Ligand Docking of Lincomycin and *Escherichia Tenella*

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Abstract

For many years, ligand docking has been used for predicting the interactions of ligands with biomacromolecules. With this, scientists have been able to utilize this procedure to test the effectiveness of drugs, the toxicity of drugs, and etc. AutoDock and AutoDockTools are two kinds of automated software that assists researchers in determining the biomolecular complexes of ligands and macromolecules. In this paper, the association of Lincomycin and *Escherichia Tenella*, the parasite that causes Coccidiosis, was achieved to determine the effectiveness of Lincomycin to treat Coccidiosis.

Introduction

Escherichia Tenella is a parasite derived from class Sporozoa. It causes cecal Coccidiosis in poultry. Coccidiosis causes economic loss in the poultry industry because it causes death in young chickens that are raised for meat consumption. Death or infection occurs once the intestines are infected due to rapid production and an epidemic occurs. "Coccidiosis caused by *Escherichia Tenella* is noticeable within three days of being infected. Chickens droop, stop feeding, huddle together, and blood appears in droppings. By day eight or nine, the bird is either dead or on the way to recovery." ***(Agri-Facts)*** Treatment of Coccidiosis has been aimed by the use of several antibiotics such as Roxarsone, Bacitricin, Tylosin, Virginiamycin, and Lincomycin; whereas Lincomycin being one of the most therapeutic forms of treatment due to it's multiple forms of dosage. ***(USPO)***

Lincomycin is a lincosamide antibiotic that comes from the actinomyces *Streptomyces lincolnensis*. ***(Wiki)*** It can be administered in pure form or with the combination of water or feed. The dosage is determined by the animals' species and weight. In previous studies, chickens have been given between five to fifty doses of twenty milligrams of Lincomycin prepared in feed. ***(USPO)*** This feed preparation has shown to help effectively treat Coccidiosis caused by *E. Tenella*.

Ligand docking is process of building a molecular complex with two molecules. It could be amongst two proteins, a ligand and a protein, a protein and DNA, and two ligands. This association is based off free energy calculations from an unbound state to a bound state. This method provides detailed and definitive information for rational drug-design projects; more of a qualitative aspect. However, this method is computationally expensive and limits its practical use. ***(Lybrand)*** The theory for ligand binding is based off "the assumption that a binding site can accommodate many molecules and, in fact, that it *should* rearrange itself with relatively little penalty to complement the small-molecule drug." ***(Carlson)***

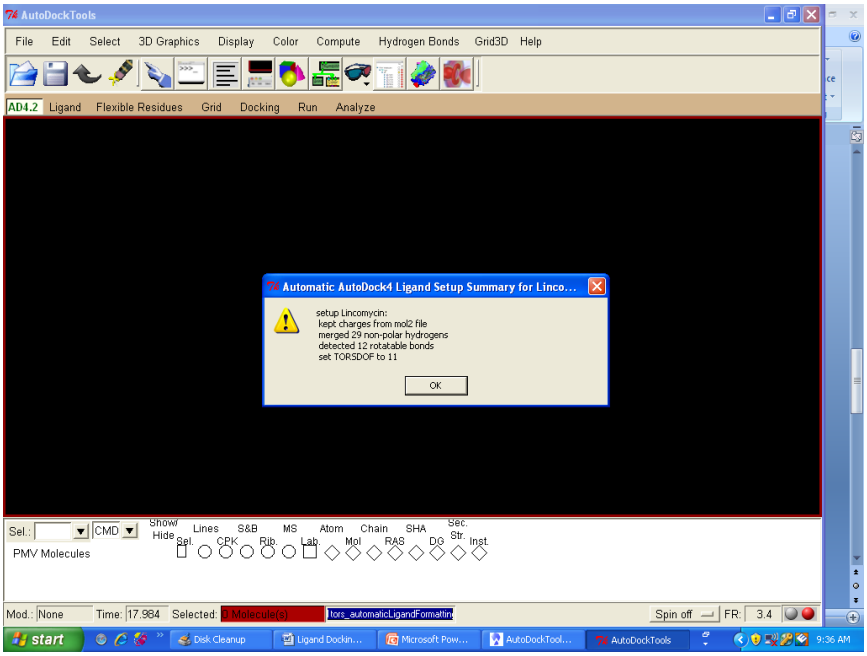
Ligand binding has brought about many advances to drug design. Specifically, it contributes to creating visualizations for biologists to discover new drugs for diseases, and it also helps test current drugs and their effects on developing diseases. By testing a protein's flexibility, scientists are able to make calculations that will help show a ligand's effect on the host in multiple ways. For example, if a protein's side chains are the only atoms tested, this neglects the possible changes in the protein's backbone. With the both, the side chains and backbone, being tested, this is full flexibility being accomplished and gives more accurate results of docking. ***(Carlson)*** Its purpose in this experiment is to show how ligand binding helps to cure Coccidiosis by binding Lincomycin to *E. Tenella* for treatment.

