IN SILICO IDENTIFICATION OF NOVEL DRUG TARGETS FOR *Campylobacter jejuni* AND POTENTIAL DRUG MOLECULES

Submitted in fulfilment of the requirements for the degree of

MASTER OF SCIENCE

IN

BIOTECHNOLOGY

By:

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DECLARATION BY STUDENT

I hereby declare that the thesis work entitled "In silico identification of novel drug targets for *Campylobacter jejuni* and potential drug molecules" submitted to the Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology Solan (H.P.), is a bonafide record of the original work done by me. The work was carried out under the supervision of Dr. Saurabh Bansal and the co-guidance of Dr. Raj Kumar.

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SUPERVISOR'S CERTIFICATE

This is to certify that the thesis work titled "**In silico identification of novel drug targets for *Campylobacter jejuni* and potential drug molecules**" by **Ms. Ginni Khullar** during the end semester in May 2022 in fulfilment for the award of the degree of Master of Science in Biotechnology of Jaypee University of Information Technology, Solan has been carried out under our supervisions. This work has not been submitted partially or wholly to any other University or Institute for awarding this or any other degree or diploma of recognition.

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Date:

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ABSTRACT

Campylobacter jejuni is the main responsible organism of Campylobacteriosis, a diarrhoea-like condition involving symptoms like nausea, vomiting, enteralgia and, in critical cases, reactive arthritis and Guillain-Barré syndrome. Usually, this disease is treated without antibiotics, but antibiotic therapy is important for patients with severe sickness as well as post-infection problems. *Campylobacter* is a gram-negative bacterium that has acquired antibiotic resistance due to drug overuse and misuse, which has led to a search for new antibiotics against *C. jejuni*, as it continues to remain untreated with the already available drugs on the market, like, Azithromycin, Erythromycin, Ciprofloxacin, Tetracycline, etc. The search for new antibiotics is carried out with the help of Computer-Aided Drug Discovery, a computational technology for discovering new drugs targeting various medicinal targets or virulence factors of *C. jejuni*.

Keywords: *Campylobacter jejuni*, Campylobacteriosis, antibiotic resistance, virulence factors, Computer-Aided Drug Discovery, docking, virtual screening.

CHAPTER 1: INTRODUCTION

Campylobacter jejuni is a microaerophilic, gram-negative organism belonging to the family *Campylobacteriacae*. It is a food and water-borne pathogen that causes Campylobacteriosis, a diarrhetic condition accompanied by nausea, vomiting, stomach cramps, and fever [1], [2]. The post-infection complications include reactive arthritis and Guillain-Barré syndrome or GBS. The Centers for Disease Control and Prevention (CDC) suggests that in individuals with weak immune systems, this bacterium sometimes spreads to the bloodstream and causes a deadly infection. Campylobacteriosis can be lethal among very young children, old & immunocompromised individuals. Around 1.5 million residents in the U.S.A. are affected every year, and 200 deaths are reported per year. In India, there is a 4.5% incidence rate of campylobacteriosis in the Southern part and a 10.28-13.5% diarrheic rate in the North [3]. *Campylobacter* is found in the intestines, liver, and other organs of many poultry animals like chickens, turkeys, and cows, showing no sign of the disease caused by the bacteria. It is spread by eating undercooked poultry, drinking unpasteurized milk and untreated water, and coming in contact with farm animals.

Campylobacteriosis is an infection that can be treated without antibiotics, but patients with severe illness and post-infection complications are required to undergo antibiotic treatment. Antibiotics such as fluoroquinolones, quinolone, macrolides, and aminoglycosides are used for the treatment of campylobacteriosis. Still, the bacteria, throughout treatment, have started developing resistance against the clinically available drugs, which has become a serious concern for the health of humans [4]. *C. jejuni* has developed resistance against many antibiotic drugs like fluoroquinolones such as levofloxacin, ciprofloxacin, and moxifloxacin, macrolides like erythromycin, clarithromycin, azithromycin, and aminoglycosides like gentamicin, neomycin, and streptomycin. The main reason for the increasing antibiotic resistance is the unsystematic use of antibiotics by humans for their treatment and therapy, growth and off-label use in animal husbandry [5].

Because of the emergence of multidrug-resistant bacteria, the utilization of medicines for the effective treatment of Campylobacteriosis has been significantly hampered [6]. It is, therefore, necessary to search for novel drug targets and potential drug molecules. This can be achieved by Computer-Aided Drug Discovery (CADD), a computational approach used for the

discovery of new drugs against different potential drug targets[7]. Potential drug molecules are selected based on their virulence. Many virulence factors are considered relevant for Campylobacteriosis, including epithelial cell adhesion and invasion, motility, serum resistance, chemotaxis and bile salt resistance [8]. Factors responsible for cell adhesion and invasion are CadF and JlpA protein, for bacterial chemotaxis are CheY, CheZ, CheA, CheB, CheR, and CheW proteins, for motility are FlgP and FlgQ and flagellin proteins like FlaA, FlaB, FlaC [9]. These factors or proteins can act as possible drug targets for developing antibiotics against *C. jejuni*. Natural ligands that are biological compounds of various herbs have been chosen for docking against the selected novel protein targets.

CHAPTER 2: REVIEW OF LITERATURE

2.1 *Campylobacter jejuni*

Campylobacter jejuni is a zoonotic pathogen that causes Campylobacteriosis [1]. It is a microaerophilic, capnophilic, gram-negative bacterium of the family *Campylobacteriacae*. The name *Campylobacter* is acquired from the Greek words **kampylos**, which means curved and **baktron**, which means rod. It is a motile organism that can be spiral, curved, and rod-shaped, having amphitrichous flagella [10].

Classification of *Campylobacter jejuni* as given by Waite et. al. [11]

Domain: Bacteria

Phylum: Campylobacterota

Class: Campylobacteria

Order: Campylobacterales

Family: Campylobacteraceae

Genus: *Campylobacter*

Species: *C. jejuni*

The unique corkscrew motility of *Campylobacter jejuni* is due to its helical morphology, which, together with its amphitrichous flagella, results in a quick rotation around the axis, which is ideal for moving through the highly viscous mucus layer that surrounds the wall of the intestine [12], [13]. It does not metabolize carbohydrates, relying instead on amino acids or intermediates of the Krebs cycle for energy [14]. The cells' width and length range from 0.2 to 0.9 mm and 0.5 to 5 mm, respectively. Because *C. jejuni* does not exhibit real thermophily, it is referred to as "thermotolerant" [15]. According to CDC, *Campylobacter* infection is identified when *Campylobacter* germs are found in faeces (poop), body tissue, or bodily fluids. The identification of *Campylobacter* can be made either by a cultivation method that isolates the bacterium or a quick diagnostic test that detects the bacteria's genetic material. *Campylobacter* has both oxidase and catalase enzymes (gives a positive catalase and oxidase test) [16]. Hippurate and indoxyl acetate are hydrolyzed, and nitrate is reduced by *Campylobacter jejuni* [17].

Table 1: Basic characteristics and biochemical tests of *C. jejuni* [15]

Basic characteristics/ Biochemical tests	**Result**
Gram staining	Negative
Flagella	Amphitrichous
Motility	Motile
Growth temperatures	37℃, 42℃
Catalase	Positive
Oxidase	Positive
Nitrate reduction	Positive
Nitrite reduction	Negative
Indoxyl acetate hydrolysis	Positive
Hippurate hydrolysis	Positive
Carbohydrate fermentation	Negative

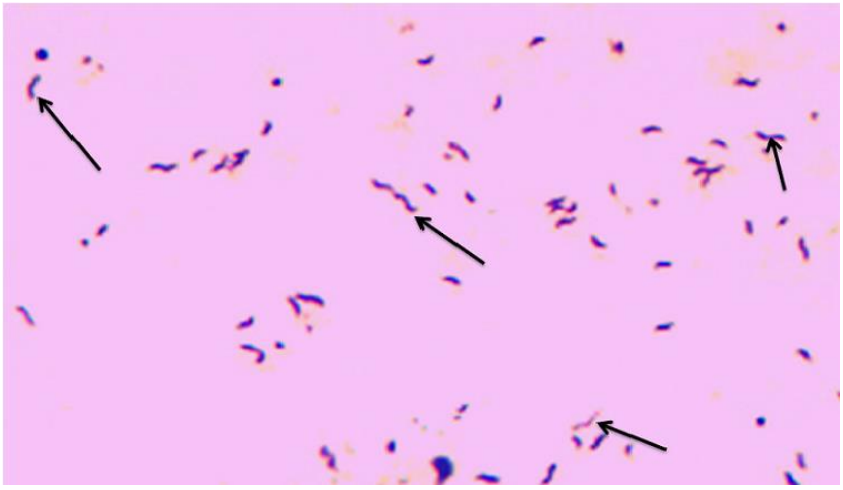


Figure1: Gram-staining showing *C. jejuni* [18]

2.2 Campylobacteriosis

Campylobacteriosis is a diarrhetic condition accompanied by nausea, vomiting, stomach cramps, and fever [7]. The post-infection complications include Reactive Arthritis, Erythema nodosum, a painful illness of the skin's fatty layer that most commonly affects the legs, and Guillain-Barré syndrome (GBS), which begins several weeks after a person falls ill. The body's immune system begins to destroy nerves, producing muscle weakness and in some cases,

paralysis. The CDC suggests that this bacterium can spread to the bloodstream and cause a deadly illness in people with compromised immune systems. Campylobacteriosis can be lethal among very young children, the old & immunocompromised individuals. According to WHO, approximately 1.5 million residents in the U.S.A. are affected every year and 200 deaths are reported per year. There is a 4.5% incidence rate of campylobacteriosis in the Southern part of India, and a 10.28-13.5% diarrheic rate is reported in the North [3]. *Campylobacter* is present in the intestines, liver, and other organs of numerous poultry animals, including chickens, turkeys, and cows, exhibiting no signs of infection. Eating uncooked poultry, drinking unpasteurized milk and untreated water, and coming into contact with farm animals are all ways to spread this bacterium. The majority of campylobacteriosis patients will heal on their own. As long as the diarrhoea lasts, people with campylobacteriosis should drink enough fluids to avoid it. Antibiotics are occasionally used to treat severe cases or persons at greater risk of developing severe diseases, like those with impaired immune systems.

2.3 Drugs available

Many antibiotics have been used for the treatment of Campylobacteriosis. Among the most common are azithromycin, erythromycin (macrolides), ciprofloxacin (fluoroquinolones), tetracycline, chloramphenicol and cephalosporins. In severe cases of Campylobacteriosis, intravenous usage of aminoglycosides is also suggested [19]. Antibiotics have proven to be a boon in saving billions of lives throughout medicine and disease treatment. These antibiotics help in inhibiting the growth and proliferation of many bacteria. The action mechanisms of some antibiotics against *C. jejuni* are discussed below.

2.3.1 Azithromycin

Azithromycin ($C_{38}H_{72}N_2O_{12}$), also available commercially by the brand name of Azomycin, Azasite, Zithromax, and Zmax, is a macrolide of the azalide subclass which is used to treat Campylobacteriosis. But the bacteria have now raised resistance against this macrolide. To treat the infection caused by *C. jejuni*, it's advised to take a dose of azithromycin 500 mg/d for three days. Azithromycin is a 15-membered ring in which the nitrogen at the 9a position has a methyl group substituted on the aglycone ring. Bacteria, for its multiplication, requires a unique protein synthesis mechanism mediated by ribosomal proteins. Azithromycin interacts with the 23S rRNA of the 50S ribosomal subunit of bacteria. It inhibits the transpeptidation or translocation process of protein synthesis as well as blocks the assembly of the 50S ribosomal

subunit, slows cell development, and kills cells [20]. As a result, numerous bacterial infections are controlled[21].

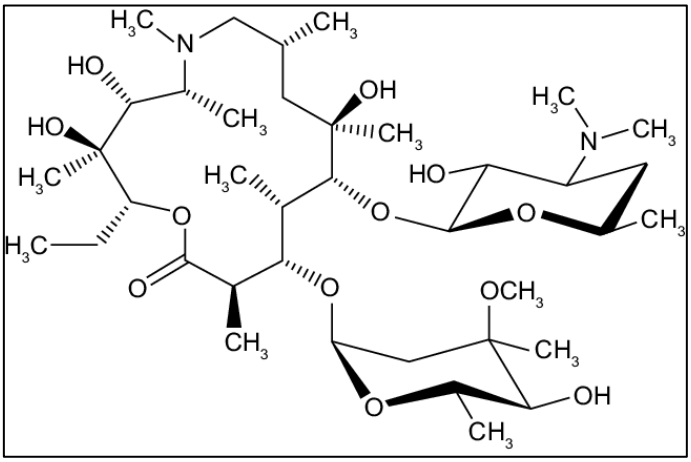


Figure 2: Azithromycin chemical structure [21]

2.3.2 Erythromycin

Erythromycin ($C_{37}H_{67}NO_{13}$) is the antibiotic of choice for the treatment of Campylobacteriosis. It belongs to the class of macrolides, which is available by the brand names Apo-Erythro-S, Benzamycin, Erygel, Erythro, Erythrocin, Erythrocin Stearate, etc. This drug is produced by the bacteria *Saccharopolyspora erythraea* and was discovered in 1952 [22]. It works by inhibiting cytochrome p450 3A4 and P-glycoprotein. In bacteria (for example, *C. jejuni*), erythromycin reversibly binds to the 50S ribosomal subunit and suppresses the translation of proteins, thereby killing the bacteria and reducing the infection [23].

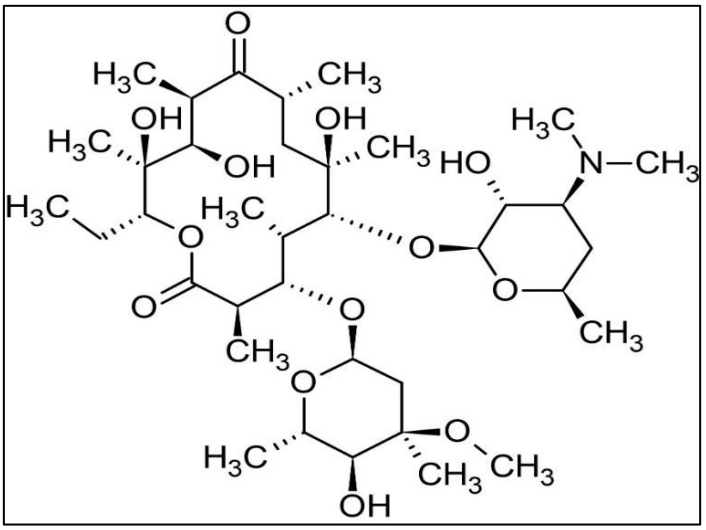


Figure 3: Erythromycin chemical structure [23]

2.3.3 Ciprofloxacin

Ciprofloxacin ($C_{17}H_{18}FN_3O_3$) is a second derivative fluoroquinolone antibiotic. It acts against both Gram-negative and Gram-positive bacteria. It is available by many brand names like Cetraxal, Ciloxan, Cipro, Cipro HC, Ciprodex, Ciprofloxacin, etc. It works by obstructing the enzymes topoisomerase II and topoisomerase IV in bacteria *C. jejuni* [24]. Ciprofloxacin has a 100-fold affinity for bacterial DNA gyrase compared to mammalian DNA gyrase. Ciprofloxacin targets DNA gyrase's alpha subunits, preventing it from supercoiling bacterial DNA and thereby preventing DNA replication in *Campylobacter* [25], [26].

