## Supporting Information of

# CHARMM Force-fields with Modified Polyphosphate Parameters allow Stable Simulation of the ATP-Bound Structure of $Ca^{2+}$-ATPase

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**Table S1.** Summary of the simulation systems.

(a) MD simulations of E1•ATP

| | |
|---|---|
| Number of atoms | 378177 |
| Number of water molecules | 96099 |
| Number of lipid molecules | 535 |
| Number of counter ions | $295K^+$ / $272Cl^-$ |
| ATP binding site | $1ATP + 1Mg^{2+}$ |
| $Ca^{2+}$ binding site | $2Ca^{2+}$ |
| P-domain | $1K^+$ |
| Box size (Å$^3$) | 140 × 140 × 200 |

(b) MD simulations of four ATP-bound proteins. In each protein, hydrogen atoms were added to the crystal structure using the VMD 1.9.1 tool set.[1] Cavities inside the protein were filled with water molecules using DOWSER.[2] The systems were solvated and neutralized with 150 mM KCl.

| | Histidine permease (PDB: 1b0u) | RNA editing ligase MP52 (PDB entry: 1xdn) |
|---|---|---|
| Number of atoms | 42410 | 41793 |
| Number of protein atoms | 4106 | 4232 |
| Number of nucleotides | 1ATP | 1ATP |
| Number of water molecules | 12727 | 12480 |
| Number of ions | $41K^+$, $39Cl^-$ | $42K^+$, $35Cl^-$, $1Mg^{2+}$ |
| Box size (Å$^3$) | 70 × 83 × 79 | 79 × 70 × 81 |
| | Phosphoribosylamidoimidazole-succinocarboxamide synthase (PDB entry: 1obd) | α-skeletal muscle Actin (PDB entry: 2fxu) |
| Number of atoms | 53229 | 63642 |
| Number of protein atoms | 4795 | 5617 |
| Number of nucleotides | 1ATP, 1AMP | 1ATP |
| Number of water molecules | 16086 | 19287 |
| Number of counter ions | $51K^+$, $45Cl^-$, $2Mg^{2+}$ | $63K^+$, $54Cl^-$, $4Ca^{2+}$ |
| Box size (Å$^3$) | 91 × 77 × 81 | 73 × 94 × 99 |