Method

As a guide through the ligand docking process, in AutoDockTools, the "Using AutoDock4 with AutoDockTools: A Tutorial" by Ruth Huey and Garrett M. Morris was utilized. The .PDB files utilized for the ligand docking procedure were taken from the Protein Data Bank online and through another Google search. Most of the process was carried out in AutoDock Tools.

First, the .PDB files were prepared for coordinate calculation by converting them into .PDBQT files. Converting the ligand, Lincomycin, into a .PDBQT file first was done. In AutoDockTools, input the ligand using the ligand > input > Quick Setup. In this step, the .PDB file's calculations are converted over into .PDBQT calculations. Loose non-polar hydrogens are

bonded together, any rotatable bonds found are calculated and, the TOSDAF is set for the ligand file.

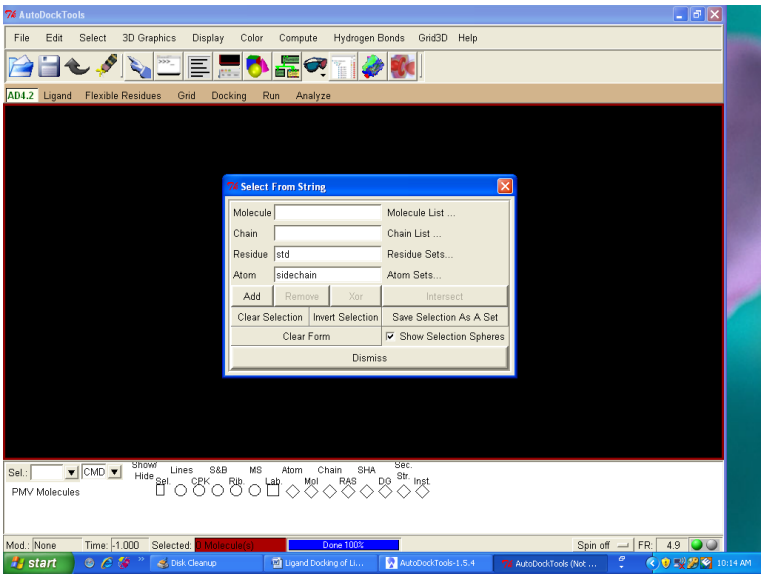


This picture is showing the results of converting the ligand file to a .PDBQT file. The warning box is displaying the setup calculations for file Lincomycin.out.pdbqt

After the .PDBQT file is produced for Lincomycin, Lincomycin.out.pdbqt, the ligand must be prepared as a rigid ligand. To do this, the root must be detected and its root expansions. In the ligand tab, torsion tree>detect root, detects the root automatically in the ligand. Torsion tree>Show Root Expansion, shows all the atoms that are fond in the root. The atoms are represented by small spheres. Torsion Tree>Choose Torsions, allows the user to choose rotatable bonds for the ligand. Rotatable bonds are green, non-rotatable bonds are red, and bonds that are rotatable but are treated as rigid are magenta. Once all of these settings have been saved, the ligand file is hidden by command on the PMV Molecules dashboard. From here, the macromolecule, *E. Tenella*, in this experiment, can be prepared.

