



# Free radical mechanism for the $\gamma$ -glutamyl carboxylase reaction: A computational study

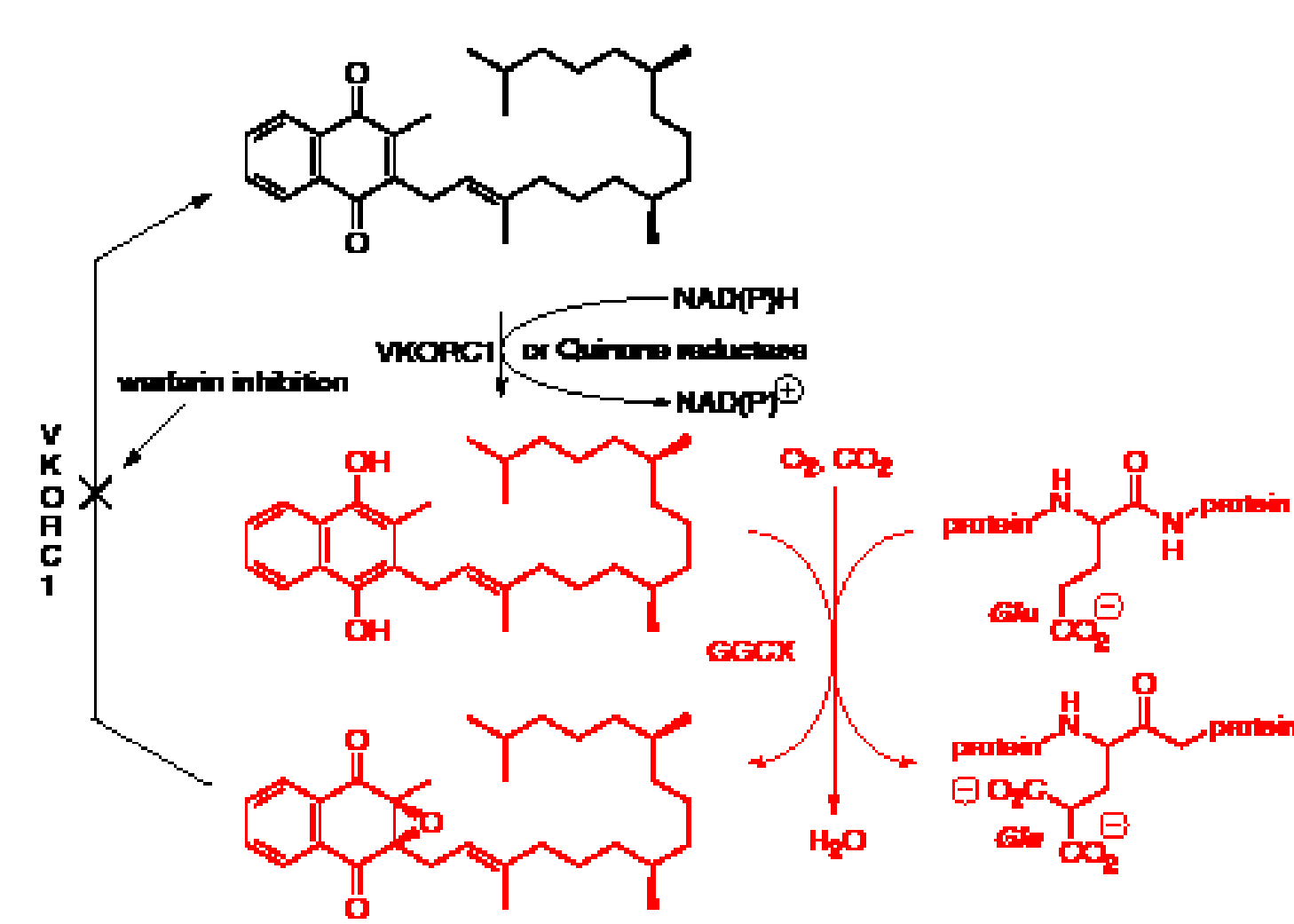
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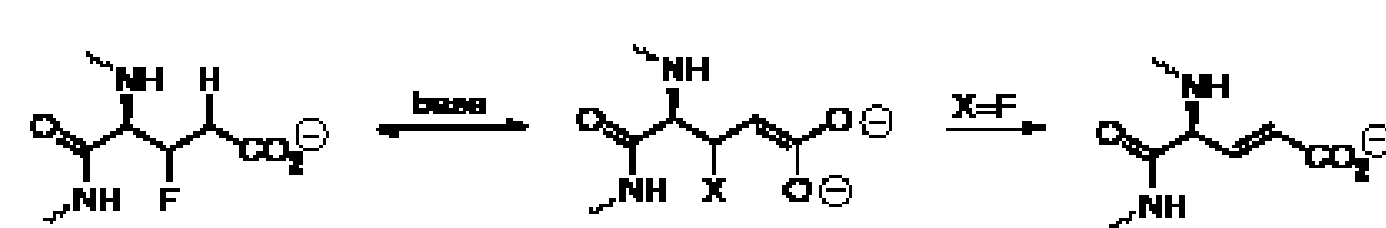
## Background

