QM/MM Study of the Reaction Mechanisms of Biocatalyst

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Simulation of Biomolecules

QM (Quantum Mechanics)
chemical reaction, photochemistry, …

MM (Molecular Mechanics)
structure and dynamics of proteins

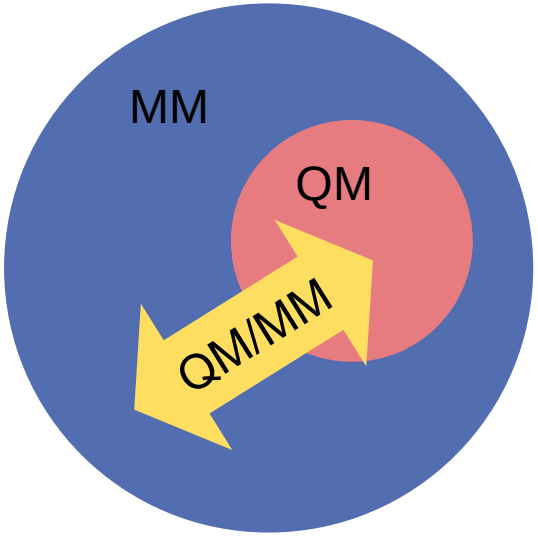
CS (Coarse-Grained)
Protein complexes

Multiscale Modeling

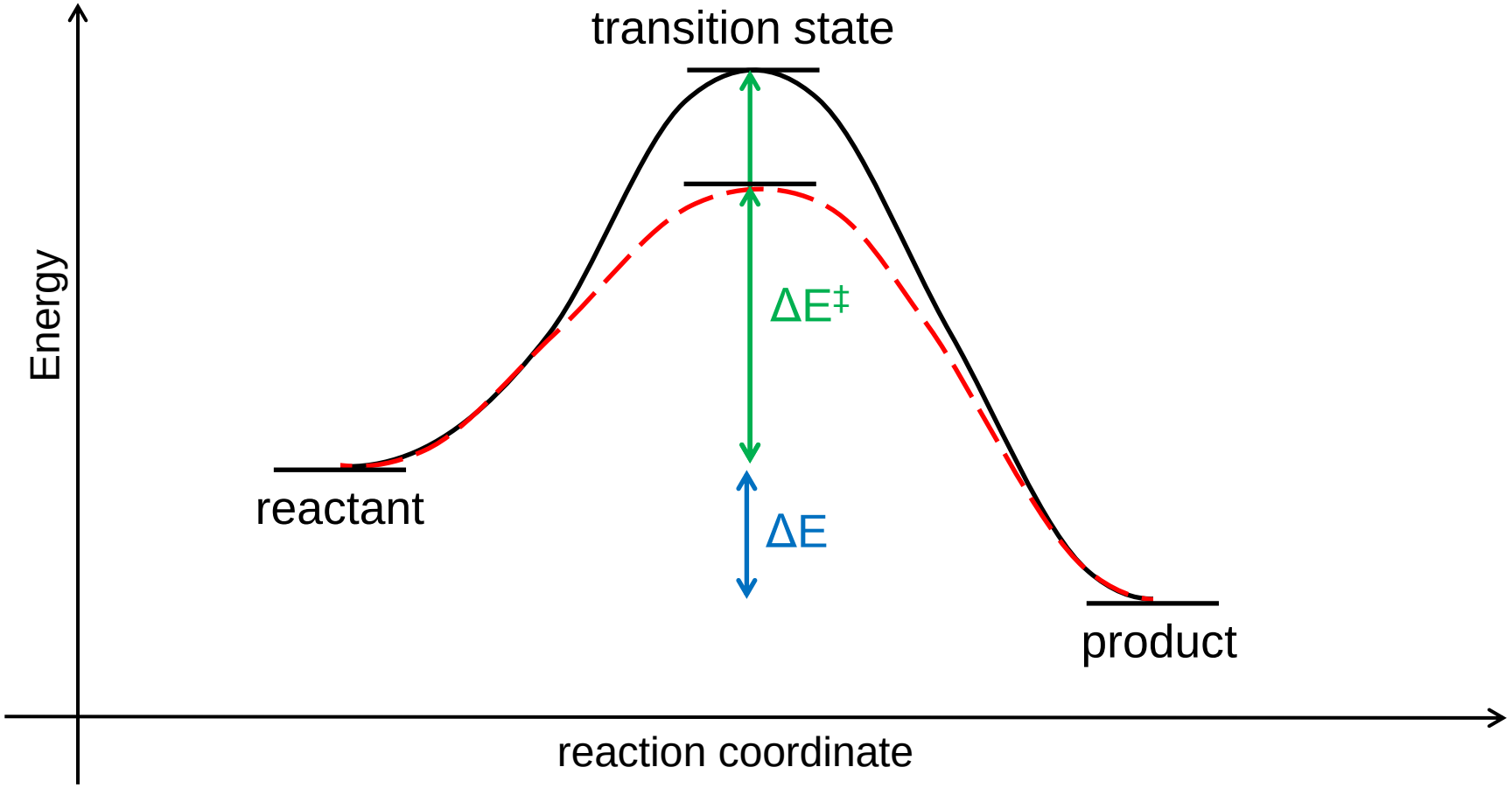
QM/MM method

- Quantum chemical model for region involved in chemical reaction
- Classical model (MM) for other parts
- Interaction between QM and MM regions

