

## Supporting Information

for

### Diversity of mechanisms in Ras-GAP catalysis of guanosine triphosphate hydrolysis revealed by molecular modeling

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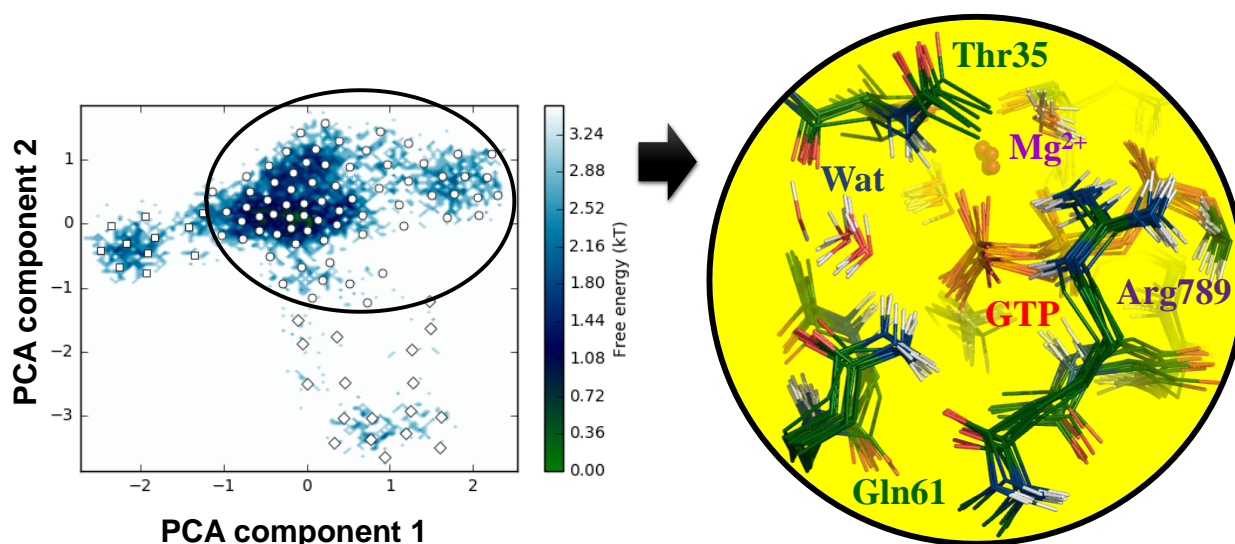
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## S1. Results of the principal component analysis

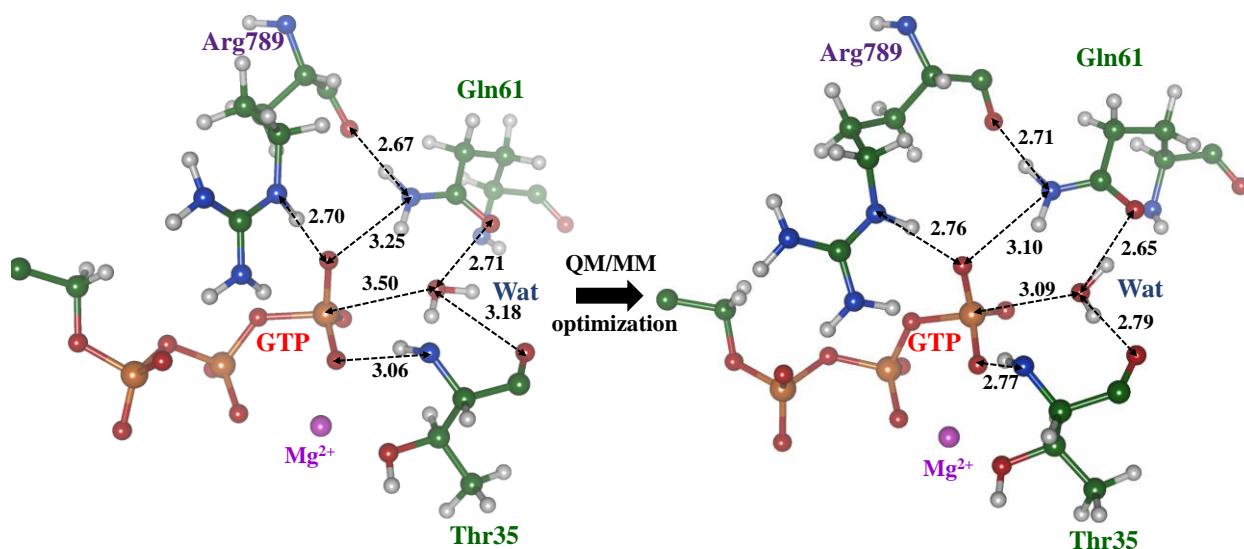
Principal component analysis (PCA) of atomic fluctuations has been carried out taking QM/MM MD trajectory data. Cartesian coordinates of all heavy atoms of the QM subsystem were selected to extract the most relevant states with the largest variance two-dimensional PCA. The left panel in Fig. S1 illustrates projection of MD data on the first and second principal components. The right panel in Fig. S1 shows variety of conformations in the vicinity of the Ras-GTP-H<sub>2</sub>O-GAP complex with the lowest free energy.



