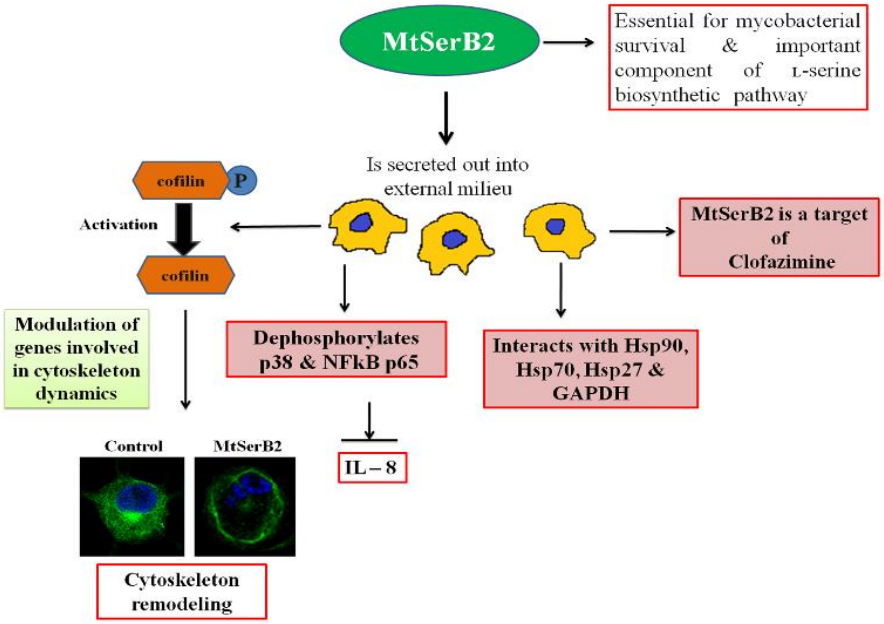
Statement of Excellence in research work of Prof. Ravishankar Ramachandran

Prof. R. Ravishankar secured Ph.D in the area of Structural Biology from *Indian Institute of Science*, Bangalore. He subsequently carried out excellent work at *Max-Planck Institute for Biochemistry* as a Humboldt Fellow and worked on the prokaryotic equivalent of the 20S proteasome in the laboratory of the Nobel laureate, Prof. Robert Huber. He returned to India in 2002 to start an independent research group at CSIR-Central Drug Research Institute, Lucknow. Ravishankar has been working in the broad areas of 'Early target discovery, Disease biology, and Discovery of new therapeutic avenues against drug resistant pathogens'. He has made fundamental contributions in the disease area of TB that has been important for CDRI right from its inception. The fundamental knowledge is subsequently translated into development of innovative therapeutic strategies for human diseases. This has brought him International recognition and National awards. Some innovative/ translational examples from his body of recent work is given below.

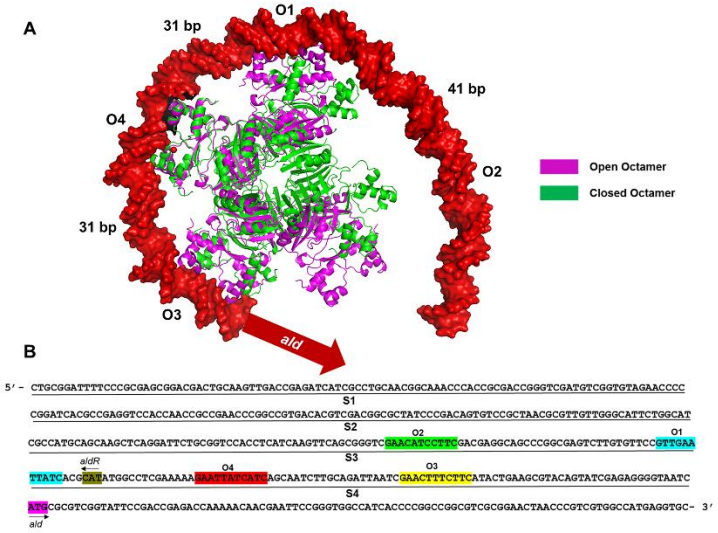
I. *M. tuberculosis* SerB2 is a target for Clofazimine and exhibits novel host-interacting functions