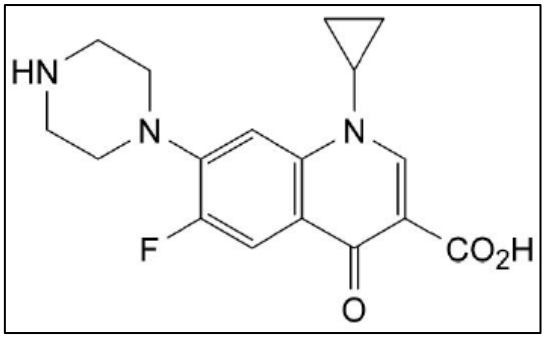


Figure 4: Chemical structure of Ciprofloxacin [27]

2.3.4 Tetracycline

The *Streptomyces* genus of Actinobacteria produces Tetracycline ($C_{22}H_{24}N_2O_8$), a broad-spectrum polyketide antibiotic. It is available by the brand names Achromycin, Pylera, and Sumycin. Tetracycline works by preventing *C. jejuni* from synthesizing proteins. It attaches to the 30S ribosomal subunit and stops amino-acyl tRNA from interacting with the ribosome's A site. It interacts with the 50S ribosomal subunit to some extent as well. The nature of this bond is that it can be reversed. Tetracycline may also cause intracellular components, such as nucleotides, to leak out of bacteria's cytoplasmic membrane, thereby killing *C. jejuni*.

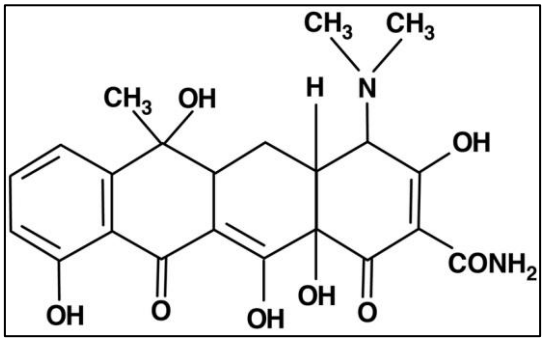


Figure 5: Tetracycline Chemical Structure [28]

2.4 Issues & challenges with the antibiotics already available

The already available antibiotics are not efficient against the bacteria due to the acquired antibiotic resistance. Hence there is a need to look for new drug molecules to control the spread of Campylobacteriosis. Because of innate competence and hypervariable genomic sequences of *Campylobacter jejuni*, there are a lot of genomic plasticities which help raise resistant bacterial progeny [29]. *C. jejuni* has notably developed antibiotic resistance to fluoroquinolones and macrolide antibiotics, raising questions regarding how these organisms acquired resistance traits and the implications for human and animal treatment [30].

2.4.1 Bacterial resistance mechanisms

Due to the overuse and misuse of these antibiotics, many bacteria have developed antibiotic resistance. *Campylobacter* has also developed resistance against the already available antibiotics like azithromycin, gentamycin, ciprofloxacin, etc. The mechanisms of antibiotic resistance generally followed by the bacteria are (i) alteration of the drug target, (ii) deactivation of the antibiotic, (iii) alteration of multidrug efflux pumps, and (iv) the antibiotic's inability to reach its intended target [5], [31]. These mechanisms may be found in the bacteria themselves or acquired from other microbes.

2.4.1.1 Modification of the drug target

C. jejuni follows many antibiotic resistance mechanisms, like decreasing its membrane permeability against the drug molecule. It alters the membrane permeability by changing the expression of porins, which are transmembrane proteins that allow chemical substances, that would otherwise be unable to pass the cell membrane into the periplasmic and intracellular environment [32]. By altering the expression pattern of porins, antibiotic diffusion is reduced into the intracellular and periplasmic sites. These porins do not allow the antibiotics, with molecular weight >360 KDa, to penetrate inside [31]. *C. jejuni* modifies the target site (ribosomal A site) of tetracycline by TetO binding.

2.4.1.2 Inactivation of the antibiotic

The pathogenic *C. jejuni* acquires antibiotic resistance by inactivating the antibiotic using enzymatic mechanisms by hydrolysis or generation of inactive derivatives [33]. The β-lactamases are an extensive drug hydrolyzing enzyme class that inactivates the beta-lactam

class of antibiotics by hydrolysis. Hydrolysis of tetracycline antibiotics results in its inactivation by the *tetX* gene [34]. The antibiotic inactivation also occurs by transferring a chemical group to the antibiotic molecule by the transferase enzyme [35]. Aminoglycosides are modified by aminoglycoside-modifying enzymes and hence become inactive.

2.4.1.3 Alteration of multidrug efflux pumps

Microorganisms use efflux pumps to manage their internal environment by eliminating harmful chemicals like antibiotics from the cytoplasmic environment and transporting them out of the cell [36], [37]. *Campylobacter* removes fluoroquinolone, macrolide, tetracycline, aminoglycosides, and beta-lactam using the efflux pump CmeABC. CmeABC is composed of three proteins, making a tripartite efflux system that eliminates a comprehensive range of various classes of antibiotics [38]. These proteins are:

- the inner membrane protein CmeB,
- periplasmic fusion protein CmeA,
- outer membrane protein CmeC.

2.4.1.4 The antibiotic's inability to reach its intended target

The antibiotic is sometimes unable to reach its target site because of mutations in the site. These mutations alter the affinity and avidity of the site for the antibiotic, rendering it useless[39]. Fluoroquinolone cannot bind to its target site because of a point mutation in the DNA gyrase target site. Due to this, the binding affinity of the antibiotic fluoroquinolone decreases for its target site. *C. jejuni* develops this resistance naturally against the antibiotics, but environmental factors can either favour or discourage it [40]. New bacterial progeny naturally develops antibiotic resistance due to the mutation of the target site and is favoured in the environment [31].

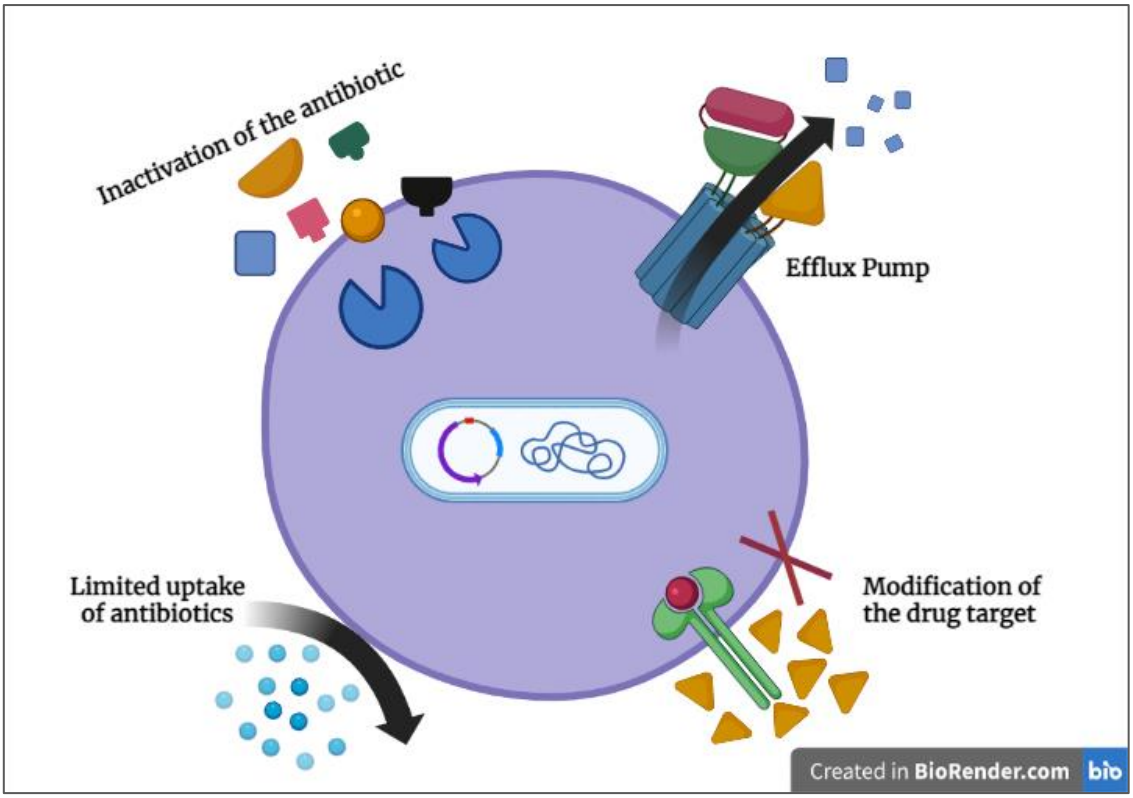


Figure 6: Antibiotic resistance mechanisms

(This figure is created by https://app.biorender.com/)

2.5 Virulence factors of *Campylobacter jejuni*

Microbial pathogens use virulence factors, which include cellular structures, chemicals, and regulatory systems, to colonize, invade, and infect the host organism. *C. jejuni* has several virulence factors that are essential for *C. jejuni* to induce campylobacteriosis, such as [8],

- Motility
- Epithelial cell adhesion and invasion
- Resistance to bile salts
- Serum resistance

The entire genome sequences of various *Campylobacter* strains and plasmids have signalled a new beginning in the research area of *C. jejuni*. These studies have uncovered possible ways through which *C. jejuni* interacts with the host [29], [41]. The ability to colonize the digestive tract of animals is aided by motility and the existence of the flagellum in *C. jejuni* [42]–[44].

2.5.1 The flagellum

Various characteristics of *C. jejuni* biology, including host colonization, pathogenicity, secretion, and host-cell invasion, rely on the amphitrichous flagella and flagellar motility. A basal body, hook, and filament makes up the flagellum of *C. jejuni*. The filament is composed of two flagellin proteins, FlaA and FlaB. The flagellar σ factors in *C. jejuni*, σ^{28} (encoded by *fliA*) and σ^{54} (encoded by *rpoN*), regulate the genes *flaA* and *flaB*, respectively [45], [46]. FlaC, which is necessary for invasion and shares very little homology with the major and minor flagellins (FlaA and FlaB), is secreted by *C. jejuni*'s flagellar export apparatus[9]. Thus, in *C. jejuni*, the flagellar export apparatus represents a crucial secretion mechanism essential for host-cell invasion. This flagellar export apparatus is necessary for the secretion of Cia proteins, which are essential for epithelial cell invasion in culture [47]. Konken et. al. demonstrated whether the flagellar apparatus of *C. jejuni* served as the Cia proteins' export apparatus. Five genes encoding three structural components of the flagella, the flagellar basal body, hook, filament genes, and genes whose products are required for flagellar protein export, were mutated. Non-motile filament assembly mutations were discovered and did not produce Cia proteins. These findings imply that the Cia proteins of *C. jejuni* are secreted from the flagellar export machinery [46]. By providing the essential motility, *Campylobacter* flagella allows the cells to perforate and pass through the viscid intestinal mucus coating of the host in a very efficient manner[48].

2.5.2 Motility/ Chemotaxis

Bacterial chemotaxis is the influenced bacterial migration toward extracellular signals with higher concentrations of helpful chemicals or lower concentrations of harmful chemicals[49]. Another sort of taxi, known as energy taxis, is a response to an intracellular signal, such as the proton motive force or the electron-transport system's redox status. The key chemoattractants are L-Fucose, L-aspartate, L-cysteine, L-glutamate, and L-serine and the intermediates of the Krebs cycle[50]. In *C. jejuni*, the chemotactic system is intimately related to the flagellum and consequently to motility. The chemotactic signalling system for specific attraction or repulsion is based on external stimulating chemicals attaching to corresponding receptors on the outer membrane of bacteria. Signalling via protein phosphorylation transmits the information to the flagellar motor. Chemotaxis is mediated by several kinases from the Che family, including CheY (a response regulator) and CheA (a histidine kinase) [51]. Transmembrane methyl-accepting chemotaxis proteins (MCP) and transducer-like proteins are the receptors for external

inputs. An MCP-like protein controls energy taxis [2]. These MCPs are split into three categories in *C. jejuni*: A, B, and C. A periplasmic sensory domain, a cytoplasmic signal mediator, and a transmembrane domain make up Group A receptors. Group B receptors have a signalling region that is membrane attached, while group C receptors are cytoplasmic proteins [52].

2.5.3 Bacterial Adhesion

Campylobacter colonization requires adhesion to the host intestinal epithelium, a vital phase in a bacterial infection. CadF, JlpA, and CapA, among other C. jejuni proteins, have been shown to bind cultured epithelial cells. The most studied adhesin is the outer membrane protein (OMP), **CadF** (*Campylobacter* adhesion protein to fibronectin), having a molecular mass of 35,997 Da and an amino acid length of 319. CadF binds to fibronectin, which is present on the basolateral surface of epithelial cells [53]. CadF's fibronectin-binding domain (FRLS) contains amino acids 134–137 [54]. CadF binds to the fibronectin of the epithelium of the host intestine. The epidermal growth factor receptor is phosphorylated after CadF binds to the fibronectin, activating a β- integrin receptor. Cia proteins attract and activate the GTPases Rac1 and Cdc42, which induce the internalization of *Campylobacter* via cytoskeleton rearrangement and subsequent membrane ruffling [55]. Young et. al. suggested that CadF, combined with CiaB and JlpA, enters host cells via fibronectin-mediated adhesion. Another adhesin called **JlpA** (*jejuni* lipoprotein A) is a surface-exposed lipoprotein and an adhesin which is encoded by the gene *jlpA*. It has a molecular mass of 42,215 Da and an amino acid length of 372. At the N-terminus, JlpA has a typical signal peptide and a lipoprotein-processing site [56]. JlpA activates NF-κB and p38 mitogen-activated protein (MAP) kinase in response to Hsp90 binding, both of which contribute to proinflammatory responses. This suggests that JlpA-dependent adherence may be involved in part of the inflammation seen during the pathogenesis caused by *C. jejuni* [57]. Another surface-exposed lipoprotein, CapA (*Campylobacter* adhesion protein A), which is an autotransporter, influences the capacity of the bacteria to bind to and penetrate human epithelial cells [58].