### The vitamin K cycle

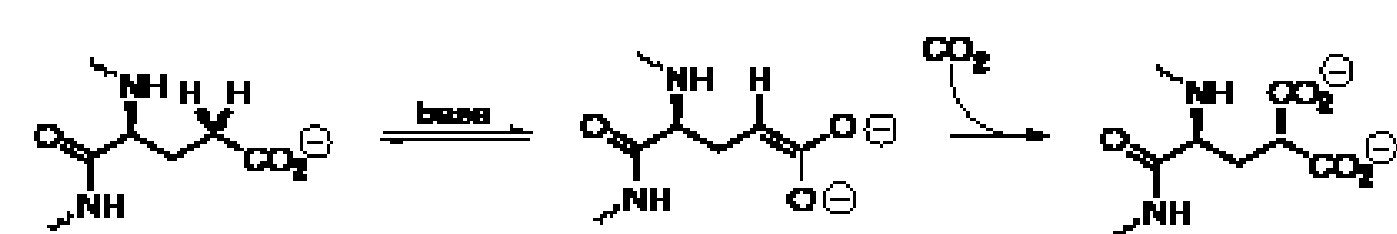


### What is known about this reaction?

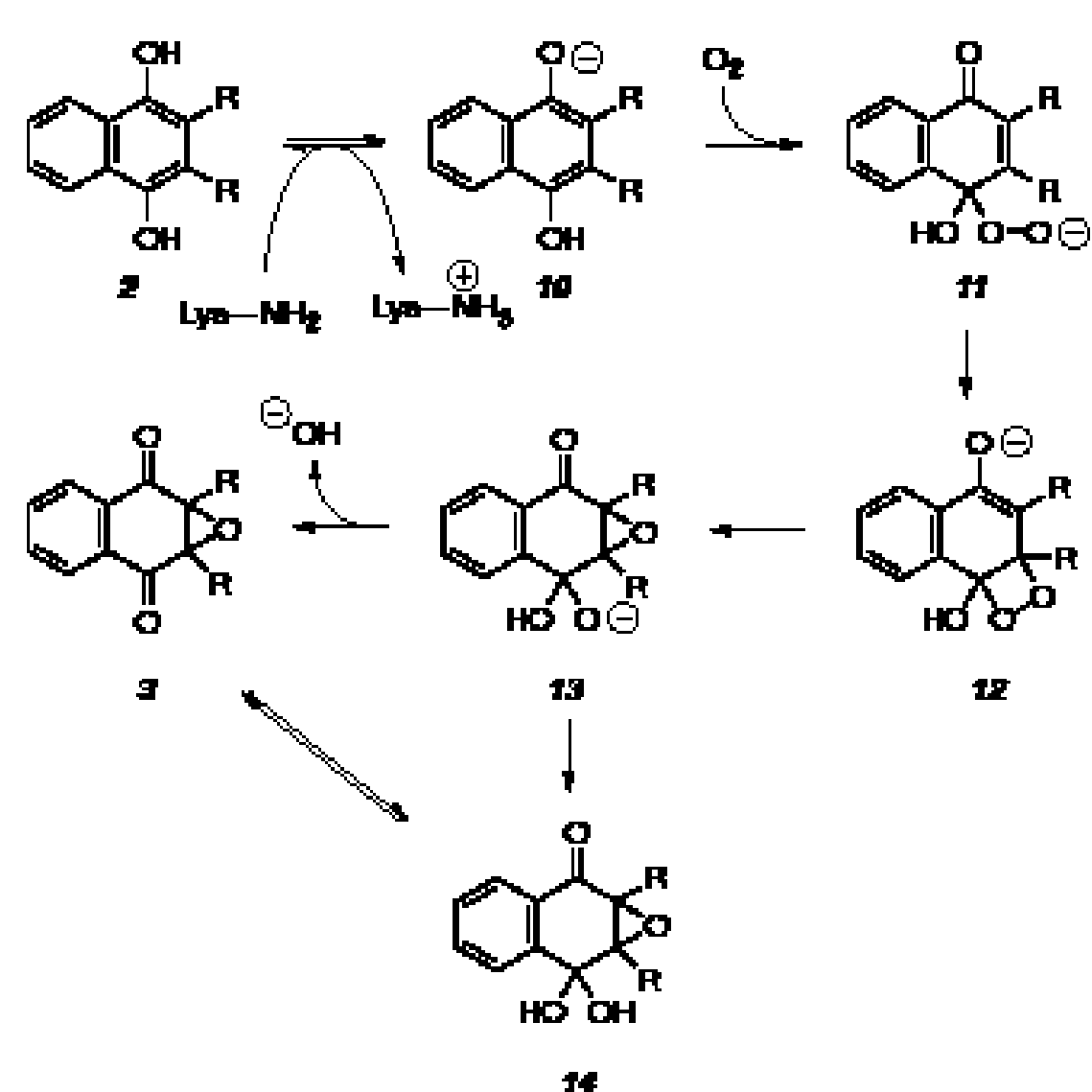
The carboxylation of a substrate analog containing a 3-fluoroglutamate residue leads to loss of hydrogen fluoride. The treatment of a similar substrate analog (without the fluorine) in tritiated water under conditions of  $\text{CO}_2$  starvation leads to stereospecific incorporation of tritium into the Glu side chain.



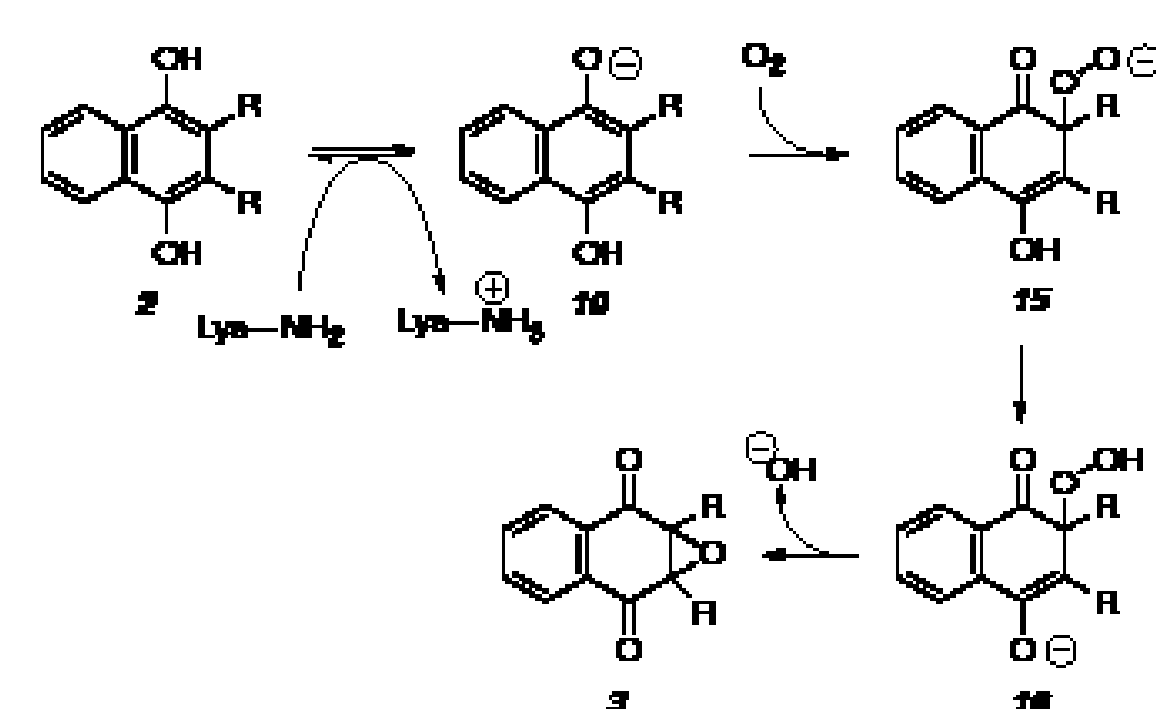
**Conclusion:** there is a very strong base generated in the reaction, and this base deprotonates the Glu side chain to generate a carboxylate dianion that reacts with  $\text{CO}_2$  to give the carboxylated (Glu) residue.



