Electronic Supplementary Material (ESI) for Chemical Science.

Supplementary Material for

State-Selective Frustration as a Key Driver of Allosteric Pluripotency

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SUPPLEMENTARY FIGURES

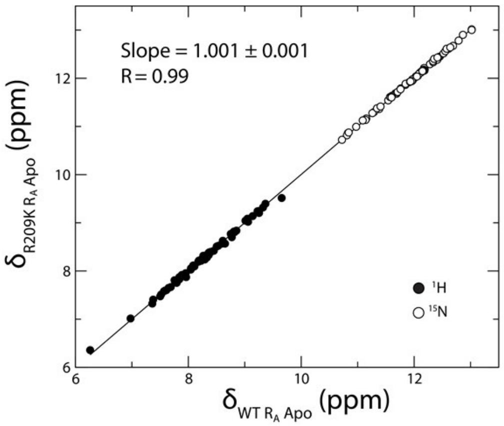


Figure S1. Correlation plot of chemical shifts of WT R_A apo *vs.* R209K R_A apo. The closed and the open circles represent the chemical shift for ^{1}H and ^{15}N (downscaled by 0.1), respectively.

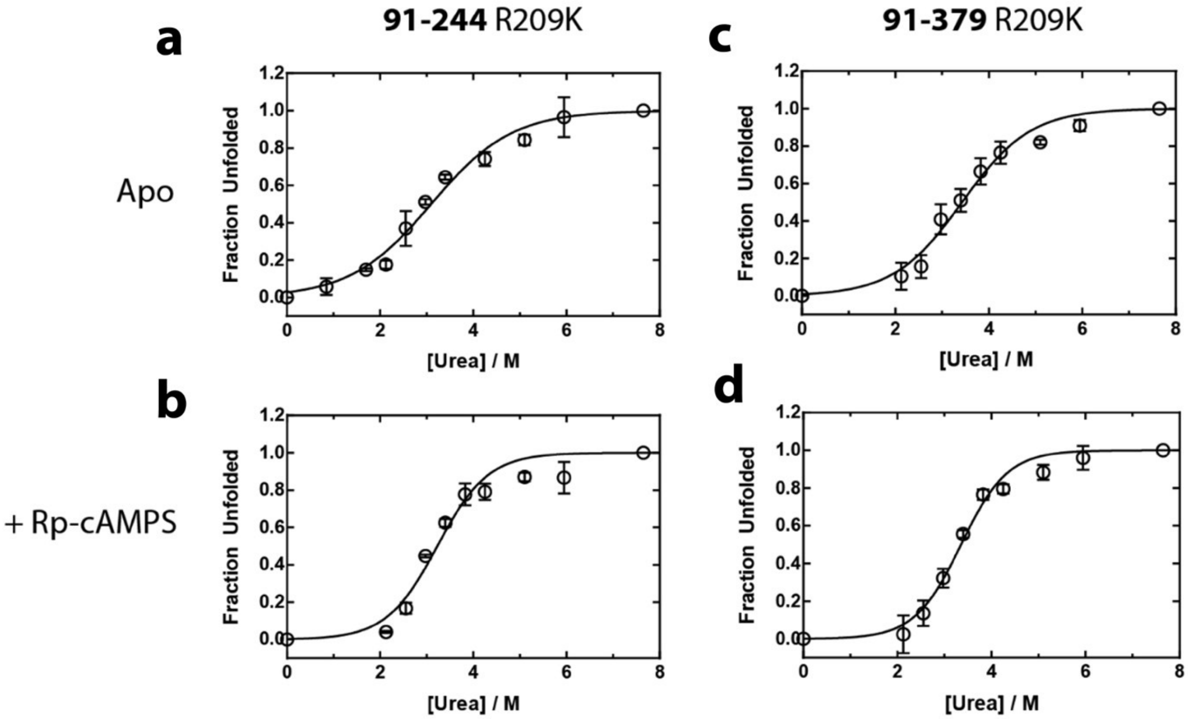


Figure S2. Urea-induced unfolding curves for R209K. **(a)** Urea-induced unfolding curve for apo R209K R_A (91-244). **(b)** Similar to (a) but in the presence of excess Rp-cAMPS (2 mM). **(c)** Similar to (a) but for the R_{AB} (91-379) construct. **(d)** Similar to (b) but in the presence of excess Rp-cAMPS (500 μM).

