In bacteria, nucleoid associated proteins (NAPs) represent a prominent group of global regulators that perform the tasks of genome compaction, establishing chromosomal architecture and regulation of various DNA transactions like replication, transcription, recombination and repair. HU, a basic histone like protein, is one of the most important NAPs in Eubacteria. *Mycobacterium tuberculosis* produces a homodimeric HU (MtbHU), which interacts with DNA non-specifically through minor groove binding. Exploration for essential genes in Mtb (H37Rv) through transposon insertion has identified HU coding gene [Rv2986c, *hupB*; Gene Id: 15610123; Swiss-Prot ID: P95109)] to be vital for the survival and growth of this pathogen.

MtbHU contains two domains, the N-terminal domain which is considerably conserved among the HU proteins of the prokaryotic world, and a C–terminal domain consisting of Lys-Ala rich multiple repeat degenerate motifs. Sequence analysis carried out by the thesis candidate showed that MtbHU exhibits 86 to 100 percent identity within the N-term region among all the mycobacterium species and some of the members of actinobacteria, including important pathogens like *M. tuberculosis, M. leprae*, *M. ulcerans*, *M. bovis*, *Nocardia*; while C term repeat region varies relatively more. This strikingly high cross species identity establishes the MtbHU N-terminal domain ($MtbHU^N$) as an important representative structural model for the above mentioned group of pathogens.

The thesis candidate has solved the X-ray crystal structure of $MtbHU^N$, crystallized in two different forms, P2 and $P2_1$. The crystal structures in combination with computational analyses elucidate the structural details of MtbHU interaction with DNA. Moreover, the similar mode of self assembly of $MtbHU^N$ observed in two different crystal forms reveals that the same DNA binding interface of the protein can also be utilized to form higher order oligomers, that HU is known to form at higher concentrations. Though the bifunctional

interface involved in both DNA binding and self assembly is not akin to a typical enzyme active site, the structural analysis identified key interacting residues involved in macromolecular interactions, allowing us to develop a rationale for inhibitor design. Further, the candidate has performed virtual screening against a vast library of compounds, and design of small molecules to target MtbHU and disrupt its binding to DNA. **Various biochemical, mutational and biological studies were performed in the laboratory of our collaborator Prof. V. Nagaraja, MCBL, IISc., to investigate these aspects.** After a series of iterations including design, synthesis and validation, we have identified novel candidate molecules, which bind to MtbHU, disrupt chromosomal architecture and arrest *M. tuberculosis* growth. Thus, the study suggests that, these molecules can serve as leads for a new class of DNA-interaction inhibitors and HU as a druggable target, more so because HU is essential to Mtb, but absent in human. Our study proposes that, targeting the nucleoid associated protein HU in Mtb can strategize design of new anti-mycobacterial therapeutics. Perturbation of MtbHU-DNA binding through the identified compounds provides the first instance of medium to small molecular inhibitors of NAP, and augurs well for the development of chemical probe(s) to perturb HU functions, and can be used as a fundamental chemical tool for the system level studies of HU-interactome.

Section I: "Crystal structure of *Mycobacterium tuberculosis* histone like protein HU and structure based design of molecules to inhibit MtbHU-DNA interaction: Leads for a new target." of this thesis presents an elaborate elucidation of the above mentioned work.

The candidate has additionally carried out structure based computational and theoretical work to elucidate the interaction of amino acid based metal complexes which efficiently bind to DNA via minor-groove, major-groove or base intercalation interaction and display DNA cleavage activity on photo-irradiation. This understanding is crucial for the design of

molecules towards Photodynamic Therapy (PDT). PDT is an emerging method of non-invasive treatment of cancer in which drugs like Photofrin show localized toxicity on photoactivation at the tumor cells leaving the healthy cells unaffected.

The work carried out in our group in close collaboration with Prof. A.R. Chakravarty of Inorganic and Physical Chemistry Department elaborates the structure based design of Amino acid complexes containing single Cu (II), such as $[Cu(L\text{-}trp)(dpq)(H_2O)]^+$, $[Cu(L\text{-}arg)_2](NO_3)_2$, Amino acid complexes containing oxobridged diiron Fe(III), such as $[\{Fe(L\text{-}his)(bpy)\}_2(\mu\text{-}O)](ClO_4)_2$, $[\{Fe(L\text{-}his)(phen)\}_2(\mu\text{-}O)](ClO_4)_2$, and Complexes containing Binuclear Cu(II) coordinated organic moiety, such as $[\{(dpq)\ Cu^{II}\}_2(\mu\text{-}dtdp)_2]$, which bind to DNA through minor groove/major groove/base intercalation interactions. Docking analysis was performed with the X-ray crystallographic structure of DNA as receptor and the metal complexes as ligands, to study the mode of binding to DNA and to understand the possible mode of DNA cleavage (single/double strand) when activated with laser.

