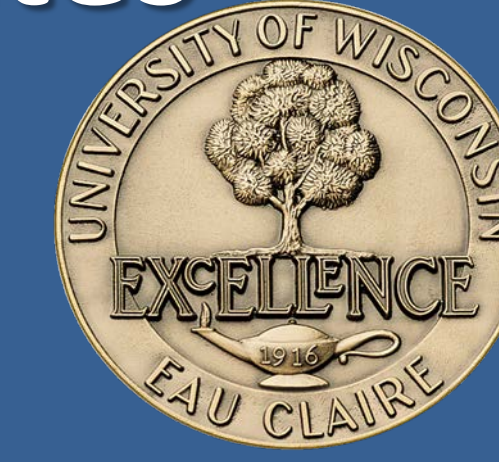
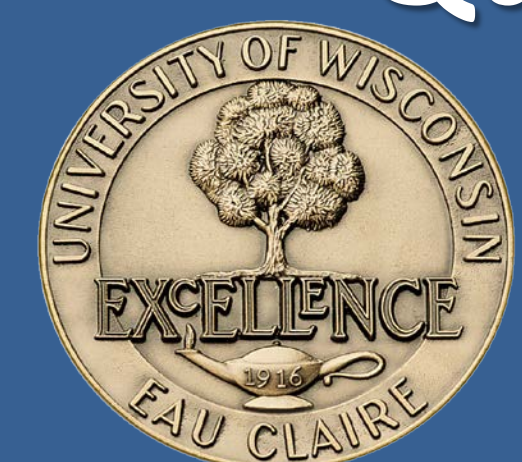


# Quantum Chemical Studies of the Binding and Catalytic Hydride Transfer Reaction of Flavin with Aromatic Substrates

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## Abstract

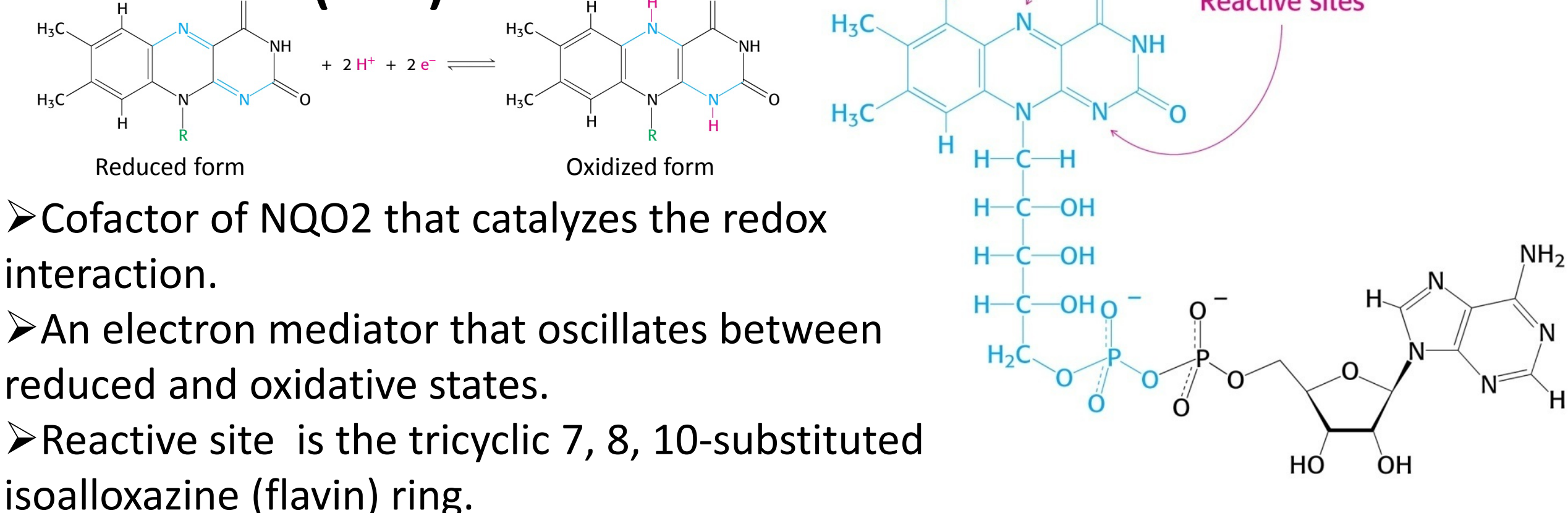
A large number of chemical reactions are catalyzed by flavoenzymes- a group of enzymes that utilizes flavin ring as a cofactor. The versatility arises mainly due to the ability of enzyme matrix to modulate flavin's redox potentials by altering non-covalent interactions. These interactions include hydrogen bonding with flavin ring atoms, hydrophobic interactions due to aromatic side chains, and  $\pi$ - $\pi$  stacking interactions with aromatic substrates (e.g. quinone reductases). Although, recent theoretical studies were successful to model hydrogen bonding interactions,<sup>1,2</sup> the effects of aromatic interactions on the redox properties of flavins have remained only partially revealed. The difficulty in modeling these interactions arises because of limitations in various theoretical treatments to reproduce van der Waal's interactions. In the present study, the  $\pi$ - $\pi$  stacking interactions of flavin and other aromatic molecules have been studied using improved semi-empirical and density functional theories. The effect of these aromatic interactions on binding as well as catalysis will be presented.

