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Computational Docking Simulations of Nitroanisole and Nitrophenol with CYP2E1

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SENIOR THESIS APPROVAL

This Honors thesis entitled

“Computational docking simulations of nitroanisole and nitrophenol with CYP2E1”

written by

Kasa B. Cooper

and submitted in partial fulfillment of
the requirements for completion of
the Carl Goodson Honors Program
meets the criteria for acceptance
and has been approved by the undersigned readers.

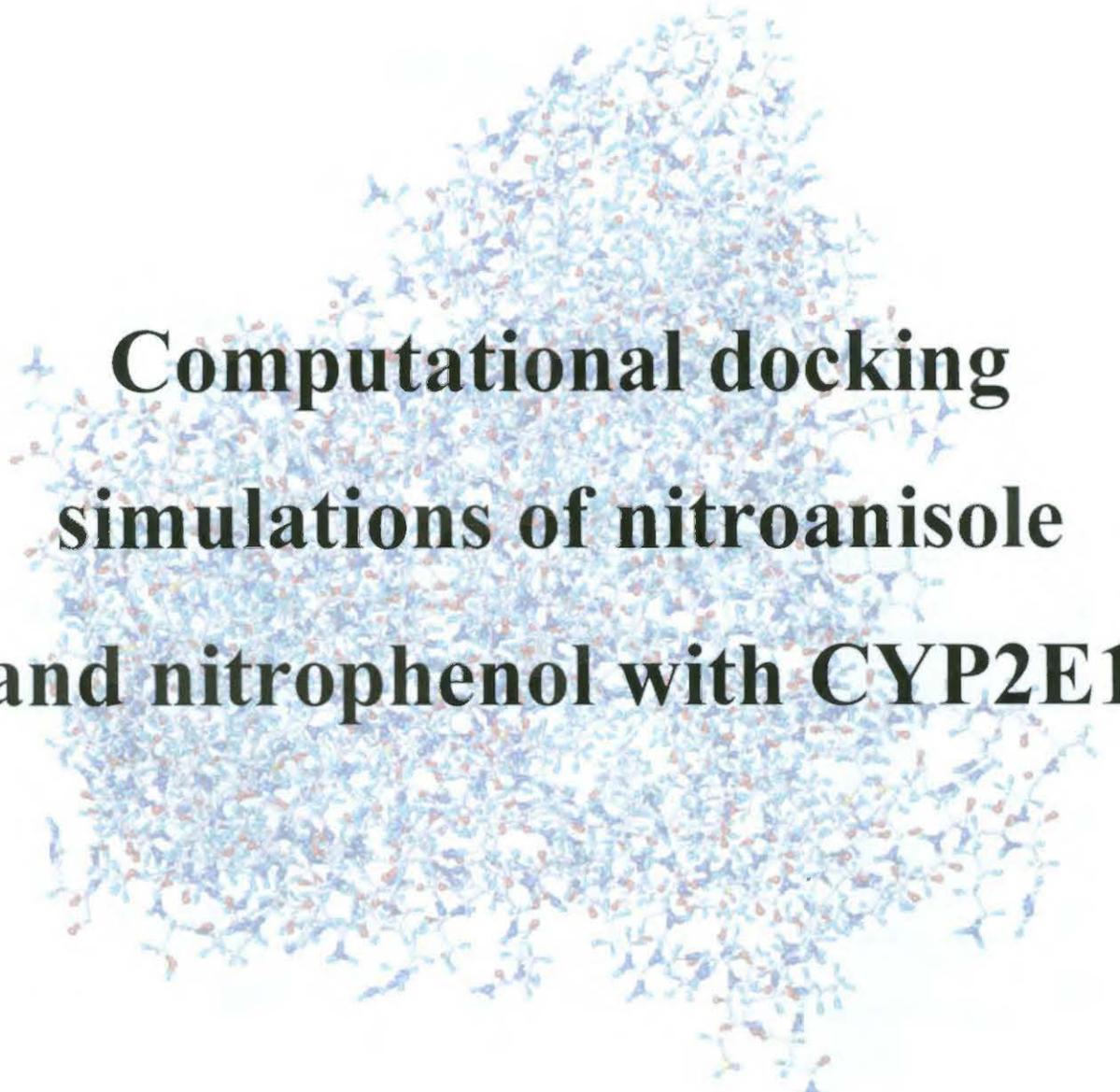
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April 16, 2012



Computational docking simulations of nitroanisole and nitrophenol with CYP2E1

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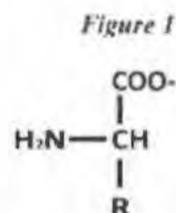
Abstract

Studies have shown that CYP2E1, a cytochrome P450 enzyme containing two primary binding sites, plays a substantial role in the oxidative metabolism of many foreign substances, including the detoxification reaction of 4-nitroanisole to 4-nitrophenol. Through the advancements of the computational docking software Tripos Sybyl7.2, it has been possible to investigate the mechanism of oxidation of 4-nitroanisole. Docking modules of Sybyl7.2 software, including Surflex and molecular dynamics, have created the ability to ascertain the likely binding configurations of 4-nitroanisole and its constitutional isomers in either the distal or proximal binding sites of CYP2E1. Knowing the relative binding relationships of 4-nitroanisole to the heme of the CYP2E1 enzyme, further experimentation may be conducted to determine the actual role that CYP2E1 plays in human oxidative metabolism of xenobiotic substances. The goal in this research was to observe the configuration of nitroanisole and its derivatives in relation to the heme of the enzyme and possibly determine a cause for the unconventional reaction kinetics of CYP2E1.

Introduction

Enzymes

Enzymes, like other proteins, are composed of amino acids linked together by peptide bonds. A chain of amino acids is called a polypeptide. The basic amino acid structure consists of an amine group, a carboxyl group, a hydrogen atom, and a variable R group attached to a carbon atom (Figure 1). The different natures of the R groups determine the characteristics of the amino acid, such as whether an amino acid is polar, nonpolar, charged, or aromatic. Because each class of amino acid behaves differently in aqueous environments, the sequence of amino acids in a protein is critical to the behavior of the polypeptide structure and ultimately its function.

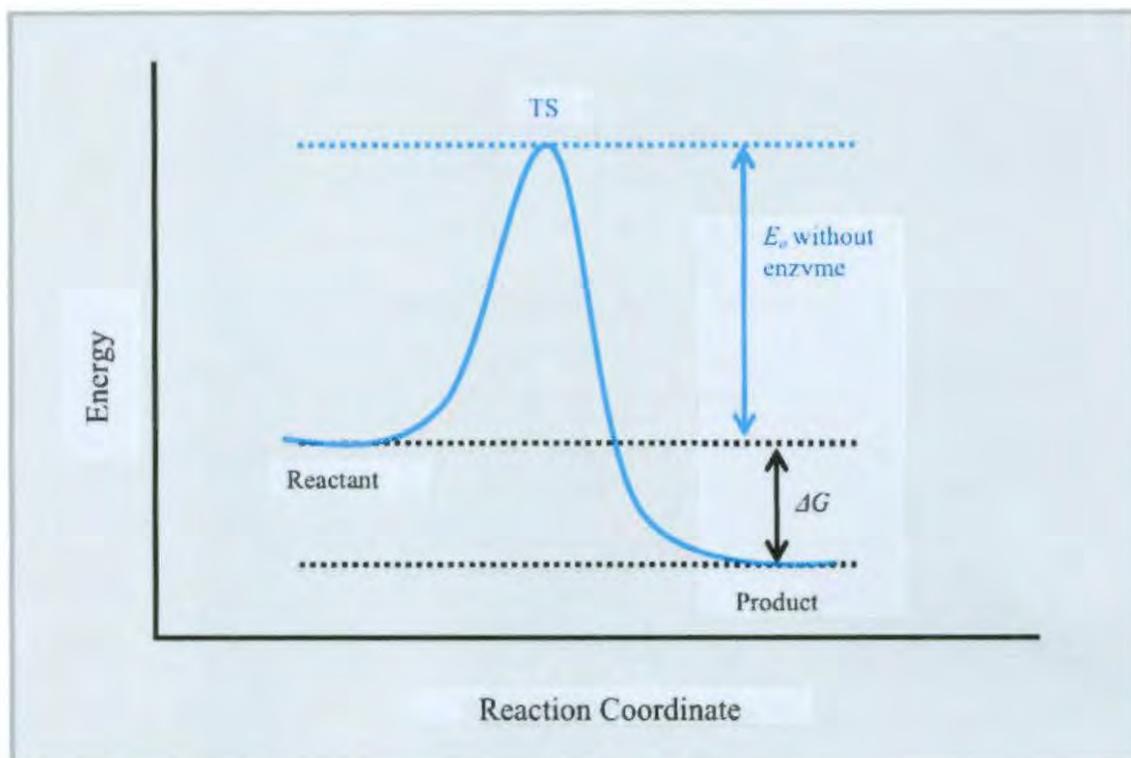


Generic structure of an amino acid.

