Towards Development of a Computational Screening Tool for Quinone Oxidoreductase Inhibitors

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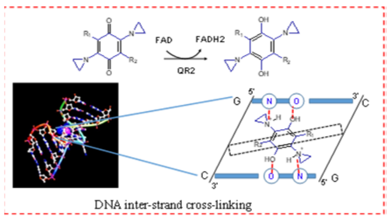
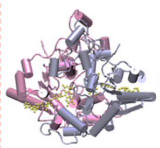
The Power of AND

Abstract

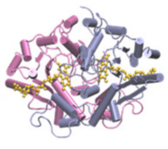
NAD(P)H:quinone oxidoreductase 1 (NQO1) and its paralog NRH:quinone oxidoreductase 2 (NQO2) function by reducing quinones into hydroquinones via a two-electron reduction, which is mediated by the cofactor flavin adenine dinucleotide (FAD). These enzymes also catalyze a similar reduction of nitroaromatic compounds to produce cytotoxic drugs. This study details the development of a computational protocol designed to screen many molecules in order to accurately predict the binding orientations of various small aromatic molecules to NQO1 and NQO2. The protocol consists of geometric positioning of molecules on the flavin ring, calculating binding free energy, and scoring these ligand molecules bound to the active site. Electronic structure calculations were performed on small model system. In parallel, a larger model with enzyme was built up and treated by a hybrid quantum mechanical/molecular mechanical approach. This was done by applying approximated quantum mechanics only to the flavin ring and ligand atoms. The computational setup and preliminary results including the binding energies and predicted orientations is presented.

Biological Significance of Quinone Reductases



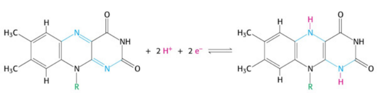


NRH: Quinone Oxidoreductase 1 (QR1)



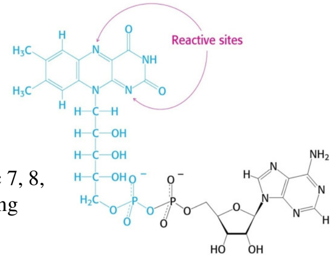
NRH: Quinone Oxidoreductase 2 (QR2)

Flavin Adenosine Dinucleotide (FAD)



Oxidized form

Reduced form

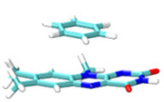


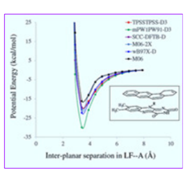
- Redox-active site contains the tricyclic 7, 8, 10-substituted isoalloxazine (flavin) ring
- FAD, the cofactor of QR2 serves an electron mediator capable of oscillating between two states by coupled electron proton additions

Electronic and Dynamic Effects in Binding

Electronic Effects:

- π-π stacking interactions
- Electrostatic forces
- Hydrogen bonding





Dynamic Effects

- Change in active site conformation upon substrate binding
- Change in collective motion upon substrate binding

QM/MM Setup

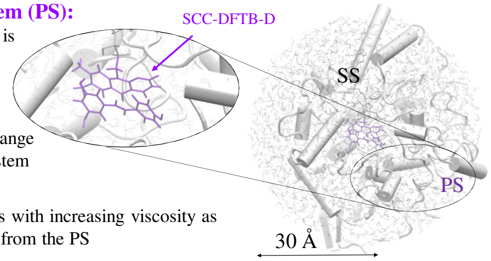
Treatment of Quantum Mechanical Atoms

Primary Subsystem (PS): Where the chemistry is occurring

Secondary Subsystem (SS): Responding to the change in the primary subsystem

Within 24-30 Å
Langevin dynamics with increasing viscosity as one moves further from the PS

Beyond 30 Å
All atoms fixed. Generalized Born's model of continuum electrostatics was used



Relative Binding Energy Calculation with Flavin

Ligand Displacement Reaction

$$\boxed{S2(g)} + \text{Fl} ::: S1\,(g) \xrightarrow{\Delta\Delta G^{o}_{disp}(g)} S1(g) + \boxed{\text{Fl} ::: S2\,(g)}$$

