Supplementary Material

Crystal structure and molecular dynamics simulations of a promiscuous ancestor reveal residues and an epistatic interaction involved in substrate binding and catalysis in the ATP-dependent vitamin kinase family members.

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Running title: Structural and kinetic analysis of a promiscuous ancestor.

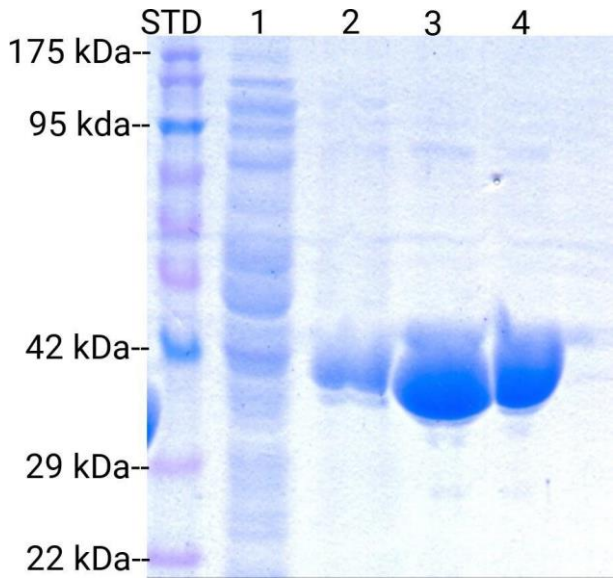


Figure S1. 12% SDS-polyacrylamide gel electrophoresis for purification analysis of AncC Gln45Met mutant. Nickel NTA immobilized metal affinity chromatography was used for purification. STD: Standard protein marker. 1: Flow through. 2: Washed fraction of 20mM of imidazole. 3: Elution fractions with enzyme activity for gradient of 20 to 500 mM of imidazole. 4: Purified AncC Gln45Met in storage buffer (Tris-HCl 50mM, 500mM NaCl, 5mM MgCl2, 1mM DTT, 1 mM ATP and 10 % glycerol).

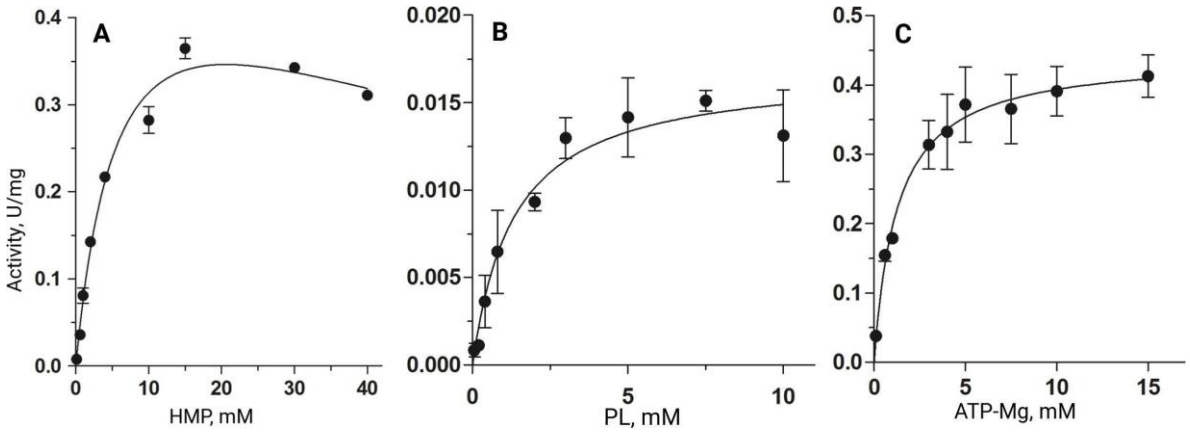


Figure S2. Saturation curves for AncC-Gln45Met mutant. A) saturation curve of HMP fitted to substrate inhibition equation. B) saturation curve of PL fitted to Michaelis-Menten equation. C) saturation curve of ATP-Mg fitted to Michaelis-Menten equation. The error bar shows the standard error of the mean (SEM) of 3 measurements from different purifications.