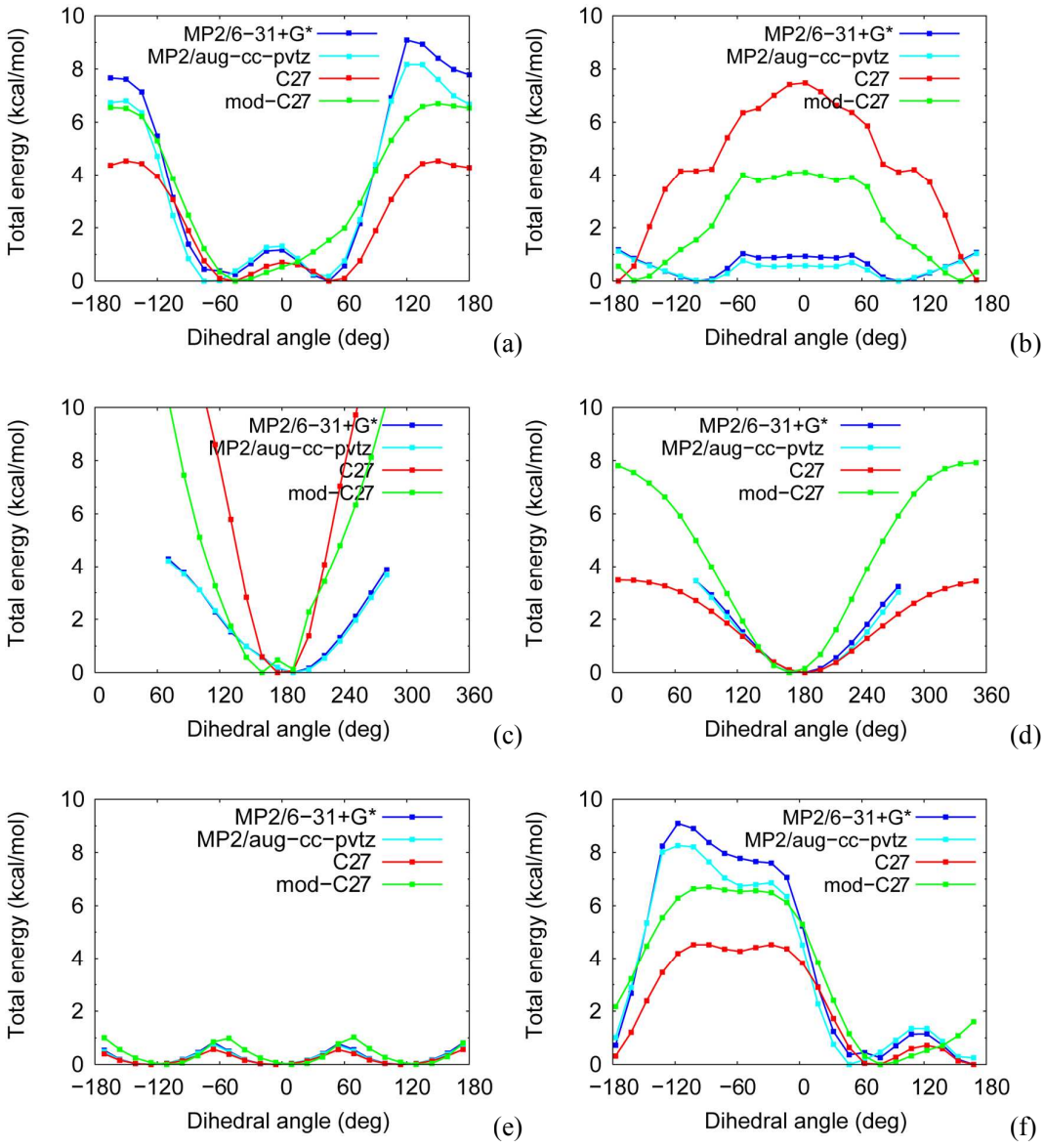
(c) REMD simulations of ATP in solution

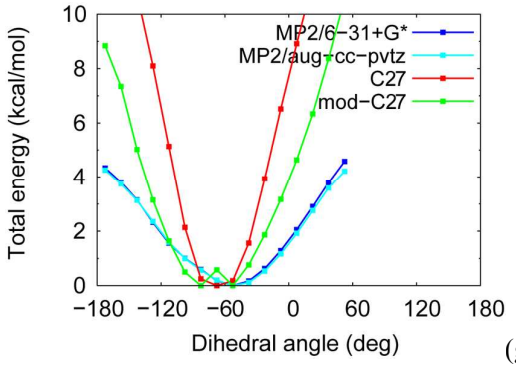
| | ATP in solution |
|---|---|
| Number of atoms | 3404 |
| Number of nucleotides | 1ATP |
| Number of water molecules | 1117 |
| Number of counter ions | $7K^+$, $3Cl^-$ |
| Box size ($Å^3$) | 34 × 34 × 39 |

**Table S2.** Atom names, types and partial charges of methyl triphosphate (MTP).

| Methyl triphosphate | | |
|---|---|---|
| Atom name | Atom type | Partial charge |
| O1G | ON3 | -0.90 |
| O2G | ON3 | -0.90 |
| O3G | ON3 | -0.90 |
| PG | P2 | 1.10 |
| O1B | ON3 | -0.82 |
| O2B | ON3 | -0.82 |
| O3B | ON2 | -0.86 |
| PB | P2 | 1.50 |
| O1A | ON3 | -0.82 |
| O2A | ON3 | -0.82 |
| O3A | ON2 | -0.74 |
| PA | P | 1.50 |
| O5’ | ON2 | -0.62 |
| C5’ | CN9 | -0.17 |
| H15 | HN9 | 0.09 |
| H16 | HN9 | 0.09 |
| H17 | HN9 | 0.09 |

**Figure S1.** Potential energy profiles along the selected dihedral angles of MTP calculated using both empirical force fields and *ab initio* methods: Modified CHARMM27 force-field (green), CHARMM27 force-field (red), MP2/6-31+G* (blue), and MP2/aug-cc-pVTZ//MP2/6-31+G* (cyan). Seven dihedral angles were considered: (a) C5'-O5'-PA-O3A. (b) O5'-PA-O3A-PB, (c) PA-O3A-PB-O3B, (d) O3A-PB-O3B-PG, (e) PB-O3B-PG-O1G, (f) C5'-O5'-PA-O1A, (g) PA-O3A-PB-O1B. The entire conformational space of the molecule was relaxed except for the dihedral angle of interest..




