Supporting information

Uncoupling Catalytic and Binding Functions in the cAMP-Dependent Protein Kinase A

*Jonggul Kim[1], Geoffrey Li[1], Michael A. Walters[3], Susan S. Taylor[4] and Gianluigi Veglia[1,2]**

[1] Department of Chemistry, University of Minnesota, Minnesota, 55455 United States.

[2] Department of Biochemistry, Molecular Biology and Biophysics, University of Minnesota, MN, 55455 United States.

[3] Institute for Therapeutics Discovery and Development, Department of Medicinal Chemistry, University of Minnesota, Minnesota, 55455 United States

[4] Department of Chemistry and Biochemistry, University of California at San Diego, La Jolla, CA 92093, USA

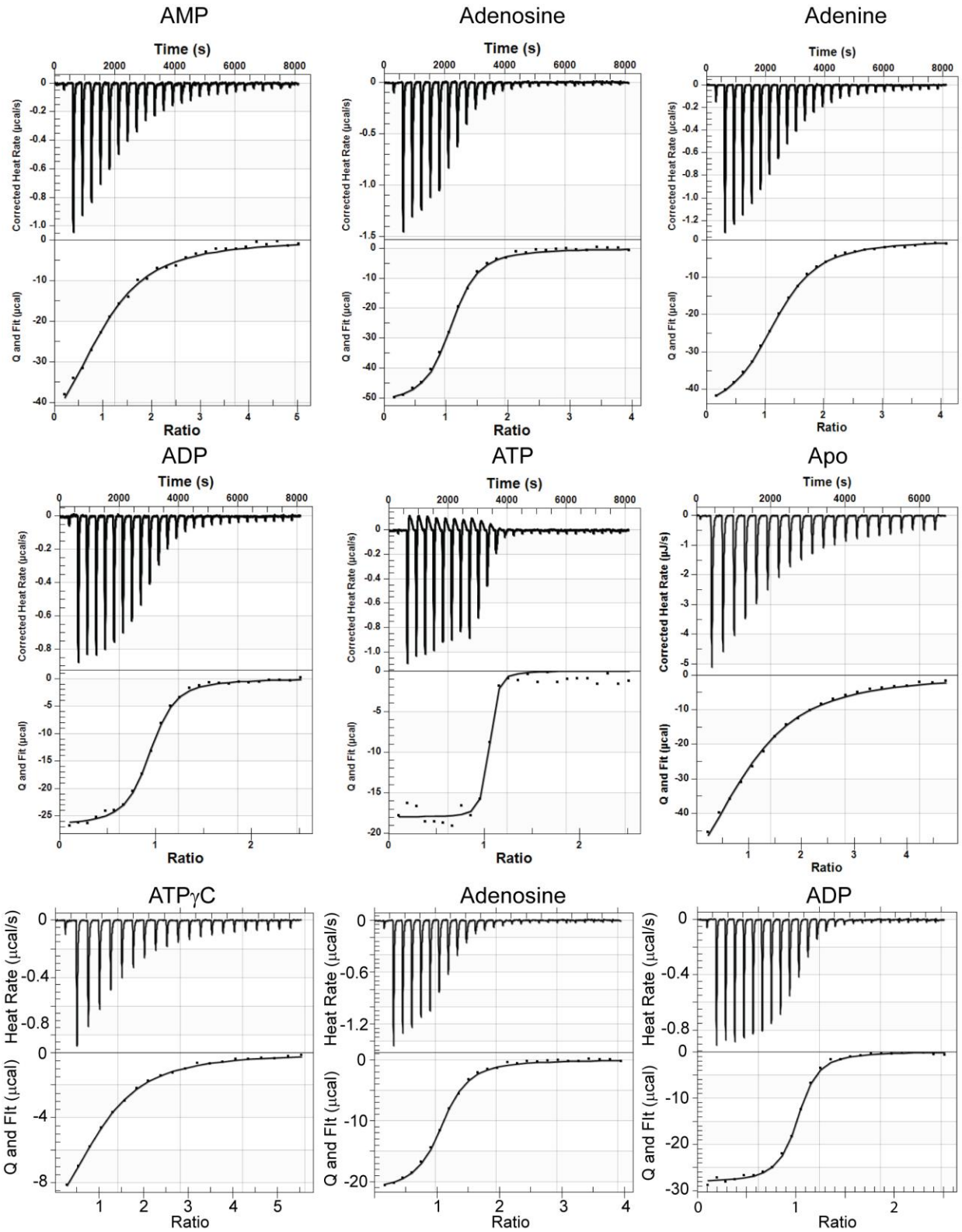

Figure S1. The ITC isotherms and their fits of PKI_{5-24} titrating to PKA-C under different nucleotide saturating conditions (2 mM). (related to Figure 2)

