Supporting Information

Structural basis for GTP versus ATP selectivity in the NMP kinase AK3

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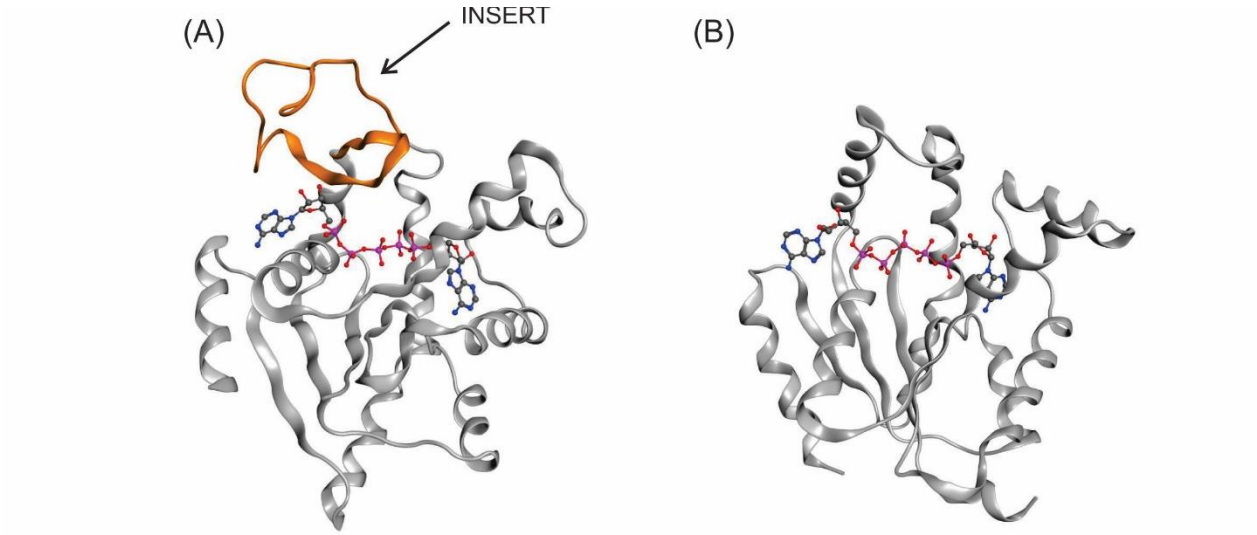

Figure S1. Structures of long and short monomeric NMP kinases. (**A**) Example of a long monomeric NMP kinase with the INSERT segment indicated and colored in orange. Adenylate kinase from *Escherichia Coli* in complex with the inhibitor Ap5A[1] (1AKE). (**B**) Example of a short monomeric NMP kinase. Adenylate kinase from the Antarctic fish *Notothenia coriiceps* in complex with Ap5A[2] (5XZ2).

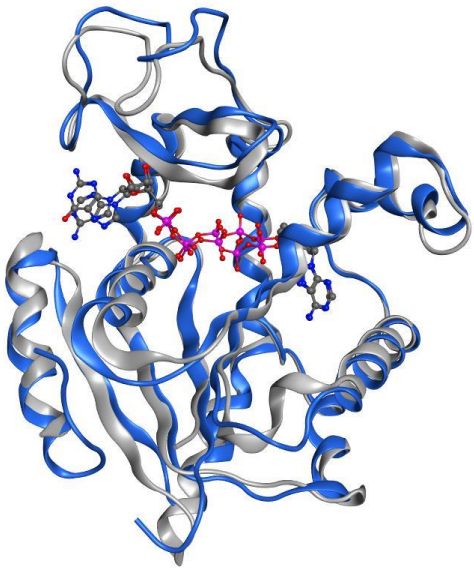

Figure S2. AK3 in a closed conformation in complex with the inhibitor. Shown is a superimposition of AK3 (blue ribbon) in complex with Gp5A with AK_{eco} (gray ribbon) in complex with Ap5A[1] (1AKE).

