Supporting Information for

Physicochemical Insights into Microscopic Events Driven by GTP Hydrolysis Reaction in Ras-GAP system

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SI 1. System setup

For the Ras-GTP-GAP system, the atomic coordinates were energetically relaxed by the following molecular mechanics (MM). First, steric clashes in the system were removed by the three-step MM simulation. The initial MM simulation was for Mg^{2+} and GTP. The next one was for all hydrogen atoms in proteins and ligands. The last one was for all atoms in the system. Each MM simulation consists of 1500 steps of steepest descent method followed by 48500 steps of conjugate gradient method. Atoms except for the Mg^{2+} and GTP and those except for all hydrogen atoms in proteins and ligands were restrained by harmonic potential with force constant of 100 kcal/mol around the initial atomic coordinates of the simulation during the first and second MM simulations, respectively.

SI 2. MD simulations for structure relaxation

The temperature and density of the Ras-GTP-GAP system were relaxed through the following five MD simulations: NVT (0.001 to 1 K, 0.1 ps) → NVT (1 K, 0.1 ps) → NVT (1 to 300 K, 20 ps) → NVT (300 K, 20 ps) → NPT (300 K, 300 ps, 1 bar). In each of the five MD simulations, the atomic coordinates except for water and Na^{+} molecules were restrained by harmonic potential with force constant of 100 [kcal/mol/$Å^2$] around the initial atomic coordinates of the simulation.

The first two NVT MD simulations and the other MD simulations were performed

using 0.01 fs and 2 fs for the time step of integration, respectively. The first two NVT simulations and the following three ones were performed using Berendsen thermostat[1] with a 0.001-ps coupling constant and Langevin thermostat with 1-ps^{-1} collision coefficient, respectively. In the first, second and third NVT simulations, the reference temperature was linearly increased along the time course. The NPT simulation was performed using Langevin thermostat with 1-ps^{-1} of collision coefficient and, Berendsen barostat[1] with 2-ps coupling constant. A set of initial atomic velocities was randomly assigned from the Maxwellian distribution at 0.001 K at the beginning of the first NVT simulation.

Using a set of the atomic coordinates derived from the above relaxation simulation, an Ras-GTP-GAP complex was structurally relaxed in aqueous solution through the following 7-step MD simulations: NVT (0.001 to 1 K, 0.1 ps, 10 kcal/mol/Å) → NVT (1 to 300 K, 0.1 ps, 10 kcal/mol/Å) → NVT (300 K, 10 ps, 10 kcal/mol/Å) → NVT (300 K, 40 ps, 5 kcal/mol/Å) → NVT (300 K, 40 ps, 1 kcal/mol/Å) → NVT (300 K, 40 ps) → NPT (300 K, 1 bar, 20 ns). The last 20-ns NPT simulation was used for the following analyses.

The first two NVT MD simulations and the other MD simulations were performed using 0.01 fs and 2 fs for the time step of integration, respectively. In the first two NVT

simulations, the reference temperature was linearly increased along the time-course. In the first 4 steps, Ras, GAP, GTP and Mg^{2+} were restrained by harmonic potentials, whose force constants are shown in the above parentheses, around the initial atomic coordinates. In each NVT simulation, temperature was regulated using Langevin thermostat with 1-ps^{-1} collision coefficient. In the last 20-ns NPT simulation, temperature and pressure were regulated by Berendsen thermostat[1] with a 5-ps coupling constant, and Berendsen barostat[1] with a 0.1-ps coupling constant, respectively. The initial atomic velocities were randomly assigned from the Maxwellian distribution at 0.001 K. The initial atomic velocities were randomly assigned from a Maxwellian distribution at 0.001 [K]. MD trajectories were recorded every 10-ps interval for the following analyses. For each of Ras-GTP-GAP and Ras-GDP-GAP complex systems, this procedure was repeated 50 times with different set of atomic velocities.

Supporting Tables

Table S1. Atomic charges of $H_2PO_4^-$.

atom name†	atom type†	RESP charge [a.u.]
O1	oh	-0.835395
H1	ho	0.448002
P1	p5	1.627536
O3	o	-0.926375
O4	o	-0.926375
O2	oh	-0.835395
H2	ho	0.448002

† Atom name and type follow the style of General Amber Force Field[2].

Supporting Figures

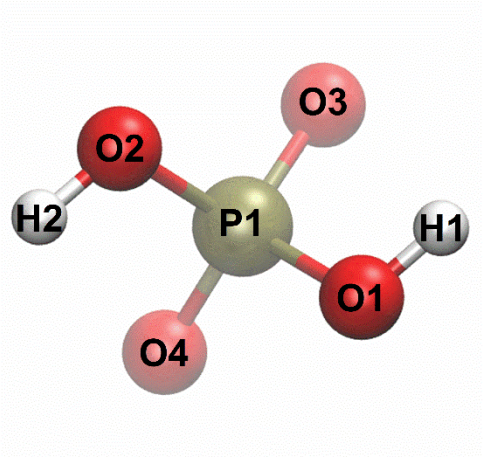

Figure S1. Atomic structure of $H_2PO_4^-$, calculated in vacuum with Hartree-Fock/6-31G* level of theory. Red, white and gold spheres are for hydrogen, oxygen and phosphorus atoms. Characters found inside these spheres denote atom name given as General Amber Force Field style.