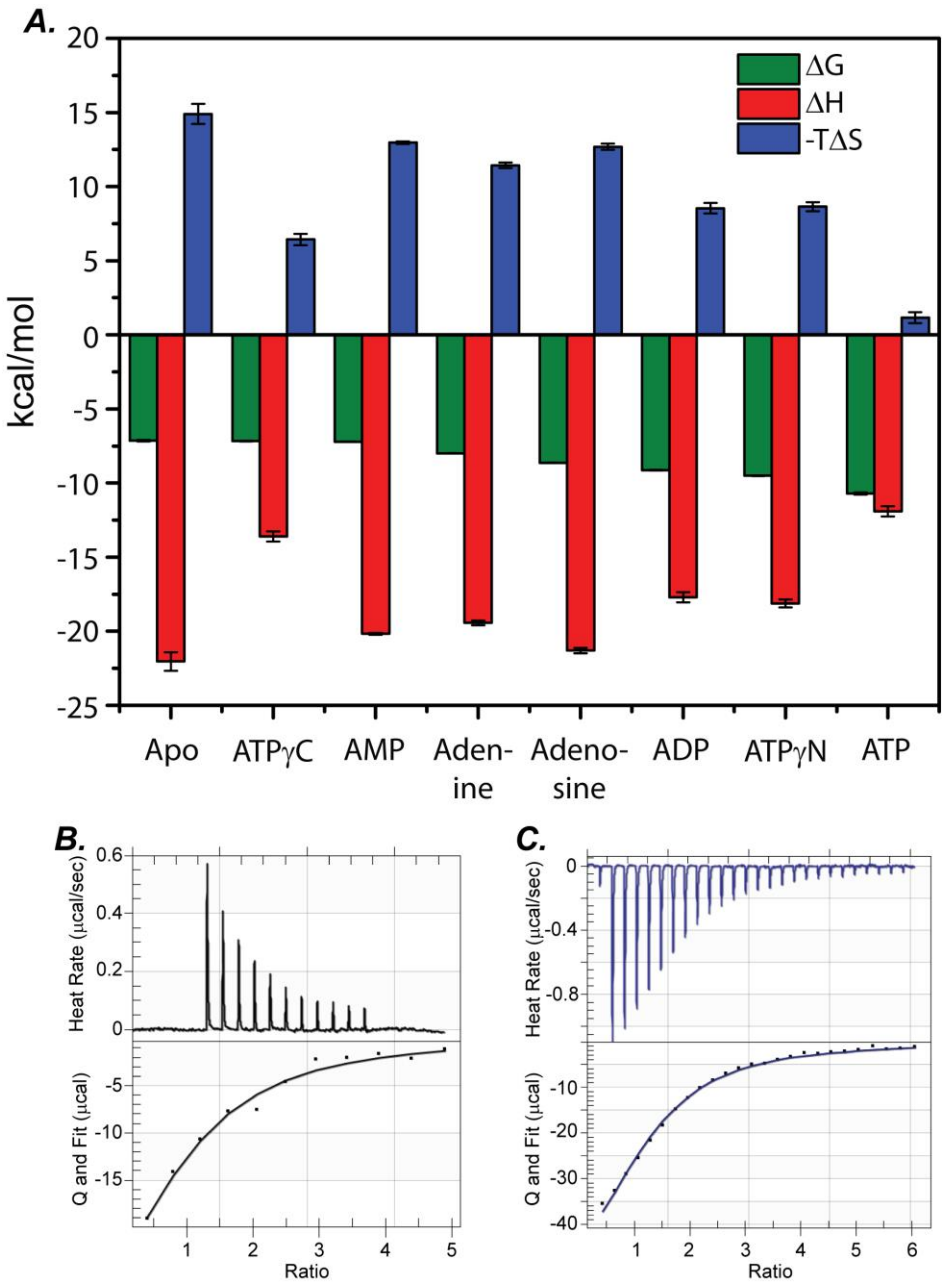

Figure S2: A. Full thermodynamic profile of PKI_{5-24} binding in the presence of various nucleotides. The entropic contribution was calculated as described in the methods section. **B.** ITC isotherm of AMP binding to PKA-C. A K_d value of 250 ± 57 μM was found. **C.** ITC isotherm of PKI_{5-24} binding at 6 mM AMP. Fit to a single-site binding model gave a K_d value of 6.2 μM (related to Figure 2).

