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Molecular-Level Insights into the Catalytic Mechanism of Human Guanylate-Binding Protein 1 (hGBP1) from Accelerated QM/MM Simulations

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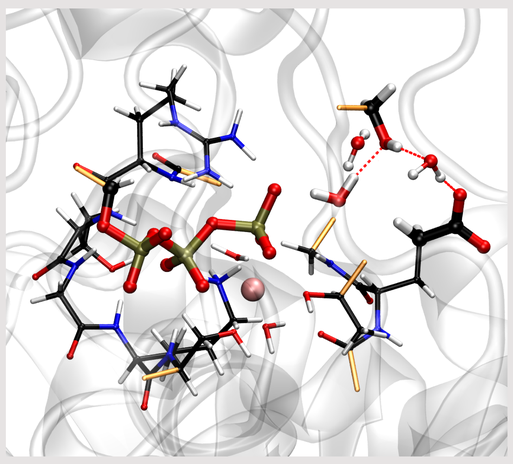
1. Introduction

- Hydrolysis of GTP (guanosine triphosphate) and other nucleotides through specific enzymes is important in such processes as signal transduction and protein biosynthesis in all living cells.[1]
- Of particular theoretical interest is this GTP-cleavage mechanism in the GTPase hGBP1 (human guanylate binding protein 1) as it exhibits biochemical properties not found in other families of GTP-binding proteins, such as nucleotide-dependant dimerisation and fast cooperative GTPase activity.[2]
- hGBP1 also has an additional property by which its active center hydrolyses GTP first to GDP and finally to GMP in two consecutive cleavage reactions,[2,3] thus making it a suitable system on which to study such catalytical processes.
- The aim of our project is to gain an insight into the unknown mechanisms of both the GTP and GDP hydrolysis reactions in hGBP1 making use of QM/MM simulations.[4]

2. Protein System - hGBP1 Dimer in Water

QM/MM simulations using CP2K code:

- MM system
  - MM system consists of the hGBP1 dimer, two GTP(GDP) molecules and circa 67 000 waters.
  - also includes 148 Na^+ and 136 Cl^- ions.
- QM system
  - QM system of triphosphate backbone of GTP(GDP) molecule, 5 water molecules, magnesium ion cofactor plus several amino acid side-groups.



QM fragment of the system utilized in our study

  - Computational details :
  - OPLS force field employed.
  - Timestep of 0.5 fs; NVT ensemble.
  - $(128\ \text{Å})^3$ MM cubic cell;
  - Temperature 300 K; Nosé thermostat.[5]
- Results of extensive parameter study led to the following optimized simulation parameters:
  - Quickstep method: GPW.[6]
  - TZV2P-(3s3p2d/s2p) basis setfor valence electrons; analytical dual-space (GTH) pseudopotentialsfor core electrons; BLYP functional.
  - 360 Ry density cutoff; outer SCF (SCF convergence $= 10^{-6}$).
  - Minimizer DIIS (stepsize $= 0.1$).
  - MT Poisson solver;$(24.0\ \text{Å})^3$ QM cell.

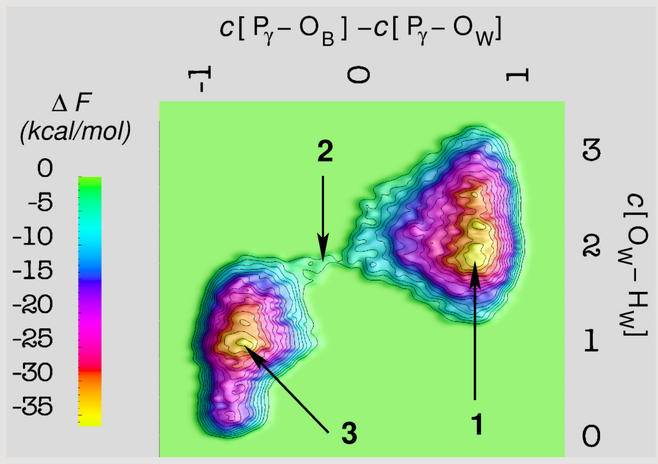
3. Reference System: GTP in Water

- Reference system used to obtain reaction barriers:
  - System of a methylated triphosphate molecule plus magnesium ion cofactor and 111 water molecules.
  - Full QM DFT simulation using CP2K code.

