

IDENTIFICATION OF NOVEL DRUGGABLE TARGET IN LEGIONELLA PNEUMOPHILA USING SUBTRACTIVE PROTEOMICS AND DRUG REPURPOSING APPROACH

Submitted in partial fulfillment of the requirements for the award of

Master of Science in Bioinformatics and Data Science

By

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DEPARTMENT OF BIOINFORMATICS

SCHOOL OF BIO AND CHEMICAL ENGINEERING

SATHYABAMA

INSTITUTE OF SCIENCE AND TECHNOLOGY

(DEEMED TO BE UNIVERSITY)

Accredited with Grade “A++” by NAAC | 12B Status by UGC | Approved by AICTE

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April - 2024



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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **R SALIGA (42739021)**, who carried out the final year project entitled **“IDENIFICATION OF NOVEL DRUGGABLE TARGET IN LEGIONELLA PNEUMOPHILA USING SUBTRACTIVE PROTEOMICS AND DRUG REPURPOSING APPROACH”** under my supervision from September 2023 to April 2024.

Dr. D.ALEX ANAND

Internal Guide

Dr. H. JEMMY CHRISTY

Head of the Department

Submitted for Viva voce Examination held on

Internal Examiner

External Examiner

DECLARATION

I **R SALIGA (42739021)** hereby declare that the Project Report entitled **“IDENTIFICATION OF NOVEL DRUGGABLE TARGT IN LEGIONELLA PNEUMOPHILA USING SUBTRACTIVE PROTEOMICS AND DRUG REPURPOSING APPROACH”** done by me under the guidance of Dr. H. JEMMY CHRISTY at Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Salai, Chennai –600 119, Tamil Nadu, India, and is submitted in partial fulfillment of the requirements for the award of Master of Science in Bioinformatics and Data Science.

DATE:

PLACE: CHENNAI

A handwritten signature in black ink, consisting of a series of loops and a long horizontal stroke extending to the right.

SIGNATURE OF THE CANDIDATE

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I am pleased to acknowledge my sincere thanks to the **Board of Management of SATHYABAMA** for their kind encouragement in doing this project for completing it successfully. I am grateful to them.

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ABSTRACT

Legionella pneumophila, the causative agent of Legionnaires' disease, poses a significant public health threat worldwide. With increasing antibiotic resistance, there is an urgent need to identify novel druggable targets within *L. pneumophila* to develop effective therapeutic strategies. This study employs a subtractive proteomics approach coupled with drug repurposing to identify potential drug targets within *L. pneumophila*. Legionnaires' disease, caused by *L. pneumophila*, presents a severe challenge due to its ability to survive and replicate within host cells, particularly within alveolar macrophages. Traditional antibiotic therapies are becoming less effective due to the emergence of drug-resistant strains, emphasizing the necessity for novel therapeutic approaches. Subtractive proteomics, a powerful technique to identify proteins unique to pathogens, combined with drug repurposing, offers a promising avenue for discovering new drug targets. The unique proteins of *Legionella Pneumophila* lens were identified by comparing the metabolic pathways of host (*Homo sapiens*) and organism (*Legionella Pneumophila* lens). The subtractive proteomics approach involves comparing the proteome of *L. pneumophila* with that of its host or closely related organisms to identify proteins specific to the pathogen. This differential proteomic analysis highlights potential drug targets crucial for the survival and pathogenicity of *L. pneumophila*. Additionally, drug repurposing involves screening existing drugs approved for other indications to identify compounds with potential efficacy against *L. pneumophila*. Drug repurposing is the technique of using an existing drug or drug candidate for a new treatment or medical condition for which it was not indicated before. The crystal structure Penicillin binding protein (MRCA) was used for docking in this study were originally obtained from the Protein Data Bank (www.rcsb.org). The downloaded protein was prepared for docking such as adding hydrogen, removal of water, elimination of all non-standard residues, adding Kollman charges, and removal of ligands if necessary. Initially, a subtractive proteomics analysis was carried out to compare the proteomic profiles of pathogenic and non-pathogenic strains of *Legionella pneumophila*.

Keywords: *LegionellaPneumophila*, Legionnaires disease, Subtractive Proteomics, Unique protein pathway, Druggable targets, Drug Reproposing, Molecular Docking.

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CHAPTER-1

INTRODUCTION

Legionnaires' disease, caused by the gram-negative bacterium *Legionella pneumophila*, is a severe form of pneumonia that poses a significant threat to public health globally. First identified during an outbreak in 1976, this disease continues to present challenges due to its ability to thrive in various water sources and human-made environments, leading to sporadic cases and outbreaks. (Khan et al, 2022)

Despite advances in diagnostics and supportive care, treatment options for Legionnaires' disease remain limited, with antibiotics such as macrolides and fluoroquinolones representing the primary therapeutic approach. However, concerns over antibiotic resistance and treatment failure highlight the urgent need for alternative strategies to combat this infectious disease.

In this context, subtractive proteomics, which involves the comparative analysis of pathogen and host proteomes to identify pathogen-specific proteins, emerges as a promising strategy for pinpointing potential drug targets in *L. pneumophila*. By elucidating the distinct molecular signatures of the pathogen, subtractive proteomics offers a means to selectively target essential virulence factors and metabolic pathways while minimizing collateral damage to the host.

Moreover, drug repurposing, the process of identifying new therapeutic applications for existing drugs, provides a cost-effective and expedited approach to drug discovery. By leveraging the vast repertoire of compounds already approved for human use, drug repurposing accelerates the translation of pre-existing knowledge into clinical practice, circumventing many of the hurdles associated with traditional drug development.

Identified 37 proteins unique to *Legionella pneumophila*, with potential roles in pathogenicity and survival.

Among these, 12 proteins were prioritized based on their involvement in virulence and evasion of host defenses.

Proteins showing homology to human proteins were filtered out to avoid potential off-target effects.

L. pneumophila is emerging as an antibiotic-resistant strain, and controlling LD is now difficult. Hence, developing novel drugs and inhibitors against *L. pneumophila* is a major research priority. Here, attempts were taken through a subtractive proteomics approach on the complete proteome of *L. pneumophila* to address the challenges of multidrug resistance

Subtractive proteomics is a **computational method for identifying potential vaccine and drug targets by excluding proteins that are not useful for vaccine and drug designing.**

We particularly considered the key essential or survival proteins of the pathogen, which are nonhomologous to the host as well as host microbiota.

Recent epidemiological research found that *L. pneumophila* strains display a high prevalence of tolerance (50 to 100%) to widely used antibiotics such as azithromycin, ceftriaxone, rifampicin, tigecycline, ciprofloxacin, moxifloxacin, doxycycline, erythromycin, levofloxacin, and clarithromycin.

By comparing the proteomes of the pathogen and its host, subtractive proteomics provides a robust method for identifying putative therapeutic targets and identifying specific bacterial proteins necessary for virulence or survival. Utilizing a subtractive proteomics approach, we were able to minimize the risk and discover new druggable targets that are unique to *Legionella pneumophila*.

Integration of subtractive proteomics with drug repurposing can enhance the identification of potential drug targets by prioritizing those proteins essential for the pathogen's survival and virulence.

In this study, we propose to integrate subtractive proteomics and drug repurposing approaches to identify novel druggable targets in *Legionella pneumophila*.

we will prioritize these proteins based on their structural and functional characteristics, as well as their potential as drug targets. Leveraging existing drug databases and bioinformatics tools, we will then perform virtual screening to identify candidate drugs that could inhibit the identified targets.

Legionella pneumophila is anaerobic, pleomorphic, flagellated, nonsporeforming, Gram-negative bacterium of the genus *Legionella*. *L. pneumophila* is the primary human pathogenic bacterium in this group. In nature, *L. pneumophila* infects freshwater and soil amoebae of the genera *Acanthamoeba* and *Naegleria*. This pathogen is found commonly near freshwater environments and will then invade the amoebae found in these environments, using them to carry out metabolic functions.

Drug repurposing, also known as drug repositioning, is the process of investigating existing drugs for new therapeutic purposes. It involves identifying new therapeutic uses for existing or investigational drugs. Drug repurposing is an effective strategy in discovering or developing drug molecules with new pharmacological/therapeutic indications.

Recent epidemiological research found that *L. pneumophila* strains display a high prevalence of tolerance (50 to 100%) to widely used antibiotics such as azithromycin, ceftriaxone, rifampicin, tigecycline, ciprofloxacin, moxifloxacin, doxycycline, erythromycin, levofloxacin, and clarithromycin.

Despite the success of macrolides and fluoroquinolone, resistance developed against these drugs resulted in treatment failures.

1.1 BACKGROUND

Drug repurposing can be a powerful tool for identifying new antibiotics, as there is a large pool of existing drugs to screen from. In this context, It could be used to identify proteins that are specifically expressed by *L. pneumophila* within amoeba hosts compared to when they are growing freely. (Shakhinur Islam Mondal, 2021)

Drug repurposing

The process of finding new uses for existing drugs.

By identifying proteins that are essential for *L. pneumophila*'s growth within amoeba hosts, researchers can look for existing drugs that target those proteins.

This approach can be faster and less expensive than developing new drugs from scratch. To identify new targets for drug development against *L. pneumophila*.

By focusing on proteins that are essential for growth within amoeba hosts, the researchers hope to find targets that are not present in human cells.

This could lead to the development of new antibiotics that are effective against Legionnaires' disease.

Scientific classification [Edit this classification](#)

Domain:	Bacteria
Phylum:	Pseudomonadota
Class:	Gammaproteobacteria
Order:	Legionellales
Family:	Legionellaceae
Genus:	Legionella
Species:	<i>L. pneumophila</i>

1.2 TREATMENT

Antibiotic Therapy: Antibiotics are the cornerstone of treatment Legionnaires'.