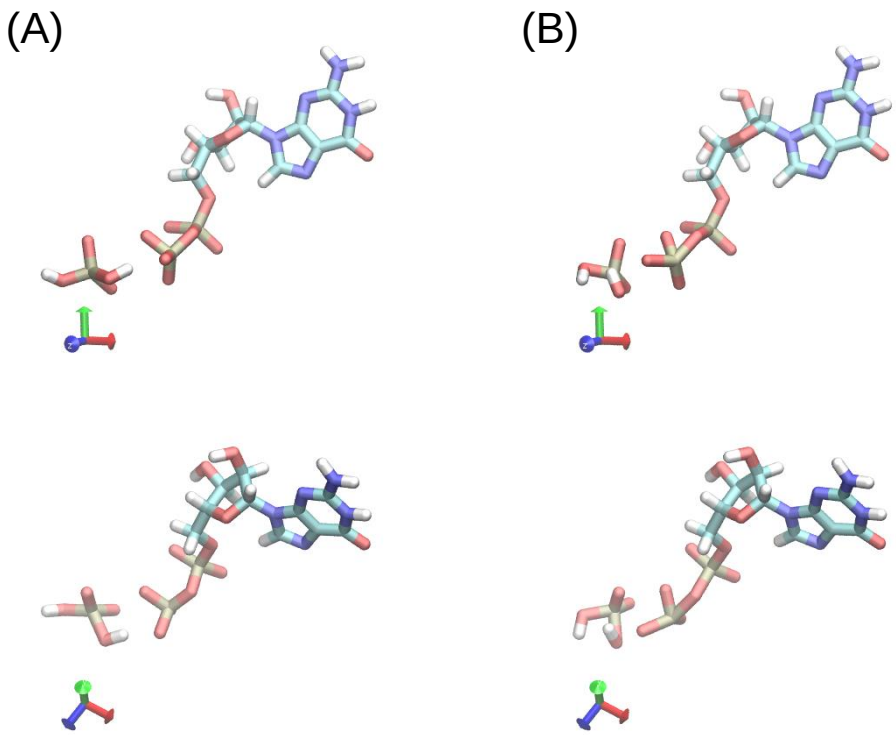

Figure S2. Modeling GDP with $H_2PO_4^-$ from GTP with reactant water molecule in Ras-GTP-GAP system by using (A) QM/MM simulation with PM3 of theory and (B) MM simulation.

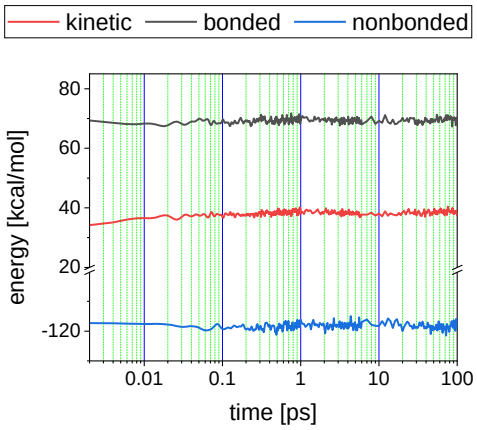

Figure S3. Time course analyses of mechanical energy for Ras-GTP-GAP system. Kinetic, bonded and nonbonded energies were shown with red, black and blue lines, respectively. Subtle increase of kinetic energy should result from thermal equilibration of $H_2PO_4^-$, whose atomic velocities were set to zero at the initial condition.

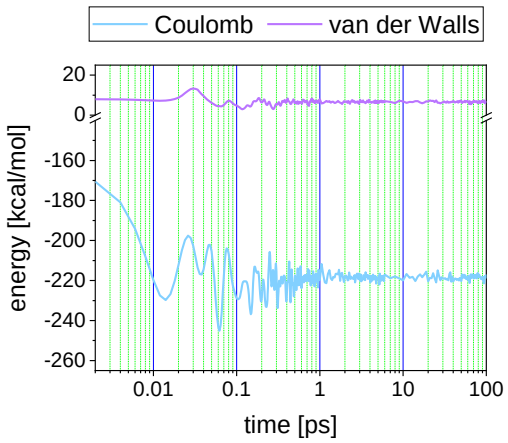

Figure S4. Time course analyses of nonbonded energy acting between GDP and $H_2PO_4^-$ in Ras-GDP-GAP system. Coulomb energy and van der Waals energy are individually shown with blue and purple lines, respectively.