Similar steps are used to convert *E. Tenella*'s file to a .PDBQT file. As in the first step, utilize the ligand tab> Input> Quick Setup procedure to convert the .PDB file to a .PDBQT file. A similar image, like the previous one for Lincomycin, should show the setup for *E. Tenella*'s file, 1HKY. The new .PDBQT file for *E. Tenella* that will be utilized for the remainder of the process is 1HKY.out.pdbqt.

From here, the ligand tab is no longer utilized to prepare the macromolecule. To display the macromolecule, the Flexible Residues tab is utilize to prepare the 1HKY file for docking calculation. Flexible Residues>Input>Open Macromolecule, allows the user to open the new .PDBQT file in the ADT viewer. Next, the flexible residues are chosen by using the Select tab on the ADT toolbar at the top of the screen. Select> Select from String, allows the user to access an interactive menu that allows the flexible residues to be chosen for calculation. In this experiment, calculations were set for the “small” residue sets and the “sidechain” atom sets of the macromolecule.



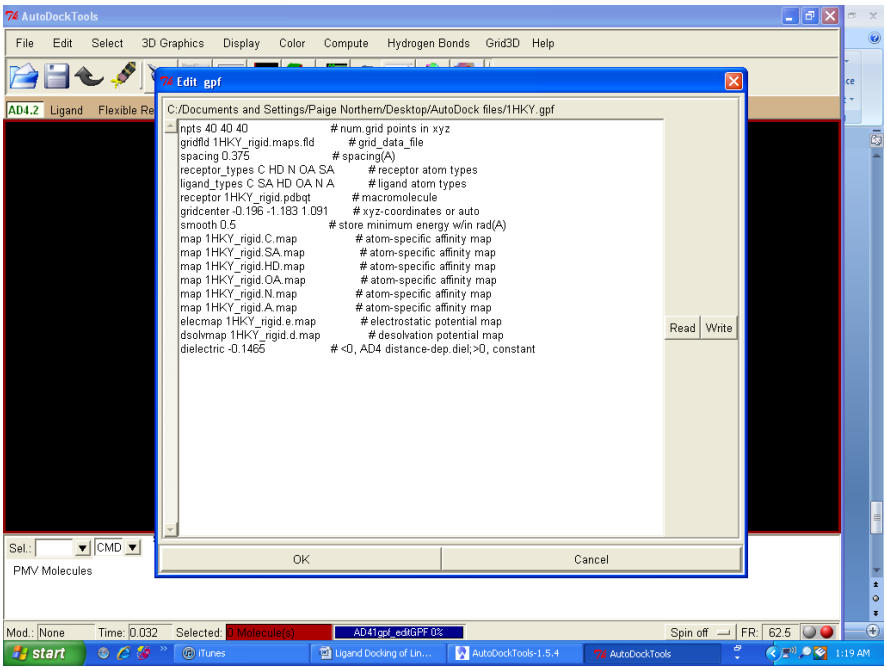
The “Select from String” panel is displayed. Residue line “small” is selected and atom set “side chain” is selected for calculation.

Once these settings were in place and added, yellow atom highlighters designate the atoms in each set for calculation. Next, Flexible Residues> Choose Torsions In currently Selected Residues, sets the assigned residues to be flexible. From here, both, the Flexible and Rigid .PDBQT files can be written for AutoGrid calculation. Flexible Residues> Output> Save Rigid PDBQT saves the Rigid file, 1HKY_rigid.pdbqt, and Flexible Residues> Output> Save Flexible PDBQT saves the flexible file, 1HKY_flex.pdbqt.