Macrolides (e.g., azithromycin, erythromycin)

Fluoroquinolones (e.g., levofloxacin, moxifloxacin)

Early Initiation: Treatment should be initiated promptly upon suspicion of Legionnaires' disease, even before laboratory confirmation, to improve outcomes.

Duration of Therapy: Antibiotic therapy typically lasts for **10-21** days, depending on the severity of the illness and clinical response.

Supportive Care: Supportive measures may include supplemental oxygen therapy, hydration, and management of complications such as respiratory failure or septic shock.

Hospitalization: Severe cases of Legionnaires' disease often require hospitalization for close monitoring and intensive care, especially if complications arise.

Prevention: Preventive measures focus on identifying and controlling sources of Legionella contamination, such as water systems in buildings, hospitals, and cooling towers. Regular maintenance, disinfection, and temperature control of water systems are crucial for preventing Legionella growth and transmission.

Vaccines: While no licensed vaccines are currently available for Legionnaires' disease, research into vaccine development continues, with promising candidates undergoing clinical trials.

Antimicrobial Resistance: Surveillance for antimicrobial resistance patterns in Legionella strains is essential to guide antibiotic selection and ensure effective treatment.

Limited Treatment Options: Current treatment options for Legionnaires' disease primarily rely on antibiotics, such as macrolides and

fluoroquinolones. However, antibiotic resistance is a growing concern, and treatment failures are increasingly reported. This highlights the need for alternative therapeutic approaches to improve patient outcomes.

Complex Pathogenesis: The pathogenesis of Legionnaires' disease involves intricate interactions between *L. pneumophila* and host cells, including mechanisms for invasion, replication, and evasion of the host immune response. Understanding these complex processes is essential for developing targeted therapies but remains a significant challenge.

Start by comparing the proteome of *Legionella pneumophila* with that of its host cells (e.g., macrophages) to identify bacterial proteins that are essential for survival and virulence but absent or significantly different in the host.

Use bioinformatics tools to prioritize potential drug targets among the identified bacterial proteins based on criteria such as essentiality for bacterial growth, surface exposure, and druggability.

Analyze the identified proteins to assess their druggability, focusing on targets with known structural features amenable to small-molecule inhibition.

Prioritize targets based on their essentiality for bacterial survival and their potential to disrupt virulence mechanisms.

Validate the efficacy of selected compounds in inhibiting *Legionella pneumophila* growth and virulence in vitro using cell culture models.

Assess the therapeutic efficacy of promising compounds in relevant animal models of Legionnaires' disease.

1.3 BOTTLENECK AREAS

Delayed Diagnosis: Legionnaires' disease can present with nonspecific symptoms resembling other respiratory infections, leading to delays in diagnosis and initiation of treatment. Improving diagnostic methods, such as rapid antigen testing and PCR assays, is crucial to expedite diagnosis and treatment.

Antibiotic Resistance: Increasing antibiotic resistance among *Legionella* strains poses a significant challenge to treatment efficacy. Surveillance for antimicrobial resistance patterns and judicious antibiotic use are essential to guide treatment decisions and prevent further development of resistance.

Complexity and time consumption: The technique itself can be laborious and involve multiple steps, extending the research timeline.

Sensitivity: There's a chance of missing low-abundance proteins crucial for *Legionella*'s survival within amoeba due to limitations in detecting faint signals.

Protein prioritization: Subtractive proteomics generates a vast amount of data.

Pinpointing the most promising targets from this data for further drug repurposing efforts can be challenging.

Drugs approved for one use might have unforeseen side effects when repurposed for *Legionella* infections. Rigorous testing is necessary to ensure safety.

As with any antibiotic, *Legionella* has the potential to develop resistance to repurposed drugs over time, rendering them ineffective.

CHAPTER-2

LITERATURE SURVEY

Legionella pneumophila is the causative agent of a severe type of pneumonia (lung infection) called Legionnaires' disease. It is emerging as an antibiotic-resistant strain day by day. Hence, identifying novel drug targets and vaccine candidates is essential to fight against this pathogen. Here, attempts were taken through a subtractive genomics approach on the complete proteome of *L. pneumophila* to address the challenges of multidrug resistance.

A total of 2,930 proteins from *L. pneumophila* proteome were investigated through diverse subtractive proteomics approaches, e.g., identification of human nonhomologous and pathogen-specific essential proteins, druggability and "anti-target" analysis, subcellular localization prediction, human microbiome nonhomology screening, and protein-protein interaction studies to find out effective drug and vaccine targets.

Legionella transmission from human to human generally does not occur or is rare and can only be acquired from environmental sources (17). The pathogen grows and localizes in hot, moist, and nutrient-rich sedimentary environments (18). A potable water distribution system was the culprit behind hospital-acquired Legionnaires' disease (19).

Macrolides (azithromycin) and fluoroquinolones (levofloxacin, moxifloxacin, and ciprofloxacin) are the standard treatment for *Legionella* infection (20, 21).

A combination of fluoroquinolone and azithromycin is recommended in the case of drug resistance or severe LD pneumonia (23).

(Khan et al.)2022

There is still a low level of clinical awareness regarding Legionnaires' disease 25 years after it was first detected. The causative agents, legionellae, are freshwater bacteria with a fascinating ecology. These bacteria are intracellular pathogens of freshwater protozoa and utilize a similar mechanism to infect human phagocytic cells.

Most diagnostic tests are directed at the species that causes most of the reported human cases of legionellosis, *L. pneumophila* serogroup 1. For this reason, information on the incidence of human respiratory disease attributable to other species and serogroups of legionellae is lacking.

Fields BS, Benson RF, Besser RE. 2002. *Legionella* and Legionnaires' disease: 25 years of investigation. *Clin Microbiol Rev* 15:506–526.

Drug repurposing explores previously approved drugs of known safety and pharmacokinetics profile for possible new effects, reducing the cost, time, and predicting prospective side effects and drug interactions.

Drug repurposing, defined as researching new indications for already approved drugs or advancing previously studied but unapproved drugs, is a core approach in drug development. Some reports state that about 30–40% of new drugs and biologics approved by the US Food and Drug Administration (FDA) between 2007 and 2009 can be considered repurposed or repositioned products .

Some reviews state about 30% of repurposing efforts are successful and result in a product approved for marketing, in comparison to about 10% for new drug applications more generally.

While docking studies consider the flexibility of the ligand as a rigid structure, molecular dynamic (MD) simulation takes the ligand–protein complex as a dynamic module.

Essential proteins are considered fundamental for cell survival. These proteins were retrieved by using an online software Geptop 2.0 server. The essentiality cutoff score was set at 0.24. Geptop is a web server that provides a forum for identifying essential genes for bacterial organisms, comparing query protein orthology and phylogeny with the experimentally developed essential gene datasets (from the Database of Essential Genes DEG). Geptop can be utilized for any species of bacteria that has a sequenced genome .

For the detection of the non-homologous protein sequences of *Legionella Pneumophila*, essential proteins were submitted against the human proteome with default values of the parameter to BlastP

Non-homologous essential proteins were computed for comparative analysis of metabolic pathways. This analysis is conducted to identify drug targets based on pathway enzymes that are both common and essential to bacteria [20]. The metabolic pathways of *Legionella Pneumophila* were identified by comparing the pathways of both the *Legionella Pneumophila* and *Homo sapiens* through the (KEGG) database [21].

Protein sequences with a high degree of homology with the human proteome were eliminated, and human nonhomologous pathogen proteins were prioritized. The resultant nonhomologous proteins were scanned against human proteins. The threshold criteria for this BLASTp analysis were $\leq 35\%$ sequence identity, $\leq 35\%$ query coverage, and an E-value cut off 10^{-4} .

(Farah Shahid,¹ and Mohsin Khurshid et al, 2020 May 22)

Molecular docking was performed using an online server CB-Dock that adopted the docking and calculation algorithms based on the Auto Dock Vina (version 1.1.2).

The blind docking was performed without defining the grid box to ensure that the best binding site with the highest binding affinity was identified.

The post docking analysis was performed by Discovery Studio software.

Molecular modeling approach is a useful method for drug discovery.

(Mohamed Fadlalla-et al) Published: 01 April 2022

Several computational software were utilized to recognize the essential proteins that are necessary for the survival of bacteria. The analysis of both the metabolic pathways and host non-homology was performed to prevent the cross-reactivity of potential drugs with bacterial and host proteins, along with the involvement of bacterial proteins in various metabolic processes of the host, respectively.

Retrieval of Essential Proteins

Essential proteins are considered fundamental for cell survival. These proteins were retrieved by using an online software Geptop 2.0 server. The essentiality cutoff score was set at 0.24. Geptop is a web server that provides a forum for identifying essential genes for bacterial organisms, comparing query protein orthology and phylogeny with the experimentally developed essential gene datasets (from the Database of Essential Genes DEG). Geptop can be utilized for any species of bacteria that has a sequenced genome .

Essential Non-Homologous Protein Identification

For the detection of the non-homologous protein sequences of *Legionella pneumophila*, essential proteins were submitted against the human proteome with default values of the parameter to BlastP.

Metabolic Pathway Analysis

In this approach, the initial protein targets were those involved in the metabolic pathways of *Legionella pneumophila* . These pathways were retrieved from the Kyoto Encyclopedia of Genes and Genomes (KEGG).

Subtractive Proteomics:

Can be complex and time-consuming.

May not be able to identify all differentially expressed proteins, especially low-abundance ones.

Drug Repurposing:

Existing drugs may not have the ideal properties for targeting *Legionella* within amoeba. They might be ineffective due to poor penetration into the host cell or lack of potency against the target protein.

