Integrating mechanism-based screening paradigm into homology and de novo modeling exemplified by *Mycobacterium Tuberculosis* 30S ribosomal structure and its potential application as a screening target

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Abstract— *Mycobacterium tuberculosis*, the causative agent of the tuberculosis, has infected more than 33% of the total populace to date. It is known to be considerably aggressive and profoundly impervious to current medications that target it. Aminoglycosides, for example, viomycin and capreomycin have been shown to bind to important functional regions of the *M tuberculosis* ribosome thus its process of protein synthesis is inhibited subsequently affecting the cell viability. Current techniques for studying drug interaction through generation of three-dimensional macromolecular structures are slow and tedious. This study exhibits a more proficient and cheap method for producing structure models in silico by utilizing both de novo and homology modeling strategies.

Here, a three dimensional 3Å high-resolution crystal structure of the 30S ribosomal subunit from *Mycobacteria tuberculosis* through structure prediction modeling methods is reported. This ribosomal structure is practically identical in quality to experimentally determined crystalline structures. This technique gives novel chances to drug target screening, and will be critical in the advancement of new classes of anti-bacterial compounds. Screening of ligands In-silico can be done to distinguish compounds that show binding potential on ribosome structures. This study demonstrates *Mycobacteria Tuberculosis* 30S ribosomal subunit generation that provides a structural scaffold allowing in silico design of drugs.

Index Terms— Ribosome; 30S subunit, rRNA, comparative analysis, three-dimensional modelling, RMSD, Docking.

Graphical Abstract

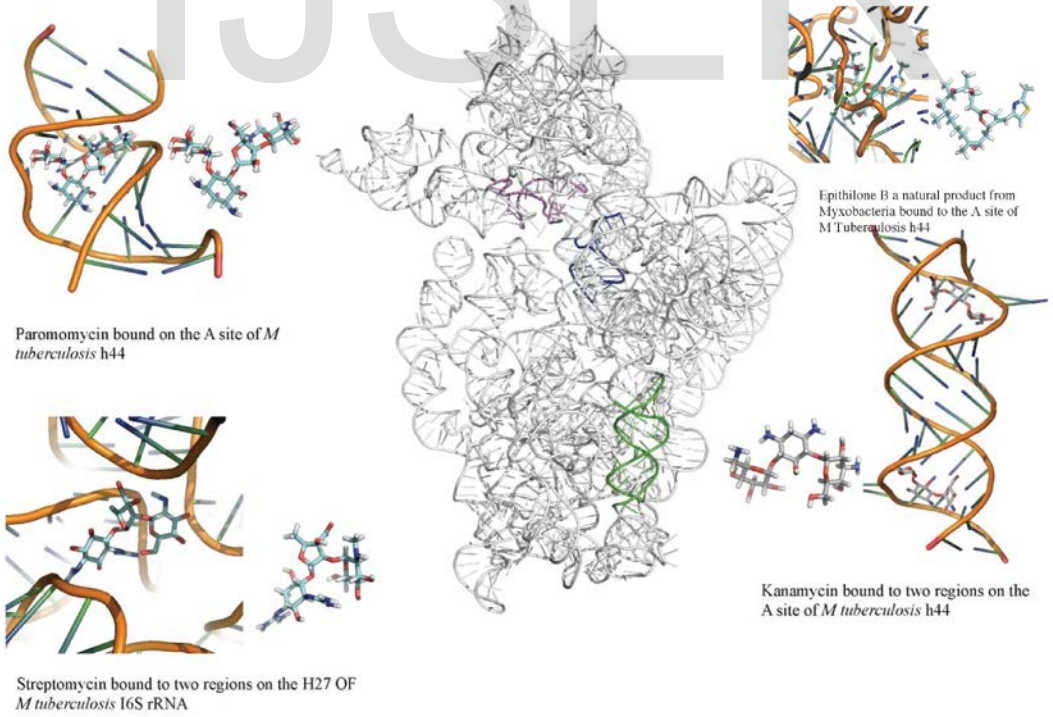

Figure 1: Graphical summary showing homology and De novo modeled *Mycobacterium tuberculosis* 16S rRNA motifs bound to antibiotics

1. INTRODUCTION

1.1 Homology modeling of *M tuberculosis* ribosome

A critical problem in combating Tuberculosis(TB) infections is that many of the existing TB drugs target processes common to both the bacteria and mammalian cells[1]. For several currently used anti-TB pharmaceuticals there are no known target molecules. The drug toxicity of these is readily apparent as these drug targets are likely to be homologous between molecules or structures of both pathogens and mammals. Selecting new targets is complicated due to the numerous similarities at the cellular as well as biochemical levels. These observations demonstrate the need to identify and utilize newly identified TB targets that are absent in humans if we are to develop more specific, effective and safer anti-TB agents[2, 3] . Meanwhile, the identification of specific targets for new TB pharmaceuticals are difficult to validate given the significant gaps in our knowledge of pathogen biology[4,5] . To overcome these challenges, the study developed a novel synthetic ribosome chemical screen that opens new avenues for the identification of specific ribosome inhibitors and for the validation of ribosome drug targets[6]. This study has moved the TB ribosome into the computer and back, developed both digital and synthetic ribosome chemical screen to identify inhibitors with a known novel mode of action targeting ribosome function. The future treatment of many diseases lies in understanding newly emergence of molecular mechanisms that or which are still to be discovered in the pathogen[7]. The preeminent approach to help stop the spread of this deadly pathogen is to focus on breakthrough science and develop novel, targeted therapies that interact in a specific manner so as to terminate the pathogen survival.

The platform technology used in this study is based on a software system developed by Prof. Santa Lucia at Wayne State University[8-10] that allows the homology and de novo modelling of prokaryotic ribosomes. The study employed this technology to identify and characterize new drug target sites in TB ribosomal RNA. Roughly half of entirely naturally occurring drugs affect protein synthesis, plus most target the ribosomes[2]. All living things have ribosomes composed of RNAs (rRNA) and proteins which are complex macromolecular machines that catalyze protein synthesis. Human and bacteria RNAs are adequately dissimilar to permit precise drug interactions. Demonstration by Nuclear Magnetic Resonance and X-ray crystallography show that rRNA is composed of independent architecturally subdomains that are accessible to small molecules and drugs even in the entirely folded rRNA structure. These features make rRNA an ultimate remedy target and the modular nature of rRNA enables molecular-modeling studies for drug design, structure resolve, and *in vitro* assays development.