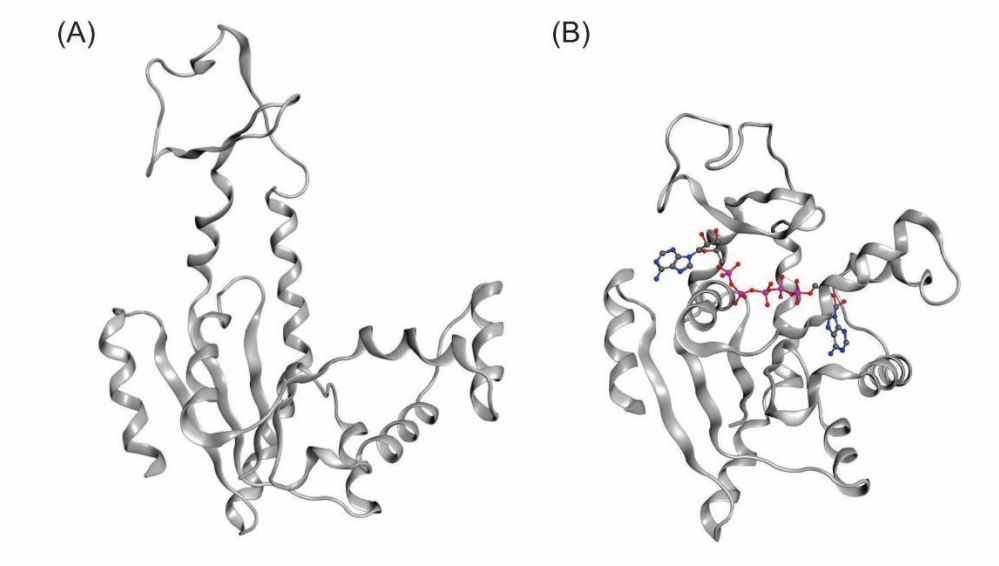

Figure S3. Structures of an open and closed NMP kinase. Crystallographic structures of AK_{eco} are used for illustration of the open and closed states. (**A**) Substrate free and open AK_{eco} [3] (4AKE). (**B**) Ap5A bound and closed AK_{eco}[1] (1AKE).

