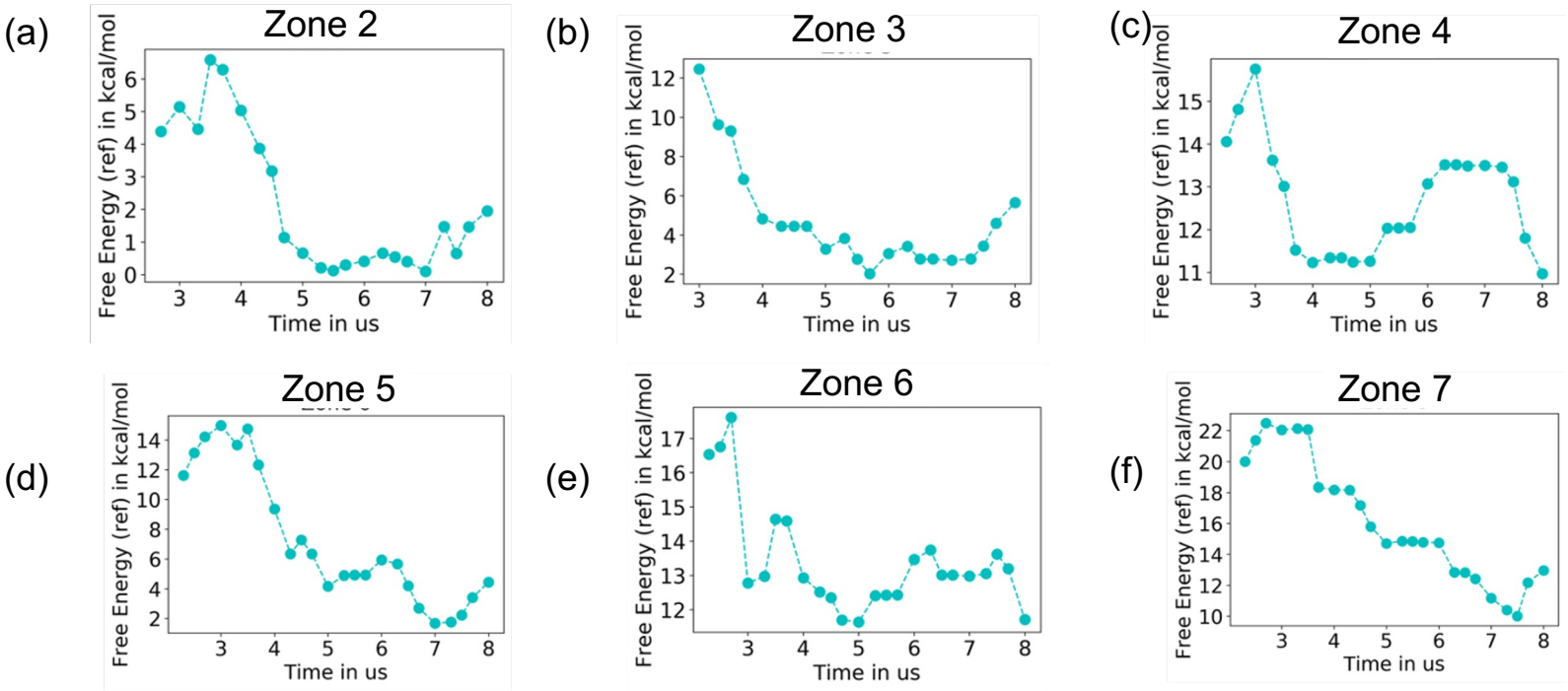


Figure S1: Convergence plots: free energies in different zones for WT system plotted against aggregate metadynamics time.

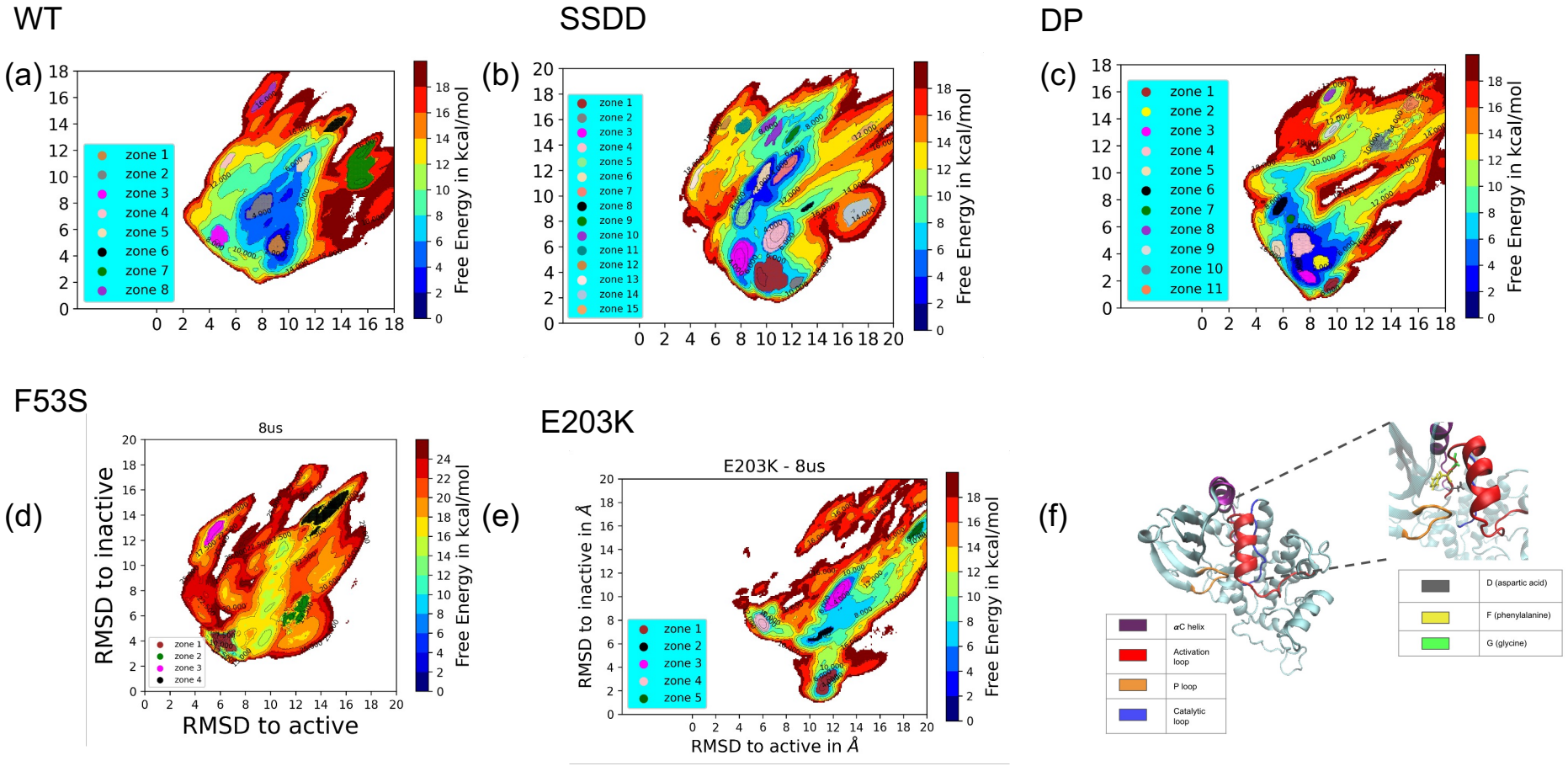
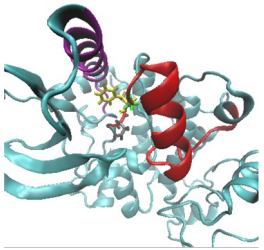
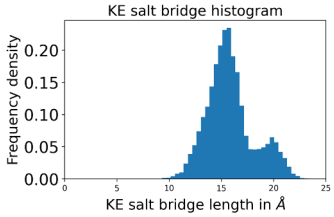
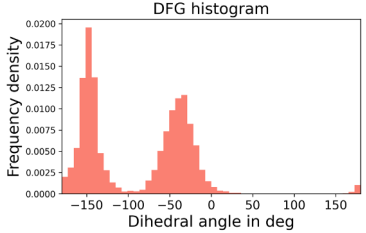
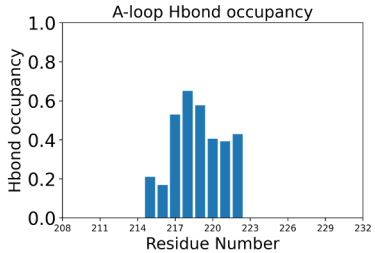
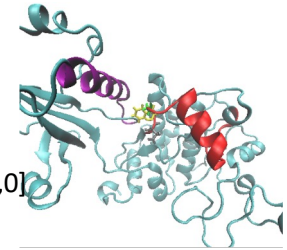
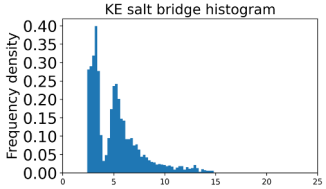
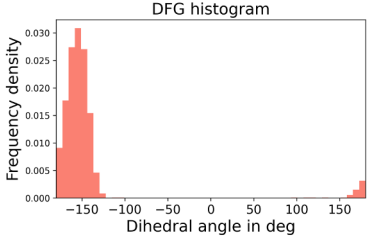
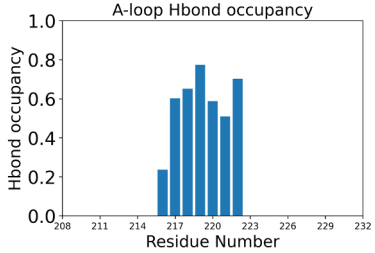
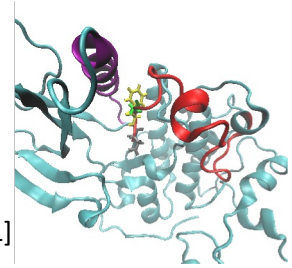
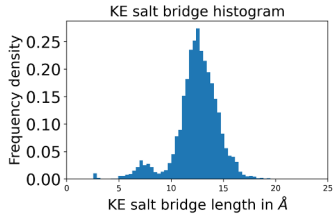
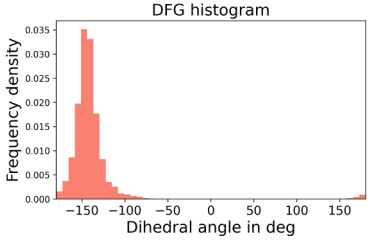
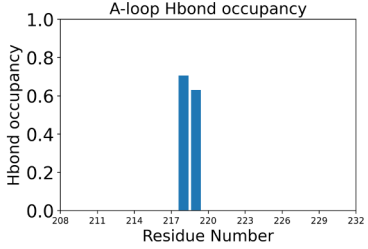


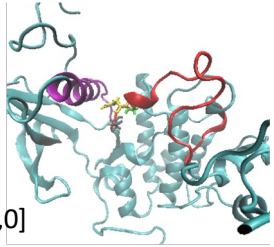
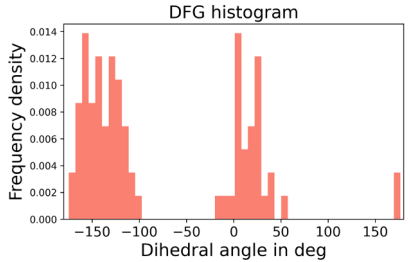
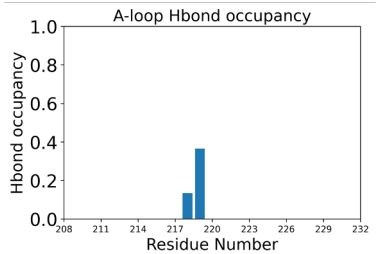
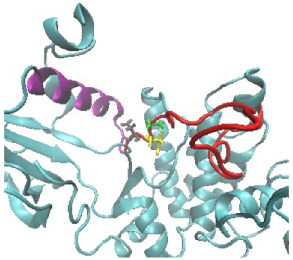
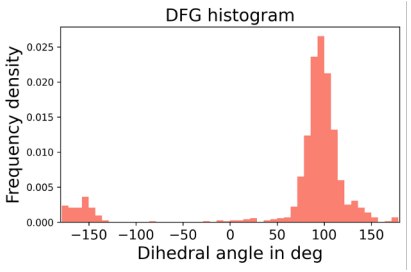
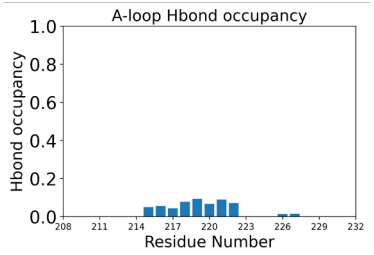
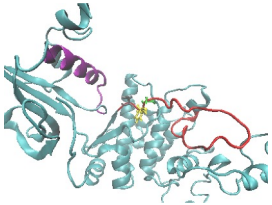
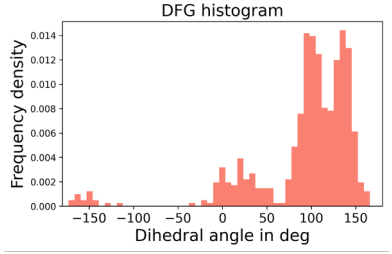
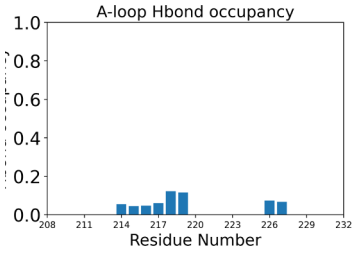
Figure S2: Free energy landscapes with zones of interest for MEK1 WT, SSDD, E203K, F53S, and di-phosphorylated systems.

