

SYSTEMS LEVEL MODELLING OF HOST PATHOGEN INTERACTION IN MYCOBACTERIUM TUBERCULOSIS- NETWORK ANALYSIS AND MATHEMATICAL MODELLING

Submitted in partial fulfillment of the requirements for the award of
Bachelor of Science in Bioinformatics and Data Science

By

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**DEPARTMENT OF BIOINFORMATICS SCHOOL OF BIO AND CHEMICAL
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SATHYABAMA

**INSTITUTE OF SCIENCE AND TECHNOLOGY
(DEEMED TO BE UNIVERSITY)**

**Accredited with Grade “A++” by NAAC ICategory-1 University by UGC I Approved by AICTE
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BONAFIDE CERTIFICATE

This is to certify that this Project Report is the bonafide work of **ENOS JADLIN.G (41738003)**, who carried out the final year project entitled "**SYSTEMS LEVEL MODELING OF HOST PATHOGEN INTERACTION IN MYCOBACTERIUM TUBERCULOSIS - NETWORK ANALYSIS AND MATHEMATICAL MODELING**" under my supervision from January 2024 to April 2024.

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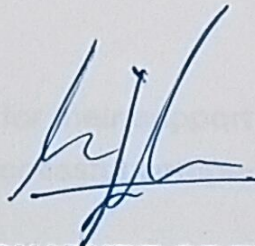
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DECLARATION

I, Mr. ENOS JADLIN.G (41738003) hereby declare that the Project Report entitled **SYSTEMS LEVEL MODELING OF HOST PATHOGEN INTERACTION IN MYCOBACTERIUM TUBERCULOSIS - NETWORK ANALYSIS AND MATHEMATICAL MODELING** done by me under the guidance of **Dr. Daniel Alex Anand** at Sathyabama Institute of Science and Technology, Jeppiaar Nagar, Rajiv Gandhi Road, Chennai-600119, Tamil Nadu, India, is submitted in partial fulfillment of the requirements for the award of Bachelor of Science in Bioinformatics and Data Science.

DATE: 27/4/2024

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ABSTRACT

Tuberculosis (TB) remains a significant global health challenge, necessitating a deeper understanding of the molecular interactions underlying host-pathogen interactions. This provides a comprehensive overview of key molecular players and pathways implicated in TB pathogenesis and host response. This work highlights key molecular interactions and pathways relevant to host-pathogen interactions, particularly in the context of tuberculosis (TB). It discusses the roles of various receptors, DNA repair enzymes, mutated proteins, and signalling complexes in immune recognition, DNA repair, and cellular signalling. While specific interactions are not detailed, the abstract emphasizes the interconnectedness of these molecules and processes, indicating their importance in TB pathogenesis and host response. Mathematical modelling using KEGG pathway analysis further supports the understanding of TB-related molecular networks. This computational approach provides valuable insights into the interconnectedness of molecular pathways involved in TB pathogenesis, offering a systems-level understanding of host-pathogen interactions and facilitating the identification of potential therapeutic targets for TB treatment and prevention.

TABLE OF CONTENTS

CHAPTER NO	TITLE	PAGE NO
	ABSTRACT	I
	LIST OF ABBREVIATIONS	VIII
	LIST OF FIGURES	X
1	INTRODUCTION	1
	1.1 OUTLINE	2
	1.1.1 TUBERCULOSIS	2
	1.1.1.1 TUBERCULOSIS EPIDEMIOLOGY	3
	1.1.1.2 TUBERCULOSIS PATHOGENESIS	4
	1.1.2 NETWORK BIOLOGY	6
	1.1.2.1 DEFINITION OF BIOLOGICAL NETWORKS	7
	1.1.2.2 TYPES OF NETWORKS	7
	1.1.2.3 PROPERTIES OF BIOLOGICAL NETWORKS	9
	1.1.2.4 METHODS OF NETWORK CONSTRUCTION AND ANALYSIS	9
	1.1.3 MATHEMATICAL MODELLING	9
	1.1.3.1 SIGNIFICANCE OF MATHEMATICAL MODELLING	10
	1.1.3.2 SCOPE OF MATHEMATICAL MODELLING	10
2	LITERATURE SURVEY	12
3	AIM AND OBJECTIVES	17
4	METHODOLOGY	19
	4.1 PATHWAY COLLECTION	19

4.1.1 REACTOME	19
4.1.2 PATHWAY SELECTION	20
4.1.2.1 INFECTION WITH MTB	22
4.1.2.2 SUPPRESSION OF PHAGOSOMAL MATURATION	22
4.1.2.3 ESCAPE OF MTB FROM THE PHAGOCYTE	23
4.1.2.4 PHAGOCYTE CELL DEATH CAUSED BY CYTOSOLIC MTB	23
4.1.2.5 SUPPRESSION OF APOPTOSIS	23
4.1.2.6 PREVENTION OF PHAGOSOMAL- LYSOSOMAL FUSION	23
4.1.2.7 BLOCKAGE OF PHAGOSOME ACIDIFICATION	24
4.1.2.8 TOLERANCE OF OXYGEN PRODUCED BY MACROPHAGES	24
4.1.2.9 RESPONSE OF MTB TO PHAGOCYTOSIS	24
4.1.2.10 MANIPULATION OF HOST ENERGY METABOLISM	25
4.1.2.11 INHIBITION OF MEMBRANE REPAIR	25
4.1.2.12 MODULATION BY MTB OF HOST IMMUNE SYSTEM	25
4.1.2.13 CELL REDOX HOMEOSTASIS	25
4.1.2.14 INHIBITION OF NITRIC OXIDE PRODUCTION	26
4.1.2.15 ANTIMICROBIAL ACTION AND ANTIMICROBIAL RESISTANCE	26
4.1.2.16 LATENT INFECTION – RESPONSES OF MTB TO PHAGOCYTOSIS	26
4.1.2.17 MTB IRON ASSIMILATION BY CHELATION	27
4.1.2.18 TOLERANCE BY MTB TO NITRIC OXIDE BY MACROPHAGES	27
4.1.2.19 SUPPRESSION OF AUTOPHAGY	27
4.1.2.20 INFECTIOUS DISEASE	

4.1.2.21 CD (DC - SIGN) SIGNALLING	28
4.1.2.22 C – TYPE LECTIN RECEPTORS	28
4.1.2.23 STING MEDIATED INDUCTION OF HOST IMMUNE RESPONSE	29
4.1.2.24 BACTERIAL INFECTION PATHWAYS	29
4.1.2.25 PURINERGIC SIGNALLING IN LEISHMANIASIS	30
4.1.2.26 DISEASE	30
4.2 NETWORK FORMATION AND INTEGRATION	32
4.2.1 CYTOSCAPE	32
4.2.1.1 NETWORK FORMATION	33
4.2.1.2 NETWORK INTEGRATION	33
4.3 NETWORK ANALYSIS	34
4.3.1 ANALYSIS OF NETWORK	34
4.3.2 CYTOHUBBA	36
4.4 MATHEMATICAL MODELLING	37
4.4.1 KEGG PATHWAY	37
4.4.2 PATHWAY SELECTION	38
4.4.3 EQUATION	39
4.4.3.1 BACILLI GROWTH AND KILLING	40
4.4.3.2 MACROPHAGE DYNAMICS	41
4.4.3.3 ACTIVATED IMMUNE CELL FORMATION	42
4.4.3.4 INFECTED CELL DYNAMICS	43
4.4.4 PARAMETERS	44
4.4.4.1 GROWTH RATE OF TB BACILLI	45
4.4.4.2 NATURAL DEATH RATE OF TB BACILLI	45

4.4.4.3 RATE OF PHAGOCYTOSIS OF TB BY MACROPHAGES	45
4.4.4.4 RATE OF TB KILLING BY ACTIVATED IMMUNE CELLS	45
4.4.4.5 RECRUITMENT RATE OF MACROPHAGES	46
4.4.4.6 DEATH RATE OF MACROPHAGES	46
4.4.4.7 RATE OF INFECTED CELL CONVERSION BACK TO MACROPHAGES	46
4.4.4.8 ACTIVATION RATE OF ACTIVATED AND T-CELLS	46
4.4.4.9 DEATH RATE OF ACTIVATED IMMUNE CELLS	47
4.4.4.10 DEATH RATE OF INFECTED CELLS	47
4.4.5 GRAPH PLOT	47
4.4.5.1 IMPORTING LIBRARIES	48
4.4.5.2 DEFINING PARAMETERS	48
4.4.5.3 DEFINING THE DIFFERENTIAL EQUATION	49
4.4.5.4 SETTING INITIAL CONDITIONS	49
4.4.5.5 SETTING TIME POINTS	50
4.4.5.6 SOLVING THE ODE's	50
4.4.5.7 PLOTTING THE RESULTS	51
5 RESULTS AND DISCUSSION	52
5.1 NETWORK ANALYSIS OF MOLECULAR INTERACTIONS IN HPI	52
5.1.1 AVERAGE SHORTEST PATH LENGTH	52
5.1.2 BETWEENNESS CENTRALITY	54
5.1.3 DEGREE	55

5.1.4	ECCENTRICITY	57
5.1.5	NEIGHBOURHOOD CONNECTIVITY	58
5.1.6	OUT DEGREE	60
5.1.7	STRESS	61
5.1.8	TOPOLOGICAL COEFFICIENT	64
5.2	MATHEMATICAL MODELLING	66
5.2.1	EQUATION DEFINING	66
5.2.1.1	BACILLI GROWTH AND DEATH	66
5.2.1.2	MACROPHAGE DYNAMICS	67
5.2.1.3	ACTIVATED IMMUNE CELL DYNAMICS	68
5.2.1.4	INFECTED CELL DYNAMICS	68
5.2.2	PARAMETERIZATION	69
5.2.2.1	INITIAL CONDITION	70
5.2.3	GRAPH PLOT	71
6	SUMMARY AND CONCLUSION	74
6.1	SUMMARY	74
6.2	CONCLUSION	74
	REFERENCES	75

LIST OF ABBREVIATION

TB	Tuberculosis
MTB	Mycobacterium Tuberculosis
NAD⁺	Nicotinamide Adenine Dinucleotide
LAM	Lipoarabinomannan
VPS33B	vascular protein sorting-associated protein 33B
RAB5	Ras-related protein
PI3P	Phosphatidylinositol 3-Phosphate
SodB/SodC	Superoxide Dismutases
DHAP	Dihydroxyacetone Phosphate
NO	Nitric Oxide
HIV	Human Immunodeficiency Virus
HCMV	Human Cytomegalovirus
DC-SIGN	DC-specific intracellular adhesion molecule-3-grabbing non-integrin
TLR	Toll-like receptor
PAMPs	Pathogen-Associated Molecular Patterns
PRRs	Pattern Recognition Receptors
KEGG	Kyoto encyclopedia of genes and genomes

DC	Dendritic Cell
RIG-I	Retinoic acid-inducible gene I
CLR	C-type lectin receptor
NLR	Nucleotide-binding domain and Leucine-rich-repeat-containing
CRD	Carbohydrate Recognition Domains
NF-κB	Nuclear Factor- κ B
ODE	Ordinary Differential Equation
TDM	Trehalose-6,6'-dimycolate
PPI	Protein Protein Interaction
ER	Endoplasmic Reticulum
CTNNB1	Beta-Catenin

LIST OF FIGURES

FIGURE NO	TITLE	PAGE NO
1.1	HPI and Granuloma Formation	5
1.2	Main Features of Tuberculosis	6
1.3	Gene regulatory network	7
1.4	Protein-Protein interaction network	8
1.5	Metabolic network	8
1.6	Ecological Network	8
4.1	Reactome Database	20
4.2	Merged complex host pathogen network of Mtb.	34
4.3	Network Analyzer result panel	35
4.4	Ranking of Nodes using Degree algorithm in Cytohubba tool	36
4.5	KEGG PATHWAY Database	38
4.6	Pathway of Mtb taken from KEGG database	39
4.7	Importing Libraries	48
4.8	Defining the Parameters	48
4.9	Defining the Differential Equation	49
4.10	Initial Conditions	49
4.11	Setting the Points	49
4.12	Solving the ODE	50

4.13	Plotting the Result	51
5.1	Simulation of TB infection and Immune Response Graph Plot	71

CHAPTER-1

INTRODUCTION

Tuberculosis (TB) is a global health threat caused by *Mycobacterium tuberculosis*, affecting millions worldwide. Its impact is particularly profound in developing countries with limited healthcare resources (World Health Organization, 2020). The disease primarily targets the lungs but can disseminate to other organs, leading to diverse clinical presentations.

Transmission occurs through the inhalation of airborne droplets containing the bacteria expelled by infected individuals during coughing or sneezing (Gandhi et al., 2020). Once inhaled, *M. tuberculosis* can establish infection, leading to latent or active disease. Latent TB infection is characterized by the presence of the bacteria without clinical symptoms, while active TB disease manifests with symptoms such as cough, fever, weight loss, and chest pain.

Diagnosis of TB involves a multifaceted approach, including clinical evaluation, radiographic imaging (e.g., chest X-ray), microbiological examination of sputum samples, and molecular tests for drug resistance (World Health Organization, 2020). Treatment typically consists of a combination of antibiotics administered over several months. However, the emergence of drug-resistant strains poses a significant challenge to effective TB management.

Despite concerted efforts to control TB, several factors contribute to its persistence. Socioeconomic disparities, inadequate access to healthcare, and the high prevalence of comorbid conditions such as HIV/AIDS fuel TB transmission and hinder treatment outcomes (Gandhi et al., 2020).

1.1 OUTLINE

1.1.1 *Tuberculosis:*

Mycobacterium tuberculosis (Mtb) is a highly specialized intracellular pathogen responsible for tuberculosis (TB), an ancient infectious disease that continues to pose significant global health challenges (Gupta et al., 2018). As an obligate aerobe with a slow growth rate, Mtb has evolved intricate strategies to evade host immune defenses and establish persistent infections. One of its defining features is the complex structure of its cell envelope, which consists of a lipid-rich outer membrane, mycolic acids, and unique glycolipids such as trehalose dimycolate (TDM) (Jackson et al., 2018). These components contribute to the impermeability of the cell wall, providing protection against environmental stresses and antimicrobial agents.

Mtb's ability to survive within host macrophages, the primary phagocytic cells involved in innate immune responses, is central to its pathogenesis. Upon phagocytosis, Mtb prevents phagosome-lysosome fusion and replicates within the phagosome, creating a niche conducive to its survival (Gupta et al., 2018). This intracellular lifestyle allows Mtb to evade detection by the host immune system and establish latent infections, which can reactivate years later under conditions of immune compromise.

Furthermore, Mtb employs a diverse array of virulence factors, including secreted proteins, lipoproteins, and cell wall-associated molecules, to manipulate host cell functions and modulate immune responses (Gupta et al., 2018). These virulence factors facilitate Mtb's ability to subvert host defenses, evade immune surveillance, and establish chronic infections.

Understanding the complex interplay between Mtb and the host immune system is essential for developing effective interventions to combat TB. By elucidating the molecular mechanisms underlying Mtb pathogenesis, researchers can identify novel drug targets and vaccine candidates to enhance TB control efforts.