## Background

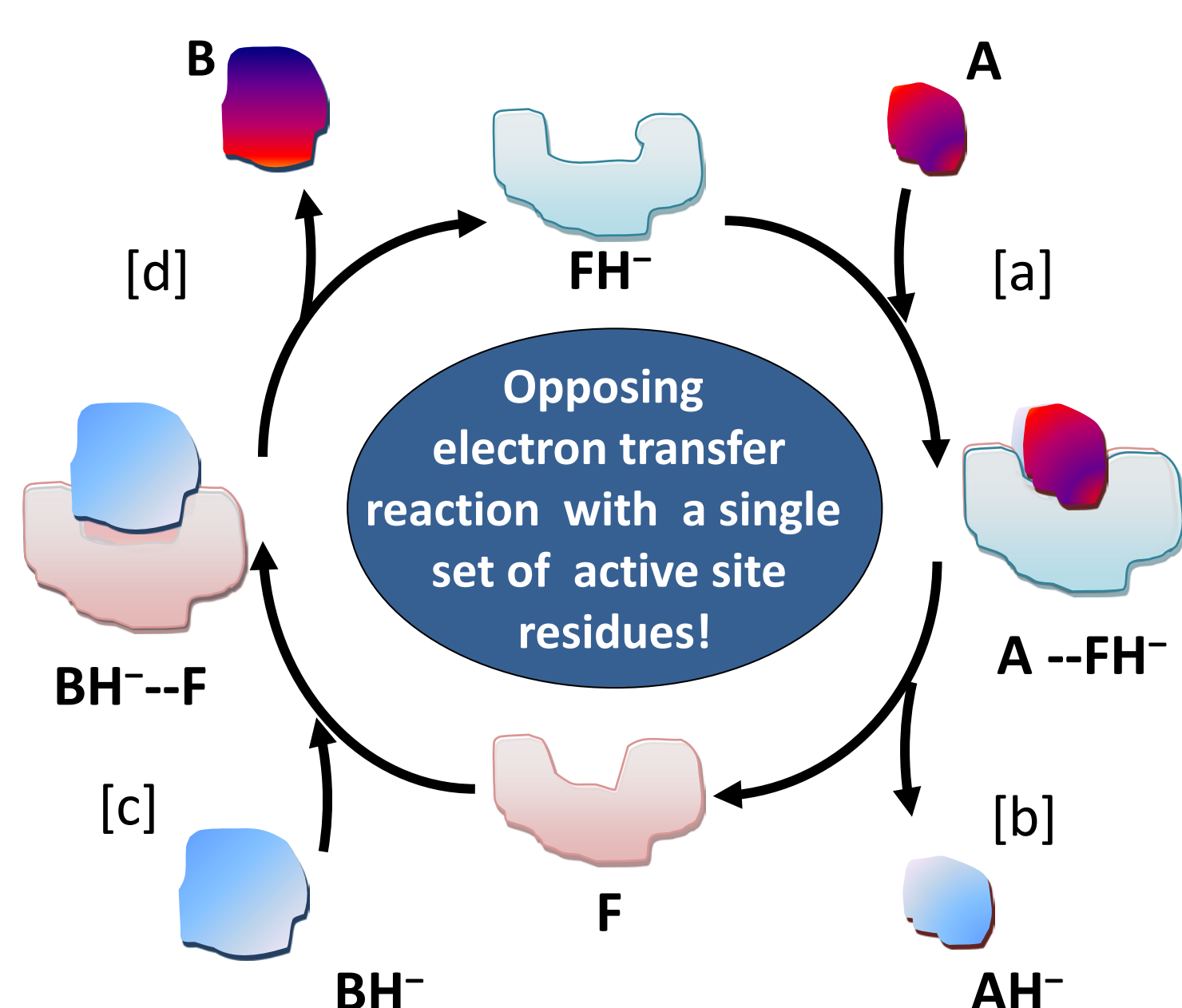
### NRH: Quinone Oxidoreductase 2 (NQO2)

