

Supporting Information for Hole Hopping through Cytochrome P450

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Comparing Absolute Reduction Potential to Experiment

Electrochemical experiments determine the reduction potential of tryptophan (Trp) with respect to some reference. We have calculated the absolute reduction potential, ΔE , of Trp. In order to compare these two numbers we look at the half cell reaction, for which we have calculated the absolute reduction potential:



To compare with experiment we need another half cell reaction with a known reduction potential vs. the NHE. We have chosen ferrocene, Fc/Fc^+ , as the reaction reference:



By using the same method as for the Trp calculations, we find the absolute reduction potential of this reaction to be 5.56 eV. This being the left half cell reaction we can calculate the reduction potential of Trp vs. Fc/Fc^+ as follows:

$$\Delta E_{vs.Fc} = \Delta E_{Trp} - \Delta E_{Fc} \quad (3)$$

The reduction potential of Trp vs. normal hydrogen electrode (NHE), $\Delta E_{vs.NHE}$, can then be calculated by adding the standard potential (in aqueous solution) of Fc/Fc^+ which is: $E_{Fc}^\ominus = 0.400 \text{ V vs. NHE}$.¹

Solvation

Using Cluster C we have studied the effect of calculations in vacuum or using a solvation model. We studied the difference in the absolute reduction potential between several cytochrome P450 (P450) geometries. Figure 2 shows the absolute reduction potential of the cluster for various geometries in vacuum and in a polarizable continuum model (PCM) dielectric medium.

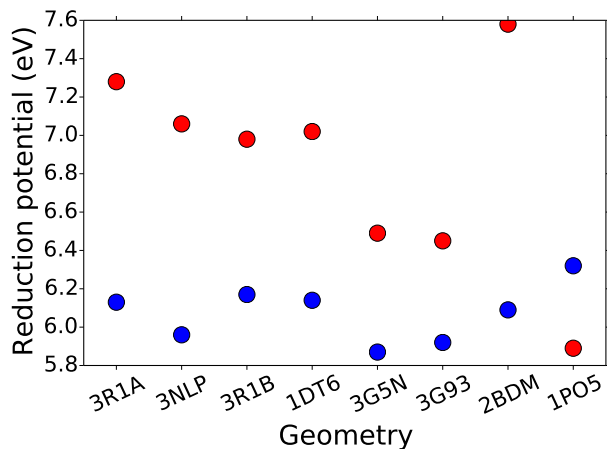


FIGURE. 1: The absolute reduction potential (eV) of various wild type geometries of P450 in Cluster C in both vacuum (red) and a PCM (blue).

In all cases, except one, adding a dielectric medium decreased the reduction potential. A dielectric medium adds a shielding between the molecules.

Cluster D

We have studied the distance dependence of the absolute reduction potential, for all three clusters containing arginine (Arg). Here We present the results for Cluster D.

