Supplementary information

Molecular simulations reveal an unresolved conformation of the Type-IA Protein Kinase A regulatory subunit and suggest its role in the cAMP regulatory mechanism

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Methods

Molecular Dynamics using GPU accelerated AMBER 12

We identified $R^{Flipback}$ as part of a series of molecular dynamics (MD) simulations studying the stability of the WT R^{Holo} conformation (residues 113-379) in the absence of the catalytic subunit. The atomic coordinates for the starting conformation of the regulatory subunit (R^{Holo}) were taken from the heterodimeric structure of the R333K mutant, RC^{Holo}. As a starting conformation for R^{Holo}, we used the Protein Databank structure, 2QCS.[1] Residue K333 was changed back to R333 to undo the mutation and simulate wild-type (WT) R subunit. The system was prepared using Schrodinger's Maestro (Suite 2012: Maestro, version 9.3, Schrödinger, LLC, New York, NY, 2012) PDB prep and Desmond system preparer. Terminal residues were capped to remove charges. Titratable residues were protonated at pH 7.0 using Maestro integrated PROPKA.[2-5] The system was solvated in cubic water box with 10Å buffer using TIP3 waters[6] and 0.15M NaCl. Using xleap from Ambertools[7] the systems parameterized using AMBER ff99SB force field[8] and periodic boundaries were applied. The system was minimized in the following four stages: 1) 2,000 steps of hydrogen only minimization, 2) 4,000 steps of solvent minimization, 3) 20,000 steps with constrained backbone, and 4) 40,000 steps full minimization. MD simulations were performed as an NTP ensemble, at 310K and 1 bar, with a 2 fs time step and a 10 Å non-bonding interaction cutoff with a partial mesh Ewald approximation for long-range electrostatics interactions. The system was heated and then equilibrated over 1ps with harmonic constraints on the backbone. Production was performed as five parallel runs with new initial starting vectors for 200 ns each. The Flipback structure ($R^{Flipback}$) was observed in one of the five runs and was identified through visual inspection with VMD.[9]

Preparation of Structures for Brownian Dynamics

The atomic structure of R subunit from the cAMP-bound structure (R^{Bound}; PDBID:1RGS)[11] was used to determine the encounter complex. The RC^{Holo} heterodimer (PDBID:2QCS)[1] was used for BD simulations of and RC^{Holo}. The $R^{Flipback}$ structure was a configuration taken from one of five molecular dynamics simulations starting from the R^{Holo} conformation. The Susan Taylor lab at the University of California, San Diego, provided the structural model of heterotetrameric $R_2C_2^{Holo}$ to us. This structure was validated through mutational experiments and published in 2011[12]. We created the $R_2C_2^{Flipback}$ tetrameric structure by aligning the A-domain of the $R^{Flipback}$ conformation with the CBD-A with of the regulatory subunit of the $R_2C_2^{Holo}$ structure using PyMol[13]. Residues 113 to 376 of the regulatory subunit were used for all simulations. Residues 13-350 were used for the C_α subunit. Titratable residues were assigned a protonation state using PROPKA 3.1 pKa prediction software[3-5, 14] on the PDB2PQR server [2-5] and manually inspected for accurate state assignment at a pH of 7.0.

To facilitate the crystallization of the heterotetramer trapped in the inactive conformation, RC^{Holo} was crystallized with phosphoaminophosphonic acid-adenylate ester (ANP) and Manganese ions (Mn^{2+}) in the active site of C subunit. To ensure the accurate electrostatic description of C subunit, we replaced ANP with adenosine triphosphate (ATP) and Mn^{2+} with Magnesium ions (Mg^{2+}). Parameters for ATP and Mg^{2+} were obtained from the Bryce Group AMBER parameters database.[15, 16] The edited PDB files were fed through tleap[17] to create parameter topology and coordinate files consistent with the Amber ff14SB forcefield[8]. PQR files consistent with the addition of Mg^{2+} and ATP in the structures were exported and formatted using ambpdb.[18] The atomic coordinates of cAMP from the R^{Bound} crystal structure and parameterized using Antechamber.[19]