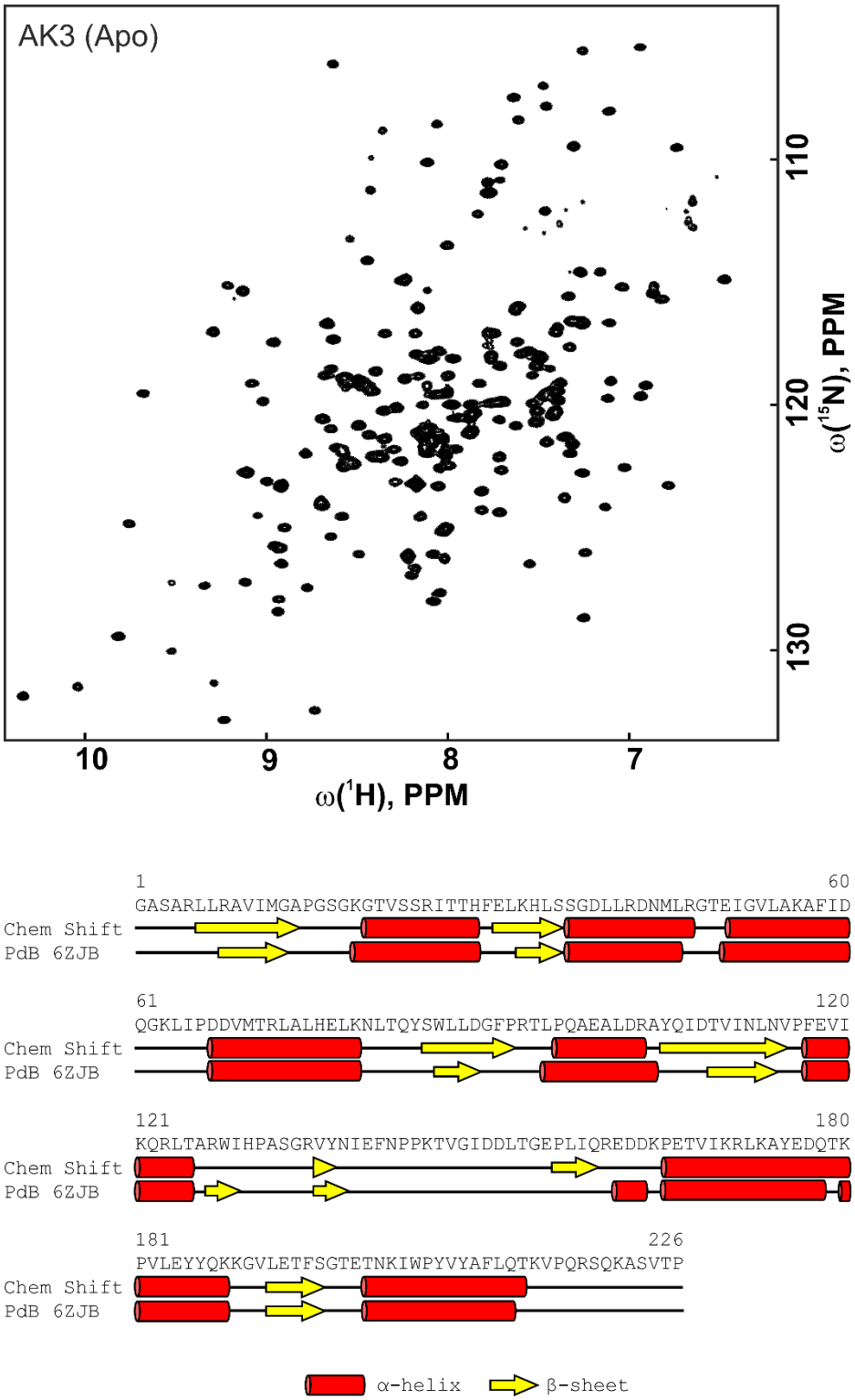

Figure S4. Top; Trosy-HSQC spectra of human AK3 (apo form) and bottom; a comparison between predicted secondary structure based on the chemical shifts and the secondary structure of the high resolution AK3-Gp5A X-ray structure (6ZJB.pdb).