Recently, the author demonstrated that the essential SerB2, involved in mycobacterial Serine metabolism, is exported and interacts with human host factors like MAPK-p38 and NF-kappa B p65 that play crucial roles in inflammatory and immune responses. Using Broncho Alveolar Lavage (BAL) samples from TB patients, he showed that it is secreted and mediates host-pathogen interactions. He elegantly used a combination of phenotypic comparison, biophysics, and biochemistry techniques to show that it is a target for Clofazimine, a drug that is being evaluated clinically against MDR- and XDR-TB. Recently his group signed an agreement with the *Global TB Alliance* for collaborative work on this target. The author has also identified 2 known drugs that target SerB2 and the potential for repurposing them in new anti-drug-resistant TB therapy is being evaluated.



Schematic diagram summarizing the multifunctional properties of MtSerB2: *MtSerB2 is secreted out in extracellular milieu by M. tuberculosis and induces significant cytoskeletal rearrangements via cofilin activation and modulation of genes involved in cytoskeletal dynamics. MtSerB2 also dephosphorylates MAPK p38 and NF-kappa B p65 proteins and this in turn leads to down-regulation of Interleukin 8 (IL-8). It also interacts with HSPs 90, 70 and 27 respectively. Inhibition studies show that MtSerB2 is a target of Clofazimine, a drug being evaluated as a new therapeutic against MDR and XDR TB infections.*
Innovation: Ravishankar used a combination of phenotypic comparison, biophysics, and biochemistry techniques to identify that Mtb rv3042c is a drug target for Clofazimine, a drug being tested in MDR-TB clinical trials. An agreement with the 'Global TB Alliance' was signed to exploit this fundamental innovation and discovery further.

II. Feast/ famine regulatory proteins (FFRPs) are global regulators that apparently help mycobacteria to switch/ adapt from 'Feast' to 'Famine' state.
Ravi's work demonstrates that the rarely observed 'open' quaternary is important for the protein to bind to non-symmetric target DNA sites, His work suggests how FFRPs can form nucleosome-like particles, and how effector-binding events can trigger specific regulatory outcomes. Ravi used a novel strategy involving X-ray crystallography based fragment screening which allowed him to distinguish between relatively weak but specific binding of amino acids to the FFRP and draw conclusions for functions and for new therapeutic development.



(A) Superposition of *open* (PDB: 2W29; MtbFFRP) and *closed* octamers (PDB: 4PCQ; MtbAldR). This was modelled onto MtbAldR binding sites O1-O4 respectively located upstream to the *ald* gene. The numbers between the two adjacent AldR-binding sites indicate the distances between the respective central T nucleotides in the base pairs. The model clearly suggests that the protein has to adopt the *open* quaternary structure in order to bind to the complete region.
Innovation: First-in-class small-molecule inhibitors *(tetra hydroquinoline carbonitrile derivatives)* have been identified by the author's group against AldR, the FFRP that regulates the *ald* gene, that has been suggested to be a top-3 target against TB

persistence. The latter molecules represent the very first inhibitors of an FFRP from any source.

III. Innovation in Drug Repurposing against Chronic Myeloid Leukemia

In another important translational effort, Ravishankar has worked in repurposing of a drug against Chronic Myeloid Leukemia (CML) as also finding out the mode of action. The drug identified by the team has several advantages over existing Bcr-abl tyrosine kinase inhibitors (TKI) like Dasatinib, Imatinib, etc. Excitingly, it eliminates drug resistance arising from Leukemia stem cells (LSC) when used in combination with TKI.

Innovation in Drug repurposing: Clofazimine was identified to act synergistically with Imatinib to act against drug-resistant Chronic Myeloid Leukemia. This also represents affordable treatment for such patients. International patents have been granted and Phase II clinical trials are being proposed presently.