Enzymes in the human body are proteins that are capable of catalyzing biological reactions. Reactants and products in a reaction both have characteristic free energies, G' . The change in free energy of a reaction, $\Delta G'$, represents the difference in the free energy of the products and reactants, $\Delta G' = G'_{\text{products}} - G'_{\text{reactants}}$. If the change in free energy of a reaction is positive, the reaction is not spontaneous, or will not occur naturally. If the free energy of a reaction is negative, the reaction will occur; it will result in a release of energy and occurs spontaneously.

However, even if a reaction is spontaneous, the reaction may not occur quickly. Many biological reactions, though spontaneous, do not occur at a sufficient rate to support life. For example, the breakdown of glucose to carbon dioxide and water has a very negative change in free energy (-2840 kJ/mol), and is therefore considered spontaneous. But the half-life of this reaction for sugar on a shelf is extremely long. Sugar can sit on a shelf for years without any appreciable breakdown. Living organisms require that the breakdown of sugar occur in a matter of seconds or minutes, not years. The reason the reaction does not happen quickly in the absence of enzymes is that a certain amount of energy is required to start the reaction, like an investment. There will be a net release of energy, but an initial energy source must be supplied in order to make the reaction proceed. This initial energy requirement is called the activation energy, E_a . A generic reaction coordinate diagram may be seen below in Figure 2.

Figure 2

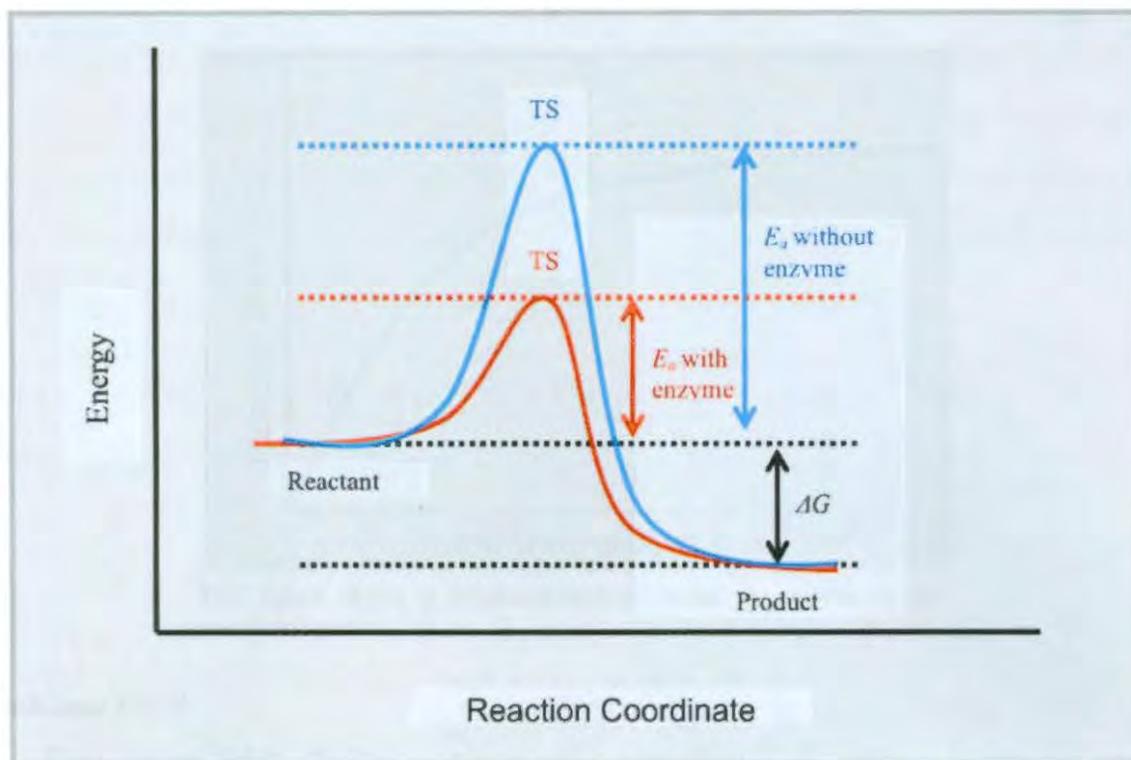


Reaction coordinate diagram detailing the energetics of a general reaction. E_a is the activation energy, ΔG is the net change in energy, and TS is the transition state of the reaction.

The state of the molecule at the peak of the energy curve, labeled TS in diagram above, is called the transition state. The transition state is the unstable conformation of the molecule undergoing reaction. This state may be unstable for many reasons. For example, the transition state may contain an atom with more bonds than spatially allowed, or it may contain a radical atom.

The transition state is energetically stabilized in the presence of a catalytic enzyme, lowering the activation energy of the reaction. Van der Waals interactions, hydrogen bonding, and ionic interaction between the ligand and protein stabilize the transition state. When the transition state is more easily reached, the reaction is more likely to proceed. Figure 3 shows a generic reaction coordinate diagram with reactions in the presence and in the absence of a catalytic enzyme.

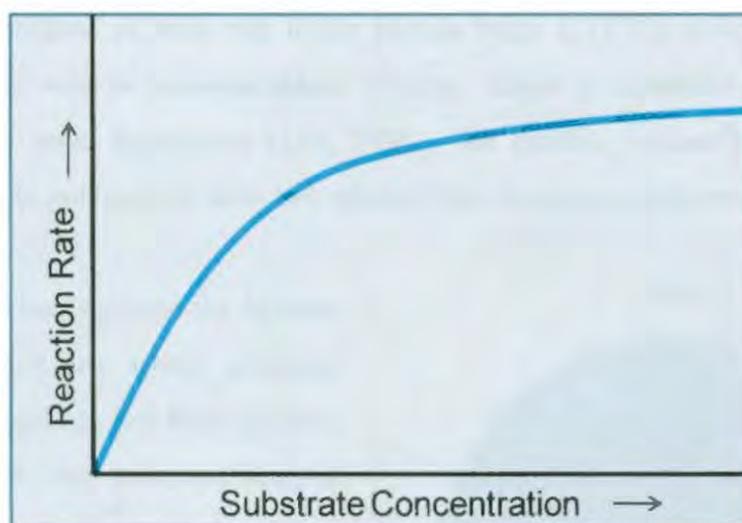
Figure 3



Reaction coordinate diagram detailing the energetics of a general reaction. E_a is the activation energy, ΔG is the net change in energy, and TS is the transition state of the reaction. Blue indicates reaction in the absence of enzyme, and red indicates reaction in the presence of enzyme.

Reactions in the presence of enzymes usually follow a characteristic pattern with increasing substrate concentration. Increasing the substrate concentration results in an increase in reaction rate because more substrates are available to the enzyme for catalysis. However, as substrate begins to saturate the enzyme, the rate of reaction reaches a plateau because the enzyme is unable to catalyze the substrate faster. Figure 4 shows a general Michaelis-Menten diagram of reaction rate versus substrate concentration.

Figure 4



This figure shows a Michaelis-Menten model for enzyme-ligand reaction kinetics.

Cytochrome P450s

Cytochrome P450 (P450s) enzymes are a superfamily of enzymes known to catalyze oxidation reactions of organic substances, including lipids and hormones that exist in the body as metabolic intermediates. P450 enzymes also take part in gluconeogenesis, which is the production of sugar. Gluconeogenesis can help to combat periods of starvation.

Other substances whose oxidation is catalyzed by the P450 enzymes include xenobiotic compounds, which are compounds found in an organism that are not normally expected to be present or compounds present in unusually high quantities. Xenobiotic substances include drugs, food additives, environmental contaminants, and toxins (Winn 2002). One major motivation for the study of P450 enzymes over the past twenty years has been “the quest for information to better understand and predict the metabolism and toxicity of drugs and other chemicals” (Guengerich, 2008).

In many cases, P450s metabolize toxic compounds into more water-soluble compounds, which are then more easily excreted from the body through urinary and renal pathways (Gonzalez, 2005). P450 enzymes are present in nearly all organisms; in humans they are concentrated in the liver, where they are usually attached to surface of the endoplasmic reticulum of cells (Goodsell, 2001).

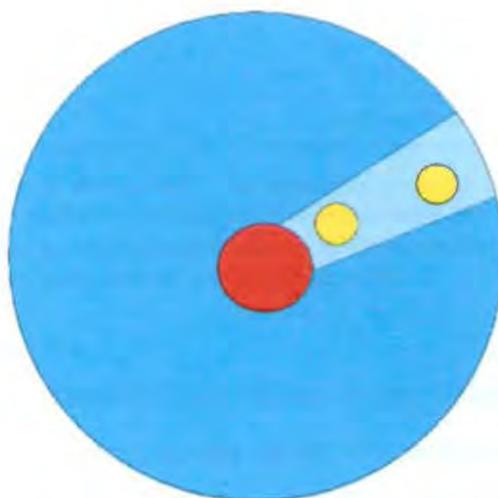
CYP2E1

The enzyme CYP2E1, a P450 cytochrome, and its protein-ligand interactions are of particular interest because of their role in the human body. CYP2E1 is known, perhaps most prominently, for its role in acetaminophen toxicity, where it catalyzes the metabolism of acetaminophen into toxic byproducts (Lee, 1996). The protein consists of 476 amino acids arranged into a single polypeptide with two chains. The secondary structures of the peptides are mostly α -helices.