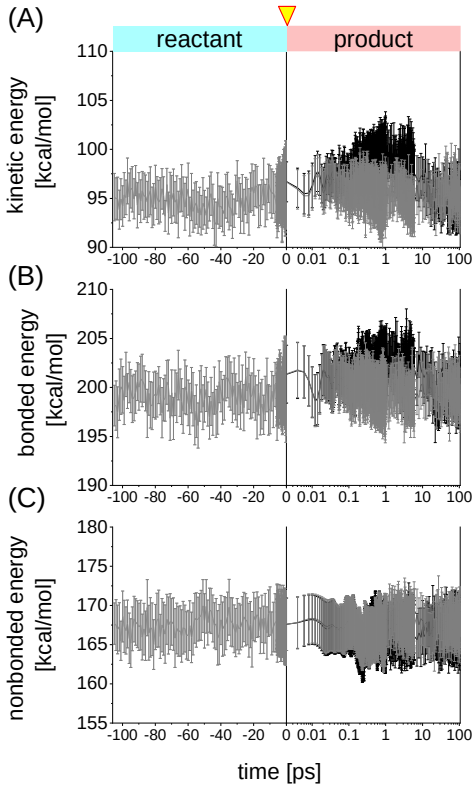

Figure S5. Energetic changes of Switch I region in Ras after GTP-GDP conversion. (A), (B) and (C) are for temporal changes of kinetic, bonded and nonbonded energies, respectively. Ras-GTP-GAP system and Ras-GDP-GAP system are represented by grey and black lines, respectively.

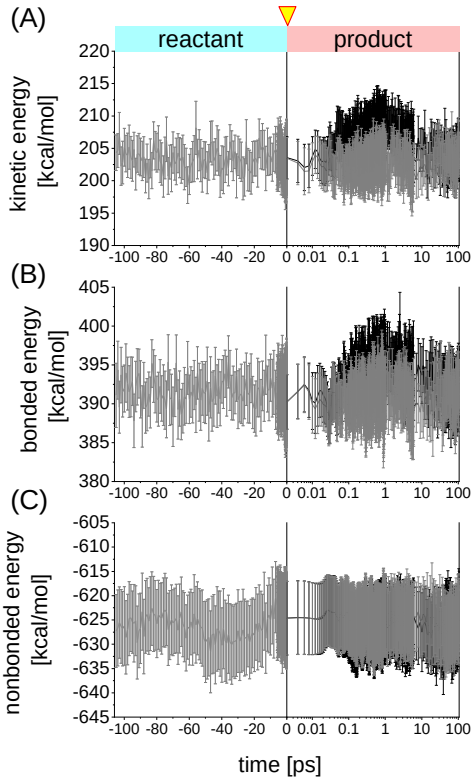

Figure S6. Energetic changes of Switch II region in Ras after GTP-GDP conversion. (A), (B) and (C) are for temporal changes of kinetic, bonded and nonbonded energies, respectively. Ras-GTP-GAP system and Ras-GDP-GAP system are represented by grey and black lines, respectively.

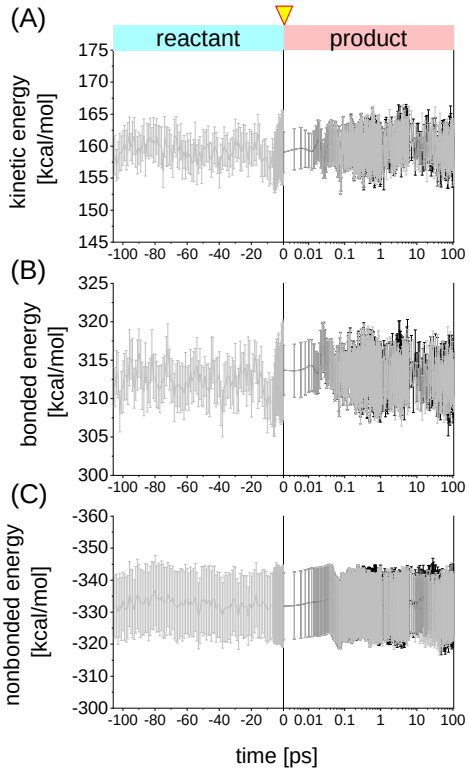

Figure S7. Energetic changes of α-helix 3 in Ras after GTP-GDP conversion. (A), (B) and (C) are for temporal changes of kinetic, bonded and nonbonded energies, respectively. Ras-GTP-GAP system and Ras-GDP-GAP system are represented by grey and black lines, respectively.

References

1. Berendsen, H. J. C.; Postma, J. P. M.; Vangunsteren, W. F.; Dinola, A.; Haak, J. R., Molecular-Dynamics with Coupling to An External Bath. *J. Chem. Phys.* **1984**, *81*, 3684-3690.

2. Wang, J. M.; Wolf, R. M.; Caldwell, J. W.; Kollman, P. A.; Case, D. A., Development And Testing of A General Amber Force Field. *J. Comput. Chem.* **2004**, *25* , 1157-1174.