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

### Flavin Adenosine Dinucleotide (FAD)

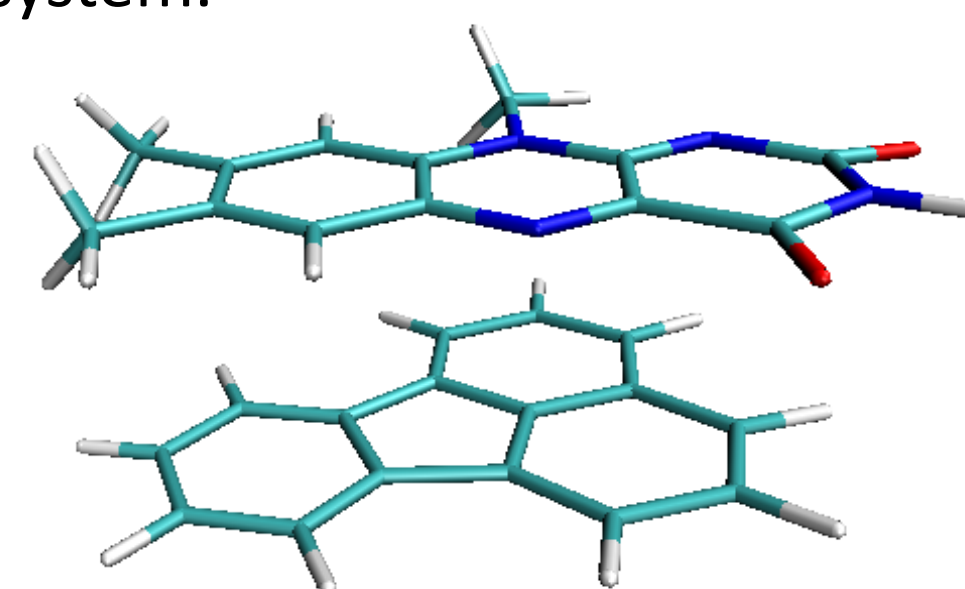


## Ping-pong Kinetics



## Objective

- Examine different functionals to determine which best characterizes the interaction between lumiflavin and known aromatic substrates.
- Determine the hydride transfer reaction energetics between flavin and 1-methyl nicotinamide in aqueous system.



## Density Functional Theory and Density Functional based Tight Binding

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

### DFTB

- Incorporates self consistent-field treatment of electron-electron interactions into tight-binding theory
- The molecular electronic orbital is expressed as a linear combination of the atomic orbitals of all the atoms in the molecule
- The calculation of electron-electron interactions is simplified by treating them as point charges

### DFT

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*.

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

Polarizable Continuum Model: to calculate solvation free energy changes.