MEK_WT

	Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 1 [0,0,0]					Free energy = 0.66 kcal/mol Remarks: Most Inactive (most stable) KE salt bridge broken**(I)** DFG out**(I) (Dout,low, Fin) I** A-loop partially helical**(I)**
Zone 2 [1,0.5,0]					Free energy = 2.35 kcal/mol Remarks: KE salt bridge formed **(A)** DFG out**(I) (Dout,low, Fin** A-loop partially helical**(I)**
Zone 3 [0.5,0,1]					Free energy = 6.28 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I) (Dout,low, Fin)** A-loop unfolded**(A)**

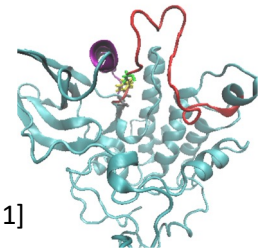
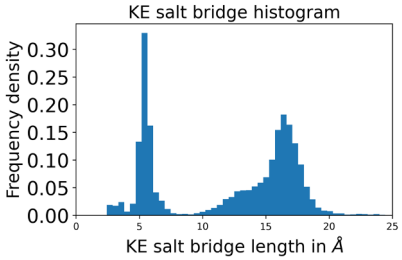
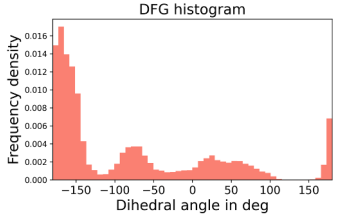
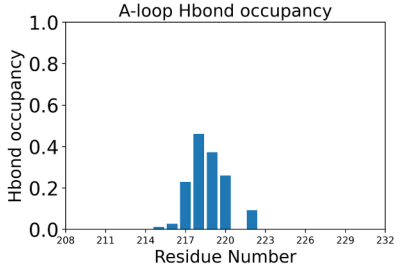
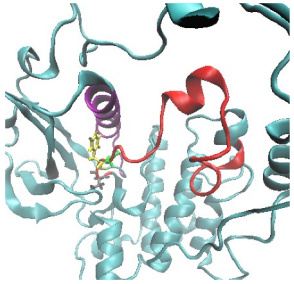
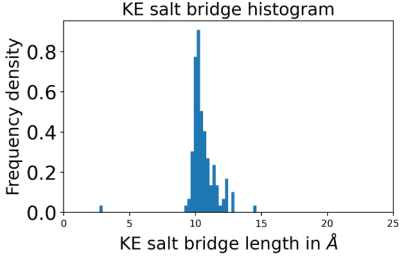
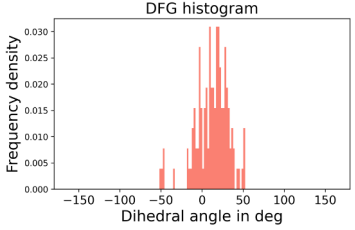
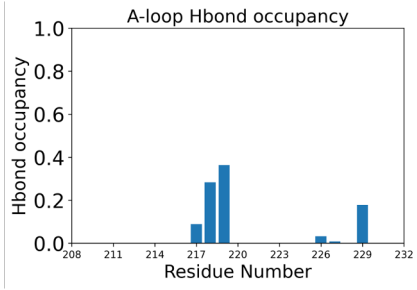
Note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_WT

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 4 [0.5,0.5,0]				Free energy = 11.6 kcal/mol Remarks: KE salt bridge broken**(I)** DFG mid**(I/A)** **(Din,low, Fin)** A-loop unfolded**(A)**
Zone 5 [0,1,1]				Free energy = 5.08 kcal/mol Remarks: KE salt bridge broken**(I)** DFG in**(A)** **(Din,up, Fout)** A-loop unfolded**(A)**
Zone 6 [0.5,1,1]				Free energy = 12.36 kcal/mol Remarks: KE salt bridge broken**(I)** DFG in**(A)** **(Din,up, Fout)** A-loop unfolded**(A)**

Note: **(I) denotes inactive feature and (A) denotes active feature**

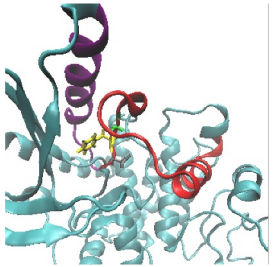
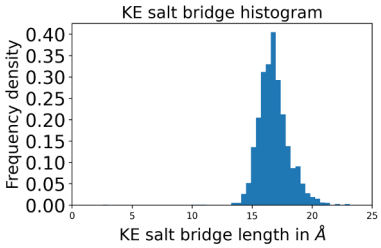
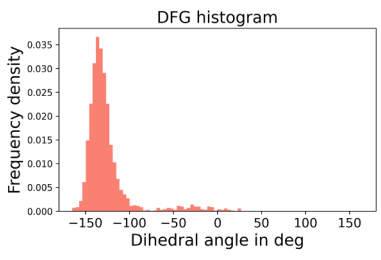
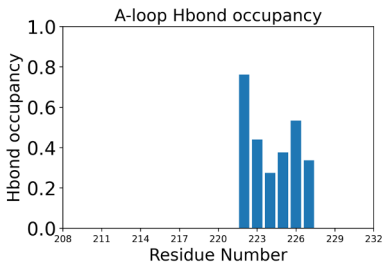
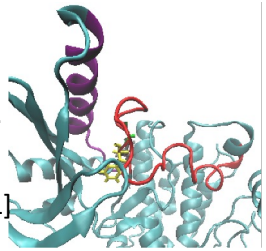
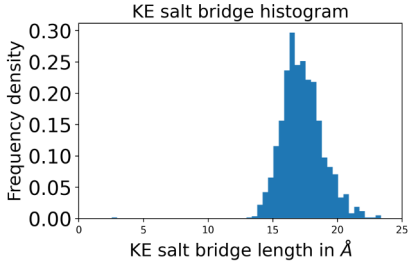
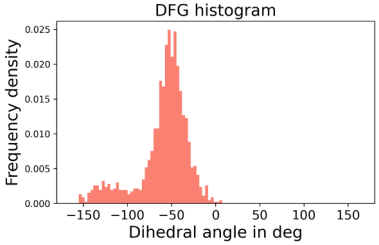
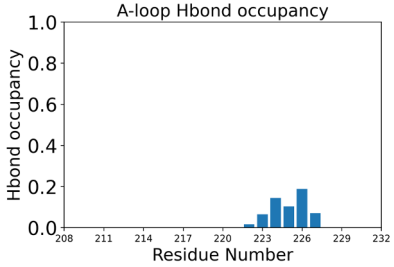
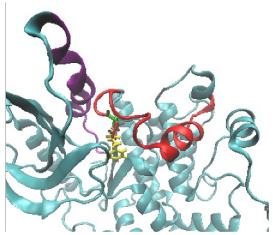
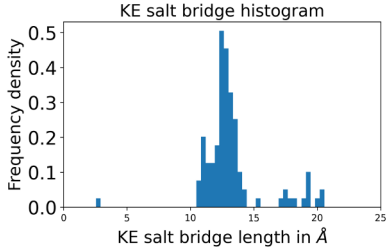
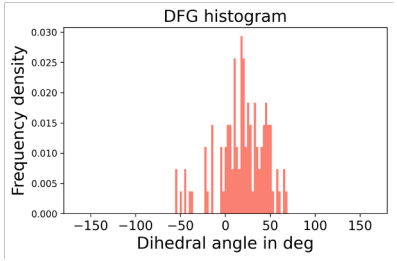
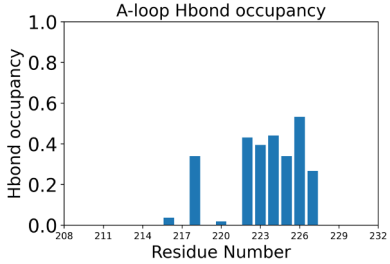
MEK_WT

	Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 7 [0.5,1,1]					Free energy = 13.51 kcal/mol Remarks: Most active KE salt bridge broken/formed**(I/A)** DFG in**(A) (Din,low, Fout)** A-loop unfolded**(A)**
Zone 8 [0,1,1]					Free energy = 15.08 kcal/mol Remarks: KE salt bridge broken**(I)** DFG in**(A) (Din,low, Fout)** A-loop unfolded**(A)**

Note: **(I) denotes inactive feature and (A) denotes active feature**

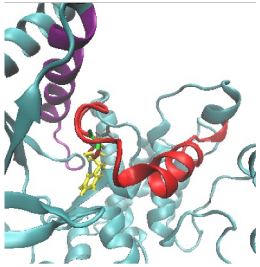
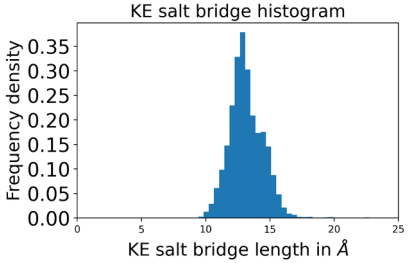
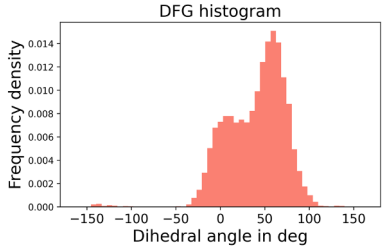
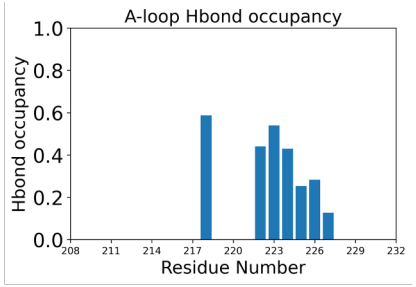
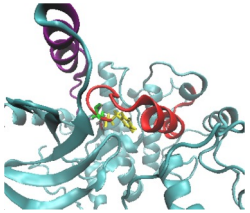
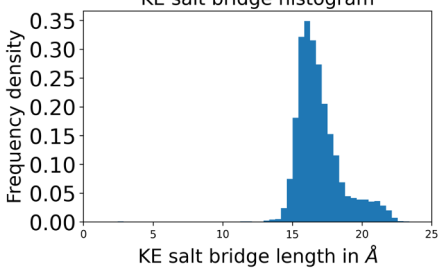
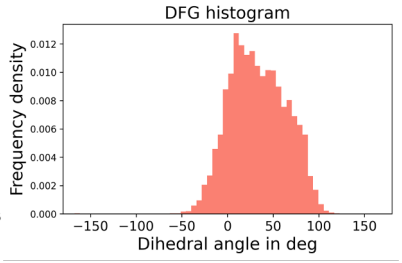
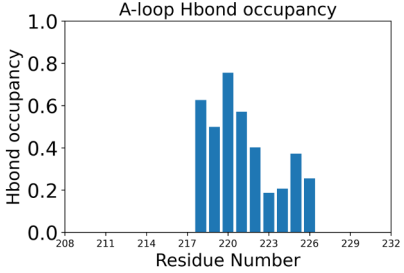
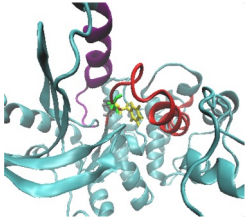
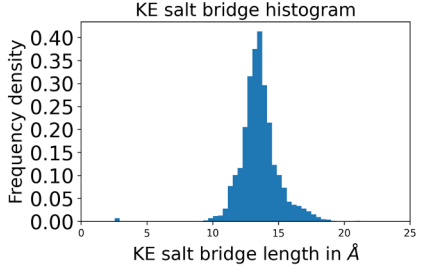
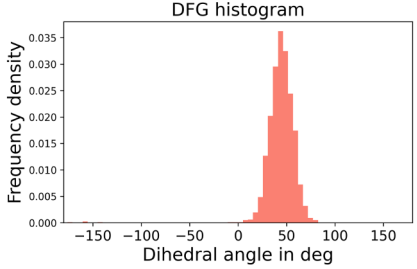
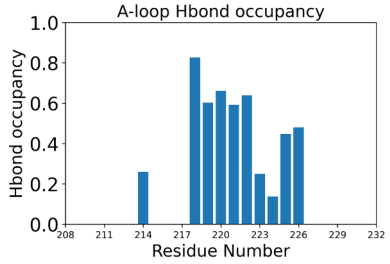
Fig. S3: MEK1 WT structures and their structural characteristics across zones

