Uncovering potential Drug Targets for Tuberculosis using Protein Networks

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Abstract:
The emergence of HIV-TB co-infection and multi-drug resistant strains of Mycobacterium tuberculosis (Mtb) drive the need for new therapeutics against the infectious disease tuberculosis. Among the reported putative TB targets in the literature, the identification and characterization of the most probable therapeutic targets that influence the complex infectious disease, primarily through interactions with other influenced proteins, remains a statistical and computational challenge in proteomic epidemiology. Protein interaction network analysis provides an effective way to understand the relationships between protein products of genes by interconnecting networks of essential genes and its protein-protein interactions for 5 broad functional categories in Mtb. We also investigated the substructure of the protein interaction network and focused on highly connected nodes known as cliques by giving weight to the edges using data mining algorithms. Cliques containing Sulphate assimilation and Shikimate pathway enzymes appeared continuously inspite of increasing constraints applied by the K-Core algorithm during Network Decomposition. The potential target narrowed down through Systems approaches was Prephanate Dehydratase present in the Shikimate pathway this gives an insight to develop novel potential inhibitors through Structure Based Drug Design with natural compounds.

Key Words: Protein interaction network, Mycobacterium tuberculosis, Shikimate pathway, Systems approach

Background:
During recent years, simulations of biological systems have been spurred by the massive acquisition and availability of data in molecular and cell biology. It is increasingly becoming evident that simulations can be paired with experiments, and in fact, they are customarily used by computational scientists to understand the quantitative behavior of many complex biological systems. Additionally, in-silico simulations are also successfully employed in the design of new Biomolecular experiments thus driving experimentalists. Although the gap between in vivo and in-silico biology has been remarkably reduced, there are still many limitations hindering the adoption of computational approaches in everyday Biomolecular research. Filling in this gap with Systems level approaches will help for a better understanding of mechanisms and operation of cellular processes in the Tuberculosis (TB) bacterium. TB continues to be a devastating public health problem. With the first cases of Total Drug Resistant strains reported in India during January 2012 and the mortality rate of Multi-Drug Resistance (MDR), Extremely Drug Resistance (XDR) and Total Drug Resistance (TDR)-TB is 30%, 60% and 100% respectively, there is an urgent need to identify novel targets and to develop new drugs [1].

In this paper, we create a network of Molecular Interaction Map (MIP) from a list of 141 possible targets reported in the comprehensive in-silico target identification pipeline, TargetTB [2, 3]. The proteins span across 5 broad functional categories 1) Cell Wall Biosynthesis; 2) Lipid Metabolism; 3) Intermediary Metabolism and Respiration; 4) Information Pathways; 5)
Regulatory proteins. MIP Interactome was increased using the STRING database version 9.0, with confidence scores as edge weights. The MIP now includes the interactions among these 141 targets and their interactions across other pathways. The network captures different types of interactions such as (a) physical complex formation between two proteins required to form a functional unit; (b) genes belonging to a single operon or to a common neighbourhood; (c) proteins in a given metabolic pathway and hence influenced by each other; (d) proteins whose associations are suggested based on predominant co-existence, co-expression, or domain fusion. Network Decomposition through K-Core analysis gave rise to the most influential target among the 141 selected targets and their interacting neighbors.

**Methodology:**
The interacting partners of all the 141 reported protein targets were selected from the STRING database and a network was constructed with Cytoscape 2.8.0 [9], a network visualization and analysis software. The shortest paths between all pairs of proteins in the network were computed. For every node in a network, the Network Analyzer computes its degree, its clustering coefficient, the number of self-loops, and a variety of other parameters. ClusterOne plugin used on the network strives to discover densely connected and possibly overlapping regions within the Cytoscape network. It essentially looks for groups of high cohesiveness based on the parameters, minimum size, minimum density, edge weights, merging and seeding methods. The minimum size of the cluster for all the proteins in the network was set to 15 resulting in 19 clusters (Figure 1).

The minimum sizes for the individual networks were set to 7. The highly interacting nodes in the cluster was identified by molecular complex detection (MCODE) algorithm [10], by keeping K-Core = 4 - 8, node score cutoff = 0.2 and max depth up to 100. At each level topological properties were studied to justify the important nodes/hubs playing crucial role in the functional pathways of the TB bacterium.

**Discussion:**
An MIP with 141 targets and their interacting protein partners depicting 344 molecules as nodes and 587 edges is a mathematical graph, permitting analysis with graph theoretical algorithms. Molecules like genes, proteins, transcriptional factors are denoted as nodes in the graph and interactions between them are called as edges. This MIP is a scale free network which obeys power law distribution of connectivity.

![Figure 1](image-url)

**Figure 1:** Interactome of all the 141 proteins showing 144 nodes and 587 edges in the protein-protein interaction map encompassing all 5 functional categories of protein targets in *Mycobacterium tuberculosis*.

