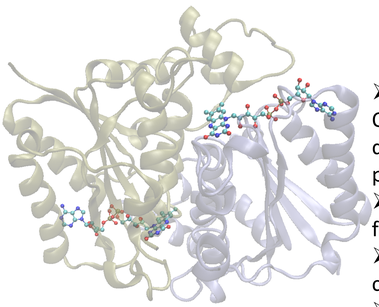
Energetics of Hydride Transfer Reactions in Quinone Reductase 2

Yer Yang, Thomas G. Bartholow, Michael A. North, Sanchita Hati and Sudeep Bhattacharyya
Department of Chemistry, University of Wisconsin-Eau Claire, WI 54702

Abstract

Quinone Reductases belong to the class of flavin-dependent oxidoreductases. With its redox active cofactor flavin adenine dinucleotide (FAD), quinone reductases are known to utilize a ping pong kinetic mechanism during catalysis in which a hydride is swapped back and forth between flavin and its two substrates.[1] In the first step, one substrate binds the active site and donates the hydride to the flavin. The oxidized substrate is then released followed by binding of the second one (in the same active site) which accepts the hydride producing the oxidized flavin and the redox cycle continues.[2,3] We are using quantum mechanical/molecular mechanical (QM/MM) simulations to explore the ping-pong kinetics in NQO2. The flavin ring atoms are treated with density functional theory, while embedded in molecular mechanically-treated enzyme active site atoms. In cellular environment, NQO2 catalyze the conversion of prodrug into drug and understanding the energetics involved in this ping pong kinetics will aid in mechanism-based drug design. The QM/MM setup, methods, and some results will be presented.

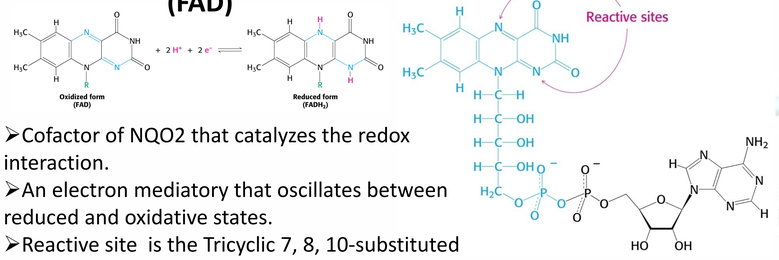
Background



NRH: Quinone Oxidoreductase 2 (NQO2)

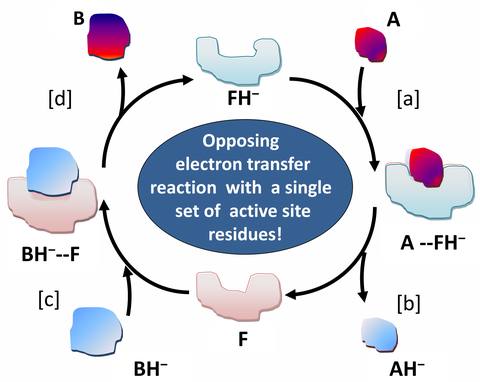
- Flavoenzyme that belongs to the family Quinone Reductases (QR) and reduces quinones to hydroquinones in a 2e⁻ reduction process.
- Helps protect cells against oxidative damage from reactive oxygen species.
- Role in preventing the production of cancer cells.
- Catalytic converter of prodrug to drug.

Flavin Adenosine Dinucleotide (FAD)



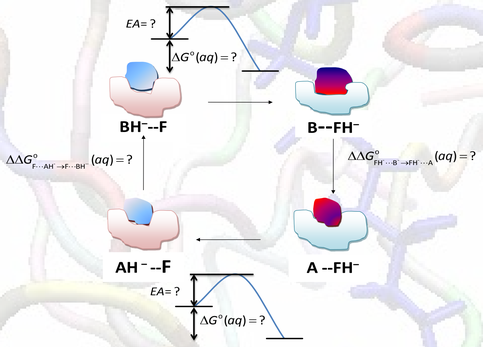
- Cofactor of NQO2 that catalyzes the redox interaction.
- An electron mediatory that oscillates between reduced and oxidative states.
- Reactive site is the Tricyclic 7, 8, 10-substituted isoalloxazine ring.