Next, AutoGrid parameter files can be created. To do this, a .GPF file is created by utilizing the grid submenu. Grid>Macromolecule> Open displays the macromolecule that will be

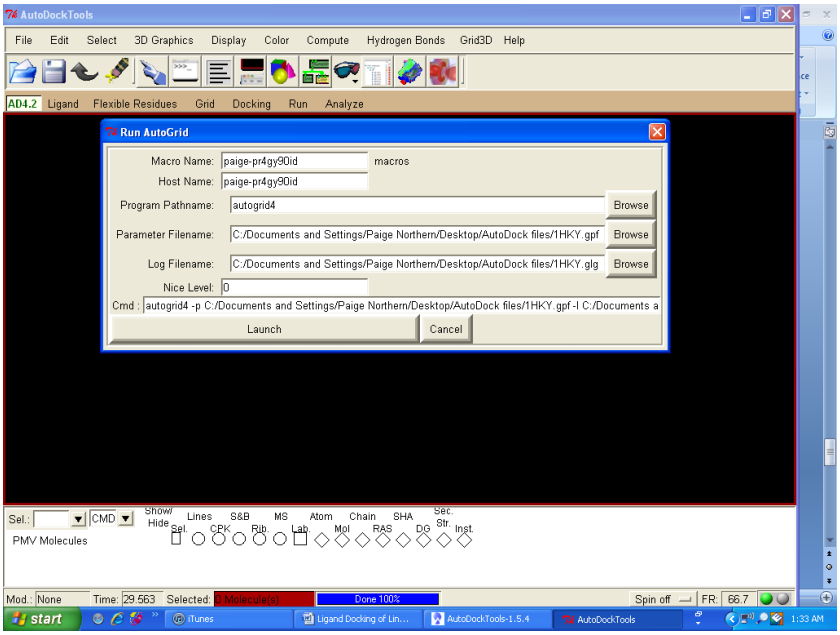
ran for calculation. Grid> Set Map Types displays another submenu that allows the remainder of tasks to be set. Before the .GPF file is created, the following tasks must be completed:

- Grid> Set Map Types> Choose Ligand
    - Select Lincomycin.out.pdbqt
- Grid> Set Map Types> Choose Flexible Residues
    - Select 1HKY_flex.pdbqt
- Grid> Set Map Types> Directly
    - Type A C HD N NA OA SA and accept. (This sets the types that will be created in the AutoGrid output file)
- Save> Output (Saves .GPF)
- Edit GPF allows the user to see the settings created for AutoGrid



The "Edit GPF" panel is displayed. The details are all the commands that have been requested in the above steps. The expected results of AutoGrid should have calculated parameters for both, ligand and protein, atom types.

Once the .GPF has been saved as 1HKY.gpf, AutoGrid can run successfully in ADT. This step is done in the following step: Run> AutoGrid. The following picture shows the panel that appears to set the directories from which the files will be ran from and saved to.



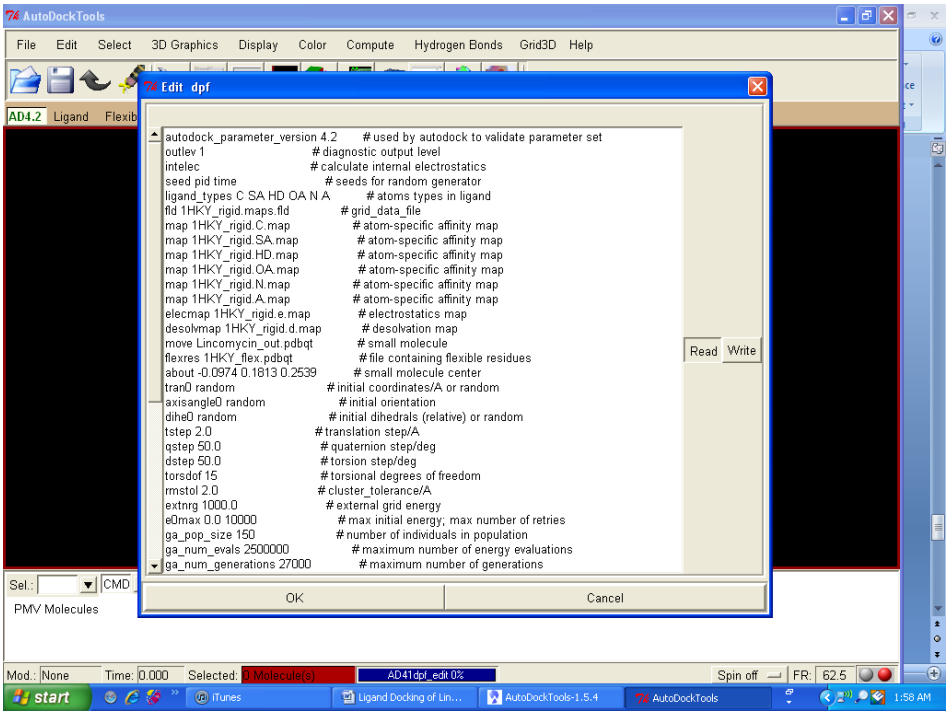
The "Run AutoGrid" panel is displayed in the image. The parameter filename is set to 1HKY.gpf in the home directory and the log filename is automatically set to 1HKY.glg, which is also found in the home directory. "Cmd" is the command line that runs AutoGrid in Putty command.