Three-dimensional structures can be modeled through several methods; experimental methods which includes nuclear magnetic resonance, cryo-electromagnetic spectroscopy, and X-ray crystallography. These experimental methods are limited by the time it takes to generate a model and also by the size of the molecule itself[11,12]. Other methods of protein and the RNA three-dimensional structure prediction include; the comparative method and *de novo* modeling[13]. These methods are often more rapid than the experimental methods and frequently lead to structures of comparable quality. In addition, they allow structures to be generated for molecules that are not easy or impossible to crystallize or create.

Homology modeling is the process by which a bio macromolecule three dimensional structure is generated through a series of computational steps[14]. This requires the use of an existing homologous structure as a template and then threading in the sequence to be modeled. In comparative (homology) modeling, two conditions have to happen; mainly, a detectable sequence similarity between template and query must be met. Secondly, an accurate alignment computed between the template structure and the query sequence [15,16]. *De novo* methods on the other hand allow tertiary structure prediction from the sequence data alone, without relying on similarity between any known structure fold level and the template[17]. These two methods usually complement each other during structural modeling, as regions that are not similar to both query and template have to be built in from sequence information.

Homology modeling is mostly preferred because it can be accurate and faster if a highly homologous template is identified, as compared to other physics based approaches in the comparative modeling[18]. Homology and de novo modeling methods were employed in the modeling of the 30S ribosomal sub unit of *Mycobacteria tuberculosis*. The control and management of TB has been complicated by the emergence of a drug resistant strain, and latent infection[19]. The bacterial ribosome is a validated drug target[20], and thus an excellent candidate for the study of new anti-bacterial anti-infective agents. Structural studies on the *M. tuberculosis* ribosome may help in understanding mechanistic models that interpret collected biochemical data[20].

Protein synthesis is executed by complex of apparatus including of ribosomes, transfer RNA (tRNA) and the messenger RNA (mRNA)[21]. Ribosomes are made up of two segments that come together during protein synthesis and dissociate after the protein is synthesized[22].

In the prokaryotic ribosome, the small subunit (30S) contains a single copy of a ribosomal RNA (16S rRNA) and 21 proteins[23]. 16S rRNA varies in size from organism to organism, for instance, in *Escherichia coli* is 1531 nucleotides long while in *Mycobacteria tuberculosis* it is 1538 nucleotides long. The larger subunit (50S) contains two ribosomal

RNAs (5S and 23S) and 31 proteins while in eukaryotic ribosomes, the lager subunit, and (60S) consist of three ribosomal RNA (rRNA) molecules (25S, 5.8S and 5S) and 46 proteins. The small subunit (40S) is comprised of one rRNA chain (18S) and 33 proteins [24]. The ratio of ribosomal RNA residue to protein residue in the ribosome has been maintained at two to one (2:1) with the exception of mitochondria ribosome[25]. Due to the large size of the 16S rRNA it is sub divided into four domains which fold independently of each other; the 3' minor domain, 3' major, central domain, and the 5' domain during the process.

Proteins on the other hand are made up of peptides or several polypeptides joined together. They are classified according to their domains; the distinct components of three-dimensional structure that is able to carry out certain molecular function including binding, catalysis[26,27]. The protein function is determined by its structural folding rather than its sequence thus; protein with low sequence similarity may have similar folding and function[27,28,29].

1.2 *M. tuberculosis* Ribosome as Drug Target

Around half of all naturally occurring drugs influence synthesis of protein, and a large portion of these target ribosomes [2]. Ribosomes are mind boggling macromolecular machines in all living things made out of RNAs (rRNA) and proteins that catalyze protein synthesis [30]. The rRNAs are the most useful parts of ribosomes[31]. Bacteria and human RNAs are adequately different to permit drugs particular and distinct interaction[32, 33]. X-ray crystallography and NMR have shown that rRNA is made out of fundamentally independent subdomains that are open to small molecules and drugs even inside the completely folded rRNA structure[2, 32]. These attributes make rRNA a perfect target for drugs and the particular nature of it boosts development of structure determination, in vitro assays and molecular studies for drug designs [34]. There is a pressing requirement for novel and effective medications to treat tuberculosis on the grounds mechanism of resistance for all as of now utilized therapeutics has been resolved [35]. There are a few ways that cells can get to be distinctly impervious to drugs. These incorporate (1) lessening the take-up of the medication into the cell: i) decrease of antibiotic take-up, ii) transport of the antibiotic out of the cell, iii) antibiotic enzymatic inactivation, iv) utilization of an option metabolic pathway, v) overproduction of the target to titrate the antibiotic, and vi) target adjustment with the goal that it is no longer perceived by the drug [36]. Of these components, target change is the most widely recognized mechanism used to handle resistance for recently developed antibiotics. The specificity of medication target binding includes the structure in addition to the sequence of the target [36]. Mutations that influence the structure or sequence of the target without affecting capacity may reduce or wipe out antibiotic binding resulting in resistance[6 For instance, aminoglycoside drugs focus on the A-site of bacterial 16S ribosomal RNA and increase the translational blunder rate[6,36]. Insights into molecular aminoglycoside resistance and action have shown that a solitary A1408G transformation decreases ribosome work by around 30% yet totally disturbs binding of certain aminoglycoside antibiotics[36]. Therefore, focusing on a drug to specific ribosomal region every single conceivable mutant that keeps up function would eliminate this resistance mechanism [35,36]. The ultimate danger for new classes of drugs is transformation of the target as, alterations happen actually in all cells. In the event that a alteration in the drug-target keeps the drug from binding then cells with mutant types of the target may turn into the transcendent life forms if the change gives insurance from the drug without meaningfully influencing the destructiveness of the pathogen [37].