The channel leading from the enzyme surface to the active site where chemical reactions are catalyzed has two binding sites, only one of which, the proximal site, is catalytic (Figure 5). A distal site is located farther up in the channel, away from the active site. Binding has been observed in each site in previous computational docking simulations of CYP2E1. At the end of the channel is the heme, which directly interacts with the substrate during oxidation. The substrate travels down the channel and binds in one of the two binding sites. Binding in the proximal site may potentially result in metabolism of the substrate.

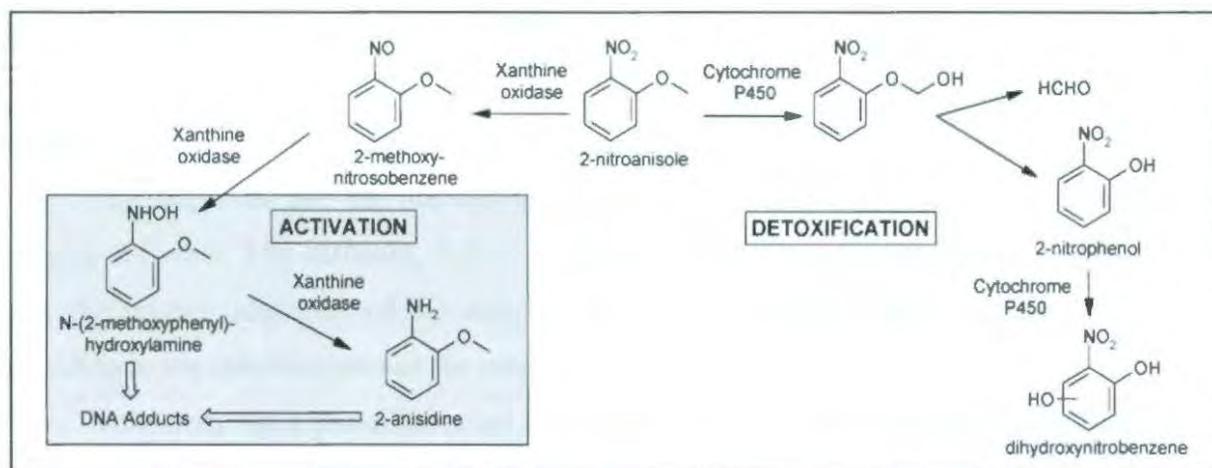
In this study, CYP2E1 was studied for its interactions with nitroanisoles and nitrophenols. Nitroanisoles may be introduced to the body through environmental pollutants and are potentially carcinogenic. Through oxidative metabolism, CYP2E1 breaks nitroanisoles down into nitrophenols and then to less harmful, more water-soluble molecules, as seen in Figure 6.

Figure 5



Cartoon outline of CYP2E1. Dark blue indicates the residues of the enzyme. Red indicates the location of the heme. Yellow indicates the locations of the proximal and distal sites in the channel (light blue).

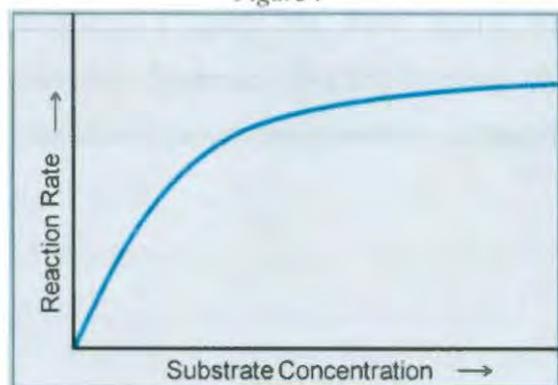
Figure 6



Reaction diagram of activation and detoxification pathways of nitroanisole. The activation pathway takes place in the absence of cytochrome P450s and results in the formation of DNA adducts, compounds that may result in DNA mutations and cancer. The detoxification pathway results in the oxidation of the nitroanisole to nitrophenol and then to dihydroxynitrobenzene, which is more water-soluble and easily excreted.

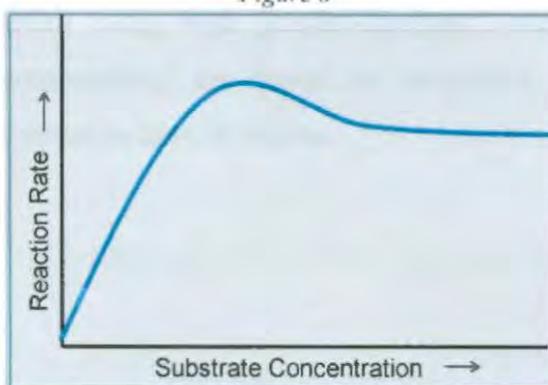
Wet labs indicate that CYP2E1 does not follow the Michaelis-Menten model of enzyme-ligand reaction kinetics when catalyzing the oxidation of nitroanisoles. In the Michaelis-Menten model, as the substrate concentration increases, the reaction rate increases in a hyperbolic manner. CYP2E1 also experiences an increase in the reaction rate with substrate concentration increase, but only to a certain point. At this point, reaction rates decrease asymptotically. The difference in general Michaelis-Menten reaction kinetics and the kinetics observed with CYP2E1 may be seen by comparing Figures 7 and 8.

Figure 7



This figure shows a Michaelis-Menten model for enzyme-ligand reaction kinetics.

Figure 8



This figure shows a CYP2E1 model for enzyme-ligand reaction kinetics.

The goal in this research was to observe the configuration of nitroanisole and its derivatives in relation to the heme of the enzyme and possibly determine a cause for the unconventional reaction kinetics of CYP2E1.

Methods

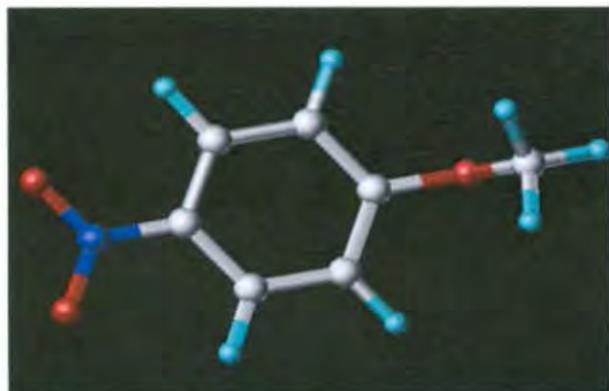
The inquiries into the mechanisms of CYP2E1 were carried out using computational docking software. The software, Sybyl7.2, employs three-dimensional modeling. The program uses the protein sequence of the enzyme and assigns partial charges and hydrogen atoms according to the specifications of the user.

This study used pre-constructed coordinates from the RCSB Protein Data Bank (PDB) (www.pdb.org) to investigate the docking tendencies of CYP2E1, specifically the 3E4E structure. The RCSB PDB is a large online database where crystal structures of proteins may be downloaded for use in modeling investigations. Preparation of the crystal structure involved removal of one chain of the protein, removal of water molecules, addition of hydrogen atoms, and application of appropriate charges. The imidazole in the crystal structure active site was removed for molecular dynamics simulations.

The CYP2E1 crystal structure was used to examine the interaction of selected substrates and the enzyme. Computational docking simulations by other students have indicated the presence of multiple binding sites. These two sites, the distal site and the catalytic proximal site, are located in a major channel extending from the enzyme surface to the heme active site of the enzyme.

The ligands docked in the CYP2E1 structure are 4-nitroanisole (Figure 9) and 4-nitrophenol (Figure 10). Each ligand was studied using both Surflex-Docking Suite and molecular dynamics. Surflex-Docking Suite computations are based on energetics while molecular dynamics computations are based on Newtonian laws of motion.

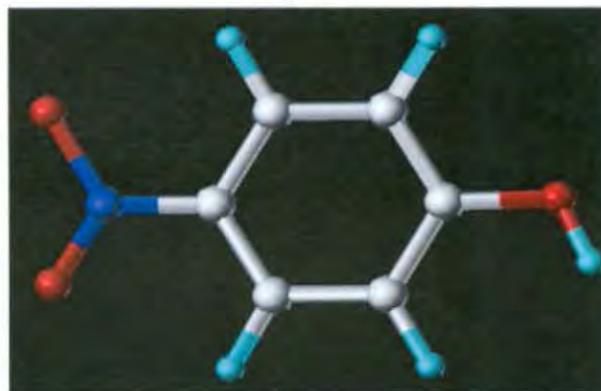
Figure 9



4-nitroanisole

The nitro group is on the left and the methoxy group is on the right. Red is oxygen, blue is nitrogen, white is carbon, and aqua is hydrogen.

Figure 10



4-nitrophenol

The nitro group is on the left and the hydroxy group is on the right. Red is oxygen, blue is nitrogen, white is carbon, and aqua is hydrogen.

In Surflex-Docking Suite, the substrate was placed into a defined area in the enzyme called a protomol. The protomol was generated based on the location of the ligand 4-methyl imidazole, which existed in the enzyme when downloaded from the Protein Data Bank. After the specified ligand was placed into the protomol, the program gave ten optional orientations of the substrate in relation to the heme based on energetics. The orientation used for comparison of results was the one given the best energy rating.

