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ABSTRACT

The uses of Diels – Alder reactions in chemistry are numerous. Diels – Alder reactions are one of many carbon-carbon bond forming reactions giving a new ring. Many natural products can be made with Diels-Alder reactions. However, Diels – Alders reaction have not been well-established in biology. Through an enzyme design, proteins can catalyze Diels – Alder reactions. Using computational chemistry, an enzyme will be designed for a specific Diels – Alder reaction. Gaussian 03 will be used to calculate an active site know, known as a theoretical enzyme, a theozyme. By using Gaussian, activation barrier of uncatalyzed reaction is calculated to be 28.7 kcal/mol in gas phase. Currently, the project involves designing the best theoretical enzyme. Activation barriers will be calculated with and without amino acid side chain mimics. Other programs like RosettaMatch and EDGE will be used to find protein scaffolds that match the theozyme design. After finding the best way to incorporate the designed active site into a stable protein, the active site will be further modified to bind the transition state, and the design will be evaluated with the Molecular Dynamics/EDGE program. Once the modified design is complete, the protein will be made in E. Coli from the appropriate gene. Catalysis will be tested on designed protein. If a protein can be used to catalyzed Diels – Alder reactions, this shows that enzymes of other non-reactions can be designed to help with other synthetic methods.

INTRODUCTION



Figure 1 – Simplest Diels – Alder reaction

- Diels – Alder
 - Form new carbon – carbon bonds
 - Reaction that is not found in nature
 - Could be used in many synthetic processes

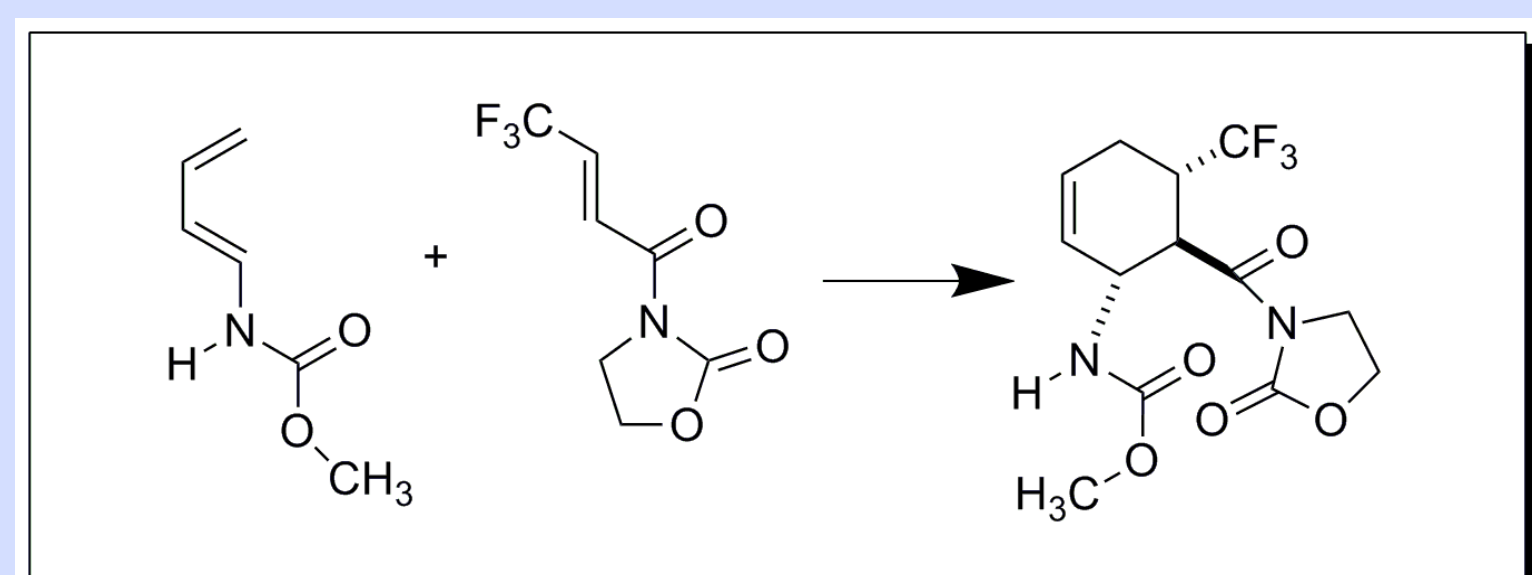


Figure 2 - The Diels – Alder reaction that is being studied for an enzyme design

- Why pick the specific Diels – Alder reaction (Figure 2)
 - Selectivity of an adduct
 - Lower the activation energy by ~10 kcal/mol
 - Dienophile was studied by previous group member¹