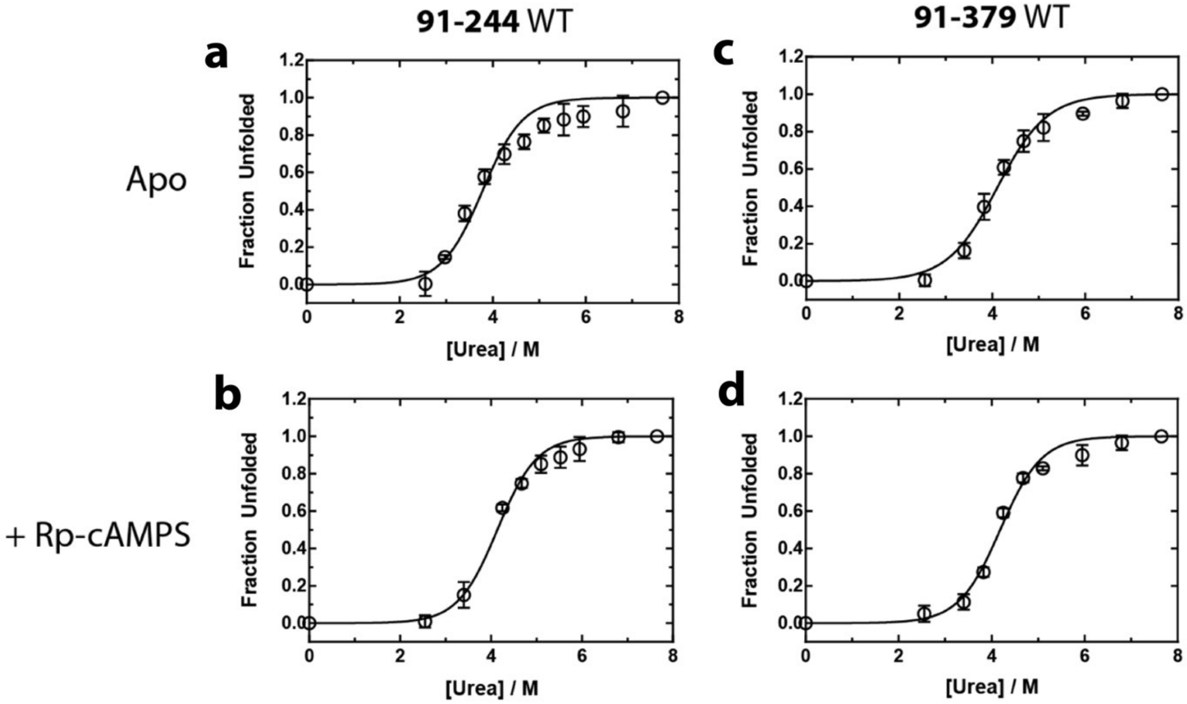
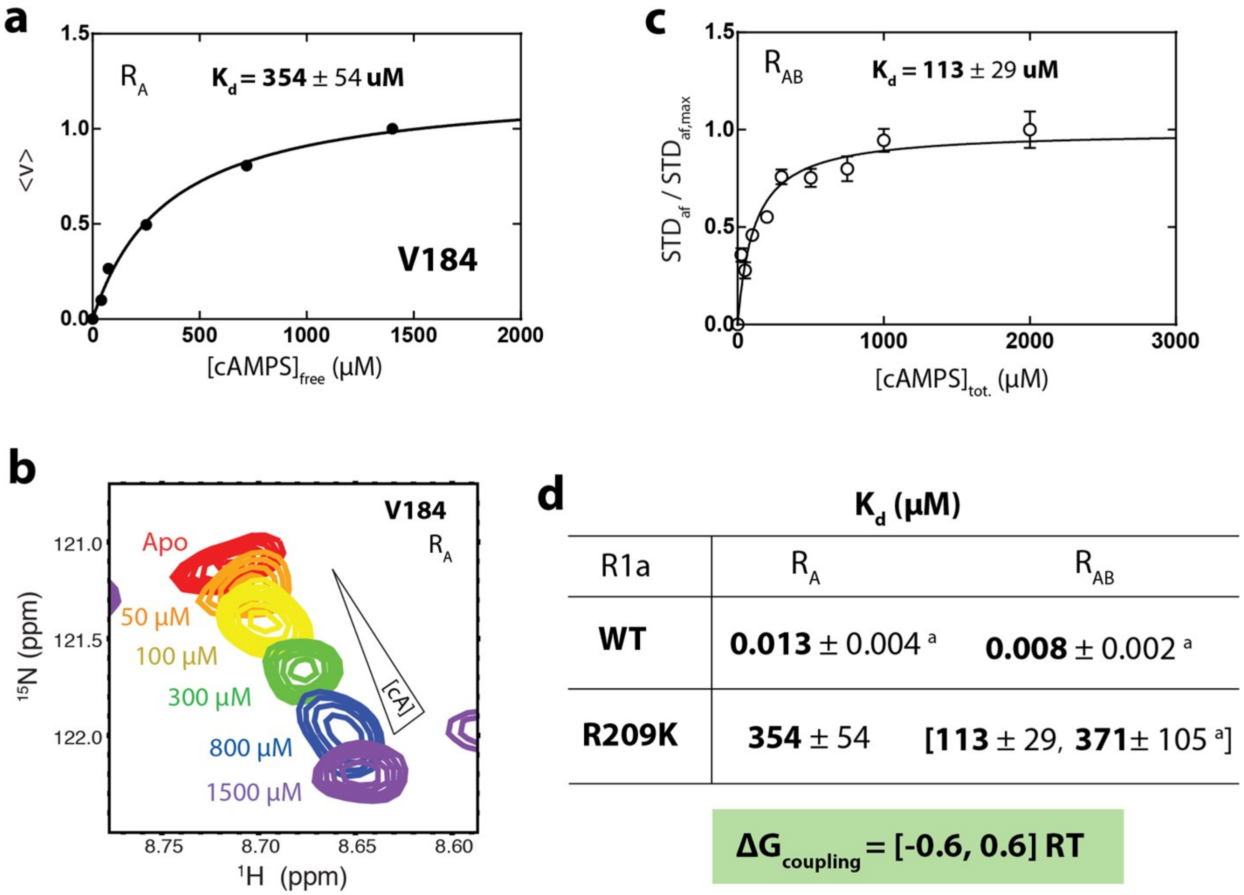