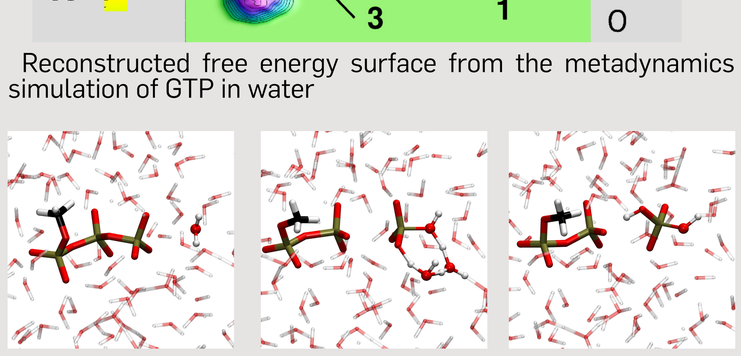
4. Metadynamics Study : GTP in Water

- Metadynamics simulation was performed utilizing 2 CVs :

  1: coordination number of the γ-phosphate (P_γ) to the bridging oxygen (O_B), minus coordination number of P_γ to the catalytic water oxygen (O_W), 2: coordination number of O_W to all water protons (H_W).



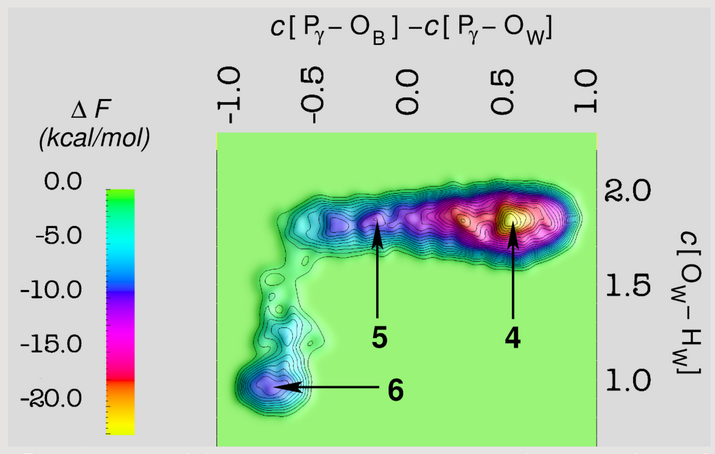
Reconstructed free energy surface from the metadynamics simulation of GTP in water



- The mechanism and the free energy barrier obtained here are in good agreement with the results of glaves et al.[7]

5. Metadynamics Study : GTP in hGBP1

- The similar set of CVs, as used in the above study, was utilized here.

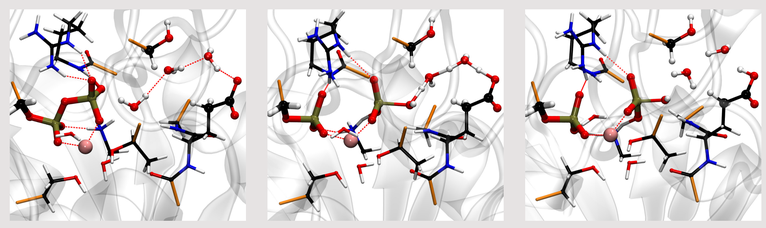


Reconstructed free energy surface from the metadynamics simulation of GTP in enzyme

- The net free energy barrier is calculated to be 21 kcal/mol, which is substantially less than the one obtained with the reference system (33 kcal/mol).
- A distinct intermediate was formed on the FES and the hydrolysis reaction proceeded via an uncouple S_N1 mechanism.

6. Metadynamics Study : GDP in hGBP1

- The free energy barrier of the GDP hydrolysis is obtained to be 25 kcal/mol.
- Glutamate99 was found to act as a base which activates catalytic water via a proton-relay process.



Snapshots from the metadynamics study of GDP hydrolysis

- No distinct intermediate on the FES was obtained, as seen in the case of GTP hydrolysis.

7. Conclusion

✓ The GTP hydrolysis in the protein proceeded with formation of an intermediate which was followed by a proton transfer from the catalytic water to one of the oxygen of the resulted metaphosphate ion (PO_3^-).

✓ Serine73, with the help of a water molecule and glutamate99, catalyzed the proton transfer process.

✓ The subsequent GDP $\rightarrow$ GMP transformation involves a proton-relay mechanism where glutamate99, with the help of 2 water molecules, activates the catalytic water.

✓ The catalytic roles of serine73 and glutamate99 during GTP/GDP hydrolysis are in accordance with the experimental study[2] where mutation of these residues resulted in huge reduction of the rate of GTP and GDP hydrolysis.

References

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