Calculation of Binding Free Energies of NAD(P)H:Quinone Oxidoreductase 1 Inhibitors

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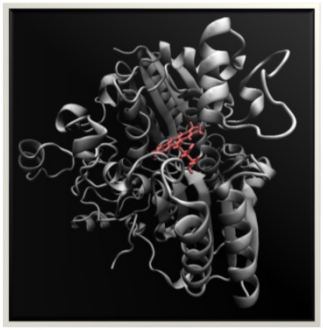
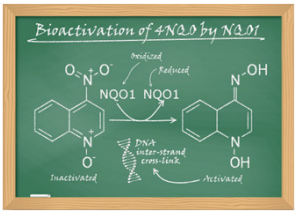
Abstract

NAD(P)H:quinone oxidoreductase 1 (NQO1) plays a key role in cellular defense in humans. It is known to reduce quinones to hydroquinones, inhibiting their ability to become a free radical semiquinone state, and cause cellular damage. NQO1 also has the ability to stabilize the tumor suppressor protein, p53, by inhibiting proteasomal degradation. In particular, it has been observed that NQO1 is activated by the reduction of the cofactor flavin – an event that triggers binding and subsequent stabilization of p53. Due to this mechanism, NQO1 holds promise for drug-targeted cancer therapy. In the proposed work, a comparative study will be conducted to explore the binding characteristics of various ligands that have the potential to be used in the drug design, including menadione, resveratrol, melatonin, and other quinone analogs that have potential binding affinity to the active site of NQO1. The docked structures of these ligands bound to the active site pocket of the enzyme have been developed in a previous project. Dynamic simulations will be run using hybrid quantum mechanical/molecular mechanical methods, and will be accompanied by free energy calculations to determine the binding characteristics of these ligands. Preliminary results of energetics from these simulations will be presented.

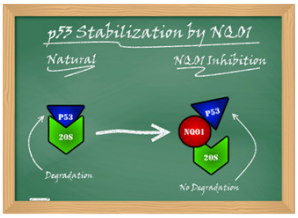
NAD(P)H:quinone oxidoreductase 1





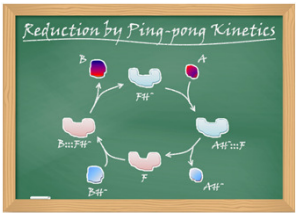
NQO1 and Cancer Treatment

- NQO1 is upregulated in and around certain cancerous cells[1]
- Bioactivation of certain ligands may cause cytotoxicity within these cancerous cells[2]



NQO1 and Protein Stabilization

- p53 is a protein that suppresses tumorigenesis
- p53 is naturally degraded by a proteasomal pathway
- NQO1 inhibits this degradation, and stabilizes p53[3]



NQO1 and Oxidative Stress Protection

- Quinones can be reduced to an unstable free radical state, that causes oxidative stress[4]
- NQO1 inhibits quinones from entering this unstable state by a flavin-dependent $2e^- / 2H^+$ reduction, known as *Ping-pong Kinetics*[5]

References

1. Ma Y, Kong J, Yan G et al., NQO1 overexpression is associated with poor prognosis in squamous cell carcinoma of the uterine cervix. *BMC Cancer.* 2014, *14*, 414.
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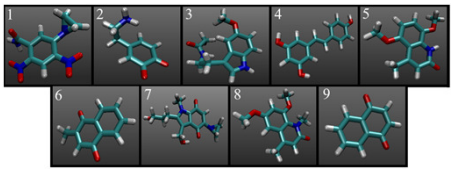
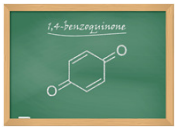
5. Kucera, H. R.; Livingstone, M.; Moscoso, C. G.; Gaikwad, N. W., Evidence for NQO1 and NQO2 catalyzed reduction of ortho- and para-quinone methides. *Free Radic. Res.* 2013, *47*, 1016-1026.

Ligands

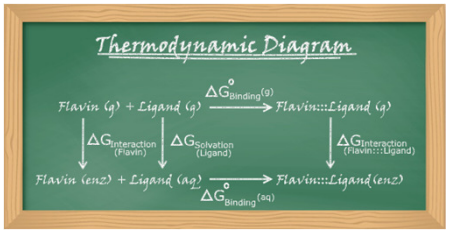


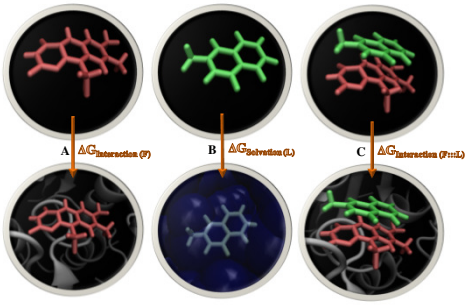
What makes a good candidate for binding?