2.5.4 Invasion

C. jejuni invades cells mostly through endocytosis, necessitating Campylobacter-induced cytoskeleton remodelling via microfilaments and microtubules [59]. Membrane protrusion, mediated by the tiny Rho-GTPases Rac1 and Cdc42, is the first stage in the invasion process

[60]. Moreover, proteins produced through the T3SS machinery are thought to have a role in the invasion by the flagellum. The secreted proteins, for example, the Cia proteins, are delivered into the cytoplasm by the flagellar secretion system and are required for colonization and invasion[46], [61]. Cia proteins (e.g., CiaB, CiaC, CiaI), not only aid invasion and colonization but also contribute to intracellular survival. *C. jejuni* secretes approximately 18 Cia proteins when it comes into touch with epithelial cells[62]. CiaC is essential for *C. jejuni* to fully invade host cells and is partly responsible for cytoskeletal rearrangements that cause membrane ruffling. CiaC transport is dependent on bacteria-host cell interaction, and Cia proteins are carried to host cell cytosol via the flagellum [63].

2.6 Tools for drug discovery

The tools used for drug discovery come under a common approach, known as Computer-Aided Drug Discovery (CADD), a computational approach used for drug discovery against different potential drug targets [7]. This approach is divided into two categories: structure-based and ligand-based. Ligand docking, pharmacophore, and ligand design are examples of structure-based approaches. Only ligand information is used in ligand-based approaches to predict activity based on its similarity/dissimilarity to previously known active ligands[64].

2.6.1 Tool for retrieval of protein sequence

The tool used for protein sequence retrieval is UniProt. UniProt (Universal Protein Resource) is a publicly available protein sequence and functions database, with several entries coming from genome sequencing efforts. It contains details about the biological function of proteins culled from scientific publications[65].

Figure 7: UniProt [65]

2.6.2 BLAST

BLAST also called as Basic Local Alignment Search Tool is a web-based tool which offers a comparison between primary biological sequencing data. Standard protein-protein BLAST (blastp) is used to detect similar amino acid sequences in protein databases as well as to identify a query amino acid sequence. Blastp, like other BLAST programmes, seeks out local regions of similarity[66]. Blastp analysis is done to check the similarity of proteins with the human proteome.

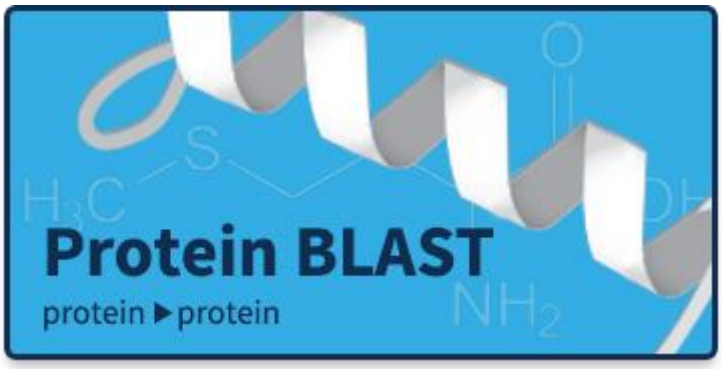


Figure 8: BLASTp [66]

2.6.3 Tool for structure prediction or retrieval and validation

The structures for the proteins can be modelled or predicted using tools like SWISS-MODEL, Phyre2, and AlphaFold.

- SWISS-MODEL is a service for automated three-dimensional (3D) protein structure homology modelling. Homology modelling is the most accurate way of generating credible three-dimensional protein structure models at the moment, and it's employed in a wide range of applications [67], [68].

Figure 9: Logo of SWISS-MODEL server [67]

- Phyre2 is a set of web-based tools for predicting and analyzing any mutations present in the protein structure. Phyre2 is designed to give biologists a simple and intuitive interface to cutting-edge protein bioinformatics tools[69].

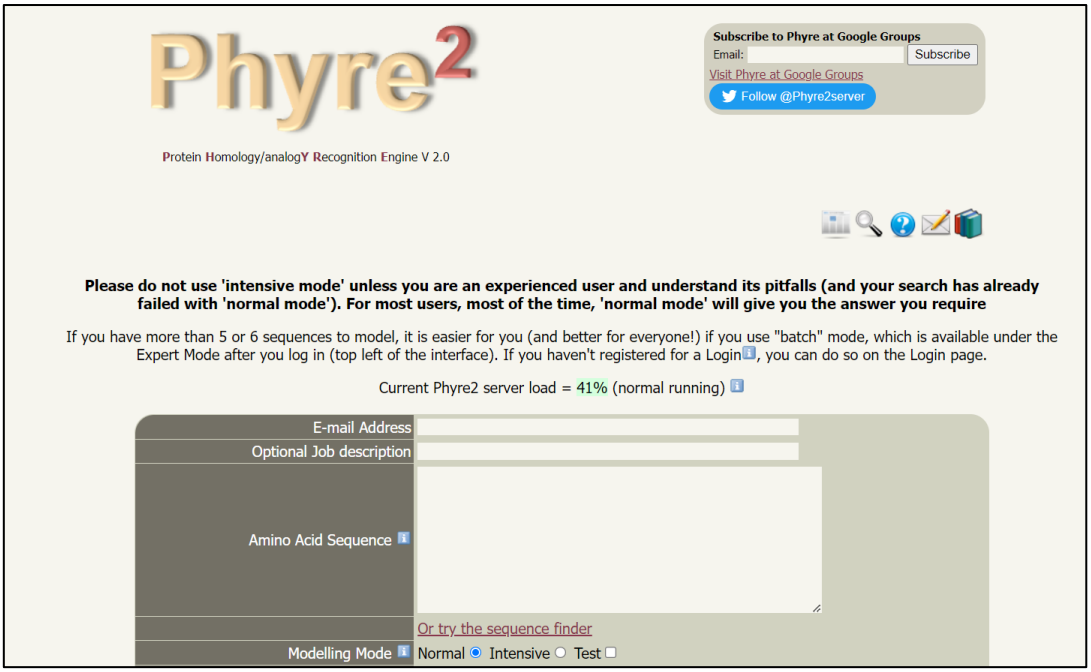


Figure 10: Phyre2 server [69]

- AlphaFold, a cutting-edge AI system developed by DeepMind, can predict protein structures computationally with incredible accuracy and speed. These forecasts are being made freely and openly available to the scientific community, paving the way for new and interesting research directions [70].

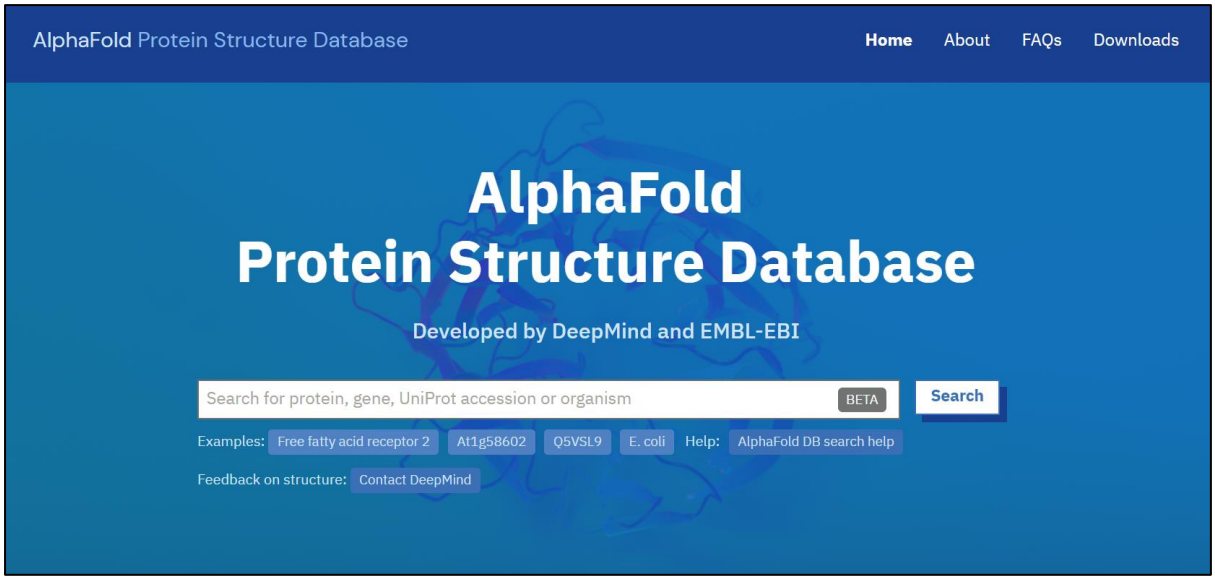


Figure 11: AlphaFold Server [70]

The proteins whose structures are available on UniProtKB are retrieved and those not available are modelled using the above-mentioned modelling tools and software. The protein structures are further validated using PDBsum [71], which checks the Phi and Psi angles with the help of the Ramachandran Plot and the Z-score is checked by ProSA [72].

Figure 12: PDBsum and ProSA servers for protein validation [71], [72]

2.6.4 Retrieval of ligands

The ligands can be downloaded from various databases available online, like

- The ZINC[12] database is a curated list of commercially accessible chemical substances that have been produced specifically for virtual screening. Researchers at pharmaceutical businesses, biotech companies, and research universities use ZINC[12] [73].

Figure 13: Logo of ZINC[12] Database [73]

- PubChem is a database of chemical molecules and their activities against biological assays. The system is maintained by the National Center for Biotechnology Information[74].

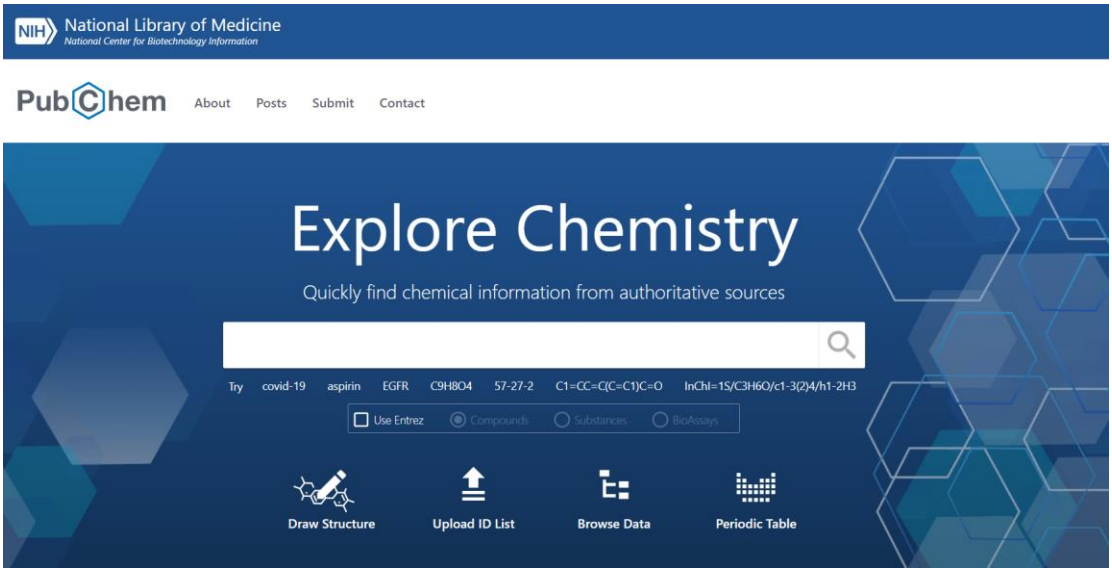


Figure 14: PubChem Database of chemical compounds [74]

Some natural compound databases are also available. They are:

- Natural Ligand Database (NLDB) is a collection of 3D interactions of ligands and proteins for enzymatic activities in KEGG-registered metabolic pathways that are automatically collected and predicted[75].

Figure 15: Logo of Natural Ligand DataBase

- The NPASS database contains 446,552 activity records on 5,863 targets and 35,032 distinct natural products extracted from 25,041 source species. Chemical components of TCM (Traditional Chinese Medicine) herbs and their biological functions are likewise covered in NPASS data [76].

Figure 16: NPASS Database

2.6.5 Binding Site Prediction Tool

The binding site prediction tool CASTp (Computed Atlas of Surface Topography of proteins) helps in the prediction of the probable binding site present in the protein. CASTp is based on contemporary Computational Geometry theoretical and algorithmic discoveries. It has numerous benefits: 1) analytical identification of pockets and cavities, 2) the margins between the bulk solvent and the active site are exactly defined, and 3) all procured parameters are rotationally invariant and do not need grid points[77].

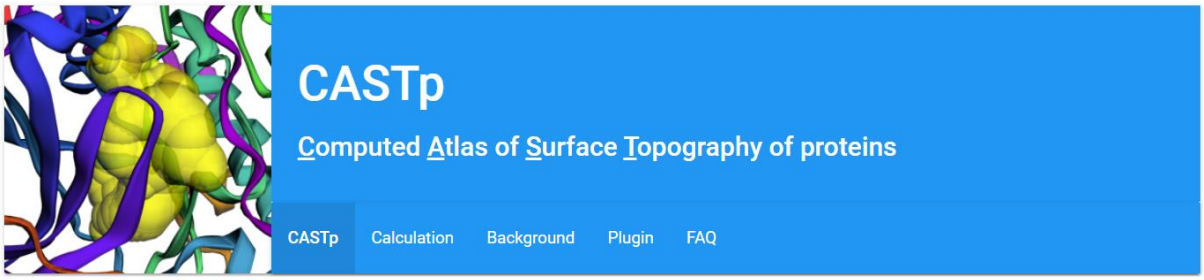


Figure 17: CASTp binding site prediction tool [77]

2.6.6 Tools for Virtual Screening of natural ligand database against proteins

Before the virtual screening is done, the ligands and proteins are prepared for the screening procedures separately. This can be done using Chimera, which is used for the interactive display and analysis of molecular structures and related data, such as density maps, trajectories, and sequence alignments[78]. Chimera is used for energy minimization of the downloaded structures. The purpose of energy minimization is to discover a set of coordinates that represent the structure's lowest energy conformation [79].

Figure 18: UCSF Chimera [78]

After the minimization, the next step is docking between the ligand and the protein, which is a molecular modelling technique. This docking aims to anticipate a ligand's position and

orientation when bound to a protein receptor. Docking can be done using various tools and software, such as,

- AutoDock is a collection of docking automation tools. Its goal is to anticipate how tiny compounds, such as substrates or medication candidates, bind to a 3D-structured receptor. AutoDock 4 comprises two primary programmes: autodock docks the ligand to a set of grids describing the target protein, and autogrid creates these grids beforehand. The atomic affinity grids can be shown in addition to being used for docking. This could aid organic synthesis chemists in designing better binders [80].

Figure 19: AutoDock Logo

- AutoDock Vina is a free molecular docking software. Dr. Oleg Trott of The Scripps Research Institute's Molecular Graphics Lab (now CCSB) created and implemented the system. AutoDock Vina eliminates the need to select atom kinds and calculate grid maps ahead of time. Instead, it internally calculates the grids for the atom types that are necessary, and it does so almost instantly [81].