$$\hat{H}_{eff} = \hat{H}_{QM} + \hat{H}_{MM} + \hat{H}_{QM/MM}$$



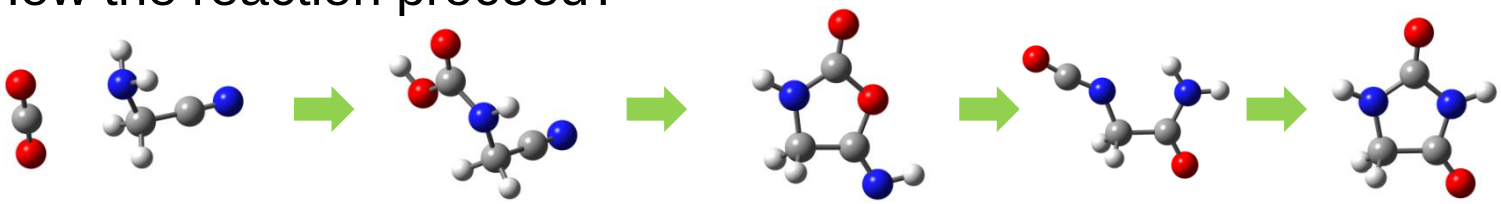
Chemical Reactions



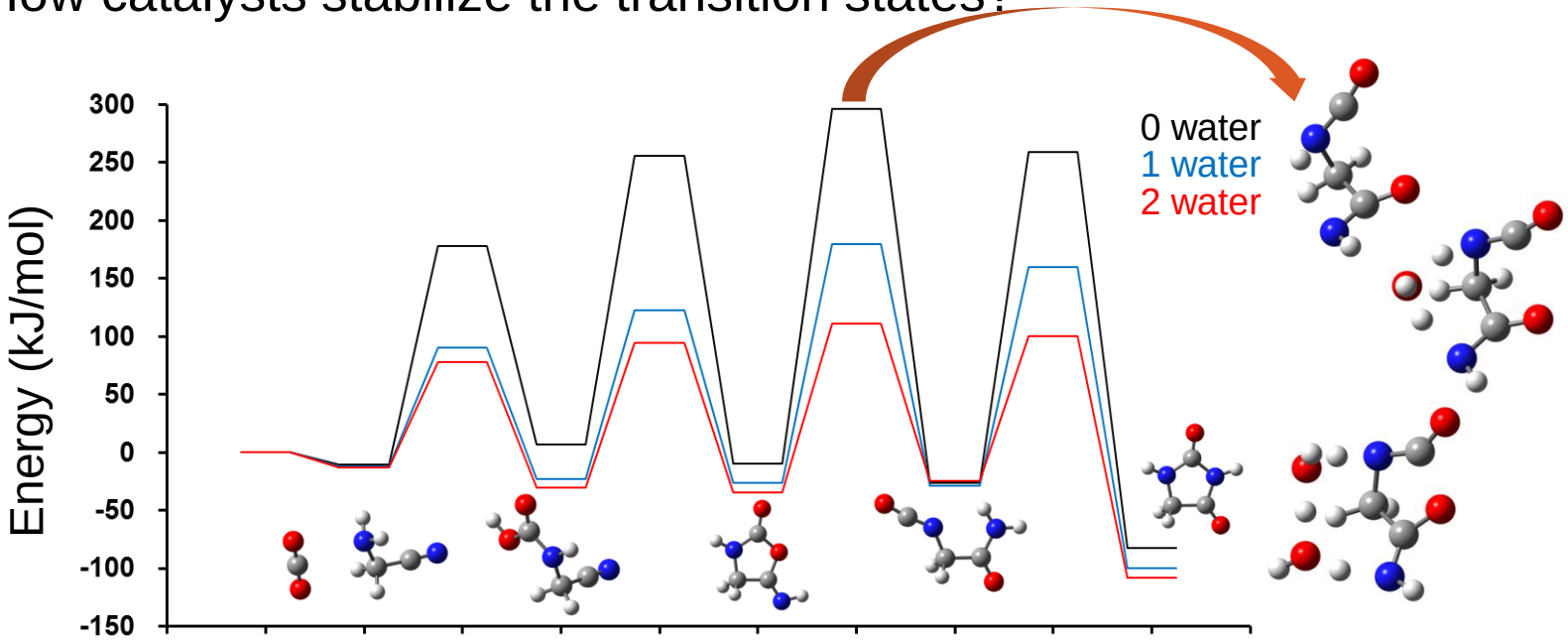
Catalysts stabilize the transition state or create new reaction path
→ accelerate the reaction

Analysis of Reaction using QM Method

- How the reaction proceed?