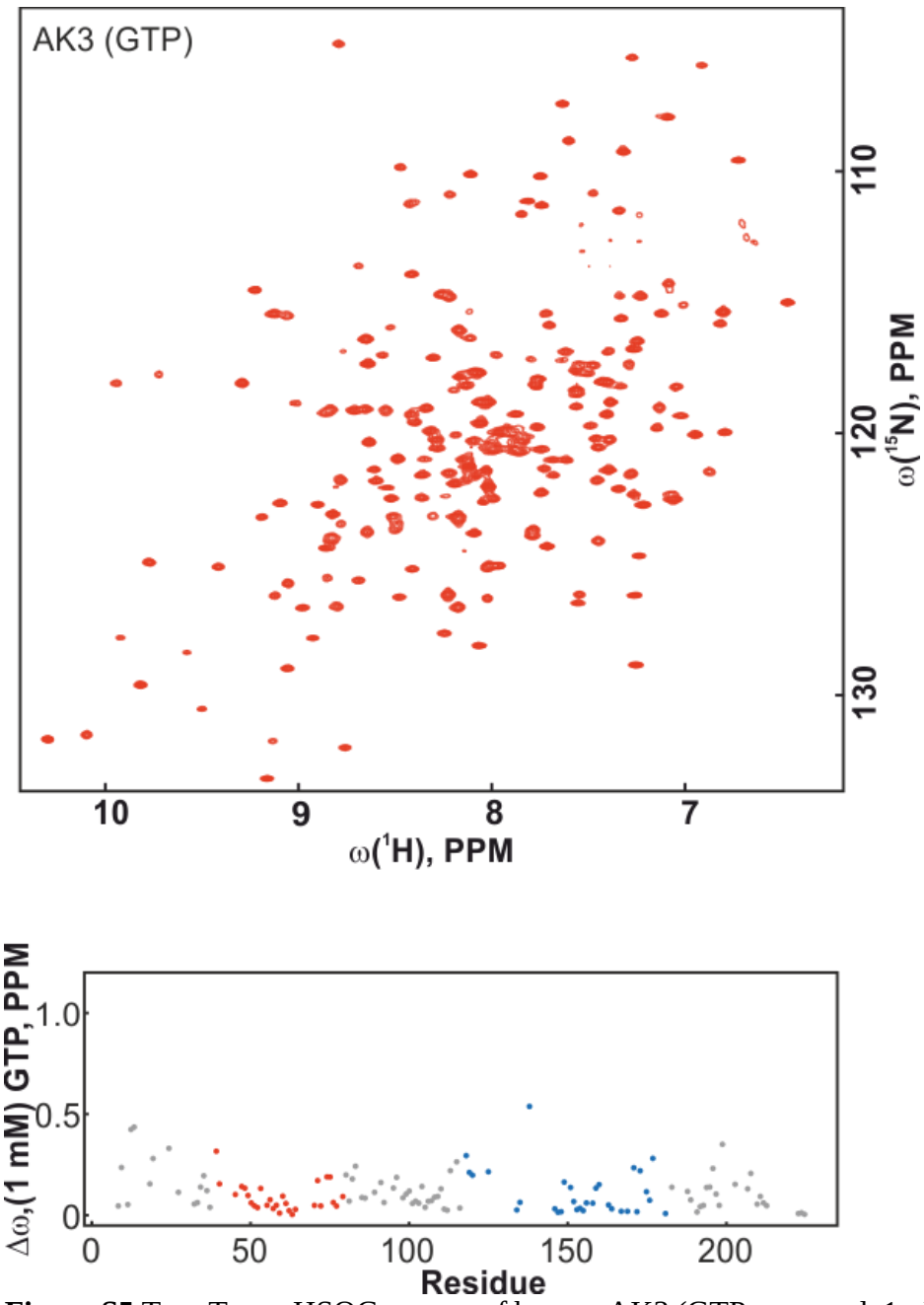

Figure S5 Top; Trosy-HSQC spectra of human AK3 (GTP saturated, 1mM) and bottom; chemical shift differences ($|^{1}H|+0.2*|^{15}N|$) between apo AK3 and AK3 saturated with 1 mM GTP. Residues in the GTPlid are colored red and residues in the AMPbd are colored blue.