Ping-Pong Kinetics



Objective

- To explore the energetics of the Ping Pong Kinetics in NQO2.
- To examine the conformational changes and free energy change due to substrate shuttling.
- To determine the best functional to calculate the electron interaction .



- How much energy is required to overcome the activation barrier in the reduction and oxidation of the enzyme in this mechanism?
- What is the free energies of these hydride transfer?
- What is the change in free energies due to substrate shuttling?

Density Functional Theory Simulation

DFT is used to calculate the electronic interaction of substrate and flavin.

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

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

E^T = kinetic energy of electrons,
E^V = nuclear-electron and nuclear-nuclear interactions;
E^J = electron-electron repulsion;
E^{XC} = electron correlation represented by the *quantum mechanical exchange energy*.

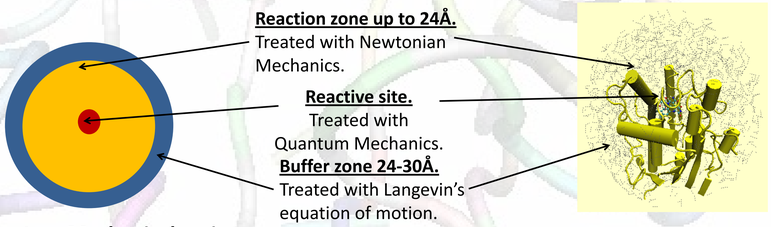
Electronic Structure Calculations: to calculate gas-phase free energy changes.

Polarizable Continuum Model: to calculate salvation free energy changes.

Functionals: M06-2X, M06, M06HF, MO5, and WB97X-D
Basis set: 6-31G + (d,p)

Employs a new exchange-correlation functional with improved medium-range (2-6 Å) correlation energy that appreciates the electron correlation significantly better.

QM/MM Simulations



Quantum Mechanical region:

- Takes into consideration the electronic interactions in the active site.
- The dynamics are computed with Density Functional Theory simulations.
- The amino acids and ligands have a dependence on the kinetic energy density therefore require a higher level of calculation.

Molecular Mechanical region:

- Calculated using Amber force field to perform molecular dynamic simulation.
- The amino acids and molecules in MM are treated as molecules on springs therefore only require low level calculations incorporating spring constants.

References-

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(2) Bhattacharyya, S.; Stankovich, M. T.; Truhlar, D. G. and Gao, J. ***J. Phys. Chem. A*** **2007**, *111*, 5729-5742.
(3) Mueller, R. M., North, M. A., Yang, C., Hati, S., Bhattacharyya, S. ***J. Phys. Chem. B*** **2011**, *115*, 3632-3641.
(4) Porcal, G.; Bertolotti, S. G.; Previtali, C. M.; Encinas, M. V. ***Phys. Chem. Chem. Phys.*** **2003**, *5*, 4123.

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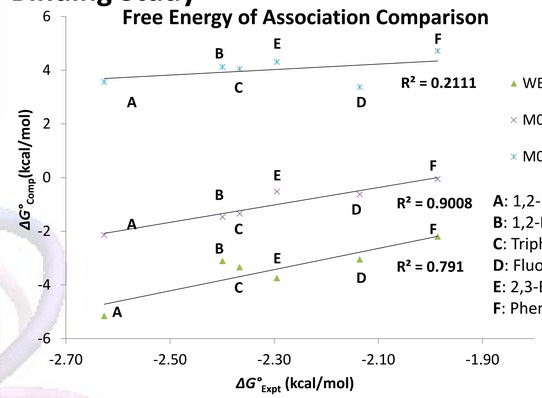
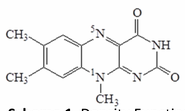
Results