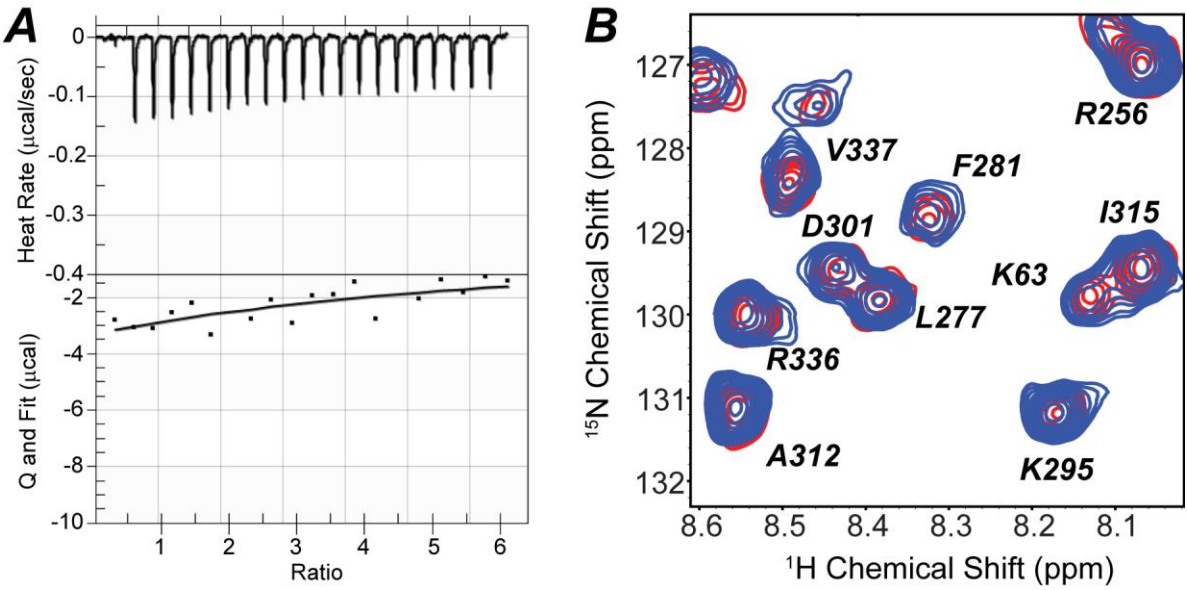

Figure S3: **ATPγC abrogates native substrate binding.** A) ITC isotherm of PLN_{1-19} binding to ATPγC saturated PKA-C. B) Overlay of [^{1}H,^{15}N]-TROSY-HSQC spectra of the complexes PKA-C/ATPγC (green) and PKA-C/ATPγC/PLN_{1-19} (red) showing no detectable chemical shift changes (related to Figure 2).