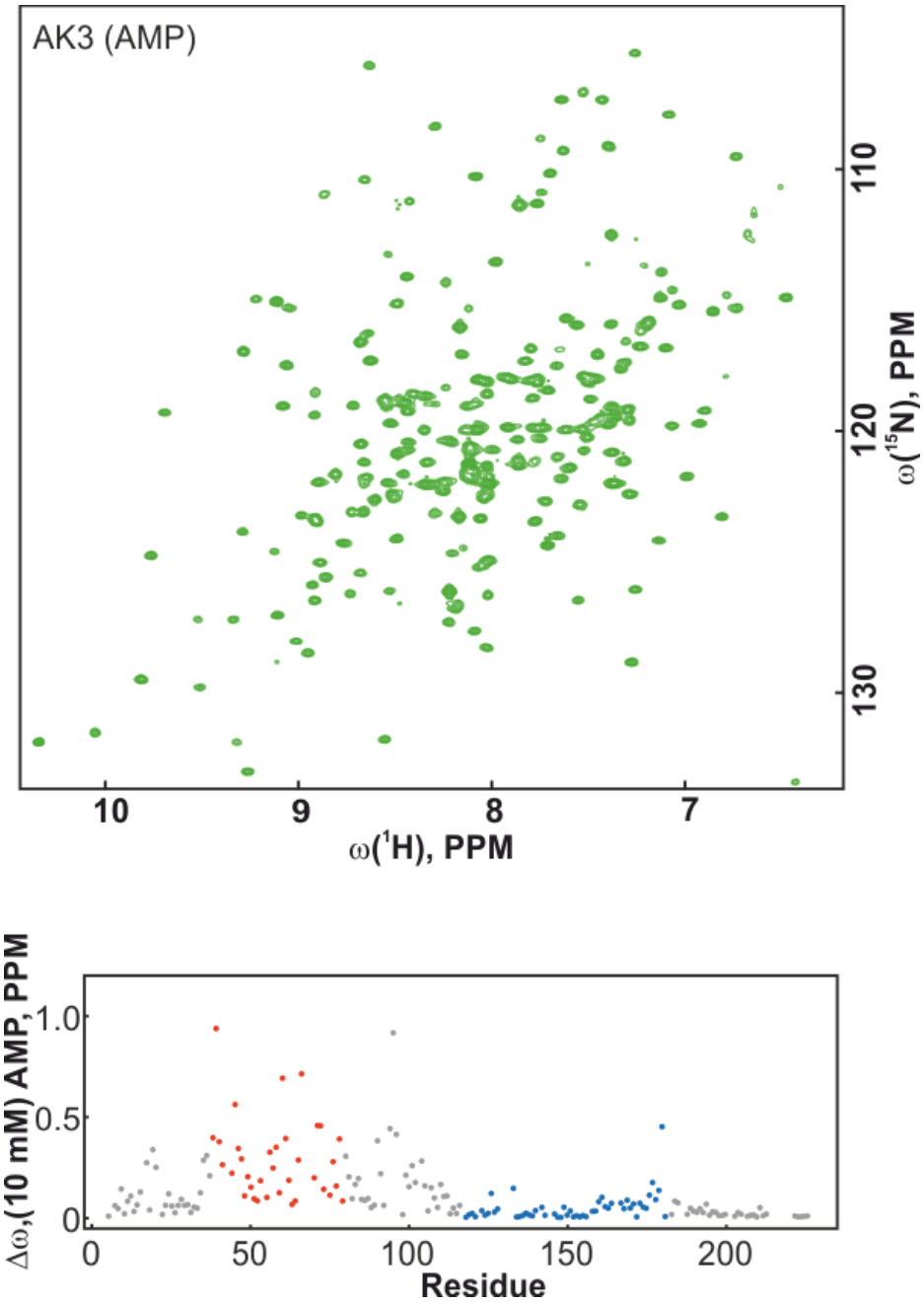

Figure S6 Top: Trosy-HSQC spectra of human AK3 (AMP saturated, 10 mM) and bottom; chemical shift differences ($|^{1}H|+0.2*|^{15}N|$) between apo AK3 and AK3 saturated with 10 mM AMP. Residues in the GTPlid are colored red and residues in the AMPbd are colored blue.

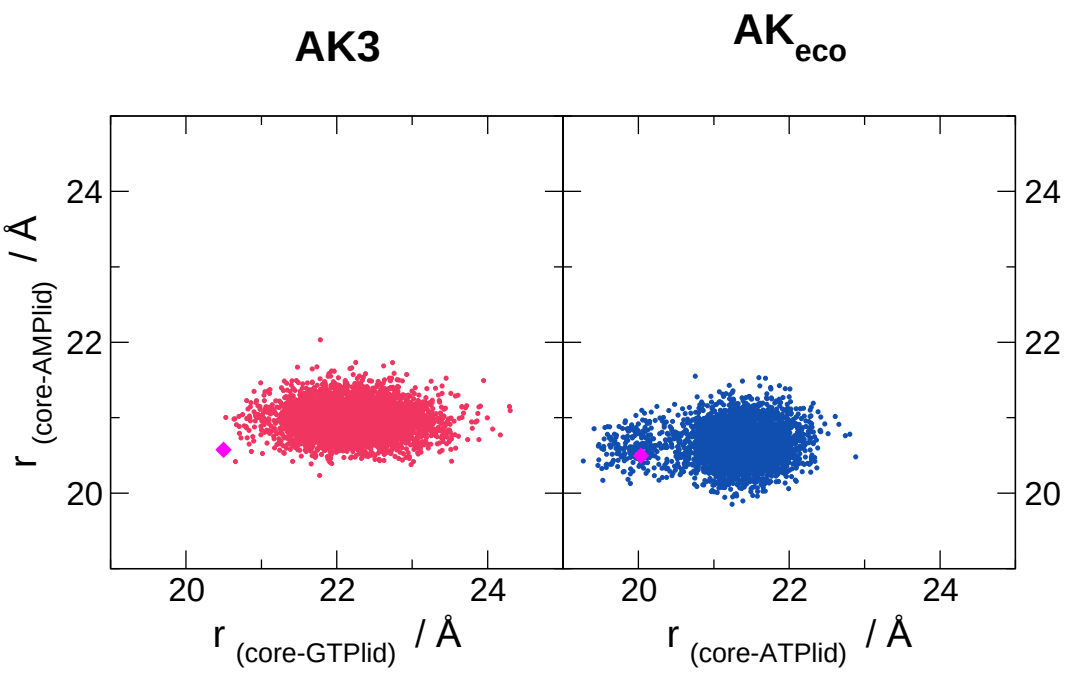

Figure S7. Displacement of the nucleotide binding domains relative to the protein core in AK3 and AK_{eco} during the course of an 500 ns MD simulation.