**Figure S1.** Left: two-dimensional PCA plot. Right: visualization of highly populated conformations showing molecular groups in the QM part.

## S2. Illustrative example of QM/MM optimization from a QM/MM MD structure

Optimization of geometry parameters of ES complexes with the QM(DFT(PBE0)/cc-pVDZ)/MM approach was performed by starting from every representative conformation generated in QM(BLYP/GWP/DZVP)/MM MD calculations. Fig. S2 demonstrates that even a QM/MM MD conformation with the “most unfavorable” orientation of Wat converges to a qualitatively correct structure of ES.

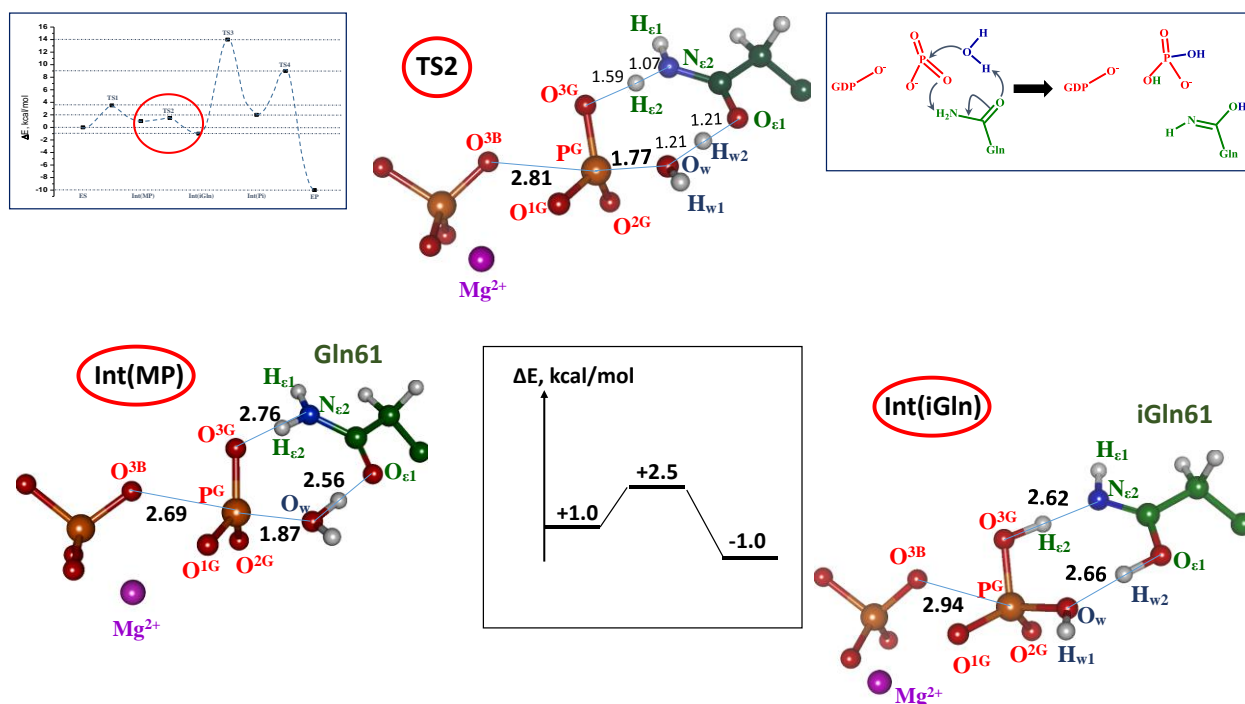


**Figure S2.** Convergence of an “unfavorable” QM/MM MD conformation (left) to the QM/MM minimized structure of ES (right).