### The Dowd base strength amplification mechanism



### The Zheng and Bruice mechanism



The primary difference between this mechanism and the Dowd base strength amplification mechanism is in the product of the oxygenation of the naphthyl oxide anion. In the Zheng and Bruice mechanism, the attack of the anion on oxygen is at the 2-position; in the Dowd base strength amplification mechanism, it is at the 4-position. Both generate hydroxide anion in a highly hydrophobic environment.

## Density Functional Theory

A quantum mechanical method used to calculate the energetics and approximate the geometry. The total energy of the system is expressed as a functional of the electron density. The Born-Oppenheimer approximation makes the calculation of the wave function feasible for large systems.

The electronic structure calculations were done using Kohn-Sham DFT scheme, where the total energy of a multi-electronic system is expressed as a functional of the electron density.

The partition of electron energy according to Kohn-Sham equation:

$$E = E^T + E^V + E^I + E^{XC}$$

$E^T$  = kinetic energy of electrons  
 $E^V$  = nuclear-electron and nuclear-nuclear interactions

$E^I$  = electron-electron repulsion

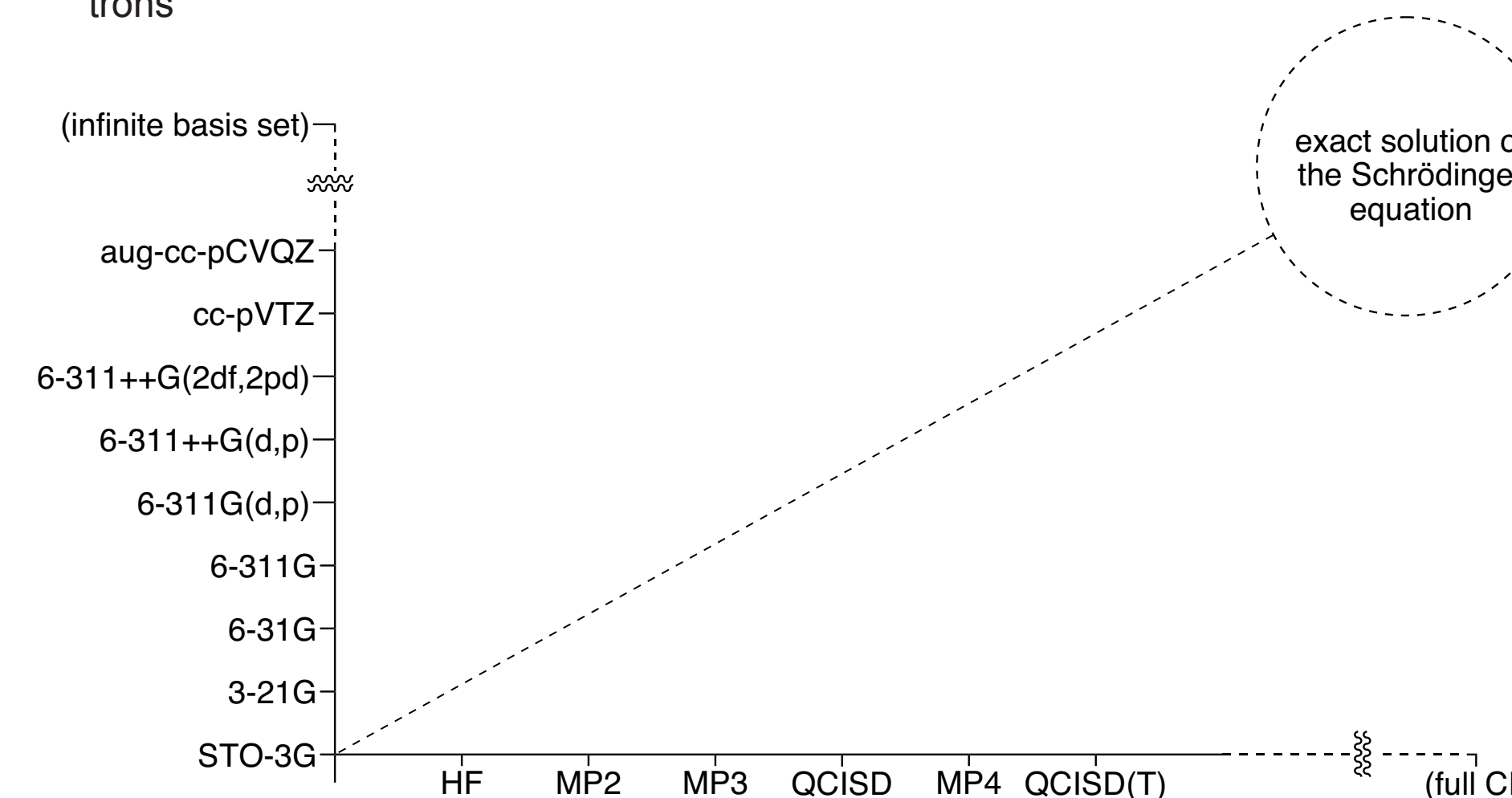
$E^{XC}$  = electron correlation represented by the quantum mechanical exchange-correlation energy.