Total energy (kcal/mol)
Dihedral angle (deg)
MP2/6−31+G*
MP2/aug−cc−pvtz
C27
mod−C27
10
8
6
4
2
0
−180
−120
−60
0
60
120
180

(g)

**Figure S2.** Time course of the selected structural parameters in 20 ns-MD simulations using mod-C27(ATP). Ion parameters of Merz, Jr.[3] were used for $Mg^{2+}$ and $Ca^{2+}$. (a) salt bridge (SB) distances: SB1 between α-phosphate and Arg489 (red line for C27(ATP) and green one for mod-C27(ATP)) and SB2 between β-phosphate and Arg560 (magenta for C27(ATP) and blue for mod-C27(ATP)). (b) RMSD of tri-phosphate moiety, (c) PA-O3A-PB bond angle, (d) O5'-PA-O3A-PB dihedral angle. The black lines in (c) and (d) show the corresponding distances observed in X-ray structure.

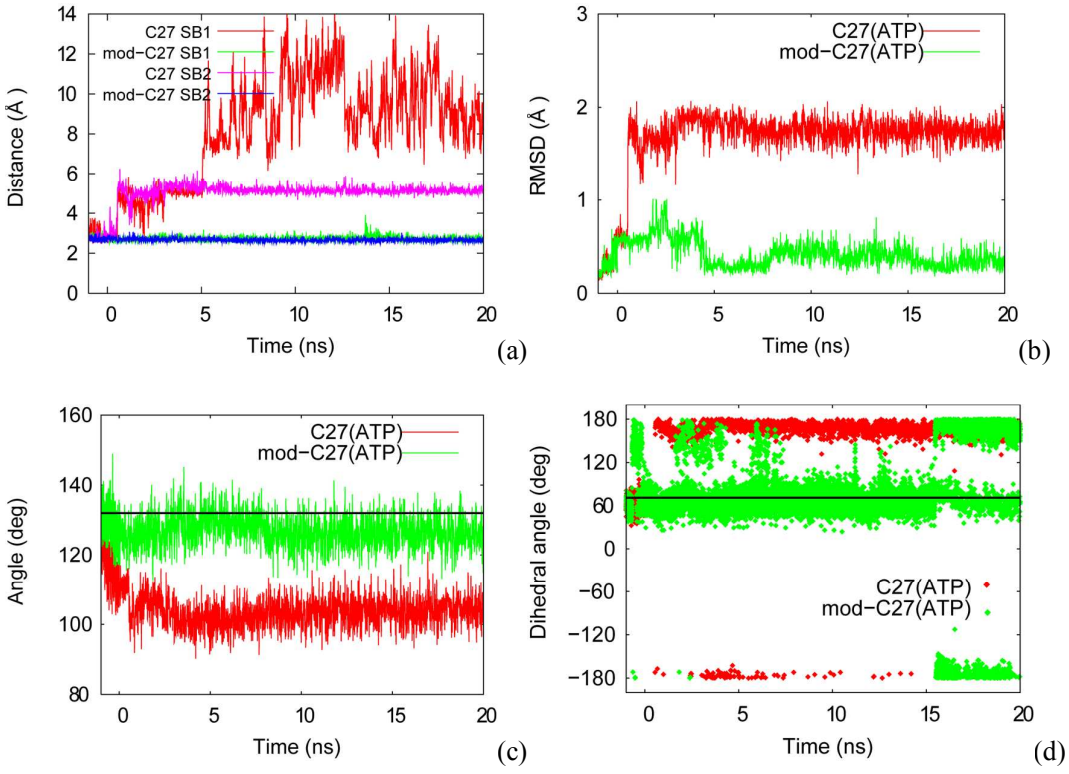


**Figure S3.** A snapshot of ATP-binding site after 20ns-MD simulation using mod-C27(ATP). Ion parameters of Merz, Jr.[3] were used for $Mg^{2+}$ and $Ca^{2+}$. The X-ray crystal structure of ATP bounded to $Ca^{2+}$-ATPase (gray) is shown for comparison.