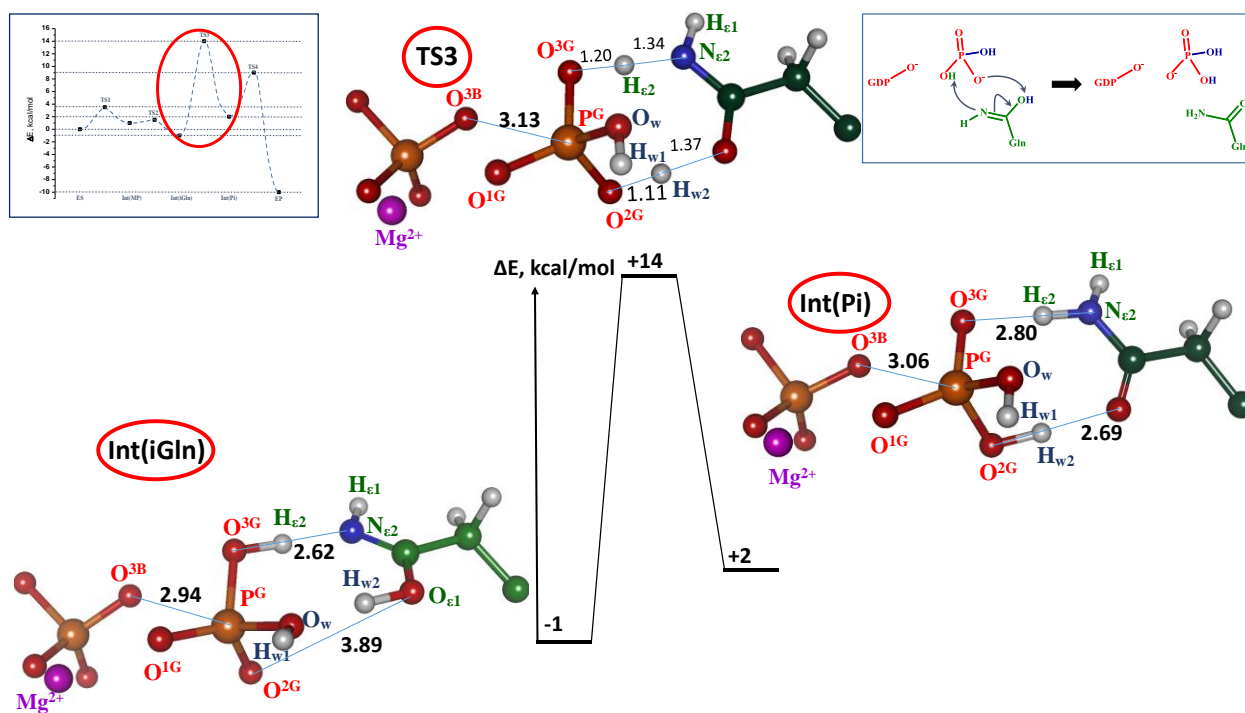
### S3. Energy profile computed in the QM(Gln)/MM variant

The first step along this route  $ES \rightarrow TS1 \rightarrow \text{Int}(\text{MP})$  is illustrated below (Section S4) where the results of QM(Gln)/MM and QM/MM(Gln) variants are compared. The next step  $\text{Int}(\text{MP}) \rightarrow TS2 \rightarrow \text{Int}(\text{iGln})$  corresponds to a shallow energy relief (Fig. S3) leading from  $\text{Int}(\text{MP})$  to the reaction intermediate with the imide tautomer of Gln61,  $\text{Int}(\text{iGln})$ . Synchronized transfer of protons,  $H_{w2}$  from the water molecule to  $O_{\epsilon1}(\text{Gln61})$  and  $H_{\epsilon2}$  from Gln61 to  $O^{3G}$ , facilitates attack of  $O_w H_{w1}^-$  on the phosphorus atom  $P^G$ . Two-dimensional scan along the gradually increased  $O_w - H_{w2}$  and  $N_{\epsilon2} - H_{\epsilon2}$  distances was used to locate vicinity of the transition state TS2.