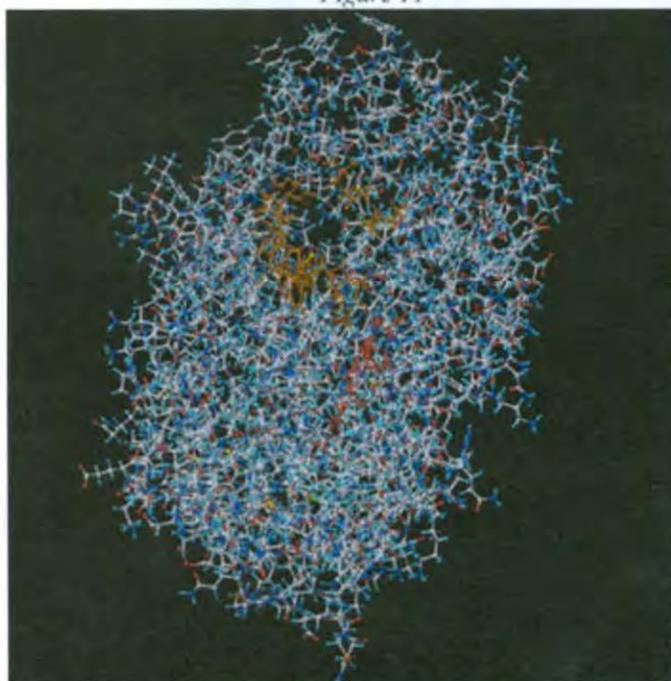
Molecular Dynamics began with the positioning of the substrate at the mouth of the channel. The structures were then allowed to interact freely for 2 picoseconds to equilibrate. The next step involved forcing the substrate down a channel into the enzyme to eventually arrive within the proximal site over a period of 200 picoseconds. The mouth of the channel used in these studies is surrounded by the following amino acid residues: ASN 219, GLN 75, GLN 216, LEU 103, LEU 368, PHE 478, PRO 104, VAL 364, and VAL 388 (Winn 2002). After the substrate arrived in the active site, all constraints were released for 200 more picoseconds to allow the substrate to move into its most likely position in relation to the heme. The process of releasing the molecules to find their lowest energy state is called minimization. After minimization, the structures were viewed to detect hydrogen bonding in the active site between the substrates and amino acid residues.

The following details the test sequence in molecular dynamics simulations:

1. Ligands were positioned in the mouth of the channel

2. Three-step docking sequence where ligands are forced to travel down the channel of the enzyme:
 - a. 2 picoseconds to equilibrate structure at 50K
 - b. 200 picoseconds at 50K with a constraint between the OCH₃/OH group and the Iron atom of the heme
 - c. 200 picoseconds at 100K with constraints removed
3. Minimized docked complexes to achieve configuration with least energy
4. Analyzed by distance from heme, position in relation to the heme, and existence of hydrogen-bonding in the docked complex

Figure 11



View of CYP2E1 3E4E crystal structure in Sybyl7.2. The heme is colored red, and the residues surrounding the entrance to the channel are colored orange.

The channel used was outlined in orange for all molecular dynamics simulations to insure that the same channel was used throughout the modeling runs.

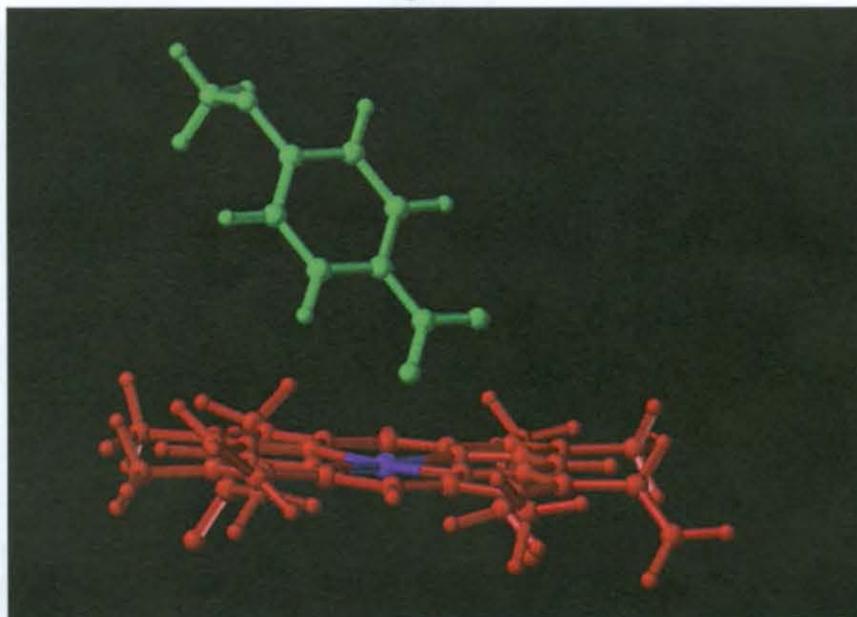
Results

Both Surflex-Dock and Molecular Dynamics results were analyzed by measuring the distance between the substrate and the iron atom of the heme group (shown as a purple iron in a red heme in result figures). These measurements were taken between the nitrogen atom of the nitro group and the oxygen atom of the methoxy or hydroxy group of nitroanisole and

nitrophenol, respectively. The first ligand is colored green, the second is colored magenta, the third is colored yellow, and the fourth is colored purple in the results section in all result images.

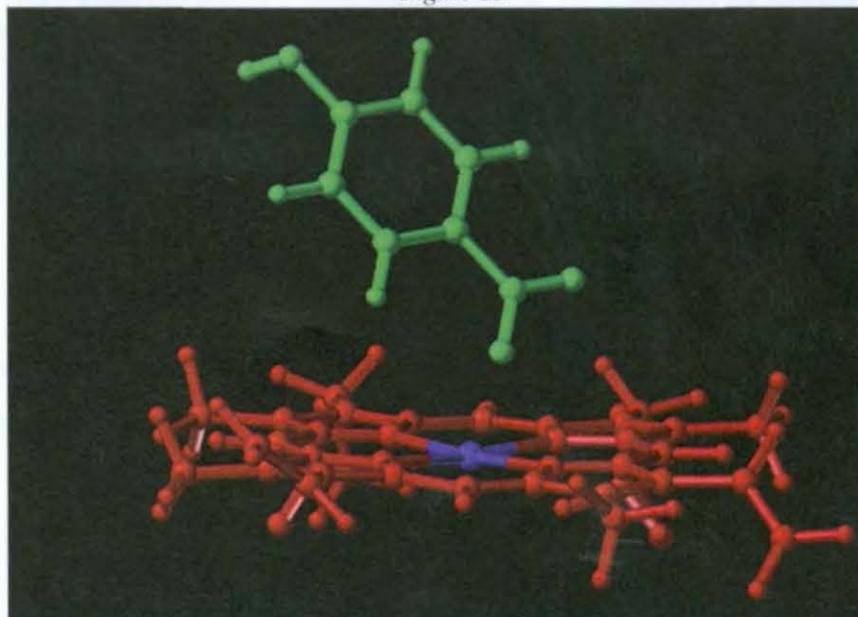
SURFLEX-DOCK

Figure 12



This figure shows a singly docked 4-nitroanisole and the heme. The distance of the oxygen from the heme is 7.768 Å and the nitrogen is 3.137 Å.

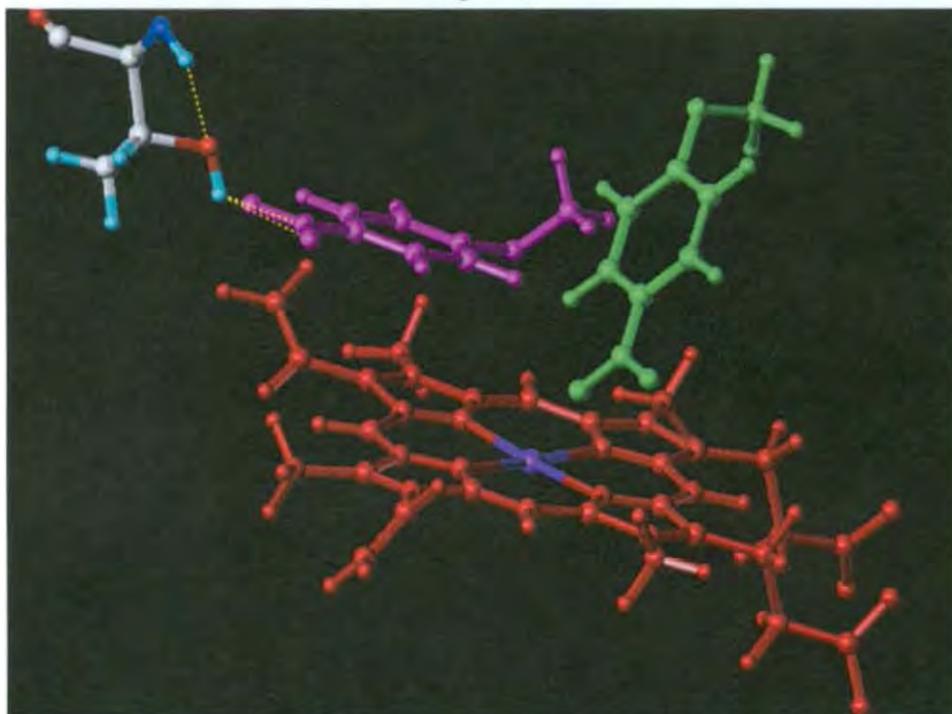
Figure 13



This figure shows a singly docked 4-nitrophenol and the heme. The distance of the oxygen from the heme is 7.656 Å and the nitrogen is 3.111 Å.