It is fundamental to understand the function and structure of the ribosome with the purpose of appreciating different states of the ribosome in synthesis of proteins [38]. Some antibiotics such as capreomycin are targets to the *M. tuberculosis* 16S rRNA that is highly conserved [38,39]. Since most of the antibiotics target the protein synthesis process, getting the ribosome's three-dimensional structure and understanding how it functions, is crucial in the process of understanding how antibiotics function[13]. It will also help in understanding how mutations are induced example in the case of resistance to streptomycin[40]. This study demonstrates a more efficient and improved way of modeling a large molecule such as the bacterial ribosome using homology and *de novo* modeling. It further focuses on the *in silico* modeling of the 30S ribosome from *Mycobacteria tuberculosis*, one of the leading opportunistic killer pathogens in the world, and its viability as a screening tool. We have employed this technology for identification and characterization of new drug target sites in TB ribosomal RNA.

1.3 Screening

The invention of homology and de novo modeling of biological macro molecules high resolution crystal structures such as the *M. tuberculosis* 30S offers another novel process that uses the three dimensional structure for identifying ribosome-related ligands and for designing ligands. These ligands have specific ribosome binding properties that may act as protein synthesis inhibitors. Thus the combination of this unique process may be used to produce ligands that could be designed to specifically inhibit or kill any target organisms growth especially the causative agents of tropical neglected diseases. In addition, this process could be used to realign prior used drugs to specific target that have developed resistance to others that could be possible targets.

2. METHODOLOGY

2.1 Proof of concept

To validate the use of *RNA123* software [8] in the homology and *de novo* modeling of the 30S ribosomal subunit. The study was initially designed to model the three dimensional structure of a bacteria whose structure had already been solved using X-ray crystallography. Ribosomal RNA structure of *Escherichia coli* (PDB ID 4V4Q) *was* modeled using the template crystal structure of *Thermus thermophilus* (PDB ID 5IWA).

In order to simplify the modeling process, 16S rRNA template structure was subdivided into four main domains the 5', central, 3' major and 3' minor. This was accomplished based on the secondary structure of the template and query. As for the protein modeling, the process followed is as described in detail in the section below.

2.2 rRNA and protein for proof of concept

The structures modeled were super imposed on the original structure and the root mean square deviation calculated. The 16S rRNA RMSD was 3Å in average. While for protein ranged from 1.51 Å and 2.83Å. It is important to note that despite a lesser accurate template structure the software refines the coordinates for the query to provide an accurate final model with both homology, de novo and super energy minimized structure.

Modelled Domains	*Thermus thermophilus* (PDB ID **5IWA**).	*Escherichia coli* (PDB ID **4V4Q**)
Domains	RMSD'S	RMSD'S
3' Minor	1.64 Å	3.72 Å
3' Major	1.98 Å	3.24 Å
Central	2.94 Å	2.98 Å
5' Major	1.48 Å	3.92 Å

Table 1: RMSD results for the modeled *Thermus thermophilus* domains superimposed with structure generated with x-ray crystallography structure of the same and from the template *Escherichia coli* respectively.

2.3 Generating protein template and the query

The sequence of the *Tuberculosis* strain H37Rv (gene bank (gi|448814763:1471846-1473382)) was used as the query in the modeling process[41]. *E. coli* (PDB ID 4V4Q) as the ideal template was used, since it has almost equal sequence length, its crystal structure is available, and its secondary structure is similar to *M. tuberculosis.*

Protein modeling of the *M. tuberculosis* query proteins was determined by homologs to those of *E.coli*, which were obtained from searches through BLAST (Basic local alignment tool[42]. The query sequence versus the template Protein co-ordinates obtained from protein data bank (RSCB)was submitted for modeling in the Swiss model server for protein homology modeling[43-45]. Sequence alignment was carried out in the server and template had a sequence similarity of above 75% to the query sequence which was good. Sequence identity of 50% between the template and the query were also used. Then finally Model coordinates are generated.

2.4 RNA Modeling

16S rRNA of *M. tuberculosis* was first dissected into different domains according to the secondary structure that had same loops as that of *E. coli* template. Each domain was modeled separately by the use of the RNA123 software [8-10]. The different generated parts then were joined together using RNA 123 and energy minimization done to form one complex the 16S ribosomal RNA structure. Figure 3. An important note is that 16S rRNA is highly conserved and the sequence alignment of both template and query are highly similar. With that information in mind RNA 123 does homology modeling in areas that are similar to each other and in areas that aren't similar, it has capability to de novo model that section by building a consensus sequence and produce a final model (see Supplementary Figure 1 and 2 of structure alignments).

2.5 Validation of the structure models

After modeling, the structures acquired were checked for structure consistency, correct fold formations and general structure viability. This process was accomplished by running the models through analysis tools such as PROCHECK[46] and MOLPROBITY[47]. MOLPROBITY checks for the flipped atoms in the structure, presence of the hydrogen's if not available it adds them. It's also able to generate a Ramachadrianplot, which shows whether the protein falls out of allowed conformational ranges during modeling or not[47,48]. PROCHECK is mostly used to access the general protein structure by checking the stereochemistry of the structure and root mean square deviation (RMSD)[49].