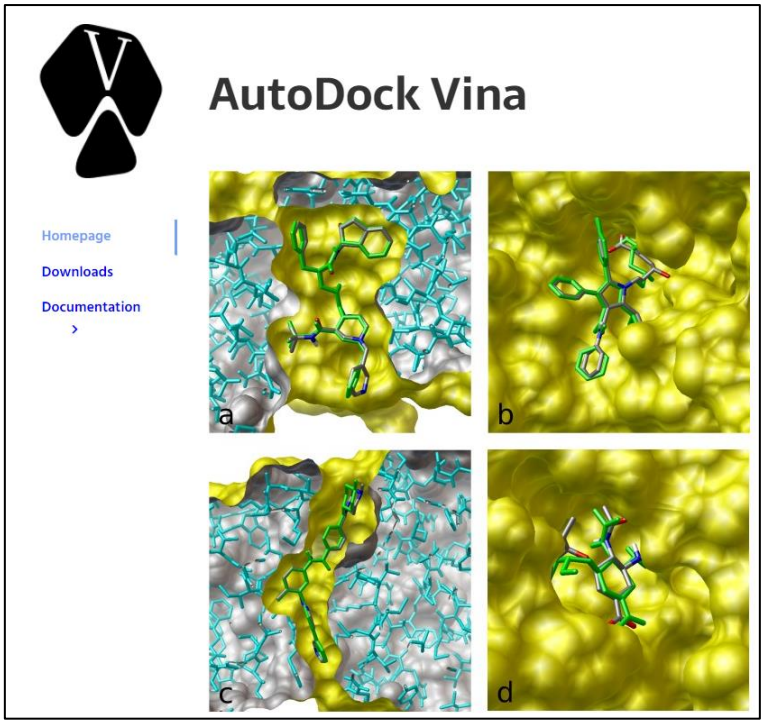


Figure 20: Homepage of AutoDock Vina

- PyRx is a Computational Therapeutic Discovery virtual screening software that can be used for the virtual screening of different libraries of compounds against prospective drug targets. PyRx allows Medicinal Chemists through all the procedure steps, from preparing the data to submitting the job and doing the analysis. PyRx is a useful tool for CADD and has a docking wizard and a simple user interface. PyRx additionally features a strong visualization engine and chemical spreadsheet-like functionality for structure-based drug creation [82].

Figure 21: PyRx- Tool for Virtual Screening

- iScreen is a small web-based tool that allows the docking of TCM ligands and custom de novo drug creation. For customers unfamiliar with command-line systems, iScreen is built with a user-friendly graphic interface. Multiple docking services are applied for

customized docking, including standard, in-water, pH environment, and flexible docking modes. For the researcher's benefit, iScreen provides several molecular descriptors[83].

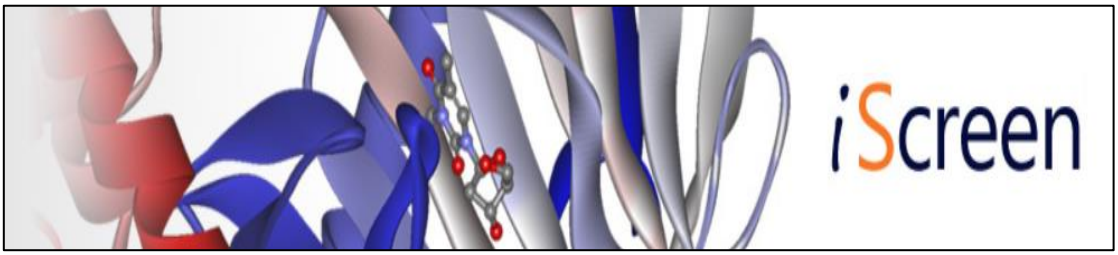


Figure 22: iScreen Web server

CHAPTER 3: METHODOLOGY

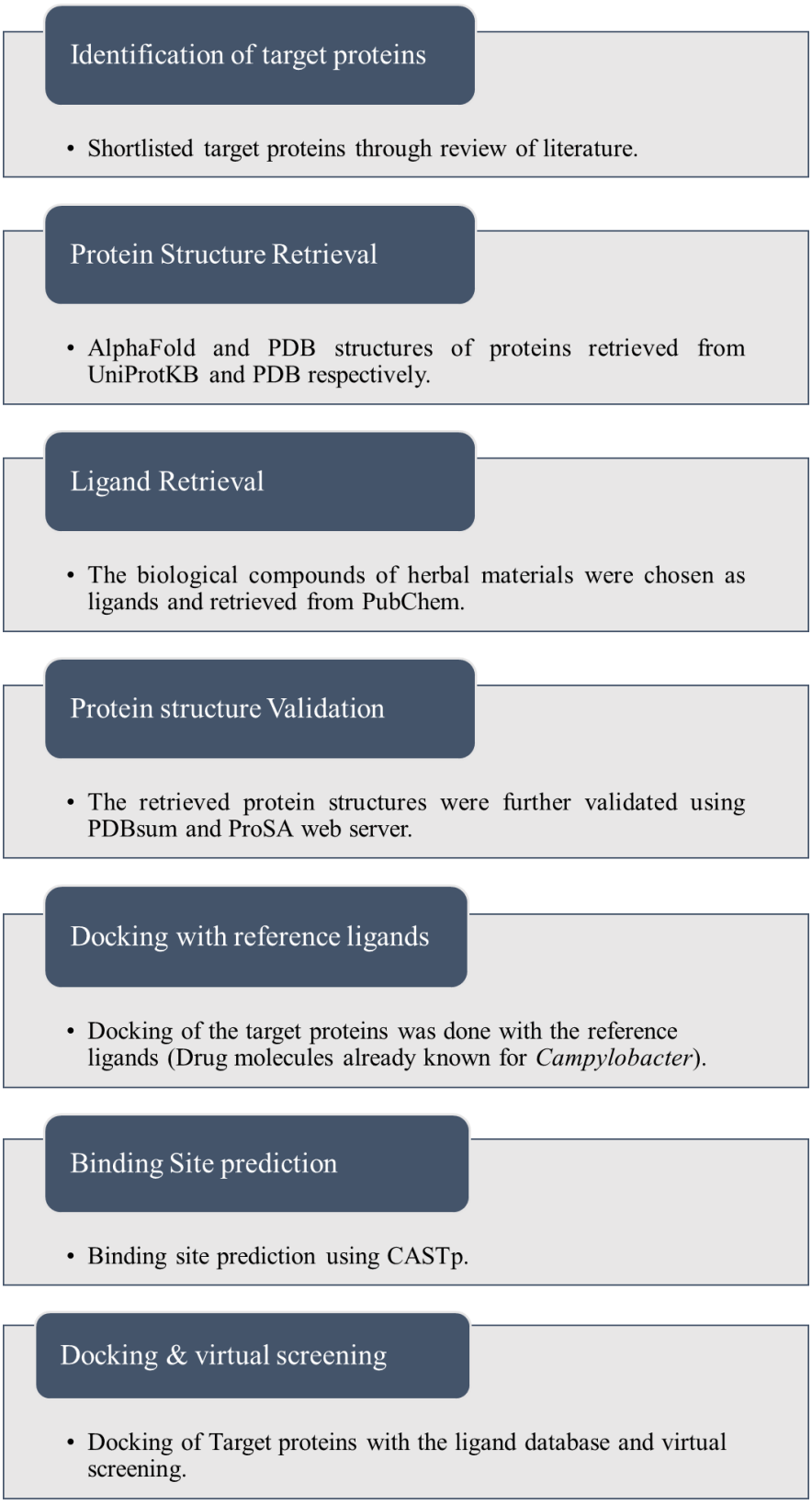


Figure 23: Schematic methodology for the virtual screening of ligands against target proteins of *Campylobacter jejuni*.

3.1 Identification of target proteins

Through an extensive review of the literature (research and review articles), six novel target proteins were identified, CadF, JlpA, FlaA, FlaC, CheV, and CheY. The sequences of these proteins were analyzed against the Human Proteome using BLASTp to rule out any similarities with the humans.

3.2 Protein structure retrieval

The protein structures of CadF, FlaC, FlaA, CheV and CheY were retrieved from the AlphaFold structures available on the UniProtKB. The structure of the JlpA protein was taken from PDB. Phyre2 tool was also used for structure prediction. The predicted structures were further validated using ProSA and PDBsum.

3.3 Ligand retrieval

Fifteen natural ligands were downloaded from PubChem. These ligands are the biological compounds of herbal materials like Clove, Portulaca, Cinnamon, Turmeric, Ginger, Thyme, Camomile, Garlic etc [84]. The database contains the 3D structures of all the natural ligands, which can be visualized with Discovery Studio[85]. The following table shows the natural ligands along with their PubChem IDs and the herbs from which they can be derived.

Table 2: Natural ligands and their respective herbs [84]

PubChem ID	Ligand Name	Herbs
3314	Eugenol	Clove
5280443	Apigenin	Portulaca, Chamomile
5280863	Kaempferol	Portulaca
5280343	Quercetin	Portulaca
637511	Cinnamaldehyde	Cinnamon
558173	Tumerone	Turmeric
64685	Borneol	Turmeric
92776	Zingiberene	Turmeric
443160	(+)-alpha-Phellandrene	Turmeric
10364	Carvacrol	Thyme
6989	Thymol	Thyme
65036	Allicin	Garlic
5280489	Beta- carotene	Mallows
5325830	(-)-Terpinen-4-ol	Tea Tree
4837	Piperazine	Black Pepper

3.4 Protein Structure Validation

Using the PDBsum and ProSA tools, the chosen model was validated by inspecting phi/psi angles with the help of the Ramachandran plot and comparing energy criterion comparisons [86]. The Phyre2 structures showed increased disallowed regions and were not selected for virtual screening. The structures retrieved from UniProt and PDB showed much better results and hence were selected for further procedures.

3.5 Docking with Reference Ligands

Docking of the six selected target proteins was done with the reference ligands. The reference ligands are the drug molecules already known for the treatment of Campylobacteriosis. These are Azithromycin, Ciprofloxacin, Clindamycin, Levofloxacin, Norfloxacin and Streptomycin. This was accomplished using the PyRx tool.

3.6 Binding Site Prediction

Based on the results from the reference docking using PyRx, different poses were analyzed and compared with the binding sites predicted using the CASTp tool. The binding site showing greater similarity with CASTp predicted sites were chosen.

3.7 Docking and Virtual Screening

The natural ligands obtained from herbal compounds and retrieved from PubChem were used for docking and virtual screening against the six target proteins. This was done using the PyRx tool.

CHAPTER 4: RESULTS & DISCUSSION

4.1 BLASTp analysis of proteins against Human proteome

Using *C. jejuni* as a background organism, a similarity search for essential protein identification was carried out. All the proteins were analyzed using BLASTp to rule out any similarities of the target proteins with the Human Proteome. No significant similarities were found which suggests that these proteins can be used for further procedures.

4.1.1 CadF

BLASTp of CadF protein sequence against Human Proteome shows no significant similarity.

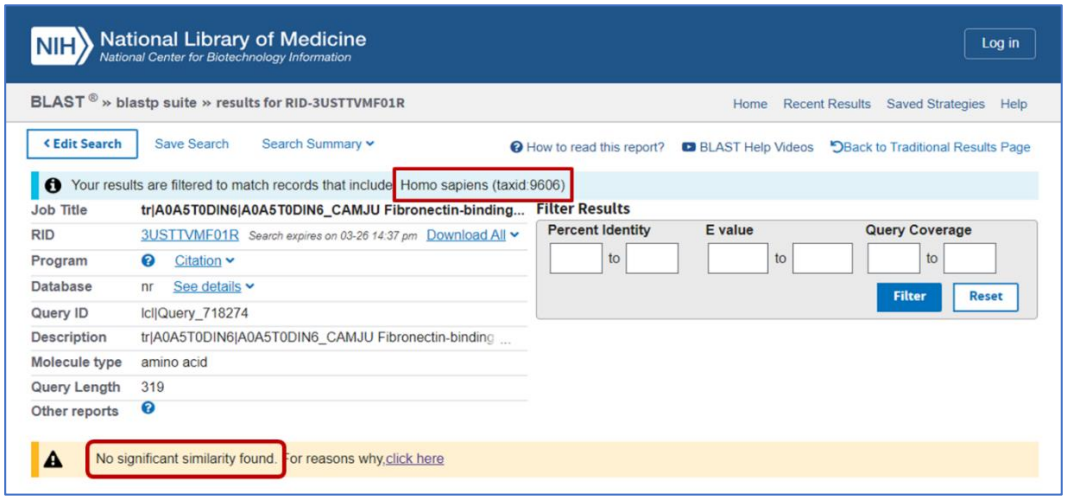


Figure 24: BLASTp between CadF protein and human proteome.

4.1.2 JlpA

BLASTp of JlpA protein sequence against Human Proteome shows no significant similarity.

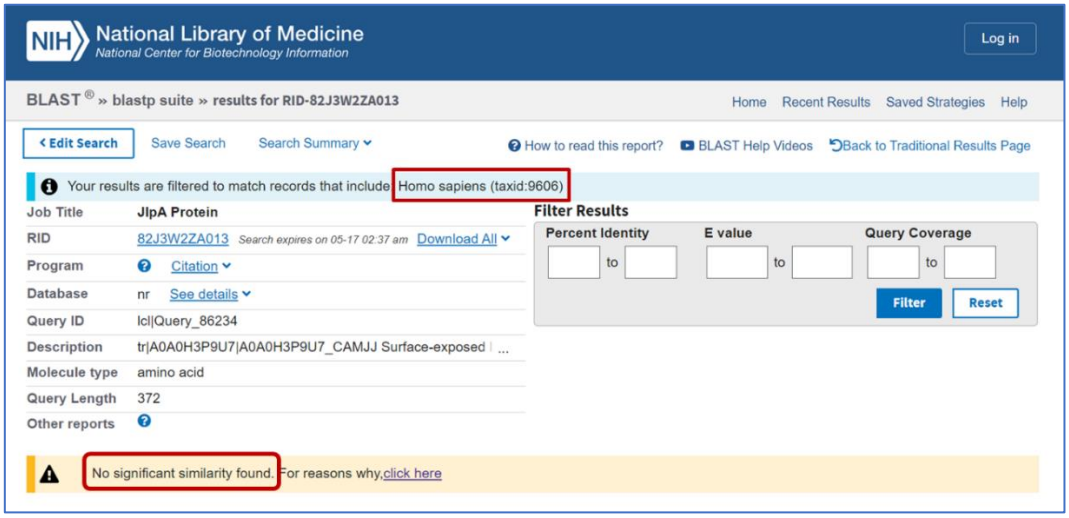


Figure 25: BLAStp between JlpA protein and human proteome.

4.1.3 FlaA

BLASTp of FlaA protein sequence against Human Proteome shows no significant similarity.