MEK_SSDD

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 1 [0,0,0]					Free energy = 3.46 kcal/mol Remarks: Most Inactive KE salt bridge broken**(I)** DFG out**(I) (Dout,low, Fin)** A-loop partially helical**(I)**
Zone 2 [0,0.5,1]					Free energy = 5.38 kcal/mol Remarks: KE salt bridge broken**(I)** DFG mid**(I/A) (Din, up, Fin)** A-loop unfolded**(A)**
Zone 3 [0,0.5,0]					Free energy = 0.79 kcal/mol Remarks: Most stable KE salt bridge broken**(I)** DFG out**(I/A) (Din,up, Fmid)** A-loop partially helical**(I)**

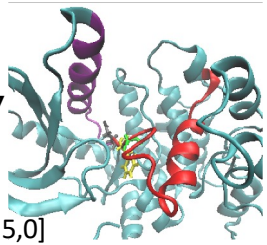
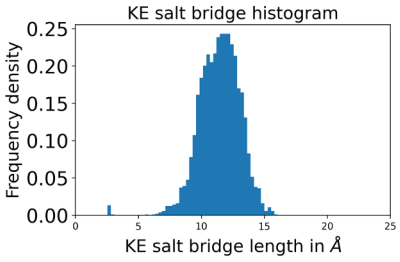
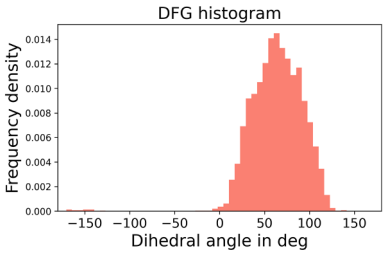
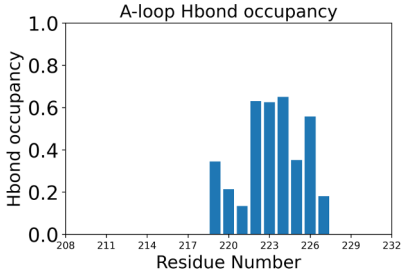
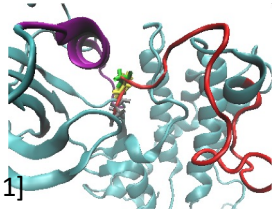
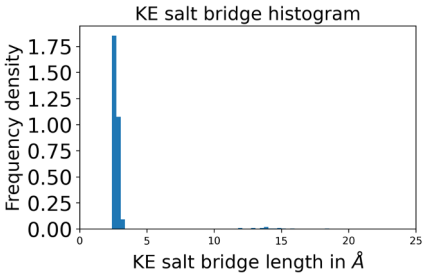
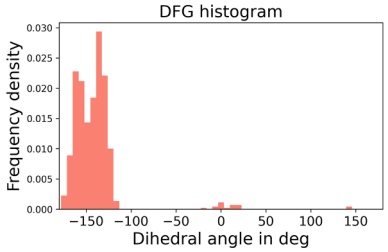
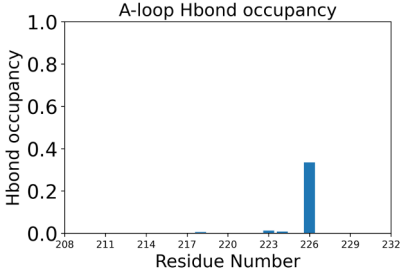
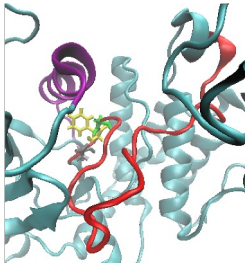
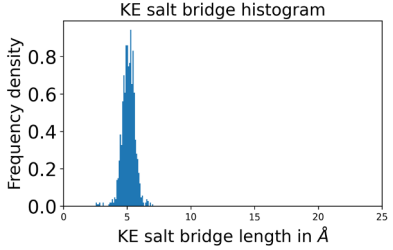
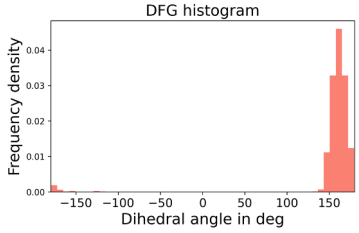
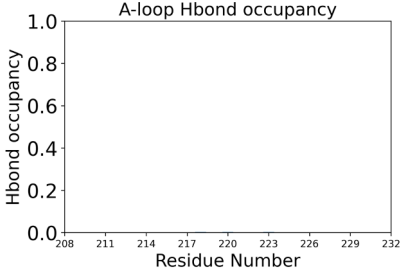
Note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_SSDD

	Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 4 0,0,0]					Free energy = 0.90 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I) (Dout,up, Fin)** A-loop partially helical**(I)**
Zone 5 [0,1,0]					Free energy = 1.22 kcal/mol Remarks: KE salt bridge broken**(I)** DFG in**(A) (Din,up, Fout)** A-loop partially helical**(I)**
Zone 6 [0,1,0]					Free energy = 0.85 kcal/mol Remarks: KE salt bridge broken**(I)** DFG in**(A) (Din,up, Fout)** A-loop partially helical**(I)**

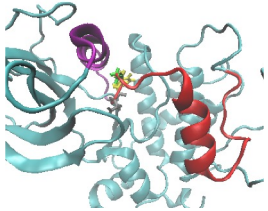
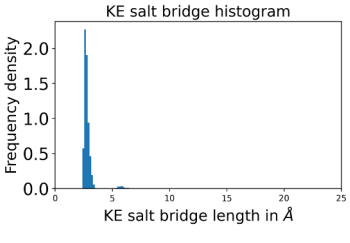
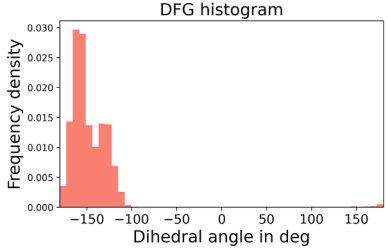
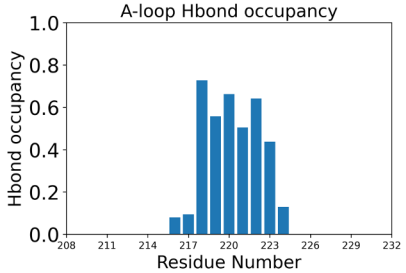
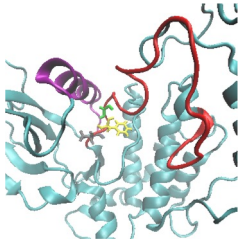
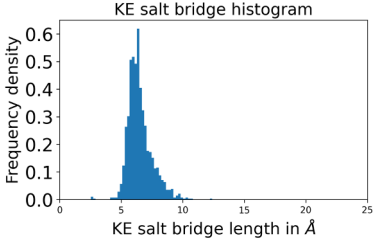
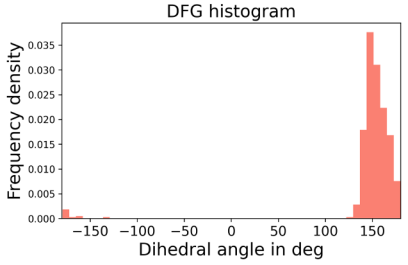
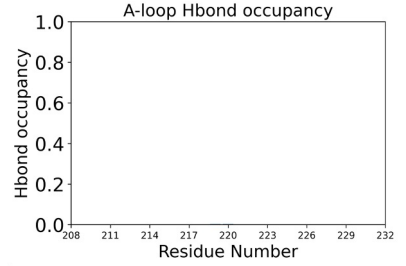
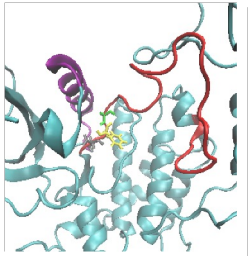
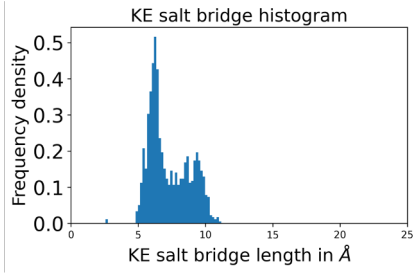
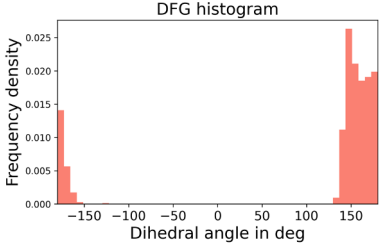
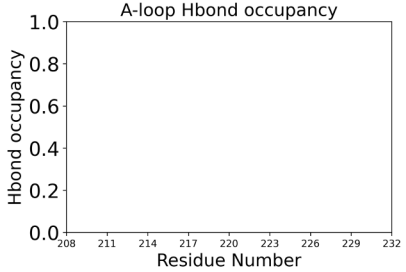
Note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_SSDD

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 7  [0.5,0.5,0]				Free energy = 1.87 kcal/mol Remarks: KE salt bridge broken**(I)** DFG mid**(I/A)** **(Din,up, Fmid)** A-loop partially helical**(I)**
Zone 8  [1,0.5,1]				Free energy = 8.4 kcal/mol Remarks: KE salt bridge formed**(A)** DFG mid**(I/A)** **(Din,low, Fmid)** A-loop extended**(A)**
Zone 9  [1,0.5,1]				Free energy = 5.23 kcal/mol Remarks: KE salt bridge formed**(A)** DFG mid**(I/A)** **(Din,low, Fin)** A-loop extended**(A)**