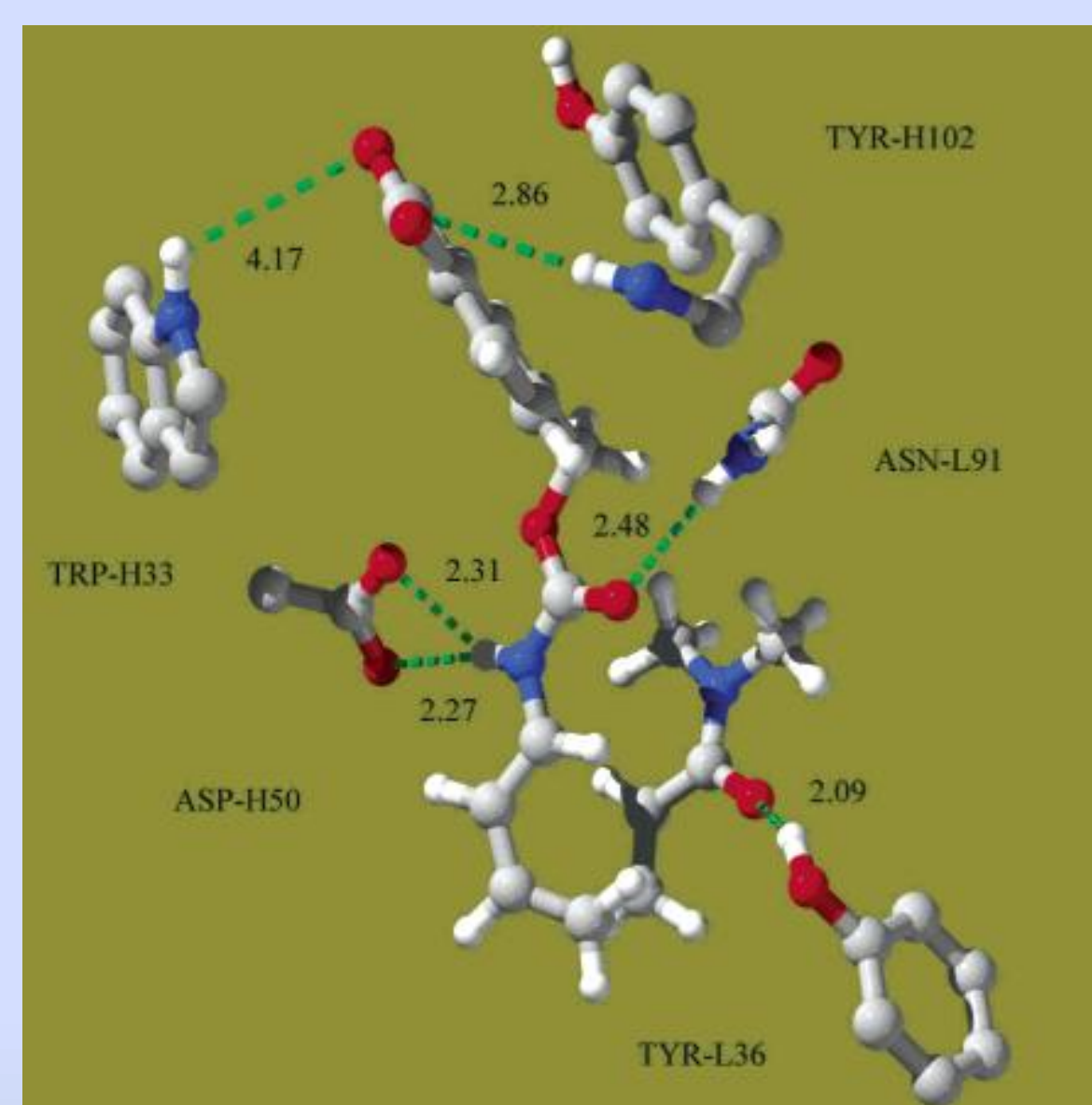


Figure 3 – Active Site of antibody 13G5²

- Antibody 13G5 Active Site enclosing a transition state²
 - Selectivity towards exo-(S,S) form
 - Similar diene to studied reaction