2.6 Docking and binding

Docking refers to computational simulation of a receptor site binding to a potential ligand, which in our case could be the receiving molecule the ribosome or any of its content. Once the binding has occurred the positioning of the small molecule (ligand) relative to the receptors well as the complex conformation when bound to each other is determined by the pose which is the candidate binding mode. This is then scored by evaluating particular poses by calculating the favorable intermolecular interactions such as the hydrophobic contacts and hydrogen bonds number. Ranking is then done by classifying the ligands that are highly probable to interact constructively to precise receptor founded on projected free-energy required in binding itself. Finally, a docking

valuation is p

performed to measure the predictive capability of the whole process. See Figure below

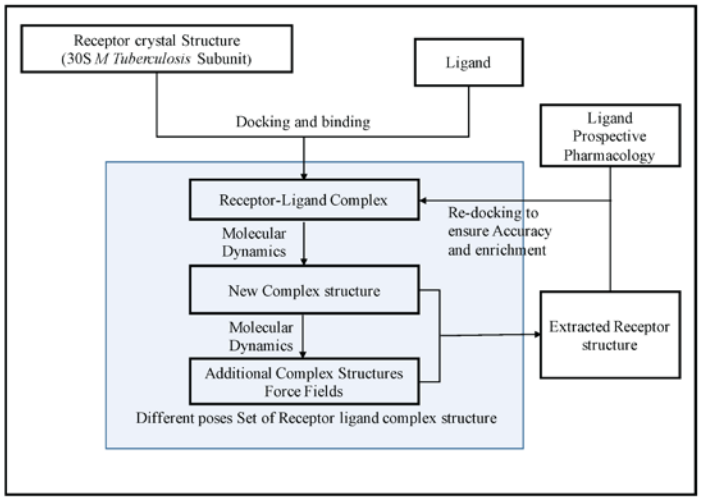

Figure 2: Docking and binding flow chart showing the different processes of *in silico* screening

2.7 *In Vitro* assay analysis to validate *in silico* approach

Pure colonies of *Mycobacterium tuberculosis* were isolated from sputum as specimen from patiens as described in the WHO report of 2007 and later processed using the NaOH-Nacl method. Zieln-Neelsen staining technique was used on a sample colony to confirm presence of t he tuberculosis bacteria microscopically. The pure colonies were further cultured together within a Mycobacterium growth indicator tube on a Lowenstein-Jensen solid culture. After 8 weeks of gro wth the development of a mRNA screening assay platform by extraction of t he genomic DNA was performed as described by Kate Wilson, 1987. DNA analysis by TAE/Agarose/Ethidium bromide gel electrophoresis was performed latter followed by PCR amplification of 16S rDNA. The quality of the PCR products was checked on a 1% agarose gel and later on Gel band purification kit and gel purification using ILLUSTRA GFX PCR DNA. JM109 *E. coli* cells transformation was then performed by preparing and assessing competent cells with recombinant plasmid containing the 16S rRNA insert. The plasmid DNA was latter extracted and the negative and positive plasmid quality determined by electrophoresis on agarose gel. T7 and Sp6 promoter PCR amplification was carried out with replacement of t he primers with T7 and Sp6 and rplasmid taken for sequencing. Linearization of recombinant DNA and negative control plasmid DNA was confirmed by running 5µl of plasmid on a 1% agarose gel electrophoresis. The plasmid was nicked with Not1 which cleaves downstream of the T7 promoter.

2.8 In vitro RNA labeling transcription analysis with DIG RNA of 16S DNA to validate *in silico* approach

The TB 16S DNA to be transcribed was cloned into the polylinker site of transcription vector, which contains a promoter for SP6, T7 or T3 RNA polymerase (Dunn and Studier, 1983; Kassavetis, 1982). Synthesis of the DIG labeled run-off transcripts was performed following the protocol of Milligan *et al.,* 1989. that creates a 5-overhand b efore transcription employed to linearize the template. The RNA products were denatured using the Glyoxal method before running on a gel in turn was followed by a Glyoxal/DMSO electrophoresis of the RNA.

Lead compounds were generated by preparing of azide functionalized paper strip a protocol according to Aminova *et al.,* 2008. The compound (ligand) array was spotted onto the azide functionalized surface, incubated at room temperatures. The array was later washed to remove the uncoupled antibiotic and the coupling reagents and left to dry.

Hybridization and detection of the labelled RNA was performed by denaturing a chilled probe and quickly added to a hybridization buffer. The pre hybridization solution was then poured off and the solution add3d to a hybridization bag. The antibiotic strip was incubated with the hybridization solution at 50 ^{0}C for about 16hours with gentle agitation. The strip was later removed and washed twice at room temperature in ample low stringent buffer (2× SSC, 0.1% SDS), and twice for 15 minutes in pre-heated high stringency buffer (0.5× SSC, 0.1% SDS) at 65ºC under constant agitation. Chromogenic detection method was used to localize the probe-target hybrids on the strip using the Block buffer set kit and DIG wash. Wash buffer was discarded and the membrane with a blocking solution was incubated with constant agitation. Afterwards the blocking solution prepared by diluting Anti-Digoxigenin-AP 1:5000 (150 mU/mL) in blocking solution discarded, and then with gentle shaking antibody solution was added. The membrane was washed twice in a washing buffer after discarding the antibody solution then equilibrated in 20ml of detection buffer.

Finally the strip was flooded with 10 m L of s ubstrate colour solution prepared by adding 200µl stock solution of NBT/BCIP to 10 mL of detection buffer and incubated for 16 hours in the dark at room temperature without shaking. When the colour reaction had produced bands of required intensity the reaction was stopped by dipping the membrane in 50 mL of TE.

3. Results

Through the process of m odeling the 30S ribosomal subunit of *M. tuberculosis,* 21 proteins and the 16S ribosomal RNA domains was modeled. The rRNA four dom ains were joined to form one major structure the 16S rRNA that was later combined with the 21 ribosomal

proteins to form the total 30S ribosomal subunit of *M. tuberculosis*. Shown in figure 3. (See in supplementary information the energy minimization and optimization graphs Sup figure 3-5)

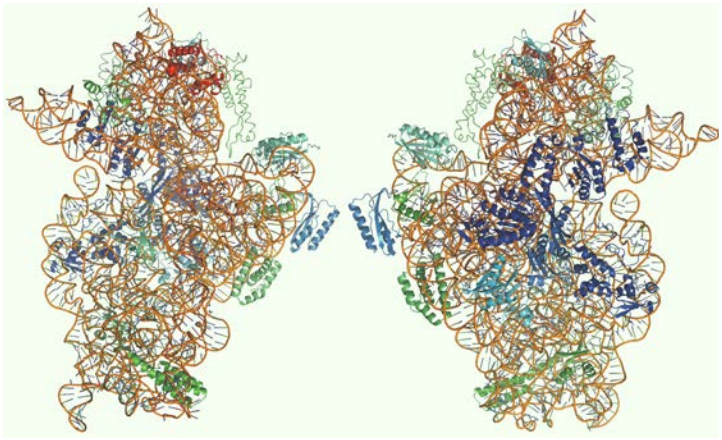

Figure 3: Front and back view of the combined 30S*M. tuberculosis* ribosomal subunit.