1.1.1.1 Tuberculosis Epidemiology:

Tuberculosis (TB) continues to be a major global health concern, with approximately 10 million new cases and 1.4 million deaths reported annually (Houben & Dodd, 2016). The epidemiology of TB exhibits geographical variations, with a higher burden observed in low- and middle-income countries, particularly in regions with limited access to healthcare services and high prevalence of risk factors such as poverty and malnutrition.

- **Global Distribution:**

TB incidence rates vary significantly across different regions and countries, influenced by factors such as population density, socioeconomic status, and prevalence of comorbidities like HIV/AIDS. High-burden countries such as India, China, and Nigeria contribute substantially to the global TB burden, highlighting the need for targeted interventions in these regions (Wang et al., 2018).

- **Population Groups at Risk:**

Certain population groups are disproportionately affected by TB, including individuals living with HIV/AIDS, prisoners, migrants, and healthcare workers. HIV/AIDS remains a significant risk factor for TB, with HIV-positive individuals being at higher risk of developing active TB due to compromised immunity (Naidoo et al., 2018). Additionally, social determinants such as poverty, overcrowded living conditions, and limited access to healthcare contribute to TB disparities among marginalized populations.

- **Drug Resistance:**

The emergence of drug-resistant TB strains poses a significant challenge to TB control efforts. Multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB (XDR-TB) strains have been reported globally, particularly in countries with suboptimal TB treatment programs and inadequate infection control measures (Gandhi et al., 2018). Addressing drug resistance requires a multifaceted approach, including improved diagnostics, treatment regimens, and infection prevention measures.

- Challenges in TB Control:

Despite advances in TB diagnosis and treatment, several challenges hinder TB control efforts. These include limited access to quality healthcare services, inadequate funding for TB programs, stigma associated with TB, and suboptimal treatment adherence. Additionally, the COVID-19 pandemic has disrupted TB care services, leading to delays in diagnosis, treatment initiation, and increased TB transmission (Hogan et al., 2020).

1.1.1.2 Tuberculosis Pathogenesis:

The pathogenesis of *Mycobacterium tuberculosis* (Mtb) involves a complex interplay between the bacterium and the host immune system, characterized by intricate molecular and cellular interactions (O'Garra et al., 2013). Understanding the mechanisms underlying Mtb pathogenesis is crucial for developing effective interventions to control TB.

- Interaction with Host Immune Cells:

Upon inhalation, Mtb enters the lungs and encounters alveolar macrophages, which serve as the first line of defense against the bacterium. Mtb can survive and replicate within macrophages by inhibiting phagosome-lysosome fusion and resisting antimicrobial mechanisms (Russell, 2013). This intracellular survival allows Mtb to evade immune surveillance and establish chronic infections.

- Granuloma Formation:

In response to Mtb infection, the host immune system initiates the formation of granulomas, organized structures composed of immune cells, including macrophages, T cells, and dendritic cells. Granulomas serve as sites of immune containment, limiting the spread of Mtb and preventing dissemination to other organs (Flynn & Chan, 2001). However, Mtb can manipulate granuloma formation and function to its advantage, leading to persistent infection.

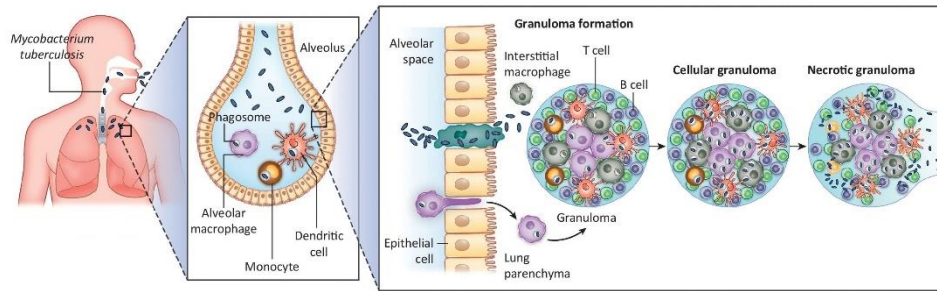


Fig 1.1 HPI and Granuloma formation (ScienceDirect)

- Immune Evasion Strategies:

Mtb employs various immune evasion strategies to subvert host immune responses and establish chronic infections. These strategies include altering the lipid composition of its cell envelope to evade detection by host immune receptors, modulating host cell signalling pathways to inhibit antimicrobial responses, and inducing T cell exhaustion and apoptosis to impair adaptive immunity (Behar et al., 2010).

- Role of Cytokines and Chemokines:

Cytokines and chemokines play a critical role in regulating the host immune response to Mtb infection. Pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF- α), interferon-gamma (IFN- γ), and interleukin-12 (IL-12) are essential for activating macrophages and promoting antimicrobial responses (Cooper, 2009). Conversely, anti-inflammatory cytokines such as interleukin-10 (IL-10) and transforming growth factor-beta (TGF- β) can suppress immune activation and promote Mtb survival (O'Garra et al., 2013).

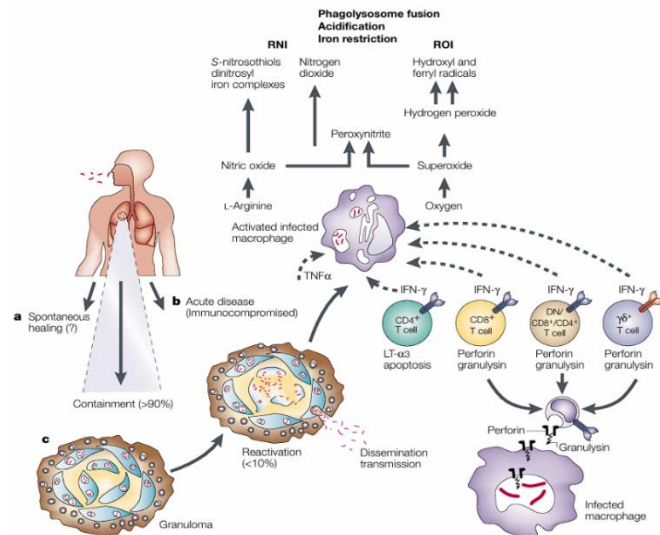


Fig 1.2 Main Features of tuberculosis: from infection to host defence (Research Gate)

1.1.2 Network Biology:

Network biology serves as a foundational framework for understanding the complex interactions and dynamics within biological systems, ranging from molecular interactions to ecological networks. This section delves into the fundamental concepts and principles underlying network biology, providing a basis for subsequent discussions on network modelling approaches. A network of interconnected, mutually reliant parts that make up a single, cohesive system is a biological system. The study of how molecules interact and combine to generate subcellular machinery—the functional units necessary for physiological functioning at the cell, tissue, and organ levels—is known as network biology (Neeru Redhu et al., 2022).

1.1.2.1 Definition of Biological Networks:

Biological networks represent the interconnectedness of biological entities, such as genes, proteins, metabolites, and species, through various types of interactions or relationships. These interactions are typically represented as nodes (vertices) connected by edges (links), where nodes represent biological entities, and edges represent the relationships between them (Barabási & Oltvai, 2004).

1.1.2.2 Types of Networks:

Biological networks encompass a diverse array of network types, each capturing different aspects of biological organization and function. Common types of biological networks include:

- Gene regulatory networks (Fig 1.3)
- Protein-protein interaction networks (Fig 1.4)
- Metabolic networks (Fig 1.5)
- Ecological networks (Fig 1.6)

Each network type reflects specific biological processes and interactions relevant to its domain (Jeong et al., 2000).

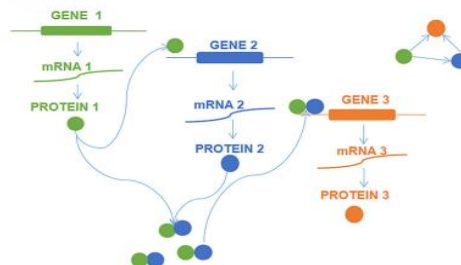


Fig 1.3 Gene regulatory network(Springer)

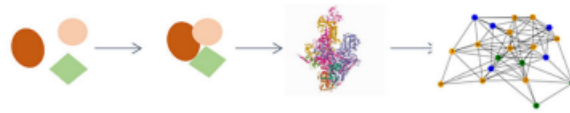


Fig 1.4 Protein-Protein interaction network(Springer)

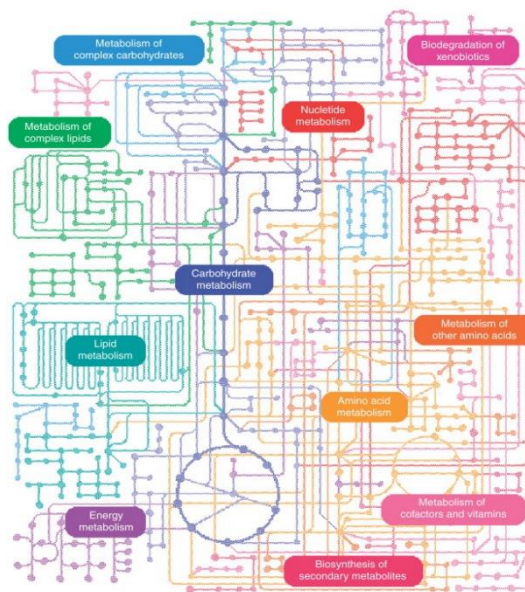


Fig 1.5 Metabolic network(Science Direct)

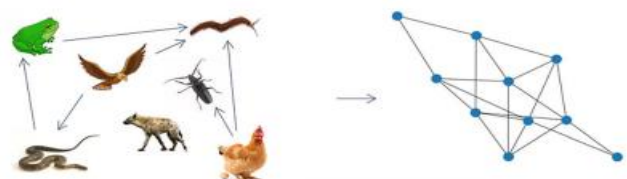


Fig 1.6 Ecological Network(Springer)

1.1.2.3 Properties of Biological Network:

Biological networks exhibit several characteristic properties that distinguish them from random networks and underlie their biological relevance. These properties include scale-free topology, small-world organization, and modularity. Scale-free networks are characterized by a few highly connected nodes (hubs) and many sparsely connected nodes, whereas small-world networks exhibit short path lengths and high clustering coefficients. Modularity refers to the presence of densely connected subnetworks or modules within larger networks, reflecting functional or structural units (Barabási & Oltvai, 2004).

1.1.2.4 Methods of Network Construction and Analysis:

Constructing and analysing biological networks involve a variety of computational and experimental techniques. Computational methods, such as graph theory and statistical modelling, are commonly used to infer network structures from large-scale omics data sets, while experimental techniques, such as yeast two-hybrid assays and mass spectrometry, provide direct evidence of molecular interactions. Integration of computational and experimental approaches enables comprehensive characterization and analysis of biological networks (Ideker & Krogan, 2012)

1.1.3 Mathematical Modelling:

Mathematical modelling plays a crucial role in elucidating the dynamic behaviour and regulation of biological pathways, offering quantitative insights into complex biological systems. This section provides an introduction to the use of mathematical modelling in studying biological pathways and highlights its significance in advancing our understanding of cellular processes (Alon, 2007).

1.1.3.1 Significance of Mathematical Modelling:

Mathematical modelling and experimental approaches synergistically advance biological research by offering complementary perspectives (Alon, 2007). Mathematical models, representing biological pathways as equations, enable the simulation and analysis of system behaviour under diverse conditions. This approach facilitates the prediction of system dynamics and the identification of crucial regulatory mechanisms. Through integration with experimental data, these models undergo refinement and validation, deepening our understanding of biological processes.

Moreover, mathematical models serve as a systematic tool for interpreting experimental data, guiding hypothesis formulation and prediction testing (Kirschner & Hunt, 1999). By simulating biological systems mathematically, researchers gain insights into phenomena not easily observable experimentally. These models aid in exploring system dynamics, identifying regulatory components, and predicting emergent properties. Consequently, the integration of mathematical modelling with experimental data enhances researchers' ability to interpret complex biological phenomena, fostering advancements in the field.

1.1.3.2 Scope of Mathematical Modelling:

Mathematical modelling in biology encompasses a diverse array of approaches tailored to address specific research questions (Le Novère, 2015). From simple mechanistic models to complex computational simulations, these approaches include deterministic models like ordinary differential equations (ODEs) for capturing continuous dynamics, stochastic models for accounting for randomness in processes such as gene expression (Gillespie, 1977), and agent-based models for simulating individual entity behaviour within systems (Anderson, 2007).

Deterministic models describe biochemical reactions and signalling pathways, while stochastic models offer insights into inherent randomness and variability. Agent-based models provide a deeper understanding of spatial organization and emergent properties, collectively enriching our understanding of biological pathways without repeating information.

CHAPTER – 2

LITERATURE SURVEY

Zak et al. (2021) underscore the urgency of understanding tuberculosis (TB) due to its significant global burden. With approximately 10 million new cases and 1.4 million deaths annually, TB remains a major public health challenge worldwide. The emergence of drug-resistant strains, coupled with socioeconomic factors and healthcare disparities, further exacerbates the TB epidemic. Zak et al. (2021) highlight the need for innovative strategies to control TB transmission and reduce disease burden. Furthermore, they emphasize the importance of interdisciplinary approaches, combining basic science research, clinical studies, and public health interventions, to address the complex challenges of TB prevention, diagnosis, and treatment.

Lee and Fujiwara (2019) emphasize the complexity of TB pathogenesis and the challenges associated with controlling the disease. Despite advances in TB research and treatment, the precise mechanisms underlying *M. tuberculosis* infection and immune evasion remain incompletely understood. Lee and Fujiwara (2019) call for continued efforts to elucidate host-pathogen interactions in TB, with the aim of developing novel interventions to combat the disease.

Patel et al. (2020) underscore the importance of elucidating host-pathogen interactions in TB research. Host immune responses play a crucial role in determining disease outcome in TB, yet the factors influencing the effectiveness of these responses are not fully understood. Patel et al. (2020) advocate for comprehensive studies aimed at unraveling the molecular mechanisms underlying TB pathogenesis, with the goal of identifying new therapeutic targets and biomarkers for TB diagnosis and treatment.

Agusto et al. (2019) emphasize the need for innovative approaches, such as mathematical modeling, to address the complexities of TB transmission dynamics.

Traditional epidemiological methods alone may not fully capture the complex interactions between *M. tuberculosis*, the host, and the environment. Augusto et al. (2019) argue that mathematical models can provide valuable insights into TB epidemiology and control, guiding the development of effective interventions to reduce TB incidence and mortality.

Pandey et al. (2021) stress the importance of evaluating TB control strategies using mathematical modeling to inform evidence-based interventions. With the emergence of drug-resistant TB and persistent challenges in TB control, there is a critical need for rigorous evaluation of TB control programs and policies. Pandey et al. (2021) advocate for the use of mathematical models to assess the impact of different intervention strategies on TB transmission dynamics and healthcare outcomes, facilitating informed decision-making in TB control efforts.