COMPUTATIONAL METHODS

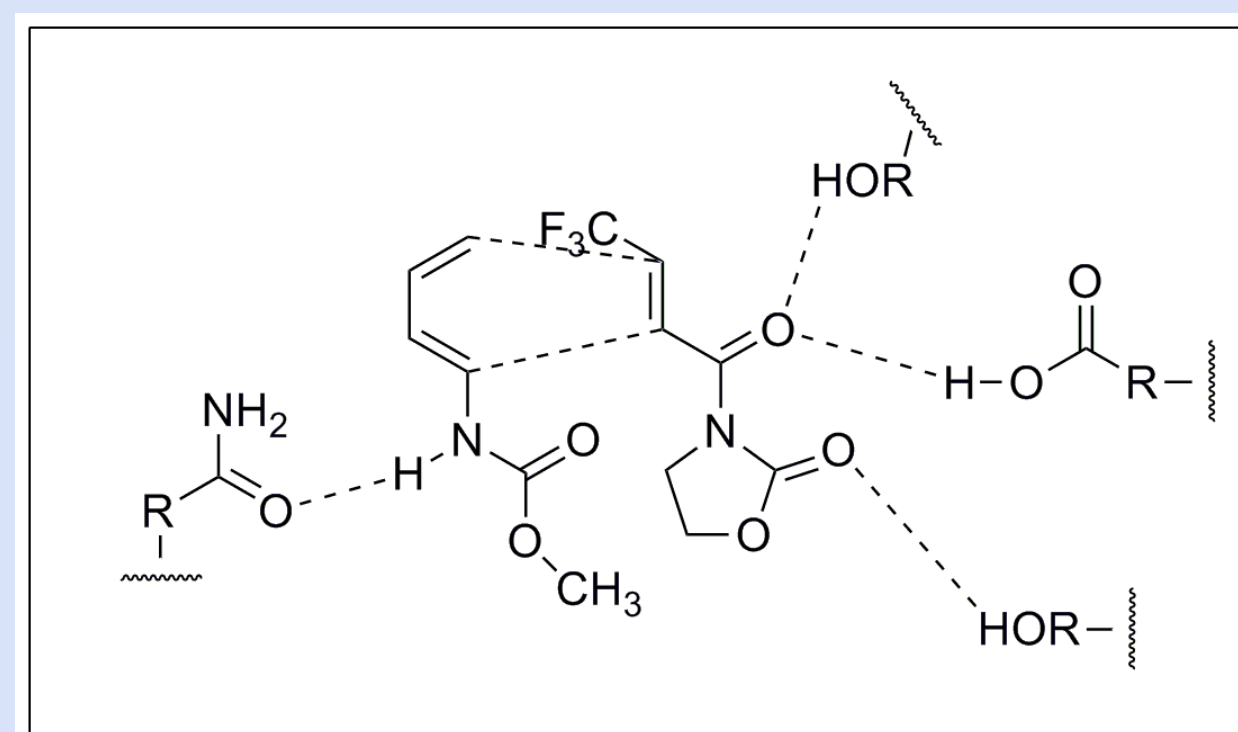


Figure 4 - Proposed Theozyme

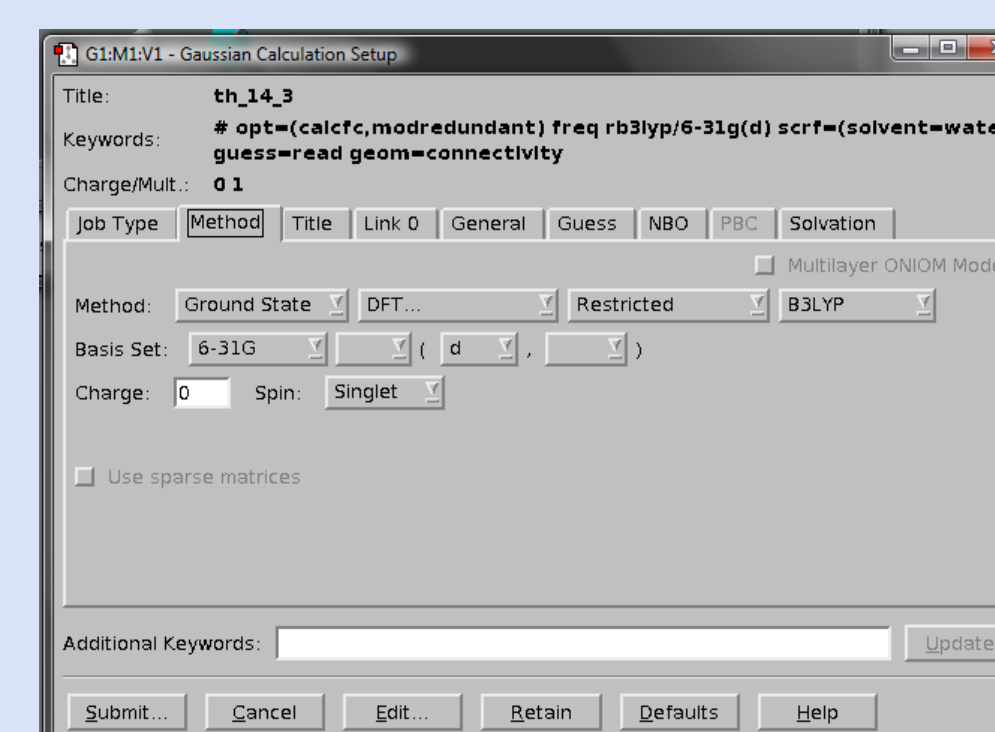


Figure 5 - Quantum Mechanics Program - Gaussian 03: the computational method

- Using Gaussian 03 for quantum mechanics calculations
- Using DFT B3LYP 6-31G(d) quantum method
- Calculate energetics of uncatalyzed and catalyzed reaction
- Use CPCM model system to model solvents conditions (water)
- Using alcohols, acids, and amides as amino acid side chain mimics since they form hydrogen bonds with specific atoms on the transition state structure