- Which reaction step require the highest energy?
- How catalysts stabilize the transition states?



M. Kayanuma *et al.*, *Chem. Phys. Lett.*, 687 (2017) 178

Biocatalyst

- Biochemistry
- Catalytic chemistry

Application

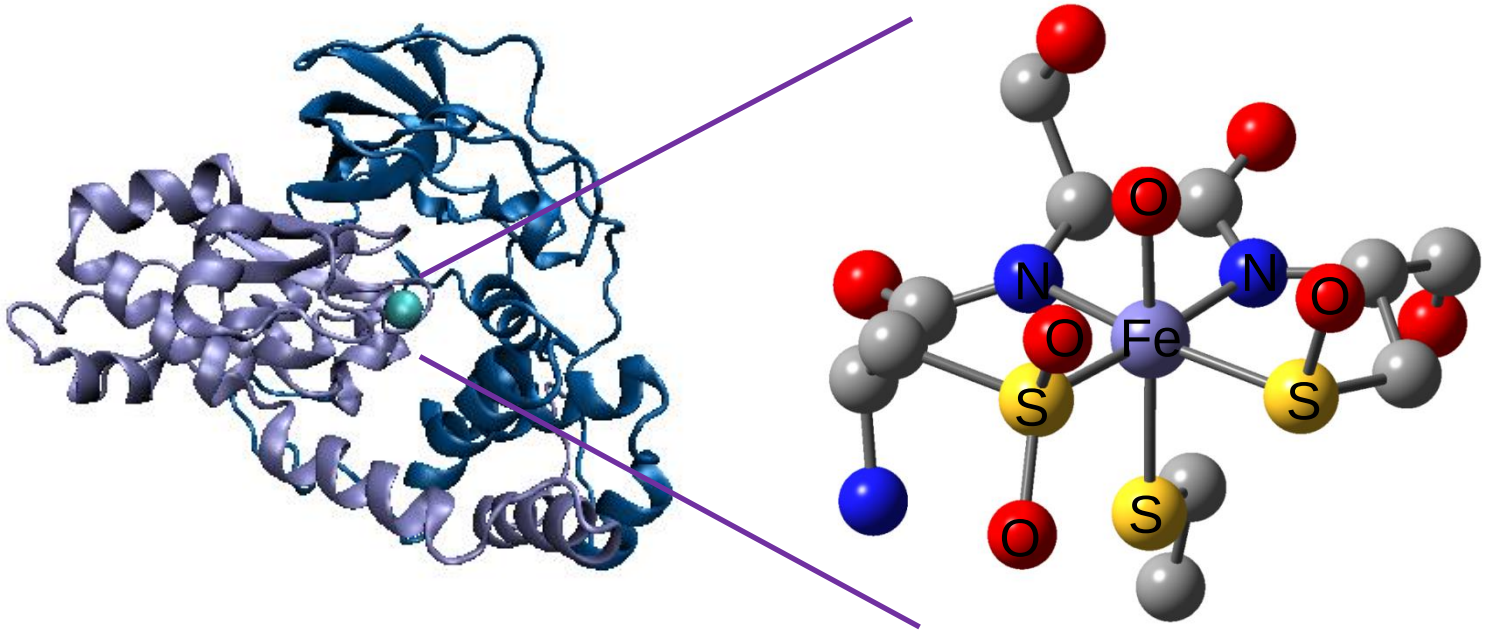
- Industry
- Bioremediation

Merits

- Turnover number
- Reaction rate
- Structural and stereochemical selectivity
- Mild condition (temperature, pH, etc.)

Nitrile Hydratase (NHase)

- catalyze hydration of nitrile to the corresponding amide
  R-CN + H_2O → R-$CONH_2$
- chemical industry (e.g. production of acrylamide)
- metal ion (Fe(III) or Co(III)) at the active site
- unique coordination structure (one -SO and one -SO_2)



Reaction Mechanism of NHase

- Time-resolved crystal structures of a mutant (βR56K) suggested the involvement of a cyclic intermediate