### Born-Oppenheimer Approximation

This assumes that the electrons follow the motion of nuclei instantaneously – i.e. the distribution of electrons remain stationary with respect to a particular nucleus while it moves. This allows to separate electronic motion from nuclear motion expressing the energy as a function of nuclear coordinates.

### M06-2X

- Is part of a group of hybrid functionals in DFT.
- A popular method for calculations in the computational community
- Developed by Prof. Truhlar's group at University of Minnesota, to further improve Kohn-Sham DFT
- Is accompanied by a set of Gaussian functions to describe the whole molecules electrons



Gas-phase optimizations of the energy and geometry for all species were carried out at the B3LYP/6-311++G(2df,2p) level.

Vitamin K and its derivatives were modeled using 2,3-dimethyl-1,4-naphthalenediol as the base structure.

The protein side chains were modeled as follows:

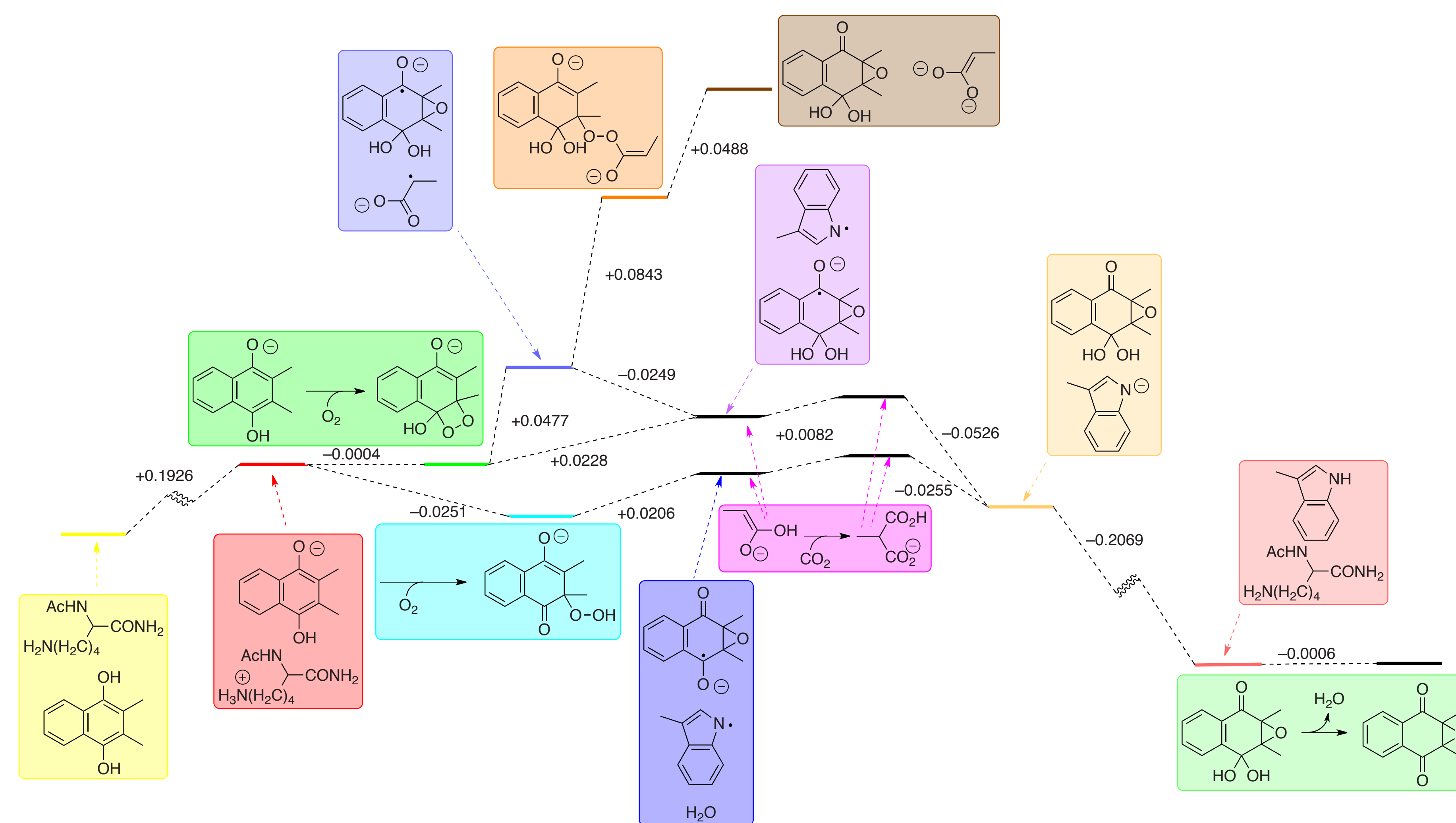
- glutamate side chain was modeled using propanoate anion;
- tryptophan was modeled using 3-methylindole;
- lysine was modeled using *N*-acetyllysine.

Optimizations of the energy and geometry for selected intermediates were carried out using the aug-cc-pvtz basis set in the gas phase, and in THF medium (the dielectric constant of THF approximates the dielectric constant in the interior of a lipid bilayer membrane).