Good candidates for inhibition can be reduced by NQO1 and bind tightly to its flavin-containing active site. They must be quinone derivatives, which have a similar skeletal structure to 1,4-benzoquinone.



Methods





Calculation

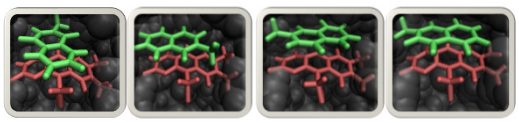
Our primary focus is calculating the interaction energy within the active site of NQO1. To do so, we calculate each individual component in a gaseous state *outside* of NQO1, and subtract this energy from each individual component *inside* of NQO1. In this way, process ***A*** determines the free energy when a no ligand is bound to NQO1, process ***B*** determines the energy of solvation of the ligand when it enters the system, and process ***C*** is used to determine the energy when a ligand is bound to NQO1.. This provides us with the following equation:

$$\Delta G_{Binding} = \Delta G_{Int.\ (F\cdots L)} - \Delta G_{Int.\ (F)} - \Delta G_{Solv.\ (L)}$$

C A B

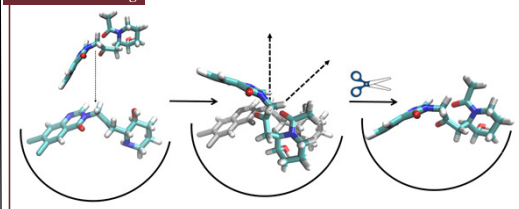
Optimal Orientations

Our ligands are rotated multiple times in an effort to find the most optimal binding orientation. For some ligands, binding does not occur at all in suboptimal positions. However, according to our simulations, *menadione* had many modes of binding, with the third orientation shown proving to be the most optimal. Dynamic simulations are run on each possible orientation, though only the most optimal orientations are presented in our preliminary results.



Dynamic Simulation Setup

Geometric Docking



1. Superimpose new ligand over previously bound inhibitor's center of mass
2. Stepwise rotations to find optimal orientation
3. Remove previously bound inhibitor

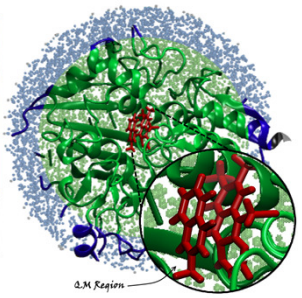
QM/MM Setup

Active Site
Center of solvated enzyme system. Region where chemistry is occurring. Ligand and FADH treated with quantum mechanics.

Secondary Subsystem
Region between active site and 24Å. Responds to change in active site. Treated with classical mechanics.

Between 24-30Å
Treated with Langevin dynamics. Increasing viscosity as atoms are further from the center of the solvated enzyme system.

Beyond 30Å
All atoms are fixed. Applied generalized Born's model of continuum electrostatics.



- **Quantum Mechanical** region atoms were treated with *Approximate Density Functional Theory*
- **Classical Mechanical** region atoms were treated with *Molecular Mechanics* using a CHARMM forcefield.

Preliminary Results

	Ligand	$\Delta E_{Potential}$	$\Delta G_{Binding}$
1	CB-1954	-20.7	-14.2
2	Dopa Quinone	-14.0	-45.2
3	Melatonin	-16.9	-15.7
4	Resveratrol	-18.3	-11.9
5	MZX	-18.3	-23.3
6	Menadione	-15.0	-16.8
7	e09	-20.4	-2.5
8	EWQ	-18.6	-4.3
9	1,4-napthoquinone	-13.6	-15.6

Conclusion

After running dynamic simulations as outlined previously, it was determined that all 9 of the tested ligands would spontaneously bind to NQO1. Particularly promising, resveratrol produced a binding free energy similar to an experimental value determined in NQO2 (-11.86 kcal/mol to -9.8 kcal/mol). Interestingly, when the ligand is removed from the system to calculate NQO1 (aq), the data is not uniform. Some structural changes may be occurring when each ligand is added/removed, and these structural differences will be investigated in future studies. Menadione and 1,4-napthoquinone had multiple orientations in the active site that successfully bound to NQO1, though each ligand clearly had an optimal orientation, which is reported here.

Future Directions

- Comparative study on experimentally tested dicoumarol analogs
- Application of high level energy correction
- Investigation into structural differences of NQO1 upon addition/removal of ligand

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