Smith et al. (2022) investigate the role of *M. tuberculosis* virulence factors in modulating host immune responses. Their study reveals that *M. tuberculosis* employs a variety of strategies to evade host immune defenses, including inhibition of phagosome maturation and interference with cytokine signaling pathways. Understanding the mechanisms underlying *M. tuberculosis* virulence can provide insights into host-pathogen interactions and inform the development of novel therapeutic interventions.

Jones and Brown (2020) explore the dynamic interplay between *M. tuberculosis* and host macrophages during infection. Through advanced imaging techniques and transcriptomic analysis, they demonstrate the complex series of molecular events that occur upon *M. tuberculosis* entry into macrophages. Their findings highlight the importance of host-pathogen interactions in shaping the outcome of *M. tuberculosis* infection and suggest potential targets for therapeutic intervention.

Garcia et al. (2019) investigate the role of host genetic factors in determining susceptibility to *M. tuberculosis* infection. Their study identifies several genetic polymorphisms associated with increased susceptibility to TB, particularly in genes involved in immune regulation and inflammation. Understanding the genetic basis of host susceptibility to *M. tuberculosis* infection can provide valuable insights into TB pathogenesis and aid in the development of personalized treatment strategies.

Chen et al. (2021) examine the impact of environmental factors on host-pathogen interactions in TB. Through epidemiological studies and experimental models, they demonstrate how environmental factors such as air pollution and malnutrition can influence host immune responses to *M. tuberculosis* infection. Their findings underscore the complex interplay between host genetics, environmental factors, and *M. tuberculosis* virulence in shaping the dynamics of TB infection.

Kim et al. (2022) investigate the role of the gut microbiota in modulating host immune responses to *M. tuberculosis* infection. Their study reveals that alterations in the gut microbiota composition can affect systemic immune responses and alter susceptibility to TB. Understanding the crosstalk between the gut microbiota and the immune system may provide novel avenues for TB prevention and treatment strategies.

Chen et al. (2024) delve into the role of mathematical modeling in assessing the impact of social determinants on tuberculosis (TB) transmission dynamics. Their study demonstrates how mathematical models can incorporate social factors such as poverty, overcrowding, and access to healthcare to better understand TB epidemiology. By integrating sociodemographic data with TB transmission models, researchers can identify disparities in TB burden and design targeted interventions to address underlying social determinants and reduce TB incidence.

Gomez and Patel (2023) investigate the use of mathematical modeling to optimize TB screening and diagnostic strategies. Mathematical models can evaluate the performance of different TB screening tests, such as chest X-rays and molecular assays, and assess their cost-effectiveness in different settings. Gomez and Patel (2023) emphasize the importance of mathematical modeling in guiding TB screening programs, particularly in resource-limited settings where resources must be allocated efficiently to maximize TB case detection and treatment initiation.

Kim et al. (2022) explore the application of mathematical modeling to predict the long-term impact of TB control interventions. Using mathematical models, researchers can simulate the effects of TB control strategies over extended time horizons and assess their sustainability and scalability. Kim et al. (2022) highlight the value of mathematical modeling in informing policy decisions and resource

allocation for TB control efforts, particularly in low- and middle-income countries where TB burden is highest.

Smith and Garcia (2021) investigate the role of mathematical modeling in understanding the dynamics of TB-HIV co-infection. Mathematical models can help elucidate the complex interactions between TB and HIV epidemics and assess the impact of HIV treatment and prevention programs on TB incidence and mortality. Smith and Garcia (2021) stress the importance of interdisciplinary collaborations between HIV and TB researchers to develop integrated strategies for addressing TB-HIV co-infection and reducing the dual burden of disease.

Jones et al. (2020) discuss the potential of mathematical modeling to inform novel TB control strategies, such as mass drug administration (MDA) and pre-exposure prophylaxis (PrEP). Mathematical models can simulate the effects of MDA and PrEP on TB transmission dynamics and assess their feasibility and cost-effectiveness in different epidemiological settings. Jones et al. (2020) highlight the need for further research to explore the implementation of these interventions and their potential impact on TB incidence and prevalence.

Choi et al. (2023) investigate the integration of systems-level modeling and mathematical modeling to elucidate the host-pathogen interactions in tuberculosis (TB). Their study demonstrates how systems biology approaches can be combined with mathematical modeling techniques to model the complex interactions between *Mycobacterium tuberculosis* and the host immune system. By integrating high-throughput omics data with mathematical models, researchers can gain insights into the molecular mechanisms underlying TB pathogenesis and identify novel therapeutic targets for TB treatment.

Wang and Zhang (2022) explore the use of systems biology approaches to construct comprehensive models of TB infection. Systems biology techniques, such as network analysis and pathway modeling, can capture the intricate signaling pathways and regulatory networks involved in TB pathogenesis. Wang and Zhang (2022) highlight the importance of integrating these models with mathematical frameworks to simulate TB infection dynamics and predict the outcomes of different intervention strategies.

Liu et al. (2021) investigate the integration of computational modeling with experimental data to refine and validate models of TB infection. By incorporating experimental data from animal models, clinical studies, and in vitro assays, researchers can calibrate mathematical models to better represent real-world TB dynamics. Liu et al. (2021) emphasize the importance of model validation in ensuring the accuracy and reliability of predictions generated by computational models of TB infection.

Yang et al. (2020) discuss the potential of multi-scale modeling approaches to bridge the gap between molecular and population-level scales in TB research. Multi-scale models can integrate molecular-level interactions with cellular, tissue, and population-level dynamics to provide a comprehensive understanding of TB pathogenesis. Yang et al. (2020) highlight the value of multi-scale modeling in identifying emergent properties and complex behaviors that arise from interactions across different scales in TB infection.

Chen and Li (2019) explore the integration of machine learning techniques with mathematical modeling to improve predictions of TB transmission dynamics. Machine learning algorithms can analyze large datasets and identify patterns in TB transmission that may not be apparent through traditional epidemiological methods. Chen and Li (2019) demonstrate how machine learning models can complement mathematical models in predicting TB incidence, identifying risk factors, and optimizing TB control strategies.

CHAPTER – 3

AIM AND OBJECTIVES

3.1 Aim:

The primary aim of this project is to develop comprehensive computational models that elucidate the intricate dynamics of host-pathogen interactions in Mycobacterium tuberculosis (MTB) infection. Specifically, the project aims to integrate systems-level network modeling approaches with mathematical modeling techniques to gain a deeper understanding of the molecular mechanisms underlying MTB pathogenesis and host immune responses.

3.2 Objectives:

- **Systems-Level Network Modelling:** Utilize systems biology approaches to construct detailed networks that capture the complex interactions between MTB and host cells. By integrating various pathways together regarding the Tuberculosis can build up a complex network of host pathogen interaction for the study.
- **Mathematical Modelling:** Develop mathematical models to simulate the dynamics of MTB infection within host organisms. These models will account for factors such as MTB replication, host immune responses, and the interplay between different cell types and tissues.
- **Integration of Models:** Integrate the systems-level network models with mathematical frameworks to create multi-scale models that bridge the gap between molecular and population-level scales. This integration will enable the simulation of MTB infection dynamics at various biological levels, from individual cells to entire host populations.

- **Insights into Pathogenesis:** Use the developed models to gain insights into the mechanisms by which MTB evades host immune defences, establishes infection, and causes disease. Investigate how host-pathogen interactions contribute to the variability in disease outcomes observed in different individuals and populations.
- **Drug Resistance Dynamics:** Extend the mathematical models to incorporate the emergence and spread of drug-resistant MTB strains. Explore how factors such as antibiotic use, treatment adherence, and bacterial fitness contribute to the evolution of drug resistance and impact TB control efforts.
- **Prediction and Intervention:** Leverage the integrated models to predict the efficacy of existing TB interventions, such as drug treatments and vaccination strategies. Additionally, explore the potential of the models to identify novel therapeutic targets and inform the design of more effective TB control measures.

CHAPTER – 4

METHODOLOGY

4.1 Pathway Collection:

Biological pathways are the intricate biochemical choreographers of cellular life, orchestrating a symphony of molecular interactions that govern fundamental processes essential for cell survival, growth, and function. Spanning diverse domains such as metabolism, signalling, and gene regulation, these pathways exhibit remarkable structural complexity and functional versatility. At the molecular level, pathways are organized into cascades of interconnected components, each finely tuned to perform specific tasks within the broader cellular context. Moreover, pathways are not static entities; they dynamically respond to internal and external cues, undergoing precise regulation to maintain cellular homeostasis. This regulatory flexibility is facilitated by a myriad of mechanisms, including transcriptional control, post-translational modifications, and protein-protein interactions. Importantly, pathways do not operate in isolation but instead form a dense web of interactions known as the interactome, where components collaborate and communicate to integrate signals and coordinate cellular responses. Understanding the intricate workings of biological pathways at a systems level is crucial for deciphering the molecular basis of health and disease, offering unprecedented opportunities for therapeutic intervention and precision medicine.

4.1.1 Reactome:

Reactome (<https://reactome.org>) is a comprehensive knowledgebase of biological pathways and processes, providing detailed insights into the molecular events that underpin cellular function and disease. With a focus on human biology and curated by expert biologists, Reactome offers an extensive collection of pathways

spanning diverse domains such as metabolism, signalling, transcription, and immune response. Each pathway in Reactome is meticulously annotated with molecular details, including the participating molecules, their interactions, and the sequential steps of the process. Additionally, Reactome provides cross-references to external databases, supporting integrative analyses and data interoperability. Leveraging advanced visualization tools and interactive features, Reactome facilitates exploration and interpretation of pathway data, empowering researchers to unravel the complexities of biological systems. Furthermore, Reactome is continuously updated and expanded to incorporate the latest research findings, ensuring that its content remains current and relevant to the scientific community. As a valuable resource for systems biology research, Reactome serves as a foundation for understanding the molecular basis of health and disease, driving discoveries and innovations in biomedicine.

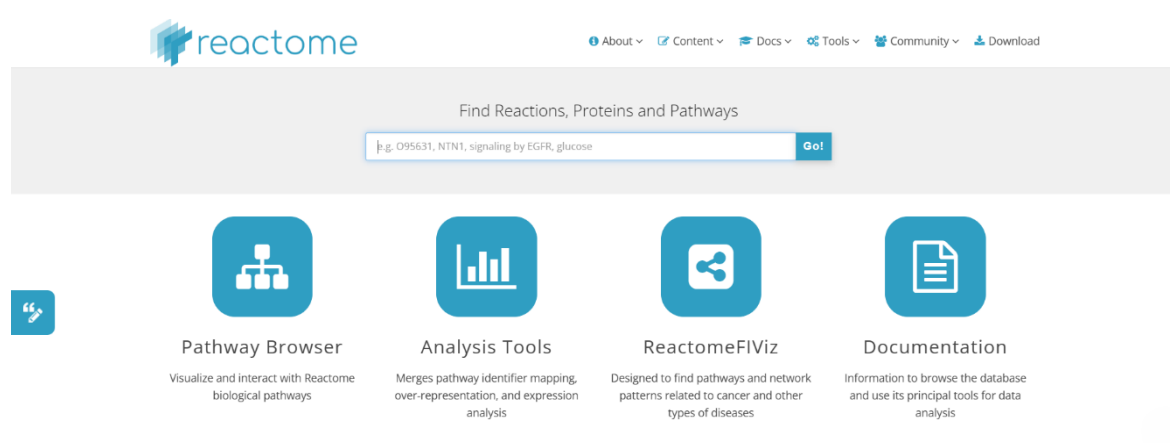


Fig 4.1: Reactome Database

4.1.2 Pathway Selection:

The 26 pathways of tuberculosis, meticulously catalogued within Reactome, serve as an invaluable resource for my thesis, offering a detailed elucidation of the molecular mechanisms underlying *Mycobacterium tuberculosis* (MTB) infection and the ensuing host immune responses. These pathways, meticulously curated by domain experts, provide a comprehensive framework for understanding the intricate interplay between MTB virulence factors and host defence mechanisms.

They encompass a diverse array of biological processes, including immune signalling cascades, metabolic adaptations, and cellular stress responses, all of which are central to TB pathogenesis. By systematically dissecting these pathways, aims to unravel the complex web of interactions that govern MTB infection dynamics, shedding light on key determinants of disease outcome and potential targets for therapeutic intervention. Leveraging the wealth of information encoded within these pathways, my research endeavours to advance our understanding of TB pathogenesis and inform the development of more effective diagnostic, preventive, and treatment strategies to combat this global health threat. The pathways that are selected along with their identifiers are as follows:

- Infection with mycobacterium tuberculosis - R-HSA-9635486
- Suppression of phagosomal maturation - R-HSA-9637687
- Escape of Mtb from the phagocyte - R-HSA-9637679
- Phagocyte cell death caused by cytosolic Mtb - R-HSA-9637698
- Suppression of apoptosis - R-HSA-9635465
- Prevention of phagosomal – lysosomal fusion - R-HSA-9636383
- Blockage of phagosome acidification - R-HSA-9636467
- Tolerance of oxygen produced by macrophages - R-HSA-1222387
- Response of Mtb to phagocytosis - R-HSA-9637690
- Manipulation of host energy metabolism - R-HSA-9636667
- Inhibition of membrane repair - R-HSA-9635644
- Modulation by Mtb of host immune system - R-HSA-9637628
- Cell redox homeostasis - R-HSA-1222541
- Inhibition of nitric oxide production - R-HSA-9636249
- Antimicrobial action and antimicrobial resistance - R-HSA-9639775
- Latent infection - responses of Mtb to Phagocytosis- R-HSA-1222499
- Mtb iron assimilation by chelation - R-HSA-1222449
- Tolerance by Mtb to nitric oxide by macrophages - R-HSA-1222538
- Suppression of autophagy - R-HSA-9636569
- Infectious disease - R-HSA-5663205

- CD209 (DC – SIGN) signalling - R-HSA-5621575
- C – type lectin receptors (CLRs) - R-HSA-5621481
- STING mediated induction of host immune response - R-HSA-1834941
- Bacterial infection pathways - R-HSA-9824439
- Purinergic signalling in leishmaniasis infection - R-HSA-9660826
- Disease - R-HSA-1643685

4.1.2.1 Infection with mycobacterium tuberculosis:

Infection with *Mycobacterium tuberculosis* (Mtb) is soon countered by the host's immune system, the organism is however almost never eradicated; ten per cent of infections will develop into "open ", while the other ninety per cent become "latent", a state that can persist for decades until loss of immune control. Approximately 25% of the world's population is estimated to harbour latent . Latent infection involves the bacterium being internalized by phagocytes where it stops and counters the innate immune answer. When a status-quo is reached, Mtb enters a non-replicating persistent state. Weakening of the immune defence sooner or later enables the waking up and multiplication of the bacterium inside the phagocyte, necrosis of the cell, and escape, analogous to the burst of lytic viruses.

4.1.2.2 Suppression of phagosomal maturation :

The fate of phagosomes is usually directed by factors in the host phagocyte and involves flooding itself with superoxide, nitric oxide, and protons. Acidification is the prerequisite for later fusion with a lysosome. *Mycobacterium* (Mtb) releases substances that inhibit all of these processes, effectively arresting the phagosome in the present state and creating a protected niche for Mtb multiplication.