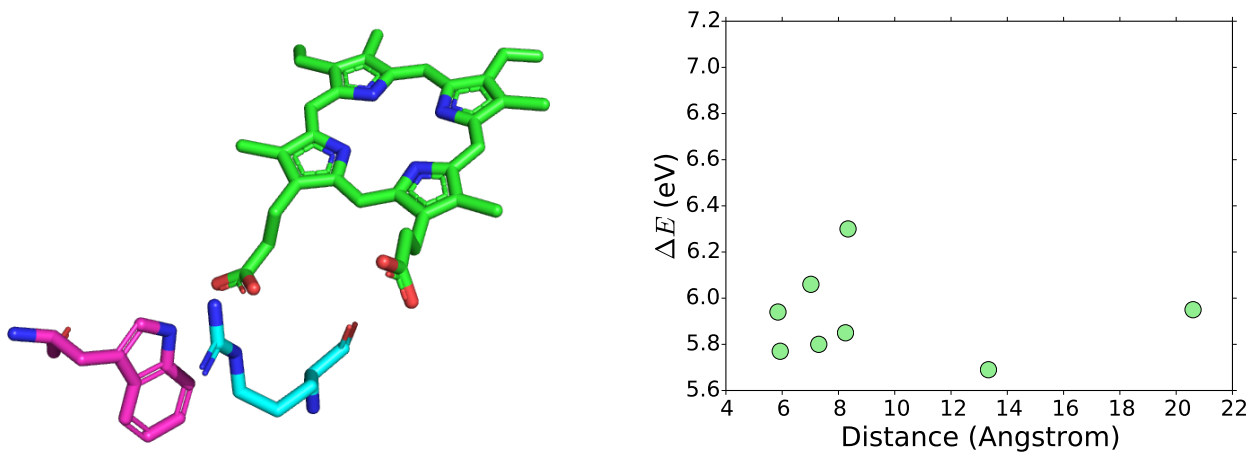


FIGURE. 2: (left) Cluster D. (Right) ΔE (eV) of Cluster D plotted against various P450 geometries shown in relation to the Arg-Trp distance (\AA).

There is no particular trend to see but generally the absolute reduction potential of Cluster D is lower than for Cluster B and Cluster C.

Reduction Potentials

All calculated reduction potentials of Trp both absolute (ΔE) and vs. NHE E_{Trp} . Calculated for all clusters, geometries, mutations, and solvations.

Table 1: Absolute reduction potential of Trp (eV), ΔE , and reduction potential (eV vs. NHE), E_{Trp} for Cluster A.

Geometry	ΔE (eV)	E_{Trp} (eV vs. NHE)
3npl	6.22	1.06
3R1A	6.24	1.08
3R1B	6.35	1.19

Table 2: Absolute reduction potential of Trp (eV), ΔE , and reduction potential (eV vs. NHE), E_{Trp} for Cluster B.

Geometry	ΔE (eV)		E_{Trp} (eV vs. NHE)	
	<i>Arg</i>	<i>His</i>	<i>Arg</i>	<i>His</i>
3npl	7.06	6.39	1.89	1.23
3R1A	7.16	6.25	2.00	1.09
3R1B	6.94	6.33	1.78	1.17
1PO5	6.53		1.37	
3G5N	6.67		1.51	
3G93	6.69		1.53	
2BDM	6.64		1.48	
1DT6	6.88		1.72	

Table 3: Absolute reduction potential of Trp (eV), ΔE , and reduction potential (eV vs. NHE), E_{Trp} for Cluster C.

Geometry	ΔE (ev)		E_{Trp} (eV vs. NHE)		
	<i>Arg</i>		<i>His</i>	<i>Arg</i>	<i>His</i>
	PCM	Vac.	PCM	PCM	PCM
3npl	5.96	7.06	5.27	0.80	0.10
3R1A	6.13	7.28	5.16	0.97	0.00
3R1B	6.17	6.98	5.12	1.00	-0.04
1PO5	6.32	5.89		1.16	
3G5N	5.87	6.49		0.71	
3G93	5.92	6.45		0.75	
2BDM	6.09	7.58		0.93	
1DT6	6.14	7.02		0.98	

Table 4: Absolute reduction potential of Trp (eV), ΔE , and reduction potential (eV vs. NHE), E_{Trp} for Cluster D.

Geometry	ΔE (ev)		E_{Trp} (eV vs. NHE)	
	<i>Arg</i>	<i>His</i>	<i>Arg</i>	<i>His</i>
3npl	5.77	5.17	0.62	0.01
3R1A	5.94	4.95	0.78	-0.21
3R1B	6.06	4.91	0.90	-0.25
1PO5	5.95	5.07	0.79	-0.10
3G5N	5.85		0.69	
3G93	6.30		1.14	
2BDM	5.69		0.52	
1DT6	5.80		0.64	

Geometry Distances

Table 5: Distance between the center of mass of molecules in Cluster B.

Geometry	Distance (Å)
	Trp-Arg
3npl	5.93
3R1A	5.85
3R1B	7.03
1PO5	20.60
3G5N	8.24
3G93	8.34
2BDM	12.87
1DT6	7.29

Table 6: Distance between the center of mass of molecules in Cluster C.