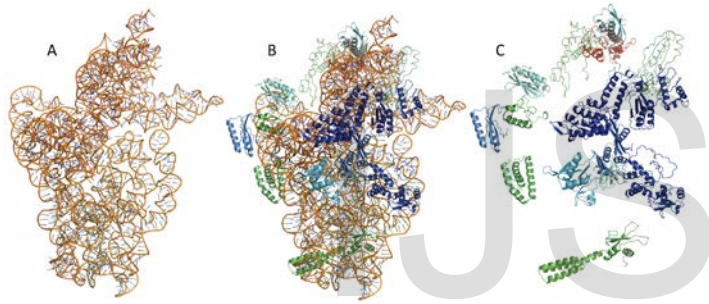

Figure 4: A) Total structure of *M. tuberculosis* 16S rRNA (left) B) Total structure of the *M. tuberculosis* 30S ribosomal subunit (Centre) C) 21 proteins of *M. tuberculosis* in space (right).

for drug target primarily due to its biological and the biochemical basis [50]. It's linked with human disease and due to its differences with the human it has well defined binding grooves and pockets [50,51]. As previously reported there are anti-tuberculosis agents that have been shown to attack the ribosome and especially the 30S unit. Both capreomycin and viomycin which are midst the best effective agents against the TB multidrug resistant from the family of tuberactinomycin bind at the same site which is the boundary between the helix 44 of the 30S subunit and 69 of the large subunit[52,53]. This suggests these drugs inhibit the translocation process in the A site of by stabilizing the tRNA[52,54]. After obtaining the 3 Å resolution 30S ribosome subunit of *M. tuberculosis*, the coordinate data could be useful for the rational modeling and design of potential drugs or inhibitors to the process of translation.

Binding and docking involves interaction examination of the 30S subunit of *M. tuberculosis* and the binding drug lead complex and eventual evaluation, using docking programs such as AUTODOCK, DOCK, GRAMM, GLIDE [55,56]. The predicted structure of *M. tuberculosis* 30S-ligand complex is obtained from various computer programs and a number can be used as mentioned above which include the procedure referred to as docking [57,58]. Minimization of repulsion and steric hinderances between the potential drug and the *M. tuberculosis* 30S ribosome can be improved greatly by applying appropriate modification of the chemical structure and shape of the ligand [56].

30S *Mycobacterium Tuberculosis* Docking Results

Molecule name	Score	Pen.	Area	ACE	Helix	Transformation
Paromomycin	6916	-2.25	819.80	-277.31	44	0.32102 -0.20217 -0.66101 -91.43852 123.35450 76.79561
Kanamycin	6296	-2.24	747.00	-324.27	44	0.70178 -0.11278 2.66706 -39.30261 79.57573 105.61857
Streptomycin	6580	-2.24	748.20	-386.88	27	-0.73033 0.84895 -0.38315 -96.77123 101.52655 82.62246
Epithilone B	7002	-2.72	871.40	-327.80	27	1.26138 -0.21315 -0.53140 -101.11861 81.60175 86.86482

Table 2: *M. tuberculosis* 30S ribosomal subunit docked to some known antibiotic drugs (Paromomycin, Kanamycin, and Streptomycin) and a novel compound epithilone B

3.1 Screening

The 30S ribosomal subunit for *M. tuberculosis* is a very important choice

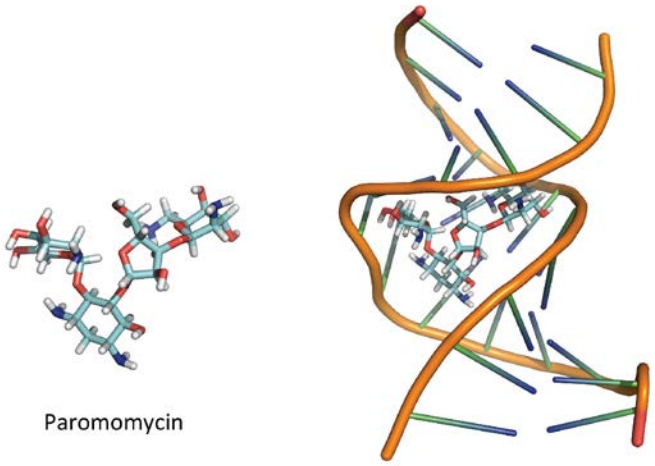

Figure 5: 30S subunit of *M. tuberculosis*16S rRNA A site considered important as binding site for antibiotics bound to Paromomycin

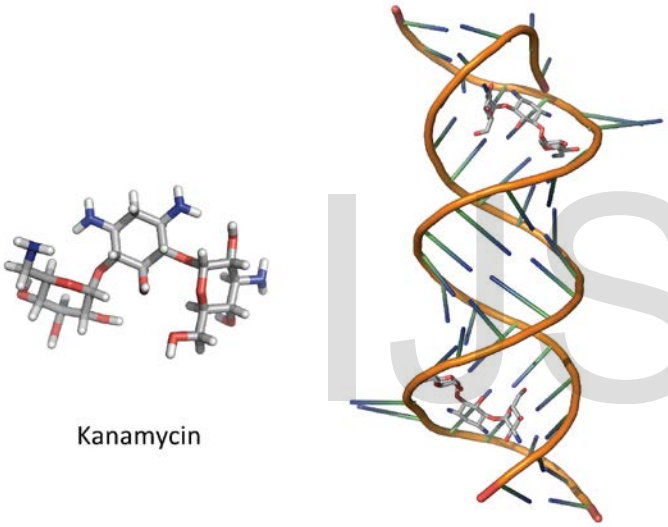

Figure 6: Kanamycin shown bound t o 2 re gions of the *M. tuberculosis*16SrRNA a site of 30S subunit