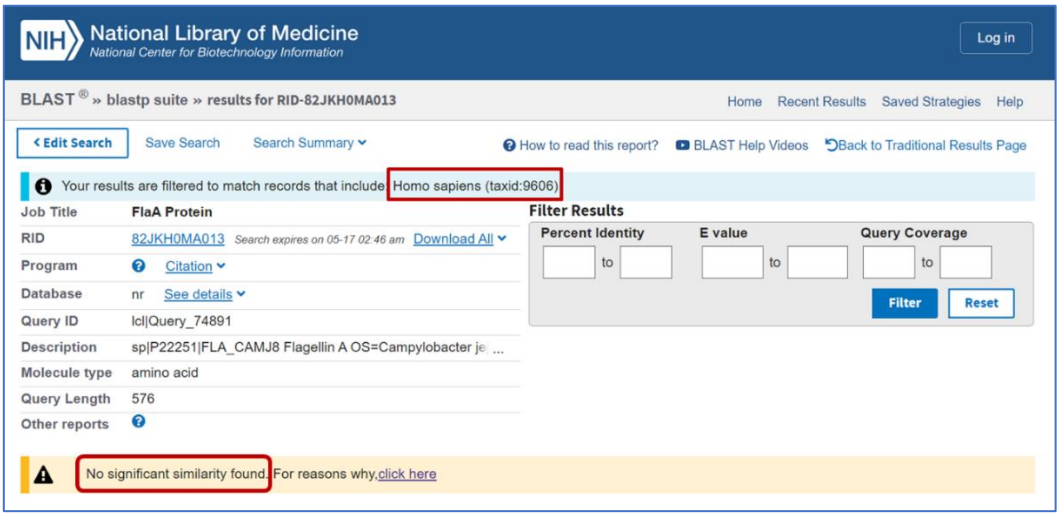


Figure 26: BLASTp between FlaA protein and human proteome.

4.1.4 FlaC

BLASTp of FlaC protein sequence against Human Proteome shows no significant similarity.

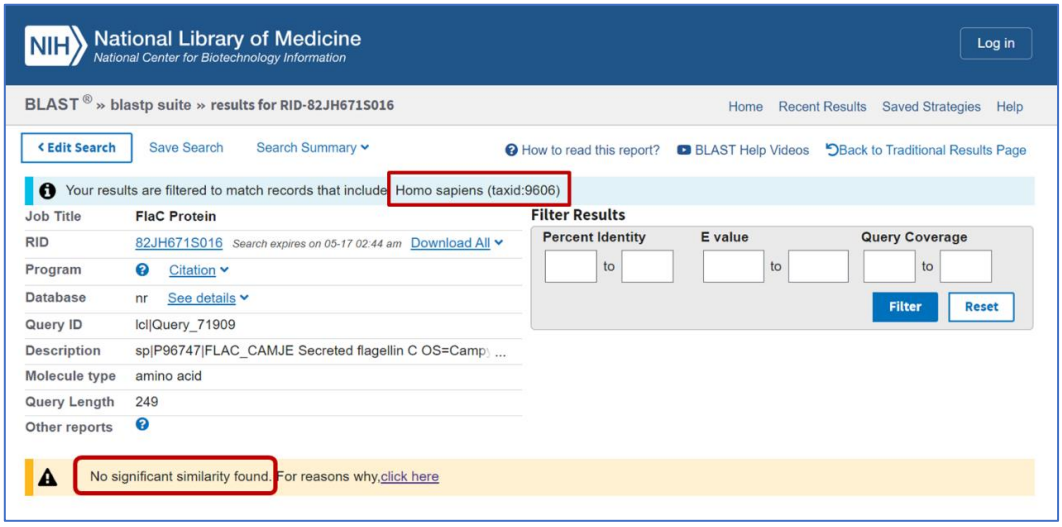


Figure 27: BLASTp between FlaC protein and human proteome.

4.1.5 CheV

BLASTp of CheV protein sequence against Human Proteome shows no similarity.

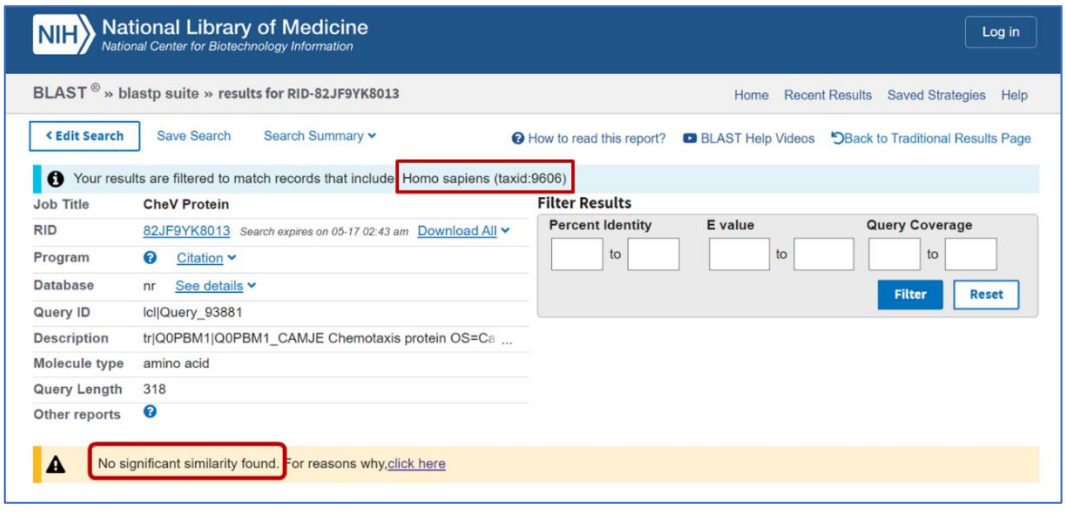


Figure 28: BLASTp between CheV protein and human proteome.

4.1.6 CheY

BLASTp of CheY protein sequence against Human Proteome shows no significant similarity.

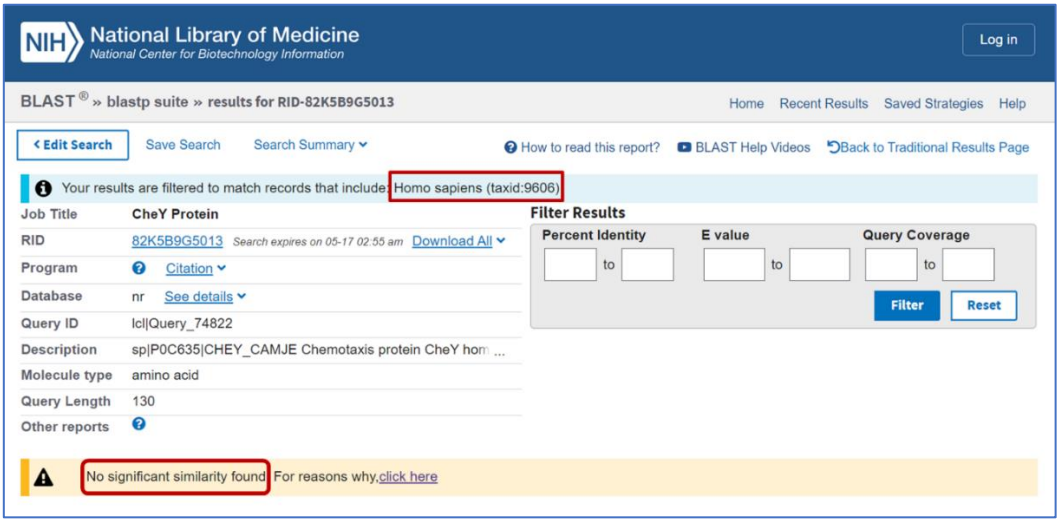
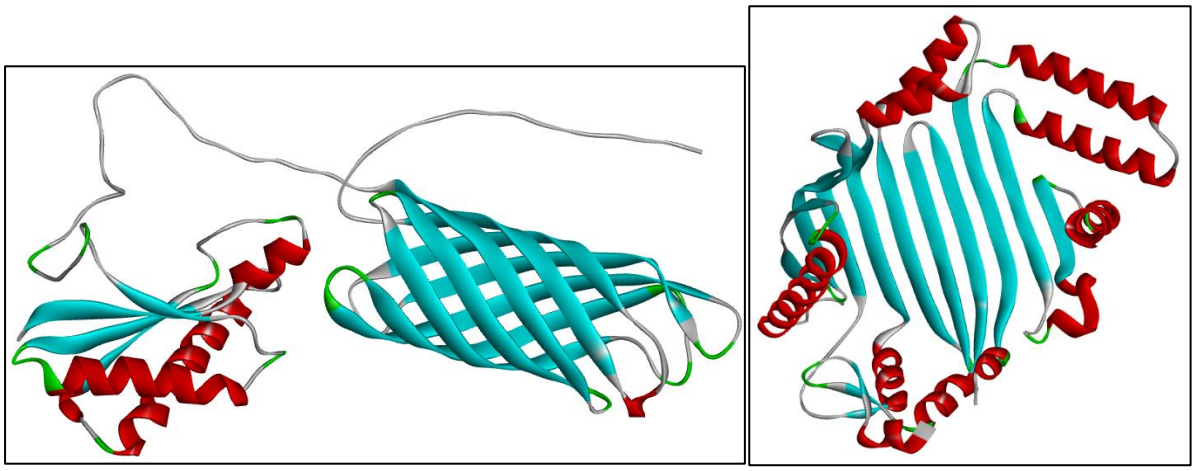


Figure 29: BLASTp between CheY protein and human proteome.

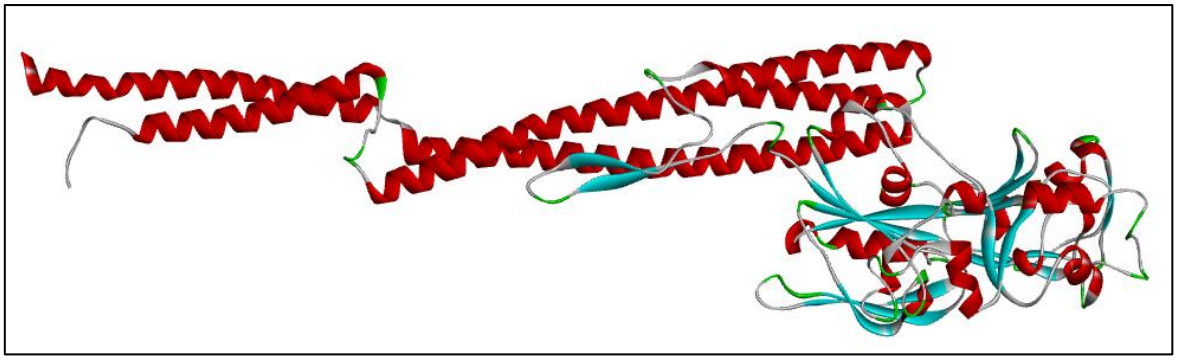
4.2 Protein structure retrieval

The following protein structures were retrieved from UniProt and PDB and were selected for further procedures.

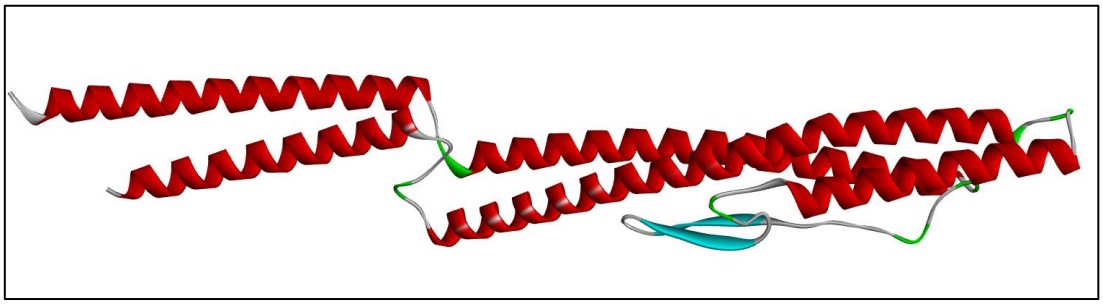


(a) CadF (UniProt ID: Q0P8D9)

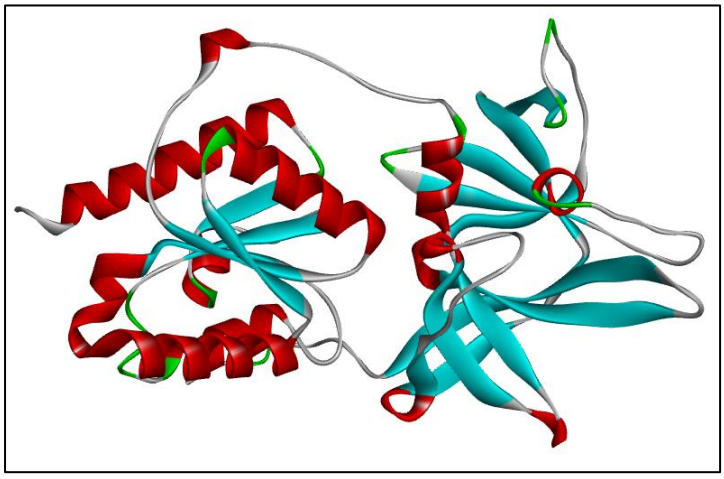
(b) JlpA (PDB ID: 3UAU)



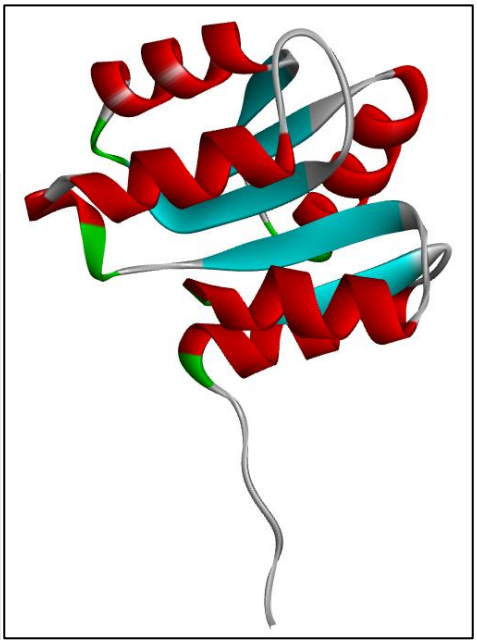
(c) FlaA (UniProt ID: P22251)



(d) FlaC (UniProt ID: P96747)



(e) FlaC **(**UniProt ID: Q0PBM1)



(f) FlaC (UniProt ID: P0C635)

Figure 30: Protein Structures from UniProt and PDB

4.3 Protein Structure Validation

4.3.1 Validation of Protein structures predicted using Phyre2

Table 3: Validation results of Phyre2 structures using PDBsum- PROCHECK

Protein Name	Total allowed regions	Disallowed regions
CadF	96.40%	3.60%
JlpA	99.40%	0.60%
FlaA	100%	0.00%
FlaC	98.30%	1.70%
CheV	94.70%	5.30%
CheY	100%	0.00%

Disallowed regions for CadF, FlaC and CheV were higher than 1.00% and hence were not suitable for virtual screening because, in most disallowed regions, obstruction exists between the side-chain methylene group and the main chain atoms. Therefore, these structures were not selected for further procedures.