Section II: "Structure based computational and theoretical analysis of metal coordinated complexes containing amino acids and organic moieties designed for photo induced DNA cleavage" of this thesis presents a detailed presentation of the above mentioned work.

The overall structure of this thesis is further resolved and explained through a chapter-wise description below:

Chapter 1 | An introduction and outline of the thesis: The chapter starts with a general introduction about the genome, as the reservoir of biological information, the importance of genome architecture in regulating the access to genome through various DNA transactions, and the proteins involved in the process. Nucleoid Associated Proteins (NAPs), such as HU in eubacteria play major roles in genome compaction, maintenance of chromosomal

architecture, recombination, replication and repair. The chapter also explains the importance of HU across various species, especially in case of *Mycobacterium tuberculosis* (Mtb), where the genome shows remarkable absence of many NAPs. The essentiality of HU for the survival and growth of Mtb, the causative organism of tuberculosis claiming millions of lives over the ages of human history, and also MtbHU being a representative model for various species of mycobacteria due to the high degrees of conservation, set the basis for the detailed structure based study of the protein, as presented through the chapters (2 to 7) of Section I. Protein crystallography and computation part of the work provide a structural basis for targeting HU, an important NAP, by inhibitors of DNA-interaction, an area hitherto unexplored, with a potential to design new chemical tools for understanding HU-interactome, and a rationale to further screen/design for inhibitors of therapeutic importance. Additionally, chapter I provides a brief introduction for the structure based computational and theoretical studies of amino acid complexes containing Cu (II) and Fe (III), important towards Photodynamic therapy, dicussed in details in Section II of the thesis. Finally, the chapter concludes on a general note, converging the work encompassed in Section I and II, to a common note, where structure based study of a macromolecule, namely MtbHU, and small molecules, such as the PDT candidate amino acid based metal complexes, in light of their interaction with DNA has opened new avenues towards identification/design of novel inhibitors of therapeutic importance.

Section I: Chapter 2 | Materials and Methods: details the crystallographic techniques, softwares and sequence/structure analyses programs used in Section I.

Section I: Chapter 3 | Purification, Crystallization, Data Collection and Processing of Mycobacterium tuberculosis histone like protein HU (MtbHU) N-terminal DNA binding domain: Rv2986c (*hupB*) was PCR amplified from *M. tuberculosis* H37Ra genomic DNA,

cloned into *E. coli* expression vector pET20b and mycobacterial expression vector pJAM2 at the laboratory of our collaborator Prof. Valakunja Nagaraja, Department of Micobiology and Cell biology, Indian Institute of Science, Bangalore. The clones were confirmed by DNA sequencing. Recombinant MtbHU was overexpressed in *E. coli* BL21 (DE3) pLysS cells. The chapter also presents the details of crystallization and data collection of MtbHU. Sparse matrix crystallization screens were used for initial crystallization trials. In parallel to the crystallization trials, dynamic light scattering experiment was also carried out to determine the optimized range of pH and buffer conditions at which MtbHU will achieve a mono disperse state. After several rounds of trials and optimization of the crystallization condition, reproducible crystals in the form of stacked plates were obtained using hanging drop vapor diffusion method, from a condition containing **3 M Sodium formate, 0.1 M Tris-HCl, pH 8.2, at MtbHU concentration of 14 mg/ml (at 295K)**.Verification of the crystallized MtbHU was done through SDS PAGE and mass spectrometry, which showed a molecular weight of around 11 KDa, suggesting a part of MtbHU was crystallized. After the data collection and structure solving, it was confirmed that, the crystals contained N terminal DNA binding domain of MtbHU ($MtbHU^N$) spanning first ~100 residues, while the C-terminal region was absent. Several biochemical studies in our collaborator's lab (Prof. V. Nagaraja, MCBL, IISc.) confirmed that, the degenerate Lys-Arg rich (AKKA, PAKKA) repeat region at the C-terminal of MtbHU gets degraded over time (both at room temeratue and 4°C). To further optimize the crystallization of $MtbHU^N$, a clone containing 100 residues from the N-terminal domain of MtbHU was constructed, overexpressed and purified. The expressed $MtbHU^N$ was crystallized at two different conditions, in which the condition containing **0.1 M Tris-HCl, pH 6.7, 3.5M sodium formate, and 10mM $MnCl_2$ (at 295K)** again gave platy crystals. The crystals of $MtbHU^N$ were initially screened at home source, but they failed to diffract to a good resolution, hence the data were collected using synchrotron

