ABSTRACT

Sirtuins are evolutionarily conserved NAD^+-dependent class III histone deacetylases (HDAC) associated with a number of age related human diseases such as neurodegenerative disorders, cancer, diabetes, and cardiovascular diseases. The need of the cofactor NAD^+ for the catalytic activity makes sirtuin a crucial regulator of a number of central cellular and metabolic functions. Silent Information Regulator 2 (Sir2) from *Saccharomyces cerevisiae* is the founding member of sirtuin family of proteins. SIR2 has been reported to extend the life span of budding yeast by suppressing the formation of extrachromosomal rDNA circles.

Since the discovery of SIR2 in yeast cells, sirtuins have now been characterized in every domains of life from bacteria to higher eukaryotes and have emerged as one of the major therapeutic targets. Seven isoforms of sirtuins (SIRTs), SIRT1-SIRT7 have been discovered in mammals which show diversity in their cellular localizations, catalytic activity and functions. These proteins share a conserved catalytic domain of ~275 amino acid residues and are flanked by distinct N- and C-terminal domains responsible for identifying binding partners and/or sub-cellular localizations.

The research in the last two decades has provided a significant amount of information on the considerable role of sirtuins in disease pathologies. This has triggered a surge of interest to search for the potent sirtuin specific modulators which could be employed in alleviating age related disorders. This thesis illustrates the use of combination of X-ray crystallography, comparative structural analyses and computational tools like molecular docking, virtual screening, and Molecular dynamics (MD) simulations to aid structure based drug design against SIRT5, SIRT6 and SIRT7. The distinct N- and C-terminal domains of sirtuins have not been structurally characterized. We have solved the crystal structure of N-terminal domain of SIRT7 ($SIRT7^{NTD}$) as eMBP fusion protein. Structural analysis of $SIRT7^{NTD}$ suggests structural similarity to regulators of transcription factors and its possible role in DNA binding as well. We hope this study would help in providing useful insights to understand the role of $SIRT7^{NTD}$ mediated physiological functions and will also aid in rational drug design. These structural insights need further investigations to validate relevance in physiological conditions.

We have also solved the crystal structure of SIRT5 in apo form (apo-SIRT5) and comparative structural analyses of apo and substrate peptide bound form suggest significant

conformational changes taking place in the zinc-binding domain and the cofactor binding loop. The observed overall loop movements and domain motion, though distinct, is yet similar to that observed for human sirtuins. We believe the apo-form crystal structure represents one of the stable conformations in solution and hence can be exploited for rational drug design approach as one of the starting models.

The comparative structural analyses and physicochemical properties of the catalytic pockets of the SIRT5, SIRT6, and homology model of SIRT7 suggest that there are significant differences in the amino acid composition of the substrate binding region in the catalytic pocket. The residues involved in NAD^+ binding region are conserved and hence will be challenging to design isoform specific modulators. Hence, in the present study the differences in the substrate peptide binding pocket were exploited for the development of isoform specific modulators. Analyses of amino acid residues lining the peptide binding pocket led us to the identification of some key residues conferring substrate specificity to SIRT5, SIRT6 and SIRT7. We performed high throughput virtual screenings to identify lead molecules for SIRT5, SIRT6 and SIRT7 using different modules of Schrödinger software suite. The stability of the most promising molecules was analyzed by MD simulations of the protein-ligand complex. Currently, it is challenging to perform experiments to further shortlist compounds obtained computationally. The top scoring compounds may not necessarily be the best binding compounds. Here, we have used a combination of fragment based screening methods to further shortlist our lead molecules obtained computationally. We exploited high throughput fluorescence based thermal shift assay to identify fragments that provided significant thermal stability to the target protein. Molecules containing such scaffolds or fragments, which promoted protein stability, were then screened by substructure search by canvas module of Schrödinger software suit. We believe this method can help us in shortlisting compound for further experimental studies to yield fruitful results. The selected lead molecules identified in the present study could be developed further to generate drug like molecules and can be tested for their effect on modulating sirtuins activity.