The nitro groups of both 4-nitroanisole and 4-nitrophenol are oriented toward the heme in the most stable docking configuration results. No hydrogen bonding was observed for the single-ligand Surflex-Dock of 4-nitroanisole or 4-nitrophenol.

Figure 14



This figure shows two docked 4-nitroanisole molecules. The distance of the nitrogen and oxygen of each ligand from the iron of the heme is listed.

First 4-nitroanisole

O: 7.768 Å

N: 3.137 Å

Second 4-nitroanisole

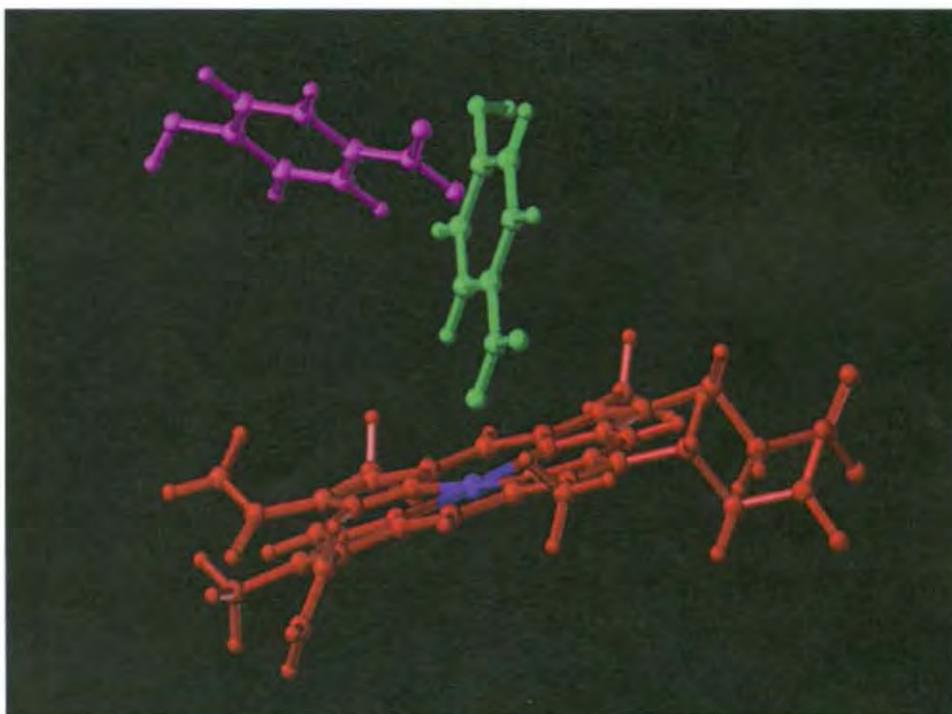
O: 7.527 Å

N: 10.283

(THR 307)

Hydrogen bonding is observed between the nitro group of the second 4-nitroanisole and the hydroxyl group of THR 307, a negatively charged amino acid residue.

Figure 15



This figure shows two docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron of the heme is listed.

First 4-nitrophenol

O: 7.656 Å

N: 3.111 Å

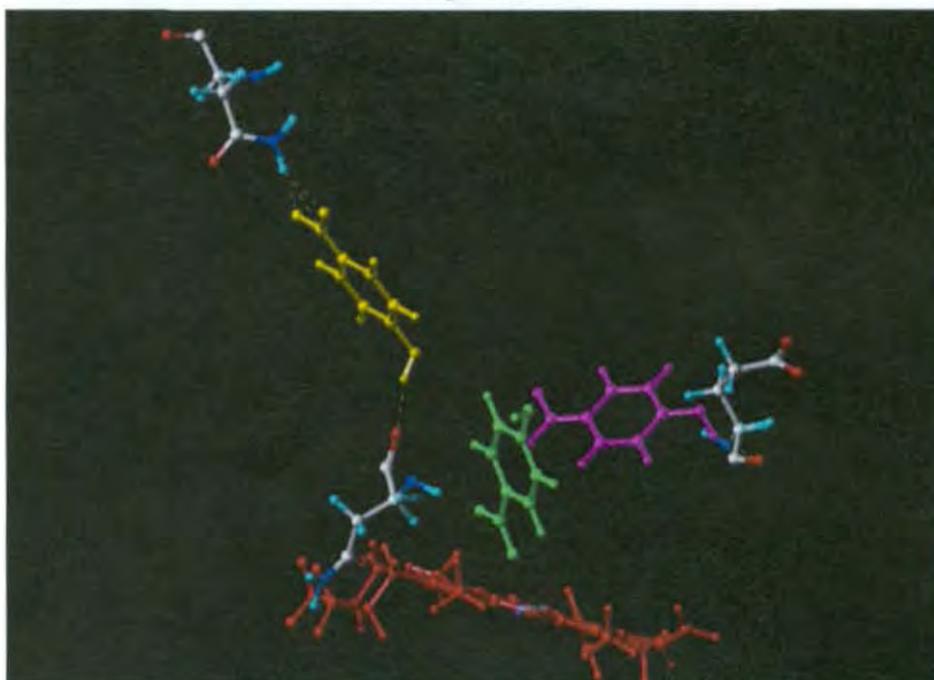
Second 4-nitrophenol

O: 9.590 Å

N: 6.541 Å

The second substrates dock farther from the heme than the first, possibly because the first substrate blocks the heme.

Figure 16



This figure shows three docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron is listed.

First 4-nitrophenol

O: 7.656 Å

N: 3.111 Å

Second 4-nitrophenol

O: 9.590 Å

N: 6.541 Å

(GLU 302)

Third 4-nitrophenol

O: 11.491 Å

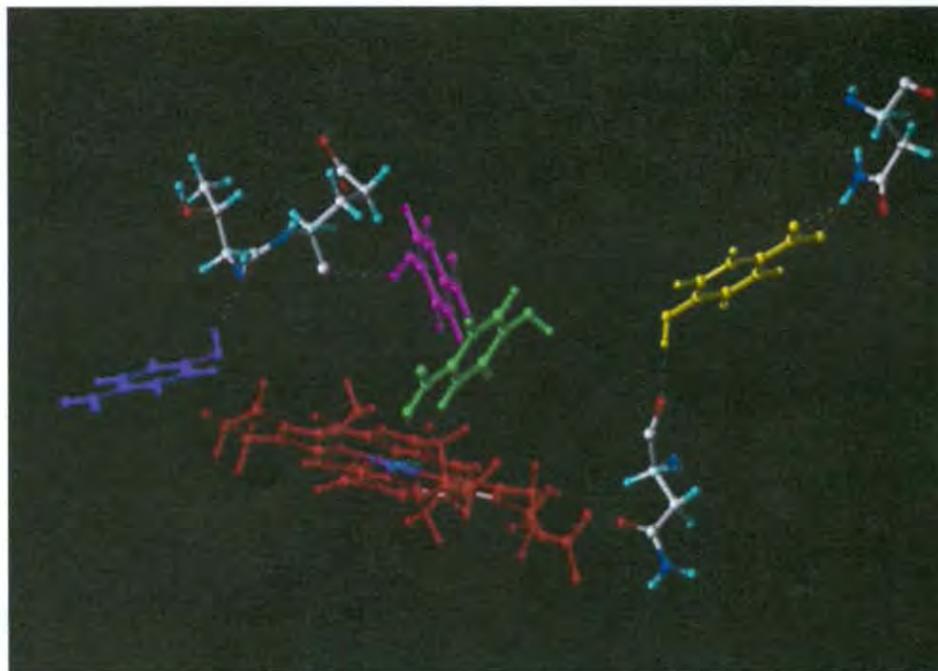
N: 16.747 Å

(ASN 220, ASN 367)

Hydrogen bonding occurs with second and third ligands

The third ligand docked in the distal site with the hydroxyl group oriented toward the heme. Hydrogen bonding was observed between the second and third ligands. The second ligand showed hydrogen bonding to GLU 302. The nitro group of the third ligand showed hydrogen bonding to an asparagine residue, as did the hydroxyl group.

Figure 17



This figure shows three docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron is listed.