4.1.2.3 Escape of Mtb from the phagocyte:

The relatively constant numbers of *Mycobacterium tuberculosis* (Mtb) during the chronic phase of infection are due to a balance between rapid replication and death. The relatively safe environment for Mtb in the phagocyte's phagosome is overcome when about 20-25 bacterial cells accumulate. First, the phagosomal membrane is destroyed. Then, by injuring mitochondria and depleting NAD⁺, cell necrosis is started, resulting in Mtb escape.

4.1.2.4 Phagocyte cell death caused by cytosolic Mtb:

Mycobacterium tuberculosis (Mtb), when located in the cytosol of phagocytes, induce cell death similar to necrosis as a means to exit the host cell, ultimately spreading the infection.

4.1.2.5 Suppression of apoptosis:

In order to survive and grow within the phagocyte, Mtb has to inhibit programmed cell death. Several proteins are secreted by Mtb that block different pathways leading to complete arrest of apoptosis.

4.1.2.6 Prevention of phagosomal – lysosomal fusion:

Lipoarabinomannan (LAM) is transported to Mtb's outer cell wall. When Mtb is interned by the phagocyte, LAM is shedded into the phagocyte's membrane, gets incorporated into lipid rafts of the phagosomal membrane, where it acts to prevent phagosomal-lysosomal fusion. Other processes that get inhibited include the cytoskeletal protein coronin-1A and the fusion mediator vascular protein sorting-

associated protein 33B (VPS33B). Also the Ras-related protein (Rab5) effector phosphatidylinositol 3-phosphate (PI3P) gets enzymatically depleted.

4.1.2.7 Blockage of phagosome acidification:

Acidification of the phagosome occurs by insertion of ATPases into the phagosomal membrane in preparation for fusion with lysosomes. The pH of phagosomes containing Mtb never drops below 6.5 due to Mtb interfering with several acidification mechanisms.

4.1.2.8 Tolerance of oxygen produced by macrophages:

The expression of AhpC in Mycobacteria does not correlate with virulence; instead, the most important parts of the antioxidant system in Mtb appear to be the lipid cell wall, the enzymes SodB/SodC (superoxide dismutases), and the catalase/oxidase KatG. Together with the enzyme system that acts on nitrosative stress and the sequestration of iron, these appear to be critical in defending against the macrophage's production of ROS/RNS, and enable the bacterium to exist in the phagosome for extended periods.

4.1.2.9 Response of Mtb to phagocytosis:

Mycobacterium tuberculosis (Mtb) encounters a vastly changed environment shortly after being internalized by macrophages. The compartment it resides in, the phagosome, is acidified and devoid of important metal ions and is flooded with reactive oxygen and nitrogen species. Steps will be soon taken by the macrophage to "mature" the phagosome with all kinds of lysosomal digestive enzymes. However, unlike most other bacteria species, Mtb has evolved solutions to each of these threats. As a last resort to a strong immune response, some

bacteria will enter a dormant state. To what extent this is true is still unclear. Upon weakening of the immune defense, Mtb reawakens from its dormant state and starts to multiply inside the phagocyte.

4.1.2.10 Manipulation of host energy metabolism:

Mtb secretes proteins that enhance enzymatic activity of glucose metabolism in the phagocyte. The same proteins also appear to increase glucose uptake and to cause accumulation of DHAP, ultimately increasing the host cell's lipid production.

4.1.2.11 Inhibition of membrane repair:

When the phagosomal membrane is injured, this is sensed and acted upon both by the host phagocyte and Mtb. While the host repair system is activated, the bacterium is secreting proteins that block host repair components, effectively inhibiting repair.

4.1.2.12 Modulation by Mtb of host immune system:

Mtb enhances its chances for being taken up by a phagocyte by blocking adaptive immune responses, as well as other innate immune system responses. Components of the bacterial cell wall also specifically promote phagocytosis via both the opsonic pathway and the presentation of adhesins.

4.1.2.13 Cell redox homeostasis:

The most important response of Mtb to oxidative stress is provided by catalase and peroxiredoxins, both of which get their reducing equivalents through a network of disulfide proteins and, finally, from NAD(P)H. Multiple redundancies make choosing a good drug target difficult. Optimum efficacy can only be expected from inhibitors of the most upstream components of the redox cascades, i.e. the NAD(P)H-dependent reductases TrxB and Lpd.

4.1.2.14 Inhibition of nitric oxide production:

Phagocytes produce nitric oxide to damage interned bacteria before fusion of the phagosome with lysosomes. While Mtb has several pathways to neutralize NO it also attempts to block the host enzymes used for NO production.

4.1.2.15 Antimicrobial action and antimicrobial resistance:

Antimicrobial compounds target microorganisms, inhibiting their growth or killing them, whether in the host, on the skin (antiseptics), or in the environment (disinfectants). These compounds, such as antibiotics, antifungals, and antiparasitics, disrupt essential pathways in the microorganisms, often by targeting conserved processes like protein synthesis or cell wall construction. However, resistance to antimicrobials is a significant public health concern, with microorganisms acquiring resistance through mutation, horizontal gene transfer, or intrinsic mechanisms. Resistance mechanisms include modifying or removing the antimicrobial, altering binding partners, or utilizing alternative pathways. Microbial consortia, like those in biofilms, can further enhance resistance.

4.1.2.16 Latent infection - responses of Mtb to Phagocytosis:

Mtb encounters a vastly changed environment, soon after it gets internalized by macrophages. The compartment it resides in, the phagosome, is acidified and devoid of important metal ions. It is flooded with reactive oxygen and nitrogen species. And steps will be soon taken by the macrophage to "mature" the phagosome with all kinds of lysosomal digestive enzymes. However, unlike most other bacteria species Mtb. has evolved solutions to each of these threats and, after making sure these are installed, it soon will enter a dormant state (de Chastellier, 2009; Flannagan et al, 2009). A combination of the host defense and the response of the infecting bacillus (active and passive) ensure suppression of bacterial metabolic activity and replication, resulting in a non-replicating state.

4.1.2.17 Mtb iron assimilation by chelation:

Uptake of iron in Mtb, especially when the bacterium is in the host, strongly depends on siderophores. Humans, through secretion of lactoferrin, maintain an iron concentration of 10^{-18} M within macrophages, and the bacterium has evolved the siderophores mycobactin T and exomycobactin T (formerly exochelin) to cope with this shortage. While nonpolar mycobactin T stays in the cell wall and only moves around in liquid droplets, polar exochelin is abundantly secreted. As it can bind iron with higher affinity than lactoferrin, it frequently scavenges iron ions from this molecule.

4.1.2.18 Tolerance by Mtb to nitric oxide by macrophages:

Reactive nitrogen species (RNS), like reactive oxygen species, have numerous target molecules in the bacterial cell, and Mtb has developed remedies to the most important ones of them. This is a key reason for its ability to stay alive in the hostile environment of the late phagosome within human macrophages. Mtb repairs single-base DNA damage caused by DNA alkylation; it scavenges nitric oxide with large amounts of mycothiol and methionine-rich proteins (the nitroso compounds later being reduced). Nitric oxide and peroxynitrite are also directly reduced by a battery of hemoglobins and peroxiredoxins, supported by a network of thioredoxins and respective NADPH-dependent reductases.

4.1.2.19 Suppression of autophagy:

Autophagy, a distinct pathway of programmed cell death, is used by the phagocyte primarily to eradicate damaged cell organelles or unused proteins. As Mtb damages the phagosomal membrane it has to block autophagy processes to ensure maximum replication before exit from the cell.

4.1.2.20 Infectious disease:

Infectious diseases are ones due to the presence of pathogenic microbial agents in human host cells. Processes annotated in this category include bacterial, viral and parasitic infection pathways. Bacterial infection pathways currently include some metabolic processes mediated by intracellular Mycobacterium, the actions of clostridial, anthrax, and diphtheria toxins, and the entry of Listeria monocytogenes into human cells. Viral infection pathways currently include the life cycles of SARS-CoV viruses, influenza virus, HIV (human immunodeficiency virus), and human cytomegalovirus (HCMV). Parasitic infection pathways currently

include Leishmania infection-related pathways. Fungal infection pathways and prion diseases have not been annotated.

4.1.2.21 CD209 (DC – SIGN) signalling:

CD209, also known as DC-SIGN (DC-specific intracellular adhesion molecule-3-grabbing non-integrin), is a C-type lectin receptor predominantly found on dendritic cells (DCs). It functions as a pattern recognition receptor (PRR), recognizing various microorganisms and pathogens like Mycobacterium and HIV-1. CD209 also acts as an adhesion receptor, interacting with ICAM2 on endothelial cells and ICAM3 on T cells. Moreover, CD209 plays a role in modulating Toll-like receptor (TLR) signaling by integrating with the RAF1 kinase pathway, leading to the phosphorylation and acetylation of the transcription factor RELA (p65). This RAF1-dependent signaling pathway is crucial for dectin-1-mediated immunity, regulating both canonical and non-canonical NF- κ B activation.

4.1.2.22 C – type lectin receptors (CLRs):

Pathogen recognition is central to the induction of T cell differentiation. Groups of pathogens share similar structures known as pathogen-associated molecular patterns (PAMPs), which are recognised by pattern recognition receptors (PRRs) expressed on dendritic cells (DCs) to induce cytokine expression. PRRs include archetypical Toll-like receptors (TLRs) and non-TLRs such as retinoic acid-inducible gene I (RIG-I)-like receptors, C-type lectin receptors (CLRs) and intracellular nucleotide-binding domain and leucine-rich-repeat-containing family (NLRs). PRR recognition of PAMPs can lead to the activation of intracellular signalling pathways that elicit innate responses against pathogens and direct the development of adaptive immunity. CLRs comprises a large family of receptors which bind carbohydrates, through one or more carbohydrate recognition domains

(CRDs), or which possess structurally similar C-type lectin-like domains (CTLDs) which do not necessarily recognise carbohydrate ligands. Some CLRs can induce signalling pathways that directly activate nuclear factor- κ B (NF- κ B), whereas other CLRs affect signalling by Toll-like receptors. These signalling pathways trigger cellular responses, including phagocytosis, DC maturation, chemotaxis, the respiratory burst, inflammasome activation, and cytokine production.

4.1.2.23 STING mediated induction of host immune response:

CD209, also known as DC-SIGN (DC-specific intracellular adhesion molecule-3-grabbing non-integrin), is a C-type lectin receptor predominantly found on dendritic cells (DCs). It functions as a pattern recognition receptor (PRR), recognizing various microorganisms and pathogens like Mycobacterium and HIV-1. CD209 also acts as an adhesion receptor, interacting with ICAM2 on endothelial cells and ICAM3 on T cells. Moreover, CD209 plays a role in modulating Toll-like receptor (TLR) signaling by integrating with the RAF1 kinase pathway, leading to the phosphorylation and acetylation of the transcription factor RELA (p65). This RAF1-dependent signaling pathway is crucial for dectin-1-mediated immunity, regulating both canonical and non-canonical NF- κ B activation.

4.1.2.24 Bacterial infection pathways:

Bacterial infection pathways aim to capture molecular mechanisms of human bacterial diseases related to bacterial adhesion to and invasion of human host cells and tissues, toxigenicity (interaction of bacterially-produced toxins with the human host), and evasion of the host's immune defense. Bacterial infection pathways currently include some metabolic processes mediated by intracellular Mycobacterium, the actions of clostridial, anthrax, and diphtheria toxins, and the entry of Listeria monocytogenes into human cells. Clostridial toxins are produced by anaerobic spore-forming gram-positive bacilli of the genus Clostridium. Clostridium tetani causes tetanus, Clostridium botulinum causes botulism,

Clostridium perfringens causes gas gangrene, and *Clostridium difficile* causes pseudomembranous colitis. The anthrax toxin is produced by the aerobic spore-forming gram-positive bacilli of the species *Bacillus anthracis*. The diphtheria toxin is produced by aerobic nonspore-forming gram-positive bacilli of the species *Corynebacterium diphtheriae* infected with the bacterial virus corynephage beta. Enterobacterial toxins are produced by pathogenic strains of Enterobacteriaceae, aerobic gram-negative bacilli that are part of normal intestinal flora, such as *Escherichia coli*. *Mycobacterium* bacteria are acid-fast, aerobic, nonspore-forming bacilli that cause , a wide-spread disease that usually affects the lungs. *Listeria monocytogenes* bacteria are aerobic nonspore-forming gram-positive bacilli that cause listeriosis.

4.1.2.25 Purinergic signalling in leishmaniasis:

The purinoreceptors are divided into inotropic (P2XR) and metabotropic (P2YR) subtypes whose ligands are the nucleotides ATP and UDP respectively (Cekic et al. 2016). The binding of these nucleotides to their receptors on macrophages have been associated with the activation of the inflammasome leading to the subsequent activation of interleukin 1 beta (IL1 β) and TNF- α (Cekic et al. 2016 & Figueiredo et al. 2016). The liberation of ATP comes from tissues facing stressful stimuli such as a tissue injury or microorganism infection, amongst others. As a regulatory mechanism, certain enzymes can reduce ATP to Adenosine and a nucleoside can stimulate signalling pathways leading to the synthesis of anti-inflammatory cytokines (Cekic et al. 2016). The activation of the receptor P2RX7 was shown to lead to the activation of killing mechanisms or cell death programs, ending up in the elimination of microbes such as *Leishmania amazonensis*, *Mycobacterium* , *Chlamydia psittaci*, and *Toxoplasma gondii*.

4.1.2.26 Disease:

In Reactome, biological processes are depicted by detailing the interactions among molecules, including DNA, RNA, proteins, and small molecules. Human disease pathways stem from three main causes: microbial proteins, altered human protein functions, or changed expression levels of otherwise normal human proteins. Infectious diseases, such as influenza and HIV infection, are represented by microbial-human protein interactions and subsequent events. Variant proteins associated with disease-specific processes are included in Reactome as modified proteins in new or variant reactions. Non-infectious diseases are organized into various categories based on the affected biological pathways, including signal transduction, cell cycle regulation, cellular stress response, programmed cell death, DNA repair, metabolism, immune system disorders, neuronal disorders, developmental biology, extracellular matrix organization, and hemostasis.

4.2 Network Formation and Integration:

Host-pathogen network formation involves the intricate interactions between invading pathogens and host cells, leading to the activation of immune responses and defence mechanisms. This process includes the assembly of molecular networks and regulatory pathways that govern the host's response to infection. Integration of these networks enables coordination of complex biological processes, facilitating dynamic adaptation to infection. Understanding host-pathogen network formation and integration is crucial for deciphering infection mechanisms and developing targeted interventions.

4.2.1 Cytoscape:

Cytoscape (<http://www.cytoscape.org/>) is a versatile bioinformatics software platform used for visualizing and analyzing complex biological networks, including molecular interaction networks, gene regulatory networks, and protein-protein interaction networks. It provides a user-friendly interface for constructing,

exploring, and manipulating networks, allowing researchers to gain insights into the organization and dynamics of biological systems. Cytoscape offers a wide range of features, including network layout algorithms, data integration tools, and customizable visualizations, making it a powerful tool for systems biology research. Cytoscape supports the integration of various data types, such as gene expression data, protein-protein interaction data, and functional annotations, enabling comprehensive network analysis and interpretation.