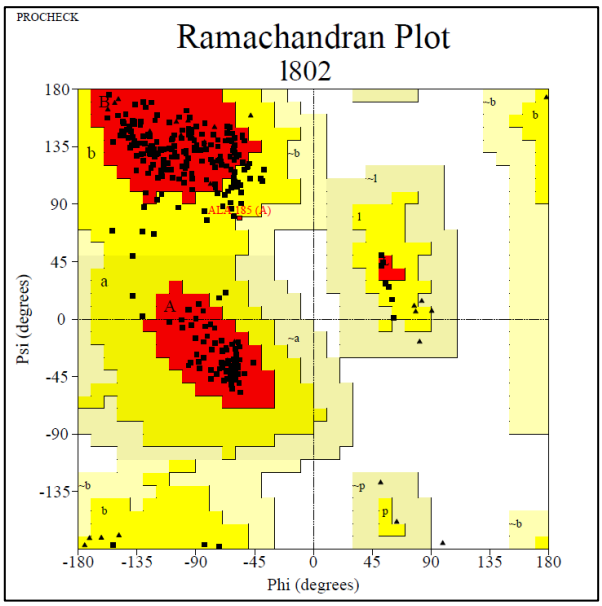
4.3.2 PDBsum-PROCHECK

Table 4: Validation results of UniProt and PDB structures using PDBsum- PROCHECK

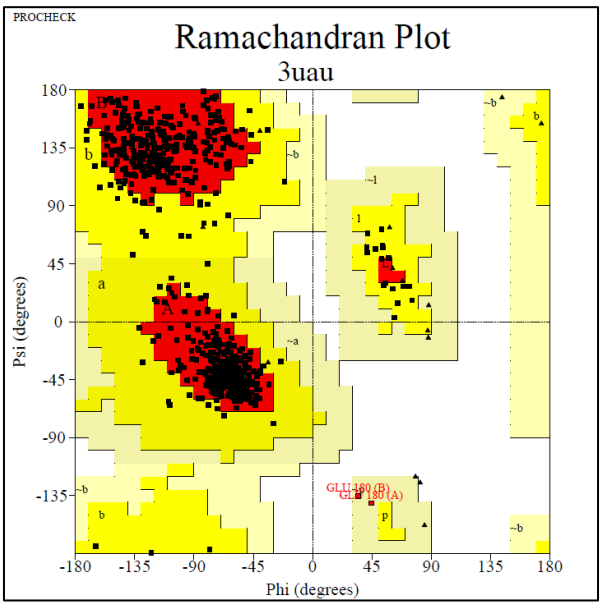
Protein Name	Source	Total allowed regions	Disallowed regions
CadF	UniProt	100%	0.00%
JlpA	PDB	100%	0.00%
FlaA	UniProt	100%	0.00%
FlaC	UniProt	100%	0.00%
CheV	UniProt	99.60%	0.40%
CheY	UniProt	99.10%	0.90%

The structures retrieved from UniProt and PDB showed negligible disallowed regions compared to the Phyre2 structures and hence were selected for virtual screening.

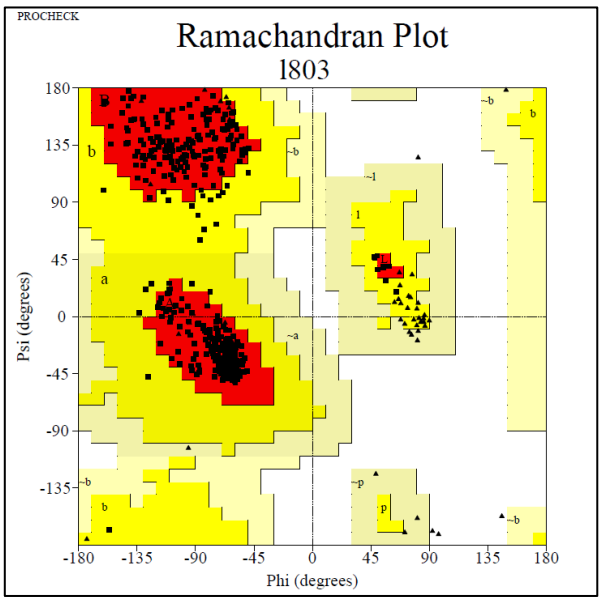
4.3.3 Ramachandran Plot



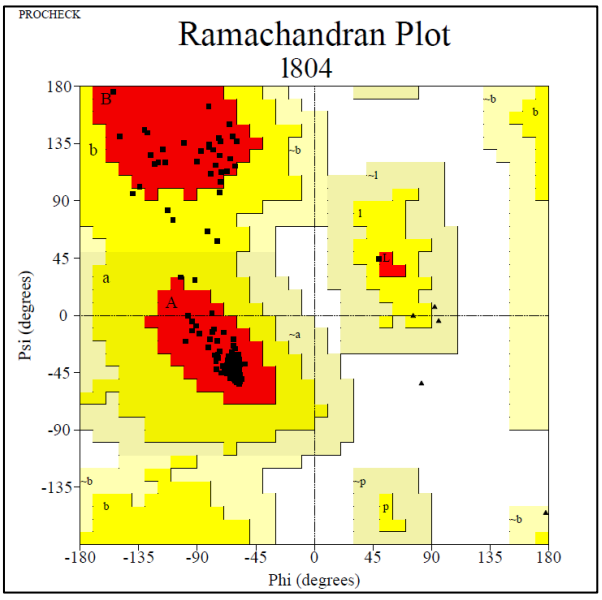
(a) CadF



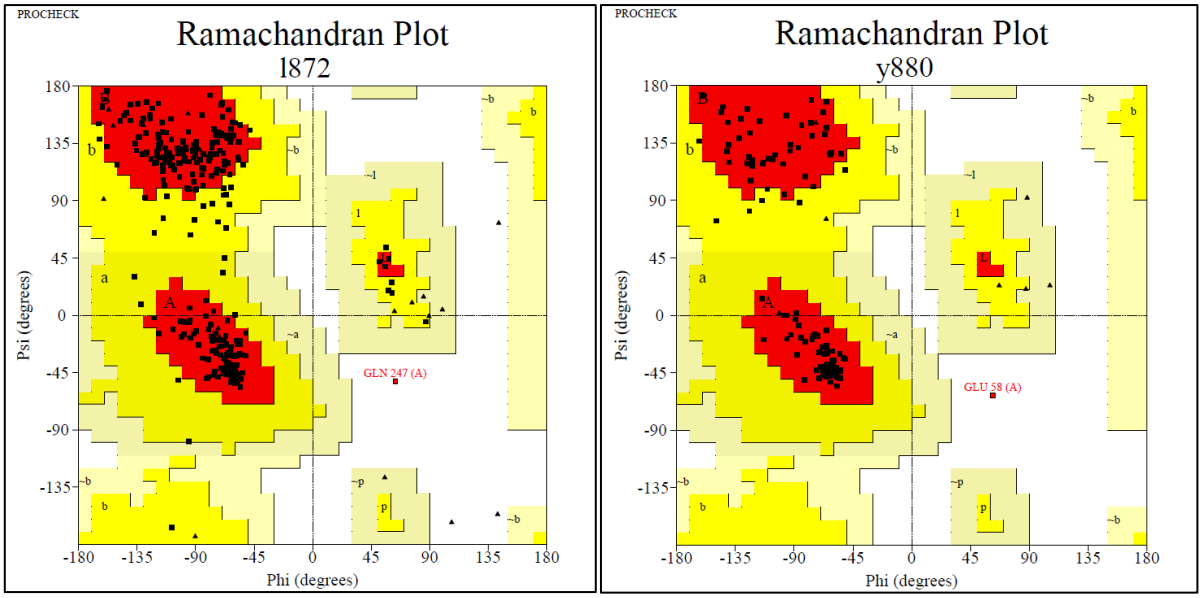
(b) JlpA



(c) FlaA



(d) FlaC

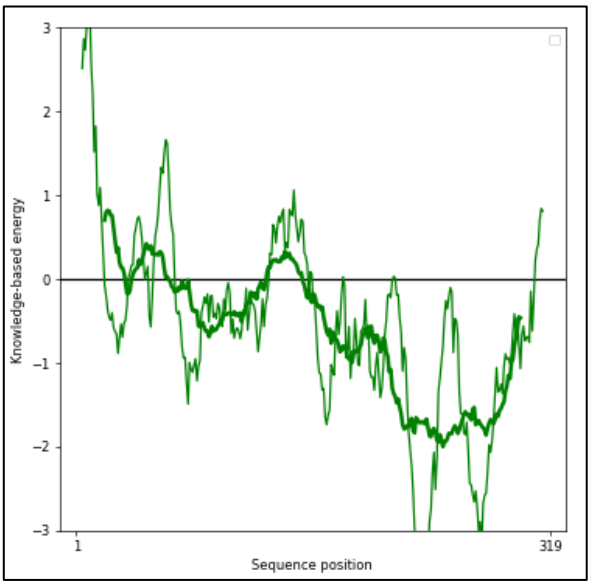


(e) CheV **(f) CheY**

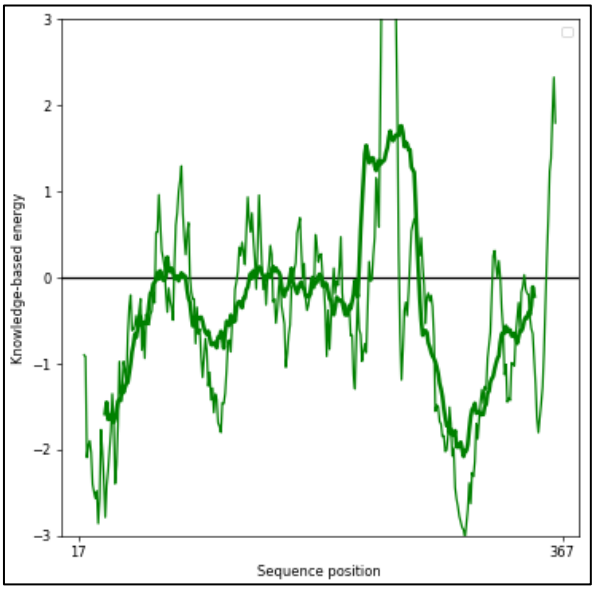
Figure 31: Ramachandran plots of all six target proteins and systematically varied phi & psi.

The white patches in the diagrams above correspond to conformations in which polypeptide atoms are closer together than the total of their van der Waals radii. Except for glycine, all amino acids are sterically forbidden in these locations. The red zones correspond to conformations with no obstruction because these are the allowed regions. Slightly shorter van der Waals radii, when used in the computation, form the yellow region in the plot.

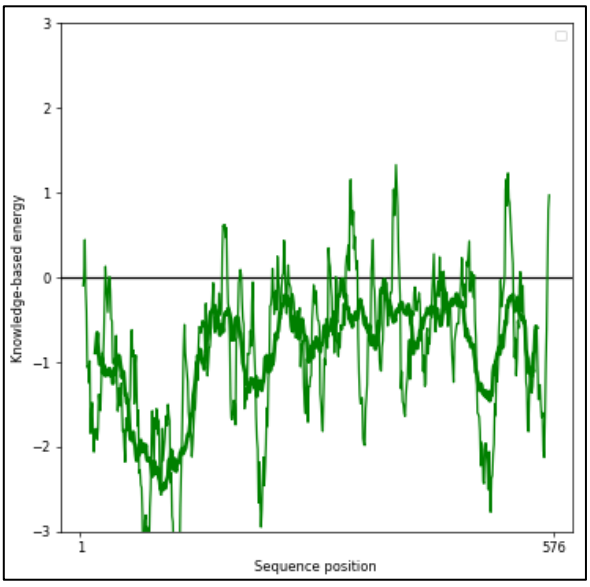
4.3.4 ProSA analysis



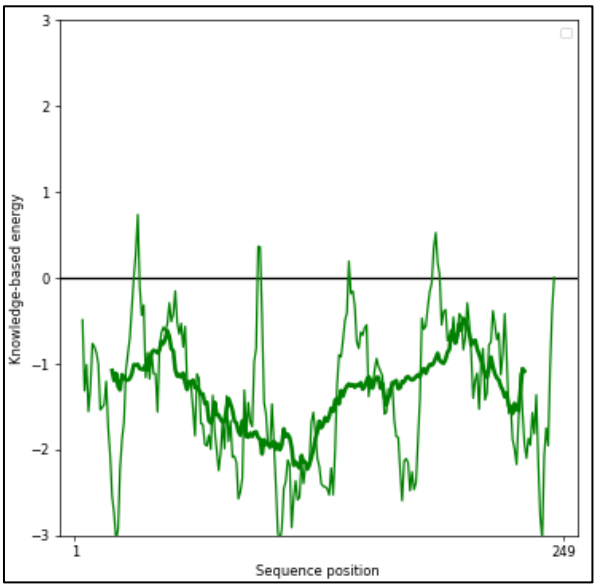
(a) CadF



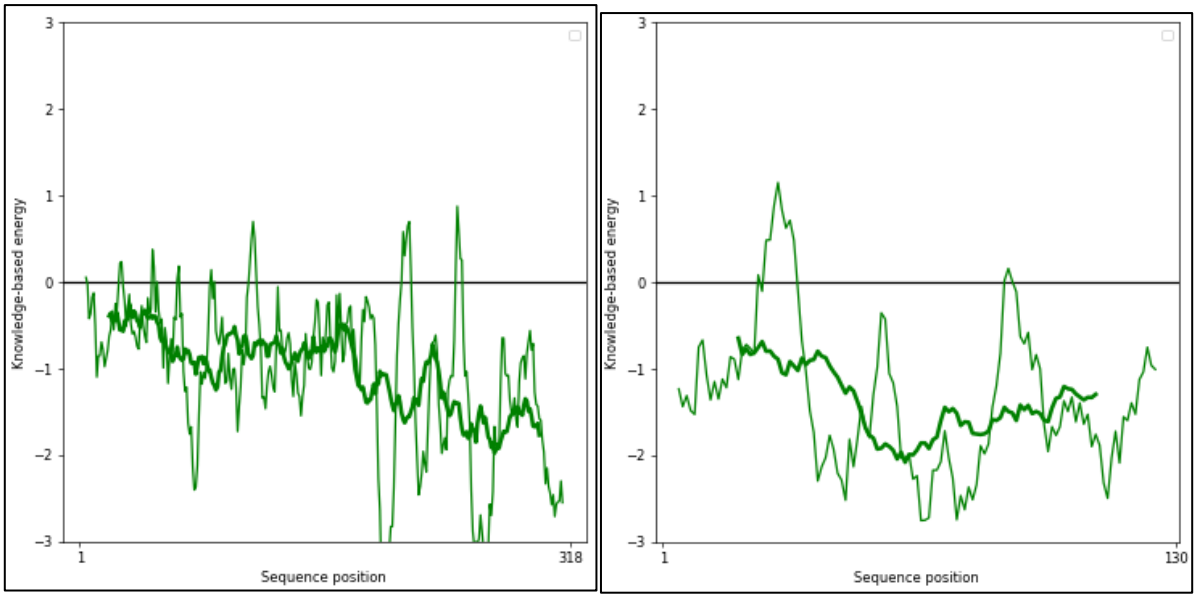
(b) JlpA



(c) FlaA



(d) FlaC



(e) CheV **(f) CheY**

Figure 32: Results of ProSA analysis

Table 5: Z-Scores of target proteins obtained from ProSA analysis

Proteins	Z-Score
CadF	-5.26
JlpA	-5.42
FlaA	-9.23
FlaC	-6.87
CheV	-9.12
CheY	-7.43

The comprehensive model quality and any deviation from the total energy of the protein structure are suggested by the z-score [72].