First 4-nitrophenol

O: 7.656 Å

N: 3.111 Å

Second 4-nitrophenol

O: 9.590 Å

N: 6.541 Å

(GLU 302)

Third 4-nitrophenol

O: 11.491 Å

N: 16.747 Å

(ASN 220, ASN 367)

Fourth 4-nitrophenol

O: 8.486 Å

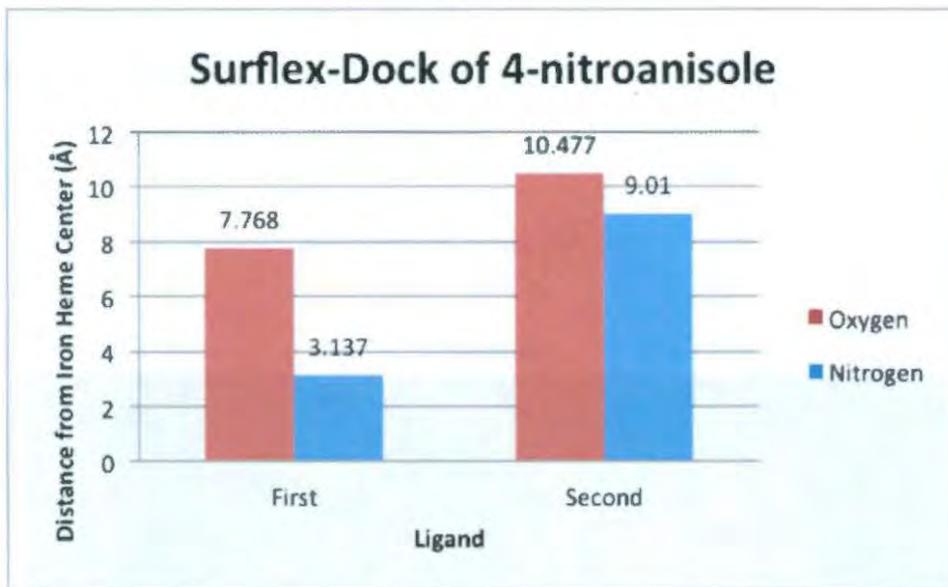
N: 11.441 Å

(THR 301)

Hydrogen bonding occurs with second, third, and fourth ligands.

The fourth ligand docked between the proximal and distal sites. Over-crowding of both sites may have resulted in the placement of the fourth ligand between sites. The fourth ligand also displays hydrogen bonding with a nearby residue, THR 301.

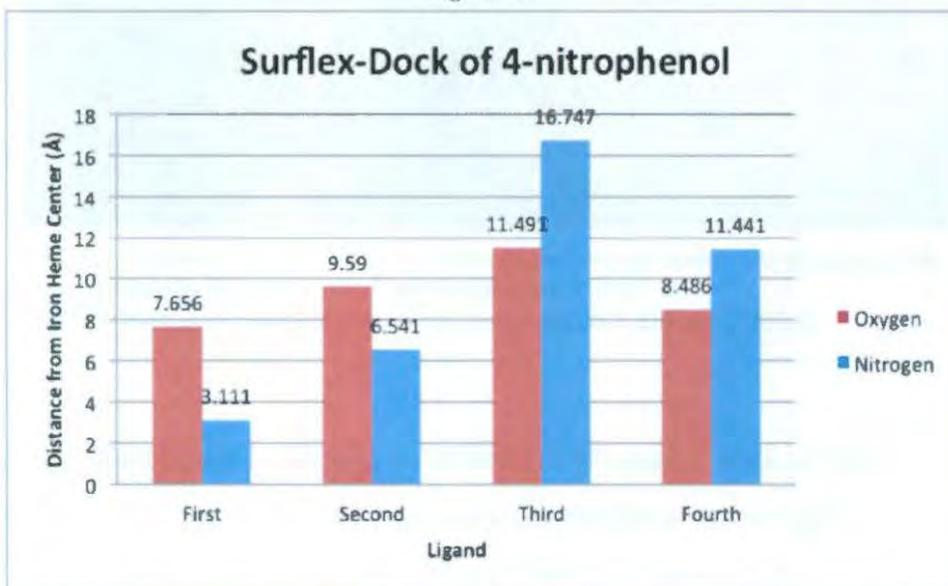
Figure 18



This figure shows the distance of each docked 4-nitroanisole ligand from the central atom of the heme.

The second 4-nitroanisole ligand docks a significant distance farther away from the heme than the first.

Figure 19



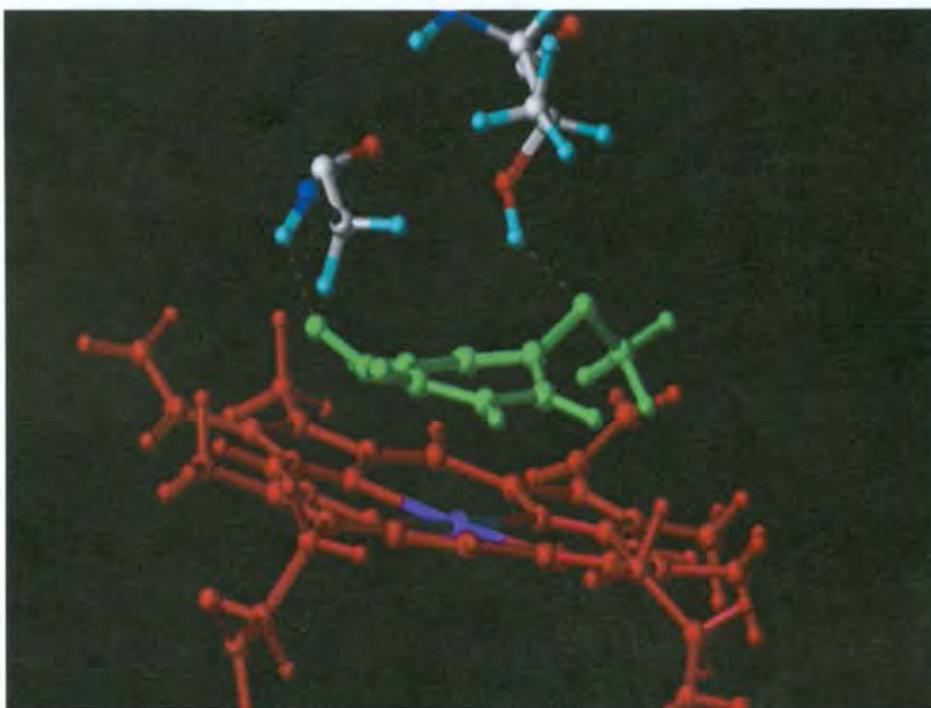
This figure shows the distance of each docked 4-nitrophenol ligand from the central atom of the heme.

The 4-nitrophenol ligands dock progressively farther away from the heme center, excepting the fourth ligand, which is forced between the second and third ligands.

Hydrogen bonding was observed in many Surflex-Dock results. Most results did not orient the methoxy or hydroxyl group close to the heme and therefore did not favor detoxification of the substrate.

MOLECULAR DYNAMICS

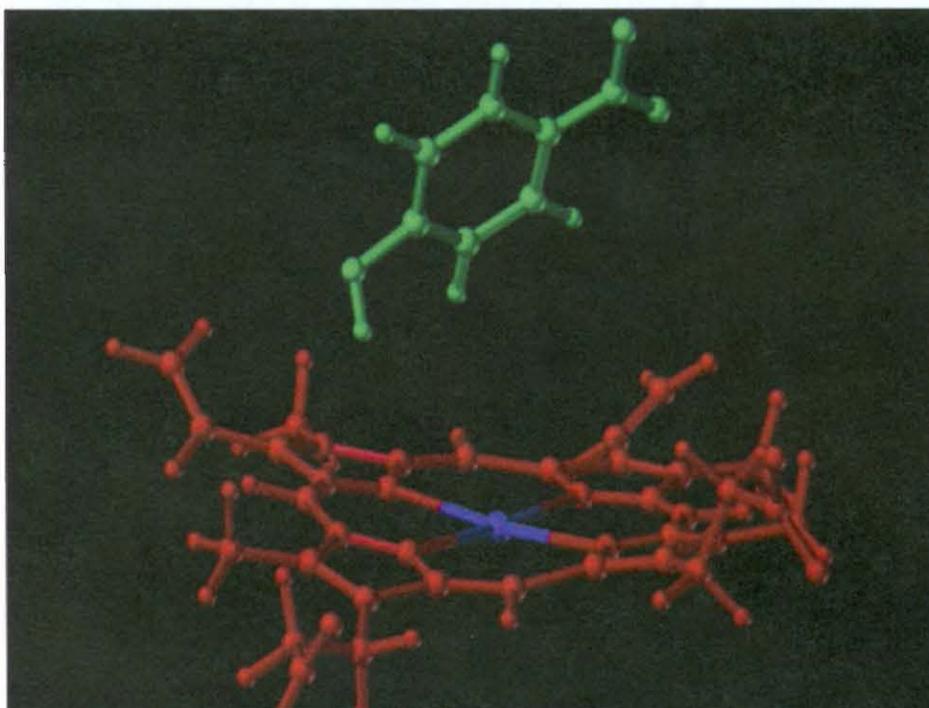
Figure 20



This figure shows a singly docked 4-nitroanisole and the heme. The distance of the oxygen from the heme is 4.362 Å and the nitrogen is 4.057 Å. There is hydrogen bonding between the ligand and GLY 300 and THR303.