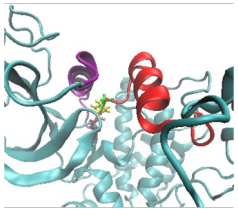
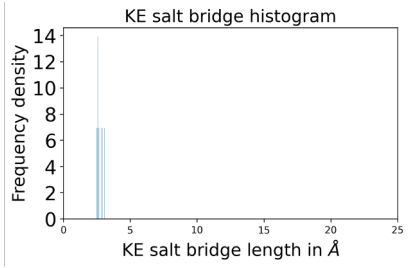
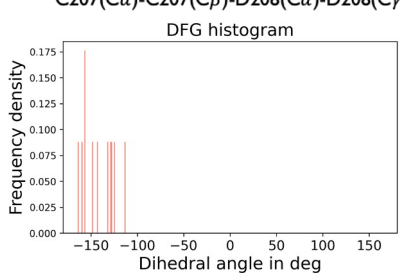
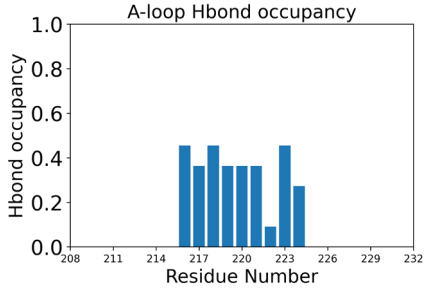
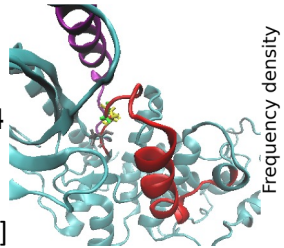
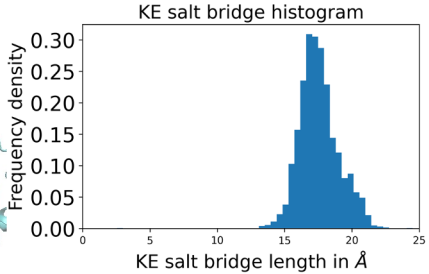
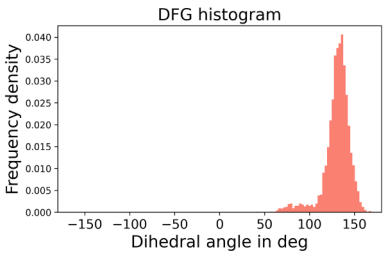
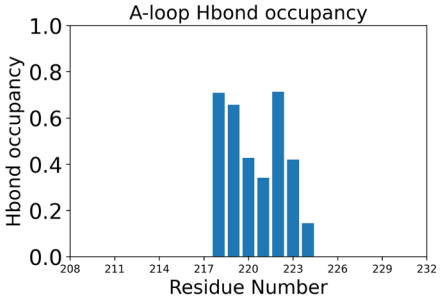
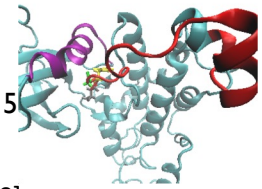
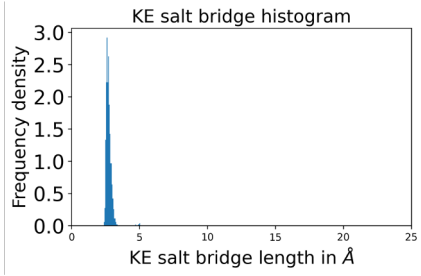
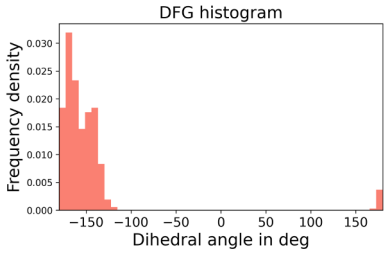
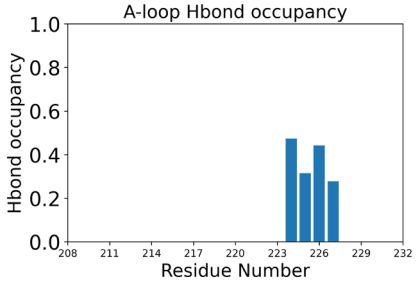
Note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_SSDD

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 10 [1,1,0]					Free energy = 6.92 kcal/mol Remarks: KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop partially helical**(I)**
Zone 11 [1,1,1]					Free energy = 11.37 kcal/mol Remarks:Most active KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop extended**(A)**
Zone 12 [1,1,1]					Free energy = 11.98 kcal/mol Remarks: KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop extended**(A)**

Note: **(I) denotes inactive feature and (A) denotes active feature**

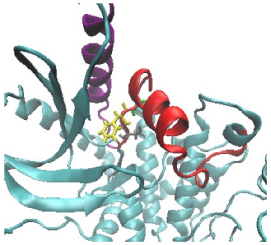
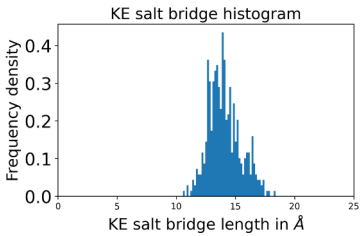
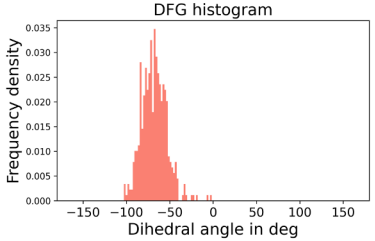
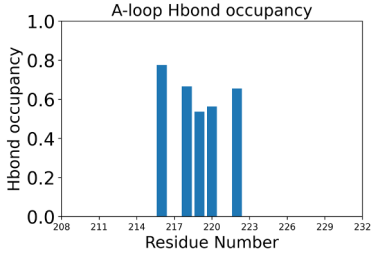
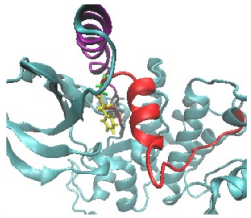
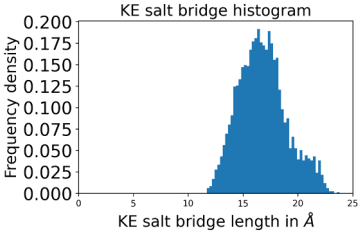
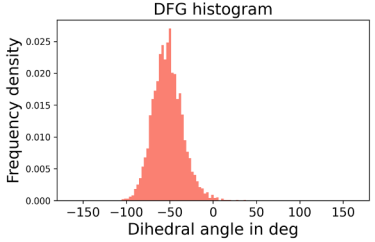
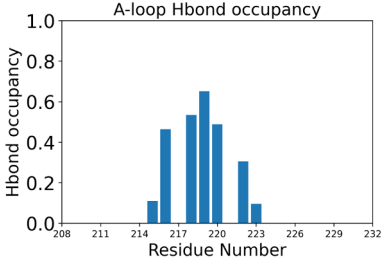
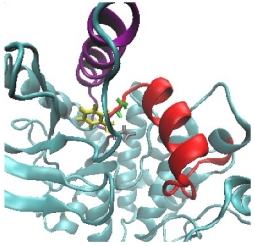
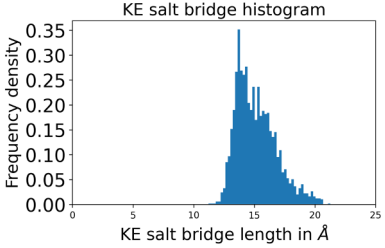
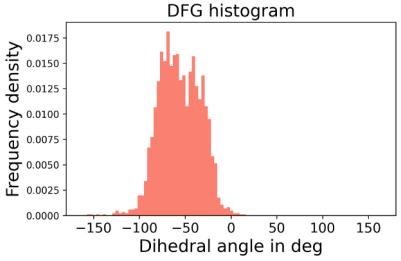
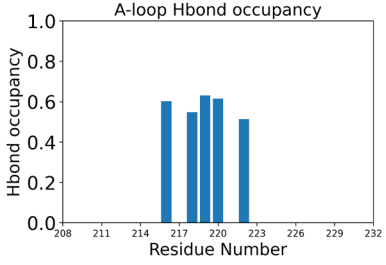
MEK_SSDD

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 13 [1,1,0]					Free energy = 11.72 kcal/mol Remarks: KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop partially helical**(I)**
Zone 14 [0,0.5,0]					Free energy = 12.19 kcal/mol Remarks: KE salt bridge formed**(I)** DFG mid**(I/A) (Din,low, Fin)** A-loop partially helical**(I)**
Zone 15 [1,1,0]					Free energy = 11.98 kcal/mol Remarks: KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop partially helical**(I)**

Note: **(I) denotes inactive feature and (A) denotes active feature**

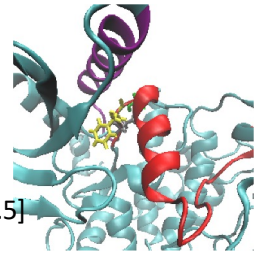
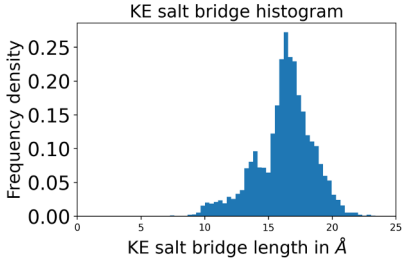
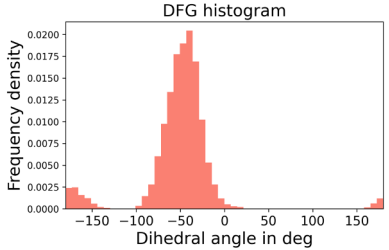
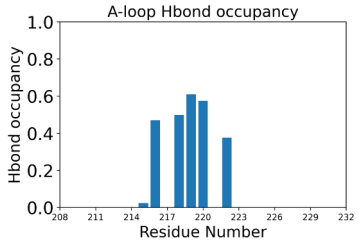
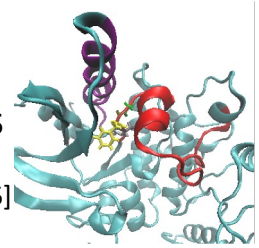
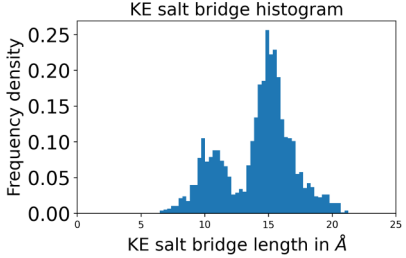
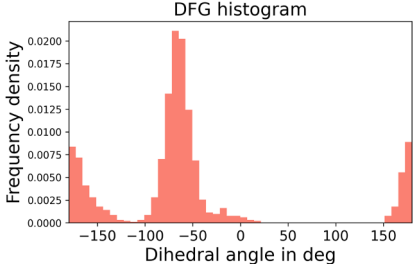
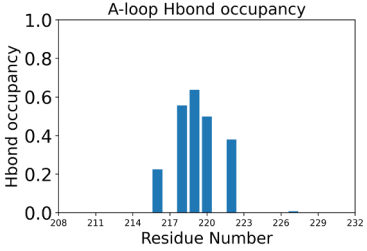
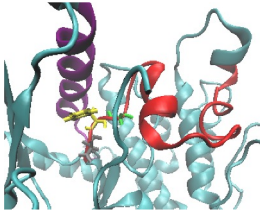
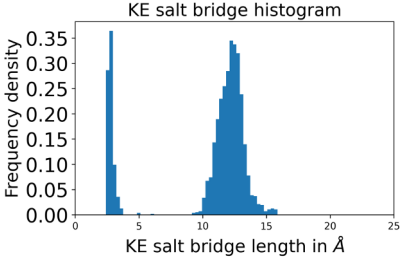
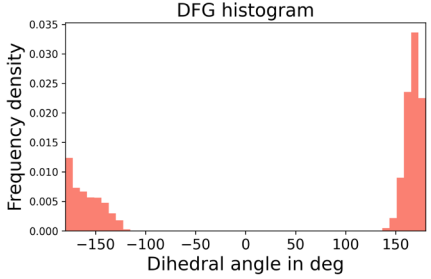
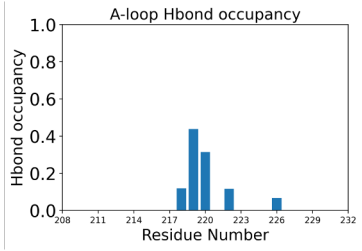
Fig. S4: MEK1 SSDD structures and their structural characteristics across zones

MEK_dp

	Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 1 [0,0,0]					Free energy = 3.70 kcal/mol Remarks: Most Inactive KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially helical**(I)**
Zone 2 [0,0,0.5]					Free energy = 1.39 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I)** **(Dout,up, Fin)** A-loop partially unfolded**(I/A)**
Zone 3 [0,0,0]					Free energy = 1.45 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially helical**(I)**