radiation source, where the platy crystals grown in Na-formate conditions, at pH 8.2 and 6.7 diffracted to the resolutions of 2.04 Å and 2.4 Å, respectively. After indexing, it was found that, $MtbHU^N$ grown at variants of Na-Formate conditions crystallized in two different crystal forms, namely monoclinic $P2_1$ and P2 (details in the table below).

Space groups	a	b	c	α	β	γ
$P2_1$	36.77 Å	53.97 Å	41.73 Å	90.00°	97.01°	90.00°
P2	55.115 Å	37.052 Å	64.854 Å	90.00°	115.29°	90.00°

The calculated self rotation function maps from the collected datasets revealed significant peaks in the kappa= 180 section, pointing to the presence of 2 fold symmetry axes, which gave peak profiles compatible with (222) symmetry. This information was utilized in validating the PHASER solution. The details of molecular replacement, model building and refinement are discussed in Chapter 4.

Section I: Chapter 4 | Structure determination and a description of the overall structure of $MtbHU^N$: The chapter details the application of molecular replacement (MR) method to the structure solution of $MtbHU^N$ homodimer at 2.04 Å and 2.48 Å resolutions from two different crystal forms, monoclinic $P2_1$ and P2 respectively, the course of iterative model building and the refinement carried out, and the quality of the final protein structure models. At first, MR was performed with the diffraction data obtained from the $P2_1$ crystal, using an ensemble containing the polyala models of truncated Anabaena HU crystal structure (PDB code: 1P51, 1P71 and 1P78) and Bacillus *stearothermophilus* (PDB code: 1HUU). The solution from PHASER located the $MtbHU^N$ dimer with its 2-fold axis of dimerization parallel to crystal axis **a**, perpendicular to the crystallographic 2-fold axis along **b.** At the end of refinement cycles, the **final $MtbHU^N$ structure at 2.04 Å resolution from $P2_1$ crystal form had R_{cryst} and R_{free} values of 20.6% and 24.97% respectively, and the model consisted of 1465 protein atoms, 4 formate ions and 84 solvent water molecules**. Refined

structure of MtbHU^{N} at 2.04 Å resolution contains 99 amino acid residues (chain A), which is by far the longest HU structure reported (**deposited as PDB code 3C4I**). For the P2 data**,** monomers derived from 3C4I were used as search models in PHASER, which gave a solution containing two monomers per asymmetric unit from two different HU dimers related by a non crystallographic diad axis perpendicular to crystallographic two fold along b, while the diad axis of dimerization is parallel to the crystallographic two fold. **The final structures from P2 crystal form at 2.48 Å was deposited with PDB id 4DKY , consisting of 1506 protein atoms, 2 Mn^{+2} ions and 42 solvent water molecules, and had R_{cryst} and R_{free} values of 18.31% and 24.79% respectively**. Each monomer of MtbHU^{N} contains two α-helices, namely α1 and α2, five antiparallel β-strands (β1 to β5) followed by a short α-helix, α3 at the C-terminal end . Two of these monomers wrap around each other to form a dimeric functional unit, giving rise to the 'HU-IHF fold. It is an assembly of a four helix bundle like dimerization subdomain S1 consisting of α1 and α2 from each monomer and a DNA binding subdomain S2 consisting of a β saddle formed by β2 and β5 of each monomer, and two extended β-arms formed by β3, β4, and connecting loops. Sequenc analysis of MtbHU has revealed that, the N terminal DNA binding region is highly conserved among different mycobacterium species including *M. leprae, M. Bovis* and other members of actinomyces, establishing MtbHU^{N} structure as a representative prototype.