The lid-to-core center-of-mass distances were calculated on the atomic coordinates saved every 0.1 ns. The elliptical distribution in AK3 (*left panel*) indicates the shifting of the GTPlid by approximately 3 Å in the direction towards and away from the protein core while the AMPbd displaces only by about 0.5 Å around the 21 Å mark. The distribution pattern for AK_{eco} (*right panel*) is more spherical suggesting that both domains exhibit a similar range of motion relative to the core, with the ATPlid domain being at a marginally farther distance from the core. Pink diamonds in both panels mark the values for the respective X-ray structures.

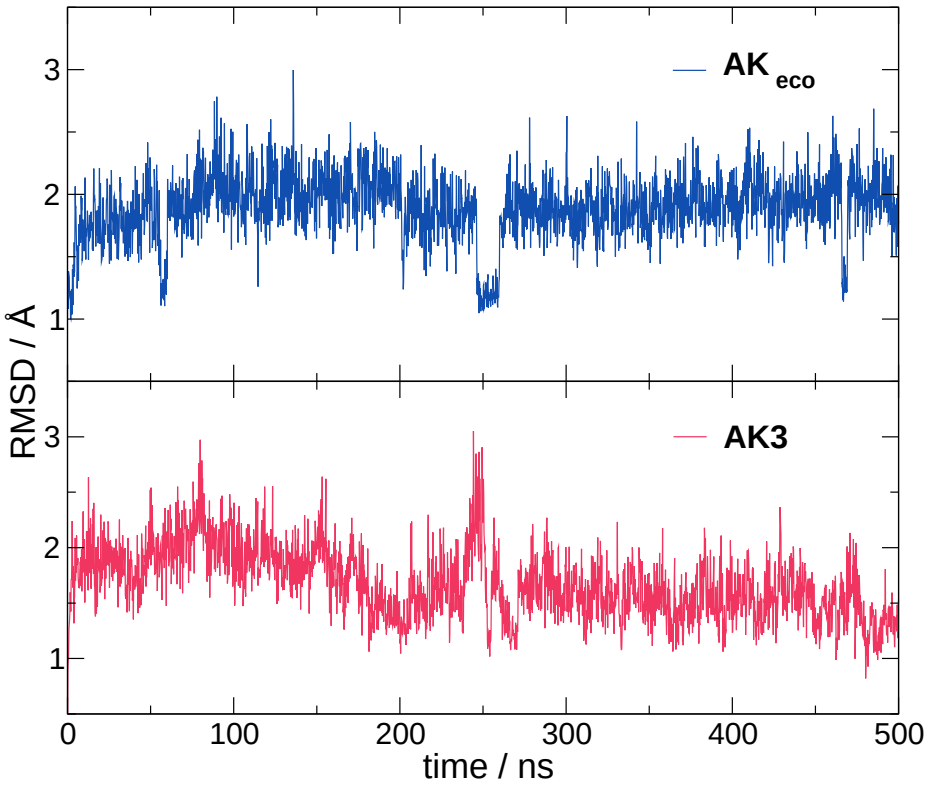

Figure S8. The root-mean-square deviation (RMSD) of the backbone atomic position during the course of a 500 ns MD simulation.

The RMSD value quantifies a relative deviation of atomic position per coordinate frame saved every 0.1 ns against the X-ray crystalline reference structure. Both adenylate kinase models demonstrate appropriate structural stability of the equilibrated system with an average displacement of 2 Å (AK_{eco}) and 1.5 Å (AK3) from the reference conformation.