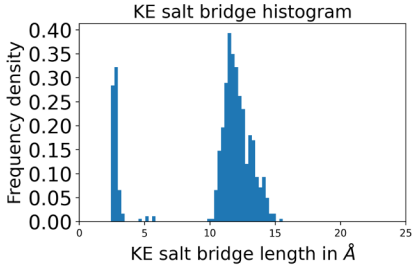
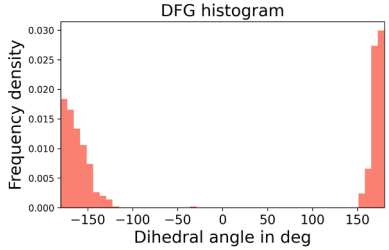
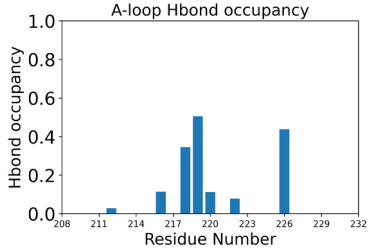
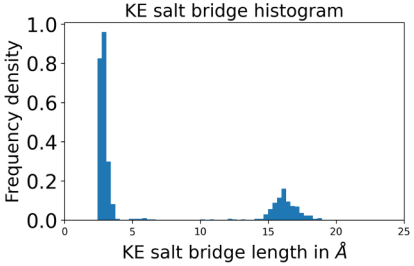
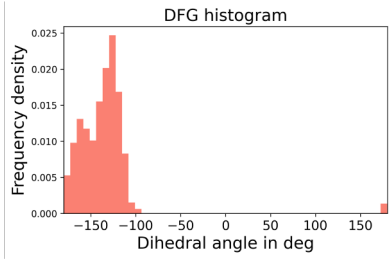
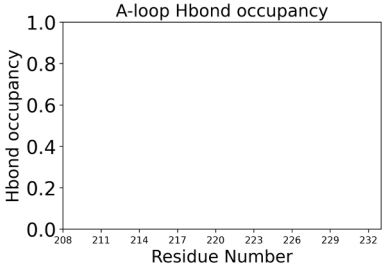
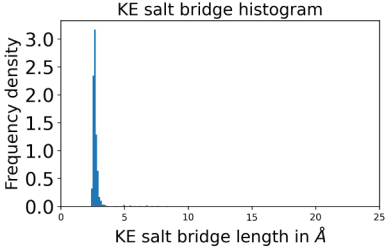
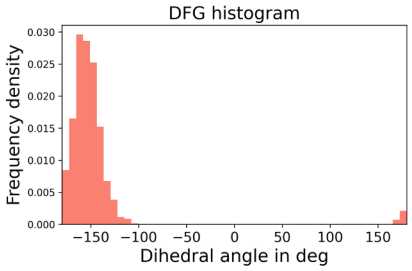
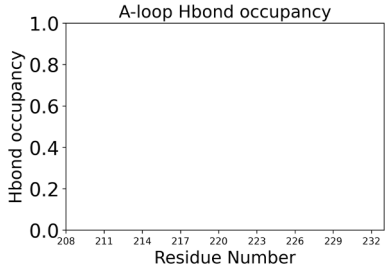
PS note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_dp

	Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 4 [0,0,0.5]					Free energy = 0.49 kcal/mol Remarks:Most Stable KE salt bridge broken**(I)** DFG out**(I) (Dout,up, Fin)** A-loop partially helical**(I)**
Zone 5 [0,0,0.5]					Free energy = 4.23 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially helical**(I)**
Zone 6 [0.5,0.5,1]					Free energy = 3.19 kcal/mol Remarks: KE salt bridge formed/broken**(I/A)** DFG mid**(I) (Din,low, Fin)** A-loop unfolded**(A)**

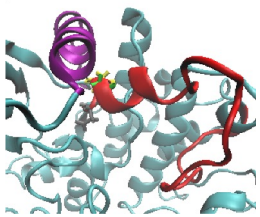
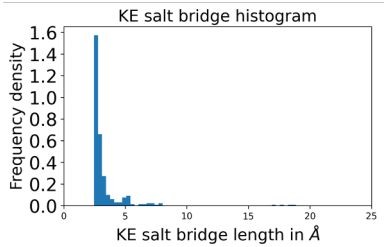
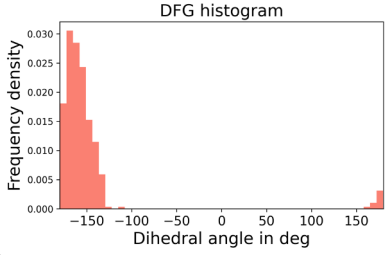
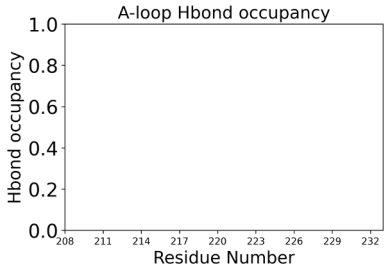
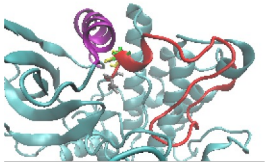
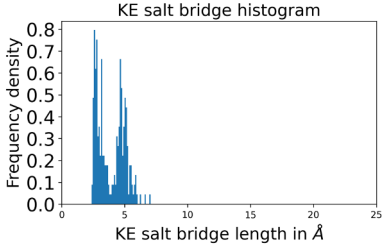
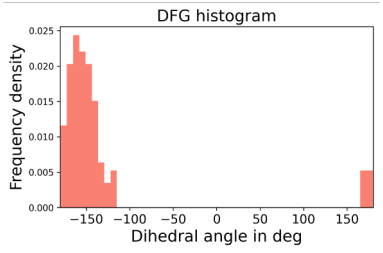
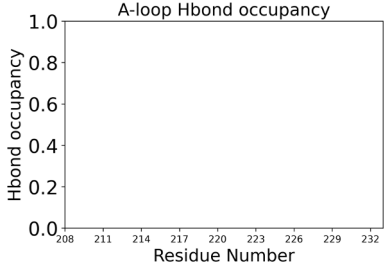
PS note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_dp

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 7 [0.5,0.5,1]				Free energy = 4.45 kcal/mol Remarks: KE salt bridge broken/formed**(I/A)** DFG mid**(I/A)** **(Din,low, Fin)** A-loop unfolded**(A)**
Zone 8 [1,1,1]				Free energy = 9.14 kcal/mol Remarks: KE salt bridge formed **A)** DFG in**(A) (Dout,low, Fin)** A-loop unfolded**(A)**
Zone 9 [1,1,1]				Free energy = 8.64 kcal/mol Remarks: Most Active KE salt bridge formed**(A)** DFG in **(A) (Din,low, Fout)** A-loop unfolded**(A)**

PS note: **(I) denotes inactive feature and (A) denotes active feature**

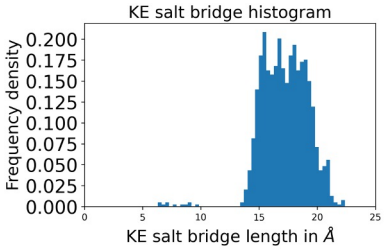
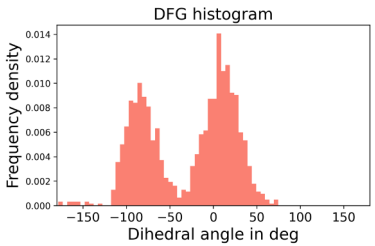
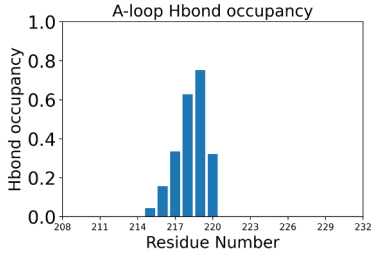
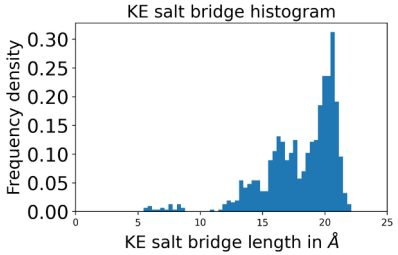
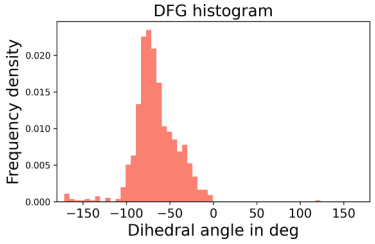
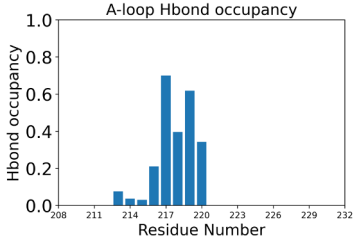
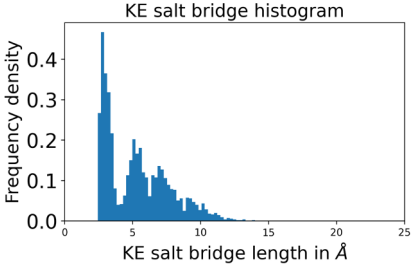
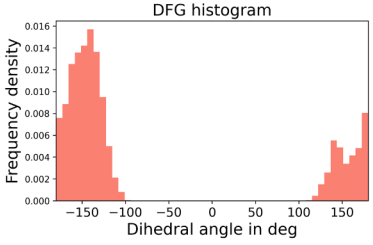
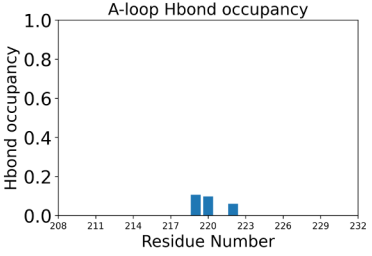
MEK_dp

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 10 [1,1,1]					Free energy = 9.24 kcal/mol Remarks KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop unfolded**(A)**
Zone 11 [1,1,1]					Free energy = 8.6 kcal/mol Remarks: KE salt bridge formed**(A)** DFG in **(A) (Din,low, Fout)** A-loop unfolded**(A)**

PS note: **(I) denotes inactive feature and (A) denotes active feature**

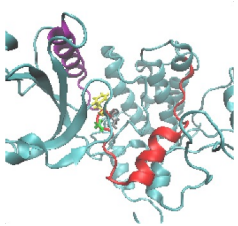
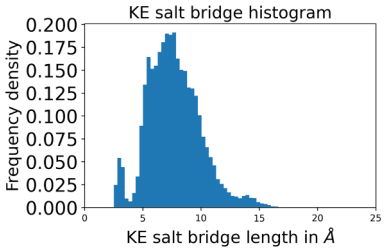
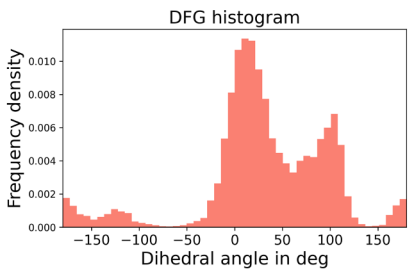
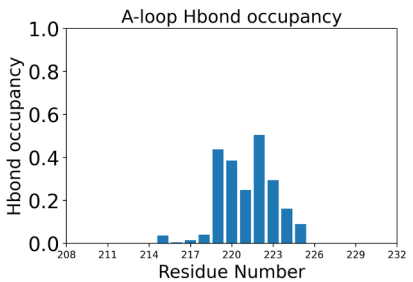
Fig. S5: MEK1 diphosphorylated structures and their structural characteristics across zones

MEK_F53S

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 1 [0.5,0.5,0.5]				Free energy = 1.83 kcal/mol Remarks:Most Stable KE salt bridge broken**(I)** DFG mid**(I/A)** **(Dout,up, Fout)** A-loop partially unfolded**(I/A)**
Zone 2 [0.5,0,0.5]				Free energy = 11.83 kcal/mol Remarks: KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially unfolded**(I/A)**
Zone 3 [1,1,1]				Free energy = 13.76 kcal/mol Remarks: Most Active KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop unfolded**(A)**