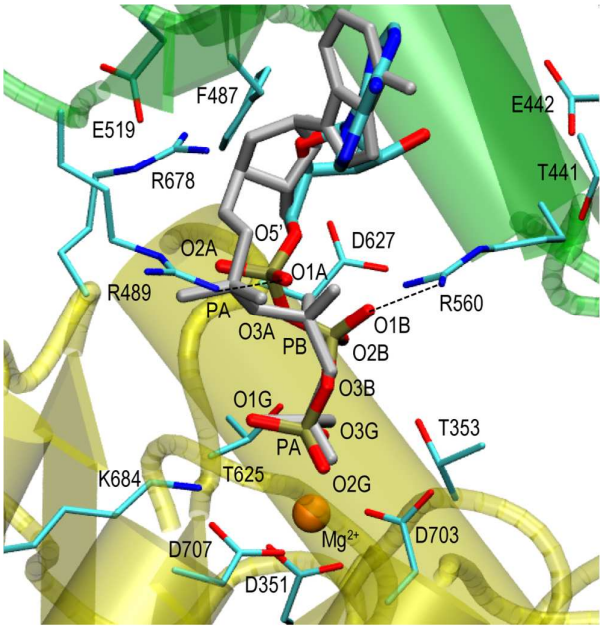


**Figure S4.** Root mean square deviations (RMSDs) of polyphosphate moiety of ATP for 5 ns MD simulations of ATP-bound proteins:
(a) Histidine permease (PDB: 1b0u), (b) RNA editing ligase MP52 (PDB entry: 1xdn), (c) Phosphoribosylamidoimidazole-succinocarboxamide synthase (PDB entry: 1obd), and (d) α-skeletal muscle Actin (PDB entry: 2fxu). RMSDs for the simulations with C27(ATP) and mod-C27(ATP) are shown in red and green, respectively.