  Y. Yamanaka *et al., Angew. Chem. Int. Ed.* 54 (2015) 10763

- However, the subsequent mechanism was not elucidated

  Disulfide intermediate formation and proton transfer from βR56

  K. H. Hopmann, *Inorg. Chem.* 53 (2014) 2760

  Water attack of the cyclic intermediate and amide formation

  Y. Yamanaka et al*., Angew. Chem. Int. Ed.* 54 (2015) 10763

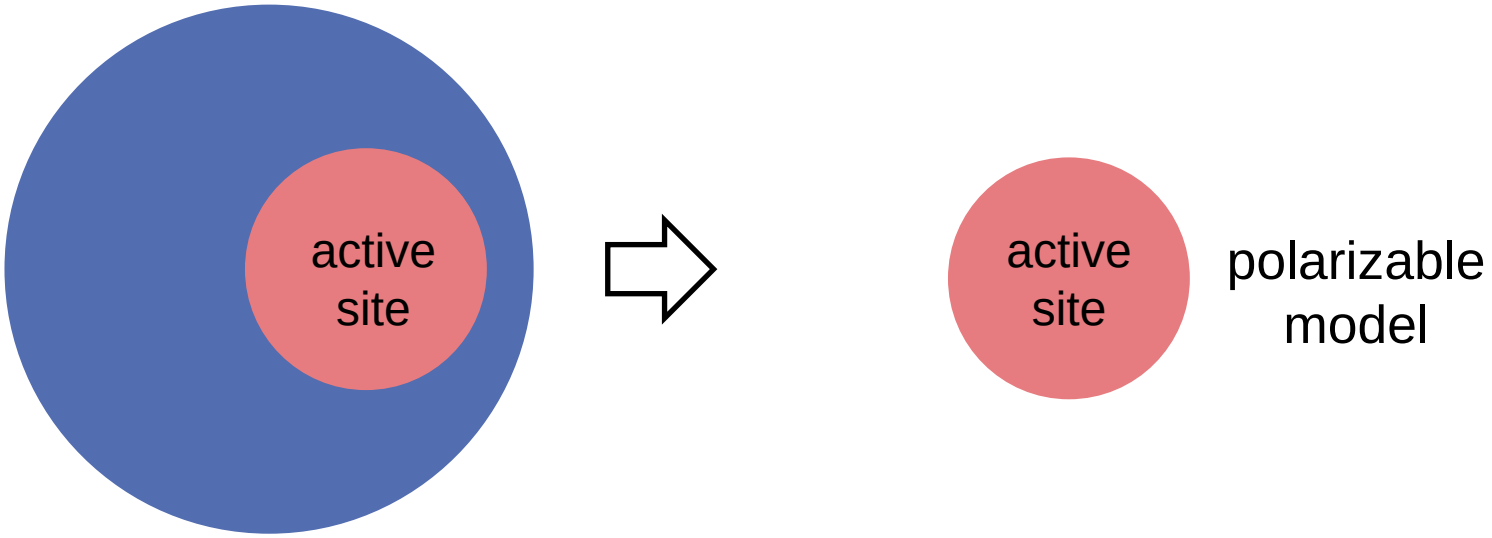
  Water attack of the cyclic intermediate and imidic acid formation

  K. M. Light et al*., Chem. Sci.* 6 (2015) 6280

Previous Theoretical Studies

Use cluster model of the active site
(protein environment was treated as polarizable model)

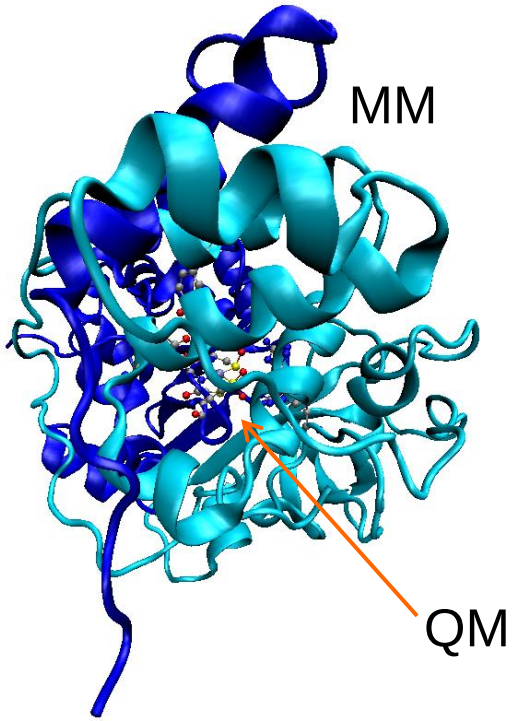
K. H. Hopmann, *Inorg. Chem.* 53 (2014) 2760

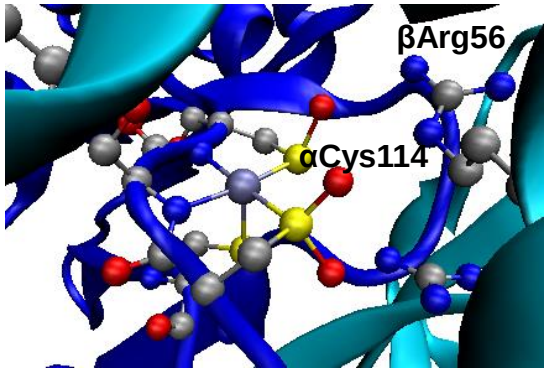


Do some specific amino acid residues affect the reaction?