May have unforeseen side effects when used in the context of a new infection.

The integration of subtractive proteomics and drug repurposing approaches holds promise for the discovery of novel druggable targets in *L. pneumophila*. By combining information on essential proteins obtained from subtractive proteomics with screening data from drug repurposing studies, researchers can prioritize potential targets for further investigation. This integrated approach accelerates the drug discovery process and increases the likelihood of identifying effective therapeutics.

Drug repurposing involves screening approved drugs or compounds with known safety profiles for activity against *L. pneumophila*. By leveraging existing libraries of compounds, drug repurposing offers a rapid and cost-effective approach to identify potential therapeutics. Recent studies have identified several compounds with activity against *L. pneumophila*, including FDA-approved drugs and natural products.

Drug repurposing, defined as researching new indications for already approved drugs or advancing previously studied but unapproved drugs, is a core approach in drug development. Some reports state that about 30–40% of new drugs and products.

Repurposing generics can have groundbreaking effects for patients: 35% of 'transformative' drugs approved by the US FDA are repurposed products. Repurposing is especially relevant for rare or neglected diseases.

Drug repositioning is a "universal strategy" for neglected diseases due to 1) reduced number of required clinical trial steps could reduce the time and costs for the medicine to reach market, 2) existing pharmaceutical supply chains could facilitate "formulation and distribution" of the drug,

Computational drug repurposing is the *in silico* screening of approved drugs for use against new indications. It can use molecular, clinical or biophysical data. Electronic health records and real-world evidence gained popularity in drug repurposing.

The growing availability of large-scale biomedical data, advances in computational biology, and increased understanding of disease mechanisms have expanded the scope and potential for drug repurposing efforts in recent years. This approach holds promise for quickly bringing new treatments to patients while maximizing the utility of existing drugs.

Aspirin (Acetylsalicylic acid):

Original use: Pain relief, fever reduction, and anti-inflammatory effects.

Repurposed use: Prevention of cardiovascular events.

(Antithrombotic Trialists,2002).

Nithya Krishnamurthy,¹ Alyssa A. Grimshaw,(et al) Published 2022 Jul 29.

Drug repurposing, also known as drug repositioning, is the process of investigating existing drugs for new therapeutic purposes. It involves identifying new therapeutic uses for existing or investigational drugs. Drug repurposing is an effective strategy in discovering or developing drug molecules with new pharmacological/therapeutic indications.

CHAPTER-3

AIM AND SCOPE

3.1 AIM

The aim of this proposed research is to identify novel druggable targets within *Legionella pneumophila*, the causative agent of Legionnaires' disease, utilizing a combined approach of subtractive proteomics and drug repurposing strategies. This research aims to address the urgent need for alternative treatment options due to the emergence of antibiotic-resistant strains of *L. pneumophila*.

Conducting subtractive proteomics analysis to compare the proteome of *L. pneumophila* with that of its host or non-pathogenic strains. This analysis aims to identify proteins that are unique to the pathogen and essential for its survival, serving as potential druggable targets.

Screening libraries of FDA-approved drugs, natural products, and compounds with known safety profiles to identify potential inhibitors of validated druggable targets in *L. pneumophila*.

Investigate the mode of action of lead compounds identified through drug repurposing approaches to understand their interactions with target proteins and their impact on bacterial physiology.

Determine the specificity and selectivity of lead compounds to ensure their efficacy against *L. pneumophila* while minimizing off-target effects.

The main goal of the project is to identify the druggable targets in *legionella pneumophila* involves using a method called subtractive proteomics, which essentially means comparing the proteome (the entire set of proteins) of *legionella pneumophila* with that of its host organism or related organisms. This comparison helps identify proteins that are unique to the pathogen and could potentially be targeted with drugs.

3.2 SCOPE

This approach combines target identification through subtractive proteomics with drug repurposing to find new ways to combat Legionnaires' disease.

It focuses on a specific niche: targeting *Legionella*'s vulnerabilities within its amoeba host using existing drugs.

The scope is not about creating entirely new antibiotics but finding new applications for existing ones, potentially accelerating the development process.

This approach might not be effective against all *Legionella* strains or other bacterial pathogens.

Subtractive proteomics has limitations in sensitivity, and some crucial proteins might be missed.

Repurposed drugs might have unforeseen side effects or require modifications for optimal efficacy against *Legionella*. Analysis of regulatory networks and metabolic pathways associated with *L. pneumophila* pathogenesis.

Identification of key druggable targets and potential synergistic interactions between targets and compounds. Comparing the proteome of *Legionella pneumophila* with that of non-pathogenic strains or host cells to identify unique bacterial proteins.

Current treatment options for Legionnaires' disease primarily rely on antibiotics, such as macrolides and fluoroquinolones. However, antibiotic resistance is a growing concern, and treatment failures are increasingly reported. This highlights the need for alternative therapeutic approaches to improve patient outcomes.

The pathogenesis of Legionnaires' disease involves intricate interactions between *L. pneumophila* and host cells, including mechanisms for invasion, replication, and evasion of the host immune response.

CHAPTER-4

METHODOLOGY

- Subtractive proteomics
- Retrieval of unique protein by metabolic pathway analysis
- Identification of essential protein
- Identification of druggable protein
- Drug repurposing approach
- Post-docking molecule

4.1 SUBTRACTIVE PROTEOMICS:

Subtractive proteomics analysis is a well-devised approach used to detect novel drug targets in the pathogen. In recent years, subtractive proteomics is efficiently used to discover species-specific vaccine candidates and in determining potential drug targets against various pathogenic bacteria .

Subtractive proteomics has been used to identify proteins that are selectively expressed or upregulated in *L. pneumophila* during infection. These proteins, which may include secreted effectors, membrane-associated proteins, or regulatory factors, are crucial for the bacterium's ability to invade host cells, replicate intracellularly, and modulate host immune responses.

Subtractive proteomics is a powerful bioinformatics approach used to identify proteins that are specific to a particular organism, such as a pathogen, by comparing its proteome to that of another organism, typically its host or a related non-pathogenic strain.

Subtractive proteomics begins with the comparison of the proteome of the target organism (e.g., a pathogenic bacterium) with that of another organism (e.g., its host or a non-pathogenic strain).

Proteins identified through subtractive proteomics may include potential druggable targets, such as enzymes, receptors, or structural proteins, that are essential for the pathogen's survival, replication, or interaction with the host.

Proteins identified through subtractive proteomics may include potential druggable targets, such as enzymes, receptors, or structural proteins, that are essential for the pathogen's survival, replication, or interaction with the host. subtractive proteomics facilitates the discovery of novel therapeutic targets for the development of antimicrobial drugs and vaccines.

Subtractive proteomics is instrumental in a wide range of applications, such as the identification of potential drug targets in pathogens, the discovery of biomarkers for disease diagnosis or prognosis, and the elucidation of the molecular mechanisms underlying various biological processes. This

technique is a powerful tool for unraveling the complexities of biological systems and advancing biomedical research and drug discovery efforts.

Subtractive proteomics is a computational method employed in bioinformatics to detect proteins that exhibit distinct characteristics or notable differences between two or more biological samples. Its primary purpose is to pinpoint potential drug targets or biomarkers.

This technique entails comparing the proteomic profiles of two conditions, such as a pathogenic and non-pathogenic strain of a microorganism.

By subtracting the proteins common to both conditions, researchers can focus on proteins that are specifically associated with the condition of interest.

Subtractive proteomics has been used in various fields, including disease biomarker discovery, drug target identification, and understanding cellular signaling pathways. (Zhao, J., & Ding, G.,2018).

Subtractive proteomics is a method used to identify proteins that are differentially expressed or present in one condition compared to another. It involves comparing the proteome of a control group to that of a treatment group or a disease state to identify proteins unique to the condition of interest.

Compare the protein profiles of different samples to identify proteins that are uniquely present or significantly differentially expressed in one condition compared to another. Statistical analysis and bioinformatics tools are often used to identify significant differences.