Restoration of the amide tautomer of Gln61 coupled with formation of the final form of Pi. As shown in Fig. S4, this step describes proton transfer between glutamine side chain and inorganic phosphate created at the preceding step. The  $O^{2G} - H_{w2}$  distance was used as a reaction coordinate at this step. As a result, Pi contains the atoms of a former nucleophilic water molecule, whereas all atoms of Gln61 return to their initial positions.

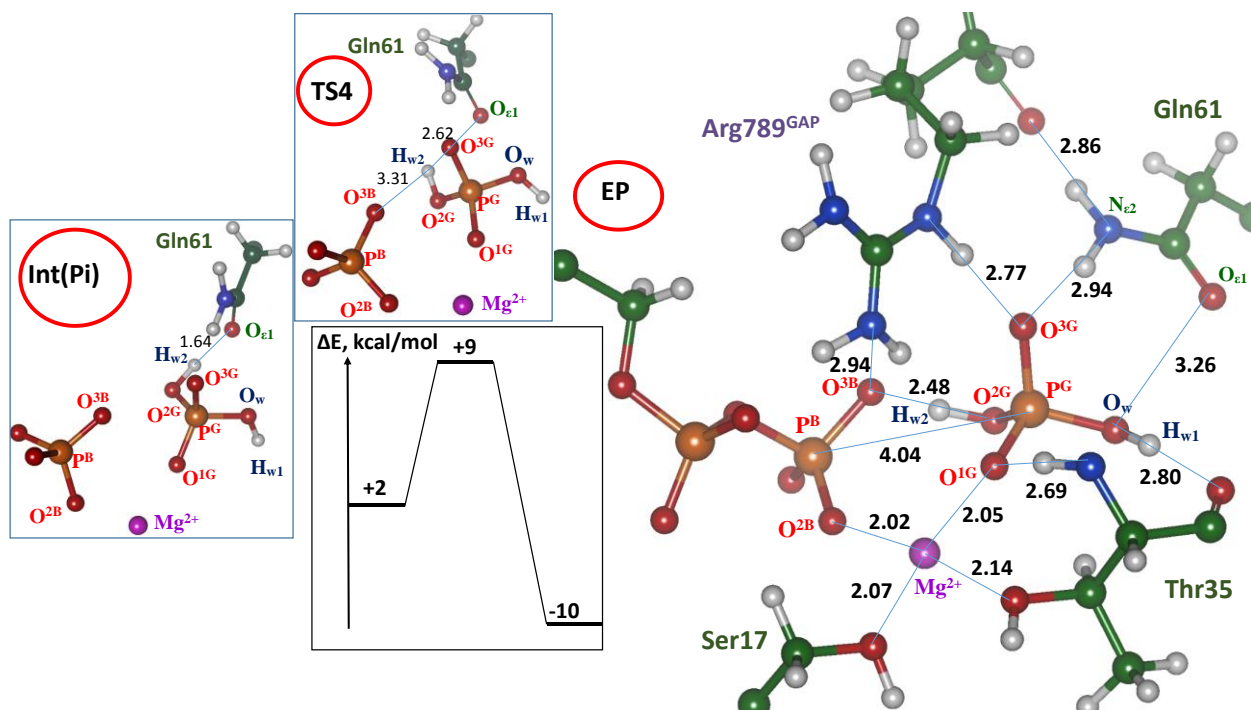


**Figure S3.** Illustration of the elementary step  $\text{Int(MP)} \rightarrow \text{TS2} \rightarrow \text{Int(iGln)}$  along the QM(Gln)/MM route. Distances are given in Å; values in bold refer to distances between the corresponding heavy atoms; a non-bold typeface is used to specify distances between heavy atoms and hydrogens. Upper left inset shows the entire pathway indicating the place of this particular step. Upper right inset illustrates the corresponding chemical transformations at this step.



**Figure S4.** Illustration of the elementary step  $\text{Int}(i\text{Gln}) \rightarrow \text{TS3} \rightarrow \text{Int}(\text{Pi})$  along the QM(Gln)/MM route. See also legend to Fig. S3.