The first 4-nitroanisole docked in Molecular Dynamics docked with the plane of the ligand parallel to the plane of the heme and in close proximity to the heme. This orientation is somewhat favorable for detoxification. The ligand shows hydrogen bonding with two adjacent residues. The methoxy group shows hydrogen bonding with THR 303, a residue thought to participate in the reaction mechanism of CYP2E1.

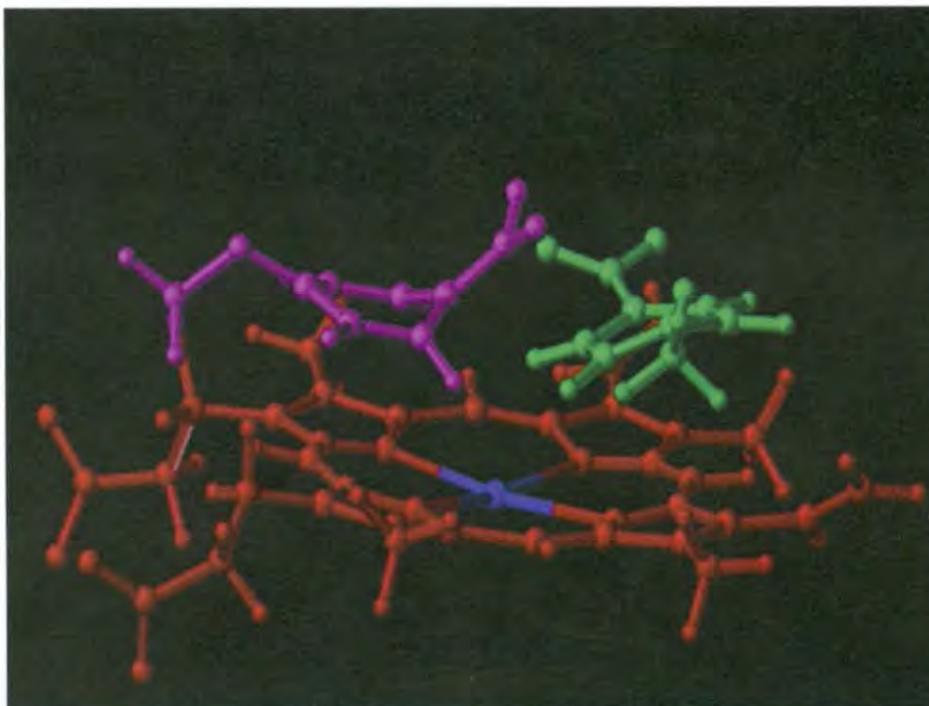
Figure 21



This figure shows a singly docked 4-nitrophenol and the heme. The distance of the oxygen from the heme is 5.726 Å and the nitrogen is 7.499 Å.

The first 4-nitrophenol docked with the hydroxyl group oriented toward the heme.

Figure 22



This figure shows two docked 4-nitroanisole molecules. The distance of the nitrogen and oxygen of each ligand from the iron of the heme is listed.

First 4-nitroanisole

O: 5.325 Å

N: 4.804 Å

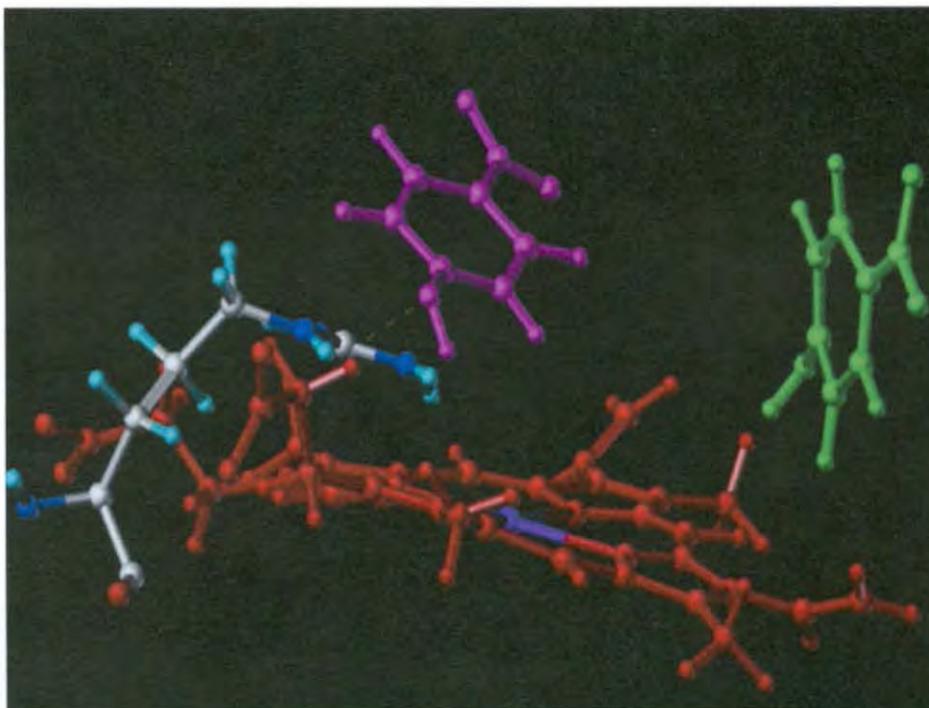
Second 4-nitroanisole

O: 6.288 Å

N: 4.026 Å

The second 4-nitroanisole also docked with the plane of the ligand parallel to the plane of the heme so that both ligands are oriented in this manner. In both cases the nitro group is slightly closer to the iron (III) of the heme than the methoxy group. No hydrogen bonding was observed with two 4-nitroanisole ligands docked in the enzyme.

Figure 23



This figure shows two docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron of the heme is listed.

First 4-nitrophenol

O: 6.666 Å

N: 9.180 Å

Second 4-nitrophenol

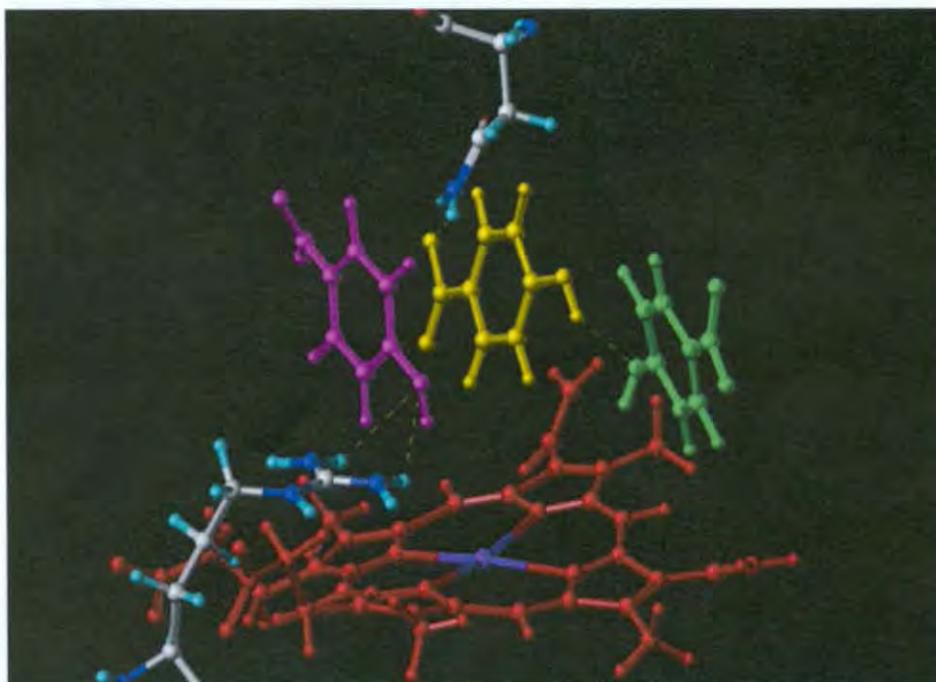
O: 4.699 Å

N: 9.451 Å

(ARG 435)

The second 4-nitrophenol docked with the hydroxyl group oriented toward the heme, favoring detoxification. Both ligands remain in the proximal site.

Figure 24



This figure shows three docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron is listed.