PS note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_F53S

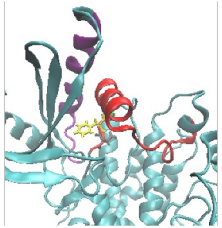
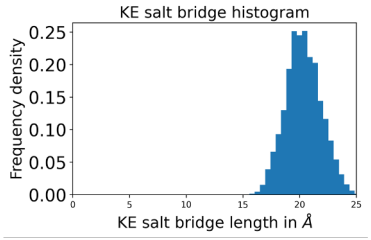
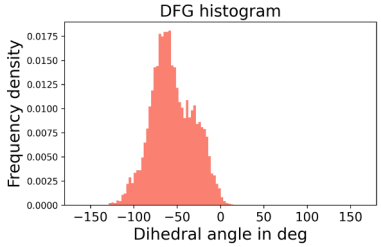
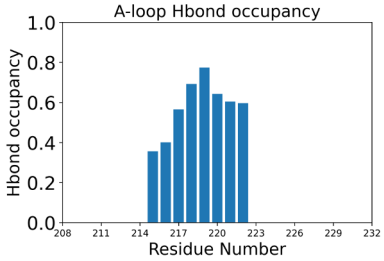
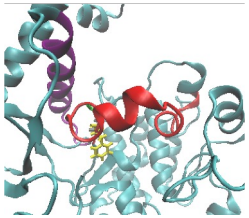
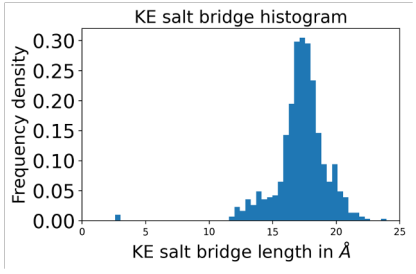
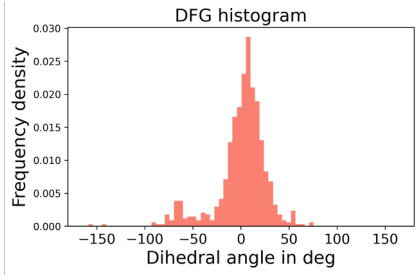
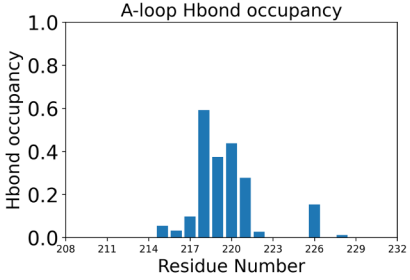
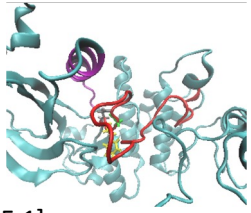
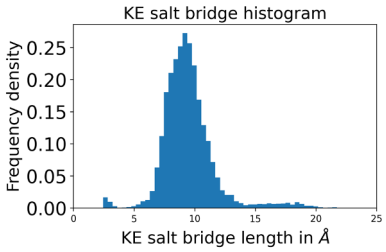
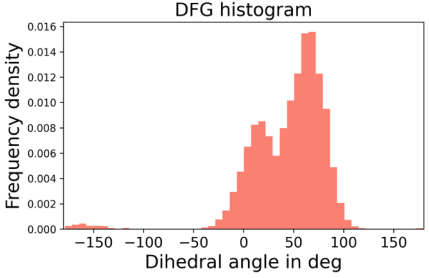
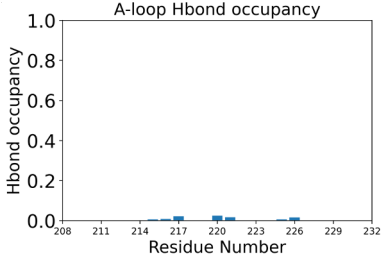
Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 4 				Free energy = 12.11 kcal/mol Remarks:Most Inactive KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially helical**(I)**

[0.5,0,0]

Fig. S6: MEK1 F53S structures and their structural characteristics across zones

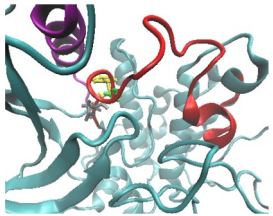
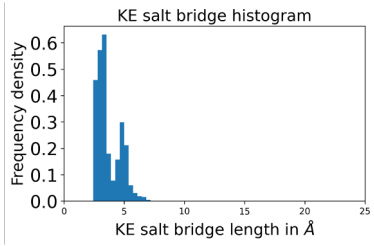
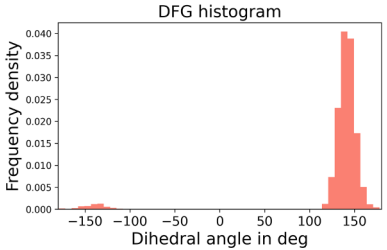
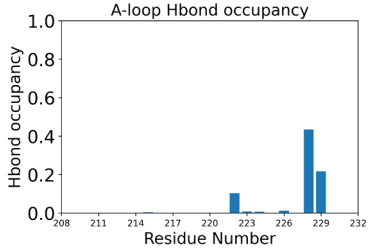
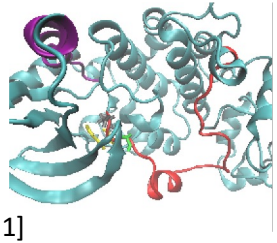
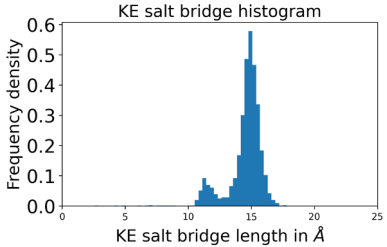
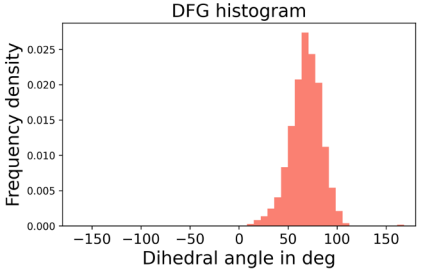
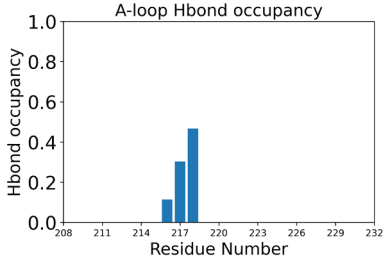
PS note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_E203K

Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 1 [0,0,0]					Free energy = 2.59 kcal/mol Remarks:Most Inactive KE salt bridge broken**(I)** DFG out**(I)** **(Dout,low, Fin)** A-loop partially helical**(I)**
Zone 2 [0,0.5,0]					Free energy = 5.71 kcal/mol Remarks: KE salt bridge broken**(I)** DFG mid**(I/A)** **(Dout,low, Fout)** A-loop partially helical**(I)**
Zone 3 [0.5,0.5,1]					Free energy = 1.11 kcal/mol Remarks: Most Stable KE salt bridge broken**(I)** DFG mid**(I/A)** **(Dout,up, Fout)** A-loop unfolded**(A)**

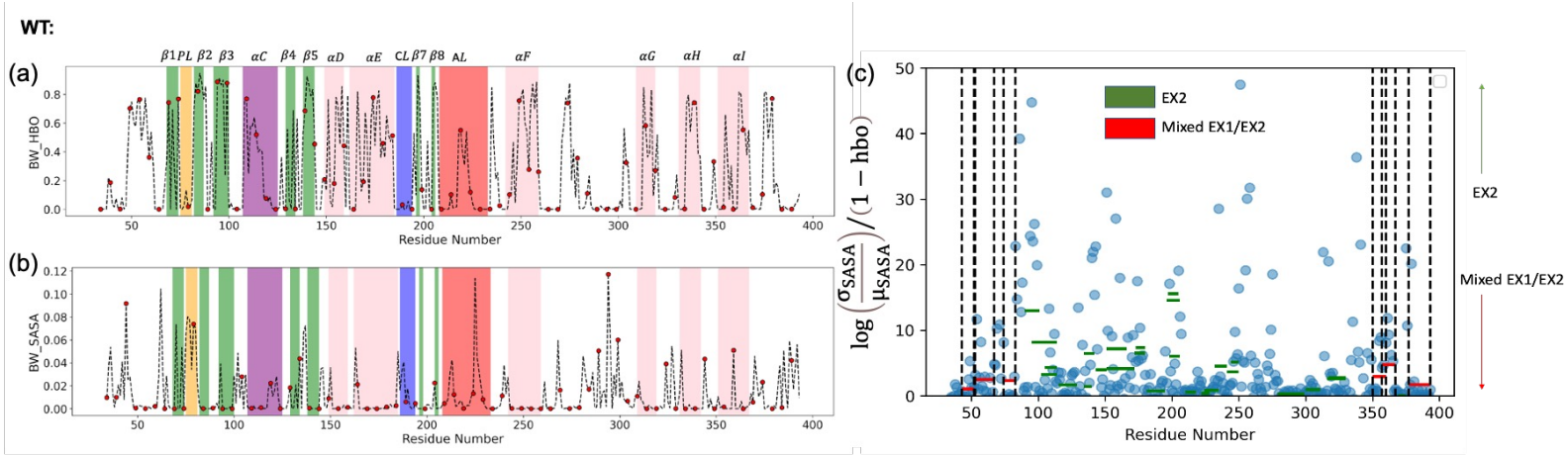
Note: **(I) denotes inactive feature and (A) denotes active feature**

MEK_E203K

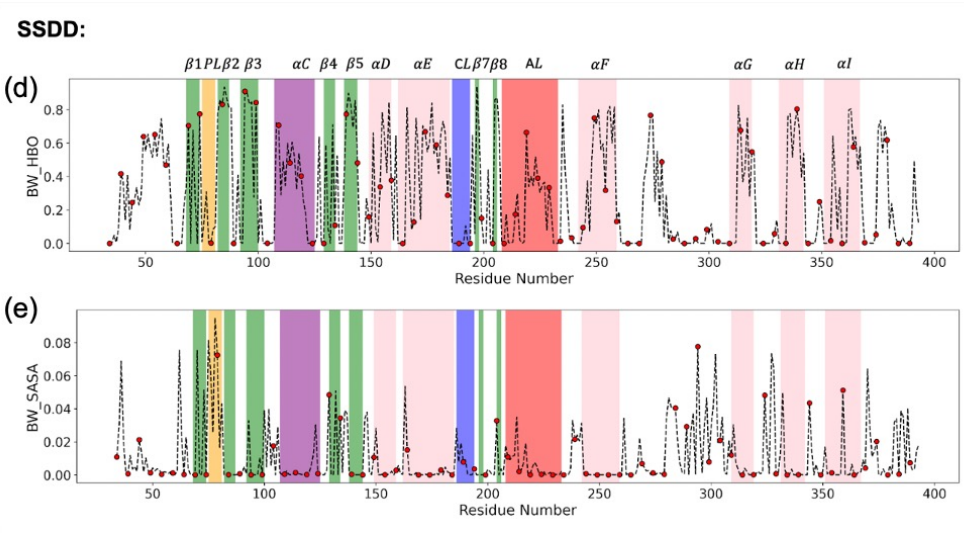
Structure from zone	KE salt bridge histogram	DFG flip C207(Cα)-C207(Cβ)-D208(Cα)-D208(Cγ)	Hbond of A-loop	Feature
Zone 4 [1,1,1] 				Free energy = 3.64 kcal/mol Remarks: Most Active KE salt bridge formed**(A)** DFG in**(A) (Din,low, Fout)** A-loop unfolded**(A)**
Zone 5 [0,0.5,1] 				Free energy = 6.0 kcal/mol Remarks: KE salt bridge broken**(I)** DFG mid**(I/A) (Din,up, Fin)** A-loop unfolded**(A)**

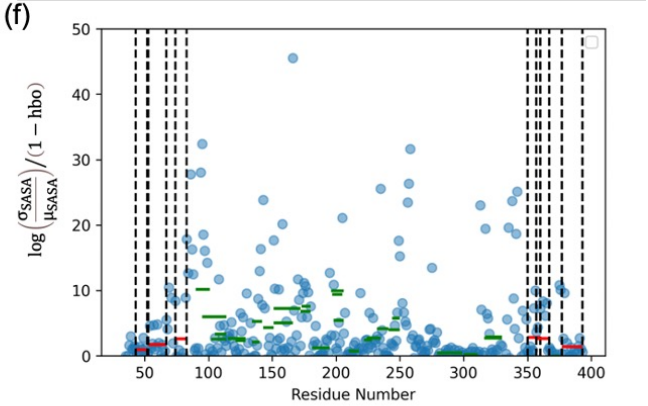
Note: **(I) denotes inactive feature and (A) denotes active feature**