The last step  $\text{Int}(\text{Pi}) \rightarrow \text{TS4} \rightarrow \text{EP}$  describes formation of the lowest energy structure of the Ras-GDP-Pi-GAP complex. To arrive to this EP structure, the  $\text{O}^{2\text{G}}\text{-H}_{\text{w}2}$  group is re-oriented towards the  $\text{O}^{3\text{B}}$  atom of GDP. The  $\text{O}^{3\text{B}}\text{-H}_{\text{w}2}$  distance was used as a reaction coordinate at this step. This final elementary step on the reaction route is illustrated in Fig. S5.

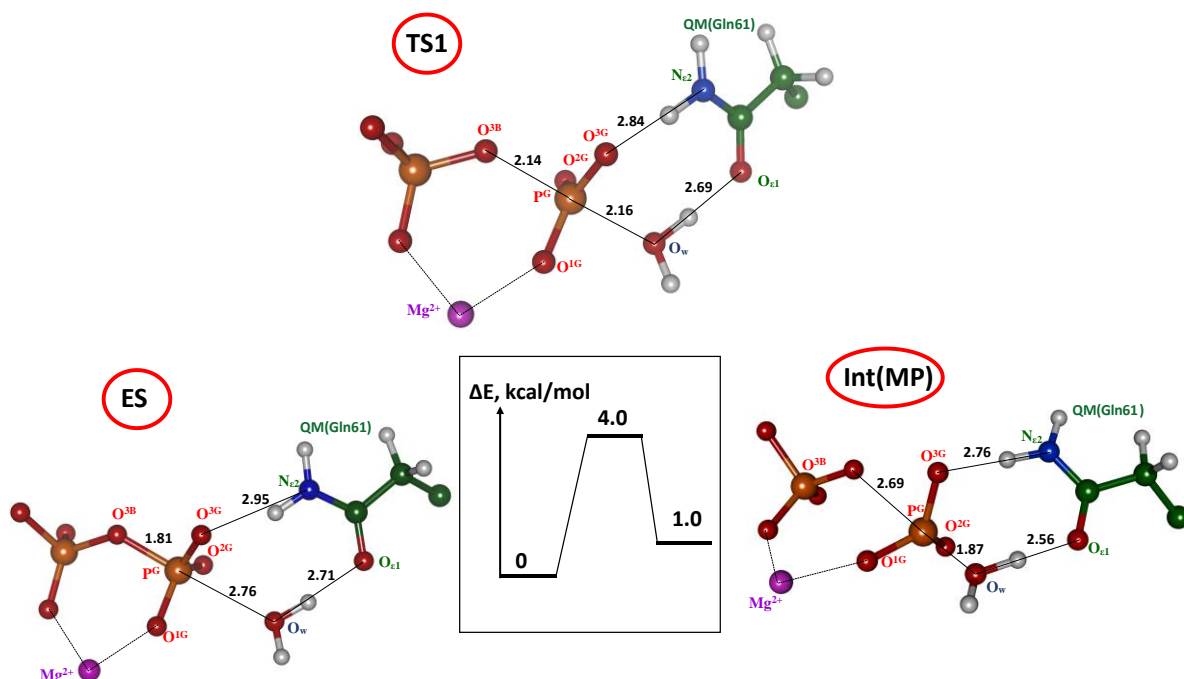


**Figure S5.** Illustration of the elementary step  $\text{Int}(\text{Pi}) \rightarrow \text{TS4} \rightarrow \text{EP}$ . Distances are given in Å; values in bold refer to distances between the corresponding heavy atoms, a non-bold typeface is used to specify distances between heavy atoms and hydrogens.