**Figure 2:** Best five interconnected cliques among all the 141 target proteins and their interactions with neighboring proteins.

**Network Analysis**
Molecular interaction map can be represented as undirected graph M (N, E), which consists of set of nodes as N and set of edges as E. The size of the graph is given by the number of its nodes. The degree of its nodes indicates the number of interaction to a single node with all the other nodes. A clique is a complete n-node sub-graph, which means that within a sub graph, each pair of nodes is connected by an edge. Using the MCODE plugin, we have found clusters (highly interconnected regions) in the networks (Figure 2). At K-core 7, 5 sub-networks/cliques from the entire 141 proteins developed Table 2 (see supplementary material).

Clique A represents enzymes of the Lipid Metabolism. Phospholipids represented by phosphatidylethanolamine (PE), phosphatidylinositol mannosides (PIMX) and cardiolipins (CL) constitute about 25 % of total lipids and 3–7 % of total dry weight of mycobacteria (Figure 2). Clique B are Cytochromes (Figure 2), the major enzymes involved in drug metabolism and bio-activation, accounting for about 75% of the total number of different metabolic reactions [12]. Clique E is represented by Menaquinones (2-methyl-3-poly prenyl-1,4-naphthoquinones) which are the predominant lipoquinones of mycobacteria. The lipoquinones involved in the respiratory chains of bacteria consist of menaquinones and ubiquinones [7], while mammals have only ubiquinone. A detailed characterization of an aerobic respiratory chain in *M. tuberculosis* showed that
Clique C represents Cys proteins (Sulphate Assimilation Enzymes) and Clique D represents Aro proteins (Shikimate pathway enzymes) and are found to repeat themselves in the network with a stringent K-core (Figure 2). The Shikimate metabolic pathways are essential for survival and the expression of virulence in many pathogenic bacteria, including Mycobacterium tuberculosis. Extracellular presentation of sulfated metabolites plays important regulatory roles in cell-cell and host-pathogen communication [5].

Mutants with defects in sulfate assimilation indicate that the fate of sulfur in Mycobacterium tuberculosis is a critical survival determinant for the bacteria during infection and suggest novel targets for tuberculosis drug therapy [6]. The Shikimate pathway leads to the biosynthesis of chorismate, a precursor of aromatic amino acids. This pathway is absent from mammals and shown to be essential for the survival of Mycobacterium tuberculosis [4, 8, 11]. PheA (Prephenate Dehydratase) is a new interacting partner appearing along with other Shikimate pathway enzymes in the MIP Table 2 (see supplementary material).

Topological analysis of the 5 functional classes of networks in TB was done through three properties of network analysis i.e. Closeness centrality, Betweenness centrality and Node degree distribution. The R-squared value (also known as coefficient of determination) gives the proportion of variability in a data set, which is explained by a fitted linear model.

Closeness centrality is a measure of how fast information spreads from a given node to other reachable nodes in the network. The closeness centrality, Cc(n) was calculated for every functional category taking into consideration, all of the shortest path for each node. Cc(n) of node n is defined as the reciprocal of the average shortest path length and is computed as follows: 

\[ Cc(n) = \frac{1}{\text{avg} (L(n,m))} \]

where \( L(n,m) \) is the length of the shortest path between two nodes n and m. Cc(n) was high for all the functional categories leaving Intermediary Metabolism and Respiration.

The Betweenness centrality of a node reflects the amount of control that this node exerts over the interactions of other nodes in the network. In undirected networks, the node degree of a node n is the number of edges linked to n. A self-loop of a node is counted like two edges for the node degree. Node degree distribution for all the functional categories is high ranging from 0.91 to 0.98 which shows high interactive networks with the edges Table 1 (see supplementary material).

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### Supplementary material:

**Table 1:** Network property analysis showing the Closeness, between’s centrality, and the node degree distribution for all proteins, and individual networks. The number of clusters in each category is given within braces. The correlation coefficient and Coefficient of Determination ($R^2$) for each property is also given.