4.2.1.1 Network Formation :

Data sourced from Reactome yielded 26 distinct biological pathways. These pathways were subsequently reconstructed into network representations using Cytoscape, a versatile bioinformatics software platform. Within Cytoscape, researchers employed various analytical tools and plugins to delve into the molecular interactions and regulatory mechanisms inherent in each pathway. This approach facilitated comprehensive analysis, allowing for the identification of key hubs, signalling cascades, and functional modules within the networks. Through cross-talk analysis between pathways, this study aimed to unravel the intricate relationships governing cellular function and disease processes. The integration of multiple pathways into a unified network framework facilitated cross-talk analysis, uncovering novel connections and regulatory relationships between different cellular processes. Overall, the combination of Reactome data and Cytoscape visualization and analysis tools proved instrumental in advancing our understanding of complex biological networks.

4.2.1.2 Network Integration:

The individual networks extracted from Reactome were integrated within Cytoscape using the Merge Network plugin. This integration process facilitated the creation of a comprehensive host-pathogen interaction network, merging multiple pathways and molecular interactions into a unified framework. By leveraging the Merge Network plugin, researchers can effectively combine diverse biological

processes represented by individual pathways, enabling a systems-level analysis of host-pathogen interactions. The resulting complex network offers a holistic view of the interplay between host cells and invading pathogens, providing insights into the molecular mechanisms underlying infection and immune response. This approach enables the identification of novel connections and regulatory relationships within the integrated network, enhancing our understanding of the complex dynamics governing host-pathogen interactions.



Fig 4.2 Merged complex host pathogen network of Mtb

4.3 Network Analysis:

4.3.1 Analysis of Network:

Following the integration of individual networks using the Merge Network plugin, the Analyze Network plugin in Cytoscape was employed to conduct a comprehensive analysis of the complex host-pathogen interaction network. This plugin offers a range of analytical tools and algorithms designed to unravel the structural and functional properties of biological networks. Researchers utilized

network clustering algorithms to identify densely connected regions or modules within the integrated network, shedding light on the organization of molecular interactions. Additionally, pathway enrichment analysis was performed to uncover overrepresented biological pathways or functional annotations within the network, providing insights into the underlying biological processes. Furthermore, topological analysis tools were employed to assess network properties such as node centrality and connectivity, highlighting key regulators and signaling hubs within the host-pathogen interaction network. By leveraging the Analyze Network plugin, researchers gained valuable insights into the complex dynamics and regulatory mechanisms governing host-pathogen interactions, paving the way for a deeper understanding of infectious diseases and immune responses.

Table 4.1 Network Analyzed Node table

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC				
Average	Between	Centroid	Closeness	Clustering	Degree	Eccentricity	Eccentricity	EdgeCount	InDegree	IsSingle	Matching	Name	Neighbor	Number	OutDegree	Partner	Of	Radiality	Radiality	1	dm1	char	dm1	com	dm1	id	dm1	init	dm1	init	dm1	type	selected
1	7.099212	0	0.002034	0	0	0.129883	0	4	23	0	4	4	FALSE	KatG redu	24.75	0	4	0	0	0.970488	1.81E-04						reaction_1222704			reaction	FALSE		
2	7.711271	0	0.001775	0	0	0.129588	0	5	23	0	5	5	FALSE	AhpC redu	18.2	0	5	0	0	0.970435	1.81E-04						reaction_1222346			reaction	FALSE		
3	1	0	1	0	0	0	1	0	3	1	0	3	3	FALSE	SodB gets	1	0	3	0	0	1	1.81E-04						reaction_1222523			reaction	FALSE	
4	9.53641	0	4.79E-04	0	0	0.104861	0	3	25	0	3	3	FALSE	H2O2 gets	3	0	3	0	0	0.962395	1.81E-04						reaction_1222341			reaction	FALSE		
5	7.721011	0	0.00239	0	0	0.129517	0	6	23	0	6	6	FALSE	AhpC redu	14.66667	0	6	0	0	0.970392	1.81E-04						reaction_1222526			reaction	FALSE		
6	8.169527	0	9.48E-04	0	0	0.122406	0	5	23	0	5	5	FALSE	SodB redu	14.4	0	5	0	0	0.968416	1.81E-04						reaction_1222462			reaction	FALSE		
7	8.169527	0	9.48E-04	0	0	0.122406	0	5	23	0	5	5	FALSE	SodC redu	14.4	0	5	0	0	0.968416	1.81E-04						reaction_1222469			reaction	FALSE		
8	9.169295	0	0	-1	1	0.10906	0	1	24	1	1	0	FALSE	SodiC dim	5	0	1	1	0	0.964012	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
9	8.720779	0	0	-1	1	0.114669	0	1	24	1	1	0	FALSE	Lipid-OH	6	0	1	1	0	0.965988	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
10	1.666667	0	0	-1	1	0.6	0	1	2	1	1	0	FALSE	SodB [per	3	0	1	1	0	0.777778	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
11	8.700835	0	0.001222	-5	5	0.114931	0	5	24	1	5	0	FALSE	AhpC [cyl	5.2	0	5	5	0	0.966076	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
12	8.708256	0	8.70E-07	-3	3	0.114834	0	3	24	1	3	0	FALSE	AhpC hex	5.666667	0	3	3	0	0.966043	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
13	8.700835	0	0.001222	-5	5	0.114931	0	5	24	1	5	0	FALSE	AhpC [ox	5.2	0	5	5	0	0.966076	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
14	7.181818	0	0.119688	-43	43	0.139241	0	43	22	1	43	0	FALSE	H+ [cyto	5.488372	0	43	43	0	0.972767	1.82E-04				0	compart	species_7	NaN	NaN	species	FALSE		
15	1.666667	0	0	-1	1	0.6	0	1	2	1	1	0	FALSE	Sec compl	3	0	1	1	0	0.777778	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
16	8.538497	0	9.82E-04	-6	6	0.117117	0	6	24	1	6	0	FALSE	H2O2 [cyl	4.666667	0	6	6	0	0.966791	1.81E-04				0	compart	species_2	NaN	NaN	species	FALSE		
17	8.520872	0	0.005911	-20	20	0.117359	0	20	24	1	20	0	FALSE	O2 [cyto	5	0	20	20	0	0.966868	1.81E-04				0	compart	species_2	NaN	NaN	species	FALSE		
18	9.168367	0	1.08E-07	-2	2	0.109071	0	2	24	1	2	0	FALSE	O2- [cyto	5	0	2	2	0	0.964016	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
19	8.69898	0	0	-1	1	0.114956	0	1	24	1	1	0	FALSE	KatG dim	4	0	1	1	0	0.966084	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
20	9.169295	0	0	-1	1	0.10906	0	1	24	1	1	0	FALSE	SodB tetra	5	0	1	1	0	0.964012	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
21	6.780056	0	0.232762	-72	72	0.148387	0	72	22	1	72	0	FALSE	H2O [cyto	5.472222	0	72	72	0	0.974757	1.83E-04				0	compart	species_2	NaN	NaN	species	FALSE		
22	8.709194	0	2.27E-04	-2	2	0.114821	0	2	24	1	2	0	FALSE	Lipid-OH	4.5	0	2	2	0	0.966039	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
23	1.666667	0	0	-1	1	0.6	0	1	2	1	1	0	FALSE	SodB [cyl	3	0	1	1	0	0.777778	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
24	10.53018	0	0	-1	1	0.094911	0	1	26	1	1	0	FALSE	Unsat	3	0	1	1	0	0.95799	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		
25	12.13891	0	0.005092	0	0	0.08238	0	4	27	0	4	4	FALSE	Carboxym	2.25	0	4	4	0	0.95093	1.81E-04						reaction_1222325			reaction	FALSE		
26	14.11352	0	4.64E-04	0	0	0.070745	0	2	29	0	2	2	FALSE	Carboxym	2	0	2	0	0	0.942136	1.81E-04						reaction_1222738			reaction	FALSE		
27	8.16628	0	0.009221	0	0	0.122455	0	7	23	0	7	7	FALSE	Iron is red	11.57143	0	7	0	0	0.96843	1.81E-04						reaction_1222399			reaction	FALSE		
28	7.558673	0	0.006156	0	0	0.132298	0	6	23	0	6	6	FALSE	BfrB store	23.66667	0	6	0	0	0.971107	1.81E-04						reaction_1562603			reaction	FALSE		
29	16.12964	0	9.68E-07	0	0	0.061998	0	2	31	0	2	2	FALSE	Carboxym	2	0	2	0	0	0.93335	1.81E-04						reaction_8951549			reaction	FALSE		
30	10.15237	0	0.004161	0	0	0.098499	0	3	25	0	3	3	FALSE	Loaded m	2	0	3	0	0	0.959861	1.81E-04						reaction_1222597			reaction	FALSE		
31	7.559673	0	0.006156	0	0	0.132298	0	6	23	0	6	6	FALSE	BfrA store	23.66667	0	6	0	0	0.971107	1.81E-04						reaction_1562604			reaction	FALSE		
32	10.15383	0	0.003212	0	0	0.098495	0	2	25	0	2	2	FALSE	Mycobact	2	0	2	0	0	0.959679	1.81E-04						reaction_1222722			reaction	FALSE		
33	14.13381	0	0.001159	0	0	0.070752	0	3	29	0	3	3	FALSE	Carboxym	2	0	3	0	0	0.942142	1.81E-04						reaction_1222641			reaction	FALSE		
34	14.13474	0	0.001159	0	0	0.070748	0	3	29	0	3	3	FALSE	LTP-2xCO	1.666667	0	3	0	0	0.942138	1.81E-04						reaction_8951552			reaction	FALSE		
35	9.070965	0	1.42E-04	-5	5	0.110242	0	5	24	1	5	0	FALSE	NADP+ [c]	6.2	0	5	5	0	0.964445	1.81E-04				0	compart	species_2	NaN	NaN	species	FALSE		
36	9.159535	0	0.002772	-2	2	0.109176	0	2	24	1	2	0	FALSE	Mycobact	4.5	0	2	2	0	0.964055	1.81E-04				0	compart	species_1	NaN	NaN	species	FALSE		

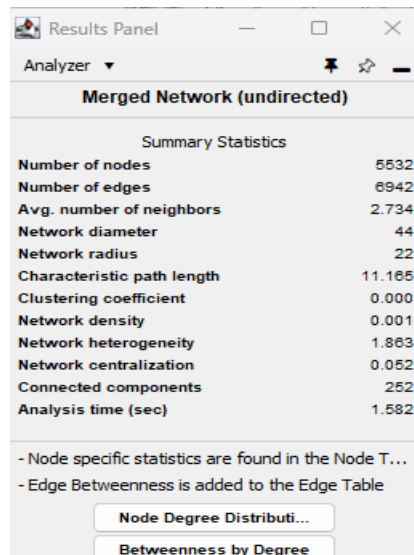


Fig 4.3 Network Analyzer result panel

4.3.2 Cytohubba:

In the analysis of integrated biological networks, the CytoHubba plugin within Cytoscape is a valuable tool for identifying key nodes or proteins critical to network dynamics. Among its algorithms, the "Degree" algorithm stands out for its ability to prioritize nodes based on their connectivity within the network. Degree centrality, a measure calculated by this algorithm, quantifies the number of edges (interactions) connected to each node. Consequently, nodes with higher degree centrality are more interconnected within the network, often serving as hubs that facilitate communication and coordination among various components. By selecting the top 5 nodes based on degree centrality, researchers can pinpoint crucial proteins central to network structure and function. These highly connected proteins typically play essential roles in biological processes and may serve as potential targets for further investigation or therapeutic intervention. Thus, the "Degree" algorithm offers valuable insights into the organization and regulation of biological networks, aiding in the understanding of complex biological systems.

The screenshot shows the CytoHubba tool interface. At the top, there's a dropdown menu for 'Network' set to 'Merged Network'. Below it, a 'Ranking Method' dropdown is set to 'Degree'. A table displays the top 5 ranked nodes, with ranks 1 through 5 and corresponding node names. The table has two columns: 'Rank' and 'Node'.

Rank	Node
1	ATP [cytosol]
2	ADP [cytosol]
3	H2O [cytosol]
4	Delayed Ear...
5	H+ [cytosol]

Fig 4.4 Ranking of Nodes using Degree algorithm in Cytohubba tool

4.4 Mathematical modelling :

Mathematical modelling is essential for understanding tuberculosis (TB) dynamics, particularly in host-pathogen interactions. These models offer a quantitative framework to simulate TB infection, incorporating factors like pathogen growth, immune responses, and drug effects. By integrating experimental data, mathematical models predict disease progression, assess intervention strategies, and guide TB control efforts. In short, mathematical modelling is a powerful tool for studying TB pathogenesis and optimizing treatment approaches.

4.4.1 KEGG Pathway:

KEGG pathways (<http://www.genome.jp/kegg/>) offer comprehensive insights into the molecular interactions and biological processes underlying various organisms, including *Mycobacterium tuberculosis* (Mtb). These pathways provide detailed maps of cellular processes, metabolic pathways, signalling cascades, and regulatory networks, curated from experimental data and literature. By organizing biological information into structured pathways, KEGG facilitates the interpretation

of genomic, transcriptomic, proteomic, and metabolomic data, aiding in the understanding of cellular functions and disease mechanisms. Researchers utilize KEGG pathways for a wide range of applications, including pathway analysis, functional annotation, comparative genomics, and systems biology modelling. Overall, KEGG pathways serve as invaluable resources for studying the complexities of biological systems and advancing biomedical research.

KEGG PATHWAY Database

Wiring diagrams of molecular interactions, reactions and relations

KEGG2 PATHWAY BRITe MODULE KO GENES COMPOUND NETWORK DISEASE DRUG

Select prefix: map Organism Enter keywords: Go Help

[New pathway maps | Update history]

Pathway Maps

KEGG PATHWAY is a collection of manually drawn [pathway maps](#) representing our knowledge of the molecular interaction, reaction and relation networks for:

- 1. Metabolism**
 - Global/overview
 - Carbohydrate
 - Energy
 - Lipid
 - Nucleotide
 - Amino acid
 - Other amino
 - Glycan
 - Cofactor/vitamin
 - Terpenoid/PK
 - Other secondary metabolite
 - Xenobiotics
 - Chemical structure
- 2. Genetic Information Processing**
- 3. Environmental Information Processing**
- 4. Cellular Processes**
- 5. Organismal Systems**
- 6. Human Diseases**
- 7. Drug Development**

The pathway map viewer linked from this page contains features of [KEGG mapping](#), especially for coloring map objects as described [here](#).