LEGIONELLA PNEUMOPHILA

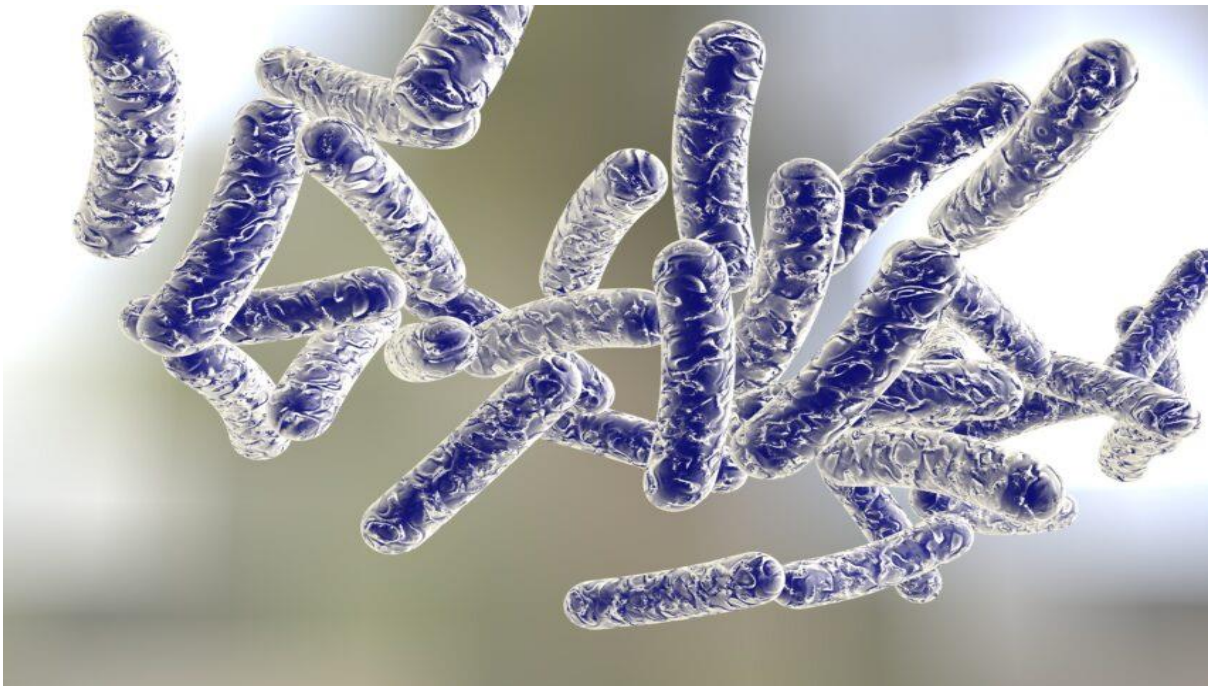


Figure 4.4.1 Legionella pneumophila

4.2 Retrieval of Unique Proteins

Retrieval of Unique proteins using KEGG Pathway Database

MONOBACTAM PATHWAY - ipf00261

>lysAC

MSGILIMQQVVIKFGGTSVSTRATWNNIAAITQKHLNTGVQPVIVCSALTQI
SNKLEKAI

EAALLDEHHSLFNDIQSSHLNLAEQLEVNHQLIANDLHQLQQWLTGISLLK
QAPAKTHAQ

ILSLGELMMTRMGHAFLEHKEIKVKWYDARELLTSTPTLGGETMNYLSAR
CESEYDPGLV

EKFLSSGAQAIITQGFFAANPHGETVLLGRGGSDTSAALLAGKLQAASCEI
WTDVPGIYT

ANPHQLPHARLLKQLNYDEAQEIASMGAKVLHPNCIPPVRKANIPMVVKY
THLPEHSGTL

ITKDIDESAPLIKSIQVKHSILLISIDTLNMWQQVGFLADVFAAFKKHGFSD
LLSSSEF

NVTLSLDVNAKIHDRPAINALLDDLNQFGRAKLIEPCSAVSLVGHHIRTVLP
HLGPALEV

FEAKQVYLMSLASNDLNLTFFVDESHADKLCQRLHHLLIESNPQIFYYSKS
WHEEFGKPN

VRPTPWWEIERDRLLTSAIHSPCYVYHSPTQAIRARQLSALESIDSLFYAV
KANPFPSI

LKTLEKEGIGFECVSIQELELVKLFPNIKKERILFTP NFAPKSEYEFALQTG
CYVTIDS

LYPLENWPELFKNREVIVRIDPGTGAGHHKHVSTGGNESKFGITQNDISKIL
SLTKSNHI

KVIGLHAHSGSGILSTDWQQTAVMLASLTDQFPEVRSINLGGGLGIVEKP
GQHPIDFAA

LDAQLMAVKSQYPGLAIWLEPGRFFVAESGVILAKVTQCCEKKGKVKFIGIE
TGMNSLIRP

SLYGAYHEIVNLTRLHEEKAGFAHIVGPICESGDTLGYDRLLPVTKEGDVILI
ANTGAYG

HCMSSHYNLRPPAQEIVLE

Access KEGG Database: Visit the KEGG website (<https://www.kegg.jp/>) and navigate to the desired pathway or search for it using the pathway name or identifier.

Select Pathway: Once you've located the pathway of interest, access its page to view the list of proteins associated with that pathway.

Removal of non-homologous proteins by BLASTp:

The protein sequences were then subjected to BLASTp against the human proteome set acquired from the NCBI database to identify host nonhomologous proteins.

Protein sequences with a high degree of homology with the human proteome were eliminated, and human nonhomologous pathogen proteins were prioritized. The resultant nonhomologous proteins were scanned against human proteins.

The threshold criteria for this BLASTp analysis were $\leq 35\%$ sequence identity, $\leq 35\%$ query coverage, and an E-value cut off 10^{-4} .

4.3 Identification of essential protein

Essential proteins have been identified by biological experimental approaches and computing methods. The advantage of biological experimental methods, such as gene knockout, conditional knockout, and RNA interference, is high reliability, but the disadvantages are that they are time-consuming and expensive.

The protein sequences that were recovered from the distinct metabolic pathways were then tested using BLASTp (with an E-value of 10^{-3}) against Homo sapiens. "Hits" (host-pathogen homologous sequences) and "No-Hits" (non-homologous sequences) were the outcomes of the BLASTp. The non-homologous sequences that displayed no resemblance to the Homo sapiens were chosen for additional examination.

Identification of essential proteins can help us uncover the mechanisms of cell aging and aging-related diseases and is of great significance to disease diagnosis and drug design. Essential proteins have been identified by biological experimental approaches and computing methods.

Essential proteins are **indispensable to the development and survival of** cells. The identification of essential proteins not only is helpful for the understanding of the minimal requirements for cell survival, but also has practical significance in disease diagnosis, drug design and medical treatment.

It is of utmost importance to identify the vital proteins in pathogens such as *Legionella pneumophila* in order to comprehend their biology and pathogenesis. By targeting these essential proteins with drugs or other therapeutic interventions, it becomes possible to disrupt crucial cellular processes, hinder bacterial growth, and potentially pave the way for the creation of innovative antimicrobial agents to treat infections.

4.4 Identification of druggable protein

The identification of druggable proteins has always been the core of drug development. Traditional structure-based identification methods are time-consuming and costly. Utilize computational tools and algorithms to predict the druggability of the protein based on structural and physicochemical properties. For every pharmacological target, the DrugBank database was used to assess the druggability of prospective proteins.

The concept of druggability is most often restricted to small molecule (low molecular weight organic substances) but also has been extended to include such as therapeutic .Selected proteins were subjected to BlastP using pre-established parameters against a list of chemicals that could be located in the DrugBank database. 4323 non-redundant protein sequences comprising the drug target, enzyme, or transporter carrier associated with 6712 drug entries are included in this database.

We discovered a new protein target that is unique to *Legionella pneumophila* through the use of subtractive proteomics. We highlighted its potential as a therapeutic target by characterizing its druggability by structural biology and computational research.

This finding addresses the pressing need for alternate treatment approaches against this disease and presents a possible path for the development of novel medications to treat *Legionella* infections.

A druggable protein, also known as a drug target, is a protein molecule that can be modulated by a drug to achieve a desired therapeutic effect. These proteins are typically involved in key biological processes or pathways that are associated with disease, making them potential targets for pharmacological interven.

4.5 Drug repurposing approach

Drug repurposing is the technique of using an existing drug or drug candidate for a new treatment or medical condition for which it was not indicated before.

It was initially developed to treat a different medical condition.

It has been described as a serendipitous process that happens unexpectedly.

Pharmaceutical organizations typically take one of three approaches to drug repurposing as part of a systematic strategy: drug, disease and target centric. They each explore the relationships between drugs, diseases and targets in different ways based on the therapeutic action of a drug.

Drug repurposing explores previously approved drugs of known safety and pharmacokinetics profile for possible new effects, reducing the cost, time, and predicting prospective side effects and drug interactions.

This step involves screening existing drugs, either approved or in clinical development, to identify compounds that exhibit activity against the target disease or condition of interest.

Drug repurposing, also referred to as drug repositioning or drug reprofiling, is a strategy within the field of drug discovery that focuses on discovering novel therapeutic applications for drugs that have already been approved or are currently being studied for different medical conditions.

DATA RETRIEVAL:

The unique proteins of *Legionella pneumophila* Lens were identified by comparing the metabolic pathways of Host (*Homo sapiens*) and Organism (*Legionella pneumophila* Len

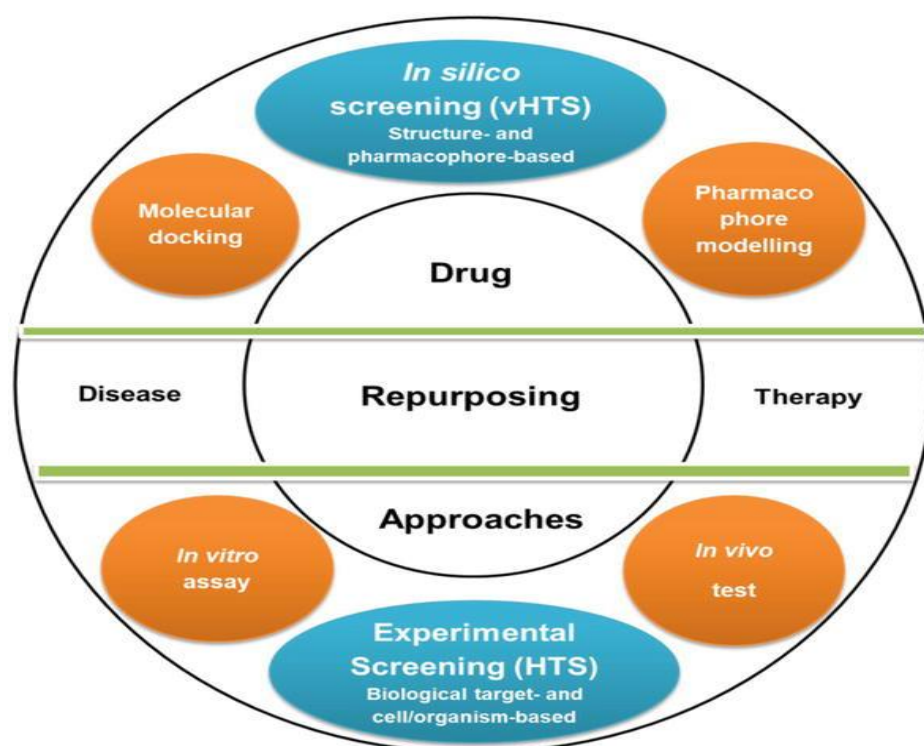


Figure 4.4.2 Drug Repurposing Approach

4.6Molecular Docking Analysis

The crystal structure Penicillin binding protein (MRCA) was used for docking in this study were originally obtained from the Protein Data Bank (www.rcsb.org).