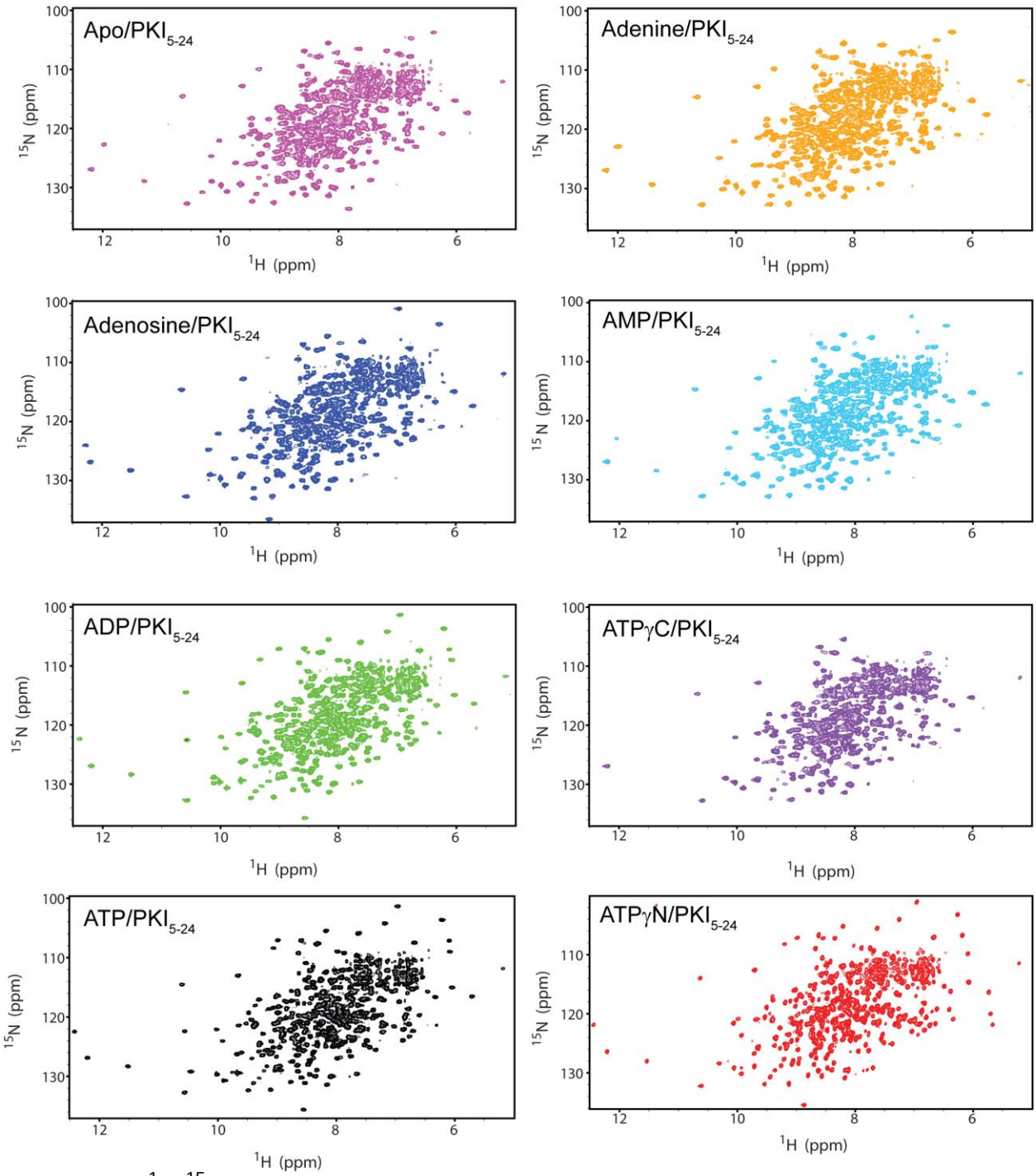

Figure S4. ^{1}H-^{15}N TROSY-HSQC of all the binary (PKA-C and PKI_{5-24}) and ternary complexes of PKA-C with PKI_{5-24} (related to Figure 3 and 5).