Geometry	Distance (Å)		
	Trp-Arg	Trp-acid	Arg-acid
3npl	5.92	5.83	3.94
3R1A	5.85	5.96	3.91
3R1B	7.02	4.84	3.83
1PO5	20.60	17.88	4.51
3G5N	8.24	6.75	3.75
3G93	8.34	6.97	3.86
2BDM	13.33	12.10	3.57
1DT6	7.29	6.65	3.54

Table 7: Distance between the center of mass of molecules in Cluster D.

Geometry	Distance (\AA)		
	Trp-Arg	Trp-Por	Arg-Por
3npl	5.93	12.49	9.03
3R1A	5.85	12.50	9.14
3R1B	7.02	10.13	8.88
1PO5	20.60	21.80	9.06
3G5N	8.25	12.19	8.56
3G93	8.34	12.18	8.53
2BDM	13.33	16.32	8.92
1DT6	7.29	13.07	8.11

Gaussian Keywords

For the FCD calculations we ran a single point calculation using the keywords: `pop=full iop(3/33=1)`.² The `pop=full` keyword specifies that all orbitals should be printed. The `iop(3/33=1)` keyword indicates that one-electron integrals should be printed.

For the POD calculations we ran a single point calculation using the keywords: `pop=full iop(5/33=3,3/33=1)`. The `iop(5/33=3)` prints the Fock matrix in the AO basis.

Coupling Elements for Histidine Mutations

The FCD python script is designed to automatically choose the MOs of interest. Therefore, the bias of handpicking the MOs is reduced. The eight highest occupied and the eight lowest unoccupied MOs are chosen for investigation by the script. Now, couplings of donor MOs (DMOs) and acceptor MOs (AMOs) can be started. First of all, the DMO and AMO can not be the same. Then, a contribution function of the investigated MOs at a fragment, $\gamma(F, MO)$, is calculated. The contribution function is the fraction of a MO localized at the fragment by using the atomic orbital coefficients:

$$\gamma(F, MO) = \frac{\sum_{k \in F} c_{MO,k}^2}{\sum_l^{AO} c_{MO,l}^2} \quad (4)$$

where AO is the number of atomic orbitals and $c_{MO,k}$ is the k th atomic orbital from the linear combination of atomic orbitals to describe the MO .

The contribution function is used for a weight function, which compare the localization of the MO versus the number of the MO compared to the frontier orbitals. The weight function of a MO at a fragment, $w(F, MO)$, is defined as the contribution function with a decrease of 5% as the number of the MO increase or decrease compared to the frontier orbitals.

$$w(F, MO) = \gamma(F) - w_{FMO} |N_{MO} - N_{FMO}| \quad (5)$$

where N_{MO} is the number of the MO, N_{FMO} is the number of the closest frontier orbital either HOMO or LUMO and $w_{FMO} = 0.05$ is the decrease of the contribution compared to the difference in the number of the MO and frontier orbital.

The highest weight functions of the AMOs and DMOs are then coupled. Furthermore, the energy difference of the AMO and DMO can not be less than $E_{AMO} - E_{DMO} < -0.001$ Hartree and the charge replacement, $\sqrt{\Delta q^2 + 4\Delta q_{12}}$, from the denominator of the FCD electronic coupling Eq. must be higher than 0.100. The AMO and DMO with the highest weight

functions and which fulfill the requirements are chosen.

Table 8

Geometry	Coupling		FCD Orbitals	
	POD (meV)	FCD (meV)	Donor	Acceptor
3npl	21.54	7.30	237	236
		21.34	237	233
3R1A	72.75	32.82	237	234
		78.69	237	235
3R1B	20.60	18.96	237	235
1PO5	0.00	0.00	236	234

We saw that with another choice of FCD orbitals the coupling match very well, also for the 3npl and 3R1A geometries.

References

- (1) *CRC Handbook of Chemistry and Physics*; CRC Press, 1992.
- (2) Cioslowski, J. A new population analysis based on atomic polar tensors. *J. Am. Chem. Soc.* **1989**, *111*, 8333–8336.