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Handedness: Does it matter?

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By: Jessie Little
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Part I: The Beginning
Research Fellowships

Many research fellowships are SURF programs (summer undergraduate research fellowships). They are highly competitive. Students are selected through an application process. Preference is given to upperclassmen. Each student chosen works under a mentor, usually a member of the college faculty at the school. Every faculty member has their research project already selected. Most projects are continuations from previous years. The student becomes immersed in the project for an average of ten weeks during the summer. The duties of the student vary depending on the research project. Typically students are given a stipend to cover their living expenses for the summer. Much of their reward comes in the experience and knowledge gained throughout their research.

Feelings Before

A variety of emotions overwhelmed me, when I was told I had been chosen to participate in a research fellowship at Ouachita Baptist University under Dr. Marty Perry. I was excited about the opportunity to expand my knowledge base by doing research and the amount that I would learn. However, I was also nervous about working with a program that was unfamiliar to me. Having seen the presentation of the project from the previous year’s research student, I knew the research would be challenging to grasp conceptually.

Initially most research students have little to no knowledge about their assigned project. Thankfully, the first few weeks are devoted to gaining a firm foundation of
background information on the project. This helps each student feel comfortable and confident as they begin the experimental portion of their research.

**Different Than Expected**

When I got my acceptance letter to the summer research program at Ouachita Baptist University, I was expecting a long summer of eight hour days with a strict work schedule. What I got was a flexible relaxed environment. I could come and go as needed as long as my work was completed each day. Our little computer room became our home for the next ten weeks. My co-researcher and I played music on Pandora while we worked and ate our lunches together as our tests were running.

I was given the opportunity to take ownership of the project and become invested in it. We were able to confer with Dr. Miller’s students at UAMS regarding their own research projects. Dr. Miller and his students were working on several cytochrome p450 enzymes. We worked specifically with CYP2C9, also a cytochrome p450 enzyme. While we ran computer simulations and did the theoretical research, Dr. Miller's lab did the experimental work with the enzymes in the lab. I was pleased to discover the strong integration of the science community and how enjoyable it was sharing research results and giving helpful suggestions based on learned techniques.

**Living on Your Own**

Some research fellowships provide dorm housing for their students. At Ouachita we had to find an apartment to stay in for the summer. I stayed in the dorm apartment with a hall director. Living on campus made transportation easy. I could walk to my research lab every morning.
The first thing you learn about living on your own is that you need food. Going from food being provided for you by the school cafeteria to having to make each meal yourself can be fun and a challenge. I personally enjoy cooking. For me it was enjoyable to go grocery shopping and plan meals to make. I would typically make more than one portion, so I could have the leftovers for lunch the next day.

Since it was summer time, the majority of students were not on campus. Most of my time outside of research was spent hanging out with friends, enjoying the beautiful weather, and studying for the MCAT.

Another plus to doing research was that the weekends were wide open to travel and see friends and family. You did not have homework to do on the weekends or tests to study for unless it was by choice.

**Responsibility**

Part of having a summer research fellowship is working on a team. Each team member is responsible for doing their part of the work each day. Some students work faster than others, so some days one student would get done before the other. The main thing is to be responsible and get your own work done.

Also it is your responsibility to ask a question if you are unsure of something. Much time can be wasted if you proceed with your research when you are uncertain. Mentors understand that the information is new to students, and they want the quality of work that comes from clear comprehension.

If you are working with other students, you are responsible to help them in understanding the tasks if they are confused. Research is not a competition. Helping each other understand the project will increase efficiency and yield better results. We
are working together to learn more about the world and gain a better understanding of the way things work. Everything that we can do to help others also gain this understanding is advantageous to our group as a whole.

**Understanding**

An understanding of what you are researching comes with time. I remember my first week of research as a swarm of long words and codes that I didn’t understand. I did Sybyl (the computer program we worked with) tutorials for a week and still only had a weak understanding of all that the program could do.