Chapter 4 also reports the structure based analyses of local flexibility carried out by the thesis candidate, in terms of rmsd between MtbHU^{N} monomers, distribution of average isotropic displacement parameters derived from the crystallographic Debye-Waller factors, TLSMD and *HingeProt* analysis. The results from these different analyses showed excellent agreement with the observation that, residues 54 to 75 of S2 constitute the dynamic region of MtbHU. As the same region is also a part of the DNA binding S2 domain of MtbHU^{N}, the mobility of this region may hint towards its involvement in the conformational changes

induced by MtbHU binding to various DNA substrates. The X-ray crystal structures of $MtbHU^N$ also showed a number of surface salt bridges involving residues Lys 3, 13, Glu 26, Asp 30, Arg 34, Glu 51, Arg 53 and 80. Among these, residues involved in intra- molecular salt bridges, such as Lys3, Arg 53 and 80 are also involved in DNA interaction, as discussed further in chapter 5.

Section I: Chapter 5 | Macromolecular interaction interface of $MtbHU^N$, a structure based analysis, and a rationale for inhibitor design: The chapter gives a detailed account of various analyses carried out to elucidate the nature of MtbHU's DNA binding site, in order to understand the mode of DNA binding by MtbHU, and to perturb its DNA-interaction by screening/ design of novel inhibitors. The structural flexibility information derived from the analyses in chapter 4 was used to define substructure constraints, which was further utilized in computational analyses involving energy minimization followed by stochastic dynamics (SD) and simulated annealing (SA) cycles to produce a model for $MtbHU^N$-DNA complex. Crystal structure combined with computation reveals that MtbHU-DNA interaction primarily involves positively charged residues Lys 3, Arg 53, 55, 58, 61, 64, 80 and Lys 86 from each monomer. Except for Lys 3 (at S1), all other interacting residues are located at the DNA binding cleft of S2 and constitute the key interactions of DNA binding cleft. Pro 63 approaches the DNA through minor groove intercalation. In all, it may be surmised that a combination of electrostatic interactions, multiple hydrogen bond network and minor groove intercalation, involving a constellation of positively charged residues with flexible side chain rotamer, and main chain flexibility of MtbHU's DNA interaction interface contribute to its dynamic nature of DNA binding.

Thus, MtbHU may engage different DNA substrates differently, as dictated by its ability to induce and stabilize the requisite DNA bends, enabling the protein to bind to various DNA

substrates of varied length. The findings corroborated well with various biochemical, mutational and biological studies (Appendix A) performed in the laboratory of our collaborator Prof. V. Nagaraja, Department of Micobiology and Cell biology, Indian Institute of Science, Bangalore.

The molecular picture of $MtbHU^N$ interaction with DNA thus opens up an opportunity for inhibitor design to perturb DNA binding by MtbHU. A structure guided ligand design/ identification rationale was developed to screen for molecules with a potential to bind MtbHU at DNA binding subdomain S2, through the interaction with residues involved in DNA binding, such as, Arg 53, Arg 55, Arg 58, Arg 61, Arg 64, Arg 80, Lys 86. The first stage of inhibitor screening identified suramin and suramin analogue NF449 as inhibitors which perturbed MtbHU interaction with DNA, disrupted nucleoid architecture and curtailed the growth of Mtb. However, the major challenges in the search for more 'drug-like' inhibitors, in terms of molecular weight and specificity were rooted in the following features of the targeted DNA interaction interface:

a) Unlike the conventionally targeted enzyme active sites, the target site of MtbHU is large with several residues spanning the interface, involved in DNA interaction, such as, Arg 53, 55, 58, 61, 64, 80 and Lys 86.

b) Due to its inherent flexibility, the site is in a dynamic state of changing conformations.

This was further addressed through a thorough investigation of MtbHU crystal packing which elucidated that the DNA interaction interface of MtbHU can also act as protein-protein interaction site, facilitating self-assembly of MtbHU. The study of contributions of various amino acid residues at MtbHU's macromolecular interaction interface, involved in DNA binding, self-assembly and ligand interactions (through molecular docking studies) lead to the identification of a 'necessary and minimal' set of target residues located at the interaction

'hot-spot', hitherto unexplored, at the core of MtbHU's DNA binding subdomain S2. With this insight, an improved strategy for the next stage of inhibitor screening/ design was devised, which identified trans-stilbene derivatives as inhibitors of MtbHU-DNA interaction. Unlike suramin and suramin analogue NF449 identified in stage I of screening, the trans-stilbene derivatives are smaller in size and not poly-ionic in nature, and hence are closer to being more 'drug-like'. A detailed account of the screening methodology, along with the effect of these inhibitors on nucleoid architecture and Mtb growth is dealt with in the next chapter.