Aime

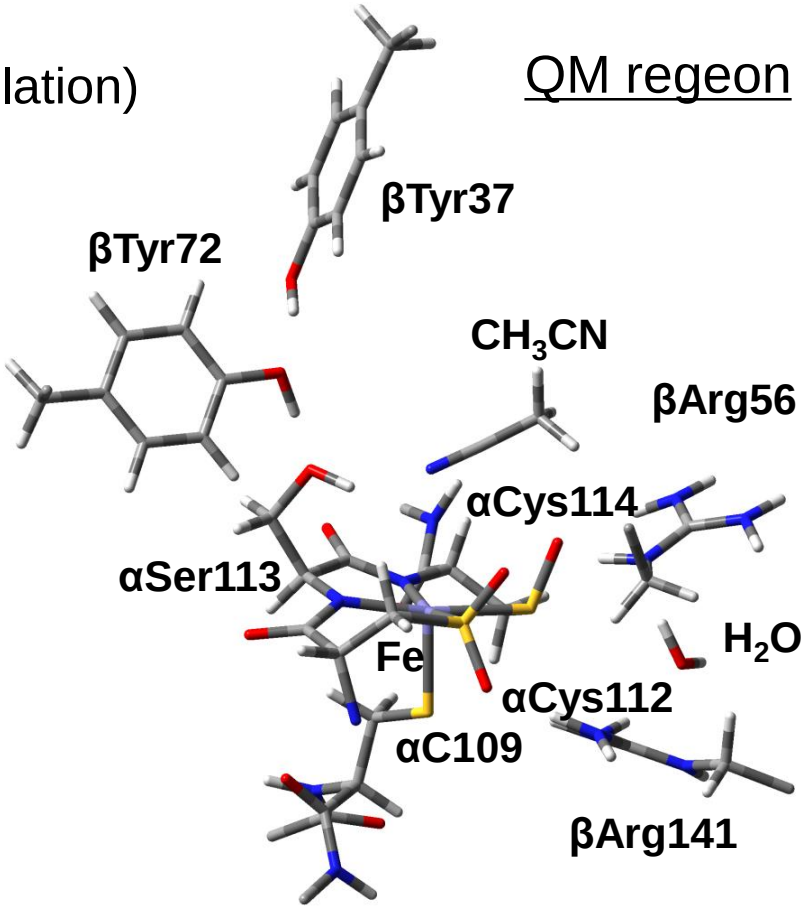
- Reveal the reaction mechanism of nitrile hydratase (NHase) subsequent to the cyclic intermediate using hybrid quantum mechanics/molecular mechanics (QM/MM) method
- Analyze the effects of protein environment on the active site





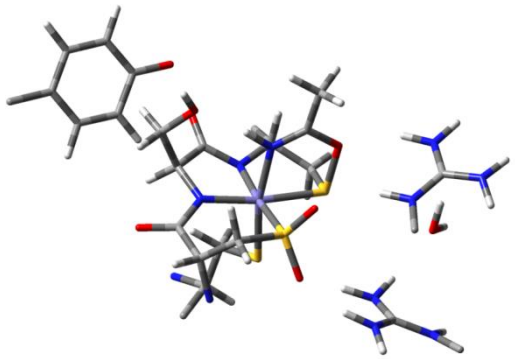
Method

- QM: B3LYP
  LANL2DZ and 6-31G(d)
  (6-311++G(d,p) for energy calculation)
- MM: AMBER99
- Initial structure: PDBID 3A8O
- Substrate: CH_3CN
- Water: $10 \times 10 \times 10$ nm^3
- Ions: $Na^+ \times 24$
- Cutoff: 9 Å
  (no cutoff for energy calculation)
- Program: NWChem 6.3
- Computer: COMA @ Univ. Tsukuba

