Docking study of Phyto Flavanones against Microcystin explored by

*Microcystis Aeruginosa*


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**Abstract**

*Microcystis aeruginosa* is the commonest cyanobacterial strain in fresh water aquatic regions during harmful algae blooms. It can release an extracellular toxin known as Microcystins, which is harmful to people and can cause liver cancer. The microcystins are the cyclic heptapeptide toxins and very difficult to remove from the water body. The problem of microcystin-contaminated water bodies is increasing due to the eutrophication of lakes and reservoirs. This study aimed to control the growth of *Microcystis aeruginosa* with the help of aquatic plant Flavanones. The root of some aquatic plant such as *Echornia crassipes* contain flavonoids especially Leuteolin, Apeginine etc. The algicidal experiments showed that it could inhibit and stop the growth of *Microcystis aeruginosa*. This study suggested that the phytoflavanone was an effective biological agent which can degrade the microcystins released by *Microcystis aeruginosa*.

**Key words**: Cyanobacteria, Microcystins, Docking, Flavanones.

**Introduction**

*Microcystis aeruginosa* is a freshwater cyanobacterium, known producer of a family of toxins termed *Microcystins*. *Microcystins* are hepatotoxic cyclic heptapeptides released into water during or on senescence of cyanobacterial blooms and infect aquatic organisms (Reena et al 2019). The peptide rings of *Microcystins* contain five nonprotein amino acids, whereas the two-protein amino acids distinguish *Microcystins* from one another. *Microcystis-LR* contains the amino acids leucine and arginine. MC-LR is one of the most commonly occurring (Watanabe et al., 1996) and the most toxic microcystin (Van Landingham, 1982). The intact cells as well as the toxins released after cellular lysis can be responsible for the toxic effects observed in many organisms, from microalgae to mammals (Puschner et al., 1998) including human (Ueno et al., 1996).
Exposure to toxic cyanobacteria or administration of MCs may cause hepatotoxic effects (Skulberg et al. 1993), oxidative stress (Angeles et al. 2005), kidney damage, growth inhibition, reproductive injury (Ding et al. 2006), hematological and biochemical alterations (Zhang et al. 2007), apoptosis and even fish death (Lindholm et al. 1999).

This study aimed to control the growth of *Microcystis aeruginosa* with the help of aquatic plant *Ecchornia crassipes*.

**STRUCTURE OF Microcystis aeruginosa**

![Structure of Microcystis aeruginosa]

**Structure of Microcystin**

![Structure of Microcystin]
Computer based approaches are becoming important and complementary to wet laboratory experiments in analyzing the structure and function of biomolecules, and play an important role in the rational design of drugs. Computational techniques have been designed to predict the properties of compounds based on their chemical structure. Several computational methods, including Lipinski filter, ADMET study and molecular docking have been designed to evaluate the behaviour of compounds as a putative future drug. Structure-based virtual screening is a promising screening method in the field of modern medicinal chemistry with wide range of applications in the analysis of molecular recognition events such as binding energetics, molecular interactions and conformational changes (Kalyaanamoorthy et al., 2011).

**Materials and Methods**

**Docking analysis using argus lab**

**Algorithm**

Docking is frequently used to predict the binding orientation of small molecules drug candidates their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.

Molecular docking can be thought of an a problem of “lock and key” where one is interested in finding the correct relative orientation of the “key” which will open up the “lock” (where an the surface of the lock is the key hole, which direction to turn the key after it is inserted, etc). Here the protein can be thought of as the “lock” and the ligand can be thought of as the “key”. Molecular docking may be defined as an optimization problem, which would describe the “best fit” orientation of a ligand that bind to a particular protein of interest. However since both the ligand and the protein are flexible, a “hand-in-glove” analogy is more appropriate then “lock and key”. During the course of the process, the ligand and the protein adjust their conformation to achieve an overall “best-fit” and this kind of conformational adjustments resulting in the overall binding referred to an “induced-fit”.

The focus of molecular docking is to computationally simulate the molecular recognition process. The aim of molecular docking is to achieve an optimized conformation for both the protein and ligand and relative orientation between protein and ligands such that the free energy of the overall system minimized.