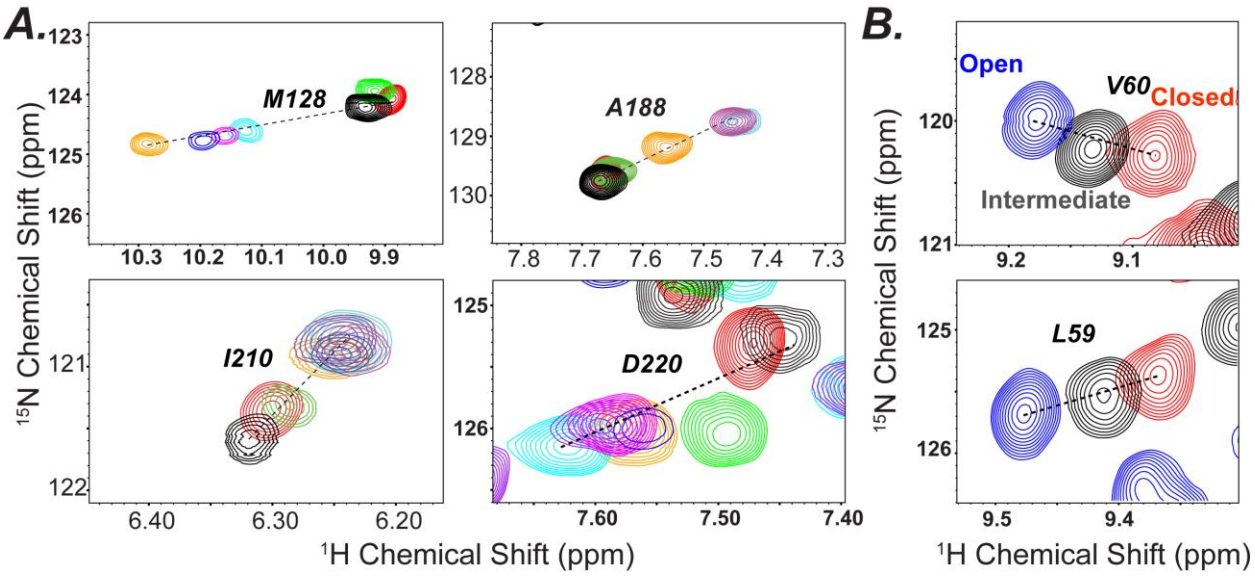

Figure S5: Linear chemical shift trajectories of PKA-C. (A) Linear chemical shift trajectories that either have some states missing or demonstrate some deviation around the linear trajectory due to local chemical changes. (B) Trajectory for the Open, Intermediate and Closed state in PKA-C (related to Figure 3 and 5).