To eliminate the effects of simulated vs. crystal structure conformations of R, all structures were solvated and relaxed into a stable conformation. The NAMD simulation package[20, 21] was used to minimize, heat, equilibrate, and simulate each system using a 2fs time-step. Every system underwent a series of separate minimization, heating, and equilibration stages in preparation for production runs in the following order. Minimization spanned five stages in 10ps intervals using the NVT ensemble: 1) 5,000 steps of hydrogen-only minimization, 2) 5,000 steps of solvent minimization, 3) 5,000 steps of side-chain minimization, 4) 5,000 steps of protein-backbone minimization, and 5) 5,000 steps of full-system minimization. The Langevin thermostat[22, 23] was used to slowly heat the system to 310K using the NVT ensemble over 250,000 steps

(500ps) following minimization. The system was then subject to three sequential equilibration stages using the NPT ensemble for 125,000 steps/stage (250ps/stage). The pressure was set to 1 atm and maintained using the Beredensen barrostat.[24] The solvent was modeled explicitly using the TIP4P water model[25] and a 0.15M sodium chloride concentration was applied after neutralizing the overall charge of the protein complexes. The Particle Mesh Ewald electrostatic summation method[26, 27] was employed to evaluate electrostatics during simulation.

Brownian Dynamics Simulations using BrownDye

Brownian Dynamics simulations with BrownDye[28] require PQR files as input, which list the position, charge, and radius of each atom in the respective molecules to be simulated. All necessary PQR files were generated using ambpdb—a program from the AMBER suite.[7] PQR files were converted to XML format through pqr2xml, an accessory program of BrownDye. APBS[29] was used to generate the electrostatic field of all molecules immersed in a 0.15M NaCl implicit solvent. Bd_top, an accessory program of BrownDye, generated all necessary input files for BD simulations. 5,000,000 single trajectory simulations were performed using nam_simulation from the BrownDye suite. Association rate constants and reaction probabilities were calculated using the compute_rate_constant function of BrownDye.

The encounter complex is described by the distance between interacting atoms in a "bound" complex. The criteria for cAMP association to R subunit was based on cAMP interactions in CBD-A of R^{Bound} crystal structure.[11] To control for the effect of sidechain orientation differences, CBD-A of R^{Bound}, R^{Holo}, and $R^{Flipback}$ were aligned and atoms with less than 1.0 Å deviation were selected for the binding criteria. The atomic interactions chosen for the encounter complex are shown in Figure S1 and listed in Table S1. A distance of 7 Å was chosen for interacting atoms, consistent with the crystal structure conformation of R^{Bound}.