RESULTS

Figure 6: Optimized structures of the reactants and product in gas phase

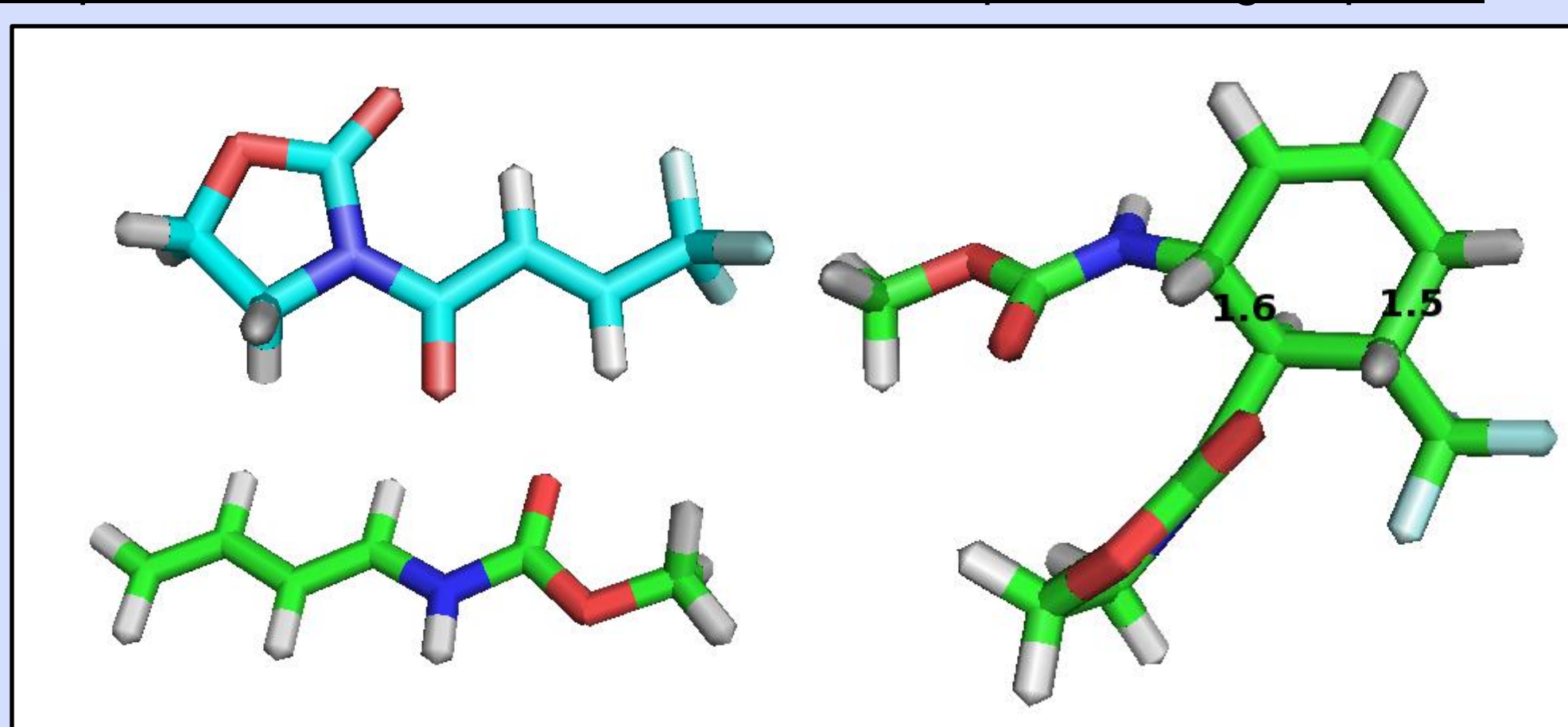


Figure 7: Asynchronous C-C bond formation and charge separation of transition state