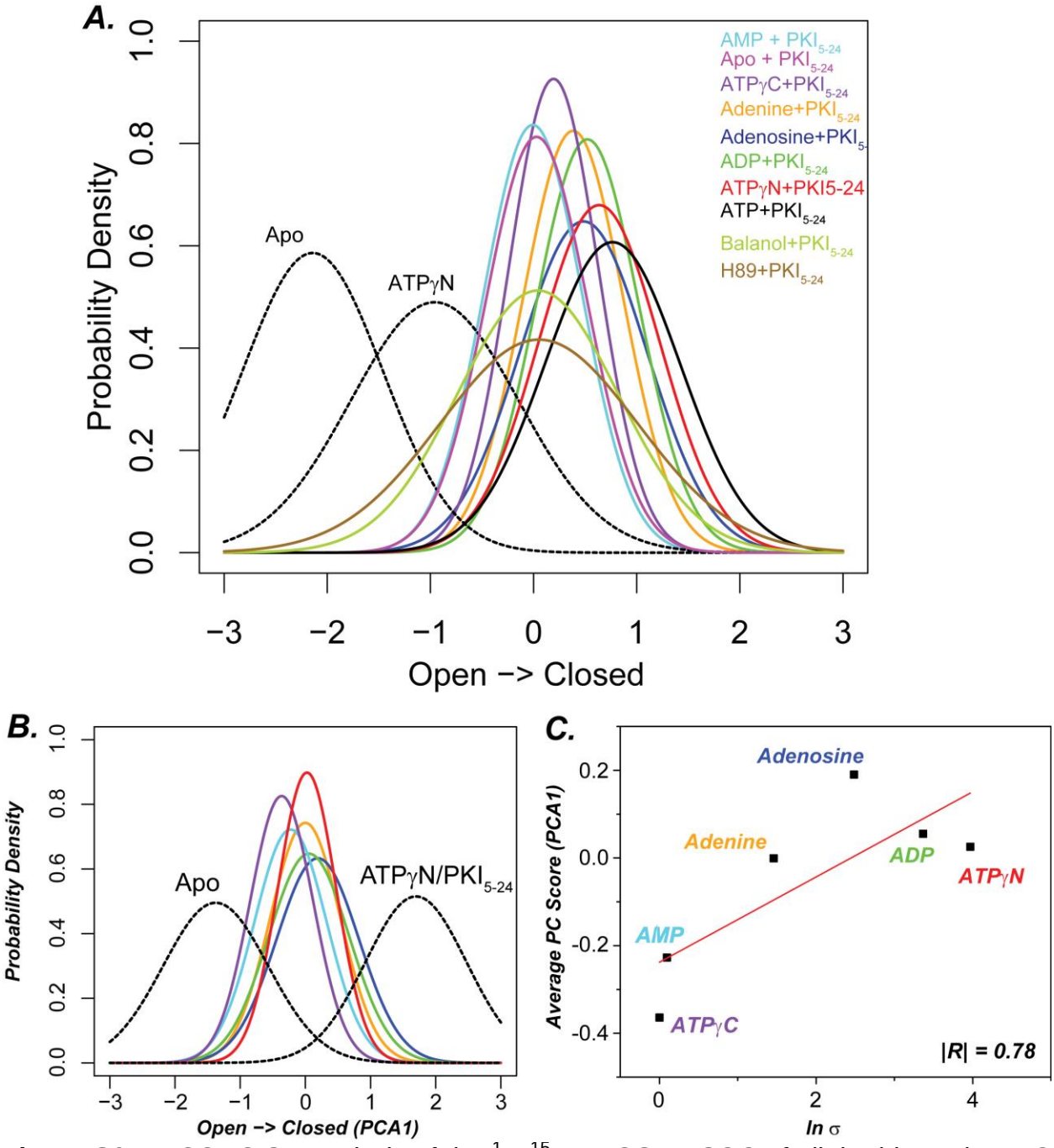

Figure S6. A. CONCISE analysis of the ^{1}H-^{15}N TROSY-HSQC of all the binary (PKA-C and PKI_{5-24}) and ternary complexes of PKA-C with PKI_{5-24}. **B.** CONCISE analysis performed on the nucleotide bound states of PKA-C (without PKI_{5-24}). **C.** A comparison of the PC score of the nucleotide bound states with the cooperativity of PKI_{5-24} binding (related to Figure 3 and 5).