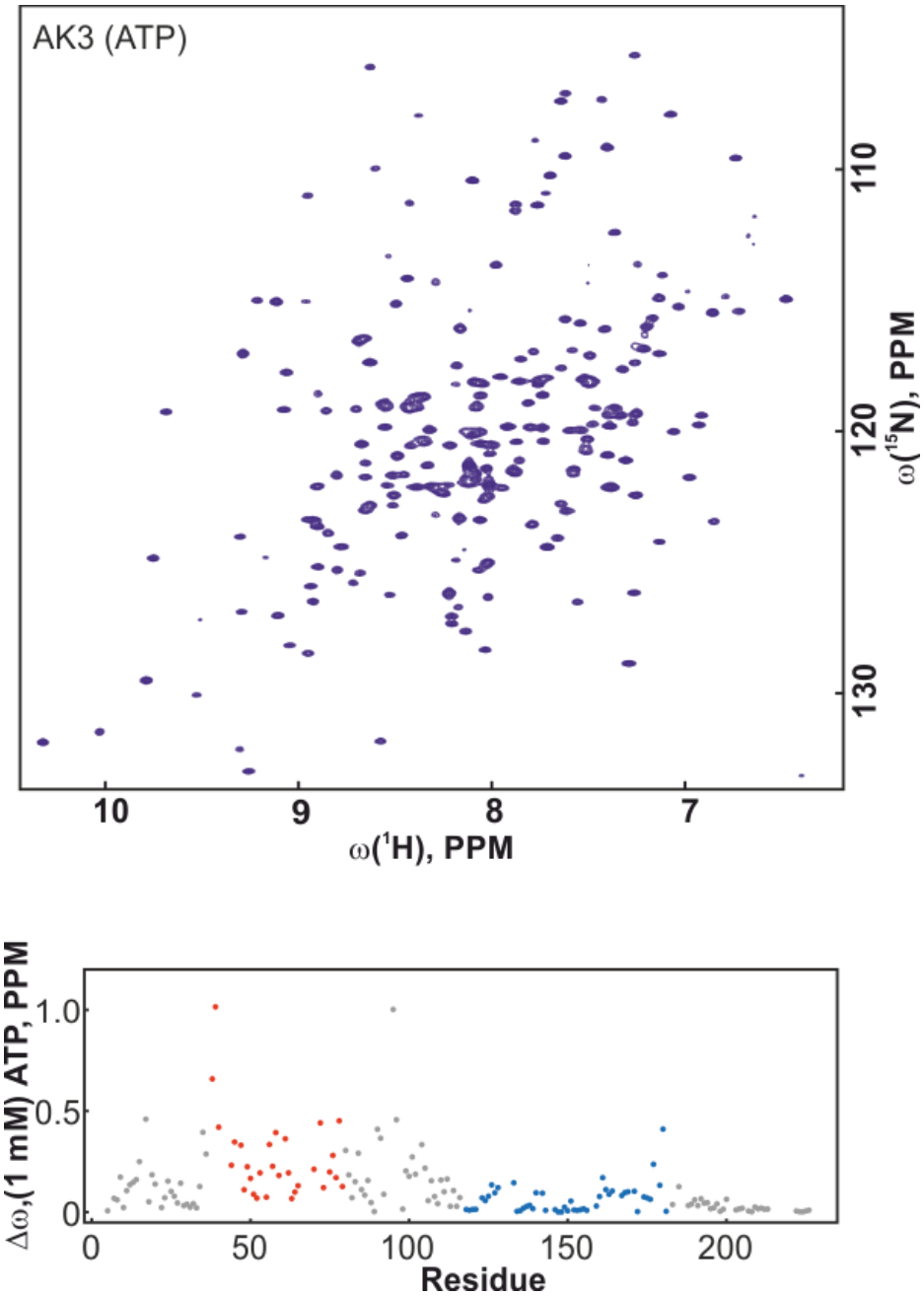

Figure S9 Top; Trosy-HSQC spectra of human AK3 (ATP saturated, 1mM) and bottom; chemical shift differences ($|^{1}H|+0.2*|^{15}N|$) between apo AK3 and AK3 saturated with 1 mM ATP. Residues in the GTPlid are colored red and residues in the AMPdb are colored blue.

References

(1) Müller, C. W.; Schulz, G. E., (1992) Structure of the complex between adenylate kinase from Escherichia coli and the inhibitor AP5A refined at 1.9 A resolution - A model for a catalytic transition-State. *J. Mol. Biol. 224*, 159-177.

(2) Moon, S.; Kim, J.; Bae, E., (2017) Structural analyses of adenylate kinases from Antarctic and tropical fishes for understanding cold adaptation of enzymes. *Scientific Reports 7*, 12.

(3) Müller, C. W.; Schlauderer, G. J.; Reinstein, J.; Schulz, G. E., (1996) Adenylate kinase motions during catalysis: An energetic counterweight balancing substrate binding. *Structure 4*, 147-156.