An Investigation of Arsenic Degradation in the 2AFN Enzyme from *Alcaligenes Faecalis*

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Abstract

Among *Alcaligenes faecalis'* versatile contributions to the environment, its most prevalent property enables it to convert the toxic derivatives of arsenic, such as arsenite, into a less toxic form, arsenate. The objective of this project was to simulate this reaction by virtually binding the enzyme (2AFN) responsible for this arsenic reduction to arsenite on its five distinct binding sites. It was hypothesized that the binding site with the largest area would display the strongest binding affinity. Schrodinger Software was the program used to determine this binding affinity using a method known as docking. Prior to advancing on to the docking phase, the enzyme 2AFN was prepared using a protein preparation wizard feature. After preparation, 2AFN underwent binding site detection using a SiteMap, which identified five specific binding sites for the arsenite to dock. The final phase before docking was the Receptor Grid Generation, which needed to be implemented to each of the five binding sites as they were individually docked to arsenite. This provided the docking values known as Glide Scores, and the binding site producing the largest values was concluded to possess the strongest binding affinity. It was observed that Binding Site 3 displayed the greatest Glide Score therefore proving that there is a significant correlation between area and binding affinity as Binding Site 3 was the second largest binding site. Obtaining these values is rudimentary as this information can be implemented into further researching arsenic and *Alcaligenes faecalis* interactions for potential degradation methods.

Background

Alcaligenes faecalis is an aerobic, gram negative bacteria generally found in moist environments. It is immensely significant in the medical field; for example, its relationship with cystic fibrosis patients. It is provided with an opportunistic respiratory tract to thrive in and is presumed to leave negative long-term impacts on these patients. Its most prevalent property however, which is the focus of our research, is its ability to reduce the highly toxic derivatives of arsenic, such as arsenite, into the less toxic form arsenate. The reduction reaction it induces releases a minute quantity of ATP which can be utilized as an alternate energy source when *Alcaligenes faecalis* is exposed to undesirable conditions, such as lack of oxygen. Arsenic poisoning is an ever increasing issue as it contaminates groundwater reserves and has deleterious effects on the consumers of that water. Though it is no longer a major threat in modern societies due to advancements in water regulation, it is still prevalent in developing countries (Emsley, 2011). Roughly 140 million people in the world are at risk of lung cancer and various diseases because of arsenic presence in groundwater (Oliver, 2007). Even some U.S. states, such as New

Hampshire, are facing issues with arsenic concentration in their groundwater, and according to Maine's Department of Agriculture, Conservation, and Forestry, it was discovered that the arsenic concentrations in the bedrock groundwater has increased by almost 40% (Yang, 2009). Understanding the relationship of arsenic and *Alcaligenes faecalis'* enzyme will allow for continued research in this recurring international issue, and optimal biological filtration methods may arise as a result of this data.

Question

The main question posed during this research and experiment was which of the five binding sites located on enzyme 2AFN from *Alcaligenes faecalis* will display the strongest binding affinity with an arsenite molecule?

Hypothesis

Schrodinger Software provided the area in atoms of each of the five binding sites (ligands) that were discovered via the Binding Site Detection process. It was hypothesized that the binding site with the largest area would have the strongest binding affinity because it would possess a greater spatial docking space for more bond interactions. After determining the areas it is clear that Binding Sites 3 or 1 respectively will most likely demonstrate the greatest binding affinity to arsenite.

Materials/Methods

To conduct this experiment, the Maestro Suite of Schrodinger Software was the crucial program primarily used to determine the binding affinity of each binding site between 2AFN and arsenite by following a rigorous, yet purely computational procedure.

Protein Preparation

The enzyme 2AFN, responsible for the arsenic degradation property in *Alcaligenes faecalis* was the focus of this experiment. In order to begin, the enzyme 2AFN underwent a Protein Preparation where 2AFN was preprocessed in the Maestro suite. The protein preparation was responsible for adding hydrogen bonds, disulfide bonds, as well as other aspects that increased the binding compatibility in the next few steps. The prepared enzyme was brought into the workspace, and both the ligand and receptor were selected so they could be viewed simultaneously on the screen. In order to proceed with the next step, the missing receptor-ligand hydrogen bonds were filled in with the 'Style' feature. This prepared the ligand and receptor for the next step in the procedure.

Binding Site Identification

After the preparation wizard, the binding site identification was run, a feature of protein analysis. This feature of Schrodinger helped to determine any potential binding

sites of the enzyme. Since this feature was used to determine potential binding sites, some binding sites could even be inactive. Fortunately, all of the binding sites found in enzyme 2AFN were active and used in the docking phase.