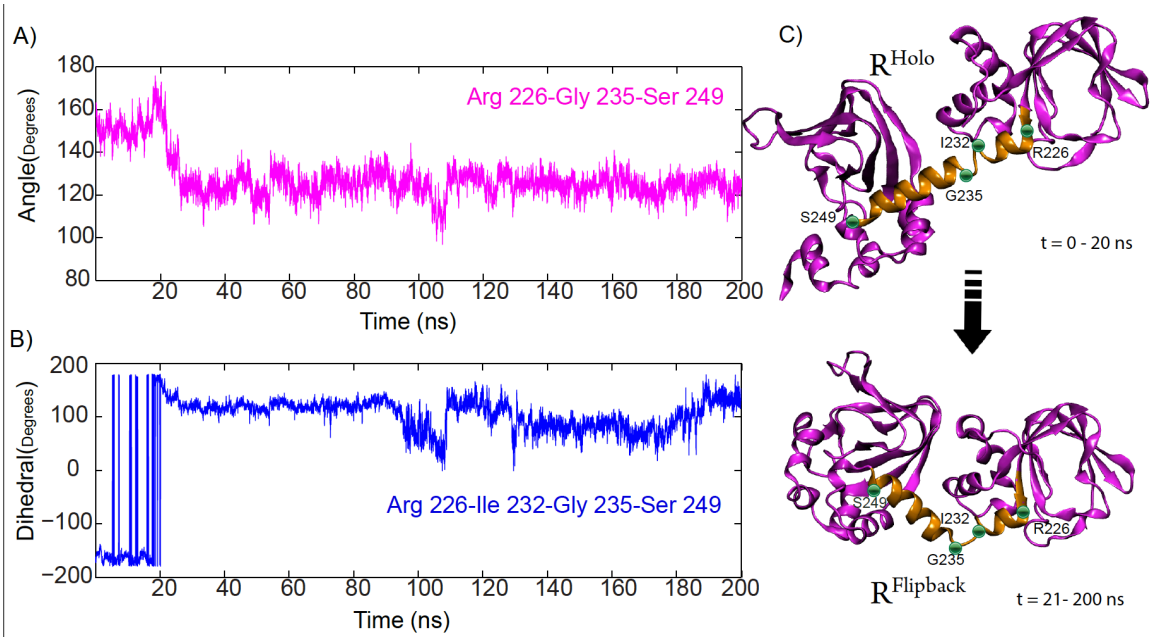

FIGURE S1. The stability of the Flipback conformation. (A) Change in B/C helix angle over the course of the MD simulation. The angle was measured between Arg226-Gly235-Ser249; (B) Change in B/C helix dihedral angle over the course of the MD simulation. The angle was measured between Arg226-Ile233-Gly235-Ser249; (C) Change in conformation of R throughout the MD simulation. CBD-A and CBD-B are shown in pink. The B/C helix is shown in gold. The position of B/C helix residues used in Figure S1A and Figure S1B are shown in green dots.

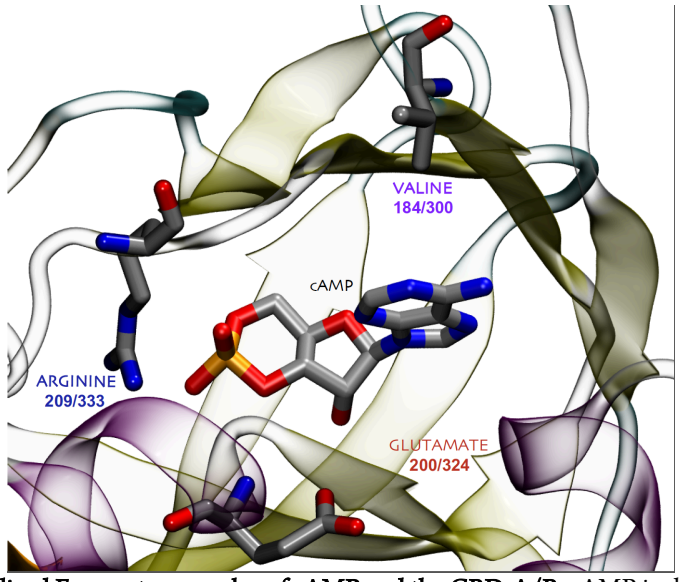

Figure S2. Generalized Encounter complex of cAMP and the CBD-A/B. cAMP is shown in the cyclic-nucleotide binding domain (CBD) of the regulatory subunit of Protein Kinase A RIα. Residue names are included next to the licorice representation of the sidechains and numbering of residues are numbered in CBD-A/CBD-B format. Hydrogen atoms are excluded. Interacting residues of the R subunit were chosen to be Valine, Arginine, and Glutamate for both CBD-A and CBD-B.

Table S1. Encounter Complex Description of R subunit and cAMP

CBD	R subunit residue number	R subunit atom name	cAMP Atom name and number
A	VAL 184	CB	C8 (imidazole ring)
A	GLU 200	CA, CB	O5 (ribose ring)
A	ARG 209	CB	O1, O2 (α, β Phosphate)
B	VAL 300	CB	C8 (imidazole ring)
B	GLU 324	CA, CB	O5 (ribose ring)
B	ARG 333	CB	O1, O2 (α, β Phosphate)

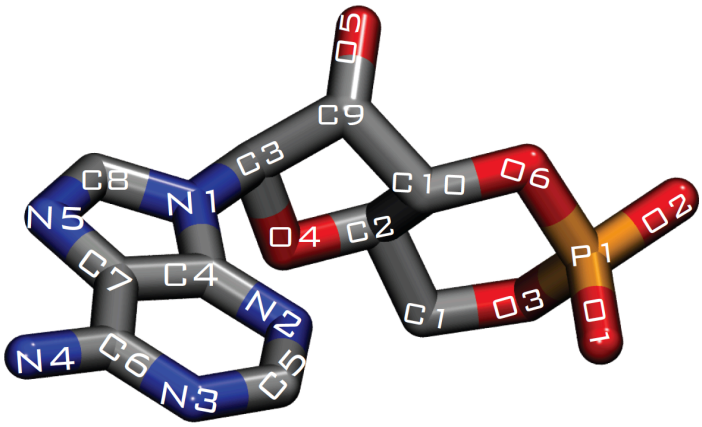

Figure S3. Atomic numbering and naming of cAMP molecule in BD simulations. cAMP atoms are named and numbered according to PDB format. Carbon (gray), Nitrogen (blue), Oxygen (red), and Phosphorous (Orange) atoms are labeled with the corresponding atom name overlaid in white