Fig. S7: MEK1 E203K structures and their structural characteristics across zones


WT:
(a)
β1 PL β2 β3 αC β4 β5 αD αE CL β7 β8 AL αF αG αH αI
BW_HBO
Residue Number
(b)
BW_SASA
Residue Number
(c)
EX2
Mixed EX1/EX2
$\log\left(\frac{\sigma_{SASA}}{\mu_{SASA}}\right)/(1-\mathrm{hbo})$
Residue Number
EX2
Mixed EX1/EX2

SSDD:


(d)
β1 PL β2 β3 αC β4 β5 αD αE CL β7 β8 AL αF αG αH αI
BW_HBO
0.0
0.2
0.4
0.6
0.8
50
100
150
200
250
300
350
400
Residue Number
(e)
BW_SASA
0.00
0.02
0.04
0.06
0.08
50
100
150
200
250
300
350
400
Residue Number


(f)
$\log\left(\frac{\sigma_{SASA}}{\mu_{SASA}}\right)/(1-\mathrm{hbo})$
0
10
20
30
40
50
50
100
150
200
250
300
350
400
Residue Number

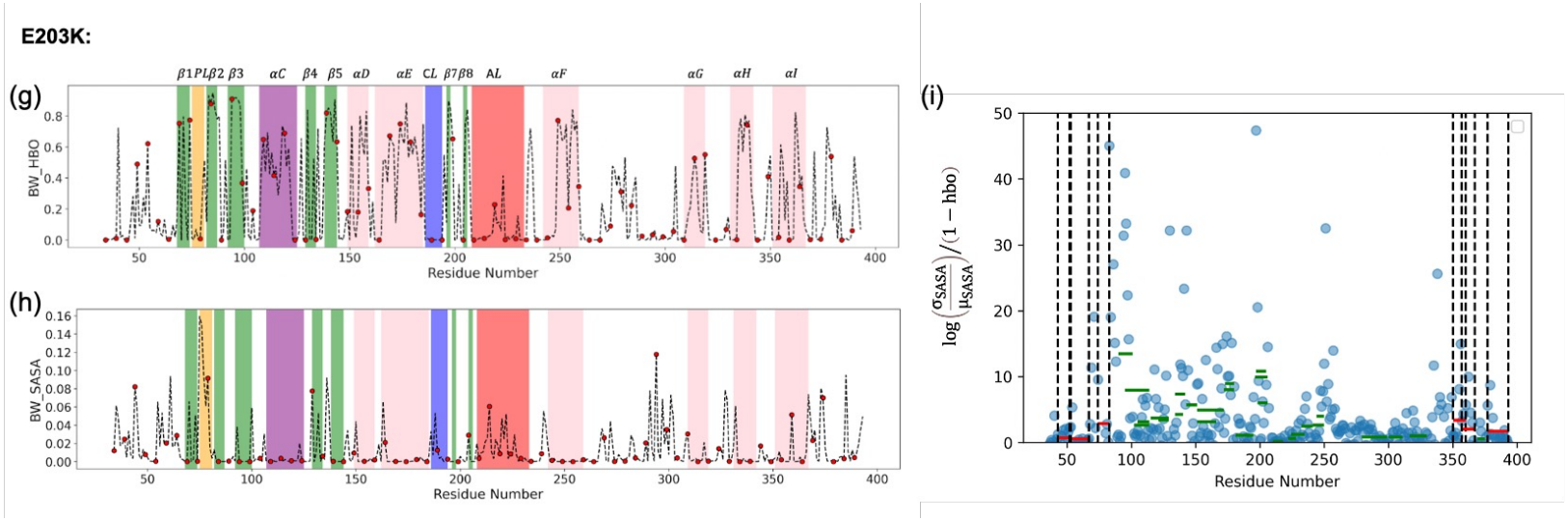


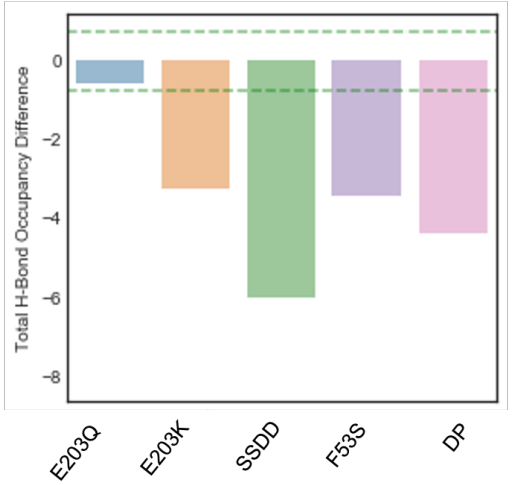
Fig. S8: Boltzmann weighted Hydrogen bond occupancy (BW-HB) in panels (a,d,g) and SASA (BW-SASA) in panels (b,e,h). Metric to compute the tendency of a peptide to have EX2 Vs Mixed EX1/EX2 exchange kinetics demonstrated for MEK1 WT: Log_{10} (norm standard deviation(SASA))/(1- hydrogen bond occupancy) in panels (c,f,i) for MEK WT, SSDD and E203K systems.

(a) **Flexibility of activation loop**

Mutant	Average RMSD (A)
WT	2.80
E203K	3.77
SSDD	3.67
F53S	4.04
DP	4.07

P-loop flexibility

Mutant	Average RMSD (A)
WT	3.17
SSDD	5.63



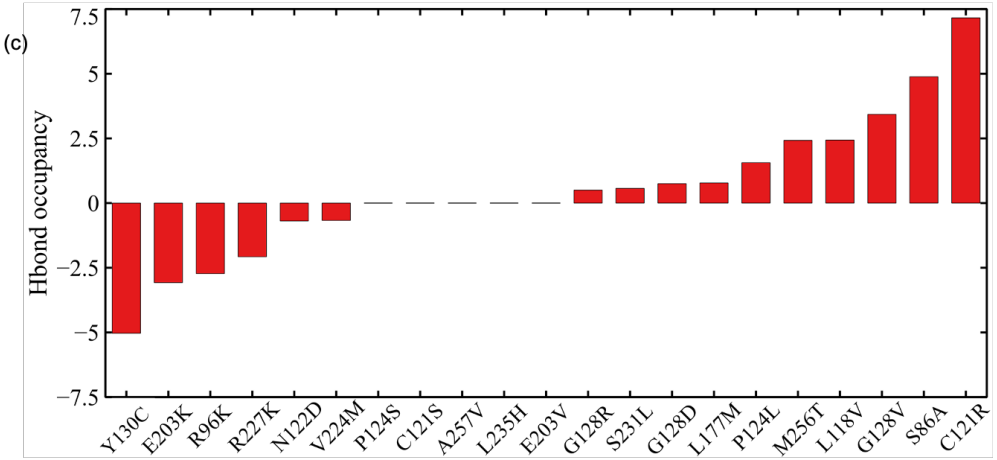


Fig. S9: Average RMSD of MEK1 systems wrt key subdomains: A-loop and P-loop, (a). Hydrogen bond occupancy difference in A-loop and αC-helix between MEK1 variant and MEK1 WT (b). Plot of accumulated hydrogen bond occupancy in the αC-helix domain and A-loop of MEK1 for inactive conformations. (Threshold 0.5) in panels (c).

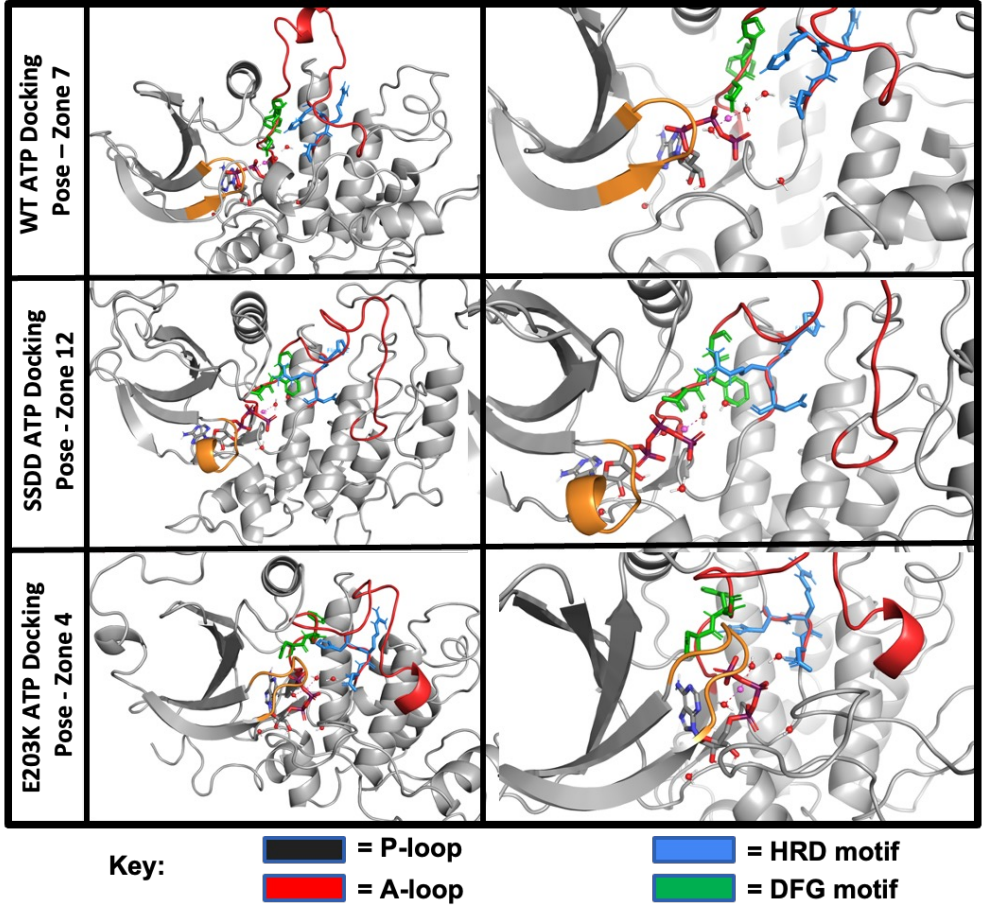


Fig. S10: Molecular docking poses (kinase and binding site views) of ATP binding to MEK1: (top) WT, (middle) SSDD, (bottom) E203K. In addition to the conserved interactions between the ATP and the protein being preserved, the Mg^{2+} ion and water molecules mediate critical interactions with the protein.