Functionals: M06-2X, M06, M06HF, M05, and WB97X-D

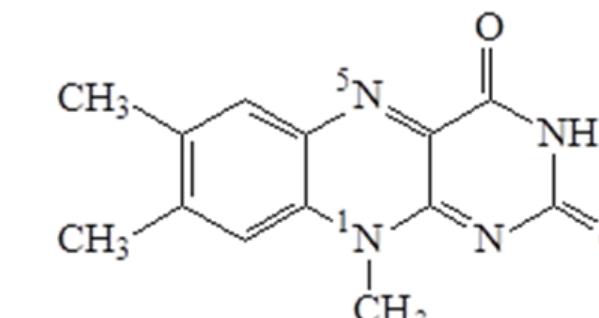
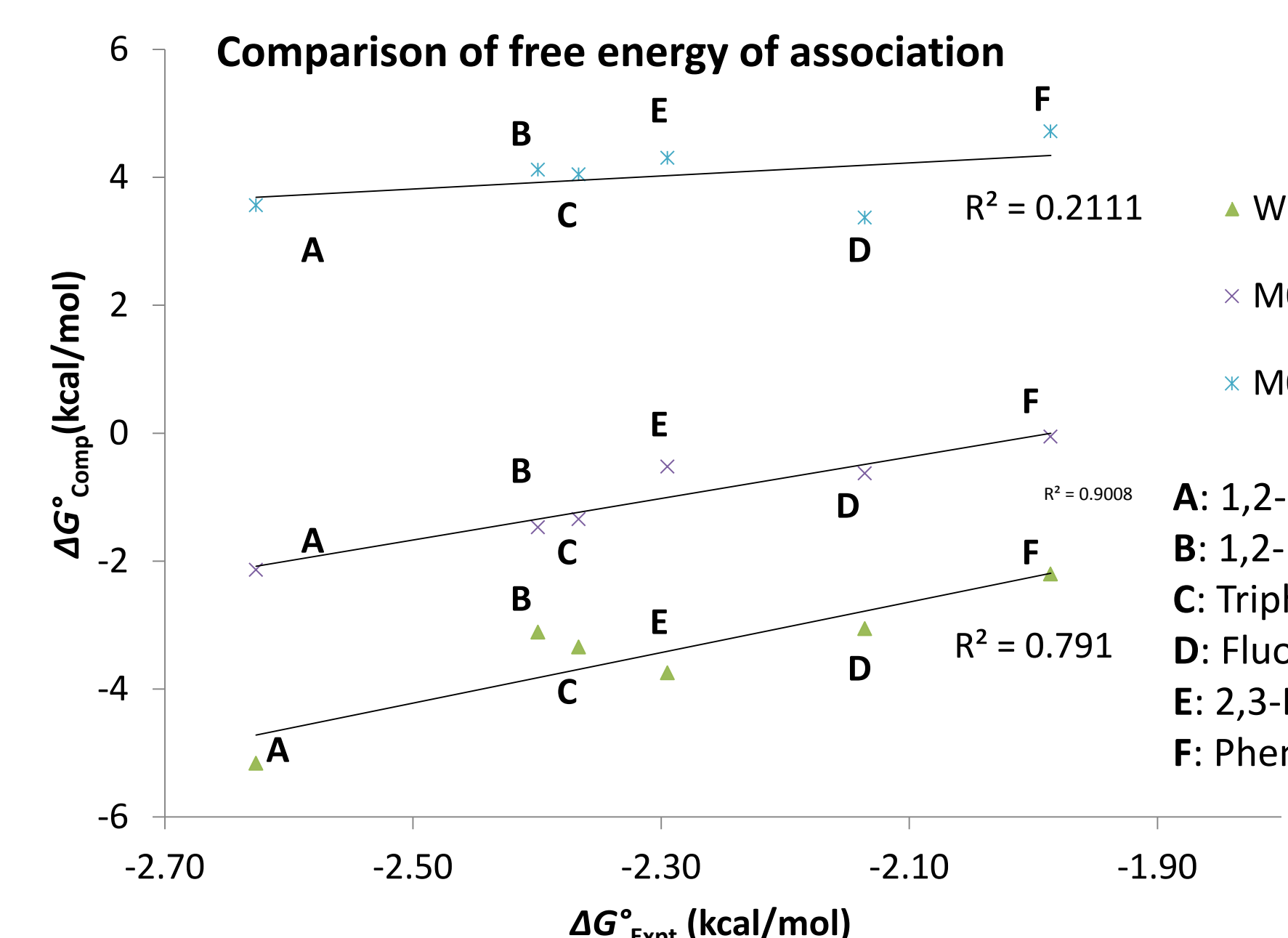
Basis set: 6-31 + g(d, p)