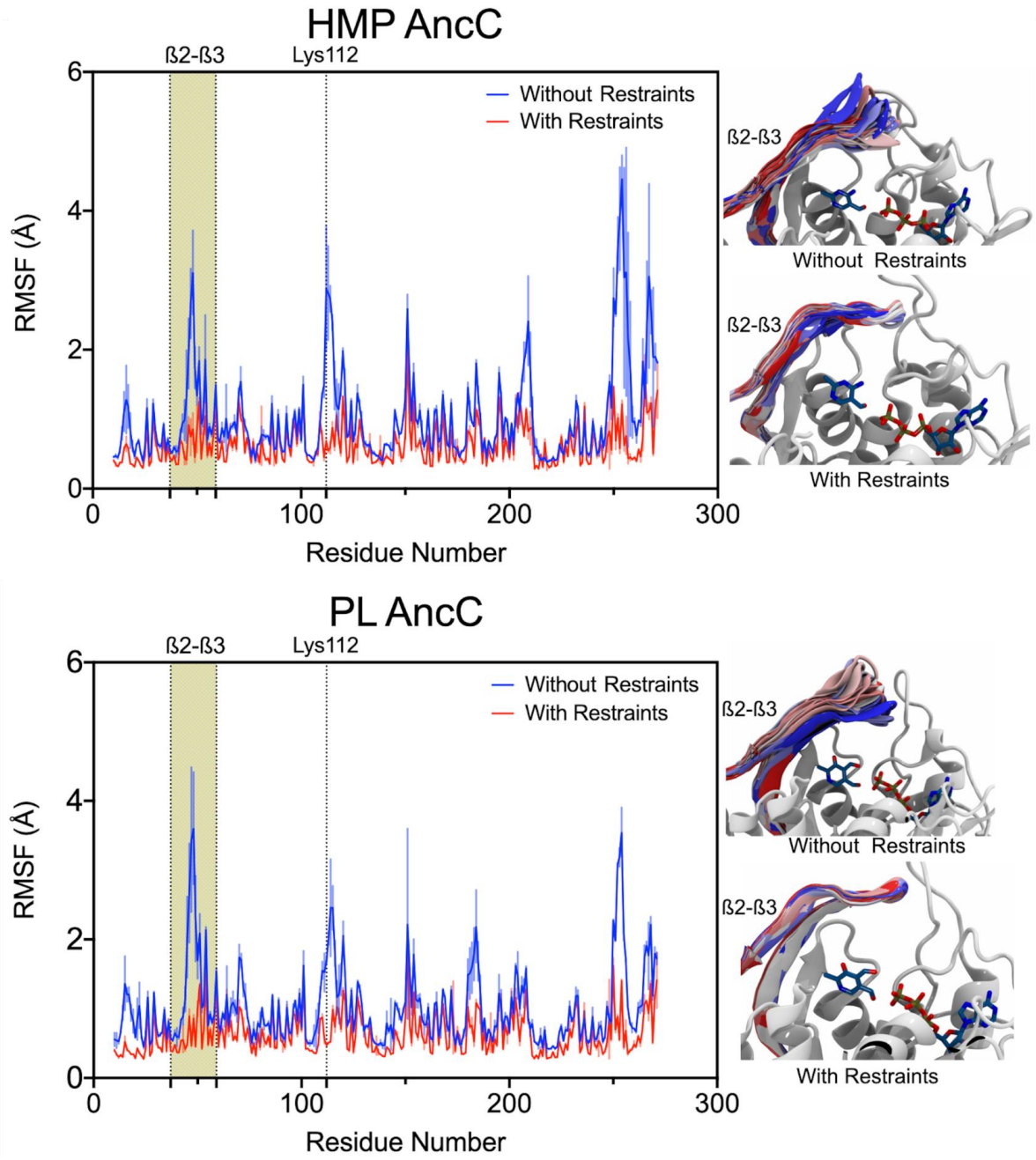


Figure S3. Conformational dynamics of ancC-HMP and ancC-PL complexes without and with positional restraint. RMSF through simulations for AncC ligand complexes with HMP (top) and PL (bottom) with (red) and without (blue) the application of a positional restraint of 0.5 kcal/mol*Å^2 in Cα atoms. The position of key regions like Lys112 and the β2-β2- hairpin are highlighted. The impact of positional restraints on the β2-β3 hairpin dynamic is shown on the right, with the hairpin colored from beginning to the end of the simulation using a color gradient from red to blue. RMSF calculation were performed over all atoms, the 10 amino acid residues from the N and C terminal were omitted owing to its high value.