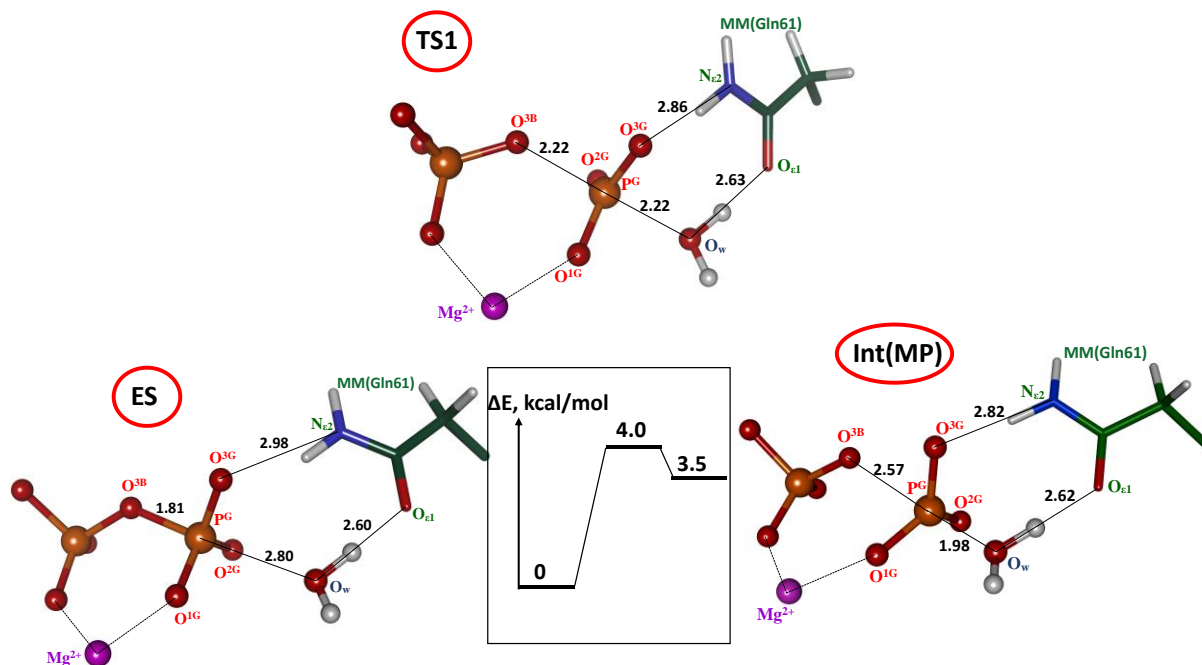
#### **S4. Reaction pathways $\text{ES} \rightarrow \text{TS1} \rightarrow \text{Int}(\text{MP})$ modeled in the QM(Gln)/MM and QM/MM(Gln) approaches**

A gradual decrease of the  $\text{P}^{\text{G}}\text{-O}_{\text{w}}$  distance from the initial value in the ES complex leads to the first transition state TS1. In this structure, the  $\text{P}^{\text{G}}\text{O}_3$  group adopts a planar configuration with the almost equal distances  $\text{O}^{3\text{B}}\text{-P}^{\text{G}}$  and  $\text{P}^{\text{G}}\text{-O}_{\text{w}}$ . Overcoming the saddle point, the system reaches the local minimum energy point, corresponding to a reaction intermediate, which is denoted

Int(MP). It should be noted that the reaction step  $ES \rightarrow TS1 \rightarrow Int(MP)$  is described similarly in the QM(Gln)/MM and QM/MM(Gln) variants (see Figs. S6, S7).



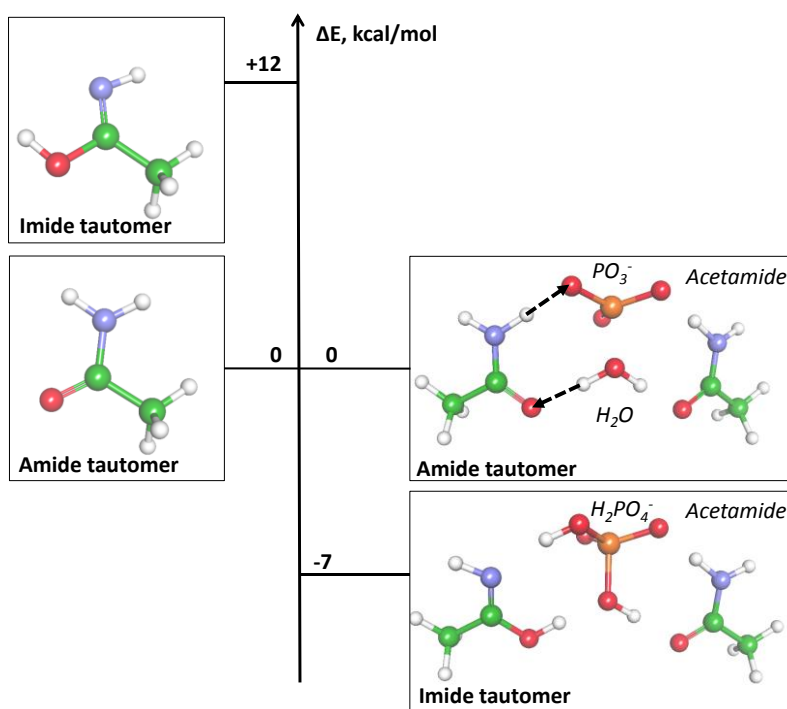
**Figure S6.** The elementary step  $ES \rightarrow TS1 \rightarrow Int(MP)$  modeled in the QM(Gln)/MM approach.



