Introduction

At the interface of a protein-protein complex, one protein has side chains that bury into a cavity, the active site, of the other protein. These side chains, called anchors, are critical to the binding process, because they give the specificity needed for protein-protein recognition.\(^1\)

The transcription factor p53 is a protein that regulates the cell cycle and has anti-cancer mechanisms. It can activate DNA repair proteins to try to fix the damage or initiate apoptosis, programmed cell death, if the DNA is beyond repair. Normally, p53 is bound to Mdm2, a protein coded by an oncogene of the same name. An oncogene is usually formed by a mutation from a normal gene and participates in the development of cancer when activated. In some cancers, there are increased levels of Mdm2 that bind to p53, which inhibits the body’s ability to fight tumors.

Docking is the \textit{in silico} method of fitting two molecules together in 3D space. It is an important tool for structure-based drug design, because it is used to identify potential drug antagonists. Antagonists are ligands that bind to a receptor and block the protein from an agonist, a substance that would normally activate the protein. A
major advantage of docking small molecules is that chemical diversity is achieved without having to put time and effort into physical screening. Mdm2 is an appealing target for small molecule docking, because an antagonist could lead to the development of drugs for cancer therapy.\(^2\) X-ray crystallography has revealed three important anchors on p53, residues Phe19, Trp23, and Leu26 (Figure 1), which interact with the binding pocket of Mdm2.\(^3\) An antagonist of Mdm2 will mimic these interactions with the binding pocket. We will dock and score four known antagonists of Mdm2.

**Methods**

![Docking Process Diagram](image)

The docking process (Figure 2) begins with obtaining the structure of a target protein. This is usually determined by x-ray crystallography or alternatively by NMR spectroscopy. The next step is to make a list of compounds that are potential ligands for the receptor. Protein complexes have an induced fit, meaning the active site molds itself around the anchors. However, our approach to docking explores a lock-and-key fit. Much like a key fits into a lock, we dock a rigid ligand to a rigid protein receptor. After docking, each ligand-protein pose is given a score, where the scoring function is a calculation of the free energy of the system. The goal is to find the system with the lowest free energy, in other words, the most stable system.

This project will utilize the molecular design software suite Moloc to dock and score four known antagonists to Mdm2 (Figure 3). Docking of our
antagonists begins with determining the protein target structure (Figure 2). We will use the Mdm2 structure from the RCSB Protein Data Bank (PDB) of the p53-Mdm2 complex. After this we choose our ligands for docking, which are the four known antagonists. Next we will generate conformations for each antagonist. Moloc achieves this by first changing any rotatable bonds in the molecule and then minimizing the energy. It records this conformation and repeats the process. If the conformation generated after the minimization is the same as the previous, this is termed a failure, and Moloc will try again. The default setting is that after four failures, Moloc will stop generating conformations for this compound. The next step is to dock each conformation to p53 using the rigid-body docking function of Moloc. Rigid-body docking performs a pair-wise matching of one molecule fragment to another, both defined by the user. The user selects three or more atoms, pair-wise, from each fragment to align, and the program will superimpose the selected atoms. After docking the fragments, an optimization can be performed in Moloc to get the best score. The Moloc scoring function is an estimate for the free energy of binding of the complex. The scores are used to find the most favorable conformation for each docked compound. The
lowest energy system will either validate a known structure or predict the co-crystal for an unknown structure.

**Goals**

The overall goal of this research is to identify an antagonist that could be used in medicinal chemistry for the development of novel compounds to fight cancer. First, we will learn the docking method and employ it to reconstruct the known co-crystal structures of two antagonists of MDM2. Second, we will dock two other Mdm2 antagonists for which the co-crystal structures are unknown. The successful docking of the two known structures will validate our predictions for the unknown.

**Limitations and Alternative Approaches**

We recognize several limitations of our small molecule docking approach. First, the sampling of conformations done by Moloc may be inadequate to find the optimal docked structure. To determine its effectiveness, we can explore the sampling of other programs and compare the outcomes. Second, we will be using the scoring function built into Moloc. We may need to use a more established function from the literature, such as ChemScore. Third, our method uses a rigid receptor from the p53-Mdm2 complex. As an alternative, we could use other structures of Mdm2 from the PDB.
References