Now that AutoGrid has been successfully run, the docking parameter files can be created. To do this, the docking submenu is utilized. The following steps are utilized to set up the .DPF file for AutoDock:

- Docking> Macromolecule> Set Rigid filename
  - Select 1HKY_rigid.pdbqt
- Docking> Macromolecule> Set Flexible Residues filename
  - Select 1HKY_flex.pdbqt
- Docking> Ligand> Open
  - Opens ligand if it's not displayed already
- Docking> Ligand> Choose
  - Selects ligand if it's displayed in the ADT viewer
- Docking> Search parameters> Genetic Algorithm
  - Genetic Algorithm is the parameter being used in this experiment. This is the only confirmation required for this file.
- Docking> Output> LaMarkian GA

- This form of Genetic Algorithm is being calculated in this experiment.

- Edit DPF
  - Allows the requested commands to be displayed for editing preferences.



The "Edit DPF" panel is displayed. These are the following files requested to be written in AutoDock. The maps and atom types are also based off commands from AutoGrid.

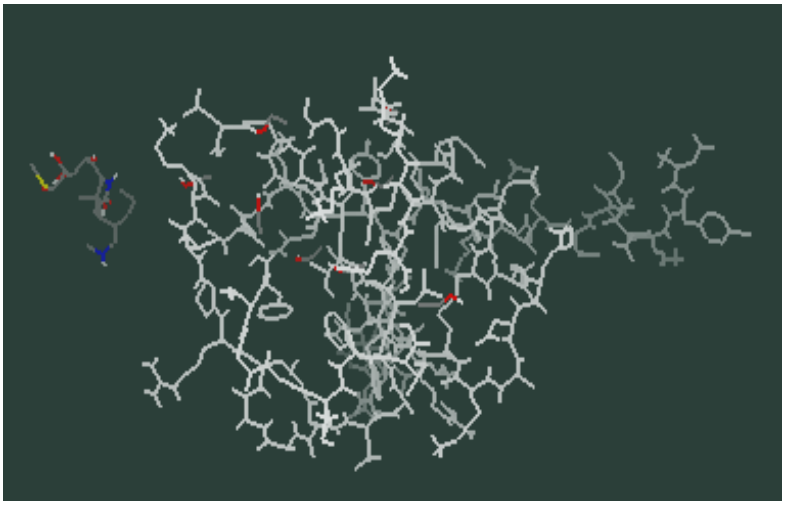
AutoDock is not directly run in AutoDockTools. It is run through LONI systems on QueenBee. All files needed to run AutoDock are safely transferred through SSH Secure Shell Client for safe file transfer. The following files are required for proper calculation:

