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Molecular Docking Study of ITPA protein substrate complex

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TRiO McNair Scholar Program

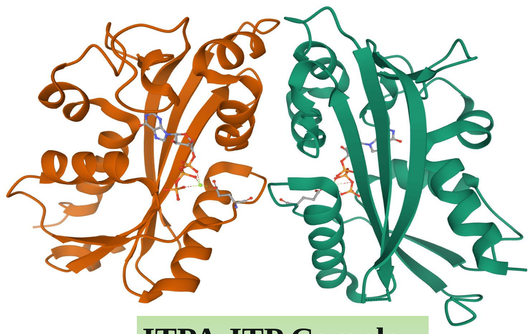
Abstract

ITPA is an enzyme that is responsible for maintaining a proper level of nonstandard nucleotides in cells. The enzyme is associated with adverse drug reactions for thiopurine or ribavirin therapy. The prediction of drug interactions sites in ITPA protein is important for modulating ITPA-related drug toxicity. There are a vast number of tools available to predict molecular interactions between receptors and ligands. The methods utilized in this study include computational docking to explore bound conformation and energy in the binding of ITP to ITPA protein. The docking results reveal how well ITPA and ITP bind together in comparison to the native complex. The root-mean-square-deviation, rmsd, is computed to analyze the similarity of the docked structures as well as the binding free energies and ligand efficiencies to rank the structures. The significance of this research study is to understand the mechanism of ITPA binding. The importance of this research pertains to visualizing operative configurations of a protein-ligand complex that will cleanse nucleotide pools and repair damaged DNA.

Introduction

Inosine Triphosphatase (ITPA), is an enzymatic molecule that works to prevent the accumulation of Inosine Triphosphate (ITP), an intermediate in the formation of purine nucleotides. The metabolic pathway of purine nucleotides includes the formation of this intermediate—ultimately leading to DNA production. Overpopulation of ITP causes mutations of DNA leading to cancers, increased Inosine levels in DNA and other immunodeficiencies. ITPA is a prominent protein in the regulation of ITP concentration. A substrate/enzyme complex created through molecular docking simulations will show how and where the receptor and ligand bind. This success also serves as a determination factor to how well the enzyme can function.

In this study, using Autodock tools, Oracle VirtualBox, and Linux software we will predict the 10-best protein-ligand complexes. Doing this will demonstrate an efficient process to eliminate hundreds of thousands of possible yet ineffective conformations. Seeing successful protein-ligand binding complexes visualizes molecular interactions that are necessary in the creation of future molecules to be used in drug development for genomic repair or mechanisms for cancer prevention.



ITPA-ITP Complex

Methods

To predict the correct protein-ligand configurations, computational docking was used in order to give a better visual of what it would look like. Docking gives us the thermodynamic work, or free energy required to bind the receptor and ligand.

Things taken into consideration when molecular modeling:

- The molecular dispersion/repulsion properties
- Electrostatics
- Torsional entropy

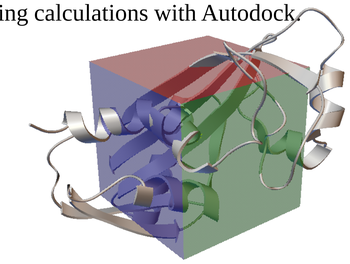
With Inosine Triphosphate being our known target molecule, we now look at how the ligand, ITP, binds to it in order to produce the most optimal configuration. Upon each competent arrangement, the free energy scoring method is necessary to rank which arrangements are most likely to occur and be successful in a protein-ligand circumstance. The scoring sheets for free energies are to be recorded for each competent arrangement.

Docking preparations:

- PDB files of the receptor and ligand were downloaded through a remote computer from the Protein Database and added to a working directory in Linux.
- The files were later extracted from the remote to the local computer using Linux command: “scp username@10.101.101.67:PathOfYourFile/TheNameOfTheFile .”.
- Water and other insignificant molecules were removed from the receptor and ligand files to eliminate any impurities or interference in the docking process .
- The receptor and ligand PDB files were loaded in the Autodock 3D view to be fitted and scaled as reassurance that the correct chain, Chain ‘A’, for both were selected.

Upon this conformation, the macromolecule was saved to be later used as the ‘input’ conformation. The Autogrid feature allowed us to manipulate the {x,y,z} positioning coordinates of the grid box. Once the coordinates were set to where the ligand was at the center of the grid box, those settings were copied and saved to the Linux working directory.