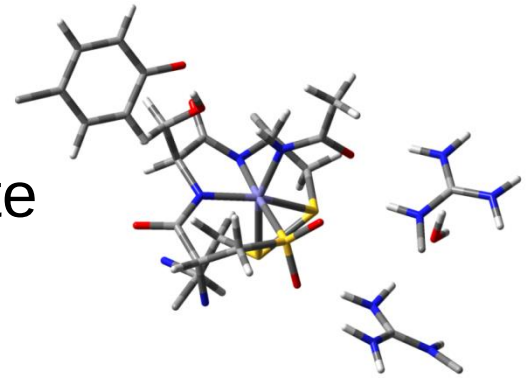


Energies are relative values to the 5-coordinated structure

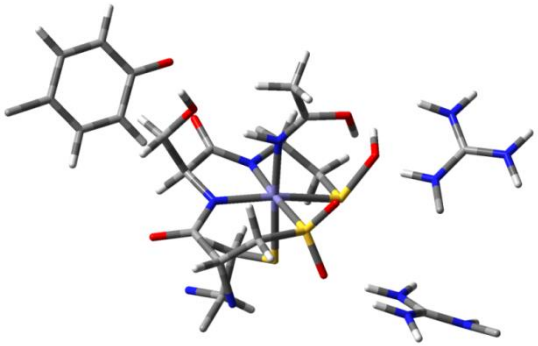
Results: Reaction Mechanism



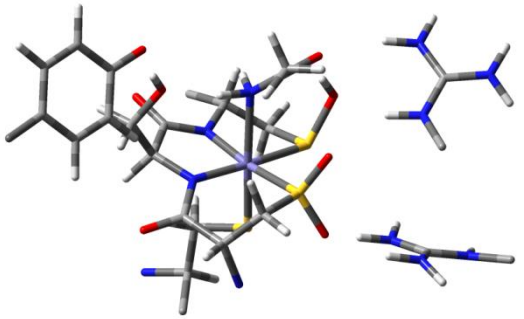
Cyclic intermediate



Disulfide intermediate



Imidic acid intermediate

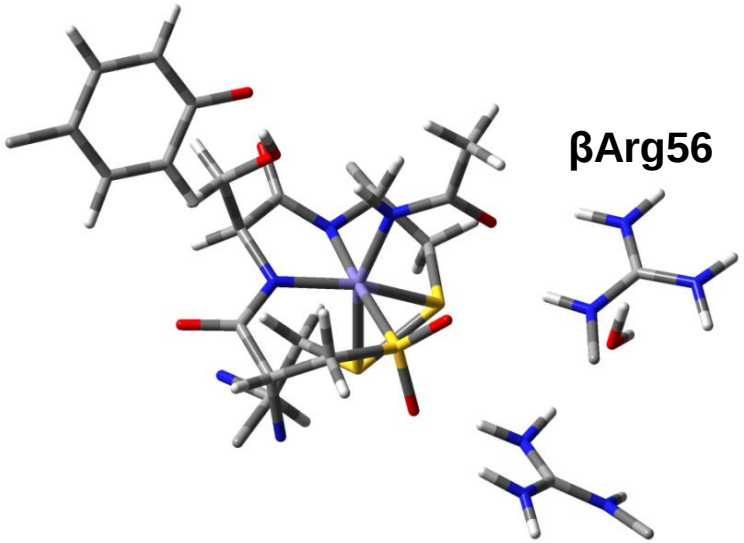


Amide product

Conflict with Experiment?

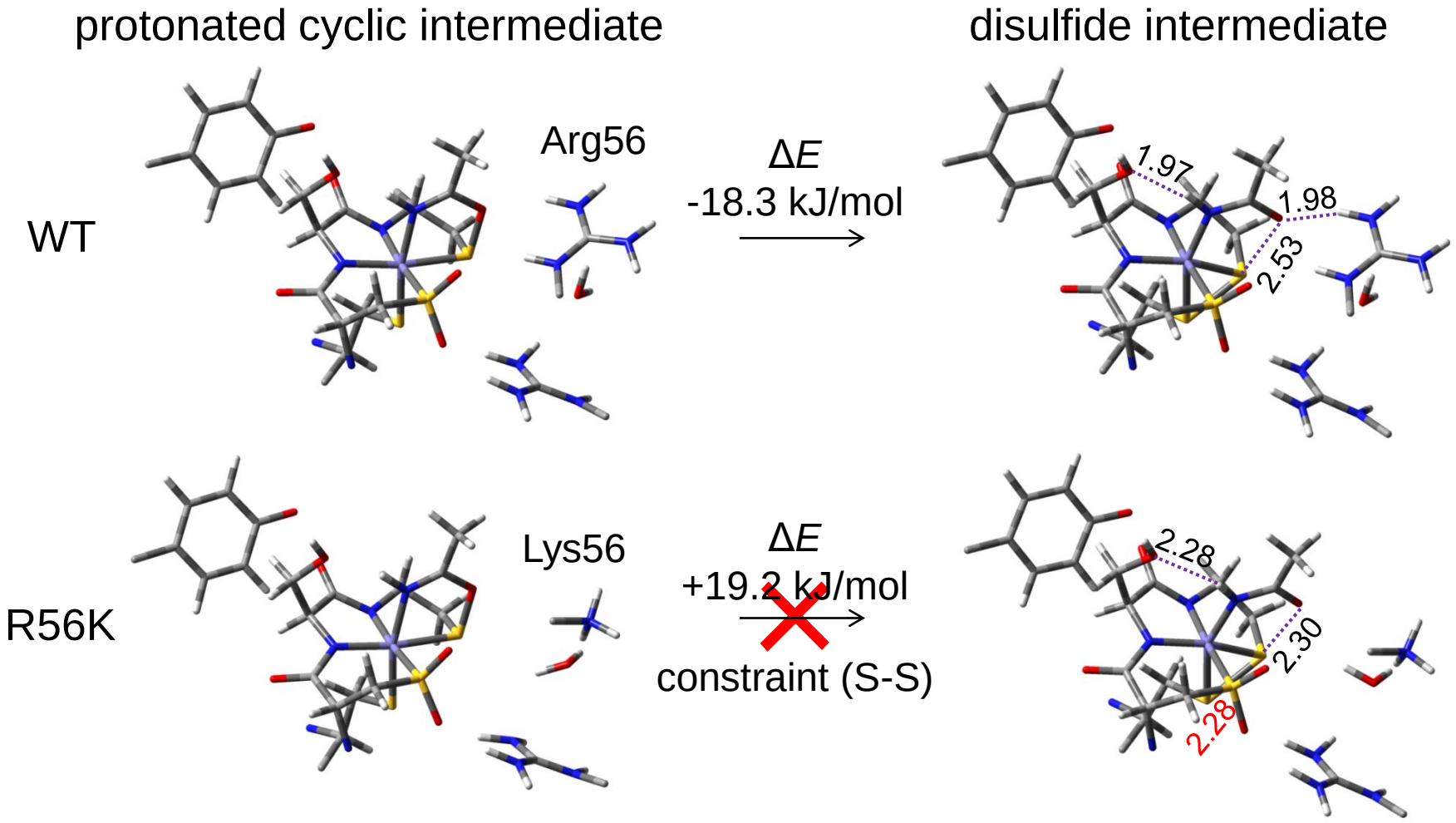
Previous and present theoretical studies
Formation of the disulfide intermediate