4.4 Docking with Reference Ligands

Docking of the target proteins with the reference ligands was done using PyRx to recognize the potential binding sites available in the proteins, which was achieved by performing blind docking. The RMSD tables with only the best binding affinities are given below:

Table 6: RMSD table for reference ligands against target proteins

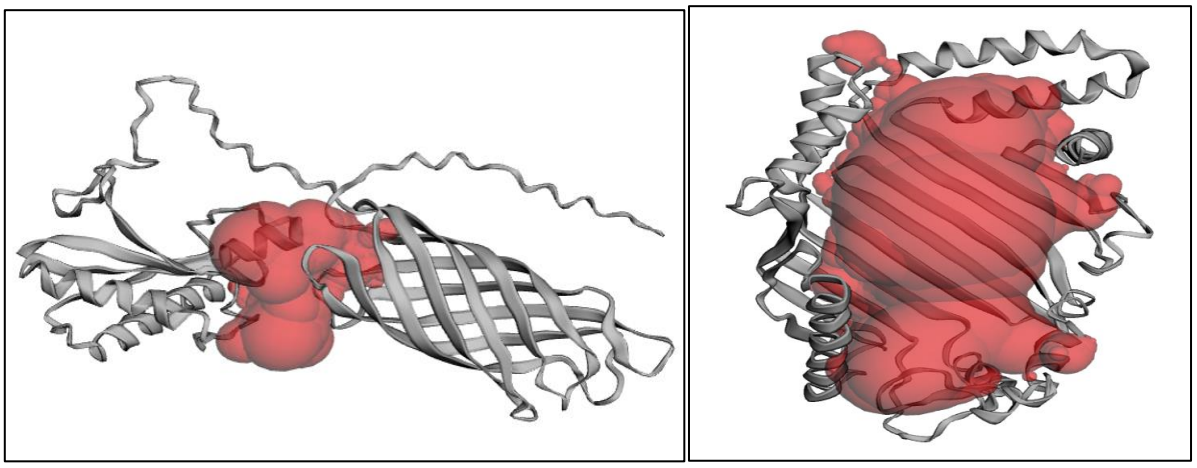
Ligands	Binding affinity	RMSD/ub	RMSD/lb
1. CadF			
CadF_Levofloxacin_uff_E=446.53	-7.1	0	0
CadF_Streptomycin_uff_E=630.68	-8.3	0	0
CadF_Ciprofloxacin_uff_E=1585.31	-7.2	0	0
CadF_Clindamycin_uff_E=476.81	-6.9	0	0
CadF_Norfloxacin_uff_E=355.30	-7.2	0	0
CadF_Azithromycin_uff_E=707.61	-7.2	0	0
2. JlpA			
JlpA_Levofloxacin_uff_E=446.53	-7.3	0	0
JlpA_Streptomycin_uff_E=630.68	-6.7	0	0
JlpA_Ciprofloxacin_uff_E=1585.31	-7.1	0	0
JlpA_Clindamycin_uff_E=476.81	-6.9	0	0
JlpA_Norfloxacin_uff_E=355.30	-6.7	0	0
JlpA_Azithromycin_uff_E=707.61	-6.9	0	0
3. FlaA			
FlaA_Levofloxacin_uff_E=446.53	-6	0	0
FlaA_Streptomycin_uff_E=630.68	-6.9	0	0
FlaA_Ciprofloxacin_uff_E=1585.31	-6.1	0	0
FlaA _Clindamycin_uff_E=476.81	-6.5	0	0
FlaA_Norfloxacin_uff_E=355.30	-6	0	0
FlaA_Azithromycin_uff_E=707.61	-7.1	0	0
4. FlaC			
FlaC_Levofloxacin_uff_E=446.53	-6	0	0
FlaC_Streptomycin_uff_E=630.68	-5.9	0	0
FlaC_Ciprofloxacin_uff_E=1585.31	-6.1	0	0
FlaC_Clindamycin_uff_E=476.81	-5.4	0	0
FlaC_Norfloxacin_uff_E=355.30	-6.2	0	0
FlaC_Azithromycin_uff_E=707.61	-6.3	0	0
5. CheV			
CheV_Levofloxacin_uff_E=446.53	-7.4	0	0
CheV_Streptomycin_uff_E=630.68	-6.1	0	0

CheV_Ciprofloxacin_uff_E=1585.31	-7.3	0	0
CheV_Clindamycin_uff_E=476.81	-6	0	0
CheV_Norfloxacin_uff_E=355.30	-6.9	0	0
CheV_Azithromycin_uff_E=707.61	-6.7	0	0
6. CheY			
CheY_Levofloxacin_uff_E=446.53	-5.7	0	0
CheY_Streptomycin_uff_E=630.68	-5.7	0	0
CheY_Ciprofloxacin_uff_E=1585.31	-5.6	0	0
CheY_Clindamycin_uff_E=476.81	-5.3	0	0
CheY_Norfloxacin_uff_E=355.30	-5.8	0	0
CheY_Azithromycin_uff_E=707.61	-6.9	0	0

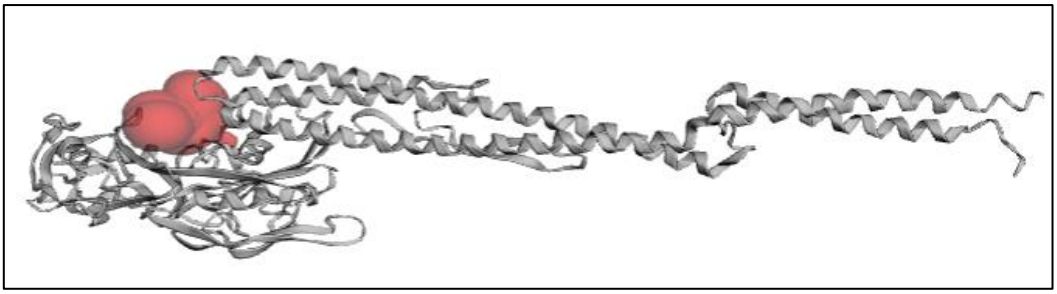
The entries with RMSD values as 0 are considered to have the best binding affinity. These respective conformations exhibited the best bonding with the active sites of the target proteins.

4.5 Binding Site Prediction

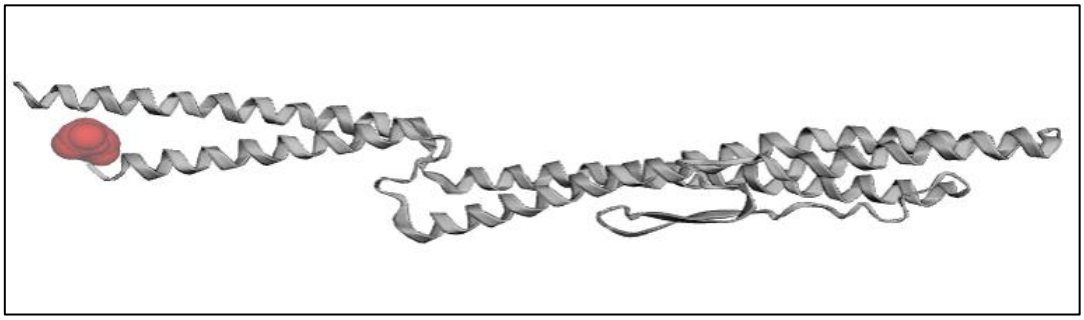
The best conformations from the above result were chosen and compared with the binding sites predicted using the CASTp tool. The proteins and their binding sites (shown in red) are given below.



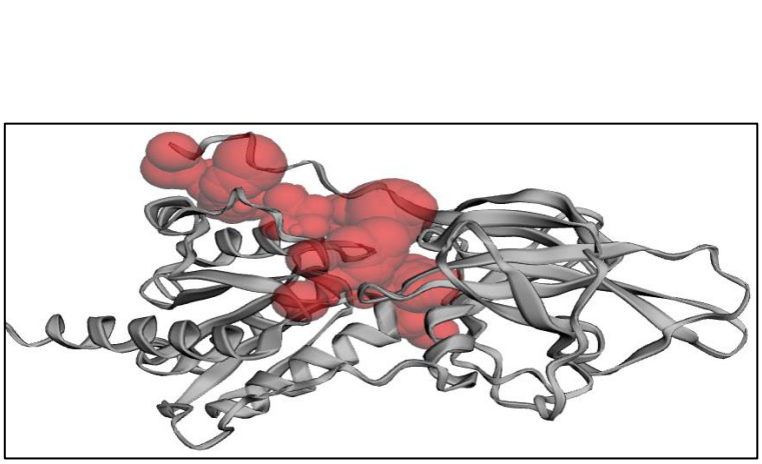
(a) CadF **(b) JlpA**



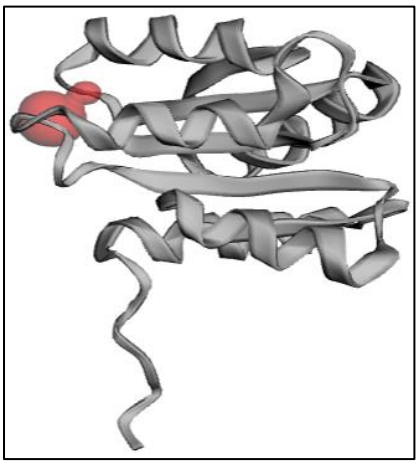
(c) FlaA



(d) FlaC



(e) CheV



(f) CheY

Figure 33: Protein structures predicted using the CASTp tool

Based on the comparison, the binding sites were further used for docking of ligands against the target proteins and virtual screening.

4.6 Docking & Virtual Screening

The results of docking of all 15 natural ligands done against the target proteins are shown below. The RMSD tables show only the best binding affinities corresponding to RMSD 0 values.

4.6.1 CadF

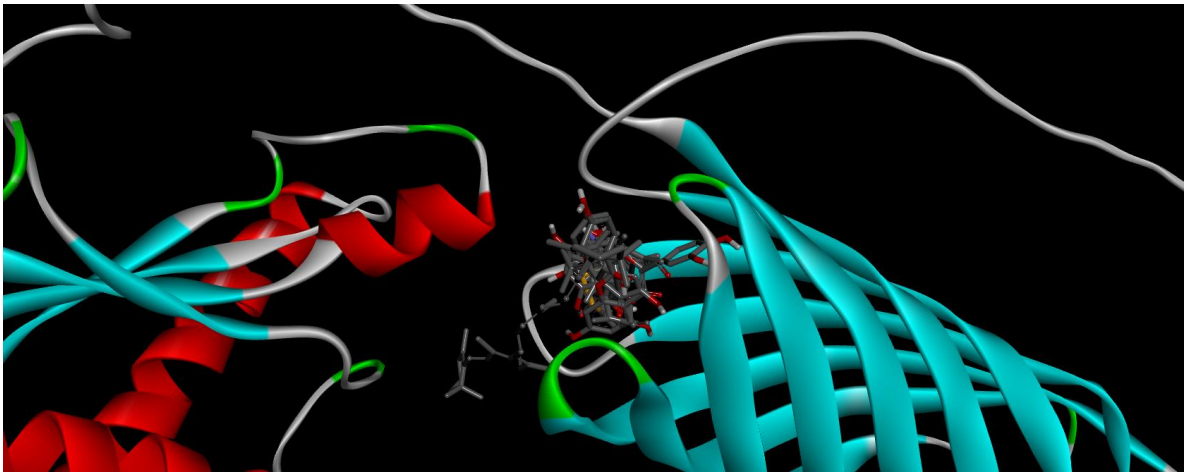


Figure 34: Ligand cluster bound to the active site of CadF protein

Table 7: RMSD table for all 15 natural ligands against CadF

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
CadF_10364_uff_E=78.47	Carvacrol	-5.3	0	0
CadF_3314_uff_E=169.59	Eugenol	-5.4	0	0
CadF_443160_uff_E=154.48	(+)-alpha-Phellandrene	-4.7	0	0
CadF_4837_uff_E=64.42	Piperazine	-3.2	0	0
CadF_5280343_uff_E=380.43	Quercetin	-7.8	0	0
CadF_5280443_uff_E=233.26	Apigenin	-7.2	0	0
CadF_5280489_uff_E=674.37	Beta-Carotene	-7.3	0	0
CadF_5280863_uff_E=362.50	Kaempferol	-7.3	0	0
CadF_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-5.1	0	0
CadF_558173_uff_E=212.14	Tumerone	-6	0	0
CadF_637511_uff_E=76.45	Cinnamaldehyde	-4.7	0	0
CadF_64685_uff_E=466.88	Borneol	-4.9	0	0
CadF_65036_uff_E=111.54	Allicin	-3.8	0	0
CadF_6989_uff_E=95.97	Thymol	-4.9	0	0
CadF_92776_uff_E=205.59	Zingiberene	-5.3	0	0

Quercitin, Apigenin, Beta-Carotene and Kaempferol can be considered as promising drug molecules against CadF protein as they have the highest binding affinities.

4.6.2 JlpA

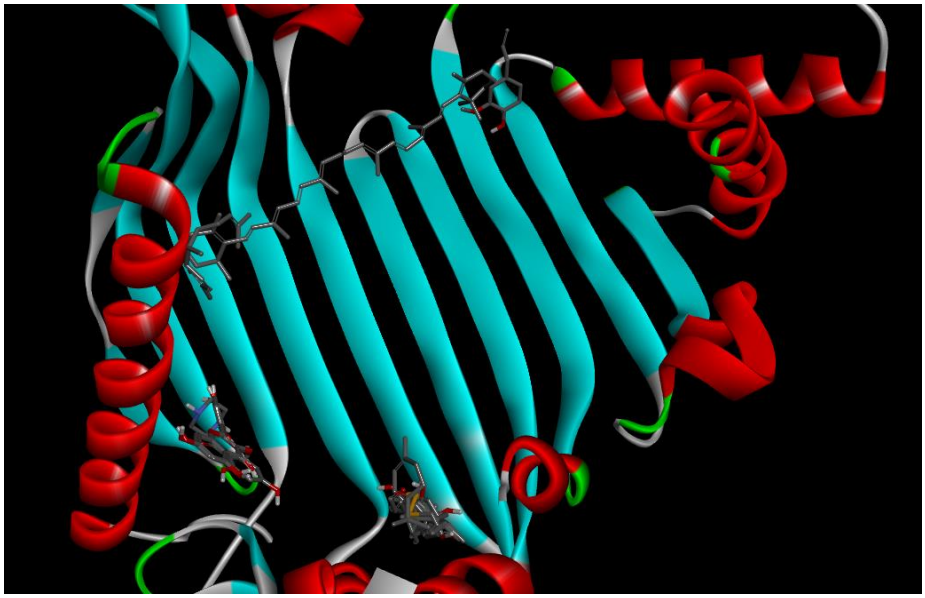


Figure 35: Ligand cluster bound to the active site of JlpA protein

Table 8: RMSD table for all 15 natural ligands against JlpA

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
JlpA_10364_uff_E=78.47	Carvacrol	-6	0	0
JlpA_3314_uff_E=169.59	Eugenol	-5.7	0	0
JlpA_443160_uff_E=154.48	(+)-alpha-Phellandrene	-6.5	0	0
JlpA_4837_uff_E=64.42	Piperazine	-3.1	0	0
JlpA_5280343_uff_E=380.43	Quercetin	-8.1	0	0
JlpA_5280443_uff_E=233.26	Apigenin	-7.7	0	0
JlpA_5280489_uff_E=674.37	Beta-Carotene	-9.6	0	0
JlpA_5280863_uff_E=362.50	Kaempferol	-7.8	0	0
JlpA_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-6.3	0	0
JlpA_558173_uff_E=212.14	Tumerone	-6.7	0	0
JlpA_637511_uff_E=76.45	Cinnamaldehyde	-5.7	0	0
JlpA_64685_uff_E=466.88	Borneol	-6.4	0	0
JlpA_65036_uff_E=111.54	Allicin	-4.6	0	0
JlpA_6989_uff_E=95.97	Thymol	-6.3	0	0
JlpA_92776_uff_E=205.59	Zingiberene	-6.4	0	0

Quercitin, Apigenin, Beta-Carotene and Kaempferol can be considered as promising drug molecules against JlpA protein as they have the highest binding affinities amongst all the ligands.