**Figure S7.** The elementary step  $ES \rightarrow TS1 \rightarrow Int(MP)$  modeled in the QM/MM(Gln) approach. The side chain of Gln61 assigned to the MM part is shown in sticks.

### **S5. Gln amide-imide tautomerization in a small molecular model**

Temporary occurrence of the imide tautomer of the Gln side chain in reaction intermediates in the QM(Gln)/MM calculations is due to a specific molecular environment. Taken away from the protein cleft, the imide tautomer is higher in energy than the amide form. To illustrate this issue we performed quantum chemical calculations at the DFT(PBE0)/cc-pVDZ level (the same as used in QM in the present QM/MM modeling) for small molecular models (Fig. S8). First, we optimized geometry configurations of the acetamide molecule (the upper left panel in Fig. S8), a precursor of the amide Gln tautomer, and of the acetimidic acid molecule (the lower left panel in Fig. S8), a precursor of the imide Gln tautomer. The energy of the latter is 12 kcal/mol higher than the energy of the acetamide molecule. Next, we designed a system mimicking some features of the Int(MP) configuration. This system, shown in the upper right panel in Fig. S8, includes acetamide, metaphosphate, water molecule and an additional acetamide molecule, which is necessary to construct a stable hydrogen-bonded molecular cluster. After proton transfer, as illustrated by dashed arrows, this cluster occurs in energy minimum point, in which the acetimidic acid molecule is hydrogen bonded to other parts of the cluster (the lower right panel in Fig. S8). The energy of this system is 7 kcal/mol lower than that of the initial cluster. Structures of the groups shown in the right panels in Fig. S8 resembles those shown in the panels in Fig. S3.



**Figure S8.** Quantum chemical calculations (DFT(PBE0)/cc-pVDZ) of amide-imide tautomerization in a small molecular model.

### S6. Coordinates of the computed structures

Coordinates of all structures in pdb format are available as separate files. The names include identification of a model system (QM(Gln61)/MM, QM/MM(Gln61), QM(NGln61)/MM, QM(Glu61)/MM) and identification of a structure (ES, EP, TS#, INT\_Pi, etc.). The first line of every file reports the corresponding QM/MM energy for all structures and the imaginary frequency for transition states.

Correspondence between names of structures and identification numbers of supplementary files is as follows.

Structure	Supplementary file ID
QM(Gln61)/MM_ES	c9ob000463g3.pdb
QM(Gln61)/MM_TS1	c9ob000463g7.pdb
QM(Gln61)/MM_INT(MP)	c9ob000463g5.pdb
QM(Gln61)/MM_TS2	c9ob000463g8.pdb
QM(Gln61)/MM_INT(iGln)	c9ob000463g4.pdb
QM(Gln61)/MM_TS3	c9ob000463g9.pdb
QM(Gln61)/MM_INT(Pi)	c9ob000463g6.pdb
QM(Gln61)/MM_TS4	c9ob000463g10.pdb
QM(Gln61)/MM_EP	c9ob000463g2.pdb



QM/MM(Gln61)_ES	c9ob000463g16.pdb
QM/MM(Gln61)_TS1	c9ob000463g19.pdb
QM/MM(Gln61)_INT(MP)	c9ob000463g17.pdb
QM/MM(Gln61)_TS2	c9ob000463g20.pdb
QM/MM(Gln61)_INT(Pi)	c9ob000463g18.pdb
QM(NGln61)/MM_ES	c9ob000463g22.pdb
QM(NGln61)/MM_TS	c9ob000463g23.pdb
QM(NGln61)/MM_EP	c9ob000463g21.pdb
QM(Glu61)/MM_ES	c9ob000463g12.pdb
QM(Glu61)/MM_TS1	c9ob000463g14.pdb
QM(Glu61)/MM_INT	c9ob000463g13.pdb
QM(Glu61)/MM_TS2	c9ob000463g15.pdb
QM(Glu61)/MM_EP	c9ob000463g11.pdb