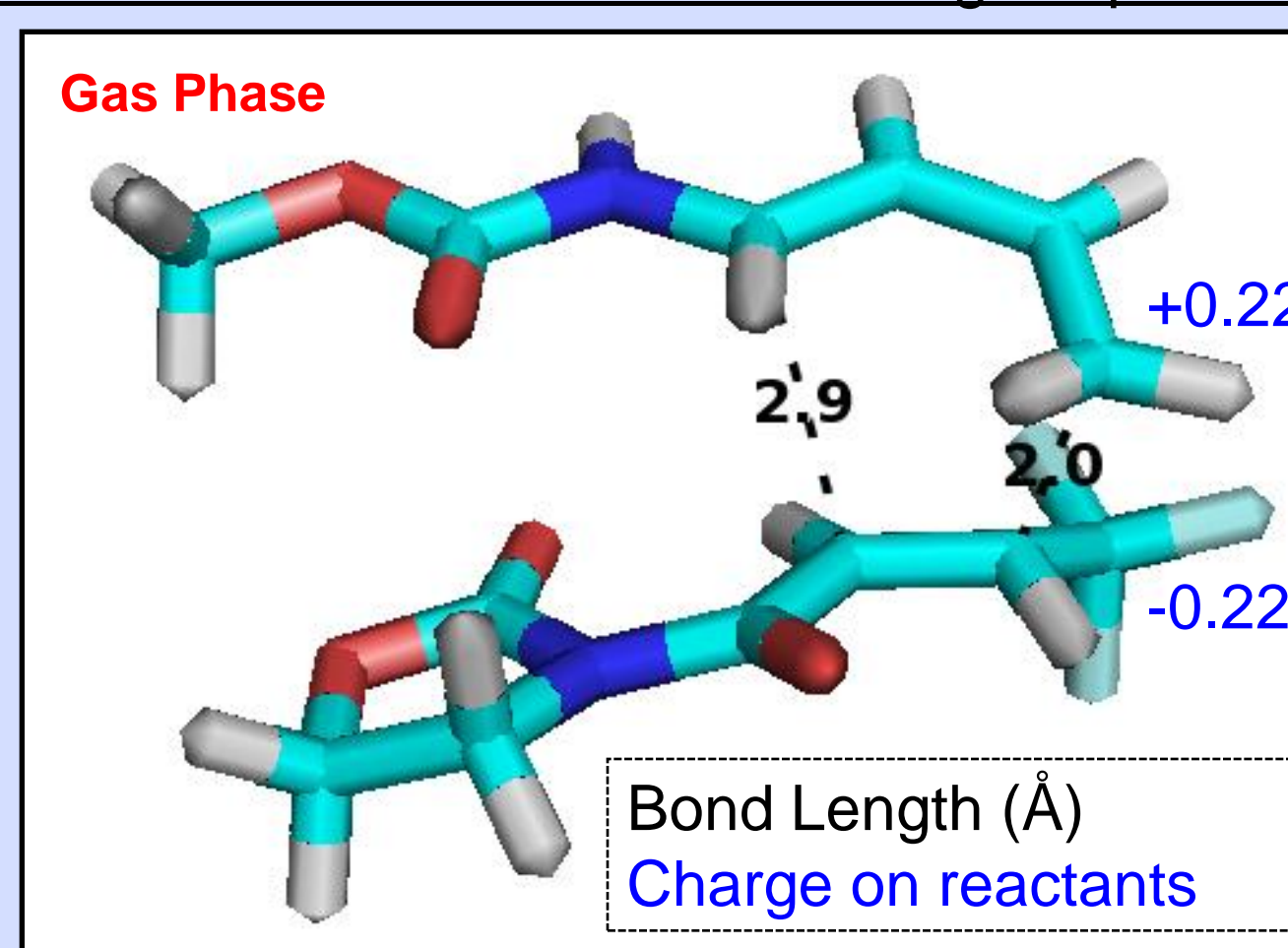