| Cluster-No. of merged clusters | Closeness centrality | Between’s centrality | Node degree distribution |
|-------------------------------|----------------------|----------------------|--------------------------|
| All clusters (19)             | Correlation coefficient=0.286 | Correlation coefficient=0.259 | Correlation coefficient=0.980 |
|                              | $R^2=0.085$            | $R^2=0.105$           | $R^2=0.961$               |
|                              | $R^2=0.5$              | $R^2=0.4$             | $R^2=0.944$               |
| Cell wall Biosynthesis(2)     | Correlation coefficient =0.7  | Correlation coefficient=0.4  | Correlation coefficient=0.959 |
|                              | $R^2=0.535$            | $R^2=0.845$           | $R^2=0.649$               |
| Lipid Metabolism(2)           | Correlation coefficient=0.755 | Correlation coefficient=0.861 | Correlation coefficient=0.959 |
|                              | $R^2=0.018$            | $R^2=0.279$           | $R^2=0.568$               |
| Intermediary Metabolism and   | Correlation coefficient=0.177 | Correlation coefficient=0.217 | Correlation coefficient=0.984 |
| Respiration(15)               | $R^2=0.018$            | $R^2=0.279$           | $R^2=0.568$               |
| Information Pathways (2)      | Correlation coefficient=0.897 | Correlation coefficient=0.892 | Correlation coefficient=0.912 |
|                              | $R^2=0.865$            | $R^2=0.865$           | $R^2=0.784$               |
| Regulatory Pathways (3)       | Correlation coefficient=0.771 | Correlation coefficient=0.217 | Correlation coefficient=0.926 |
|                              | $R^2=0.516$            | $R^2=0.025$           | $R^2=0.538$               |

**Table 2:** The 5 best Cliques generated by MCODE at K-core-7, node score cutoff = 0.2 and max depth up to 100 along with interacting protein partners and names of the proteins.

| CLIQUE No. | INTERACTING PROTEINS | SCORE | NODES | EDGES | NAME OF THE PROTEIN |
|------------|-----------------------|-------|-------|-------|---------------------|
| 1          | KasA FabG FabH        | 5     | 9     | 45    | 3-oxoacyl-(acyl carrier protein) synthase II |
|            |                       |       |       |       | 3-oxoacyl-[acyl-carrier protein] reductase |
|            |                       |       |       |       | 3-oxoacyl-(acyl carrier protein) synthase III |
| Lipid Metabolism | FabD InhA AcpM KasB MabA AccD3 |       |       |       | acyl-carrier-protein S-malonyltransferase |
|            |                       |       |       |       | enoyl-(acyl carrier protein) reductase |
|            |                       |       |       |       | acyl carrier protein; Acyl carrier protein involved in meromycolate extension |
|            |                       |       |       |       | 3-oxoacyl-(acyl carrier protein) synthase II |
|            |                       |       |       |       | fabG1 - 3-oxoacyl-[ACP] reductase |
|            |                       |       |       |       | putative acetyl-coenzyme A carboxylase |
|            |                       |       |       |       | carboxyl transferase subunit beta |
| 2          | Cyp143 Cyp142 Cyp128 | 4.429 | 7     | 32    | cytochrome P450 143 |
| Cytochrome P450’s | Cyp130 Cyp141 Cyp125 Cyp121 |       |       |       | cytochrome P450 130 |
|            |                       |       |       |       | cytochrome P450 144 |
|            |                       |       |       |       | cytochrome P450 128 |
| Sulphate Assimilation | CysD CysNC | 4.375 | 8     | 35    | sulfate-transport integral membrane protein |
| Enzymes    | CysN CysW CysH SubI   |       |       |       | ABC transporter CysT |
|            |                       |       |       |       | sulfite adenylyltransferase subunit 2 |
|            |                       |       |       |       | bifunctional sulfate adenylyltransferase |
|            |                       |       |       |       | subunit 1/adenylsulfate kinase protein |
|            |                       |       |       |       | sulfate-transport integral membrane protein |
|            |                       |       |       |       | ABC transporter |
|            |                       |       |       |       | phosphoadenosine phosphosulfate reductase |
|            |                       |       |       |       | sulfate-binding lipoprotein |
|            |                       |       |       |       | sulfate-binding lipoprotein |
|            |                       |       |       |       | shikimate kinase |
| 4          | AroK AroA             | 4.286 | 7     | 30    | 3-phosphoshikimate 1- |
| Shikimate Pathway | AroC AroB AroE PheA AroQ* |       |       |       | carboxyvinyltransferase |
| Enzymes    |                       |       |       |       | 3-dehydroquinic synthase |
|            |                       |       |       |       | 3-dehydroquinat dehydratase |
|            |                       |       |       |       | chorismate synthase |
|            |                       |       |       |       | prephenate dehydratase |
|            |                       |       |       |       | shikimate 5-dehydrogenase |
| 5          | MenA                  | 3.8   | 5     | 20    | 1,4-dihydoxy-2-naphthoate |
| Menaquinone Biosynthesis | MenE MenC MenD MenB |       |       |       | octaprenyltransferase |
|            |                       |       |       |       | O-succiny1benzoic acid-CoA ligase |
|            |                       |       |       |       | O-succiny1benzoate synthase |
|            |                       |       |       |       | 2-succiny1-5-enopyruvyl-6-hydroxy-3-cyclohexone-1-carboxylate synthase |
|            |                       |       |       |       | napthoate synthase |