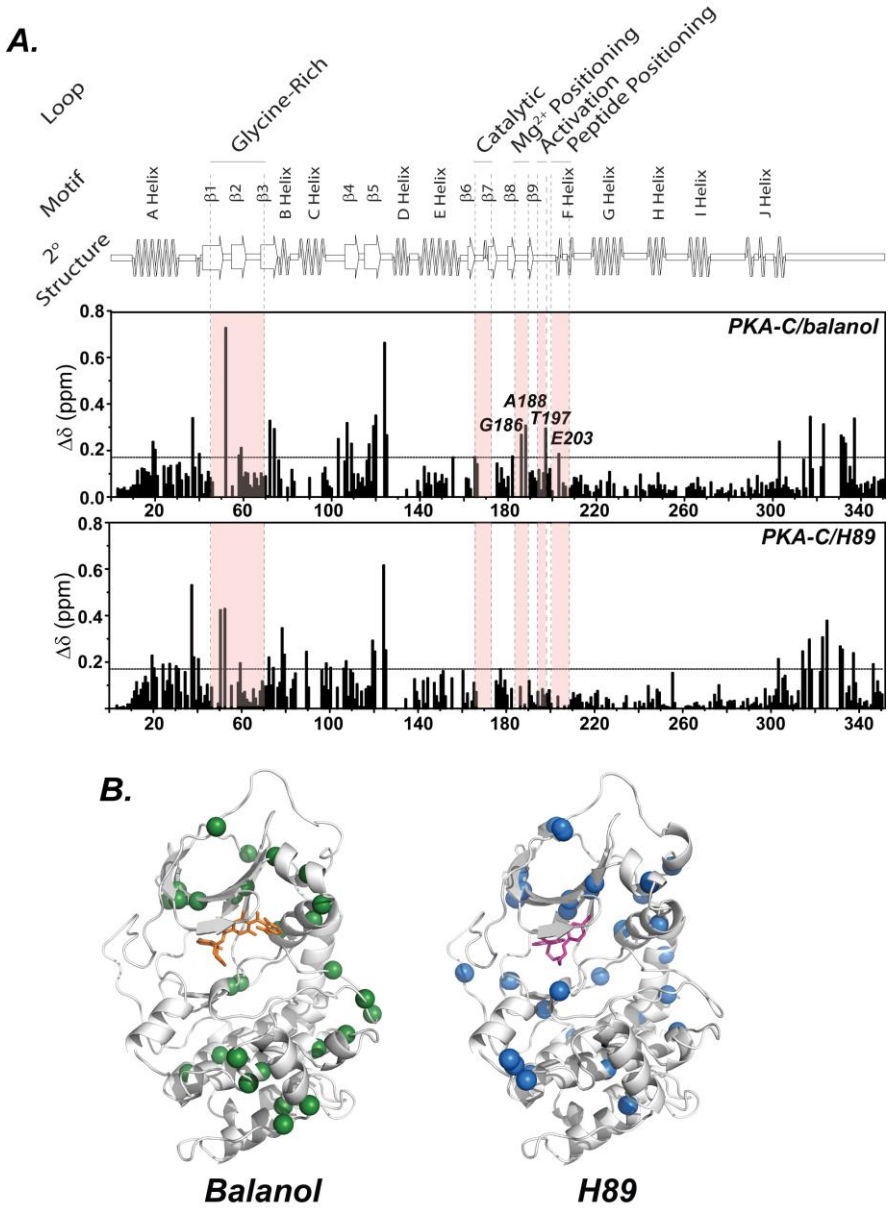

Figure S7. A. Chemical shift perturbation of PKA upon balanol or H89 binding. **B.** Residues of PKA-C that do not follow the open to closed linear trajectory upon ATP-competitive inhibitor binding (related to Figure 6 and 7).