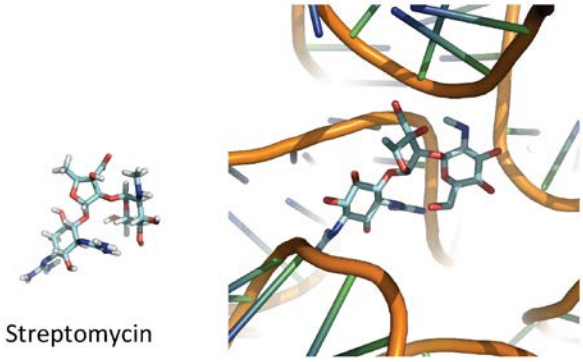

Figure 7: *M. tuberculosis*16S rRNA A site considered important as binding site for antibiotics bound to Streptomycin

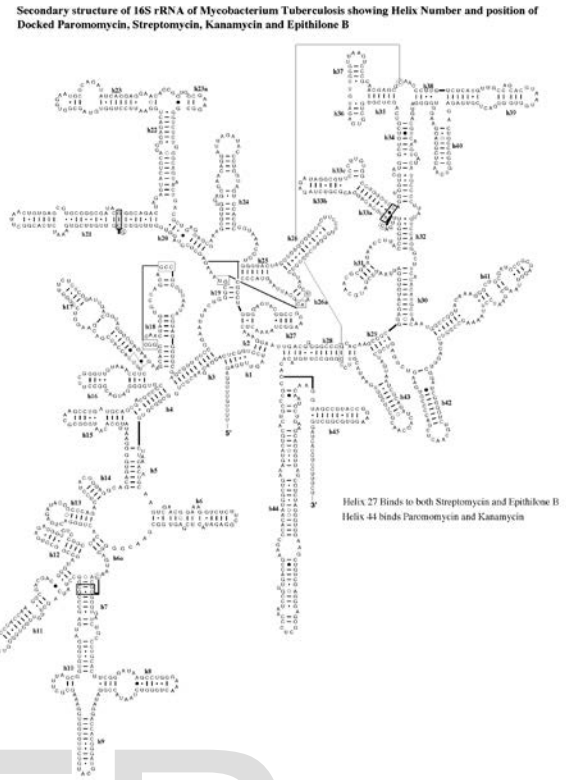

Figure 8: 30S subunit *M tuberculosis* 16S rRNA secondary structure showing the helix numbers

The 30S subunit ligand complex was further analyzed using computational tools that transform the structure of the ligand so that it could fit specifically and stably with minimal energy and strike the best pose at the active defined site of the *30S M. tuberculosis* subunit and the difference of Fourier electron density maps were recorded. It's important to note that the prediction quality is dependent on the accuracy of both the structures.

3.2 *In vitro* Screening for RNA –antibiotic interaction

Mycobacterium TB 16S rRNA corresponding RNA probes were made by run-off transcription of the vector(Invitrogen) with SP6 or T7 polymerase, respectively using DIG-RNA labeling kit (Roche Diagnostics Corp. Indianapolis Ind.). The sections were prehybridized at 37C° in 50% form amide, 5× Denhardt's solution, 5× SSC, 25 0 g/ml yeast t-RNA, 1 mg/ml salmon sperm DNA and 4 mM EDTA for 60 m in and hybridized in the prehybridization buffer (without the salmon sperm DNA) containing 2.5 to 10 ng/l DIG-labeled cRNA probes at 48 C° overnight. Sections were then rinsed in 4× SSC (2×10 min), 2× SSC (10 min) and 1× SSC (10 m in) and 0.1× SSC (30 m in at 48° C.). The DIG-labeled RNA was detected with mouse anti-DIG IgG followed with a DIG conjugated antibody to the mouse IgG Fab fragment and finally

anti-DIG IgG conjugated with alkaline phospahatase (Roche). Strips impregnated with antibiotics were processed in parallel under the same conditions and using the same batches of probes and reagents. Hybridization was done using the labelled RNA to screen for interaction of RNA and selected antibiotic. Alkaline phosphatase conjugated with fragments of polyclonal anti-DIG Fab were used to detect the probe-target hybrids. BCIP (5-Bromo-4-Chloro-3-indoyl phosphate toluidine salt) and Nitro blue tetrazolium chloride (NBT) were used as Chromogenic substrates. Detection is made more sensitive in this reaction by colour intensities due to further reaction after dephosphorylation of the AP-substrate(BCIP) as an oxidation product while NBT serves as an oxidant both giving an dark-blue dye.

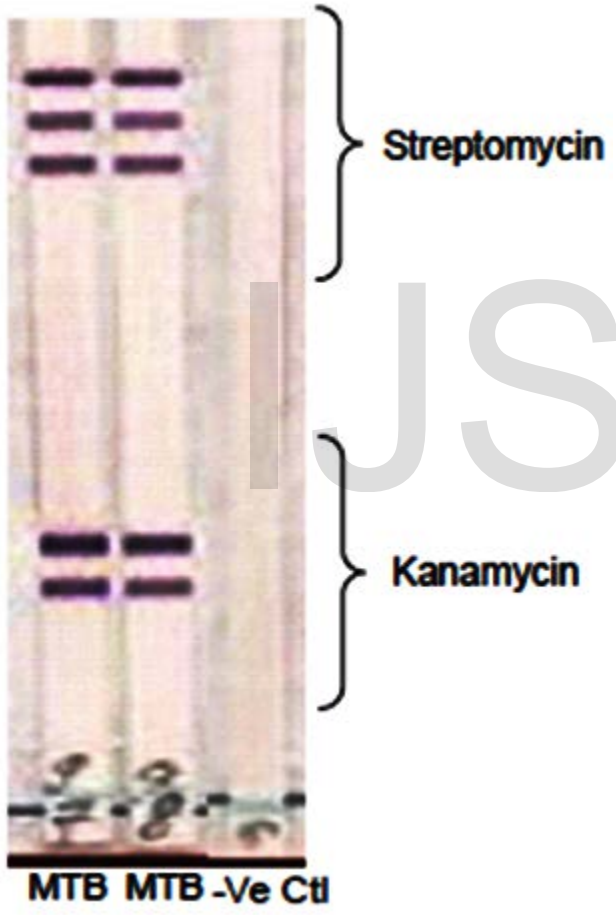

Figure 9: Hybridization of target- probe interaction (labelled MTB rRNA) on antibiotic (Kenamycin and streptomycin) platform with a negative control (–ve Ctl; water)

4. DISCUSSION

Since the homology and de novo modeling process used in the actual project is the same as the one in the proof of concept, we have confidence in the structure obtained (the 30S ribosomal subunit of H37Rv *Mycobacteria tuberculosis*).