Figure S3. Urea-induced unfolding curves for WT. **(a)** Urea-induced unfolding curve for apo WT R_A (91-244). **(b)** Similar to (a) but in the presence of excess Rp-cAMPS (500 μM). **(c)** Similar to (a) but for the R_{AB} (91-379) construct. **(d)** Similar to (b) but for R_{AB} (91-379) construct.



R1a	R_A	R_{AB}
WT	**0.013** ± 0.004 [a]	**0.008** ± 0.002 [a]
R209K	**354** ± 54	[**113** ± 29, **371** ± 105 [a]]

ΔG$_{coupling}$ = [-0.6, 0.6] RT

Figure S4. Dissociation constants of cAMP from PKA R1a CBD-A in the absence and presence of R209K and/or the inter-domain interactions. **(a)** Chemical shift-based isotherm for the binding of cAMP to R209K CBD-A in the absence of inter-domain interaction (*i.e.* R_A construct) using the NMR monitored titration shown in **(b)**. **(c)** Isotherm for the binding of cAMP to R209K CBD-A in the presence of inter-domain interaction (*i.e.* R_{AB} construct) built through the saturation transfer difference (STD) experiment. The STD amplification factors (STD_{af}) are normalized to the $STD_{af,max}$ and plotted against the total cAMP concentration. **(d)** Table of K_d values of cAMP dissociation from PKA R1a CBD-A in the absence and presence of R209K and/or the inter-domain interactions. The superscript [a] denotes K_d values computed based on reference 39 [39]. The $\Delta G_{coupling}$ computed using equation (3) and the K_d values in panel d falls in the -0.6 to 0.6 RT range.

Table S1. Summary of the Initial Structures of PKA R1a-subunit Used in the MD Simulations

Structure	**R209K Mutation Present? (Y/N)**	**Bound Ligand**		**Simulation Length (ns)**
		In CNB-A domain	**In CNB-B domain**	
R_p-cAMPS-bound wild-type	N	R_p-cAMPS	R_p-cAMPS	400
R_p-cAMPS-bound R209K mutant	Y	R_p-cAMPS	R_p-cAMPS	400