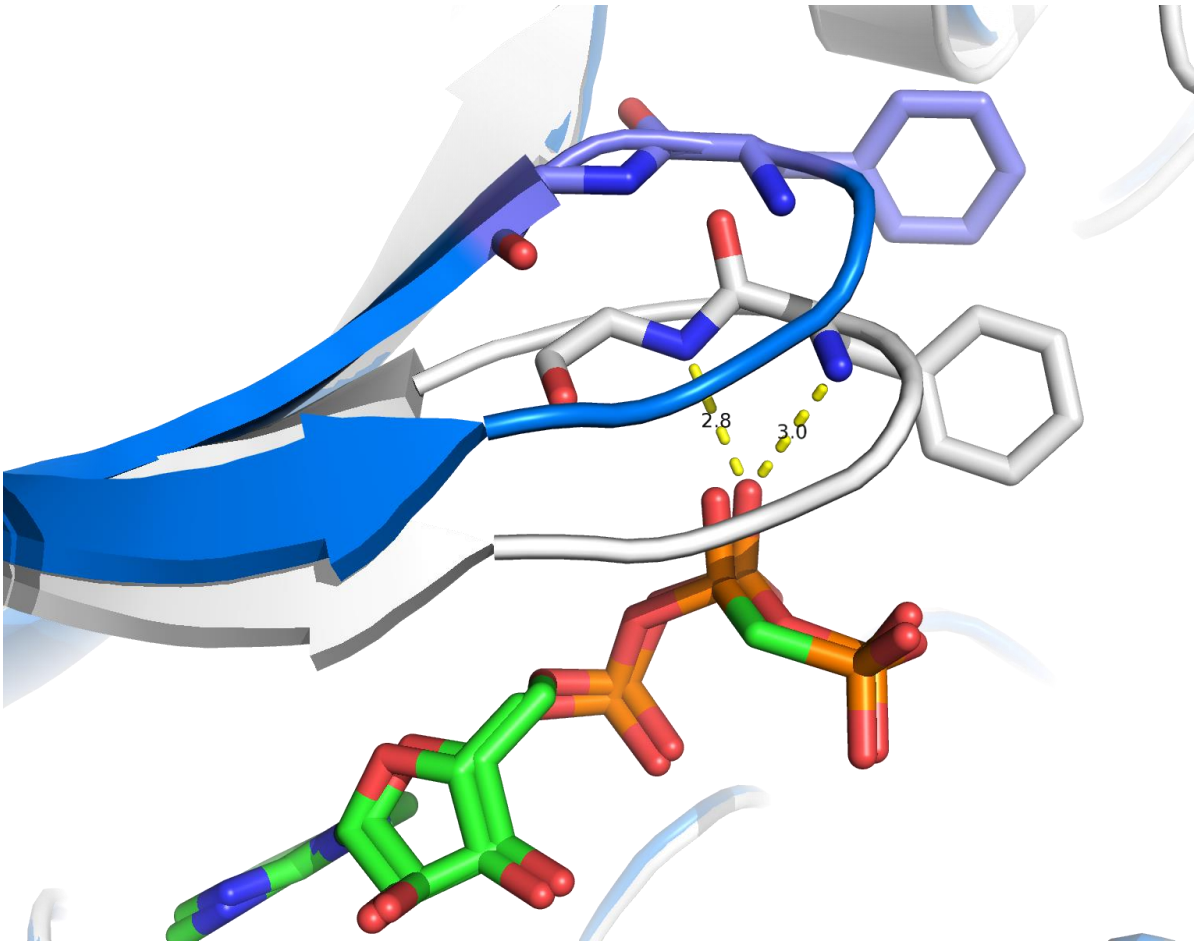

Figure S8. Structure of the glycine rich loop of PKA-C/ATP/PKI_{5-24} (PDB:1ATP, white) and PKA-C/ATPγC/PKS_{5-24} (PDB: 4IAC, blue) (related to Figure 8).

Table S1: List of residues that defined linear trajectories for the 12 states analyzed with CONCISE using the thresholds reported in the Methods section (related to Figure 5).

8	16	17	35	37	44	58	62	97	117
143	144	155	177	191	192	193	206	208	213
214	220	226	241	245	249	256	265	274	315
322	323	331	337	338	348				

Table S2: PCA and standard deviation of the CONCISE analysis of all ten structural states (related to Figure 5).

	PCA 1	Standard Deviation
Apo	-2.14	0.68
ATPγN	-0.96	0.81
AMP/PKI$_{5-24}$	-0.01	0.48
ATPγC/PKI$_{5-24}$	0.19	0.43
Apo/PKI$_{5-24}$	0.03	0.49
Adenine/PKI$_{5-24}$	0.38	0.48
Adenosine/PKI$_{5-24}$	0.48	0.62
ADP/PKI$_{5-24}$	0.52	0.49
ATPγN/PKI$_{5-24}$	0.64	0.59
ATP/PKI$_{5-24}$	0.77	0.66
H89/PKI$_{5-24}$	0.05	0.96
Balanol/PKI$_{5-24}$	0.04	0.78

SI Materials and Methods

Analysis of Chemical Shift Perturbations

We employed the COordiNate ChemIcal Shift bEhavior (CONCISE)[1] method to monitor trajectories of chemical shifts and measure the change in equilibrium position associated with each PKA-C construct (apo, ATPγN, adenosine/PKI_{5-24}, adenine/PKI_{5-24}, Apo/PKI_{5-24}, AMP/PKI_{5-24}, ADP/PKI_{5-24}, ATPγC/PKI_{5-24}, ATPγN/$PKI_{5-24,}$ balanol/PKI_{5-24} and H89/PKI=). In short, through Principal Component Analysis (PCA), the method identifies a set of residues whose chemical shifts respond linearly to the conformational transition. Each one of these residues provides a measure of the equilibrium position for every PKA-C construct in form of scores along the first principal component. The equilibrium position for a given construct is given by the average of the PC-scores over all linear residues. To identify the residues whose chemical shifts follow a linear pattern, a threshold of 1.0 to 3.0 for the ratio of the standard deviations of PC1 over PC2 was used, and residues that were affected by chemical shifts perturbations below 0.05 ppm were also discarded (see [1]for details on the threshold calibration). After this threshold was applied, a total of 36 residues formed the subset that was used to trace the equilibrium position of each state (see **Table S1-2**).

References

1. Cembran, A., et al., *NMR mapping of protein conformational landscapes using coordinated behavior of chemical shifts upon ligand binding.* Phys Chem Chem Phys, 2014. **16**(14): p. 6508-18.