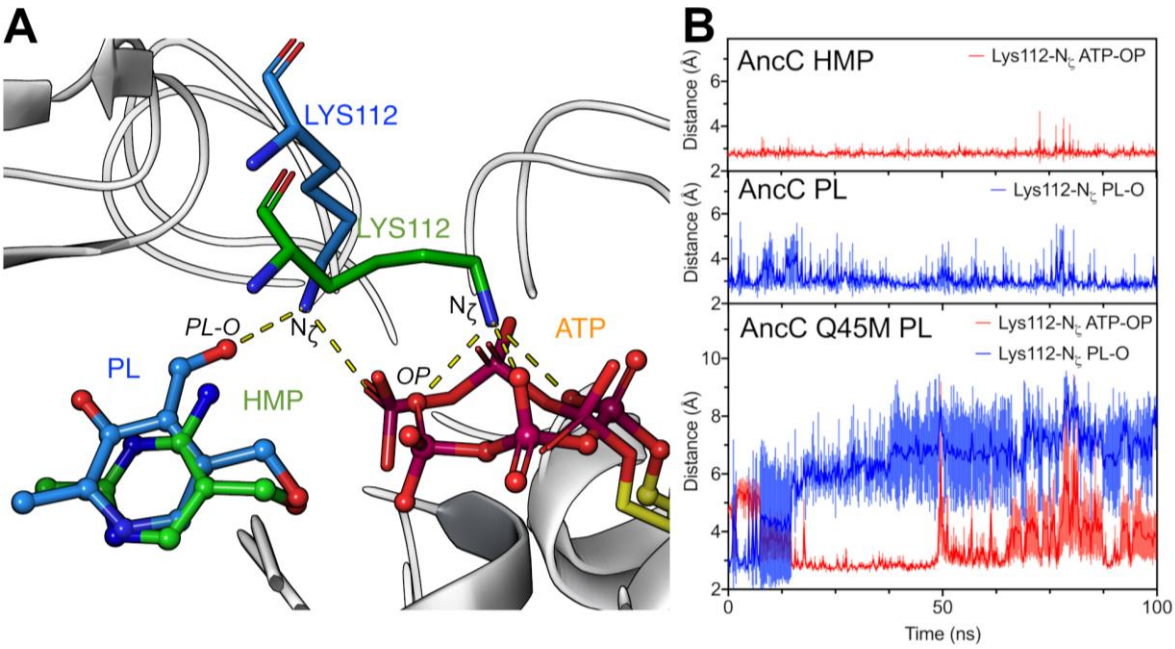