Fig 4.5 KEGG PATHWAY Database

4.4.2 Pathway Selection:

Incorporating pathways from resources like KEGG into mathematical models offers a structured framework for studying *Mycobacterium tuberculosis* (Mtb) dynamics. KEGG pathways provide detailed information on molecular interactions and cellular processes relevant to Mtb infection. By integrating these pathways into mathematical models, researchers can simulate and analyze various aspects of Mtb pathogenesis, such as metabolic pathways, virulence factors, and host-pathogen interactions. This integration enhances the accuracy and realism of

4.4.3 Equations:

Modelling the immune response to tuberculosis using differential equations and each of the steps in establishing equations. define the populations involved and then establish equations to describe how the numbers of each population change over time. The following population is considered in formation of the Equation:

- Bacilli (B): TB bacteria causing the infection.
- Macrophages (M): Immune cells that try to fight off infections by engulfing bacteria.
- Activated Immune Cells (A): Stronger immune cells activated to effectively combat the bacteria.
- Infected Cells (I): Body's cells infected by the bacteria.

4.4.3.1 Bacilli Growth and Killing:

The dynamic process by which the population of *Mycobacterium tuberculosis* (TB bacteria) changes over time within the host organism. This process involves both the proliferation (growth) of bacilli and their removal (killing) by various components of the immune system.

- **Bacilli Growth:** Bacilli growth refers to the reproduction and multiplication of *Mycobacterium tuberculosis* within the host. This process occurs when the bacteria replicate, increasing their numbers over time. Bacilli growth is influenced by factors such as the availability of nutrients, environmental conditions within the host, and the ability of the bacteria to evade immune detection. In mathematical terms, bacilli growth is represented by the term $rB \cdot B$ in the equation you provided, where r is the growth rate constant and B is the current population of bacilli. This term describes the rate at which the bacilli population increases due to replication and growth.

- **Bacilli Killing:** Bacilli killing refers to the elimination or reduction of *Mycobacterium tuberculosis* within the host, primarily by the immune system's defense mechanisms. This process involves the recognition and destruction of bacilli by immune cells, such as macrophages and activated immune cells (such as T cells). Bacilli can be killed through various mechanisms, including phagocytosis (engulfment) by macrophages, release of toxic molecules by immune cells, and activation of immune responses that target the bacteria. In mathematical terms, bacilli killing is represented by the terms $f_{MB} \cdot M \cdot B$ and $f_{AB} \cdot A \cdot B$ in the equation, where f_{MB} and f_{AB} are rate constants for the interactions between regular macrophages (M) or activated immune cells (A) and bacilli (B). These terms describe the rates at which bacilli are being eliminated by macrophages and activated immune cells, respectively.

4.4.3.2 Macrophage Dynamics:

The changes in the population of macrophages over time within the host organism during tuberculosis infection. Macrophages are key immune cells that play a central role in the defense against *Mycobacterium tuberculosis* by phagocytosing (engulfing) and destroying the bacteria. The components of the equation and their implications for macrophage dynamics:

- **Macrophage Recruitment and Production (rM):** This term represents the rate of recruitment or production of macrophages within the host. Macrophages can be derived from circulating monocytes that are recruited to the site of infection or from local tissue-resident macrophages. The rate of macrophage recruitment or production (rM) depends on factors such as the presence of inflammatory signals, cytokines, and chemokines released in response to infection. A higher value of r indicates a higher rate of macrophage recruitment or production, which may occur in response to increasing bacterial load or immune activation.

- **Macrophage Death ($dM \cdot M$):** This term represents the rate at which macrophages die or are depleted within the host. Macrophages can undergo cell death due to various factors, including direct killing by *Mycobacterium tuberculosis*, apoptosis (programmed cell death), or necrosis (uncontrolled cell death). The term $dM \cdot M$ describes the rate of macrophage death, where dM is the death rate constant and M is the current population of macrophages. A higher value of dM indicates a higher rate of macrophage death, which may occur due to factors such as bacterial virulence or host immune responses.
- **Macrophage-Bacilli Interaction ($fMB \cdot M \cdot B$):** This term represents the rate of interaction between macrophages (M) and bacilli (B). Macrophages can phagocytose and eliminate *Mycobacterium tuberculosis* through various mechanisms, including the release of antimicrobial molecules and the formation of granulomas. The term $fMB \cdot M \cdot B$ describes the rate at which macrophages interact with and phagocytose bacilli, where fMB is the interaction rate constant. A higher value of fMB indicates a higher efficiency of macrophages in phagocytosing and killing bacilli.
- **Macrophage Activation (fIM):** This term represents the rate of activation of macrophages to an activated state (A) that is more effective in killing bacilli. Activated macrophages exhibit enhanced antimicrobial activity through the release of cytokines, chemokines, and reactive oxygen and nitrogen species. The term fIM represents the rate constant for the conversion of regular macrophages (M) to activated immune cells (A). A higher value of fIM indicates a higher rate of macrophage activation, which may occur in response to inflammatory signals or interactions with other immune cells.

4.4.3.3 Activated immune cell formation:

The process by which macrophages are activated to become stronger immune cells capable of effectively combating *Mycobacterium tuberculosis* infection. These activated immune cells play a crucial role in the host defense mechanism against tuberculosis. The components of the equation and their implications for the formation of activated immune cells:

- **Macrophage Activation ($rA \cdot M$):** This term represents the rate at which macrophages (M) are converted into activated immune cells (A). Macrophages can be activated to a more potent antimicrobial state in response to various signals, such as recognition of pathogen-associated molecular patterns (PAMPs) or cytokines released by other immune cells. The term rA is the rate constant for macrophage activation. A higher value of rA indicates a higher rate of macrophage activation, which may occur in response to increasing bacterial load or immune stimulation.
- **Activated Immune Cell Death ($dA \cdot A$):** This term represents the rate at which activated immune cells (A) undergo cell death or are depleted within the host. Activated immune cells may undergo apoptosis (programmed cell death) or be eliminated by other immune cells as part of the normal immune response. The term dA is the death rate constant for activated immune cells. A higher value of dA indicates a higher rate of cell death among activated immune cells, which may occur due to factors such as immune exhaustion or excessive inflammation.

4.4.3.4 Infected cell dynamics:

The changes over time in the population of host cells that have been infected by Mycobacterium tuberculosis (MTB). These infected cells play a crucial role in the progression and transmission of tuberculosis. The components of the equation and discuss their implications for the dynamics of infected cells:

- **Bacilli-Macrophage Interaction ($fMB \cdot M \cdot B$):** This term represents the rate at which bacilli (B) interact with macrophages (M) to infect host cells. Bacilli can be phagocytosed by macrophages, leading to the intracellular replication of MTB within these host cells. The term fMB represents the rate constant for bacilli-macrophage interaction. A higher value of fMB indicates a higher rate of bacilli uptake by macrophages, which may occur in response to factors such as bacterial load or macrophage activation status.
- **Infection of Host Cells ($fIM \cdot I$):** This term represents the rate at which infected cells (I) are formed through the interaction between bacilli and susceptible host cells. Once inside the macrophages, bacilli can replicate and spread to neighbouring cells, leading to the formation of new infected cells. The term fIM represents the rate constant for the infection of host cells. A higher value of fIM indicates a higher rate of host cell infection, which may occur in response to factors such as bacterial virulence or host susceptibility.
- **Infected Cell Death ($dI \cdot I$):** This term represents the rate at which infected cells (I) undergo cell death or clearance within the host. Infected cells may be eliminated by host immune responses, such as apoptosis or phagocytosis by immune cells. The term dI represents the death rate constant for infected cells. A higher value of dI indicates a higher rate of cell death among infected cells, which may occur due to factors such as immune-mediated killing or bacterial cytotoxicity.

4.4.4 Parameters

the dynamics of tuberculosis infection and immune response, parameters are numerical values that represent various biological factors, rates, or properties influencing the behavior of the system. These parameters dictate how different components of the model interact with each other and how the system evolves over time. They can represent rates of growth, death, interaction strengths, or other biological processes relevant to the disease dynamics being modeled. Adjusting these parameters allows researchers to simulate different scenarios and understand how changes in these factors affect the progression and outcome of the disease.

4.4.4.1 Growth rate of TB bacilli (r_B):

- Represents the rate at which TB bacilli multiply in the host environment.
- Higher values indicate faster growth of the bacterial population.

4.4.4.2 Natural death rate of TB bacilli (d_B):

- Indicates the rate at which TB bacilli naturally die off, possibly due to environmental factors or immune response.
- Helps regulate the size of the bacterial population.

4.4.4.3 Rate of phagocytosis of TB by macrophages (f_{MB}):

- Defines the rate at which macrophages engulf and kill TB bacilli.
- Reflects the efficiency of the immune system in clearing the infection.

4.4.4.4 Rate of TB killing by activated immune cells (f_{AB}):

- Represents the effectiveness of activated immune cells (such as T-cells) in killing TB bacilli.
- Influences the rate at which the infection is controlled.

4.4.4.5 Recruitment rate of macrophages (r_M):

- Indicates the rate at which new macrophages are recruited to the site of infection.
- Higher values imply a faster influx of immune cells to combat the infection.

4.4.4.6 Death rate of macrophages (d_M):

- Represents the rate at which macrophages die off, possibly due to exhaustion or bacterial toxicity.
- Affects the availability of macrophages for immune response.

4.4.4.7 Rate of infected cell conversion back to macrophages (f_{IM}):

- Defines the rate at which infected cells are converted back to macrophages, possibly as part of the immune response or cellular turnover.
- Influences the balance between infected cells and immune cells in the host

4.4.4.8 Activation rate of activated and T-cells (r_A):

- Indicates the rate at which immune cells are activated in response to the infection.
- Higher values suggest a more rapid immune response to combat the infection.

4.4.4.9 Death rate of activated immune cells (d_A):

- Represents the rate at which activated immune cells die off, possibly due to exhaustion or apoptosis.
- Affects the longevity and effectiveness of the immune response.

4.4.4.10 Death rate of infected cells (d_I):

- Indicates the rate at which infected host cells die off, possibly due to the infection itself or immune-mediated killing.
- Influences the progression and severity of the disease.

4.4.5 Graph Plot:

A graph plot, in the context of a mathematical model like the one provided for tuberculosis infection and immune response, typically displays the dynamics of various components of the system over time. Each line or curve on the graph represents the changing quantities of different populations (such as bacilli, macrophages, activated immune cells, and infected cells) as the model evolves. The x-axis usually represents time, while the y-axis represents the quantity of each population. By analyzing these plots, can gain insights into how the disease progresses, how the immune system responds, and how different factors influence the outcome of the infection.

4.4.5.1 Importing Libraries:

```
import numpy as np
from scipy.integrate import odeint
import matplotlib.pyplot as plt
```

Fig 4.7 Importing Libraries

- numpy (aliased as np) for numerical computations.
- odeint from scipy.integrate for solving ordinary differential equations (ODEs).
- matplotlib.pyplot (aliased as plt) for plotting the simulation results.

4.4.5.2 Defining Parameters:

```
r_B = 0.4
d_B = 0.1
f_MB = 0.0001
f_AB = 0.001
r_M = 1e6
d_M = 0.1
f_IM = 0.1
r_A = 0.01
d_A = 0.1
d_I = 0.2
```

Fig 4.8 Defining the Parameters

These are the parameters used in the ODE system. Each parameter represents a specific rate or factor affecting the dynamics of the TB infection and immune response model.

4.4.5.3 Defining the Differential Equation:

```
def model(y, t):  
    B, M, A, I = y  
    dBdt = r_B * B - d_B * B - f_MB * M * B - f_AB * A * B  
    dMdt = r_M - d_M * M - f_MB * M * B + f_IM * I  
    dAdt = r_A * M - d_A * A  
    dIdt = f_MB * M * B - f_IM * I - d_I * I  
    return [dBdt, dMdt, dAdt, dIdt]
```

Fig 4.9 Defining the Differential Equation

This function model defines the system of ordinary differential equations. Given the current state y (a list of variables [B, M, A, I]) and time t, it computes the rate of change of each variable (dBdt, dMdt, dAdt, dIdt) based on the defined equations.

4.4.5.4 Setting Initial Conditions:

```
B0 = 100      # Initial bacilli count  
M0 = 1e6      # Initial macrophage count  
A0 = 0        # Initial activated immune cells  
I0 = 0        # Initial infected cells  
initial_conditions = [B0, M0, A0, I0]
```

Fig 4.10 Initial Conditions

Initial conditions for the variables [B, M, A, I] are specified. These values represent the initial populations of bacilli, macrophages, activated immune cells, and infected cells, respectively.

4.4.5.5 Setting Time Points:

```
time = np.linspace(0, 200, 1000) # 200 days
```

Fig 4.11 Setting the Points

Time points for the simulation are generated using `np.linspace()`, creating 1000 points evenly spaced between 0 and 200 days.

4.4.5.6 Solving the ODE's:

```
results = odeint(model, initial_conditions, time)
```

Fig 4.12 Solving the ODE

The `odeint` function is called to solve the system of ODEs (`model`) with the initial conditions (`initial_conditions`) over the specified time points (`time`). The resulting solutions for each variable (`[B, M, A, I]`) are stored in the `results` array.

4.4.5.7 Plotting the Results:

```
plt.figure(figsize=(12, 8))
plt.plot(time, results[:, 0], label='Bacilli (B)')
plt.plot(time, results[:, 1], label='Macrophages (M)')
plt.plot(time, results[:, 2], label='Activated Immune Cells (A)')
plt.plot(time, results[:, 3], label='Infected Cells (I)')
plt.title('Simulation of TB Infection and Immune Response over 200 Days')
plt.xlabel('Time (days)')
plt.ylabel('Population')
plt.legend()
plt.grid(True)
plt.show()
```

Fig 4.13 Plotting the Result

The simulation results are plotted using Matplotlib. Each population (Bacilli (B), Macrophages (M), Activated Immune Cells (A), Infected Cells (I)) is plotted against time. Legends, title, labels, and gridlines are added to make the plot informative and visually appealing.

CHAPTER – 5

RESULTS AND DISCUSSION

5.1 Network Analysis of Molecular Interactions in HPI:

Network analysis of molecular interactions in host-pathogen interactions involves examining the intricate web of interactions between host cells and invading pathogens, such as bacteria, viruses, fungi, or parasites. One common approach to analyze such interactions is through the use of network analysis tools like Cytoscape, which allows researchers to visualize and explore complex biological networks.

After using the "Analyze Network" plugin in Cytoscape to analyze the network, the top 10 nodes typically represent the most important molecules or entities within the interaction network. These nodes could include proteins, genes, metabolites, or other biomolecules that play crucial roles in mediating the interaction between the host and the pathogen.

5.1.1 Average Shortest Path Length:

Examined the average shortest path length, which provides insights into the efficiency of information flow and connectivity among nodes. Here's how the concept applies to the specific nodes identified in our study:

- **CLEC4E:** This receptor protein binds to alpha-mannan and trehalose-6-6'-dimycolate, playing a crucial role in immune recognition and response to fungal and mycobacterial pathogens. Its involvement suggests a potential pathway for efficient communication and coordination in the host's defense against these pathogens.