The downloaded protein was prepared for docking such as adding hydrogen, removal of water, elimination of all non-standard residues, adding Kollman charges, and removal of ligands if necessary.

3D structure of Pyrvinium Pamoate was obtained from the <https://pubchem.ncbi.nlm.nih.gov/database>.

Molecular modeling approach is a useful method for drug discovery.

Molecular docking was performed using an online server CB-Dock that adopted the docking and calculation algorithms based on the Auto Dock Vina (version 1.1.2).

The blind docking was performed without defining the grid box to ensure that the best binding site with the highest binding affinity was identified.

The post docking analysis was performed by Discovery Studio software.

Identify protein targets from *Legionella pneumophila* that play crucial roles in virulence, pathogenesis, or survival within host cells. These targets may include enzymes involved in essential metabolic pathways, virulence factors, or proteins critical for host-pathogen interactions.

Identify and characterize the binding site or active site of the protein where ligand binding occurs. This can be done using experimental data, structural analysis, or computational tools.

Define the binding site coordinates and dimensions to guide the docking process.

Molecular docking analysis serves as a computational method utilized in drug discovery for the purpose of forecasting and examining the interaction between a small molecule (ligand) and a target protein (receptor).

This technique is extensively utilized in structure-based drug design to pinpoint potential drug candidates, comprehend the binding mechanisms, and enhance ligand-receptor interactions.

A grid is generated around the binding site of the receptor molecule. This grid defines the search space where the ligand will be docked and allows for efficient exploration of the ligand's binding modes within the binding site.

Molecular docking analysis provides valuable insights into the binding mechanisms of ligands to their target proteins, aiding in rational drug design and lead optimization.

Removal of non-homologous proteins by BLASTp:

The protein sequences were then subjected to BLASTp against the human proteome set acquired from the NCBI database to identify host nonhomologous proteins.

Protein sequences with a high degree of homology with the human proteome were eliminated, and human nonhomologous pathogen proteins were prioritized.

The resultant nonhomologous proteins were scanned against human proteins.

The threshold criteria for this BLASTp analysis were $\leq 35\%$ sequence identity, $\leq 35\%$ query coverage, and an E-value cut off 10^{-4} .

PRELIMINARY ANALYSIS

Genome wide proteome exploration of *Legionella pneumophila* was utilized to screen and identify the novel drugGen targets through subtractive proteomics approach.

The subtractive proteomics approach is a process where non-drug targets were removed from whole proteomic profile by comparing the metabolic pathways of host and organism.

The novel druggable proteins were identified by comparing the unique metabolic pathways.

In the preliminary study, we identified 20 unique metabolic pathways that present only in *Legionella pneumophila*.

DATA RETRIEVAL

The unique proteins of ***Legionella pneumophila* Lens** were identified by comparing the metabolic pathways of Host (*Homo sapiens*) and Organism (*Legionella pneumophila* Lens).

CHAPTER-5

RESULT AND DISCUSSION

Subtractive Proteomics: This involves comparing the proteome of *Legionella pneumophila* with that of its host organism or related non-pathogenic strains. By subtracting out proteins that are common to both, researchers can identify proteins unique to the pathogen, which are potential targets for intervention.

Identification of Novel Targets: Through subtractive proteomics, researchers may identify proteins that are crucial for the pathogen's survival, virulence, or interaction with the host. These could include proteins involved in essential metabolic pathways, virulence factors, or proteins involved in evading host immune responses.

Drug Repurposing: Instead of developing new drugs from scratch, drug repurposing involves identifying existing drugs that could potentially target the identified proteins in *Legionella pneumophila*. This approach leverages existing knowledge of drug safety profiles, pharmacokinetics, and mechanisms of action to expedite the development process.

Computational analysis identified several of these proteins as promising druggable targets based on their structural properties and conservation among pathogenic strains. Virtual screening of FDA-approved drugs against the druggable targets led to the identification of candidate compounds with predicted binding affinity and therapeutic potential.

By conducting subtractive proteomics analysis, we were able to compare the proteomic profiles of pathogenic and non-pathogenic strains of *Legionella pneumophila*. As a result, we have identified a specific group of proteins that are either exclusive to the pathogenic strain or present in significantly higher levels than in the non-pathogenic strain. These proteins could serve as promising drug targets crucial for the bacterium's virulence and ability to thrive in the host organism.

5.1 UNIQUE PROTEIN LIST

>lysAC

MSGILIMQQVVIKFGGTSVSTRATWNNIAAITQKHLNTGVQPVIVCSALTQI
SNKLEKAI

EAALLDEHHSLFNDIQSSHLNLAEQLEVNHQLIANDLHQLQQWLTGISLLK
QAPAKTHAQ

ILSLGELMMTRMGHAFLEKHEIKVKWYDARELLTSTPTLGGETMNYLSAR
CESEYDPGLV

EKFLSSGAQAITQGFFAANPHGETVLLGRGGSDTSAALLAGKLQAASCEI
WTDVPGIYT

ANPHQLPHARLLKQLNYDEAQEIASMGAKVLHPNCIPPVRKANIPMVVKY
THLPEHSGTL

ITKDIDESAPLIKSIQVKHSILLISIDTLNMWQQVGFLADVFAAFKKHGFSD
LLSSSEF

NVTLSLDVNAKIHDRPAINALLDDLNQFGRAKLIEPCSAVSLVGHHIRTVLP
HLGPALEV

FEAKQVYLMASNDLNLTFVVDESHADKLCQRLHHLLIESNPQIFYYSKS
WHEEFGKPN

VRPTPWWEIERDRLLTTSIAHSPCYVYHSPTQAIRARQLSALESIDSLFYAV
KANPFPSI

LKTLEKEGIGFECVSIQELELVKLFPNIKKERILFTPNFAPKSEYEFALQTG
CYVTIDS

LYPLENWPELFKNREVIVRIDPGTGAGHHKHVSTGGNESKFGITQNDISKIL
SLTKSNHI

KVIGLHAHSGSGILSTDWQQTAVMLASLTDQFPEVRSINLGGGLGIVEKP
GQHPIDFAA

LDAQLMVKSQYPGLAIWLEPGRFFVAESGVILAKVTQCCKEKGKVKFIGIE
TGMNSLIRP

SLYGAYHEIVNLTRLHEEKAGFAHIVGPICESGDTLGYDRLLPVTKEGDVILI
ANTGAYG

HCMSSHYNLRPPAQEIVLE

>Aspartate-semialdehyde dehydrogenase

MSRHLNVAIVGATGAVGETFLTVLEERNFPIKSLYPLASSRSVGKTVTFRD
QELDVLDLA

EFDFSKVDLALFSAGGAVSKEYAPKAVAAGCVVVDNTSCFRYEDDIPLVVP
EVNPHRIAD

YTKRGIIANPNCSTIQMVVALKPIYDAVGISRINVATYQSVSGTGKKAISELV
AQVGDLL

NGRPANVQVYPQQIAFNALPHIDQFEDNGYTREEMKMVWETRKIMEDDSI
MVNPTAVRVPVIYGHSEAVHLELKKPLTADDARALLAKAPGVTVVDNLSKA
RYPTAIKNAVGHDDVFVGRIRQDISHPCGLNLWIVADNIRKGAATNAVQIAEI
LQREFL

>dihydrodipicolinate synthase

MFSGSIVALVTPMRNDSVDVHHLRELVEFHIAKGTHALVAAGTTGEAGTLS
HSEKLLVIK

TVIEQAKERVPVIAGTAMNATKDCIELTQQAMEYGAHAALIMTPAYIKPTQE
GLYLHYSH

IAQSVAIPIILYNVPGRTACDMLPETVARLAKISNIIGIKEATGQMTRLQQILRL
CEGSI

DVYSGDDLTAQWLLAGAKGVISVTANVAAKLMAKMCDLAMDDDQAGCL
RIQEQLMPLHELLFVESNPIPVKWAMNKMGLIGGELRLPMTELSEKHHQA
LEKVLKNLELI

>dapB

MRTRVIVNGANGKMGILACETLENHEQFEVVAKLSRQDNLGQSILDTKAQI
VVDLTRADC

VYENSLTIINHGARPVIGTSGLVETQIDELTKLCKIKQIGGIIAPNFSLGAILM
MMLAAK

ASEYFSEVEIIEGHHQQKLDAPSGTALKTAEMMAAARKKPKNKLPLKELTP
GARGGSHHD

INIHSLRLPGLLARQEVLFGNIGETLSITHNSIDRRCFMPGIVLACQKVLNLT
NLVYGLE

HLL

5.2 Unique Metabolic Pathways in *L. pneumophila*

Table 5.5.1 Unique Metabolic Pathways in *L. Pneumophila*

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S.NO	UNIQUE METABOLIC PATHWAY
1.	Toluene degradation
2.	Caprolactam degradation
3.	Beta-Lactam resistance
4.	Vancomycin resistance
5.	Quorum sensing
6.	Bacterial chemotaxis
7.	Flagellar assembly
8.	Bacterial secretion system
9.	Legionellosis
10.	Cyanoamino acid metabolism
11.	Acarbose and validamycin biosynthesis
12.	Polyketide sugar unit biosynthesis
13.	Lipopolysaccharide biosynthesis
14.	O-Antigen nucleotide sugar biosynthesis
15.	Exopolysaccharide biosynthesis
16.	Exopolysaccharide polysynthesis
17.	Teichoic acid biosynthesis
18.	Novobiocin biosynthesis
19.	Monobactam biosynthesis