IV. Innovative strategy involving targeting HIV-Nef for novel antiviral therapy development

HIV-1 Nef is known to play a pivotal role in HIV pathogenesis and binds to different protein partners in lower and higher oligomeric states respectively. His work on HIV-1 Nef derived from patient samples, identified the only known instance of a transition from a dimer to a tetramer with 4-fold symmetry. The functional implications of this unique transition are in an elegant mechanism where HIV-1 Nef in the dimeric state binds to a set of protein partners that are subsequently excluded from associating with the protein at higher oligomeric states due to the change in the subunit association.

Innovation: Rationally identified inhibitors with a new mode of action that work by disrupting specific HIV-1 Nef–co-protein interactions [52, 59, PCT patent granted].

V. Novel Drug Targets for therapy development against drug-resistance

An important contribution involves identification of direct evidence for a glutamate 'switch' mechanism to operate in a sub-class of aminotransferases, and structure-function studies on mycobacterial factors like Lysine ε-aminotansferase (MtbLAT) and L-Alanine dehydrogenase (MtbALD) that are thought to be important for adaptation/maintenance of tuberculosis persistence/latency [25, 35]. They have incidentally been ranked among the top-3 targets against tuberculosis persistence by the TB-Structural-Genomics Consortium [PLOS Comp. Biol. 2, 539-550, 2006]. The results were exploited to identify the very first inhibitors of MtbLAT using structure- based approaches [28, 70].

In another effort, the author has quickly developed a Surface Plasmon Resonance based assay to assess efficacy of PCSK9 based therapeutics for dyslipidemia. A strategy to develop potential Biotherapeutics exploiting this target is also being developed. The work is planned to be carried out in a joint manner with an identified pharma company partner. Other targets have also been chracterised and demonstrated to be important from therapy discovery point of view

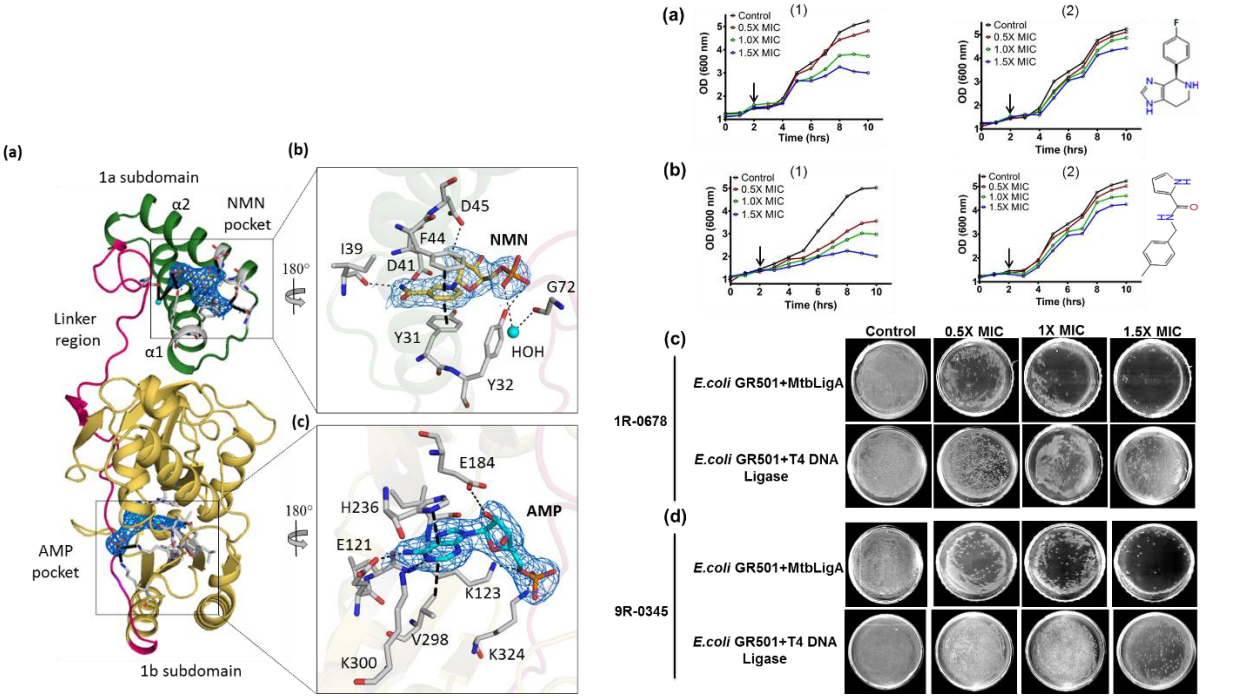
Innovation: The author is partnering with M/s AtomWise Inc, and has secured the Artificial Intelligence Molecular Screen (AIMS) Awards program by M/s Atomwise for academic researchers seeking novel compounds to treat disease. The specialist team of