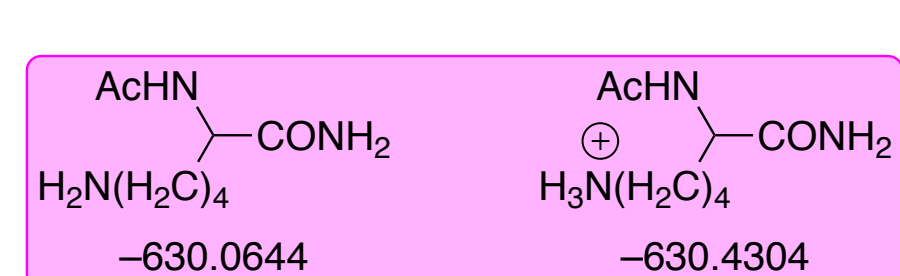
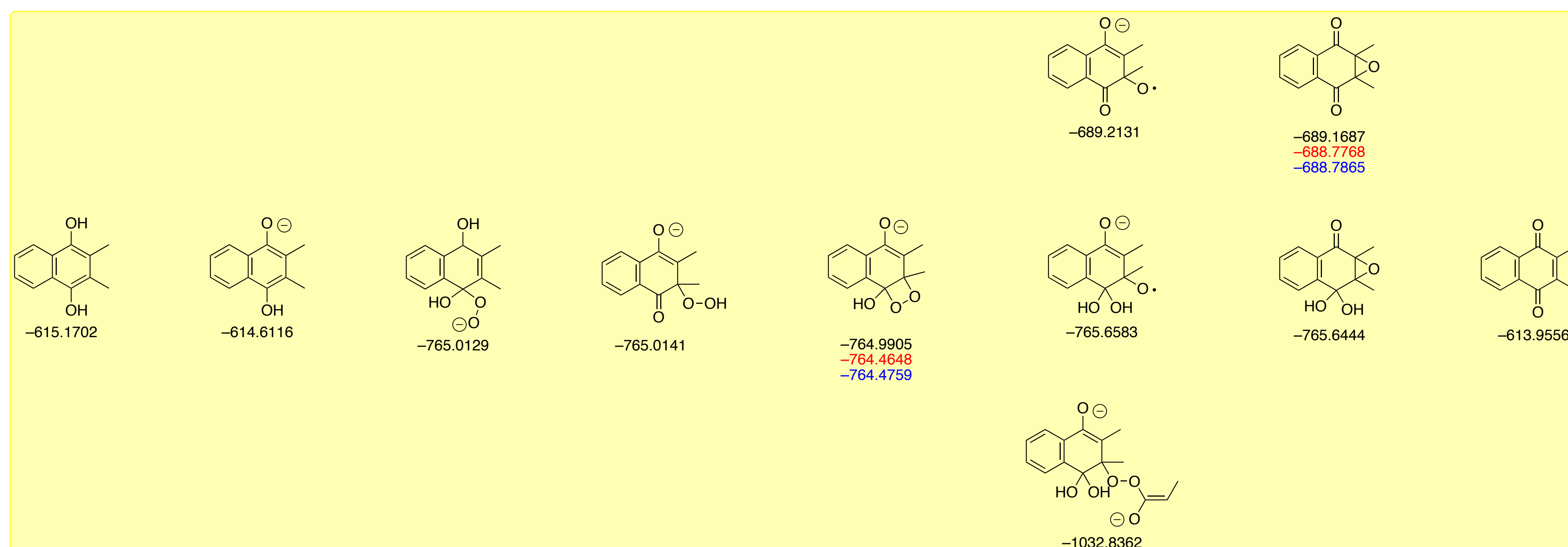
Optimizations of selected intermediates in the gas-phase were also carried out at the B3LYP/aug-cc-pvtz level.