5.5.2 List of Top Druggable Proteins predicted using Drug Bank Database Search

This 15 proteins are the essential protein. MRCA is the most druggable protein

Table 5.5.2 Druggable Proteins predicted using Drug Bank Database Search

S.NO	DRUGGABLE PROTEIN	DRUG BANK SCORE
1.	rmLa;glucose-1-phosphatethymidyltransferase	366.696
2.	algC	459.914
3.	murA; UDP -n-acetylglucosamine 1-carboxyvinyltransferase	525.013
4.	murB; UDP -n-acetylenolpyruvoylglucoasime reductase	170.629
5.	mruC; UDP-n-acetylmuramate—L-alanine ligase	457.603
6.	mruD; UDP -n-acetylmuramoylalanine –D-glutamate ligase	312.383
7.	murE	330.872
8.	ddlA;D-alanine—D-alanine ligase A	253.832
9.	murF;UDP-n-acetylmuramoyl-tripeptide—d-alanyl-d-alanine ligase	157.142
10.	uppS;Undecaprenyl pyrophosphate synthetase	226.483
11.	murG;UDP-n-acetylglucosamine-n-acetylmuramyl-(pentapeptide)pyrophosphoryl -undecaprenol n-acetylglucoasime transferase	90.5077
	MRCA	
12.	mrdA;penicillin-binding protein2	528.865
13.	ftsJ;Peptidoglycan synthetse FtsJ precursor	128.257
14.	lysAC;hypothetical protein	191.43
15.	Asd;Aspartate-semialdehyde dehydrogenase	102.064

TABLE 5.5.3 Identified druggable targets with drug names

S.N O	ACCES SION NO	PROTEIN NAME	DRUG BANK ID	DRUG NAME	BIT SCO RE	QUE RT LENG TH
1	tr Q5ZS5 2	RNA polymerase sigma factor RpoH	DB08874	Fidaxomicin	103.6 05	318
2	tr Q5ZT0 4	RNA polymerase sigma factor RpoD	DB08226 and DB08266	MyxopyroninB and methyl [(1E,5R)-5-{3- [(2E,4E)-2,5- dimethyl-2,4- octadienoyl]-2,4- dioxo-3,4-dihydro- 2H-pyran-6- yl}hexylidene]carb amate	302.3 68	248
3	tr Q5ZU P0	UDP-N- acetylmurama te-l-alanyl- gamma-d- glutamyl- meso-2,6- diaminohepta ndioate ligase	DB01673 , DB03909 , and DB04395	Uridine-5'- diphosphate-N- acetylmuramoyl-l- alanine,adenosine -5'-[β, γ- methylene]triphos phate, and phosphoaminoph osphonic acid- adenylate ester	145.5 91	398
4	tr Q5ZXL 1	Multidrug resistance protein	DB03825 , DB04209 ,and DB03619	Rhodamine 6G, dequalinium, and deoxycholic acid	427.5 57	1,020
5	tr Q5ZX1 7	Peptidoglycad ,d- transpeptidas e FtsI	DB00303 and DB00671	Ertapenem and cefixime	122.0 94	632

5.4 MRCA Protein - Amino acid Sequence

Penicillin-binding protein:

MKKMAYFWRKGLWALMSLFFLLIVAGSLLYLYLESQLPNVDSLKTVQLQVPLQIFS
KEGLLIQEYGEKKRIPVTYDEIPPTLIHALIATEDQRRFFEHPGVDVMGLGRAAVSML
KTGTKSQGGSTITMQVARNFFLSRKKTFRLKFNEIMLAIKIDRELSKEKILELYLNR
VYLGNRAYGVGAAAMVYFGKSLKDLNLAELAMIAGLPQAPSTQNPINANPLAAKKR
RDHVLRLLEEHYINEEQYQNAVNQPITAKYHGTNIKVKAPYVAEMIRQSLYDNFG
PEAYTKGYKVYTTIDGKLQNTANEVVEKNLIAYDHRHGYRGPIANIGEKDSQSPKI
RQKYLRQYPELNSLIPAVITEVREKEATVALQNGQIIIPWEGMSWARPALKRGGW
VGKSPTKAQQVVAAGDIIYVHPTEKSWQLAQIPEAESAMVAMNPKNGAIEVLVGG
FNFSKSKFNRATQSSRQPGSSFKPFVYAAALNNGYNLATLINDAPIVVDDPSQPN
LWRPHNVNLKFNGPTRLKQALVQSKNLVSIRILDDIGIDYTIDFLTRFGFNKKSLPR
ALSLALGSLSPMDLTTAYAVFANGGYKVEPYLIDHITDADGKILLQAKPSVVCDP
CDKDKVDTSTLAPRVIDEDIAFLMNTALRDVIQHGTGRAARVLNRQDIAGKTGTTN
DQVDAWFAGFNPNLVVTTWIGFDNPKSLHEYAANLALPLWIDFMKVALKDKPETE
LKQPENVI AVRIDPVSGLLAKPNQENGII EYFRHNEVPSEEDQGPVYNAHNDQQE
EELAPVEESLF