During the RNA modeling process, it was noted that some nucleotide miss match lead to distortion of the structure while others did not. This mostly depended on the position of the mismatch, in that, most mismatches in the helical stem had no effect but mismatches on the hairpin had effect on the structure. On the other hand, any gap that occurred in the alignment lead to a distorted structure. In order to avoid this, modeling of the RNA had to be carried out on short sequences that could be easily aligned to avoid gaps.

The results obtained from the protein modeling process are encouraging, since in the proof of concept where the result obtained is very similar to the original with some having an RMSD of as less as 1Å, which shows similarity between the two structures.

SUP Table 2 shows the results of the RMSD of the proteins that were obtained and are within the acceptable range; in addition, the Z-score is within the acceptable range too.

As with the structure obtained by a primary method such as X-ray crystallography, the results obtained of the 30S subunit by homology modeling shows the different grooves of the structure, reserved areas and also the different folding patterns. During the process of energy minimization, it was evident the structure energy changed from positive to the negative as given in the table that infers the structure had more biological conformation. These process ensured an adequate structure that is compact and more biologically relevant. This structure was then used to demonstrate that possible screening of drugs could be performed *in silico* to already known ligands.

4.1 Homology and *De novo* modeling studies of TB rRNA Domains.

NMR spectroscopy, Homology and de novo modeling can be utilized to decide key RNA targets functional groups and provide numerous sorts of data that is critical. First, homology modeling can be used to validate whether the rRNA structure targets reserved out of the framework of the ribosome resemble their ribosome structure and can therefore be effective targets for compound libraries screening [59]. Homology modeling can likewise be utilized to give definite mechanism of binding of RNA targets with small-molecule ligands stereochemical information. To sum up, homology-modeling studies can be used to expose fundamental changes between the TB and the human small subunit rRNAs [60]. The wild type comparison with the transformed assemblies will expose the vital functional groups and structural motifs essential for ribosome purpose, thus targeting these critical residues for designing drugs. Characterization of RNA-ligand structural complexes with antibiotics in this study discloses in what way every single compound identifies the vital target motifs. Additional, dynamics characterization of

RNA by homology modeling in the presence and absence of bound antibiotics reveals the role of induced fit in RNA recognition [6,32,60].

Importantly, the homology modeled TB structure falls within 0.8 Å RMSD with the X-ray structure of the small-subunit, demonstrating that the motifs identified used in antibiotic binding studies here are binding for use in drug screening [61]. The structures show numerous new topographies forming novel motifs and are pre-organized into a conformation that is helical, apparently for contact with the other ribosomal component to facilitate protein synthesis. Data comparison achieved using homology modeling with X-ray structural studies and NMR studies allows association of specific sequence elements in rRNA targets with purpose of the ribosome. These approaches are powerful tools in the eventual discovery and design of new antibiotics [2,35].

4.2 Antibiotics Screening Strategy

Most screening methodologies are focused on the small molecules to proteins binding identification. [6]. Although ample can be learned from the study of therapeutics that interacts with proteins, our focus is on exploiting strategies that use RNA target-based drug design. In order to recognize molecules that will bind to the nucleotides in the RNA targets favorably, we have employed specific RNA-ligand based screening assays and a combination of diverse antibiotics and compound collections, including small libraries of RNA-binding antibiotics compounds [2,6].

The theoretical crystal structure of the obtained 30S *Mycobacterium Tuberculosis* ribosomal subunit data proposes binding sites. Herein, Maestro was used to explore and scrutinize the potential sites for ligand binding in the structure. Maestro created a grid, to reveal possible binding modes of different ligands to cover all possible generated sites of binding. Consequently, the most favorable ligands binding poses adopted. Different ligands binding poses were examined and ranked based on their docking scores by the docking result report. Further analyses were performed for the selected pose with lower score that suggests the ligand binding mode was the most probable. The ligand preferable binding poses for the three known antibiotics and one novel compound against *Mycobacterium Tuberculosis* 30S ribosomal subunit were performed and the best binding position of the complex docked perfectly into the ribosome structure with the high geometric shape score, good interface area and minimal atomic contact energy(ACE) area shown in Table 2 above.

The above docked results are samples from the docking experiment. Best poses of the compounds in the active sites of the *Mycobacterium Tuberculosis* 30S ribosomal subunit. This clearly shows that the compounds are active against *Mycobacterium Tuberculosis*. In the tables above which shows the results of docked complex between the ligand and the *Mycobacterium Tuberculosis* 30S ribosomal subunit, the best solution given shows the ranking of various complexes made when the ligand is in various positions in the molecule. The score refers to the geometric shape complementary score. Each of the solution is ranked according to this score with the highest being solution shown. The area in the table refers to the complex interface area. ACE refers to the energy of atomic contact. This is the energy the ligand uses to fit in the protein. The solution with the minimal energy was ranked high as it meant that the ligand fitted perfectly well without much strain. Transformation is the 3D transformation of the ligand molecule in the complex formed, the rotational 3 angles and translational 3 parameters.