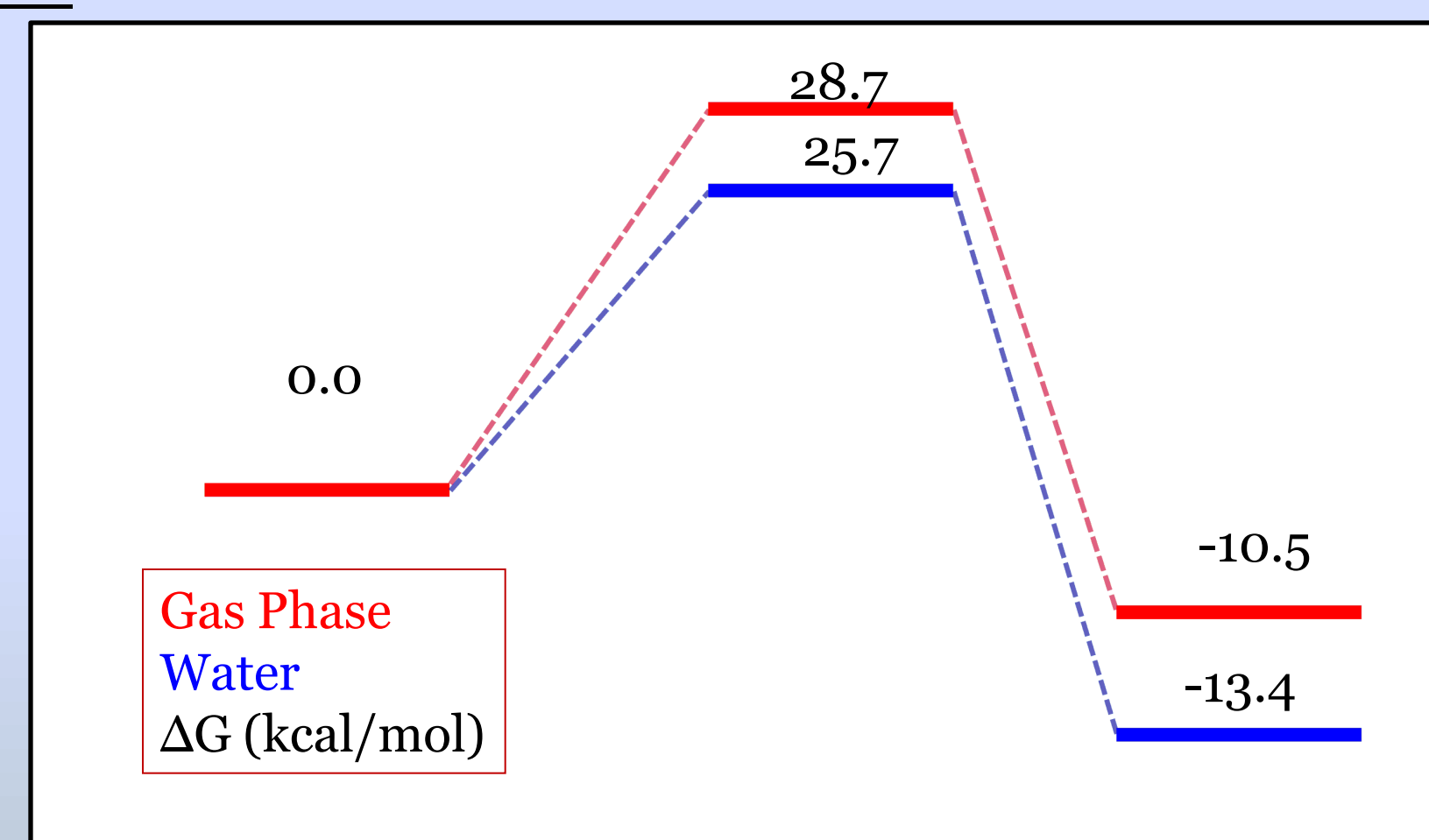