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

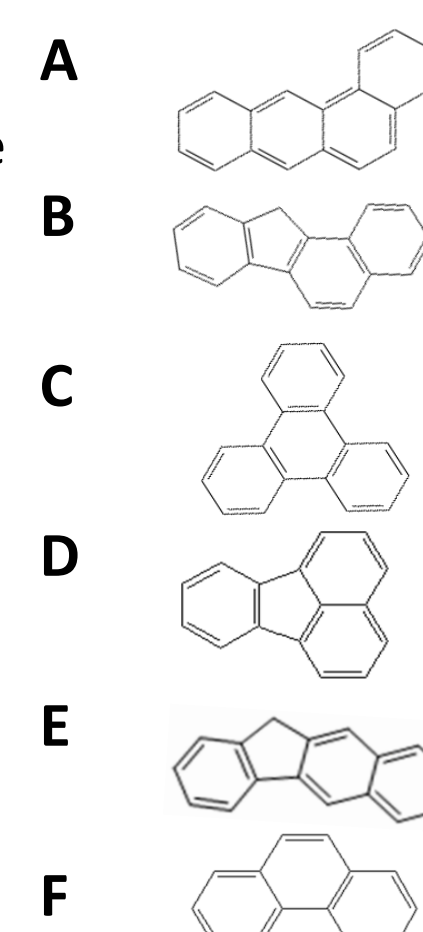
## Results

### Binding Study of Lumiflavin and Aromatics

#### DFT



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<sup>4</sup>,  $\Delta G^\circ_{Expt}$  (kcal/mol). Experimental results indicate M06-2X having the best correlation with experimental results with an  $R^2$  of 0.9008.

## DFT Calculations of Binding Free Energies in Ethanol

Compound	M06-2X	M06HF	M05	M06	WB97X-D	Experimental <sup>4</sup>
1,2-benzanthracene	-2.13	-1.05	3.56	3.09	-5.16	-2.63
1,2-benzofluorene	-1.47	-1.44	4.12	4.26	-3.11	-2.40
Triphenylene	-1.34	-2.02	4.04	2.78	-3.34	-2.37
Fluoranthene	-0.62	-1.66	3.37	2.53	-3.06	-2.14
2,3-benzofluorene	-0.52	-2.29	4.30	1.93	-3.75	-2.29
Phenanthrene	-0.05	-1.52	4.72	3.67	-2.20	-1.99