Experimental study of a mutant (βR56K)
No change in the position of the S atoms
→ Disulfide intermediate seemed improba



Why disulfide intermediate is not observed in the experiment of βR56K mutant?

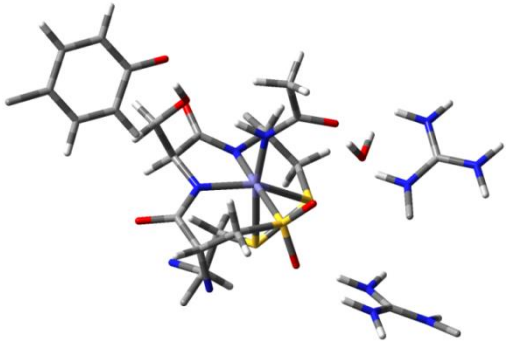
Effect of Arg56



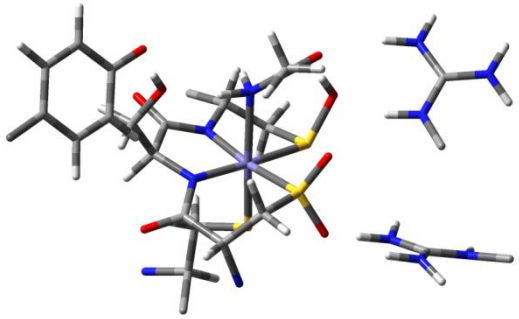
βArg56 is required for the formation of disulfide intermediate

Water attack

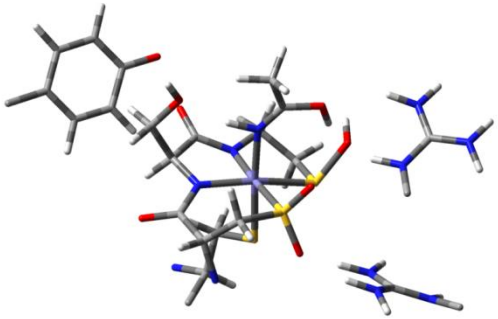
- Reaction path via imidic acid formation has lower reaction barrier
- Isomerization of imidic acid might occur outside the protein



disulfide intermediate



amide product



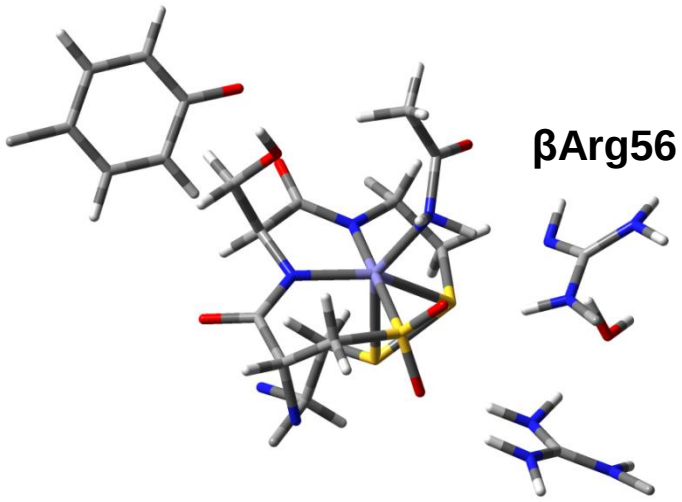
imidic acid intermediate

or isomerization occur outside protein

Effects of Protein Environment

Previous theoretical study using active site models:
proton transfer from βArg56 after disulfide intermediate formation