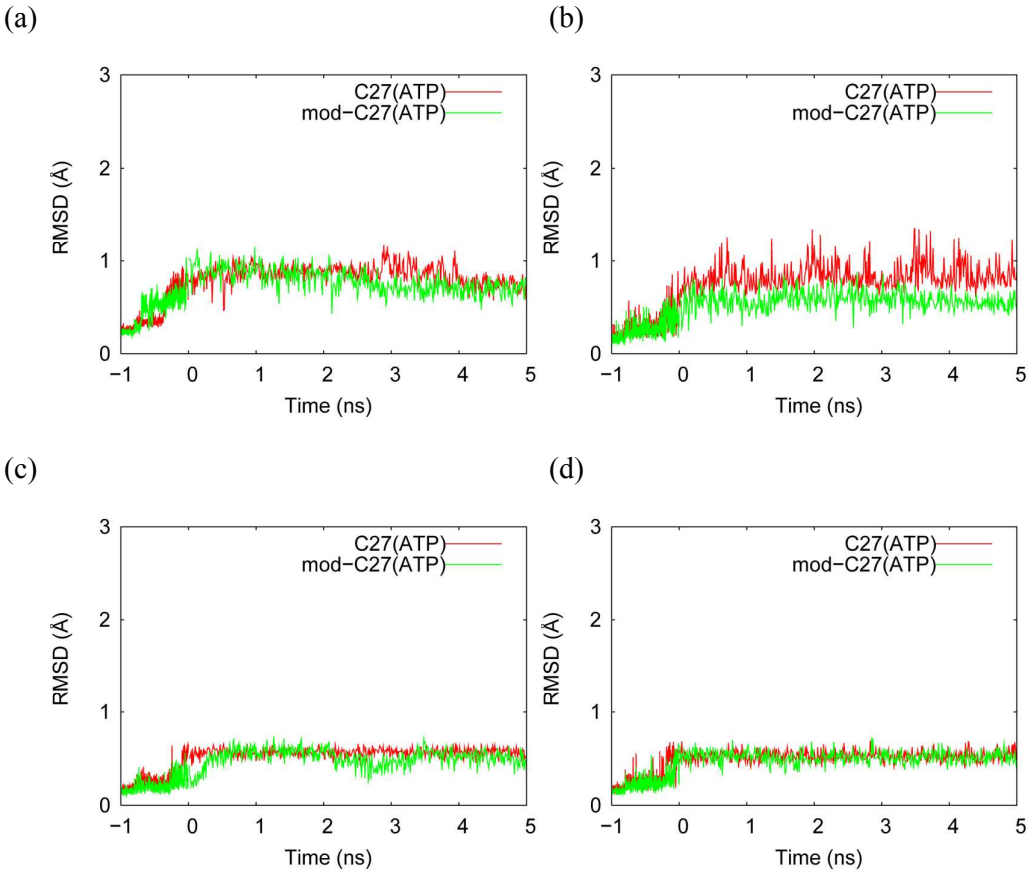
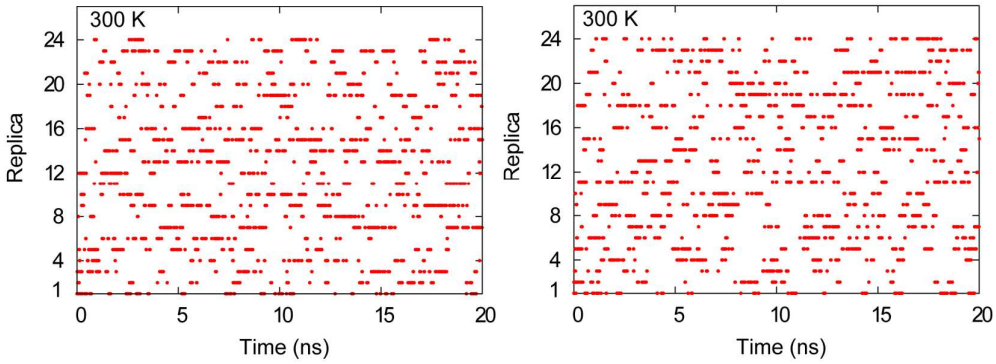
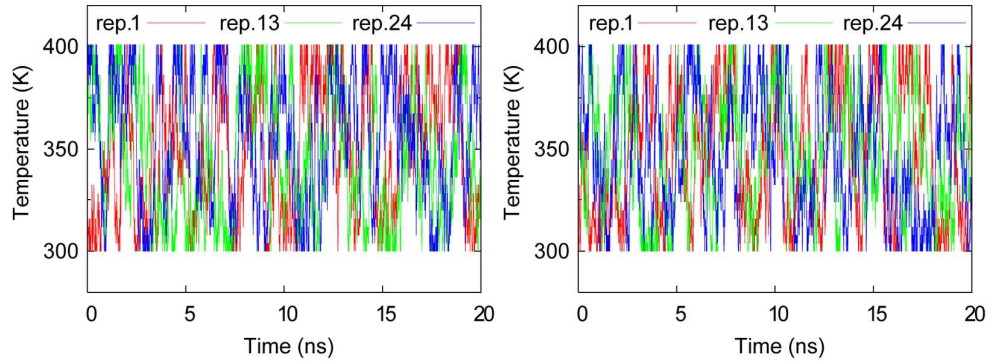


**Figure S5.** Performance of REMD simulations using C27(ATP) (left) and mod-C27(ATP) (right). (a) Time series of replica exchange at 300 K. (b) Time series of temperature exchange of three arbitrary chosen replicas (replica 1, 13, and 24). (c) Time series of total potential energy of three arbitrary chosen replicas (replica 1, 13, and 24). (d) The canonical probability distributions of the total potential energy of the system at 24 temperatures. 300 K, 350 K and 400 K are colored red, green and blue, respectively.

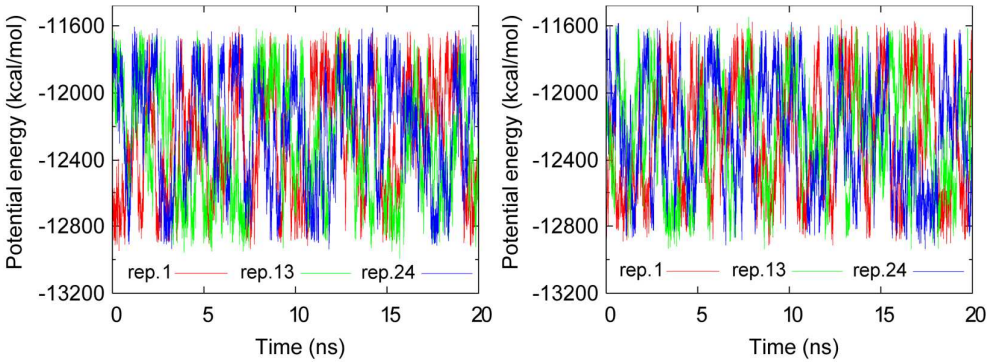
(a)