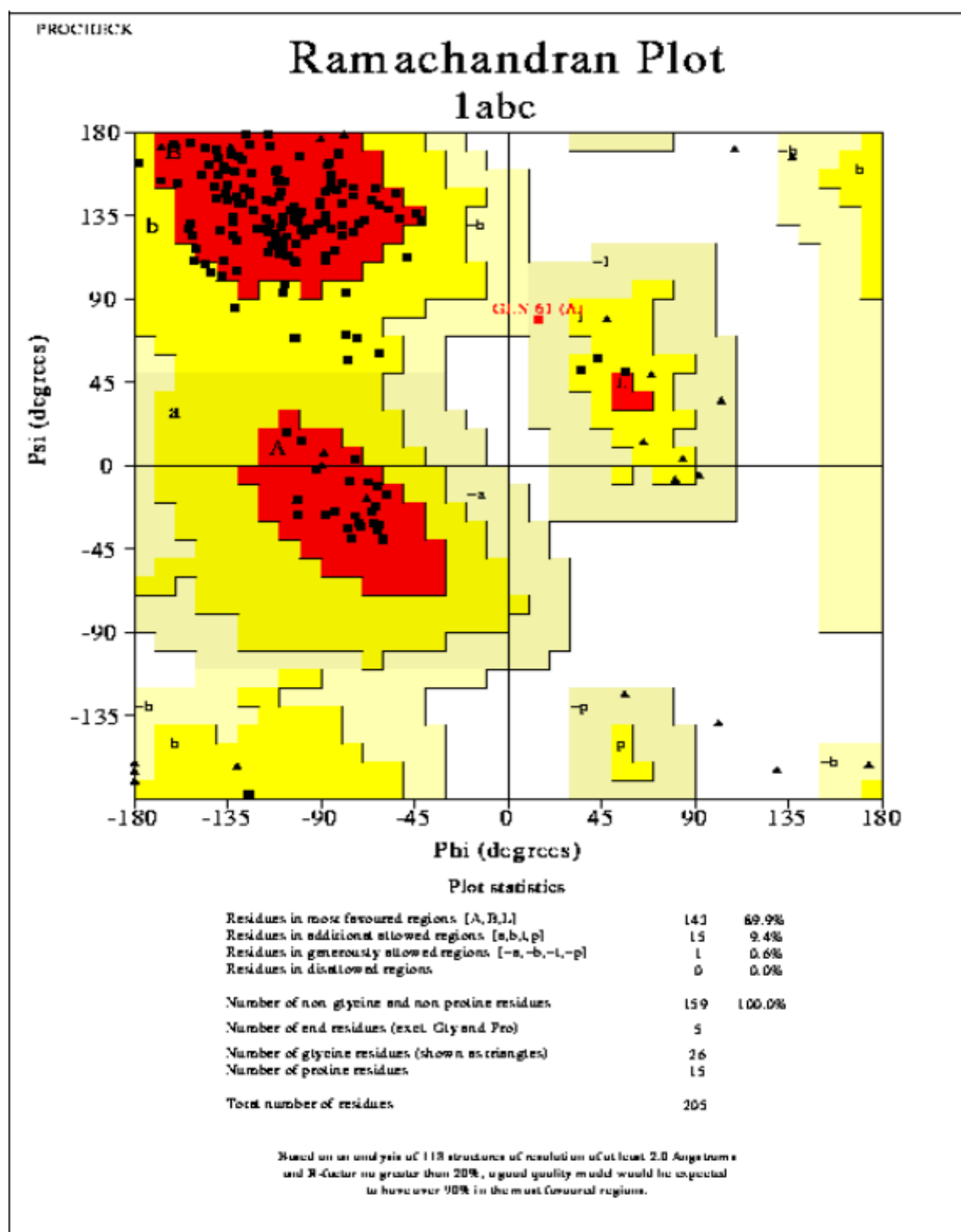


Figure 5.5.1 Ramachandra plot

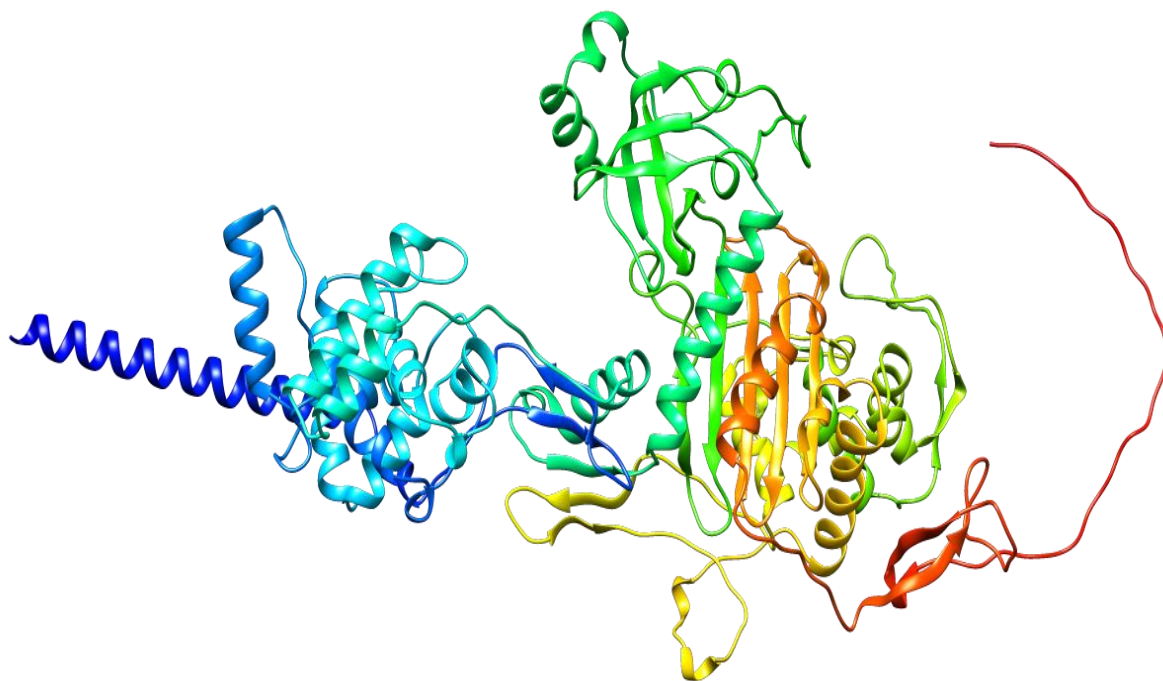


Figure 5.5.2 3D Model of Druggable Protein

3D Structure of MRCA protein was Downloaded from Alpha fold Database

5.6 Pyrvinium Pamoate - Drug Repurposing Approach:

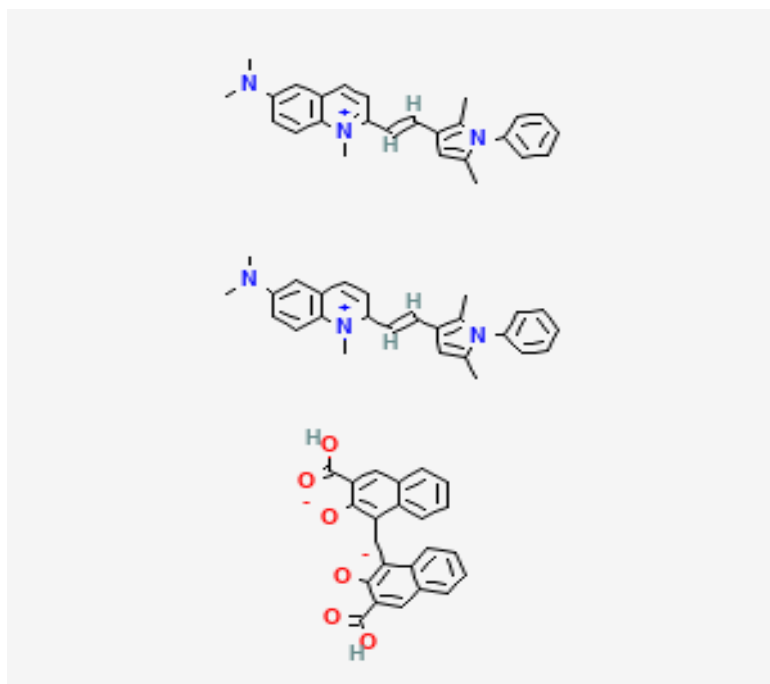


Figure 5.5.3 Pyrvinium Pamoate

Table 5.5.4 Pyrvinium Pamoate-Drug Repurposing Approach

DRUG LIKNESS PROPERTY	VALUE
Water solubility	2.04
Hydrogen Receptor Count	1
Hydrogen Donor Count	0
Polar Surface Area	12.05

Pyrvinium Pamoate:

Pyrvinium (Viprynum) is an anti-helminthic effective for pinworms. Pyrvinium is used to treat pinworms (enterobiasis).

Anti-parasitic drug, Pyrvinium Pamoate were the most studied drug for drug repurposing.

It will not work for other types of worm infections (for example, roundworms or tapeworms). Pyrvinium is an anthelmintic effective for pinworms. Several forms of pyrvinium have been prepared with variable counter anions, such as halides, tosylate, triflate and pamoate .

Pyrvinium pamoate functions by disrupting the energy metabolism of the parasite, resulting in paralysis and the subsequent removal of the worms from the intestines. Typically, it is administered orally in the form of tablets or suspension. Pyrvinium salts can also inhibit the growth of cancer cells. More specifically, the pamoate salt has been shown to have preferential toxicity for various cancer cell lines during glucose starvation.

Several studies have indicated that pyrvinium pamoate might possess anti-cancer properties, as it has demonstrated the ability to impede the growth of specific cancer cells in preclinical trials. Nevertheless, further research is required to comprehensively comprehend its potential in this aspect.