By the end of my ten weeks as a research student, I felt very comfortable with the content and methods of my research. I could explain them in simple terms to others in a way that they could also understand.
Part II: The Research Paper
Computational Docking Analysis to Increase Understanding of Chiral Drug Metabolism

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Abstract

Pharmaceutical drugs are activated through a process called xenobiotic metabolism. Out of the drugs on the market, 50% are chiral, having two enantiomers. When this drug is metabolized, one of the enantiomers is typically preferred over the other. This unique specificity is thought to be a result of the structure of the protein. Interactions between different residues and the drug being metabolized may affect the movement and orientation of the drug as it moves down the channel of the protein to be oxidized. Further understanding of the metabolism of chiral drugs could possibly be used to predict how new drugs might react within a protein. The results of this study were determined using computational methods. Molecular dynamics in Sybyl-X 1.3 were used to create a simulation of the first step in an oxidation reaction between a specific drug and its target protein. The final product was analyzed to determine the interactions of close residues and calculate their stabilizing energy. The residues with the largest difference in energy between enantiomers were determined for each of the four chosen chiral drugs. This data was compiled with previous research to pinpoint residues possibly responsible for enantiospecificity in CYP2C9.
**Introduction**

Some of the most common enzymes found primarily in the liver are cytochrome P450 oxidases. There are two main groups of these heme-containing monooxygenases: steroidogenic and xenobiotic. The first group, the steroidogenic P450s, is found in both prokaryotes and eukaryotes. These enzymes are located primarily in the mitochondria and smooth endoplasmic reticulum of eukaryotes. The functions of steroidogenic P450s are the maintenance of the cell wall for prokaryotes and cell differentiation through the metabolism of steroids and other necessary substances. The second group, xenobiotic P450s, work to metabolize foreign biological substances in eukaryotes’ smooth endoplasmic reticulum. Xenobiotic P450s are suggested to be a protein evolved to increase the survival chances of animals by allowing foreign substances to be detoxified.

The most important function of P450s in the human body is the promotion of phase 1 drug metabolism. Phase 1 of drug metabolism occurs in the gut wall or liver. During this phase the biotransformation of the drugs into polar metabolites occurs. In some instances several different enzymes can metabolize a single drug. The fewer enzymes a drug can be metabolized by, the more dependent that drug is on the functional activity of the enzyme.\(^4\) Enzymes with different characteristics are broken down into subfamilies.

CYP2C9 is one of the subfamilies of the Cytochrome P450 superfamily. CYP2C9 is known to metabolize ~15-20% of all the pharmaceutical drugs on the
market. This enzyme also synthesizes cholesterol, steroids and other lipids. It is located in the smooth endoplasmic reticulum and found primarily in cells in the liver.¹

Figure 1: CYP2C9 with drug at mouth of channel. Heme, iron and channel highlighted.

CYP2C9 substrates are drugs that are metabolized by CYP2C9 enzymes. Drugs can have several different effects on its metabolizing enzyme. First the drug may inhibit the activity of the enzyme which can potentially cause harmful results. A drug can also induce the enzyme, which reduces the effectiveness of the substrate. Inducing the enzyme can be harmful because if no reaction is seen then the concentration of the dose might be increased risking toxicity. The concentration of the substrate dose must be closely watched due to the wide range of reactions that could take place.
CYP2C9 has been discovered to be genetically different depending on the person. Some people have more active CYP2C9 enzymes than other people. Pharmacogenetic testing for CYP2C9 may be helpful in determining dosage and predicting drug outcomes for patients.²

Most of the drug metabolism in the human body occurs in the liver. According to Dr. Rolfe, “Metabolism is the enzymatic conversion of one chemical compound into another.”⁵ The metabolic process increases the polarity of the drug making the drug more water-soluble. This step is important and must occur before a drug can be eliminated from the body. The beneficial effect of the drug decreases as it goes through the process of metabolism.

Metabolism has two phases. Phase 1 most commonly is the oxidation step of the reaction. This step is catalyzed by a cytochrome P450 (such as CYP2C9). After this phase the drug metabolite that results is still chemically active. Phase 2 also increases the polarity of the drug by attaching an ionized group through conjugation. The more polar a substance is, the more water-soluble it will be. Therefore, the results of these two phases of drug metabolism cause a decrease in drug activity and allow for easy excretion of the drug from the body.