the company has selected 5 targets from the author's group for further multi-target Artificial Intelligence driven drug discovery studies. The targets are in the disease areas of Dyslipidemia, drug-resistant TB & Filariasis.

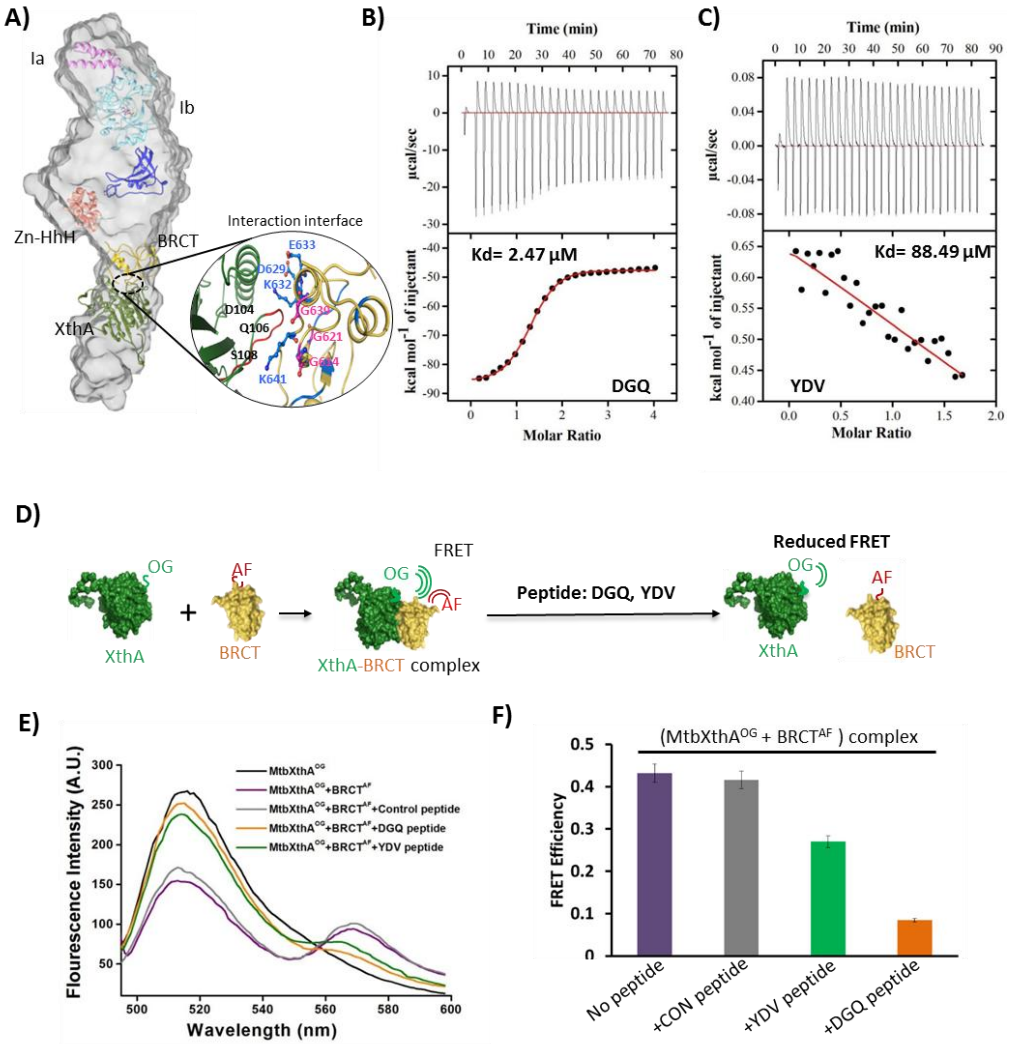
VI. Targeting of BERosomes for new therapy development that abrogates DNA Base-excision repair (BER).