- **CLEC6A:** Similar to CLEC4E, CLEC6A recognizes alpha-mannan in fungal cell walls, indicating its importance in the immune response to fungal infections. Its presence in the network may contribute to the establishment of efficient pathways for immune recognition and response.
- **CLEC10A:** This C-type lectin receptor binds to Tn-MUC1, a mucin glycoprotein associated with immune recognition, particularly in cancer and inflammation contexts. Its inclusion in the network highlights its potential role in mediating interactions and signaling pathways relevant to immune responses.
- **CLEC4D:** Another C-type lectin receptor, CLEC4D, binds to mycobacterial trehalose-6,6'-dimycolate (TDM), suggesting its involvement in immune recognition and response to mycobacterial infections. Its presence may contribute to the formation of efficient pathways for immune defense against mycobacteria.
- **OGG1 mutants, NTHL1 D239Y, MUTYH LOF mutants:** These nodes represent DNA repair enzymes involved in repairing oxidative DNA lesions. Their presence in the network underscores the importance of efficient DNA repair pathways in maintaining genomic stability and protecting against DNA damage-induced diseases.
- **Non-secretable F8 variant:** This variant of the Factor VIII protein, retained within the endoplasmic reticulum lumen, is crucial for blood clotting. Its inclusion in the network suggests its role in mediating interactions and signaling pathways relevant to blood clotting disorders such as hemophilia A.

- **pS45-beta-catenin: p-Axin:GSK3 del304-316: CK1alpha: APC: PP2A: AMER1 complex:** This complex regulates the Wnt signaling pathway, which is implicated in various cellular processes and diseases, including cancer. Its presence highlights its potential role as a central hub in coordinating signaling events within the cell.

By analyzing the average shortest path length within the network, gain insights into the efficiency of communication and coordination among these nodes involved in immune recognition, DNA repair, blood clotting, and signaling pathways. These findings contribute to our understanding of the complex interplay of molecular interactions underlying host-pathogen interactions and disease mechanisms, which is crucial for developing targeted therapeutic interventions.

5.1.2 Betweenness Centrality:

Examined the betweenness centrality of specific nodes within the network, which provides insights into their importance in mediating interactions and communication pathways. Here's how this concept applies to the nodes identified in our study:

- **CLEC4E, CLEC6A, CLEC10A, and CLEC4D:** These nodes represent C-type lectin receptors involved in immune recognition and response by binding specific components of pathogens or cellular molecules. Their high betweenness centrality values indicate their crucial roles as central hubs in mediating interactions within the network. These receptors likely play pivotal roles in coordinating immune responses to pathogens by recognizing pathogen-derived molecules and initiating signaling cascades.
- **Defective ATRX, Defective RFT1, Defective MMADHC, and FLT mutants:** These nodes represent mutated or dysfunctional proteins that fail to perform their intended functions. Their high betweenness centrality values suggest that despite their defects, they still influence communication

pathways within the network significantly. These defective proteins may disrupt normal cellular functions, potentially impacting disease pathogenesis or drug resistance mechanisms.

- **ASP-3026-resistant ALK mutants:** These mutants demonstrate resistance to ASP-3026, indicating alterations in the binding site or mechanism of action of this drug. Their high betweenness centrality values suggest their importance in mediating interactions within the network, potentially influencing drug resistance mechanisms and treatment outcomes.

Overall, the nodes with high betweenness centrality values likely serve as critical mediators of interactions within the network, influencing communication pathways and potentially serving as central hubs in host-pathogen interaction networks. These findings shed light on the complex interplay of molecular interactions underlying immune recognition, disease pathogenesis, and drug resistance mechanisms, which is essential for understanding and targeting host-pathogen interactions effectively.

5.1.3 Degree:

Focused on the degree centrality of specific nodes within the cytosol network, shedding light on their importance in cellular metabolism, gene expression, and host-pathogen interactions. Here's a detailed elaboration of findings:

- **ATP, ADP, H₂O, H⁺, GTP, and PPi:** These molecules are fundamental components involved in cellular metabolism and energy transfer. ATP, in particular, exhibits the highest degree centrality, indicating its central role in cellular processes. ATP is synthesized from ADP during cellular respiration and serves as a primary energy currency in cells. This interconversion between ATP and ADP is facilitated by enzymes such as ATP synthase and ATPases. Additionally, H₂O and H⁺ are crucial for cellular hydration and pH regulation, maintaining cellular homeostasis. GTP and PPi participate in

cellular metabolism and signaling pathways, with GTP serving as an energy source in protein synthesis and signal transduction, while PPi is generated as a byproduct of various metabolic reactions.

- **Delayed Early (DE) Gene Expression and Late (L) Gene Expression:** These nodes represent different stages of gene regulation during viral infection. They highlight the importance of gene expression dynamics in the host's response to viral pathogens. DE Gene Expression and L Gene Expression processes involve complex interactions between viral and host factors, leading to the expression of specific genes at different times during the viral life cycle. These stages play crucial roles in determining the outcome of viral infection and the host's ability to mount an effective immune response.
- **Addition of Tegument to HCMV Nucleocapsid C:** This node likely refers to a specific step in the replication cycle of Human Cytomegalovirus (HCMV), involving the interaction between viral proteins and host cellular components. This process facilitates the assembly and maturation of viral particles, contributing to the spread of infection within the host. The involvement of viral components in host-pathogen interactions underscores the complexity of viral replication and the host's response to viral infection.

The nodes with high degree centrality values represent central components or processes within the cytosol network, playing critical roles in cellular metabolism, gene expression, and host-pathogen interactions. These findings provide insights into the interconnectedness of biochemical pathways, regulatory networks, and cellular processes underlying the host's response to pathogenic challenges such as MTB infection.

5.1.4 Eccentricity:

The network analysis of key nodes reveals pivotal elements central to host-pathogen interactions, DNA repair mechanisms, and cellular signaling pathways, particularly in the context of *Mycobacterium tuberculosis* (MTB) infection. These nodes, characterized by their low eccentricity values, are fundamental in mediating interactions, pathways, and processes with minimal distance from other network components, indicating their critical roles in orchestrating molecular and cellular responses to MTB infection.

- The C-type lectin-like receptors (CLEC4E, CLEC6A, CLEC10A, CLEC4D), situated at the forefront of pathogen recognition and immune response, underscore their indispensable involvement in detecting pathogen-associated molecular patterns (PAMPs) and triggering immune activation. Their close interconnection within the network suggests collaborative signaling pathways and immune cascades, highlighting their concerted effort in combating MTB invasion.
- The DNA repair enzymes and mutants (OGG1, NTHL1, MUTYH) epitomize the cellular machinery responsible for maintaining genomic stability and combating DNA damage. Their tight integration within the nucleoplasmic network signifies their collaborative role in repairing DNA lesions, thereby safeguarding the host genome against MTB-induced genotoxic stress.
- The presence of the Non-secretable F8 variant within the endoplasmic reticulum (ER) lumen underscores its relevance to protein folding, quality control, and secretion pathways. Its proximity to other ER-resident proteins suggests its participation in ER homeostasis and its potential implications in MTB-mediated disruptions of cellular protein processing.

- The pS45-beta-catenin: p-Axin: GSK3 del304-316: CK1alpha: APC: PP2A: AMER1 complex elucidates intricate signaling networks within the cytosol, particularly in the regulation of the Wnt signaling pathway. Its central position within the network implies its pivotal role in modulating intracellular signaling cascades and potentially influencing cellular responses to MTB infection.

While the specific interactions between these nodes are not explicitly elucidated, their collective integration into a network of interconnected molecular pathways and cellular processes underscores their significance in orchestrating host responses to MTB infection. This comprehensive understanding provides valuable insights into the intricate molecular landscape underlying host-pathogen interactions, DNA repair mechanisms, and cellular signaling dynamics during MTB infection, thereby paving the way for targeted therapeutic interventions and elucidating novel avenues for combating tuberculosis.

5.1.5 Neighbourhood Connectivity:

This analysis part reveals nodes within the network characterized by high Neighbourhood Connectivity values, indicating their central roles in cellular signaling pathways and disease mechanisms, including those pertinent to MTB infection.

- Nodes 1-7 encompass mutations or defects in critical signaling proteins such as CTNNB1 (beta-catenin), ABCC8, TGFBR1, and TGFBR2, affecting their phosphorylation and downstream signaling cascades. Dysregulation of these pathways may disrupt normal cellular functions, leading to aberrant cellular responses implicated in disease pathogenesis.

- Nodes 8-10 represent various receptor autophosphorylation events, signifying the activation and regulation of receptor tyrosine kinases (EGFRvIII, ligand-responsive EGFR, and PDGFRA) within intricate signaling networks. These events play pivotal roles in orchestrating cellular responses to extracellular signals, influencing processes vital for cell proliferation, survival, and migration.

The interconnectedness of these nodes within the network suggests their participation in complex signalling networks. Here's how they may be interconnected:

- Mutations in CTNNB1 (nodes 1-7) disrupt its phosphorylation by specific kinases (GSK3beta and CK1alpha), leading to dysregulation of the Wnt signaling pathway. This dysregulation can impact cellular processes such as proliferation, differentiation, and apoptosis, potentially influencing host responses to MTB infection.
- Defects in ABCC8 (node 4) result in the inability to form functional KATP channels, affecting glucose homeostasis and cellular metabolism. Such alterations may impact host immune responses and susceptibility to MTB infection.
- Mutations in TGFBR1 and TGFBR2 (nodes 5 and 6) impair their ability to phosphorylate downstream targets (SMAD2 and SMAD3), disrupting TGF-beta signaling pathways involved in cell growth, differentiation, and immune regulation. Dysregulation of these pathways may affect host immune responses to MTB infection and contribute to disease progression.
- Autophosphorylation events in receptor tyrosine kinases (nodes 8-10) regulate cell proliferation, survival, and migration in response to

extracellular signals. Dysregulation of these events may impact host immune responses to MTB infection and influence disease outcomes.

Overall, these nodes likely form interconnected pathways that regulate various cellular processes crucial for host responses to MTB infection. Dysregulation of these pathways due to mutations or defects may contribute to disease pathogenesis and impact host immune responses, underscoring their significance in the context of MTB infection and providing potential targets for therapeutic intervention.

5.1.6 Out Degree:

This analysis highlights nodes within the network characterized by high Outdegree values, indicating their pivotal roles in mediating interactions and regulating essential cellular processes, including those relevant to MTB infection.

- Nodes 1-6 (ATP, ADP, H₂O, H⁺, GTP, PPi) encompass molecules primarily located in the cytosol, crucial for various cellular processes such as energy metabolism, ion regulation, and hydrolysis reactions. ATP and ADP serve as key components in cellular energy metabolism, participating in metabolic pathways such as glycolysis and oxidative phosphorylation. H₂O and H⁺ are integral to numerous cellular processes, including hydrolysis reactions and pH regulation, while GTP and PPi play roles in signaling pathways and biosynthetic reactions. These molecules are interconnected through intricate biochemical pathways, contributing to the overall functioning of the cell and potentially influencing host responses to MTB infection.
- Node 7 (H₂O [lysosomal lumen]) represents water molecules within the lysosomal lumen, essential for hydrolysis and degradation of macromolecules during lysosomal digestion. The hydrolytic activity

facilitated by water molecules within lysosomes is crucial for the recycling and turnover of cellular components, potentially impacting MTB survival and intracellular trafficking within host cells.

- Node 8, PI(3,4,5)P3 at the plasma membrane, plays a critical role in signal transduction pathways regulating cell growth, differentiation, and survival. Its interactions with various signaling proteins and receptors at the plasma membrane influence cellular responses to extracellular stimuli, potentially modulating host immune responses to MTB infection and pathogenesis.
- Nodes 9-10, GDP and Pi in the cytosol, participate in cellular metabolism and signaling cascades. GDP serves as an intermediate in nucleotide metabolism, while Pi plays essential roles in energy transfer and signaling events. Dysregulation of these molecules and their associated pathways may impact cellular responses to MTB infection, influencing host immune surveillance and pathogen clearance.

These nodes with high Outdegree values play central roles in cellular processes crucial for host responses to MTB infection. Their interconnectedness within the network highlights their significance in orchestrating cellular homeostasis and responding to environmental cues, providing potential targets for therapeutic intervention and further research into tuberculosis pathogenesis.

5.1.7 Stress:

Identified nodes within the network characterized by high Stress values, indicating their critical roles in maintaining network integrity and regulating essential cellular processes, including those relevant to MTB infection.

- Nodes 1-2, ATP and H₂O, serve as fundamental molecules in cellular metabolism and function, essential for energy transfer and cellular hydration. Their high Stress values underscore their central roles in maintaining cellular homeostasis, making them crucial targets for further investigation in the context of MTB infection and host-pathogen interactions.
- Nodes 3-6 encompass various proteins and processes involved in cellular signaling, protein transport, and vesicle trafficking. Their high Stress values suggest their importance in regulating these cellular processes, potentially influencing host responses to MTB infection and modulating intracellular trafficking of the pathogen.
- Nodes 7-10 involve protein interactions and modifications implicated in cellular responses to external stimuli, such as signaling pathway activation and host-pathogen interactions. Their high Stress values highlight their significance in orchestrating cellular responses to MTB infection, potentially influencing disease pathogenesis and host immune surveillance mechanisms.

Interconnections among nodes in the network reveal intricate molecular interactions, signaling pathways, and cellular processes crucial for cellular responses to MTB infection:

- ATP and H₂O are interconnected through metabolic pathways, where ATP is hydrolyzed to ADP, releasing energy, and water molecules are produced as byproducts. This interconnection underscores the tight coupling between energy metabolism and cellular hydration, essential for cellular function and response to environmental cues, including those related to MTB infection.

- Processes involving DT fragment B and CDK5:p25 likely intersect with other pathways involved in toxin transport, endosomal sorting, and cellular signaling. These interactions may modulate host responses to MTB infection by influencing intracellular trafficking of bacterial toxins and cellular responses to toxin exposure.
- Interactions between CDK5:p25 and its downstream target JUN highlight the role of cyclin-dependent kinase signaling in regulating cellular proliferation and differentiation, potentially influencing host immune responses to MTB infection and disease progression.
- The involvement of H⁺ in vesicular acidification within endocytic vesicles suggests its role in modulating intracellular trafficking of MTB and host-pathogen interactions. Dysregulation of vesicular acidification may impact MTB survival and intracellular trafficking within host cells.
- Processes involving tetX HC and tetX LC intersect with pathways involved in vesicle trafficking and toxin internalization, potentially influencing MTB dissemination and intracellular survival mechanisms. These interactions may contribute to MTB pathogenesis and host immune evasion strategies.
- Interactions between tetX LC:Zn²⁺ and its substrate VAMP2 underscore the role of toxin-mediated disruption of neurotransmitter release in MTB-induced neuropathogenesis. These interactions may contribute to the neurotoxic effects of MTB infection and modulate host immune responses in the central nervous system.

The nodes with high Stress values are central to cellular processes crucial for host responses to MTB infection, highlighting their significance in disease pathogenesis

and providing potential targets for therapeutic intervention and further research into tuberculosis.

5.1.8 Topological Coefficient:

The nodes within the network characterized by high Topological Coefficient values, indicating their central roles in facilitating efficient communication and coordination within cellular compartments, including those relevant to MTB infection.