Binding Study



A: 1,2-Benzanthrancene
B: 1,2-Benzofluorene
C: Triphenylene
D: Fluoranthene
E: 2,3-Benzofluorene
F: Phenanthrene



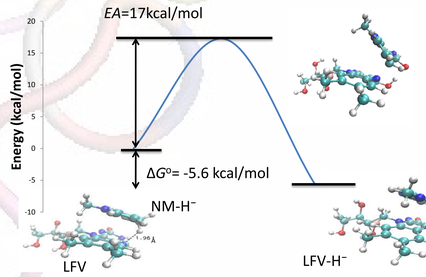
Scheme 1. Density Functional Theory simulations with Lumiflavin structure (above) with the six aromatic compounds in determining the most efficient functional.

$\Delta G^\circ_{Comp.}$ of association (kcal/mol) for the flavin-Aromatic compounds (A-F) utilizing functionals M06-2X, M06HF, M05, M06, and WB97X-D using the basis set 6-31+g(d,p) in comparison to experimental data, ΔG°_{Expt} (kcal/mol). Experimental results indicate M06-2X having the best correlation with experimental results with an R^2 of 0.9008.

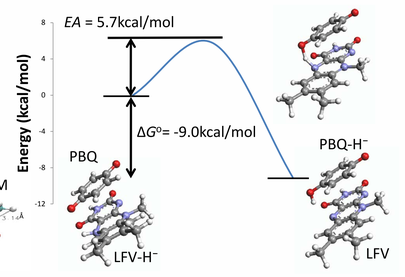
Compound	M06-2X	M06HF	M05	M06	WB97X-D	Experimental[4]
A	−2.13	−1.05	3.56	3.09	−5.16	−2.63
B	−1.47	−1.44	4.12	4.26	−3.11	−2.40
C	−1.34	−2.02	4.04	2.78	−3.34	−2.37
D	−0.62	−1.66	3.37	2.53	−3.06	−2.14
E	−0.52	−2.29	4.30	1.93	−3.75	−2.29
F	−0.05	−1.52	4.72	3.67	−2.20	−1.99

Transition State Calculation of Lumiflavin and Substrates

LFV-H⁻ (red) PBQ → PBQ-H⁻ + LFV (ox)



LFV (ox) + NM-H⁻ → LFV-H⁻ (red) + NM

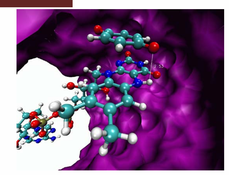


Simulations and calculations using the functional M06-2X with the basis set of 6-31+G(d,p), the energy necessary to overcome the activation barrier came out to be 17 kcal/mol and the free energy extracted from the system is calculated out to be -5.6 kcal/mol for the reduction of LFV with Nicotinamide (NMH).

Experimental results for the reduction of LFV in complex with parabenzoquinone (PBQ) in aqueous conditions suggests an energy of activation barrier of 5.7 kcal/mol and a free energy extraction from the system of -9.0 kcal/mol using the functional M06-2X and the basis set of 6-31+G(d,p).

Conclusions

- M06-2X has the greatest accuracy in computing free energies of association for flavin:aromatic complexes when compared to experimental values at an R^2 value of 0.9008.
- Hydride transfer to PBQ from the flavin is highly favorable with the free energy of reaction being -9.0 kcal/mol and has a lower activation barrier of 5.7kcal/mol.
- The hydride transfer from nicotinamide to the flavin in aqueous conditions is quite favorable with the free energy of reaction calculated out to be -5.6 kcal/mol but with a high energy of activation of 17kcal/mol.

Future Work

- Analysis of the QM/MM results of the FAD and PBQ along with FAD and NMN in enzyme.
- Investigate the cause of the unfavorable reduction of the flavin and continue further calculations to obtain smaller activation barrier.
- Determine whether water molecule in active site plays a role in substrate positioning.