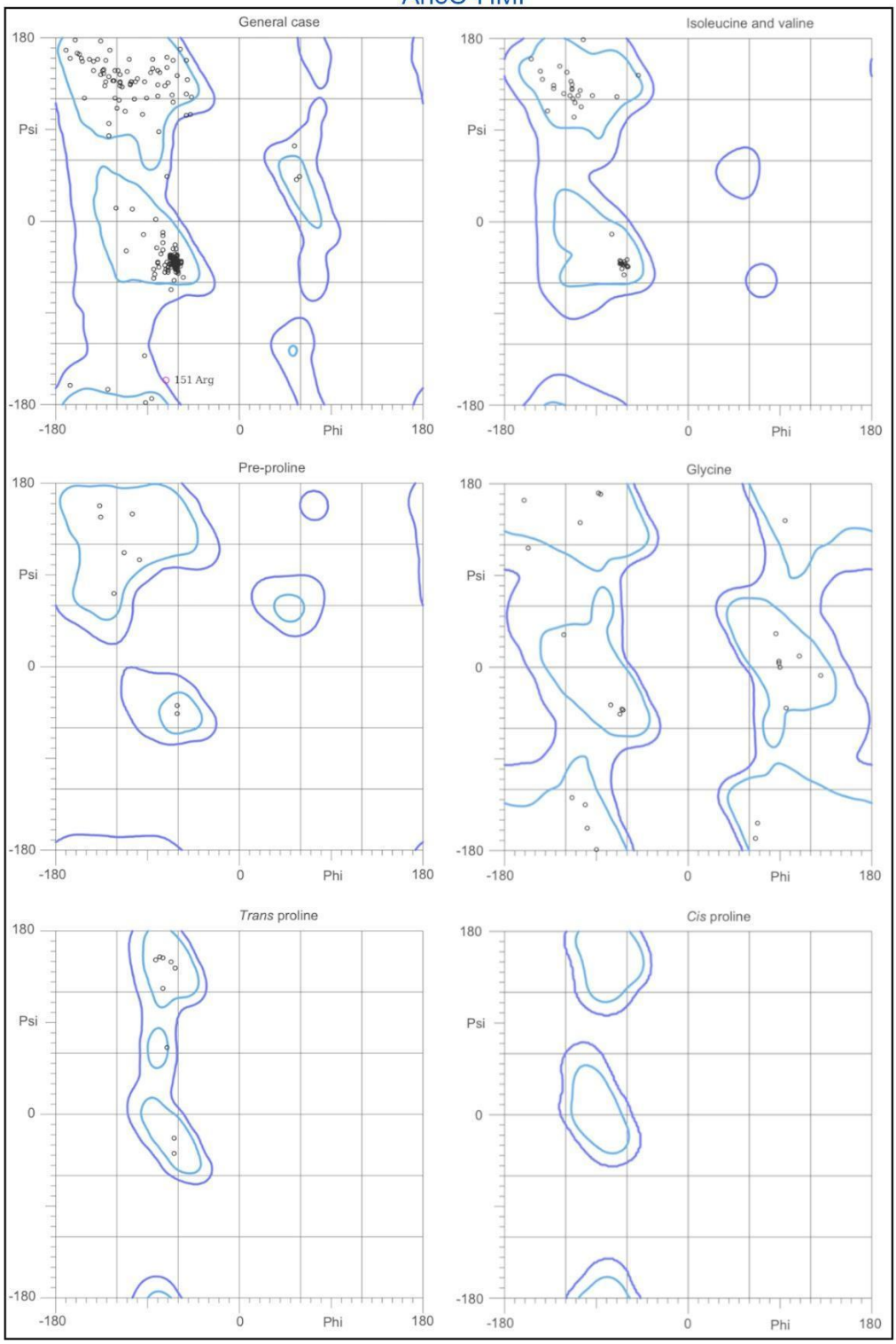