Some drugs are ingested in the inactive form, and then the drugs become active in the metabolite form after going through biotransformation. Also drugs can be toxic in their metabolite form. These toxic drugs are then detoxified in phase 2 of metabolism by joining with glutathione during conjugation.
Liver metabolism is affected by many factors such as aging, diseases, genetic deficiencies, the use of other drugs, and dietary and environmental factors. These factors can both inhibit and increase enzyme activity in the liver.\(^5\)

![The Catalytic Cycle of Iron](image)

**Figure 2: The Catalytic Cycle of Iron**\(^6\)

The catalytic cycle of iron (shown above) displays the process that the heme of the protein goes through in drug metabolism. The protein starts in its ground state with a positive three charge. Then as electrons are added and the iron becomes bonded to oxygen, the iron changes to a plus five charge. This protein is known as the activated protein. The very next step is the oxidation of the drug.

Many pharmaceutical drugs on today’s market are known to be metabolized by CYP2C9. For example, CYP2C9 metabolizes Cyclophosphamide, Mitiglinide,
Rosiglitazone, and Zileuton. Cyclophosphamide is a drug used to treat a variety of cancers such as different types of lymphoma and leukemia as well as retinoblastoma, neuroblastoma, ovarian cancer, and breast cancer. Cyclophosphamide is categorized as an alkylating agent. This term means that the drug works by slowing or stopping the growth of the rapidly dividing cancer cells. It is taken in tablet form by mouth once per day and can be used with other medications and cancer treatments. The length of this treatment depends on the body’s response to the drug and is determined by the expertise of the doctor.

Mitiglinide and Rosiglitazone are both used to treat type 2 diabetes. If diet and exercise alone do not help a patient reach normal blood sugar levels, then drugs are used simultaneously with diet and exercise. Specifically Mitiglinide works to lower the blood sugar levels in the patient’s body. Mitiglinide can be injected into the bloodstream the same way that insulin is injected. This medication can be used simultaneously with insulin.

If basic diabetes medications such as Mitiglinide show no results, then a possible solution would be to try Rosiglitazone. Rosiglitazone is a last resort medication. It has severe side effects such as leading to or worsening congestive heart failure due to fluid retention and increasing the patient’s risk of having a heart attack. Rosiglitazone is used in addition to diet and exercise and can also be used with additional medications. Rosiglitazone is characterized as a thiazolidinedione. Thiazolidinediones work by making the body more sensitive to insulin. Therefore Rosiglitazone cannot be used to treat type 1 diabetes because in type 1 diabetes the body does not produce any insulin.
This medication is taken in tablet form once or twice a day. Rosiglitazone is used only to control not cure type 2 diabetes.

Zileuton is prescribed for patients with asthma. This medication prevents the symptoms that come with asthma; it is not a treatment for an asthma attack that has already begun. Zileuton is characterized as a leukotriene synthesis inhibitor. These inhibitors stop the body from producing the natural substances that cause many of the symptoms in asthma patients: swelling, tightening, and mucus production in the airways. This medication is also taken in tablet form four times a day and is to be swallowed whole. Zileuton is used to control the symptoms that accompany asthma, but is not a cure.³

![Figure 3: R enantiomer of tested drugs](image)

**Methods**

All aspects of this research project were performed computationally within a Mac lab. The software Sybyl-X 1.3 from Tripos, Inc. was used to run the simulations. We
imported proteins into Sybyl from www.pdb.org. In this project we imported the crystal structure of CYP2C9 called 1R9O.

The first step was to choose four drugs with which to run the simulations. All the drugs tested had a single chiral center and were known to be metabolized by CYP2C9. The four drugs chosen for this research were Cyclophosphamide, Mititglinide, Rosiglitazone, and Zileuton.