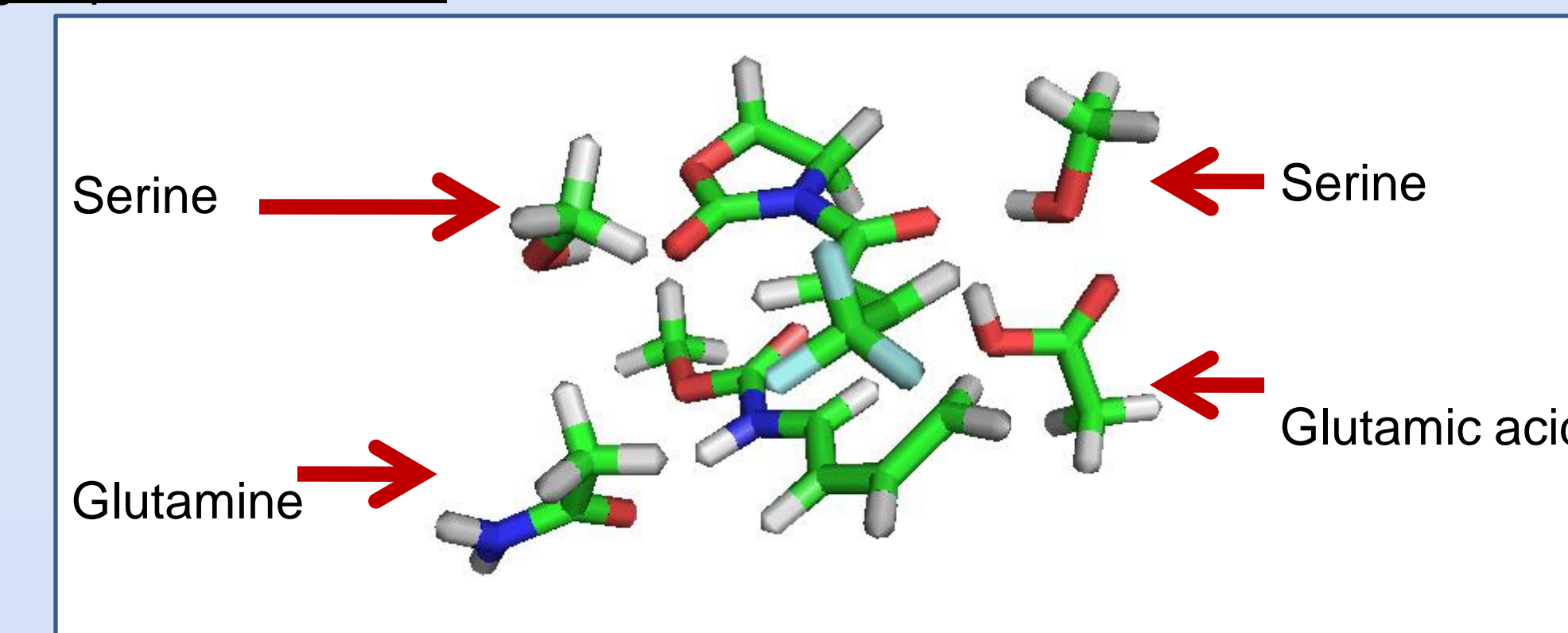