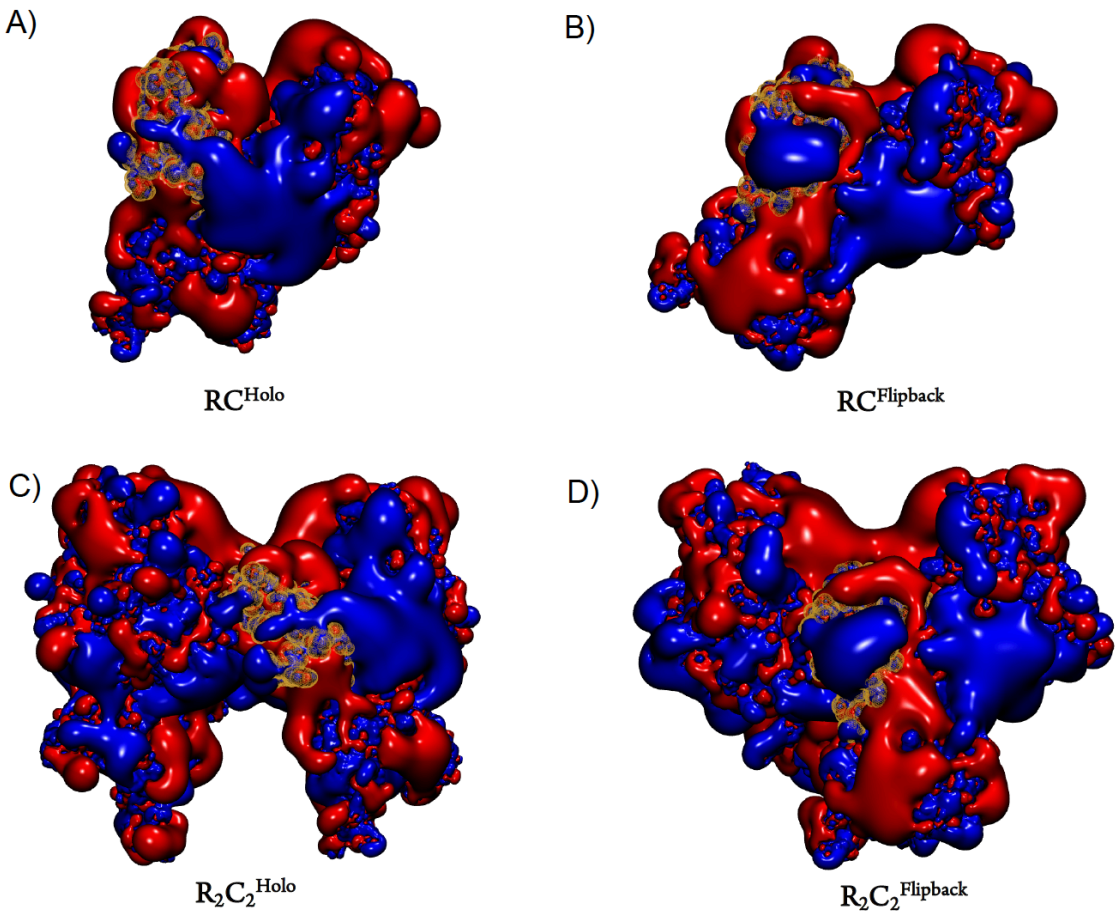

Figure S4. Electrostatic descriptions of the four systems. (A) Electrostatic description of RC^{Holo}; (B) Electrostatic description of $RC^{Flipback}$; (C) Electrostatic description of $R_2C_2^{Holo}$; (D) Electrostatic description of $R_2C_2^{Flipback}$; Domain A is highlighted in gold wire mesh. An isosurface value of 0.75 was used to create the positive and negative potentials. Electropositive potentials are shown in blue and electronegative potentials are shown in red.

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