**Preparation**

Before the simulation could begin, both the protein and the drug had to be prepared. 1R9O’s structure was imported from the protein database into Sybyl. To prepare this crystal structure of the CYP2C9 protein hydrogens were added, water and unwanted structures were eliminated, and charges were added. For this ground state protein, the charge of the iron in the heme group was 3.0. This final structure was then minimized at 5,000 iterations to achieve the lowest possible energy of the structure.

Each of the drugs was drawn in the sketch mode in Sybyl. The structures were looked up online, then replicated. Both the R and S enantiomer of each drug were exported into a file for later use. The color of the enantiomers was changed to indicate whether it was the R enantiomer (purple) or the S enantiomer (pink), and the oxidation site on each was indicated by a blue-green color. Then each of the eight enantiomers was also charged and minimized (at 5,000 iterations) to achieve their lowest possible energy. This final structure was used in the simulations that were run.
**Runs**

Each enantiomer of each drug went through the exact same process. First, the charged and minimized protein was imported into Sybyl. The channels of the protein leading to the heme were highlighted using the MOLCAD surface generation tool. Then the charged and minimized drug was imported and positioned at the mouth of the largest and most direct channel in the 1R9O protein. This background was then frozen, and the two structures were merged together.

Three molecular dynamics runs were performed for each drug enantiomer. These runs used Newton’s laws of motion as well as Coulombic forces. Run 1 was set at a length of 2,000 fs (femtoseconds) at a temperature of 50 K with snapshots every 50 fs. The force field was set at Tripos and the charges to Use Current. An aggregate was placed between the iron of the heme and the oxidation site of the ligand. This aggregate allowed the ligand to be positioned properly at the mouth of the channel. Then the run was started; it took approximately 10-15 minutes.

Directly after run 1, run 2 was performed. The length of the run was changed to 200,000 fs. The temperature was kept at 50 K. Next the aggregate was deleted and a constraint was placed between the same two atoms (the iron of the heme and the oxidation site of the ligand). The constraint pulled the ligand down the channel towards the heme using charges. The distance and constant of the constraint were changed to 3.0. This run took about 12 hours.

Again, run 3 was done right after run 2 finished. The length stayed at 200,000 fs, but the temperature was changed to 100 K. Run 3 was performed with neither an
aggregate nor a constraint. This step allowed for equilibrium between the protein and the drug to be reached. Run 3 also took approximately 12 hours.

After the three dynamics runs were complete, the final structure was minimized at 5,000 iterations a final time and then exported. This entire process was then repeated with the activated protein. The activated protein was prepared by adding a double bonded oxygen to the iron in the heme of the protein and giving the iron a +5 charge.

**Residue Energies**

The purpose of these tests was to determine which residues in CYP2C9 might be most significant in contributing to enantiospecificity. Therefore, in the analysis of our results we only looked at the residues within 4 Å (angstroms). This analysis was achieved by importing the final result of each of the runs, then selecting only the ligand and adding any residues contained within a 4 Å radius. The topography was measured in Sybyl using non-bonded lengths. The resulting data were copied into an Excel spreadsheet. The data was filtered to show only results from 0.1-4.0 Å. Data from the text file of the specified minimized protein was obtained from Sybyl and copied into a separate sheet in the same Excel spreadsheet. This data was compiled and the energies between each bonded pair were calculated using the formulas for hydrogen bonding and van der Waals forces (shown below).
The hydrogen bonds between the ligand and the surrounding residues of the protein were seen by adding hydrogen bonds to the image in Sybyl and identifying which atoms these bonds occur between. After every hydrogen bond and van der Waals force was accounted for, the total energies for each residue were calculated. The duplicates were then removed from the list of residues and placed in numerical order.