Section I: Chapter 6 | Structure guided design of inhibitors to perturb MtbHU-DNA interaction: The chapter details the rationale for screening/ design of inhibitor molecules for targeting the DNA binding S2 domain of $MtbHU^{N}$, developed through multiple iterations of virtual screening, experimental validation of DNA binding inhibition by the identified compounds, computational analyses of binding site interaction and mutational studies for validation of the proposed binding model. The effort led us to a final set of molecules, which perturb MtbHU's DNA binding, disrupt Mtb nucleoid architecture and curtail cell growth of the formidable pathogen, suggesting these molecules as leads for a new class of DNA-interaction inhibitors and HU as a druggable target; more so because HU is essential to Mtb, but absent in human. The screening process can be largely divided into two stages:

Stage I describes the identification of molecules such as suramin and suramin analogue NF449, providing the initial proof of concept that, the macromolecular interaction interface of MtbHU can indeed be targeted by medium sized molecules acting as inhibitors of DNA binding. The target site for ligand identification during the virtual screening methodology for stage I encompassed the whole DNA-binding site at S2 (details in Appendix B), including Arg 53, Arg 55, Arg 58, Arg 61, Arg 64, Arg 80, Lys 86. Electrophoretic Mobility Shift

Assays (EMSA) performed in our collaborator Prof. V. Nagaraja's lab showed, in case of suramin, 50% inhibition (IC_{50}) of DNA binding was obtained at 1.7 μM concentration, while NF449 showed an IC_{50} of 3.5 μM. The minimal inhibitory concentrations (MICs) determined through Resazurin microtitre plate assay (REMA) of suramin and NF449 against *M. tuberculosis* H37Ra strain ranged from 350-400 μM and 550-600 μM respectively.

Stage II demonstrates an improved rationale focused at a 'hot-spot' of interaction at the target interface, leading to the identification of smaller inhibitor molecules. Table I6.2 contains the details of 36 inhibitors identified in stage II. Among them, two *trans*-stilbene derivatives 4,4'-[(E)-ethene-1,2-diylbis({5-[(phenylcarbonyl) amino] benzene 2,1diyl} sulfonylimino)] dibenzoic acid and its methoxy derivative 4,4'-[1,2-ethenediylbis({5-[(4-methoxybenzoyl)amino]-2,1-phenylene} sulfonyl imino)]dibenzoic acid, referred to as compound 1 and 4 were identified through EMSA as the most potent inhibitors. The target site for stage II was restricted to a minimal set of crucial residues from a region defined within a 'bounding-box', at the base of MtbHU's DNA binding site at S2. According to the computational analysis, the compounds bind strongly at DNA binding cleft through interactions with residues Arg 55, 58, 80 and Lys 86, which was further verified through mutation studies. EMSA showed, in case of compound 1, 50% inhibition (IC_{50}) of DNA binding was obtained at 20 μM concentration, while compound 4 showed an IC_{50} of 1.7 μM. The MICs of compound 1 and 4 against MtbH37Ra strain ranged from 200-300μM and 800-900 μM respectively. These compounds are smaller than suramin and NF449 (molecular weight >1000 Da), with better pharmacological properties.

Thus, the findings have propelled the inhibitor screening/design rationale a step further, enabling identification of inhibitor molecules which not only opens up avenues for future lead design, but also provide valuable chemical probes to investigate the HU-interactome in

an important pathogen like Mtb, bridging the gap between our understanding of its basic biochemical and biophysical role and implications towards therapeutic applications.

Section I: Chapter 7 | Summary and Future direction: This is the concluding chapter of section I, which summarizes the major findings, and also outlines the future direction of the work presented in the section. Among the points highlighted here, perhaps the most important is resolving the MtbHU macromolecular interaction interface at S2, involved in DNA binding into two different regions, based on the varied degrees of contributions by different residues involved in interaction, and the structural mobility of the regions:

i) '**core of interaction**' region at the base of S2 containing Arg 53, 55, 58, 80 and Lys 86, primarily involved in docking of interaction partners, such as DNA, another HU molecule.

ii) '**Dynamic rim region**' containing Arg 61, 64 and Pro 63, which forms the DNA embracing β-arms and facilitates DNA bending through conformational changes induced by DNA binding.