4.6.3 FlaA

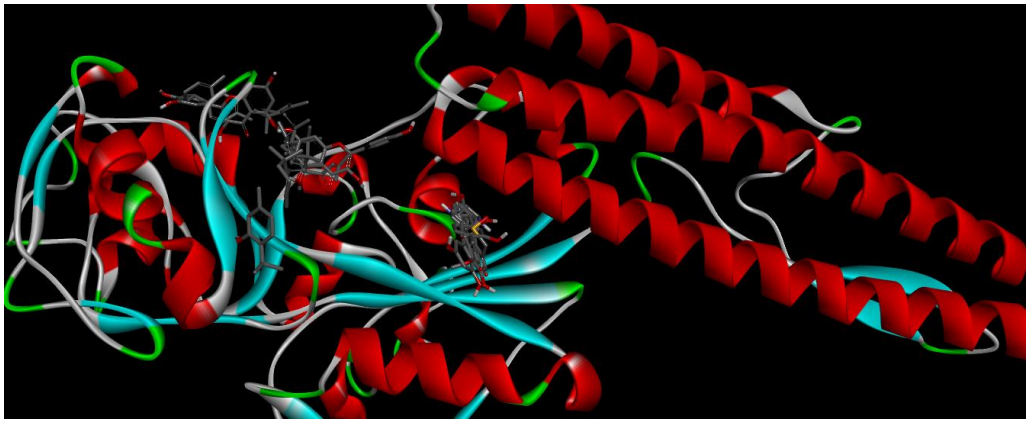


Figure 36: Ligand cluster bound to the active site of FlaA protein

Table 9: RMSD table for all 15 natural ligands against FlaA

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
FlaA_model1_10364_uff_E=78.47	Carvacrol	-4.7	0	0
FlaA_model1_3314_uff_E=169.59	Eugenol	-4.7	0	0
FlaA_model1_443160_uff_E=154.48	(+)-alpha-Phellandrene	-4.5	0	0
FlaA_model1_4837_uff_E=64.42	Piperazine	-3.2	0	0
FlaA_model1_5280343_uff_E=380.43	Quercetin	-6.5	0	0
FlaA_model1_5280443_uff_E=233.26	Apigenin	-7.4	0	0
FlaA_model1_5280489_uff_E=674.37	Beta-Carotene	-7.2	0	0
FlaA_model1_5280863_uff_E=362.50	Kaempferol	-6.4	0	0
FlaA_model1_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-4.8	0	0
FlaA_model1_558173_uff_E=212.14	Tumerone	-5.2	0	0
FlaA_model1_637511_uff_E=76.45	Cinnamaldehyde	-4.7	0	0
FlaA_model1_64685_uff_E=466.88	Borneol	-4	0	0
FlaA_model1_65036_uff_E=111.54	Allicin	-3.4	0	0
FlaA_model1_6989_uff_E=95.97	Thymol	-4.6	0	0
FlaA_model1_92776_uff_E=205.59	Zingiberene	-4.4	0	0

Apigenin and Beta-Carotene can be considered as good drug molecules against FlaA protein as they have better binding affinities than the other ligands.

4.6.4 FlaC

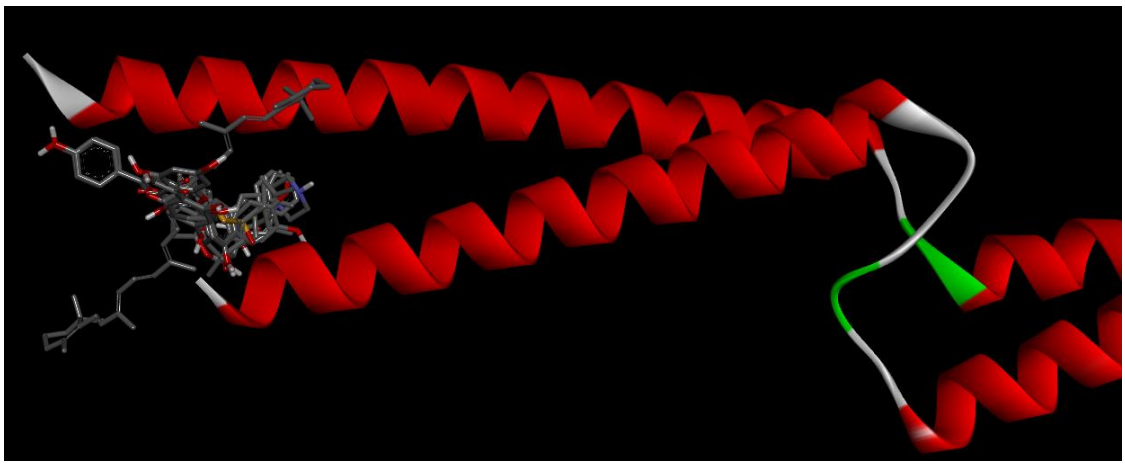


Figure 37: Ligand cluster bound to the active site of FlaC protein

Table 10: RMSD table for all 15 natural ligands against FlaC

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
FlaC_model1_10364_uff_E=78.47	Carvacrol	-4.3	0	0
FlaC_model1_3314_uff_E=169.59	Eugenol	-3.9	0	0
FlaC_model1_443160_uff_E=154.48	(+)-alpha-Phellandrene	-3.8	0	0
FlaC_model1_4837_uff_E=64.42	Piperazine	-2.7	0	0
FlaC_model1_5280343_uff_E=380.43	Quercetin	-4.9	0	0
FlaC_model1_5280443_uff_E=233.26	Apigenin	-5.1	0	0
FlaC_model1_5280489_uff_E=674.37	Beta-Carotene	-4.6	0	0
FlaC_model1_5280863_uff_E=362.50	Kaempferol	-4.9	0	0
FlaC_model1_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-3.9	0	0
FlaC_model1_558173_uff_E=212.14	Tumerone	-5	0	0
FlaC_model1_637511_uff_E=76.45	Cinnamaldehyde	-3.9	0	0
FlaC_model1_64685_uff_E=466.88	Borneol	-3.8	0	0
FlaC_model1_65036_uff_E=111.54	Allicin	-3	0	0
FlaC_model1_6989_uff_E=95.97	Thymol	-4.1	0	0
FlaC_model1_92776_uff_E=205.59	Zingiberene	-4.6	0	0

Quercetin, Apigenin, Beta-Carotene, Kaempferol, Tumerone, and Zingiberin can be considered as suitable drug molecules against FlaA protein as they have better binding affinities than the others.

4.6.5 CheV

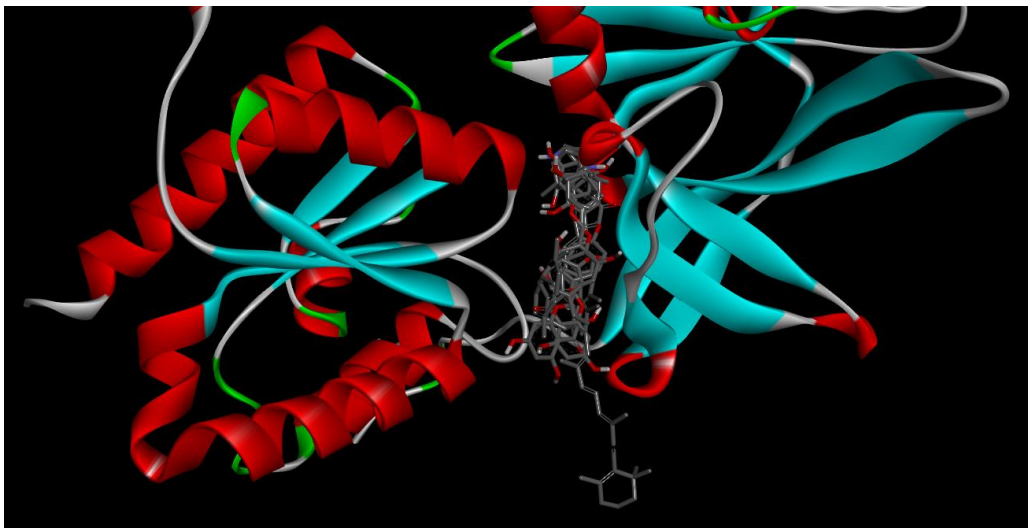


Figure 38: Ligand cluster bound to the active site of CheV protein

Table 11: RMSD table for all 15 natural ligands against CheV

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
CheV_10364_uff_E=78.47	Carvacrol	-6.1	0	0
CheV_3314_uff_E=169.59	Eugenol	-5.4	0	0
CheV_443160_uff_E=154.48	(+)-alpha-Phellandrene	-5.8	0	0
CheV_4837_uff_E=64.42	Piperazine	-3.7	0	0
CheV_5280343_uff_E=380.43	Quercetin	-8.1	0	0
CheV_5280443_uff_E=233.26	Apigenin	-8.6	0	0
CheV_5280489_uff_E=674.37	Beta-Carotene	-8.1	0	0
CheV_5280863_uff_E=362.50	Kaempferol	-8.1	0	0
CheV_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-5.9	0	0
CheV_558173_uff_E=212.14	Tumerone	-6.6	0	0
CheV_637511_uff_E=76.45	Cinnamaldehyde	-5.3	0	0
CheV_64685_uff_E=466.88	Borneol	-5.9	0	0
CheV_65036_uff_E=111.54	Allicin	-4	0	0
CheV_6989_uff_E=95.97	Thymol	-5.9	0	0
CheV_92776_uff_E=205.59	Zingiberene	-6	0	0

Quercitin, Apigenin, Beta-Carotene and Kaempferol can be considered as suitable drug molecules against CheV protein as they have better binding affinities.

4.6.6 CheY

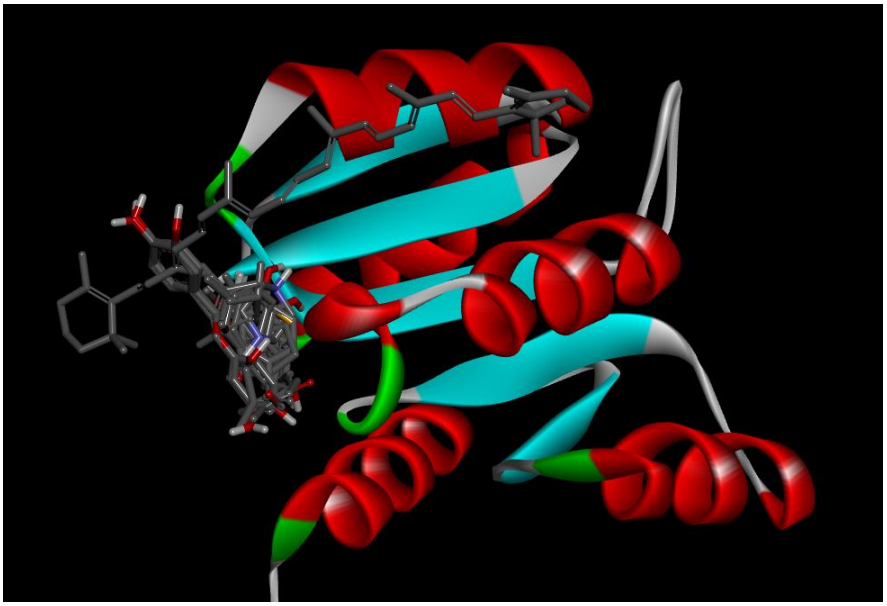


Figure 39: Ligand cluster bound to the active site of CheY protein

Table 12: RMSD table for all 15 natural ligands against CheY

Ligand	Ligand names	Binding Affinity	rmsd/ub	rmsd/lb
CheY_model1_10364_uff_E=78.47	Carvacrol	-4.3	0	0
CheY_model1_3314_uff_E=169.59	Eugenol	-4.2	0	0
CheY_model1_443160_uff_E=154.48	(+)-alpha-Phellandrene	-4	0	0
CheY_model1_4837_uff_E=64.42	Piperazine	-2.8	0	0
CheY_model1_5280343_uff_E=380.43	Quercetin	-5	0	0
CheY_model1_5280443_uff_E=233.26	Apigenin	-4.9	0	0
CheY_model1_5280489_uff_E=674.37	Beta-Carotene	4.4	0	0
CheY_model1_5280863_uff_E=362.50	Kaempferol	-4.9	0	0
CheY_model1_5325830_uff_E=155.64	(-)-Terpinen-4-ol	-4.4	0	0
CheY_model1_558173_uff_E=212.14	Tumerone	-4.9	0	0
CheY_model1_637511_uff_E=76.45	Cinnamaldehyde	-3.9	0	0
CheY_model1_64685_uff_E=466.88	Borneol	-4	0	0
CheY_model1_65036_uff_E=111.54	Allicin	-3.2	0	0
CheY_model1_6989_uff_E=95.97	Thymol	-4.2	0	0
CheY_model1_92776_uff_E=205.59	Zingiberene	-3.9	0	0

Quercitin, Apigenin, Kaempferol and Tumerone can be considered promising drug molecules against CheY protein as they have better binding affinities than the others on the list.

CHAPTER 5: CONCLUSION

Fifteen natural ligands were used for virtual screening against six target proteins identified based on various literature reviews. These ligands were docked against the target proteins using the PyRx tool. It can be observed that the ligands, Quercitin, Apigenin, Kaempferol, Beta-Carotene, Tumerone and Zingiberene have shown higher binding affinities than the other natural ligands. Hence, it can be concluded that these natural ligands, obtained from herbs like Portulaca, Chamomile, Mallows and Turmeric, can serve as promising potential drug molecules against *Campylobacter jejuni* and its potential drug targets.

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