This process was repeated for each drug enantiomer before the results were compared. A separate Excel spreadsheet was used to create the energy charts that compare the residue energy favorability of each drug. Each of the sets of residues and energies were lined up side-by-side. The residues were then ordered numerically from top to bottom with only a single residue per row. Some enantiomers did not have every residue/energy pair. Once this was complete, the energy differences were found for each drug by the following formula:

\[
E_{\text{H-bond}} = \frac{C_{ij}}{(R_{ij})^{12}} - \frac{D_{ij}}{(R_{ij})^{10}}
\]

\[
E_{\text{VDW}} = E_{ij} \left[ \frac{1.0}{(a_{ij})^{12}} - \frac{2.0}{(a_{ij})^{6}} \right]
\]

where \(E_{ij} = \sqrt{(E_i - E_j)}\), \(C_i\) and \(D_i\) are parameters for repulsive and attractive H-bonding, \(R_{ij}\) is the distance between atoms, and \(a_{ij}\) is the distance between atoms divided by the sum of the VDW radii.

The preferred enantiomer of each drug was determined by reading literature of past research on each drug. An energy chart was then made of these energy
preferences by residue eliminating all residues that did not contain at least a difference of 0.5 kcal for at least one drug.

**Surflex Docking**

In addition to molecular dynamics, Surflex docking was run for each enantiomer of each drug. The first step was to prepare the protein by adding hydrogens and charges as before. The molecule was then minimized with 5,000 iterations. Next, a protomol was generated inside the protein. A protomol is a protein docking site or the active site of the protein. The ligand route was chosen in order to generate the protomol. After the protomol was made, the ligand was loaded into the protein. Each surflex dock took about 20 minutes. The results of Surflex docking are given in a table. Important values are the total score, the crash score, and the polarity.

**Results and Discussion**

The energy charts below create a visual of the enantiomer energy difference for each drug by residue. Only residues with a difference of at least 0.5 kcal for one or more of the drugs are shown in the chart. Significant enantiospecific residues are indicated on the charts by red arrows.
Figure 4: Energy chart showing the difference in energies for drugs run with the ground state protein.
Figure 5: Energy chart showing the difference in energies for drugs run with the activated protein.

Two of the residues were seen to be significant in both the ground state and activated protein: PHE100 and PHE476.

Table I: Table of most enantiospecific residues

<table>
<thead>
<tr>
<th>Type of Protein</th>
<th>Significant Enantiospecific Residues</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ground State</td>
<td>PHE100, LEU102, LEU208, PHE476</td>
</tr>
<tr>
<td>Activated</td>
<td>PHE100, LEU366, PHE476</td>
</tr>
</tbody>
</table>

All of these residues have been determined as significant with other drugs tested in previous years.
**Table II**: Literature and computational enantiomer preference by drug

<table>
<thead>
<tr>
<th></th>
<th>Cyclophosphamide</th>
<th>Mitiglinide</th>
<th>Rosiglitazone</th>
<th>Zileuton</th>
</tr>
</thead>
<tbody>
<tr>
<td>Literature</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Computational</td>
<td>S</td>
<td>Neither</td>
<td>Neither</td>
<td>S</td>
</tr>
<tr>
<td>(Ground)</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Computational</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>(Activated)</td>
<td>S</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Most of the drugs tested also agreed with literature as far as which enantiomer are preferred in metabolism.

**Conclusions**

As hypothesized the data suggests that there are residues within the structure of the protein CYP2C9 that make it especially enantiospecific. These residues have repeatedly shown up as significant with a wide range of drugs tested in CYP2C9. Therefore we can conclude, that if these residues are mutated in a CYP2C9 protein, we can achieve more efficient metabolism for both the R and S enantiomer of a drug.

It is not our goal, however, to go inside the human body and change enzymes within the liver to increase drug metabolism. A larger and more significant application of this study is in the prediction of new drug reactions. This process of studying the enantiospecificity of CYP2C9 can allow potential new pharmaceutical drugs to be tested quickly and efficiently in order to determine which enantiomer is preferred and will yield beneficial results. This computational process will save a great deal of time and money.
Also, the FDA now requires that all drugs only contain one enantiomer of the drug. This requirement should limit the negative side effects patients have when taking the medicine.

Enantiospecificity in protein metabolism has rarely been studied before. In order to get true and accurate results and a broad understanding of how drug metabolism works, many more trials need to be run. In addition, the protein needs to be studied in all stages of the catalytic cycle of iron to see where changes occur.