For compatibility with Autodock, the PDB files were converted into ‘PDBQT’ files which reflect partial charges and atom types. The grid parameter files (GPF) was then prepared using the command: “prepare_gpf4.py -l ligand-file.pdbqt -r receptor-file.pdbqt -p npts="60,60,60" -p gridcenter=' x y z” with the previously obtained grid box settings for x,y, and z inputted. The grid parameters file uses the {x,y, z}coordinates to particularize the 3D search space, the probe atoms to use, and estimates the energy of certain atoms over the 3D space grid. Lastly, The docking parameter files (DPF) for both molecules were prepared to be used during the docking calculations with Autodock.

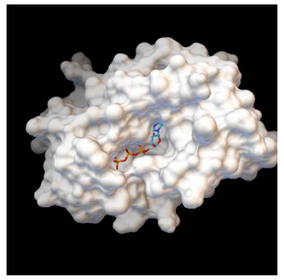


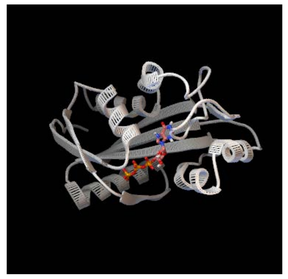
Docking Grid Box

Results/Discussion

Both the receptor and ligand pdb files were extracted from the Protein Databank and successfully converted into PDBQT files.

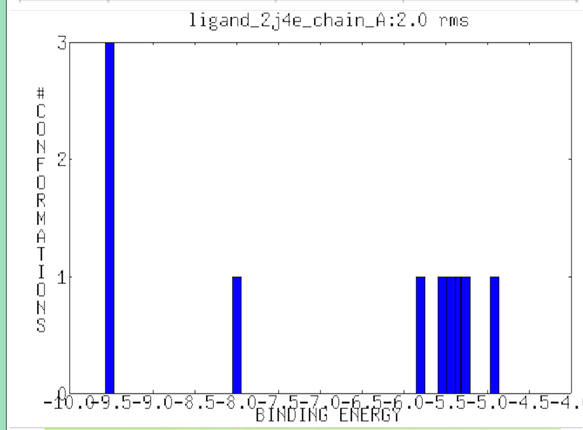
Positioning of the grid box was done to where the box equally surrounded the ligand on all three of the coordinates. This highlighted the ligand’s active site covering every possible spot where it could bind to the receptor. The grid dimensions were 44.249 Å x 24.403 Å x -28.731Å. These were copied and inputted in the grid parameter file preparation command on Linux. The docking parameter files, and docking log files were accurately utilized to conduct calculations for the rmsd, binding energies, and ligand efficiency of the top 10 docked conformations.





AutodockTools view of Conformation 1 protein-ligand complex

Rank	Binding Energy	RMSD	Ligand Efficiency
1	-9.52	1.17	-0.31
2	-6.91	1.87	-0.22
3	-5.46	1.52	-0.18
4	-8.00	6.29	-0.26
5	-5.80	3.86	-0.19
6	-5.54	4.66	-0.18
7	-5.44	5.52	-0.18
8	-5.38	4.73	-0.17
9	-5.26	3.06	-0.17
10	-4.92	5.54	-0.16



Top 10 docked conformations data

The command “adt” was used to open the docking simulations and evaluate the results. A lower value in free binding energy is preferred because it represents a higher level of stability for the arrangement between the ligand and the receptor. A lower value in rmsd is also preferred because the rmsd value is a comparison between the native complex and the docked structure; the less deviation, the closer it is to that structure and more effective. The conformations binding energy ranges from -9.52 kJ/mol to -4.92 kJ/mol. The best docked conformation had a binding energy of -9.52 kJ/mol and the tenth best docked conformation had a binding energy of -4.92 kJ/mol. In the conclusion of the results, the best docked conformation (1), had the lowest rmsd and binding energy. The results of this research show that the Autodock software can predict the native ITP-ATPA complex interactions. In these structures the protein would be able to conduct its enzymatic function effectively regulating the concentration of ITP in the body and diminishing the risk of overproduction leading to genomic damage and DNA mutations. This study validates the use of the Autodock software in predicting molecular interactions between ITPA and small molecules.