Figure S4. Different Lys112 conformations and its distance to PL and ATP atoms along MD simulation with substrates. A) Representative orientations of Lys112 through the molecular dynamics with PL and HMP. MD simulations with PL shows Lys112 interacts with aldehyde's oxygen atom of PL (PL-O), while in the simulation with HMP, Lys112 (Nζ atom) interacts mainly with phosphates' oxygens (OP) of ATP. Dashed lines indicate hydrogen bonds. B. Atomic distances for Lys112 (Nζ atom), ATP-OP and PL-O in MD simulations. Complex AncC-HMP (Upper panel), AncC-PL (Middle panel) and AncC-Q45M-PL (Bottom panel). Blue and red lines indicate distances of Lys112 (Nζ atom) with PL-O and ATP-OP, respectively. Mean and standard deviations of distances are shown as dark and shadow colors, respectively.

AncC-HMP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Glycine
Trans proline
Cis proline
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Psi
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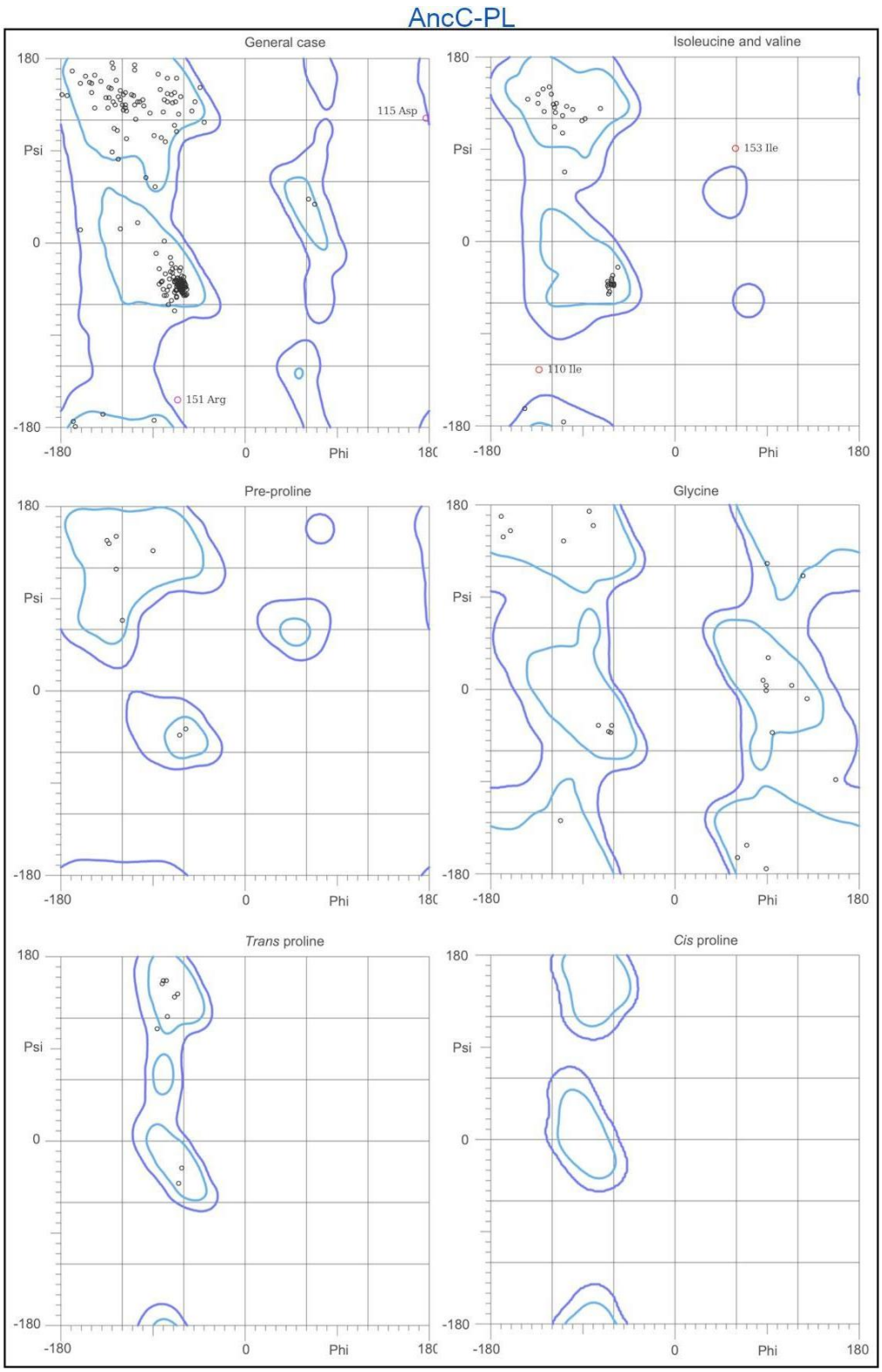

Figure S5. Ramachandran plots of AncC models with substrates. Analysis for AncC-HMP (upper) and AncC-PL complexes (bottom). Ramachandran plots were obtained with Molprobity.

Table S1. AncC residues involved in substrate binding. Residues around HMP and PL were determined from the molecular dynamic simulations and clustered in groups according to the distance between ligand and residues.

Less of 5 Å		**5 to 8 Å**	
Position	Amino acid	Position	Amino acid
11	ALA	9	THR
12	GLY	10	ILE
13	SER	15	SER
14	ASP	16	SER
19	ALA	17	GLY
20	GLY	18	GLY
21	ILE	22	GLN
43	VAL	23	ALA
45	GLN	24	ASP
50	HIS	39	ILE
78	LYS	40	THR
80	GLY	41	ALA
81	MET	42	ILE
106	ASP	44	ALA
108	VAL	46	ASN
110	ILE	49	GLY
111	ALA	51	LYS
112	LYS	52	GLY
117	LEU	53	VAL
211	HIS	79	THR
212	GLY	82	LEU
213	ALA	83	ALA
214	GLY	107	PRO
215	CYS	109	MET
		113	GLY
		140	ASN
		143	GLU
		177	LYS
		210	THR
		216	THR
		217	PHE
		218	SER
		219	ALA
		256	VAL
		259	THR