**Acknowledgements**

I would like to acknowledge Dr. Patterson and Dr. Greene for the funding they have provided this summer research program. I would also like to acknowledge the Arkansas INBRE program. Thank you to Ouachita Baptist University for the use of their facilities. Also a thank you to my mentor Dr. Martin D. Perry and Tim Horton for all of their help in answering questions and helping with the project, my coworker Mallory Burroughs and our wet lab mentor at UAMS, Dr. Grover P. Miller.
Literature Cited


Part III: The Finish
Communicating Research

After your research has been conducted and your scientific paper written, it is time to go and share your results with the community. I had several opportunities to share my research. First my fellow research students and I presented in front of the other research members and mentors in the summer research program at Ouachita. It was nice to get to present first for people with whom we were comfortable and who could give us feedback.

Then we traveled to the Clinton Library in Little Rock and presented again for our peers all over the state. Next I took my poster presentation to the Arkansas INBRE Conference in Fayetteville. We were judged at this conference, and I was awarded 2nd place in the chemistry division. This award was a great attribution to the help of my mentor and co-researcher and the work we had put in.

There was a second meeting in Little Rock, the regional meeting in which there were many more PhDs going around and asking questions. This meeting was where the depth of my knowledge really got tested. We also had the opportunity to present to congressmen at the Capitol in Little Rock. These presentations were short as their time was limited.

At the National ACS Convention in Dallas, we went to a talk on how to speak simply. They expressed the importance of sharing complicated science research with people from any and every background. When we presented, judges came around to see if we could apply what we learned. Many Ouachita students won the Speak Simply award including myself. It is important to know your audience when you present and change your presentation style accordingly.
One of my favorite trips was to the National Conference on Undergraduate Research (NCUR) in Kentucky. The difference with this one was that it was a multidisciplinary conference. We got to listen to presentations from all schools of learning.

Finally, I presented one last time at Scholar’s Day, put on by the Carl Goodson Honors Program at our university. This time I got to present to other students and professors at my school. Each of these conferences has developed my skills as a presenter and communicator and helped to prepare me for a career in pediatric medicine in which I will often need to explain scientific terms simply to both children and parents.

**Talking to the More Experienced**

The most unsettling thing about presenting your research is when you are presenting to those with much more experience than yourself in your area of research. PhDs especially love to pinpoint a small aspect of your project that they know thoroughly and drill you on it. The purpose of these questions is both to test your understanding and to gain more knowledge for their own research. The best thing to do is not to get flustered. They do not expect you to know everything that they know. Do your best to present clearly all you know on the topic, then it is ok to say “I do not know.”

Respect their knowledge while realizing that they too were undergraduate students at one time and were just beginning their research experience. Understand that they are simply searching for more knowledge.
It is better to admit you do not know something, than to try and make something up on the spot. An inaccurate statement could invalidate everything you have said previously.

**The Scientific Community**

Through presenting at different research conferences I began to see the scientific community as a whole. It stretches all across America and the globe. Communication of research is important to the continuation of research. By sharing what we have found, others have a step up to a new discovery or cure. We all work together to gain a better understanding of the world around us.

**Feelings After**

Completing summer research gives a research student new-found confidence in the skills they have and their ability to acquire new skills in the future. They can think back to their insecurity at the beginning of research and see how far they have come. Working through insecurities builds confidence and will make the student a better scientist.

My attitude also changed about research and the scientific community. At the research conferences, I was impressed by many of the techniques utilized by the researchers. I found myself intrigued by several topics that were presented and discovered that exciting things can happen in a small laboratory with one individual. I gained an even greater appreciation for my university and the research opportunities it provides for students.
Preparation for Medical School/Importance

The abilities gained doing research were invaluable. It provided a strong skill set to help me understand research-based evidence that will later be utilized in medical school. This experience helped me gain confidence. It was exciting to see how my presentation skills developed through each conference I participated in. I learned not only to convey my research but to simplify it in a way that the general public could understand.