## Gas-phase Free Energies Calculated by DFTB vs. DFT

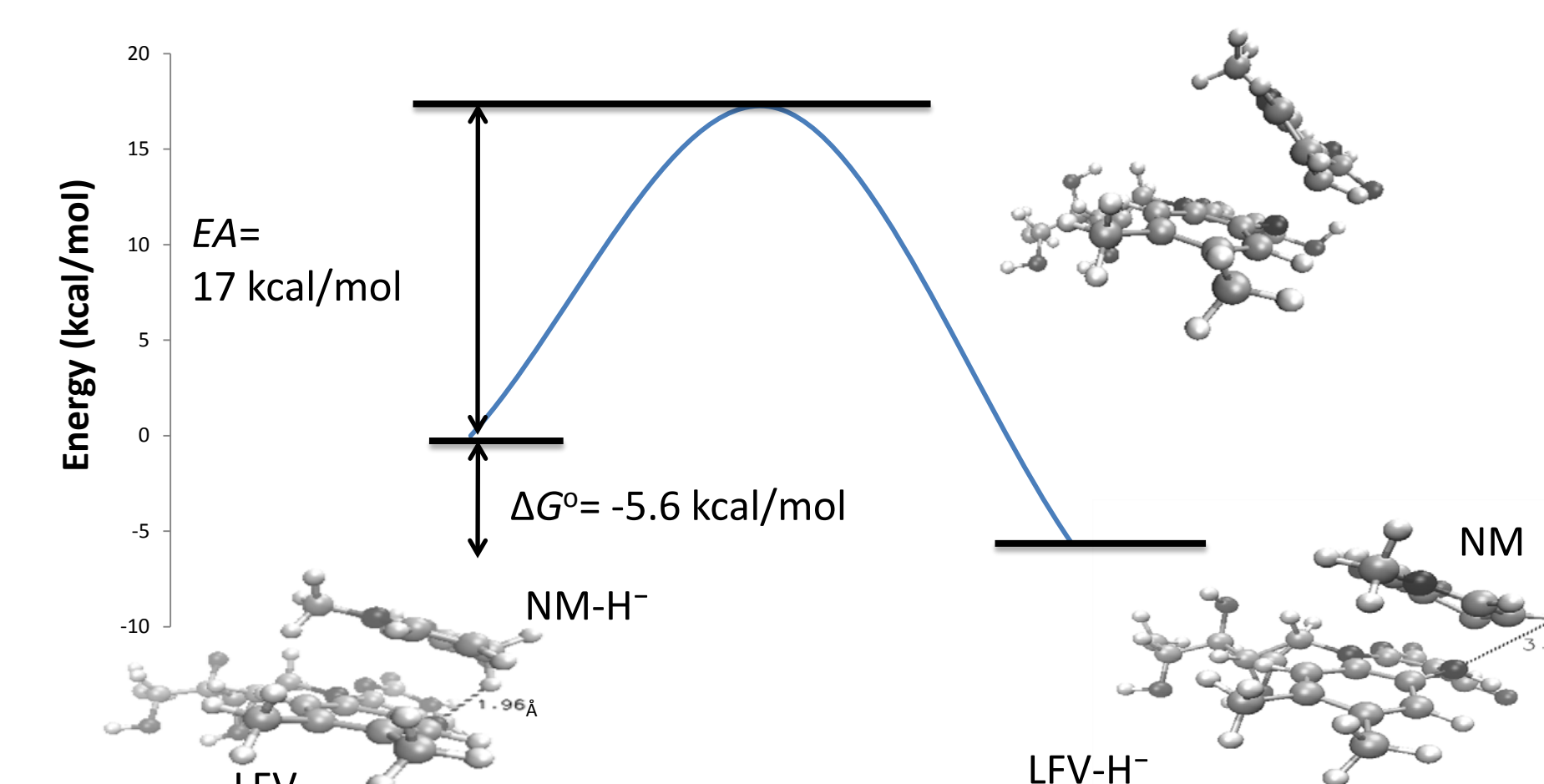
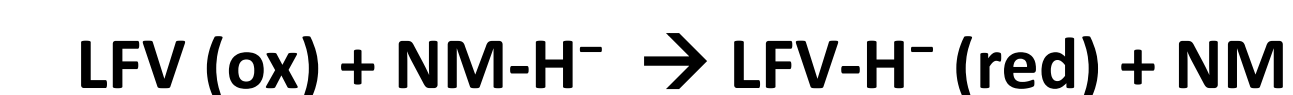
Compound	M06-2X	$\Delta G$ SCC-DFTB
1,2-benzanthracene	-5.65	8.16
1,2-benzofluorene	-6.28	8.79
Triphenylene	-6.28	5.65
Fluoranthene	-3.77	8.16
2,3-benzofluorene	-7.53	6.28
Phenanthrene	-3.14	10.0

DFTB gas-phase association energies are positive, implying the binding does not occur. SCC-DFTB may not be a preferred method to calculate binding free energies for molecules with  $\pi$ - $\pi$  stacking interactions.