(b)



(c)


Potential energy (kcal/mol)
-11600
-12000
-12400
-12800
-13200
0
5
10
15
20
Time (ns)
rep.1
rep.13
rep.24

(d)

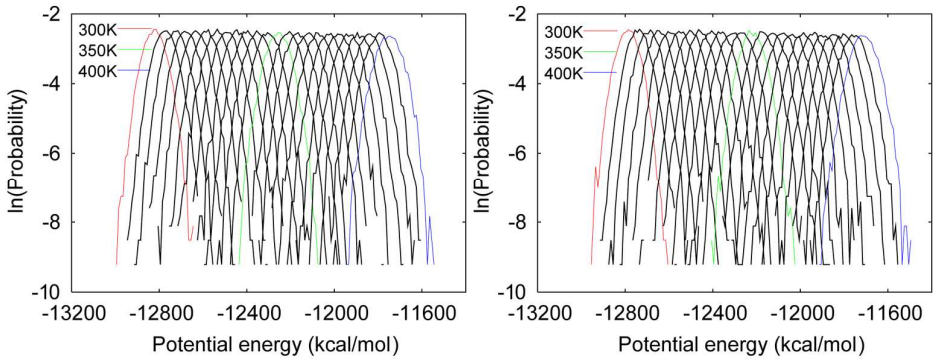

ln(Probability)
-2
-4
-6
-8
-10
-13200
-12800
-12400
-12000
-11600
Potential energy (kcal/mol)
300K
350K
400K

**Figure S6.** Convergence of REMD simulations using C27(ATP) (top) and mod-C27(ATP) (bottom). Cumulative averages of PMF landscapes along the two dihedral angles (O5'-PA-O3A-PB (**dih1**) and PA-O3A-PB-O3B (**dih2**)) are shown for every 5 ns.