Grid Generation

The next step of the procedure was 'Grid Generation', which was found under 'tasks' and 'docking'. When the window appeared, the option had to be changed to entry, where the ligand was then selected so it would be excluded from the 'Receptor Grid Generation.' The remaining items in the workspace were left deselected to be treated as part of the receptor. The rest of the options in the 'Receptor Grid Generation' feature, such as the 'Scaling Factor' and 'Van-der-Waal's radius scaling' were left at recommended values in order to allow for the most accurate results. In the second tab of the window, the 'Centroid of Workspace ligand' and 'Dock ligands similar in size to the workspace ligand' options were selected. This option was ideal and consistent with the shape and structure of the ligand. In the 'Constraint' tab, the 'Pick atoms' option was highlighted under 'H-bonds.' After these options were selected, the grid generation was run for each of the five binding sites of enzyme 2AFN. The grid generation had to be done individually on the five binding sites in order for the docking procedure to work. As each of the grid generation procedures was running, there was an option to monitor the progress for each site. This was the longest and most time consuming parts of the overall procedure. After the process was complete and the job was incorporated, a file was automatically saved in a zip file in a designated folder.

Docking

After the completion of the grid generation, the docking process to determine the binding affinity was able to run. The 'Glide Docking' option was chosen under the 'Tasks' bar, where a ligand and a receptor file had to be attached to determine the Glide Score. Since each binding site had generated a unique zip file, the files were separated based on the number of the binding site. The grid generation for the binding sites were used as the receptors. The first one was attached along with the arsenite compound, compatible with Schrodinger, which had been saved previously. Then, the second part of the docking process was run, resulting in the Glide Score value for that particular site. The procedure was then repeated with the other four binding sites while the arsenic compound remained constant throughout the rest of the procedure.

For accurate results, a second trial was run for each binding site to determine if the Glide Score was consistent in each of the trials, resulting in a total of two trials. A Glide Score in

general ranges from -10 to 2, where -10 depicts the greatest binding affinity, and a score of 2 showed little to no interaction between the receptor and the ligand.

Data/Results & Analysis

According to the results, Binding Site 3 yielded the greatest Glide Score average therefore indicating that it has the greatest binding affinity to arsenite. This is crucial as the conclusion can be made that there is a significant relationship between area and binding affinity of Binding Sites. The other Site's Glide Scores had similar outcomes as the smallest Sites in area had little to no affinity to arsenite with scores as low as -2 and -1. These results can be seen in *Figure 1*. Binding Site 1, which was the second largest did not yield the second largest binding affinity however did have significant results as well, remaining consistent with the conclusion. Binding Site 2 was the median Site area wise at around 2900 atoms. Although it was significantly smaller than Sites 1 and 3 it still yielded the second largest binding affinity with values at -6 and -5. Binding Site 5 on the other hand, which was the second smallest Site in area at around 1800 atoms was also consistent with our hypothesis as it had a binding affinity as low as -2, similar to Binding Site 4 which was the smallest. *Figure 2* is a table dividing the results by trial. This is an alternate means for data organization and display.

Determining this information is rudimentary as these results can be further implemented in understanding *Alcaligenes faecalis*' interactions with arsenite using enzyme 2AFN. This was initially the motive of this project as we planned to design a filter which will target even the smallest derivatives of arsenic such as arsenite. Other existing filters were ineffective in this field as they were made of geological factors composite iron matrices, and were permeable to the smaller molecules. Since our enzyme will be functioning on the molecular level it will be able to best target all arsenical compounds and serve as an effective alternative filtration system. Now that the enzyme has been optimized its properties can be harnessed and utilized in various fields of arsenic degradation form naturally cleansing arsenic infused waters to acting as a biological filter for arsenic contamination. This is research that can be implemented in future expansion of this project.

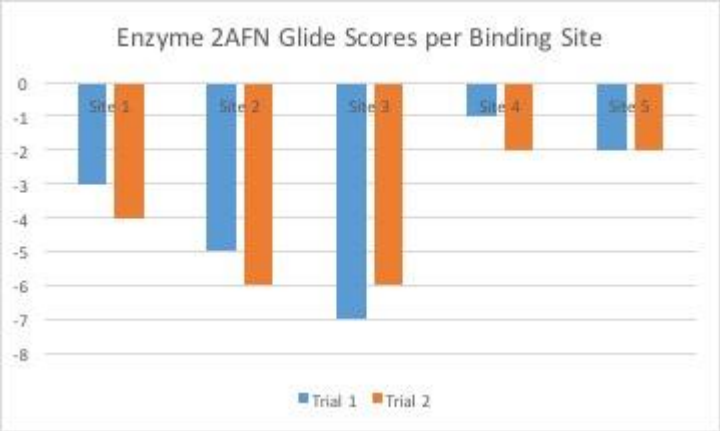

Figure 1.

Trial 1. **Enzyme 2AFN Glide Scores per Binding Site**					
	Binding Site 1	**Binding Site 2**	**Binding Site 3**	**Binding Site 4**	**Binding Site 5**
Glide Score	-3	-5	-7	-1	-2

Trial 2. **Enzyme 2AFN Glide Scores per Binding Site**					
	Binding Site 1	**Binding Site 2**	**Binding Site 3**	**Binding Site 4**	**Binding Site 5**
Glide Score	-4	-6	-6	-2	-2

Figure 2.

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