- Nodes 1, 3, 4, and 6 represent cellular structures or complexes involved in protein synthesis, quality control, and viral replication. Their high connectivity within their respective cellular compartments underscores the coordinated interactions among their components, essential for maintaining cellular homeostasis and regulating cellular processes crucial for MTB infection and host-pathogen interactions.
- Nodes 2, 7, and 9 encompass protein complexes or interactions occurring at the plasma membrane, potentially involved in immune response or membrane transport processes. Their high connectivity within the plasma membrane environment suggests their importance in modulating cellular communication, immune responses, and membrane dynamics critical for MTB invasion and host immune surveillance mechanisms.
- Nodes 5, 8, and 10 represent complexes involved in viral replication or transcription regulation within the nucleoplasm. Their high connectivity within the nuclear environment highlights their central roles in regulating viral gene expression and replication, potentially influencing host responses to MTB infection and modulating cellular antiviral defence mechanisms.

The interconnections among nodes with high Topological Coefficient values suggest close associations and interactions within their respective cellular environments:

- Nodes involved in protein synthesis and quality control likely share common regulatory mechanisms and signaling pathways related to protein folding, maturation, and trafficking, essential for maintaining protein homeostasis within the cell. These interactions may impact MTB infection by influencing the expression of host factors critical for pathogen survival and replication.
- Protein complexes or interactions occurring at the plasma membrane may participate in signaling cascades, immune responses, or membrane transport processes essential for host defense against MTB infection. Coordinated interactions within the plasma membrane environment facilitate cellular communication and function, influencing host-pathogen interactions and modulating immune responses to MTB invasion.
- Complexes involved in viral replication or transcription regulation within the nucleoplasm likely regulate viral gene expression and replication, impacting the outcome of MTB infection and host immune responses. These interactions reflect the intricate network of molecular processes occurring within cellular compartments, crucial for regulating cellular homeostasis and coordinating host responses to MTB infection.

Nodes with high Topological Coefficient values play central roles in facilitating cellular processes relevant to MTB infection, highlighting their significance in disease pathogenesis and providing potential targets for therapeutic intervention and further research into tuberculosis.

5.2 Mathematical Modelling:

The tuberculosis model is structured using differential equations to capture the basic dynamic interplay between the immune system and tuberculosis bacilli, providing insights into the progression of infection and the body's response. The equations are crafted based on standard assumptions in immunology and microbiology, grounded in biological realities and typical behaviours of both the pathogen and the immune system.

5.2.1 Equation Defining:

The differential equations used in tuberculosis model helps to capture the dynamic interaction between the immune system and tuberculosis bacilli over time.

5.2.1.1 Bacilli Growth and Death:

$$dB/dt = rB \cdot B - dB \cdot B - f_{MB} \cdot M \cdot B - f_{AB} \cdot A \cdot B$$

- dB/dt : Rate of change of the population of bacilli over time.
- $rB \cdot B$: Growth term for bacilli population, where rB is the growth rate and B represents the current population of bacilli.
- $dB \cdot B$: Natural death term for bacilli population, where dB is the death rate.
- $f_{MB} \cdot M \cdot B$: Killing of bacilli by macrophages, where f_{MB} represents the efficiency of macrophages in killing bacilli, M is the population of macrophages, and B is the population of bacilli.

- $f_{AB} \cdot A \cdot B$: Killing of bacilli by activated immune cells, where f_{AB} represents the efficiency of activated immune cells in killing bacilli, A is the population of activated immune cells, and B is the population of bacilli.

The equation governing bacilli growth and natural death reflects the standard assumption that bacteria reproduce at a constant rate under favorable conditions, while also experiencing natural mortality. This formulation aligns with our understanding of bacterial dynamics and environmental influences on bacterial growth.

5.2.1.2 Macrophage Dynamics:

$$dM/dt = rM - dM \cdot M - f_{MB} \cdot M \cdot B + f_{IM} \cdot I$$

- dM/dt : Rate of change of the population of macrophages over time.
- rM : Recruitment rate of macrophages.
- $dM \cdot M$: Natural death term for macrophages, where dM is the death rate.
- $f_{MB} \cdot M \cdot B$: Depletion of macrophages due to interaction with bacilli.
- $f_{IM} \cdot I$: Replenishment of macrophages from infected cells.

The equation describing the killing of bacilli by macrophages and activated immune cells accounts for the effectiveness of these cells in controlling infections, depending on their numbers and encounters with bacilli. This assumption is consistent with the role of immune cells in combating pathogens and regulating infection progression.

5.2.1.3 Activated Immune Cell Dynamics:

$$dA/dt = rA \cdot M - dA \cdot A$$

- dA/dt : Rate of change of the population of activated immune cells over time.
- $rA \cdot M$: Activation rate of immune cells, where rA is the activation rate and M is the population of macrophages.
- $dA \cdot A$: Natural death term for activated immune cells, where dA is the death rate.

The equation modeling the activation and death of immune cells reflects the process by which macrophages and other immune cells become activated in response to infection, with activation scaling with the severity of the infection. This formulation aligns with our understanding of immune cell activation and the dynamics of immune responses to pathogens.

5.2.1.4 Infected Cell Dynamics:

$$dI/dt = fMB \cdot M \cdot B - fIM \cdot I - dI \cdot I$$

- dI/dt : Rate of change of the population of infected cells over time.
- $fMB \cdot M \cdot B$: Formation of infected cells from macrophages and bacilli.
- $fIM \cdot I$: Clearance of infected cells by activated immune cells.
- $dI \cdot I$: Natural death term for infected cells, where dI is the death rate.

The equation governing the formation and clearance of infected cells accounts for the interaction between macrophages and bacilli, with infected cell formation influenced by the numbers of both macrophages and bacilli. This assumption

reflects the direct interaction between immune cells and pathogens during infection progression.

5.2.2 Parameterization:

- Growth Rate of TB Bacilli ($r_B = 0.4/\text{day}$): This parameter represents the rate at which TB bacilli multiply under favourable conditions within the host environment.
- Natural Death Rate of TB Bacilli ($d_B = 0.1/\text{day}$): The natural death rate reflects the mortality of TB bacilli due to factors such as aging or unfavourable environmental conditions.
- Rate of Phagocytosis of TB by Macrophages ($f_{MB} = 0.0001/\text{day/cell}$): This parameter signifies the rate at which macrophages engulf and eliminate TB bacilli.
- Rate of TB Killing by Activated Immune Cells ($f_{AB} = 0.001/\text{day/cell}$): It represents the efficiency of activated immune cells in killing TB bacilli.
- Recruitment Rate of Macrophages ($r_M = 10^6 \text{ cells/day}$): This parameter denotes the rate at which macrophages are recruited to the site of infection from the bone marrow or other tissues.
- Death Rate of Macrophages ($d_M = 0.1/\text{day}$): It indicates the natural death rate of macrophages within the host environment.
- Rate of Infected Cell Conversion Back to Macrophages ($f_{IM} = 0.1/\text{day}$): This parameter represents the rate at which infected cells revert back to being normal macrophages or die.
- Activation Rate of Activated and T-cells ($r_A = 0.01/\text{day}$): It signifies the rate at which immune cells become activated in response to the presence of TB bacilli.
- Death Rate of Activated Immune Cells ($d_A = 0.1/\text{day}$): This parameter denotes the natural death rate of activated immune cells within the host environment.
- Death Rate of Infected Cells ($d_I = 0.2/\text{day}$): It represents the mortality rate of cells infected by TB bacilli.

5.2.2.1 Initial Condition:

- **Starting with 100 Bacilli ($B(0) = 100$):** At the onset of the simulation, there are 100 TB bacilli within the host environment, initiating the infection process.
- **Healthy Number of Macrophages ($M(0) = 10^6$):** The simulation begins with a substantial population of macrophages, representing the baseline immune defense against the TB infection.
- **Initially No Activated Immune Cells ($A(0) = 0$):** At the start of the simulation, there are no activated immune cells present, indicating the absence of an immune response against the TB infection.
- **No Infected Cells at the Start ($I(0) = 0$):** Initially, there are no cells infected by TB bacilli, marking the beginning of the infection process.

By considering a timeframe of 200 days, aim to observe the progression of the TB infection, the subsequent activation of the immune response, and the potential stabilization or progression of the disease dynamics. This timeframe allows for comprehensive analysis of the infection dynamics and the host's immune response over an extended period, facilitating insights into the course of tuberculosis pathogenesis.

5.2.3 Graph Plot:

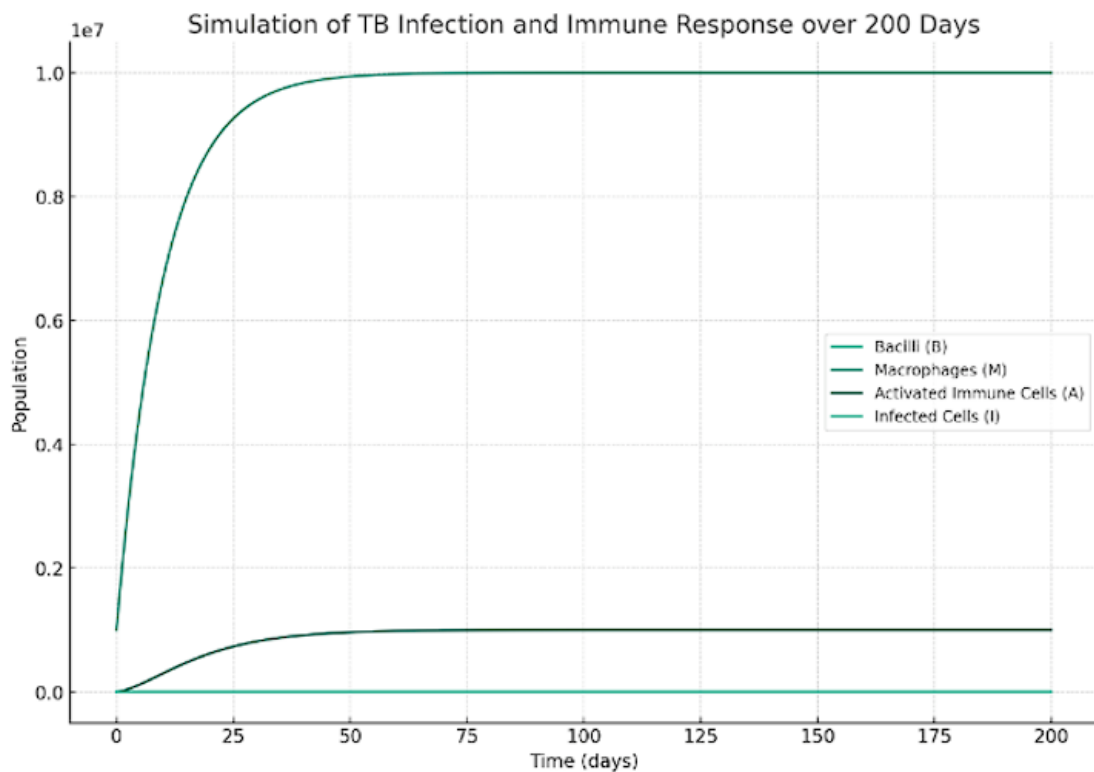


Fig 5.1 Simulation of TB infection and Immune Response Graph Plot

- **Bacilli (B) Line:**

Observation: Describe how the bacilli count initially increases, then declines.

Explanation: Explain the initial proliferation of TB bacteria and subsequent decline due to the immune response, particularly the increase in activated immune cells leading to effective bacilli killing.

- **Macrophages (M) Line:**

Observation: Discuss the slight decrease followed by stabilization in the macrophage count.

Explanation: Describe how macrophages initially engage with bacilli, leading to depletion or infection, but stabilize as the body recruits more macrophages and some infected cells revert back to macrophages.

- **Activated Immune Cells (A) Line:**

Observation: Note the gradual increase in activated immune cell count.

Explanation: Highlight the ongoing activation of immune cells in response to infection, contributing to effective control of bacilli proliferation.

- **Infected Cells (I) Line:**

Observation: Mention the initial rise followed by a decline in infected cell count.

Explanation: Explain the initial success of bacilli invasion, countered by the strengthening immune response leading to infected cell clearance or death, indicating infection control.

5.3 Conclusion:

In conclusion, the comprehensive analysis, integrating various network centrality measures, KEGG pathways, mathematical modelling, and network analysis, offers valuable insights into the complex dynamics of *Mycobacterium tuberculosis* (Mtb) infection. Through the exploration of molecular interactions, pathways, and cellular processes, we have uncovered key players and regulatory motifs critical for host-pathogen interactions, DNA repair mechanisms, and cellular signalling within the context of TB infection.

The identification of central nodes, such as CLEC receptors, DNA repair enzymes and mutants, non-Secretable F8 Variant, and signalling complexes, highlights their pivotal roles in mediating immune responses, maintaining genomic stability, and regulating cellular processes during TB infection. Their centrality within the network underscores their significance as central hubs or key mediators facilitating

interactions essential for both host defense mechanisms and pathogen evasion strategies.

By leveraging KEGG pathways, we have gained deeper insights into the underlying molecular mechanisms driving Mtb pathogenesis. Mathematical modelling has enabled the simulation and prediction of TB infection dynamics, providing valuable insights into disease progression and potential therapeutic targets. Network analysis has further illuminated critical nodes and regulatory motifs within host-pathogen interaction networks, enhancing our understanding of TB pathogenesis and guiding the development of effective interventions.

The integration of KEGG pathways with mathematical modelling and network analysis offers a powerful approach for studying TB, providing a holistic framework to unravel the intricate dynamics of Mtb infection. This multidisciplinary approach not only enhances our understanding of TB pathogenesis but also identifies novel therapeutic targets and informs the development of innovative intervention strategies to combat this global health threat.

In summary, the study underscores the importance of integrating diverse methodologies and approaches to unravel the complexities of TB infection. By combining KEGG pathways, mathematical modelling, and network analysis, we have advanced our understanding of TB pathogenesis and laid the foundation for future research aimed at addressing the challenges posed by this devastating infectious disease.

CHAPTER – 6

SUMMARY AND CONCLUSION

6.1 Summary:

The comprehensive analysis conducted in this study integrates various methodologies and approaches to unravel the complexities of *Mycobacterium tuberculosis* (Mtb) infection dynamics. By combining network centrality measures, KEGG pathways analysis, mathematical modelling, and network analysis, valuable insights into the molecular interactions, pathways, and cellular processes underlying TB infection have been gained. Key players and regulatory motifs critical for host-pathogen interactions, DNA repair mechanisms, and cellular signaling have been identified, shedding light on TB pathogenesis.

6.2 Conclusion:

This study underscores the importance of integrating diverse methodologies to advance our understanding of TB pathogenesis. The identification of central nodes within the network, such as CLEC receptors, DNA repair enzymes, and signaling complexes, highlights their pivotal roles in mediating immune responses and regulating cellular processes during TB infection. Leveraging KEGG pathways, mathematical modeling, and network analysis has provided deeper insights into Mtb pathogenesis, disease progression, and potential therapeutic targets. The integration of these approaches offers a powerful framework for studying TB and guiding the development of innovative intervention strategies to combat this global health threat. This multidisciplinary approach lays the foundation for future research aimed at addressing the challenges posed by TB infection.

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