First 4-nitrophenol

O: 5.789 Å

N: 8.885 Å

Second 4-nitrophenol

O: 4.572 Å

N: 9.935 Å

(ARG 435)

Third 4-nitrophenol

O: 6.806 Å

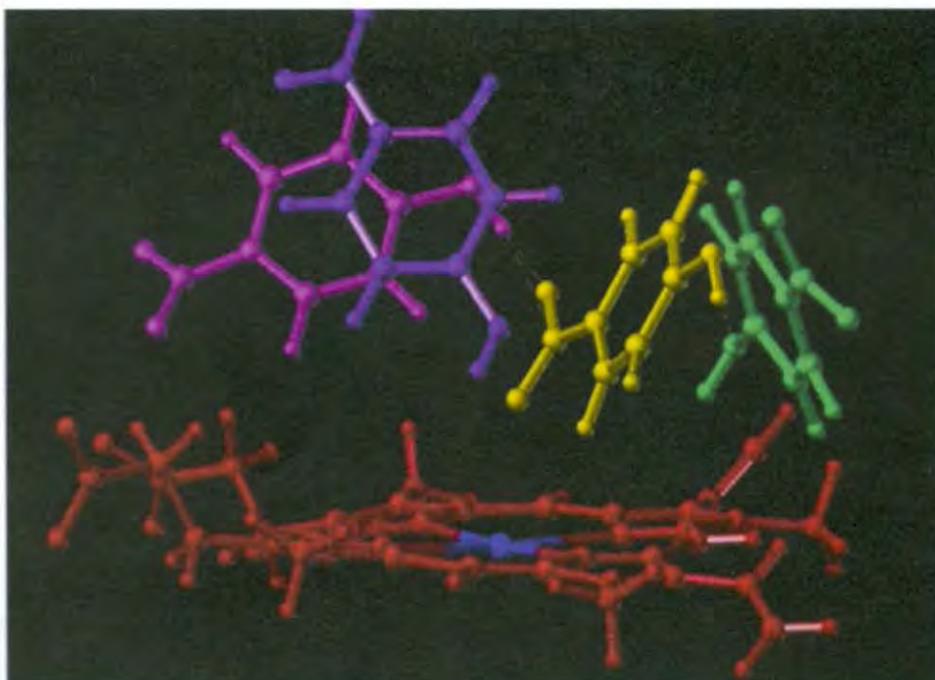
N: 8.521 Å

(ASN 117)

Hydrogen bonding occurs with all ligands.

The third nitrophenol was oriented in much the same way as the first two. The ligands appear to be stacking over the heme with the second ligand showing hydrogen bonding with ARG 435 and the third with ASN 117.

Figure 25



This figure shows four docked 4-nitrophenol molecules. The distance of the nitrogen and oxygen of each ligand from the iron is listed.

First 4-nitrophenol

O: 5.751 Å

N: 8.265 Å

Second 4-nitrophenol

O: 10.649 Å

N: 11.254 Å

Third 4-nitrophenol

O: 7.377 Å

N: 8.946 Å

Fourth 4-nitrophenol

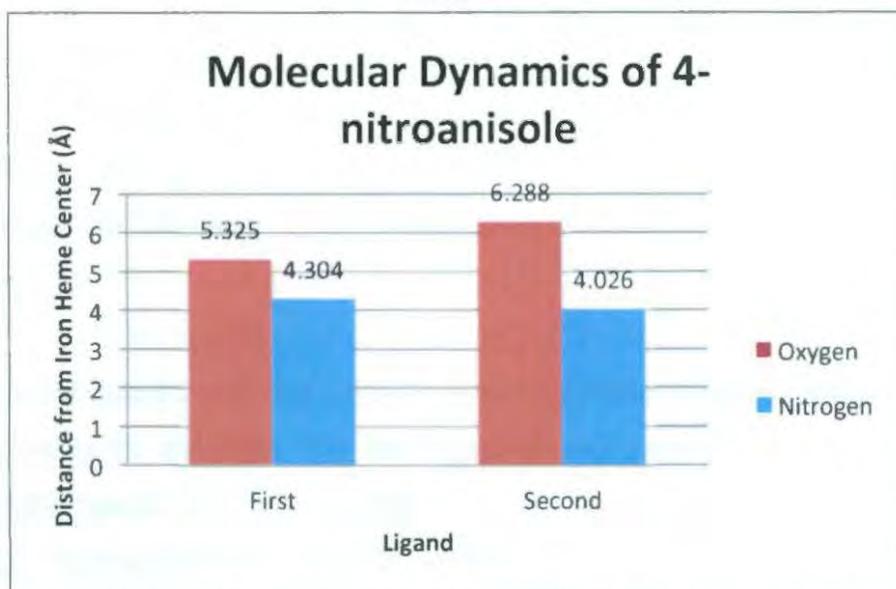
O: 5.734 Å

N: 10.506 Å

Hydrogen bonding occurs between ligands.

The fourth ligand docked over the heme like the first three, continuing the stacking pattern. The only hydrogen bonds observed at this point were between ligands.

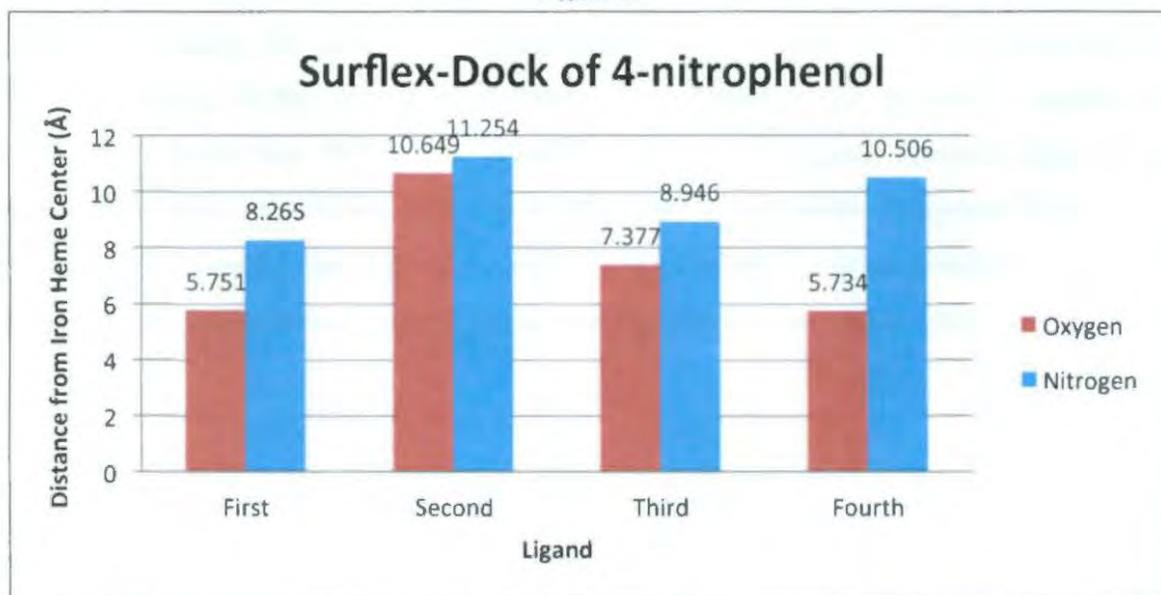
Figure 26



This figure shows the distance of each docked 4-nitroanisole ligand from the central atom of the heme.

The two 4-nitroanisole ligands docked at approximately the same distance from the heme center in approximately the same position. Both ligands were in a fairly favorable position for reaction since the methoxy groups were within a short distance from the heme.

Figure 27



This figure shows the distance of each docked 4-nitrophenol ligand from the central atom of the heme.

The first ligand docked with its methoxy group less than 6 Å from the heme, a fairly reasonable position for reaction. The second ligand appears to be docked in the distal site, with the third and fourth ligands located between the proximal and distal binding sites.

Results of molecular dynamics show orientations more favorable for detoxification of ligands, especially the nitrophenols, than Surflex-Dock orientations.

Conclusions

All docked ligands, both in Surflex-Dock and molecular dynamics simulations, preferred the proximal site to the distal site. The first ligand showed a tendency to dock in the proximal site with second ligands also binding near the heme, excepting the 4-nitroanisole molecular dynamics runs. Third and fourth ligands in molecular dynamics bound nearer to the heme than the second ligand. This result is likely due to the ability of the molecules to move in molecular dynamics simulations, as opposed to Surflex-Dock where the first ligands docked remain stationary as additional ligands are docked. The third and fourth ligands of molecular dynamics are unable to move farther up the channel because the second ligand still docked in the distal site blocks their movement.

In the molecular dynamics simulations, no ligands moved out of the channel of the enzyme once released. The hydrogen bonding of the ligands with the enzymes likely kept the ligands from exiting the channel. Hydrogen bonding indicates that the nitroanisoles and nitrophenols belong in the enzyme. Conversely, the tendency of the ligands to remain in the channel after release may indicate the possibility of existing ligands blocking entry of other molecules that might potentially enter the enzyme and be detoxified. If ligands fail to exit the enzyme quickly enough, they may block entry to the channel for other potential ligands. This might provide an explanation for the irregular reaction kinetics associated with CYP2E1.

Literature Cited

- Goodsell, David S. 2001. The Molecular Perspective: Cytochrome P450. *The Oncologist* 6(2):205-206.
- Winn, Peter J., Susanna K. Lüdemann, Ralph Gauges, Valère Lounnas, and Rebecca C. Wade. 2002. Comparison of the dynamics of substrate access channels in three cytochrome P450s reveals different opening mechanisms and a novel functional role for a buried arginine. *PNAS* 99(8):5361-5366.
- Gonzalez, Frank J. 2005. Role of cytochromes P450 in chemical toxicity and oxidative stress: studies with CYP2E1. *J Mutation Research* 569:101-110.
- Guegnerich, F. Peter. Cytochrome P450 and Chemical Toxicology. *Chem. Res. Toxicol.* 21(1):70-83.