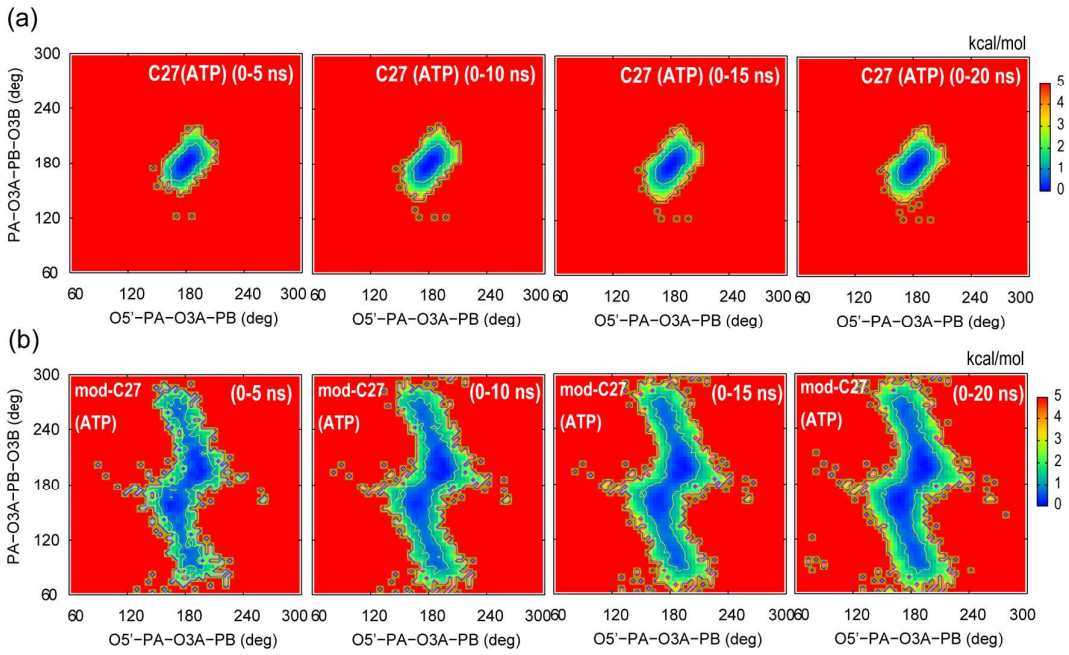
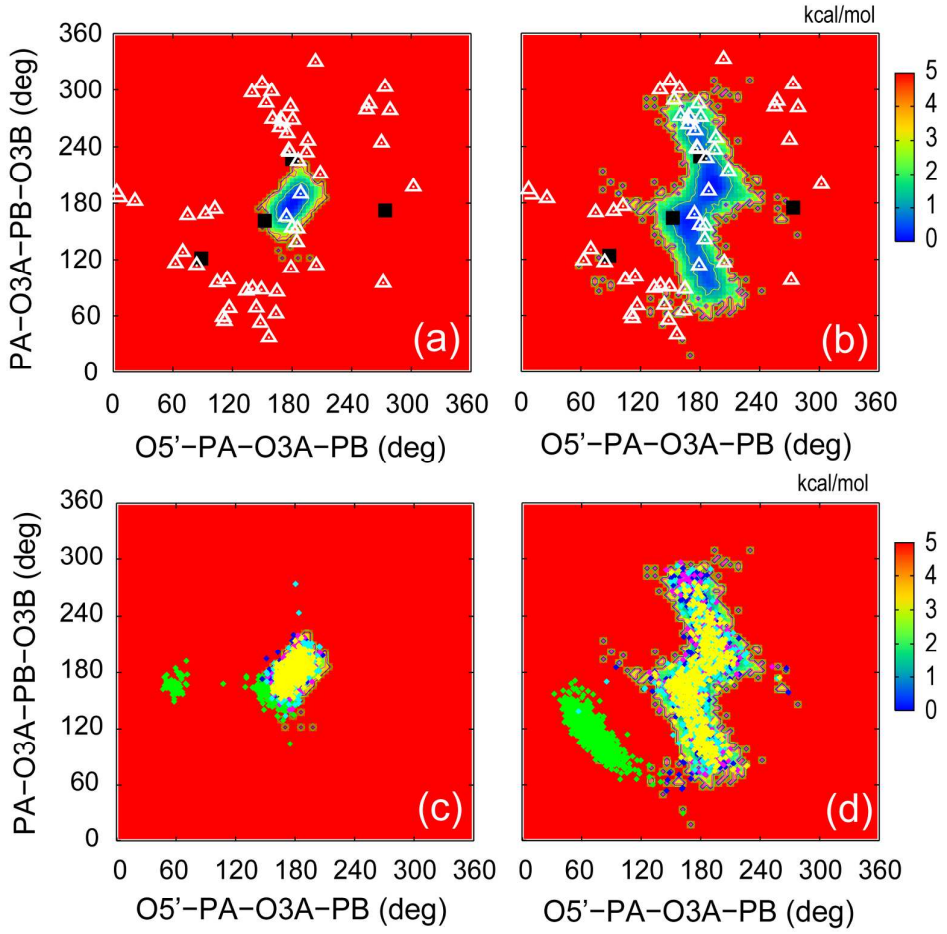


**Figure S7.** Comparison of calculated PMF with X-ray crystal structures. PMF landscapes along the O5'-PA-O3A-PB (**dih1**) and PA-O3A-PB-O3B (**dih2**) dihedral angles from the trajectory at 300 K in the REMD simulations using C27(ATP) (a) and mod-C27(ATP) (b). The corresponding values of 59 ATP structures in proteins, which were taken from Protein Data Bank (PDB), are marked on the PMF surfaces. Black squares are plotted for the crystal structures with resolutions better than 1.5 Å (4 structures), while white triangles are plotted for the structure with resolutions between 1.5 Å and 2.0 Å (55 structures). The **dih1-dihe2** distribution maps for the simulations of $Ca^{2+}$-ATPase (green) as well as additional four ATP-binding proteins (blue for Histidine permease, magenta for RNA editing ligase MP52, cyan for Phosphoribosylamidoimidazole-succinocarboxamide synthase, and yellow for α-skeletal muscle Actin) using C27(ATP) and mod-C27(ATP) are shown in (c) and (d), respectively.



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3. Li, P.; Roberts, B. P.; Chakravorty, D. K.; Merz, K. M., Jr., Rational Design of Particle Mesh Ewald Compatible Lennard-Jones Parameters for +2 Metal Cations in Explicit Solvent. *J. Chem. Theory Comput.* **2013,** *9*, 2733-2748.