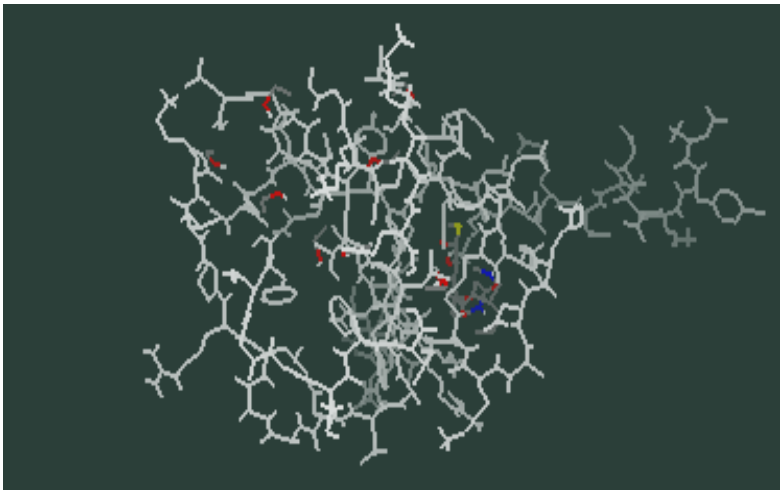
- 1HKY.dpf
- 1HKY.glg
- 1HKY.gpf
- 1HKY_flex.pdbqt
- 1HKY_model1.out.pdbqt
- 1HKY_rigid.A.map
- 1HKY_rigid.C.map
- 1HKY_rigid.d.map
- 1HKY_rigid.e.map
- 1HKY_rigid.HD.map
- 1HKY_rigid.maps.fld
- 1HKY_rigid.maps.xyz
- 1HKY_rigid.N.map
- 1HKY_rigid.OA.map
- Lincomycin.out.pdbqt
- Lincomycin_out.pdbqt
- 1hky.dlg
- 1HKY_rigid.pdbqt
- 1HKY_rigid.SA.map

The files listed above are all the files that were created in ADT. To run AutoDock on QueenBee, the user's environment must be set to respond to AutoDock software. The software can be set by "vi ${Home}/ .soft". Once this command is designated, add the software before the @default. The software entry is "+autodock-4.3-gcc-4.3.2." Save and quit this editing. From this point, the script, "submit_both", this was created to run AutoGrid and AutoDock on QueenBee simultaneously, is submitted through SSH Secure Shell Client (wireless interface).

Results

As a result, Lincomycin is proven to treat Coccidiosis. In the visualization, Lincomycin binds to *E. Tenella* by attaching to the hydrogen bonds in effort to treat the disease. Over time, the parasite dies off, as a progressive effect of Lincomycin binding to it. As per my analysis, the duration of treatment is inconclusive due to lack of tools and time length of program.





In the following images are the before and after images of the simulation. Lincomycin (colored molecule) binds to *E. Tenella* (gray molecule) by hydrogen bonding.

Conclusion

The benefits of ligand docking will bring significant technological advances to structure-based drug design. The future goal of ligand docking is to be able to bind many protein structures together to make them useful for drug development and design. As stated by Carlson in her article, *Protein Flexibility and Drug Design: How to Hit a Moving Target*, "The use of homology models in structure-based drug design. Homology models are not accurate enough for drug design because they contain errors that often result in binding sites with the wrong size and shape. Methods that allow for protein flexibility will help correct some of those errors by allowing crosstalk between the receptor and the ligand in question."

Ligand docking also contributes to deeper techniques such as Molecular Dynamics simulation. Although docking calculates a precision of likeliness that a ligand can bind with its receptor, MD simulation confirms this on a higher scale by calculating the structures based on time dependency.

Ligand docking is able to give a qualitative visualization of Lincomycin treating Coccidiosis. Through this visualization, scientists are able to see a precise pathway of Lincomycin affecting the parasite in an effort to treat it. Although the length of treatment can not be determined at this time, the results can contribute to future work with Molecular Dynamics (MD) simulation. This type of simulation will generate accurate and quantitative results than with Ligand Docking.

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