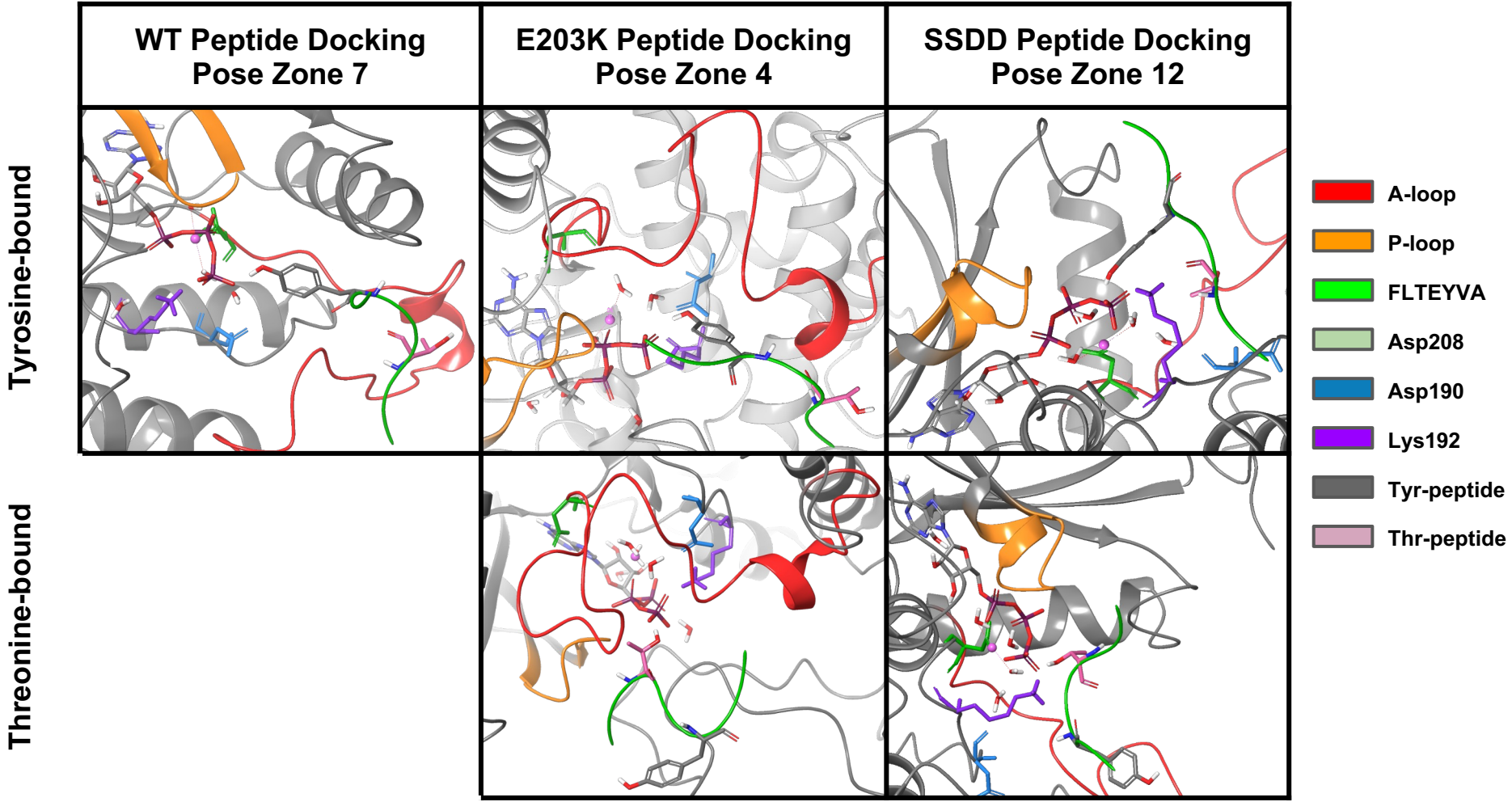


Fig. S11: Molecular docking poses (binding site view) of FLTEYVA to most active zones of MEK1 variants: (left) WT, (middle) E203K, (right) SSDD. Transition states of both Tyr (top) and Thr (bottom) phosphorylation were modeled in the reaction competent zones.

WT
Zone1

SSDD
Zone1

E203K
Zone1

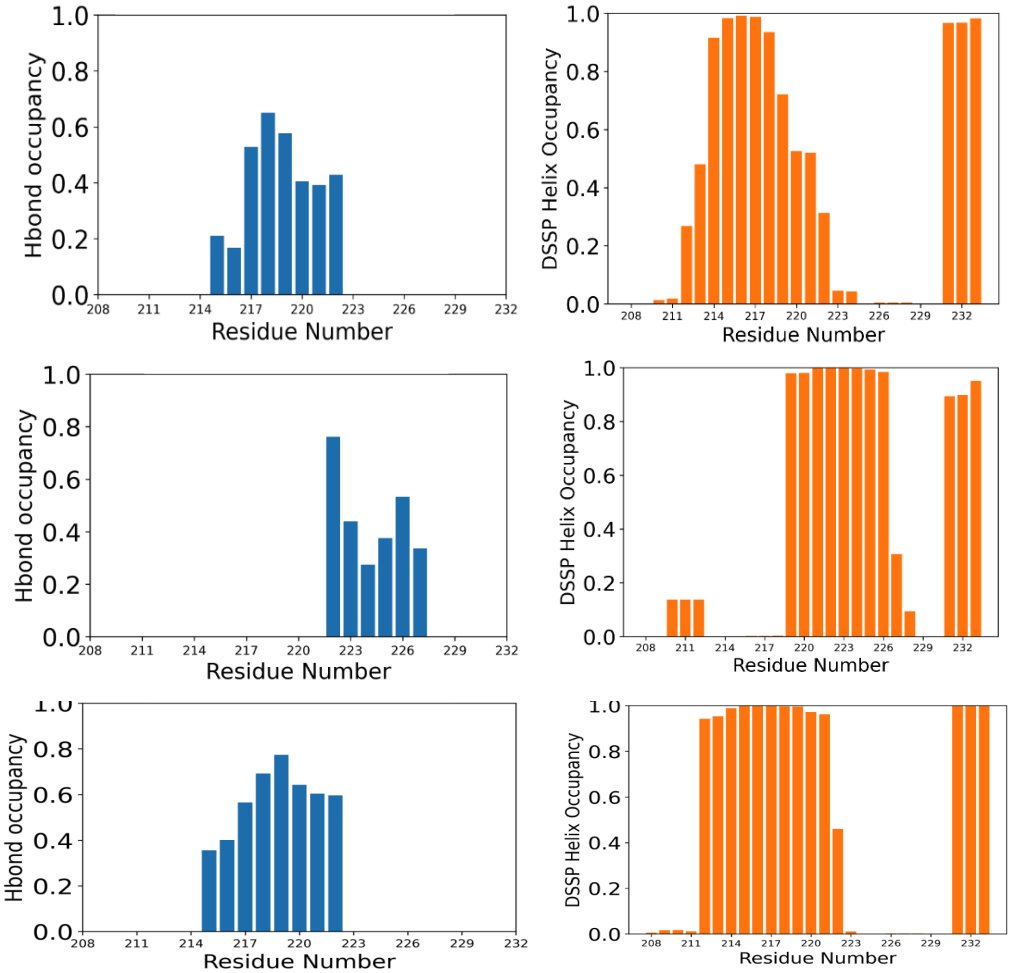


Fig. S12: Comparison of hydrogen bonding and DSSP analysis for zone 1 of 3 MEK1 variants. The results show qualitative similarity between the two analyses.

a. WT ATP Docking Results

STRUCTURE	ZONE INFO	FREE ENERGY (KCAL/MOL)	BINDING AFFINITY (KCAL/MOL)	MG^{2+} COORDINATION	D208 – MG^{2+} DISTANCE (Å)	ADENOSINE H-BONDS	REACTION COMPETENT?
Zone 1	1. KE salt bridge broken 2. DFG out 3. A-loop partially helical	0.66	-18.472	✓	9.81	✓	✗
Zone 2	1. KE salt bridge broken 2. DFG in 3. A-loop partially helical	2.35	-16.841	✓	1.39	✓	✓
Zone 3	1. KE salt bridge broken 2. DFG in 3. A-loop partially helical	6.28	-15.432	✓	1.23	✓	✓
Zone 4	1. KE salt bridge broken 2. DFG in 3. A-loop unfolded	11.62	-14.846	✓	1.96	✓	✓
Zone 5	1. KE salt bridge broken 2. DFG in/out 3. A-loop partially helical	5.08	-15.967	✓	1.40	✓	✓
Zone 7	1. KE salt bridge partly intact 2. DFG in 3. A-loop unfolded	13.6	-20.327	✓	2.12	✓	✓

b. SSDD ATP Docking Results

STRUCTURE	ZONE INFO	FREE ENERGY (KCAL/MOL)	BINDING AFFINITY (KCAL/MOL)	MG^{2+} COORDINATION	D208 – MG^{2+} DISTANCE (Å)	ADENOSINE H-BONDS	REACTION COMPETENT?
Zone 1	1. KE salt bridge broken 2. DFG out 3. A-loop partially helical	3.46	-13.482	✓	2.05	✗	✗
Zone 3	1. KE salt bridge broken 2. DFG out 3. A-loop partially helical	0.77	-14.462	✓	5.53	✗	✗
Zone 4	1. KE salt bridge broken 2. DFG out 3. A-loop partially helical	0.9	-16.956	✓	7.06	✗	✗
Zone 5	1. KE salt bridge broken 2. DFG in/out 3. A-loop partially helical	1.22	-16.984	✓	10.44	✓	✗
Zone 6	1. KE salt bridge broken 2. DFG in 3. A-loop partially helical	0.85	-16.742	✓	8.82	✓	✗
Zone 7	1. KE salt bridge broken 2. DFG in 3. A-loop partially helical	1.87	-15.245	✓	6.62	✗	✗
Zone 9	1. KE salt bridge formed 2. DFG in 3. A-loop unfolded	5.23	-15.258	✓	2.03	✓	✓
Zone 10	1. KE salt bridge formed 2. DFG in 3. A-loop partially helical	6.92	-14.138	✗	3.39	✓	✗
Zone 11	1. KE salt bridge partly formed 2. DFG in 3. A-loop unfolded	11.37	-13.656	✓	4.85	✗	✗
Zone 12	1. KE salt bridge partly formed 2. DFG in 3. A-loop unfolded	11.98	-18.038	✓	2.00	✓	✓

c. E203K ATP Docking Results

STRUCTURE	ZONE INFO	FREE ENERGY (KCAL/MOL)	BINDING AFFINITY (KCAL/MOL)	MG^{2+} COORDINATION	D208 – MG^{2+} DISTANCE (Å)	ADENOSINE H-BONDS	REACTION COMPETENT?
Zone 1	1. KE salt bridge broken 2. DFG out 3. A-loop partially helical	2.59	-20.278	✓	5.21	✓	✗
Zone 2	1. KE salt bridge broken 2. DFG out 3. A-loop unfolded	5.72	-14.421	✓	7.68	✓	✗
Zone 3	1. KE salt bridge broken 2. DFG out 3. A-loop unfolded	1.07	-14.599	✓	8.09	✓	✗
Zone 4	1. KE salt bridge formed 1. DFG in 3. A-loop unfolded	3.64	-18.914	✓	6.23	✓	✓
Zone 5	1. KE salt bridge broken 2. DFG in/out 3. A-loop unfolded	6	-17.246	✓	5.25	✗	✗

Table S1: Molecular docking of ATP to different zones of MEK1: (a) WT, (b) SSDD, (c) E203K.

a. WT Peptide Docking Results

STRUCTURE	TYR-BOUND BINDING AFFINITY (KCAL/MOL)	THR-BOUND BINDING AFFINITY (KCAL/MOL)	REACTION COMPETENT?	TYR PHOSPHORYLATION COMPETENT?	THR PHOSPHORYLATION COMPETENT?
Zone 1	-	-	✗	✗	✗
Zone 2	-3.989	-	✓	✓	✗
Zone 3	-5.872	-5.595	✓	✓	✓
Zone 4	-	-6.442	✓	✗	✓
Zone 5	-4.821	-	✓	✓	✗
Zone 7	-4.723	-	✓	✓	✗

b. SSDD Peptide Docking Results

STRUCTURE	TYR-BOUND BINDING AFFINITY (KCAL/MOL)	THR-BOUND BINDING AFFINITY (KCAL/MOL)	REACTION COMPETENT?	TYR PHOSPHORYLATION COMPETENT?	THR PHOSPHORYLATION COMPETENT?
Zone 1	-	-	✗	✗	✗
Zone 3	-6.151	-5.372	✗	✓	✓
Zone 4	-5.421	-5.001	✗	✓	✓
Zone 5	-	-	✗	✗	✗
Zone 6	-6.515	-6.649	✗	✓	✓
Zone 7	-4.518	-4.853	✗	✓	✓
Zone 9	-7.201	-6.548	✓	✓	✓
Zone 10	-	-6.315	✗	✗	✓
Zone 11	-4.789	-6.396	✗	✓	✓
Zone 12	-6.466	-5.543	✓	✓	✓

c. E203K Peptide Docking Results

STRUCTURE	TYR-BOUND BINDING AFFINITY (KCAL/MOL)	THR-BOUND BINDING AFFINITY (KCAL/MOL)	REACTION COMPETENT?	TYR PHOSPHORYLATION COMPETENT?	THR PHOSPHORYLATION COMPETENT?
Zone 1	-1.007	-	✗	✓	✗
Zone 2	-6.666	-6.447	✗	✓	✓
Zone 3	-5.414	-	✗	✓	✗
Zone 4	-6.257	-4.580	✓	✓	✓
Zone 5	-5.678	-4.939	✗	✓	✓

Table S2: Molecular docking of FLTEYVA peptide derived from ERK1 (PDB: 5UMO) to different zones of MEK1: (a) WT, (b) SSDD, (c) E203K.