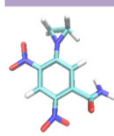
$$\Delta G_{S}(S2, aq) \downarrow \qquad \downarrow \Delta G_{S}(\text{Fl} ::: S1, enz) \qquad \downarrow \Delta G_{S}(S1, aq) \qquad \downarrow \Delta G_{S}(\text{Fl} ::: S2, enz)$$

$$\boxed{S2(aq)} + \text{Fl} ::: S1\,(enz) \xrightarrow{\Delta\Delta G^{o}_{disp}(enz)} S1(aq) + \boxed{\text{Fl} ::: S2\,(enz)}$$

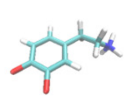
Fl = F (oxidized flavin), S1 = hydroquinone, S2 = Inh

$$\Delta\Delta G^{o}_{disp}(enz) = \Delta\Delta G^{o}_{bind}(g) + \Delta\Delta G_{S}(S2 \rightarrow S1, H_2O) + \Delta\Delta G_{S}(\text{Fl:::S1} \rightarrow \text{Fl:::S2}, enz)$$

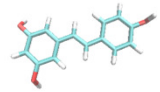
Ligands of Interest



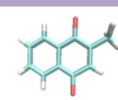
CB1954



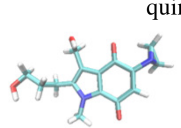
Dopamine quinone



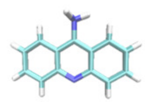
Resveratrol



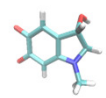
Vitamin K3 (menadione)



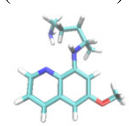
E09 (derivative of parabenzoquinone)



9-Amino acridine



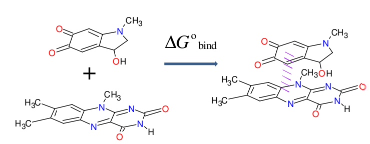
Adrenochrome

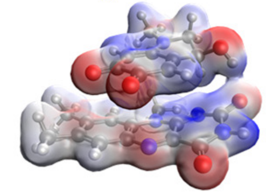


Primaquine

Preliminary Results

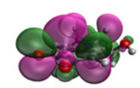
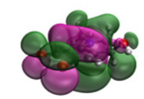
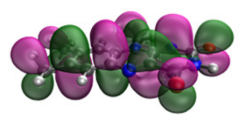
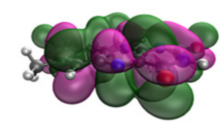
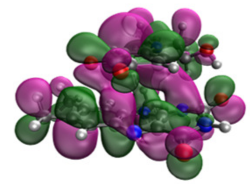
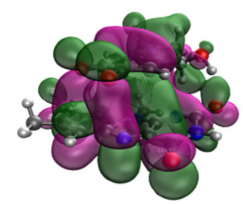
Gibbs Free Energy Calculations using DFT





flavin-bound adrenochrome

Molecular Orbitals Visualizations

Highest Occupied Molecular Orbitals or HOMO		Lowest Unoccupied Molecular Orbitals or LUMO
	Adrenochrome	
	Lumiflavin	
	Stacked complex	

Conclusions

- The Gibbs' free energy of binding in gas-phase between lumiflavin and adrenochrome is -5 kcal/mol
- Strong overlap of orbitals (HOMO and LUMO) between lumiflavin and adrenochrome

Future Directions

- Expand the calculations to include interaction energies for enzymes as well as in the aqueous phase
- Analysis of the potential energy of the complex as a function of the distance between the lumiflavin ring and the bound ligand

References-

(1) Reinhardt, C. R.*, Hu, Q. H.*, Bresnahan, C. G.*, Hati, S., Bhattacharyya, S. *ACS Catal.* **2018**, *12*, 12015-12029.
(2) Li, R.; Bianchet, M. A.; Talalay, P.; Amzel, L. M. *Proc. Natl. Acad. Sci. U.S.A.* **1995,** *92*, 8846.
(3) Bresnahan, C. G., Reinhardt, C. R., Bartholow, T. *, Rumpel, J. P., North, M. A., and Bhattacharyya, S. *J. Phys. Chem. A* **2015**, 119, 172–182.
(4) North, M.A., Bhattacharyya, S.; Truhlar, D. G. *J. Phys. Chem. B* **2010**, *114,* 14907–14915.
(5) Miyamoto, S. and Kollman, P. A. *Proteins.* **1993,** 6, 226–245.
(6) Calamini, B. et al. *Biochemical Journal.* **2010,** 429, 273–282.
(7) Wu, K.; Knox, R.; et al. *Arch. Biochem. Biophys.* **1997,** 347, 221-228.

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