The work presented in section I of this thesis underlines the fact that, to perturb MtbHU-DNA interaction, it may be sufficient to target the 'core of interaction' region containing Arg 53, 55, 58, 80 and Lys 86, which is also a region of less structural 'mobility or flexibility', compared to the dynamic rim region. The restriction of mobility at the core of interaction may be a general strategy utilized in MtbHU-DNA interaction, as can be supported from the 'salt-bridge' interaction analysis, which showed two of the conserved intra- molecular salt bridges, include residues Lys3, Arg 53 and 80, also involved in DNA interaction (as mentioned in chapter 5). The strategy of restricting the mobility of the backbone and/or the side chain atoms of Lys/ Arg may help the DNA interaction of these residues by minimizing the change in entropy on MtbHU-DNA complex formation, which

otherwise would've had a positive energy penalty resulted by the loss of entropy by these flexible surface exposed basic residues.

The adverse effect of the inhibitors on nucleoid architecture and cell growth in Mtb underline that HU is a druggable target of interest, at least in organisms like Mtb, where genome shows a remarkable absence of many NAPs (Cole *et.al*., 1998). The findings described in this thesis thus open new avenues towards future exploration, as described in chapter 7, future directions part. Some of the aspects of major interests are:

- **Use of MtbHU-DNA interaction inhibitors as chemical probes to study structural interactome with the help of microarray and RT PCR studies**
- **Development of better inhibitor molecules, such chemical moieties, peptides with higher affinity and specificity towards MtbHU^{N} DNA binding site, using the 'core of interaction' as the target site, the Achilles' heel of MtbHU.**
- **Co-crystallization with inhibitor molecules and protein engineering**
- **Co-crystallizations of full length MtbHU with DNA**

Section II: Chapter 8 | deals with the molecular docking analyses of metal complexes which efficiently bind to DNA via minor-groove, major-groove or base intercalation interaction and display DNA cleavage activity on photo-irradiation. **This work was carried out by the thesis candidate in close collaboration with Prof. A. R. Chakravarty's Laboratory, (Inorganic and Physical Chemistry Department, Indian Institute of Science.), who had synthesized, characterized and provided us the crystal structures of the metal complexes.** The docking analysis was performed by the thesis candidate using X-ray crystal structures of the metal complexes as ligands (provided by our collaborator Prof. A. R. Chakravarty, Inorganic and Physical Chemistry Department), and DNA as receptor, to

study their various modes of binding to DNA and to understand the possible mode of DNA cleavage (single/double strand) while activated with laser. Computational methods combined with crystal structure and experimental findings thus helped us to understand the possible modes of action of these complexes in terms of DNA binding and cleavage, key to design effective therapeutics for PDT. Further, the design of complexes using bio-essential metals, such as copper and iron, and amino acids as building blocks also improve the biocompatibility of the molecules, providing valuable templates for further lead design.

Metal coordinated complexes containing amino acids and organic moieties used for molecular docking:

Class I: Amino acid complexes containing single Cu (II):

1) $[Cu(L\text{-}trp)(dpq)(H_2O)]^+$
2) $[Cu(L\text{-}trp)(dppz)(H_2O)]^+$
3) $[Cu(L\text{-}phe)(dppz)(H2O)]^+$
4) $[Cu(L\text{-}arg)_2](NO_3)_2$
5) [Cu (L-arg) (phen)-Cl]Cl]

} **Netropsin mimics**

Class II: Amino acid complexes containing oxobridged diiron Fe(III) :

1) $[\{Fe(L\text{-}his)(bpy)\}_2(\mu\text{-}O)](ClO_4)_2$
2) $[\{Fe(L\text{-}his)(phen)\}_2(\mu\text{-}O)](ClO_4)_2$
3) $[\{Fe(L\text{-}his)(dpq)\}_2(\mu\text{-}O)](ClO_4)_2$

Class III: Complexes containing Binuclear Cu(II) coordinated organic moiety:

$[\{(dpq)\ Cu^{II}\}_2(\mu\text{-}dtdp)_2]$

This work in greater details has been published in various scientific journals, attached at the end of Section II, chapter 8.

Appendix A details the various biochemical assays and biological studies carried out in the laboratory of our collaborators at the Department of Microbiology and Cell biology, of our Institute. These studies provided crucial complementation, support and validation to the structure based analyses, and are presented in Appendix A for the sake of completeness of the Section I workflow (mentioned in Scheme 1.7.1, **Chapter 1).**

Appendix B gives a detailed description of the target site at $MtbHU^{N}$ DNA interaction interface used in computational screening of the inhibitor molecules.