Figure 8: Water solvent calculations showed a more stable transition state: reaction is more exothermic



RESULTS (CONTINUED)

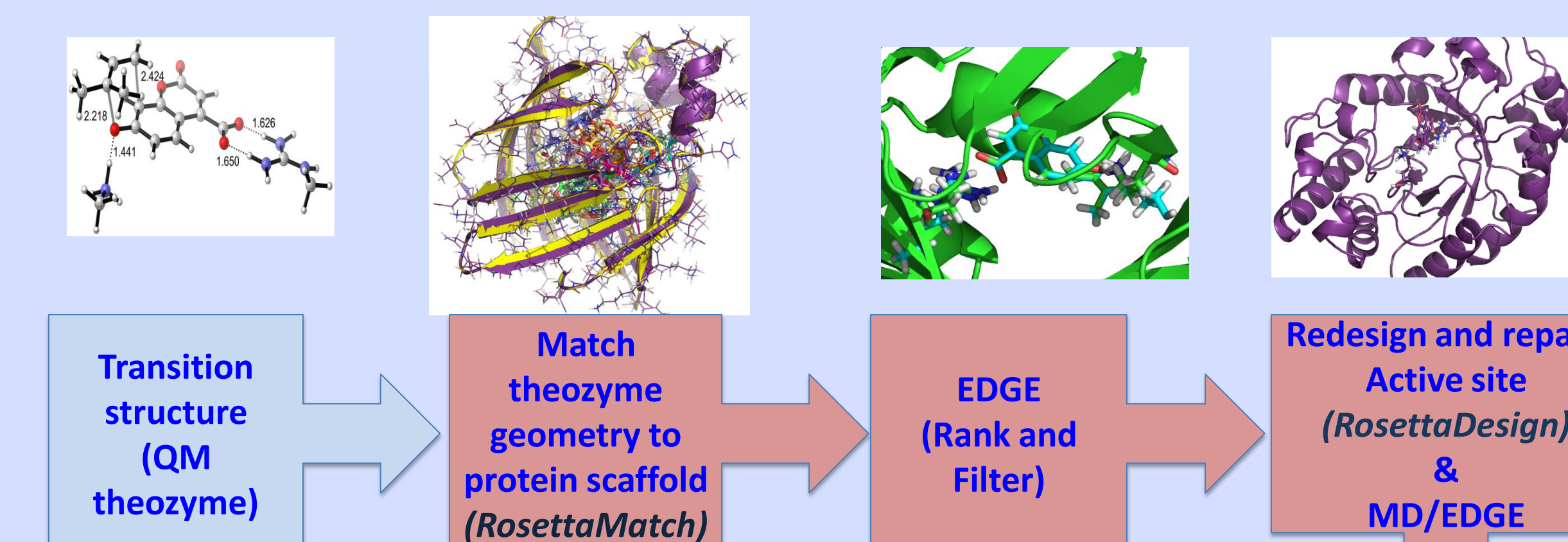
Figure 9: Theozyme being calculated: Two serines, a glutamic acid, and a glutamine side group mimics added



CONCLUSIONS

- After optimizing the reactants, product, and transition state, the activation barrier for the uncatalyzed reaction was calculated to be around 29 kcal/mol
- Reaction is exothermic
- Water helps stabilize transition state, allowing the reaction to be more exothermic
- There is a charge separation in the transition state showing a transfer of charge between the reactants.
- Since the reactants are not symmetric, the bond formation is asynchronous
- The theozyme calculations are underway due to the size and numbers of molecules.

FUTURE PLANS



- Put theozyme into ether solvent (ether has similar dielectric constant as a protein's active site)
- Find protein scaffolds using RosettaMatch and EDGE programs
- Use RosettaDesign to redesign active site
- Send results to Baker Group for gene expression for catalytic activity in E. coli
- Study of the selectivity of other adducts in the reaction

REFERENCES

1. Lam Y., Cheong P., Mata J., Stanway S., Gouverneur V., Houk K. *J. Am. Chem. Soc.* **2009** 131, 1947-1957
2. Cannizzaro C., Ashley J., Janda K., Houk K. *J. Am. Chem. Soc.* **2003**, 125, 2489-2506

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