All energies are given in (kcal/mol)

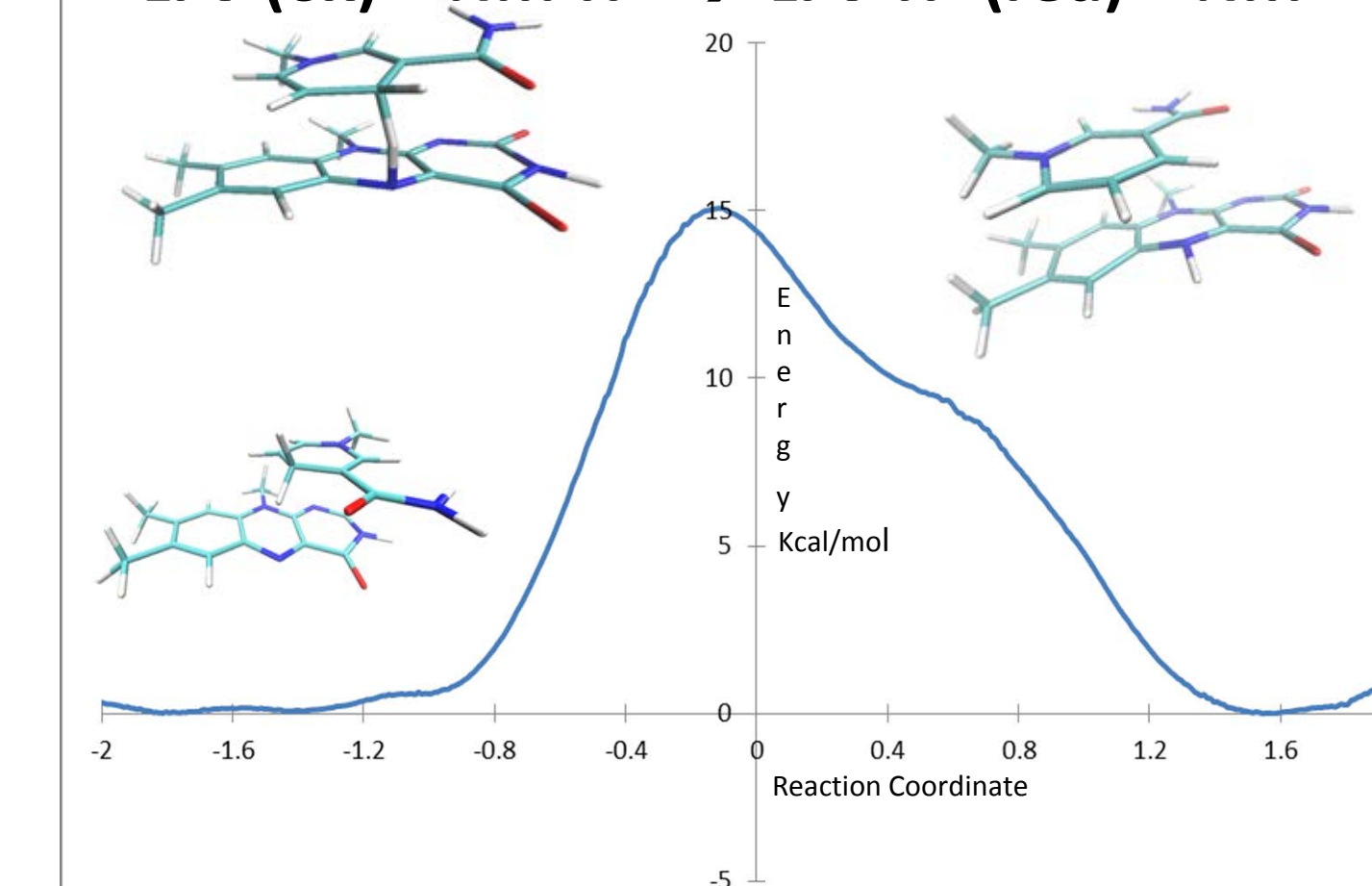
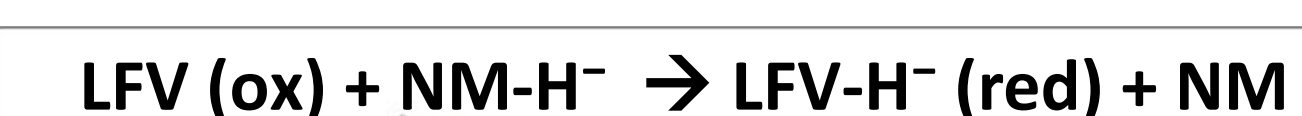
## Free Energy Calculations of Hydride Transfer