Two approaches are particularly popular within the molecular docking community. One approach uses matching techniques that describes the protein and the ligand as complementary surfaces. The second approach simulates the actual docking process in which the ligand-protein pairwise interactions energies are calculated. Both approaches have significant advantages as well as some limitations.
Mechanics of docking

To perform docking, the first requirement is the structure of the protein. Usually the structure has been determined in the lab using a biophysical technique such as x-ray crystallography, or less often, NMR spectroscopy. This protein structure and a database of potential ligands serve as inputs to a docking program. The success of a docking program depends on two components namely the search algorithm and the scoring function.

The search algorithm

The search space consists of all possible orientations and conformations of the protein paired with the ligand. With the present computing resources, it is impossible to exhaustively explore the search space. This would involve enumerating all possible distortions of each molecule since molecules are dynamic and exist in an ensemble of conformational states and all possible rotational and translational orientations of the ligand relative to the protein at a given level of granularity. Most docking programs in use account for a flexible ligand, and several are attempting to model a flexible protein receptor. Each snapshot of the pair is referred to as a pose.

The scoring function

The scoring function takes a pose as input and returns a number indicating the likelihood that the pose represents a favorable binding interaction. Most scoring functions are physics-based molecular mechanics force fields that estimate the energy of the pose where a low or negative energy indicates a stable system and thus a likely binding interaction. An alternative approach is to derive a statistical potential for interactions from a large database of protein-ligand complexes, such as the Protein Data Bank, and evaluate the fit of the pose according to this inferred potential.

Similarity search using BLAST(www.ncbi.nlm.nih.gov/blast)

BLAST is a Basic Local Alignment Tool. It is a homology and similarity search tool. It is a set of search programs designed for Windows platform and is used to perform fast similarity
searches regardless of whether the query is for protein or DNA. It is provided by NCBI. It is used to compare a novel sequence with those contained in nucleotide and protein databases by aligning the novel sequence with previously characterized genes. The emphasis of this tool is to find the regions of sequence similarity. This will yield functional and evolutionary clues about the structures and function of this novel sequence.

Methodology

- open the PDB file of the active site in ARGUSLAB
- select the molecular tree view option from Tools Menu to toggle the tree view
- Expand the tree view and open up the residues
- Then move to aminoacid and click on that folder to see all the amino residues present in the active site
- select any one aminoacid residues by right click and then select all by CTRL+A
- right click on any one of the residue and select the ‘Make a group from the selected residues’
- Then a dialog box appears.select the ‘binding site button’ from ‘Group type’
- Then open the PDB file of the ligand and expand the tree view
- click on disc in the Residue Folder and select the ligand
- Right click on the ligand and select ‘Make a group from this residue
- then from the dialog box select the 'ligand' button and give a name
- bring up the dock setting dialog box by selecting the calculation/dock ligand menu option or click on the button in the horizontal tool bar
- keep as docking engine argus dock, calculation type as dock and ligand as flexible
- click the 'start' button and the docking calculation will begin
- after the docking is completed note down the best ligand pose energy
- visualize the protein as carbon ribbons and ligand as ball and sticks using pymol visualization tool

Lipinski’s rule of five

Lipinski rule of 5 helps in distinguishing between drug like and non drug like molecules. It predicts high probability of success or failure due to drug likeness for molecules complying with 2 or more of the following rules

- Molecular mass less than 500 Dalton
- High lipophilicity (expressed as LogP less than 5)
- Less than 5 hydrogen bond donors
- Less than 10 hydrogen bond acceptors
- Molar refractivity should be between 40-130
Result

Sequence of the target from genbank in fasta format

Docked positions of ligands on Microcystin active site are demonstrated in Figure 1.2 and Figure 1.3. The ligands are represented in stick format with custom colouring (green) and binding of the key residues to the ligands are represented in line format with standard CPK colour scheme. The interactions between the ligand and the receptor residues are shown in dotted lines. The hydrogen bonds are yellow in colour. The receptor surface is enabled for the docked complex and is colour green-orange red using custom colouring option in DS 4.0.