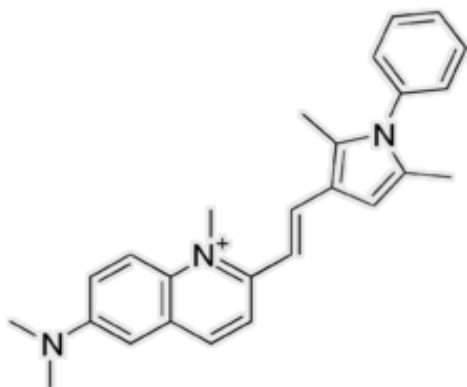


Figure 5.5.4 STRUCTURE OF PYRUVINIUM PAMOATE

5.7 MOLECULAR DOCKING ANALYSIS

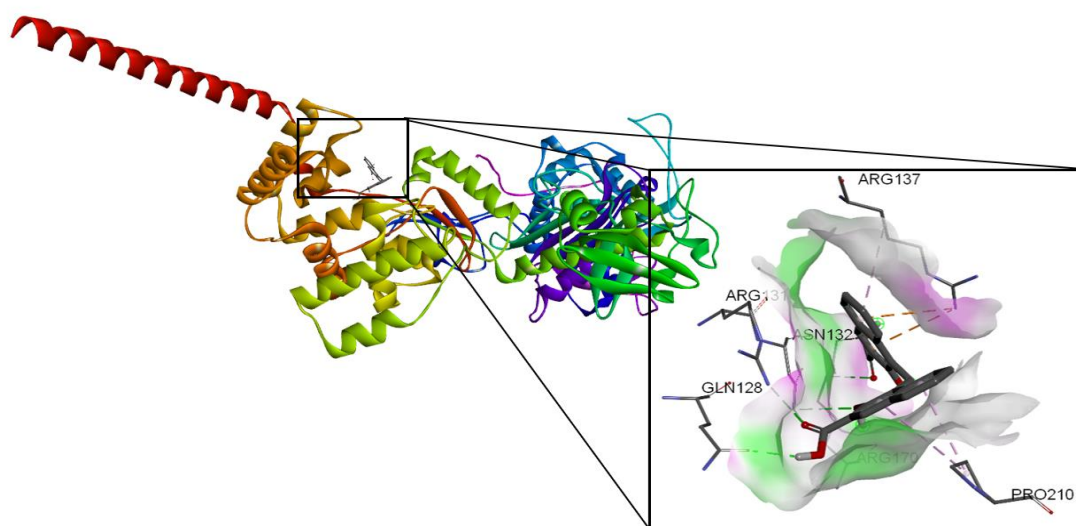


Figure 5.5.4 Molecular Docking

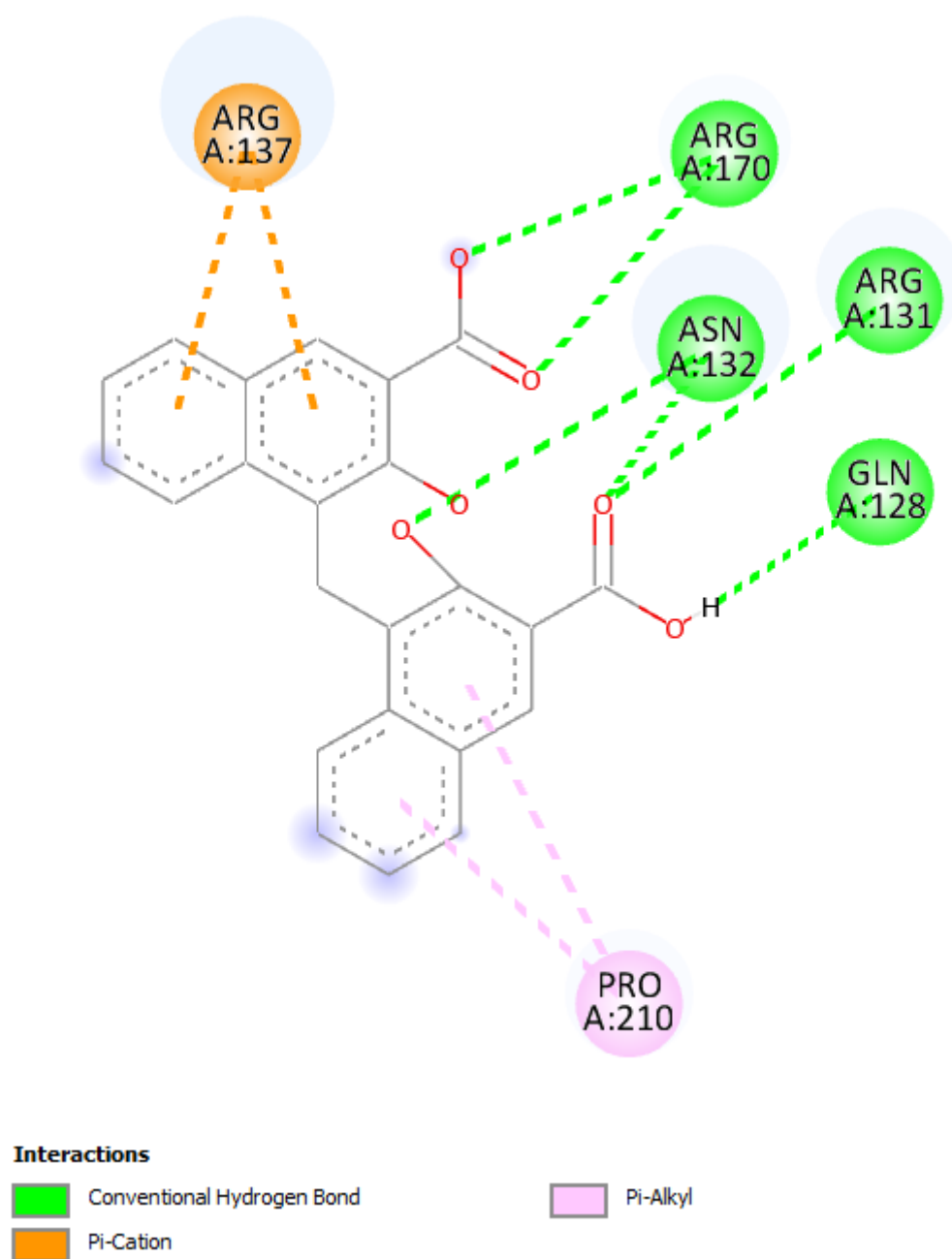


Figure 5.5.5 Hydrogen Bond Interaction

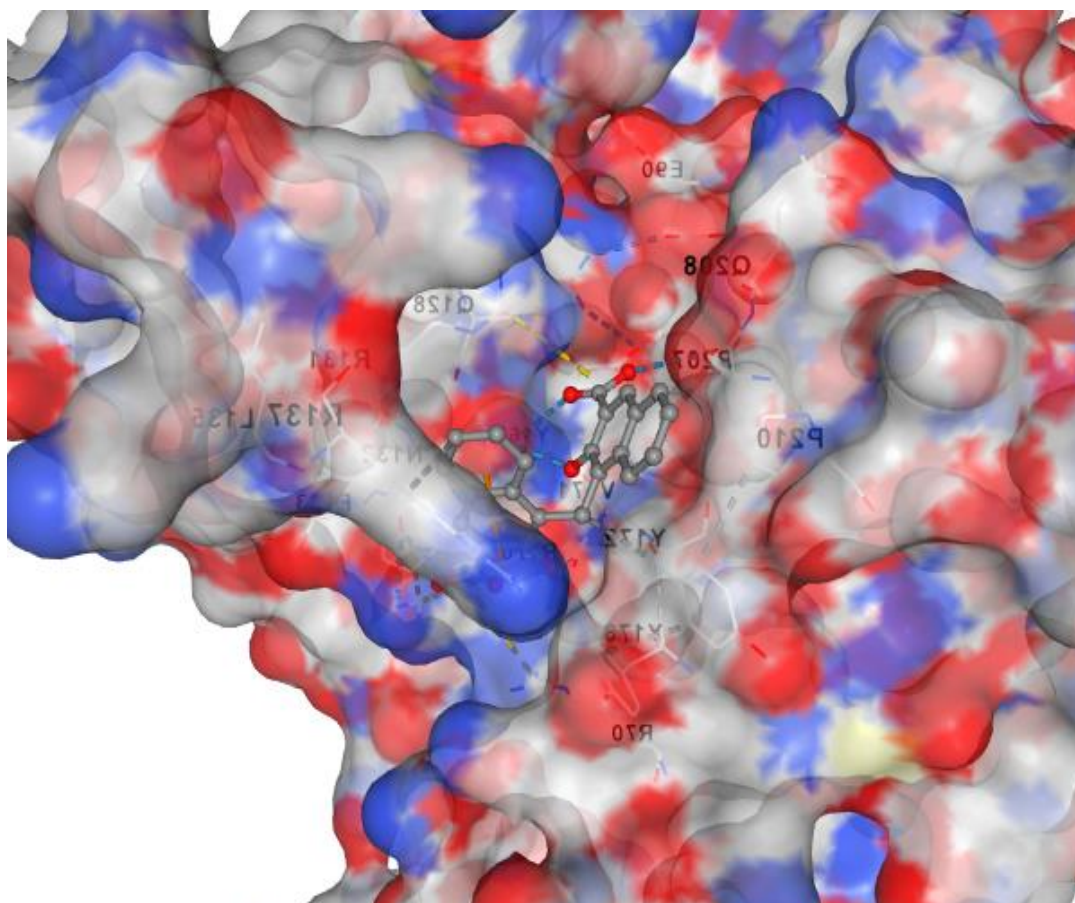


Figure 5.5.6 Hydrogen bond interaction

MINIMUM BINDING ENERGY	X,Y,Z CENTER	DOCKING CENTER
-8.2 KCAL	-37,5,25	27,21,35

Hydrogen Bond Interaction: GLN A:128, ARG A:131, ASN A:132, ARG A:170

SUMMARY

This research project is focused on the discovery of new potential targets for drug development in *Legionella pneumophila* through a comprehensive approach that combines subtractive proteomics and drug repurposing strategies. *Legionella pneumophila* is a harmful bacterium that causes serious respiratory infections, and the issue of antibiotic resistance presents a major obstacle in its treatment.

The study involves utilizing bioinformatics analysis to compare the proteomic profiles of pathogenic and non-pathogenic strains, with a focus on identifying proteins crucial for virulence and pathogenicity. Additionally, the project includes the screening of existing drug libraries to repurpose drugs that can target the identified proteins.

Through experimental validation, the effectiveness of these candidate drugs in inhibiting the growth and pathogenicity of *Legionella pneumophila* will be evaluated. Ultimately, the goal of this investigation is to offer alternative therapeutic solutions for Legionnaires' disease, while also addressing the challenges posed by antibiotic resistance. Existing antibiotics are becoming less effective due to increasing antibiotic resistance in *Legionella* strains. Identifying proteins essential for *Legionella*'s growth within amoeba hosts (a key factor in its pathogenesis). Utilizing subtractive proteomics to find these unique proteins. Repurposing existing drugs that target the identified proteins, potentially accelerating the development process.

The combined use of subtractive proteomics and drug repurposing offers a promising approach for identifying new drug targets in *Legionella pneumophila*, the bacteria responsible for Legionnaires' disease.

Subtractive proteomics helps pinpoint unique bacterial proteins crucial for its survival and virulence by comparing its proteome with that of related organisms.

CONCLUSION

To summarize, the combination of subtractive proteomics and drug repurposing shows promise in identifying new targets for treating *Legionella pneumophila*. By comparing protein profiles between pathogenic and non-pathogenic strains, we have identified key proteins that contribute to the bacterium's virulence and pathogenicity. Through drug repurposing, we have screened existing drugs to target these proteins, offering potential therapeutic interventions.

The findings of this study provide valuable insights into alternative treatment options for Legionnaires' disease, which is particularly important due to the issue of antibiotic resistance. It will be crucial to experimentally validate the effectiveness of candidate drugs in inhibiting the growth and pathogenicity of *Legionella pneumophila*, paving the way for further clinical investigations.

Anti-parasitic drug, Pyrvinium Pamoate were the most studied drug for drug repurposing.

The Penicillin Binding Protein (MRCA) was identified by using subtractive proteomics as potential drug target for combating *Legionella pneumoniae* infection.

We recommend using the Pyrvinium Pamoate in further research, including virtual screening, which could help the overall efforts in finding effective therapeutic agents against *Legionella pneumoniae*.

Our study highlights the utility of subtractive proteomics combined with drug repurposing approaches in identifying novel druggable targets and repurposed drugs for *Legionella pneumophila*. The identified targets and candidate compounds represent promising avenues for further research and development of therapeutics against Legionnaires' disease, addressing an unmet medical need and contributing to improved patient outcomes.

In this study, we successfully employed a multidisciplinary approach combining subtractive proteomics and drug repurposing strategies to identify novel druggable targets within *Legionella pneumophila*, the causative agent of Legionnaires'

disease. Through a systematic analysis of proteomic data and computational assessments, we identified proteins unique to *L. pneumophila* with potential roles in virulence and pathogenicity, prioritizing them as promising drug targets.

This not only accelerates the drug discovery process but also leverages existing knowledge to repurpose drugs for new indications. Pyruvinium pamoate could serve as an example, demonstrating the potential of repurposing compounds to target novel druggable targets in infectious diseases.

Herein, our current research concerning drug repurposing using molecular docking and other in silico drug discovery approaches in the battle against *Legionella pneumoniae*.

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