This study was therefore motivated by the fact that despite the RNA being the central and largest molecule in gene expression no drug has been produced to target the RNA of infectious bacteria. RNA cements its prominence as a therapeutic target due to its key role in a range of biological systems. Hence, developing and screening compounds that modulate RNA activity is imperative. In this study we were able to show that it is possible for RNA to bind to immobilized kanamycin and streptomycin in vitro after *in silico* analysis, these agreed with other studies that had postulated that off-target RNA could be involved in binding with antibiotics that target ribosome exact sites. It is imperative to note that RNAs are being discovered in genomic sequences. Most of these epitomize unexploited potential as target for therapeutics or chemical generation probes. Our study sought to progress a universal technique to ascertain ligands that bind to highly structured RNA scaffolds very quickly using computational tools and eventual in vitro validation of potential ligands. Selection of ligands and the use of azide based antibiotic platform based screening to score ligand library for binding were critical to this study. Antibiotic platform allows for simplistic screening of target biomolecules for binding including proteins and RNAs.

4.3 Significance of ribosome homology and *de novo* modeling technologies.

These results demonstrate the power of ribosomal RNA homology and *de novo* modeling to identify essential and functional motifs with little or no alterations in the rRNA, and to detect all of the possible alterations of a drug target with the likelihood to lead to drug resistance. The results further demonstrate the convenience of the RNA homology modeling software for structure resolve. We strategize to use these tools in further studies to choose and characterize several of the novel rRNA targets identified in this study, to guide the performance of compound screening experiments, and to determine the structures of target/hit of the identified complexes. This homology and *de novo* modeling tryouts should result in the discovery of compounds that are resistant to target site alteration

and hence refractory to antibiotic resistance. The compounds resulting from such studies can be improved to be ready for optimization into clinical candidates.

5. CONCLUSION

A key tool in computer-assisted drug design and molecular structural biology is molecular binding and docking. The idea of ligand-ribosome complex docking is to anticipate the overwhelming restricting mode(s) of a particular ligand with a natural complex of known three-dimensional structure. Fruitful docking strategies utilize a scoring capacity which effectively positions applicant complex docking postures and look for high-dimensional spaces adequately. Virtual screening of expansive compound libraries can be performed through docking which is important in lead enhancement, the outcomes positioned, and propose basic theories of how the ligands hinder the objective. Docking input structure set up is also as basic as docking itself, and analyzing the eventual outcomes of s tochastic inquiry systems can on occasion be uncertain. Modeling the 3D structure of 30S *M. Tuberculosis* ribosomal subunit and molecular docking of known antibiotics and a novel compound was achieved, this can in the future lead to determining the novel lead compounds that can be used as anti-TB drugs and have been passed on as other uses drug. Further mutational studies of all areas that they can be tolerated can be done to show drug re sistance and help combat *M. Tuberculosis* by prior prediction and design of drug leads.

The results obtained by homology and *de novo* modeling method are highly similar to the results obtained by the primary modeling method. Due to the critical need to determine the three dimensional structures of protein, RNA and organelle units (e.g. the ribosome model use to bridge the knowledge gap), homology modeling is a powerful tool. This technology is efficient and reliable and the result are similar to the one obtained from the more expensive and time consuming X –ray crystallography or other experimental processes. Further studies show that the structure is even more important to effective drug development since mutations can be introduced into the target regions of the structure and perform efficient screening to identify potential drugs of choice that could interfere with the normal pathogen ribosome function.

5.1 Application of *Mycobacterium Tuberculosis* Homology Modeled Structural Data.

Determining 30S *Mycobacterium Tuberculosis* ribosomal three-dimensional structure provides a basis for use in computational homology modeling to reveal an atomic-level view of i t pro viding information of the rRNA structure and movement that allows for design anti-invectives-like compounds that target biologically functional sites of it in a predictable manner. This information for example can be used to design different molecules by computer modeling programs expected to interact with possible functional and structural features of 30S such as confirmed RNA motifs, loops and other binding sites.

Aminoglycosides are well known groups of antibiotics used successfully over years. Both streptomycin and kanamycin and paromomycin have been shown to bind to the specific sites of t he 16S rRNA affecting the process of protein synthesis. The rRNA amino acyl-tRNA site commonly known as the A site is a preferred site due to the major differences between the prokaryotes and eukaryotes selectively killing bacteria.

Intensive studies on the adverse effects of this types of drugs have been carried out to demonstrate that the main challenge to antibiotic therapy in clinical practice relates to the rapid increase of the emergence of resistant pathogen strains and/or drug toxicity. With the method outlined in this study, modification and reconstruction of both the antibiotic important ligands will help overcome the undesirable properties of natural occurring compounds.

The 30S ribosomal model high resolution subunit provided may be used to study candidate compounds and determine the binding of anti-invectives to this biological molecular target. Such include known aminoglycosides antibiotics that help prepare screening cut off and other newer compound libraries.

This study has shown that there are a handful of bioactive compounds that can bind RNA motifs as demonstrated by binding of RNA to streptomycin and kanamycin. This information will facilitate construction of RNA motifs database that can specifically be recognized by small molecules such as antibiotics. This will lead to development of new antimicrobial compounds with less susceptibility to the development of resistance, and therefore extended clinical profiles. These would provide valuable new weapons in the arsenal of compounds used to treat infection caused by persistent Mtb and will reduce toxicity and shorten treatment time. The advantages of targeting RNA over targeting proteins include: accessibility to more RNA target sites compared to protein, difficulty to inhibit proteins specifically due to common ligand or substrate like ATP and the possibility to improve multivalent drugs to target RNA or d rugs that target mRNA sequence that is vital for encoding an important arrangement of a protein.

By increasing the results revealed herein and investigating diverse chemical spaces for binding RNA via *in vitro* studies, more scaffolds are possible to be recognized. Future development integrating chemoinformatics to compute chemical diversity approaches and quick *in vitro*-based other small molecules screening would offer benefits to rationally design libraries with broad chemical landscapes. Using statistical enquiry of b inders, general ligand features for bi nding RNA can be elucidated quickly. Further studies are therefore required to

determine the internal RNA loop-drugs affinity capacities which will necessitate production new family of antibiotics. This will not only shorten the length of treatment but also lower the cost for cure.

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