K. H. Hopmann, *Inorg. Chem.* 53 (2014) 2760

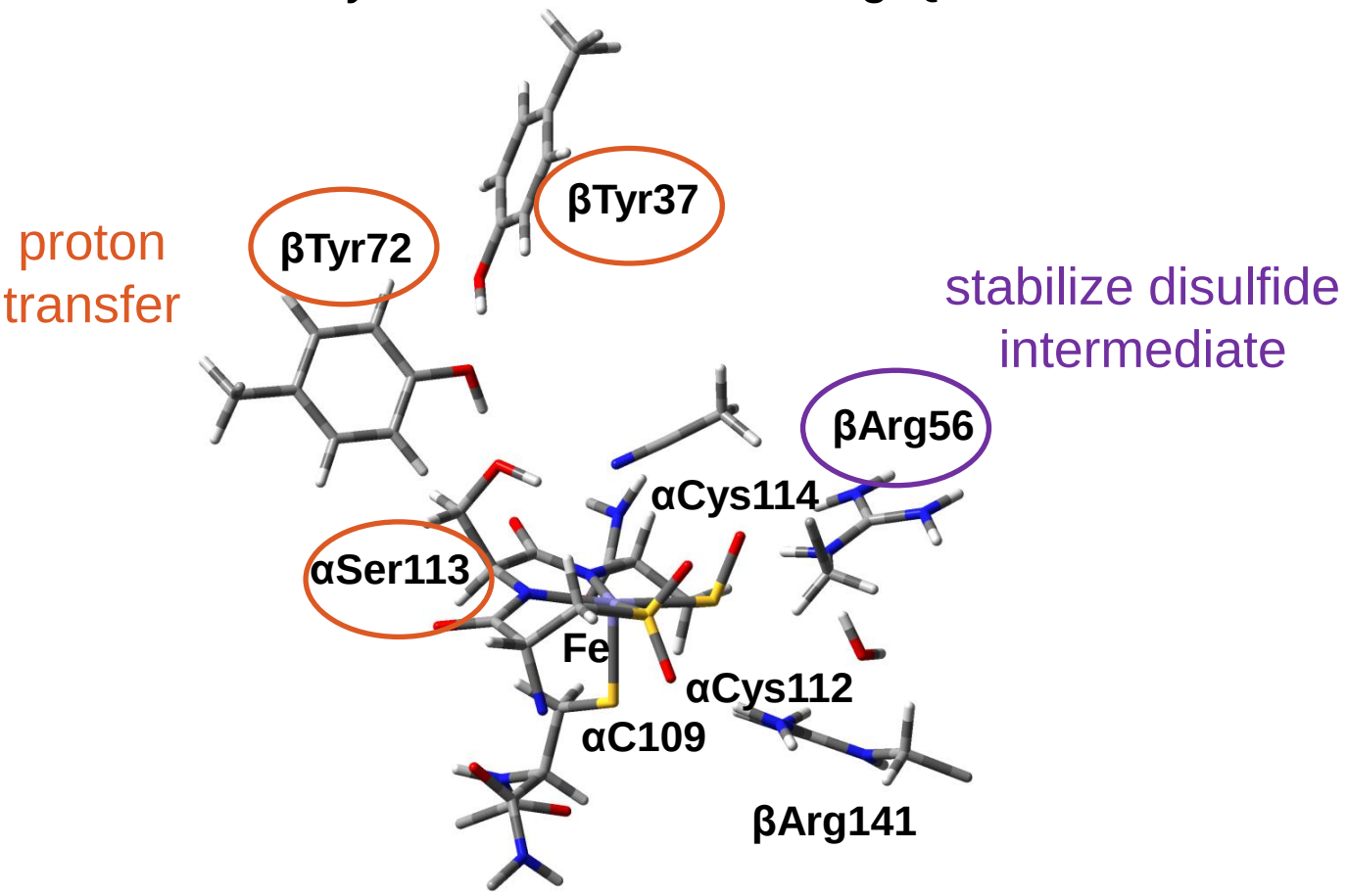


Deprotonation of βArg56 was not favorable in protein environment

Considering the effects of protein environment is important

Conclusion

We revealed the reaction mechanism of NHase subsequent to the formation of cyclic intermediate using QM/MM method



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