### DFT

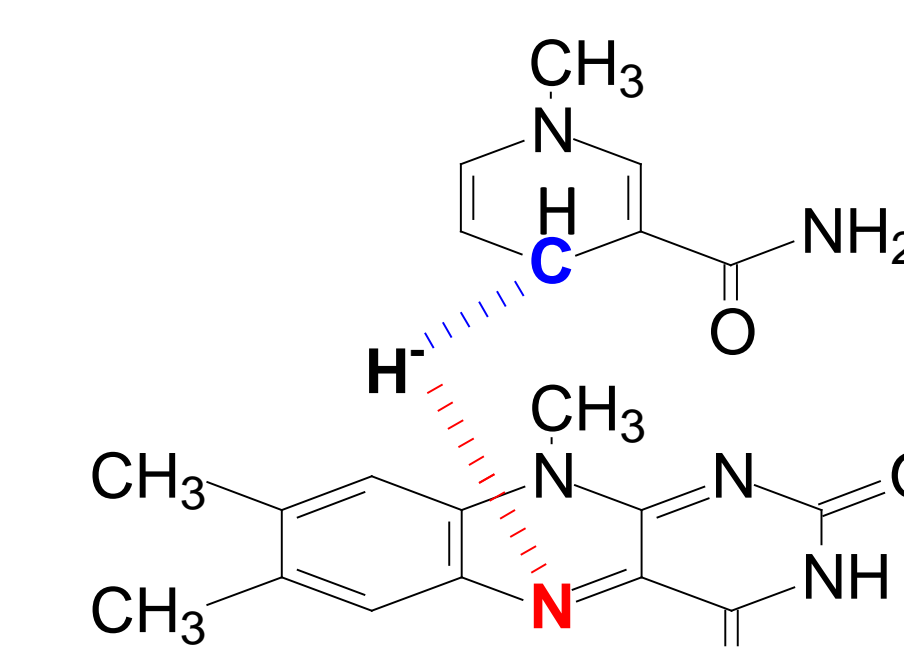


The computed Gibbs free energy of reaction (using M06-2X) was found to be equal to -5.6 kcal/mol while the Gibbs free energy of activation was 17 kcal/mol. Experimental value of the Gibbs free energy of activation barrier is 16.3 kcal/mol.<sup>5</sup>

### DFTB



The computed activation energy using SCC-DFTB resulted to be 15kcal/mol. This is 1.7kcal/mol smaller than experimental value of 16.3kcal/mol.



Rxn Coord. = [R-CH] - [R-NH]

Reaction Coordinate: the lumiflavin and the nicotinamide were set four angstroms away from each other, setting the midpoint at zero. With each bin we moved the hydride 0.4 angstroms away from the nicotinamide and closer to the flavin.

## Conclusions

- DFT studies with intermolecular complexes between lumiflavin and other aromatic molecules show that M06-2X is capable of reproducing  $\pi$ - $\pi$  (aromatic) interactions with moderate accuracy.
- Based on the DFT and DFTB calculations, the activation energy barrier for the 1-methyl nicotinamide to lumiflavin hydride transfer reaction are 17 and 15 kcal/mol, respectively. Experimental values have shown that the activation barrier energy is 17 kcal/mol.<sup>5</sup>

### Future directions-

We are working to correct the dftb-calculated activation barrier using a higher-level method such as DFT. Also, attempts are being made to extend the calculations into quinone reductase enzyme.

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