## Putting it all together: a combined energy profile for the radical mechanisms

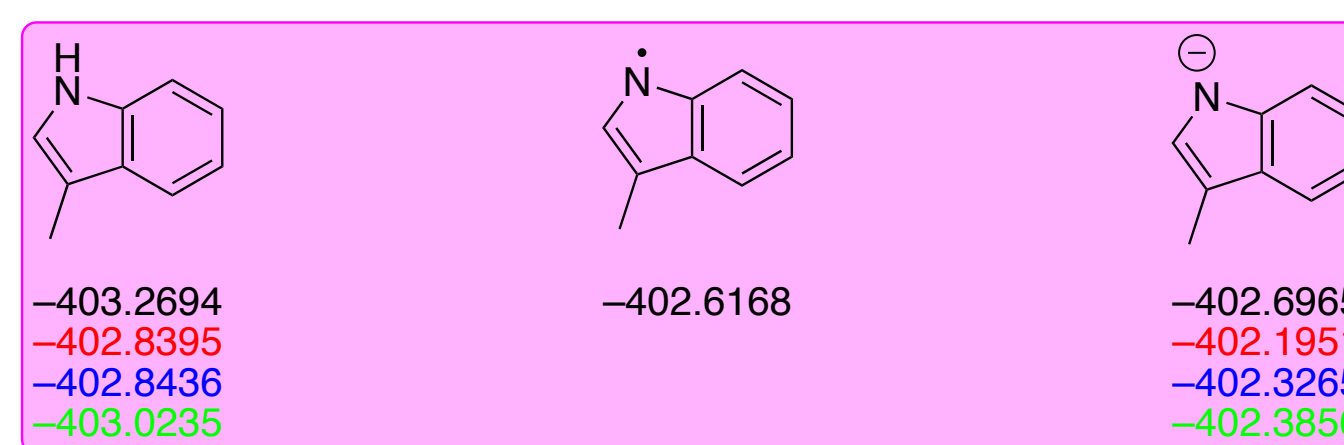
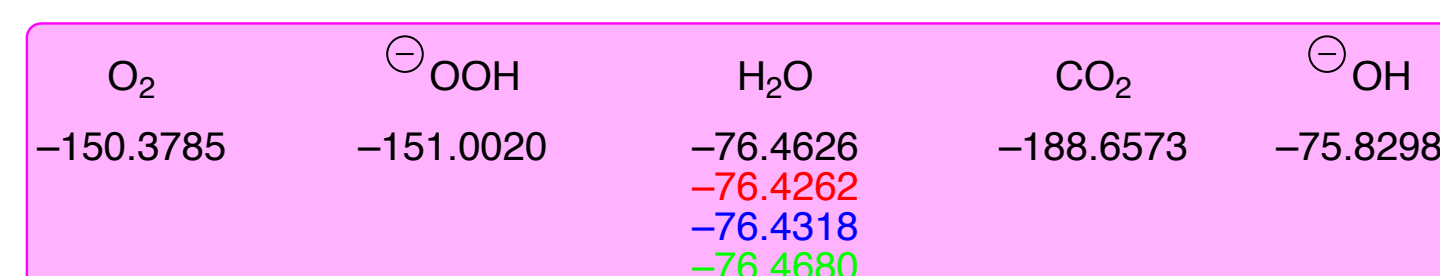
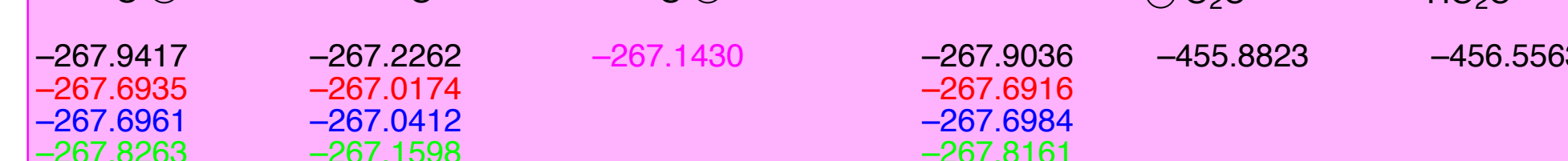
- The initial step of the reaction mechanism involves a highly endothermic deprotonation of the naphthoquinone by the free amine. This reaction is found to be much less endothermic if a carboxylate anion is located close to the amine.
- The addition of the dioxygen molecule to the 4-position of the anion is thermodynamically preferred.
- The Dowd di-oxygenation occurs as a relatively low energy intermediate, but a hydroperoxyenolate anion is lower energy still.
- The dehydration of the quinone epoxide hydrate is only slightly exothermic, which may allow it to be reversible.
- Formation of the peroxyester dianion is a strongly endothermic reaction, and probably does not occur in the cell.
- Hydrogen atom transfer from tryptophan to either the Dowd di-oxygenate or the hydroperoxyenolate lowers the energy of the reaction by at least 0.045 a.u. (28 kcal mol<sup>-1</sup>)



## Putting it all together: energy levels for species involved in the mechanism effect of basis set on the computed energy (in a.u.) of selected species



B3LYP/6-311G++(2df,p) [gas phase]  
 M06-2X/aug-cc-pvtz [gas phase]  
 M06-2X/aug-cc-pvtz [in THF]  
 B3LYP/aug-cc-pvtz [gas phase]



## Acknowledgments

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Computations using the Aug-cc-pvtz basis set were carried out using the BLUGOLD Cluster Supercomputer.

We would also like to thank Dr. Sudeep Bhattacharyay for helpful discussions, and Lewis Group alumni Michael Schmidt and Jared Gillingham, who began this project.