Docking between Microcystin and Ligand

• Receptor-ligand interaction

![Figure 1.2: Interaction between Apigenin and Microcystin](image1)

• Fig 1.3: Interaction between Apigenin and Microcystin (Surface representation)
**Fig 1.4: Interaction between Flavones and Microcystin**

**Fig 1.5: Interaction between Flavones and Microcystin (Surface representation)**
• **Fig 1.6: Interaction between Luteolin and Microcystin**

**Fig 1.7: Interaction between Luteolin and Microcystin (Surface representation)**
### Induced fit docking scores, h-bond interaction of ligand with protein

<table>
<thead>
<tr>
<th>S.NO.</th>
<th>LIGAND NAME</th>
<th>H-BOND INTERACTION</th>
<th>ENERGY VALUE (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>APIGENIN</td>
<td>N – H – - - - - O (GLN40)</td>
<td>-6.49323</td>
</tr>
<tr>
<td>2.</td>
<td>FLAVONE</td>
<td>O – H – - - - - O(THR42)</td>
<td>-6.37249</td>
</tr>
<tr>
<td>3.</td>
<td>LUTEOLIN</td>
<td>N – H – - - - - O(ARG43)</td>
<td>-7.32387</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N – H – - - - - O(GLN40)</td>
<td></td>
</tr>
</tbody>
</table>

The predicted structure was submitted in CASTP server for the identification of possible catalytic residues available in the model. The selected pocket volume is 1.4 Å and the surface area 68.4.

The parameters and patterns are identified using protparam. These information was used to characterize the function of the target of the protein.

The validated model was searched for possible ligands that may hinder the normal function of microcystin protein. The three ligands are selected from different plants. The inhibitors Apiginine, Flavonone, and Luteolin. The inhibitor Apiginine which shows the pose energy of -6.49323 and has one hydrogen bond with the residues GLN 40 of the target protein.

The inhibitor Flavonone which shows the pose energy of -6.37249 and has 1 hydrogen bond with the residues THR 42 of the target protein.

The inhibitor Luteolin which shows the pose energy of -7.32387 and has 2 hydrogen bond with the residues ARG 42 and GLN 40 of the target protein.

Of the three ligands docked the all three (Apiginine, Flavonone, and Luteolin) showed computationally best interaction with the target and they follows the Lipinski rule of five. Considering the above results, the binding of the ligands may bring about the inhibition of the function played by ligands against Microcystin toxic protein explored by *Microcystis aeruginosa.*

**Discussion**

A molecular docking study was performed to validate the binding efficiency of compounds to EGFR using LibDock module (Diller 2001) implemented in DS 4.0. Crystal structure of Microcystin protein domain was directly downloaded from the RCSB protein data bank (http://www.pdb.org/). All the bound waters and ligands were eliminated and polar hydrogen was added to the proteins. Docking was performed on the shape-based complementarity search programme. The resolution of the selected PDB structure is 2.60 and the possible binding sites from the structure were defined using macromolecule tool set (from receptor cavities) in DS 4.0. The key residues of the
receptor were identified from literature Stamos et al. 2002. The site number 1 involving the identified key residues was selected for the docking study. The scoring function of LibDock was used to measure the binding efficiency of each compound. Efficiency of ligand docking is compared with erlotinib.

1. Conclusion

All the three compounds were docked with the target protein Microcystin. To allow all possible conformational degrees of induced fit docking algorithm of Argus Lab software is used. Based on the H-bond interaction, Energy value of the interactions reduced by ligands. Thus we conclude that the aquatic plants namely Ecchornia crassipes has the ability to produce phytoflavonones responsible for inhibiting Microcystin toxin. The ligands selected from plant compounds, such as Apiginine, Luteolin, Flavonone has greater effectiveness and they do not have any side effects. It may not cause any other harmful effect.

Considering from this, these compounds have good activity against Microcystin

Reference


Watanabe, Y., Imai, K., Oishi, H., Tamai, Y. 1996 Disruption of phospholipase B gene, PLB1, increases the survival of baker’s yeast Torulaspora delbrueckii. FEMS Microbial Lett 145(3):415-20