DNA repair and recombination are essential processes for an organism. Ravi's work on important mycobacterial components from the DNA Base Excision Repair (BER) pathway has demonstrated the existence of large multimolecular assemblies (BERosomes) that function in this pathway. He showed that XthA, an exonuclease, exhibits DNA regulated modes of interaction with the β-clamp, and also that it is possible to specifically disrupt the interactions using small molecules and peptides. His studies have led to the identification of new classes of inhibitors that exhibit anti-bacterial specificity and distinguish the human enzyme several fold, both *in vitro* and in LigA-deficient strains using a novel strategy that combines *in silico* computational evaluation against 2 examples each of ATP and NAD+-dependent Ligases followed by biochemical and structural analysis leading to a robust evaluation platform.

Recently, he identified a fundamental BER interaction that *M. tuberculosis* class II apurinic/apyrimidinic-endonuclease/3′-5′ exonuclease (XthA) engages with NAD+ - dependent DNA ligase A (LigA) to counter futile cleavage and ligation cycles in Base Excision Repair. Novel inhibitor identification strategy that aims to strengthen the interaction based on non-conserved (between bacteria and humans) interface features and thereby abrogating the repair activity is being implemented based on the fundamental studies.



Crystal structures of the NMN-bound MtbLigA adenylation domain, inhibition studies and assays leading to first-in-class fragment inhibitors that target the NMN site



In a recent fragment-inhibitor discovery campaign, Ravishankar identified first-in-class fragment inhibitors using an innovative approach using Small-angle-xray scattering (SAXS), crystallography, biochemical and mutational approaches that targets the NMN-binding site of bacterial LigA. Since this site is not conserved in humans, these inhibitors have less possibility of developing drug-resistance.

VII. Translational approaches against COVID19 pandemic

a) Phase III clinical trials of Umifenovir *:- Drug repurposing against COVID19*

Dr. Ravishankar, as a Nodal scientist, is leading a team for drug repurposing studies against COVID19. The drug Umifenovir was identified and shortlisted based on the efficacy, toxicity, patent status and other favourable parameters. The interactions with COVID19 target was evaluated in vitro and computationally. Since it is not available in India, the process was implemented and transferred to a company (M/s Medizest, Goa) for manufacture under GMP conditions. Careful analysis of the dosage needed for COVID19 patients was carried out based on the reported pharmacokinetics. DCGI permission was received and presently the Phase 3 clinical trials titled "*Phase 3, Randomized, Double-blind, Placebo control trial of Efficacy, Safety and Tolerability of*

Antiviral drug Umifenovir vs Standard care of therapy in non-severe COVID-19 patients" involving 3 clinical trials centres" has been completed. It was identified that Umifenovir meets the primary and secondary endpoint criteria and exhibits statistically significant efficacy for Mild-asymptomatic patients. It is efficacious, safe and well-tolerated at the tested dosage of 800mg BID, maximum 14 days. Commercialization and patent filing are in process.

b) Drug targets for COVID19 therapeutic discovery

The author as the Nodal PI has established an assay platform involving the main protease mPro of Sars-Cov2 and has identified novel scaffolds as inhibitors for it using structure-based rational approaches. RBD-Ace2 interactions and 3CL-Pro are also being targeted. A couple of the new scaffolds are showing promising results in *in vitro* virus culture based inhibition studies too.

c) RT-PCR based diagnostic laboratory against COVID19

The author, as the Nodal PI, has helped quickly establish a diagnostic/screening facility against COVID19. Till date more than 3,00,000 patient samples from Uttar Pradesh districts have been screened in this facility successfully.

(Prof. Ravishankar Ramachandran)

Place: Lucknow