Summary

Flavins (Riboflavin, FMN, FAD) are involved inimportant metabolic processes including assimilatory iron reduction in many pathogens. It has been recently shown that the enzymes involved in riboflavin biosynthesis pathway of *H. pylori* are essential for itssurvival. Therefore the enzymes of riboflavin pathway are potential drug targets for the treatment of gastritis and gastric cancer. In *H. pylori*, the *ribBX* gene encodes for 3,4-dihydroxy 2-butanone 4-phosphate synthase domain (ribB) and carries a domain (ribX) with an unknown function. The ribBX enzyme is shown to be essential for the survival of *H. pylori* and catalyze a rate-limiting reaction in riboflavin biosynthesis pathway. Here, we attempt to characterize this ribBX enzyme biophysically and biochemically from *H. pylori*, so that this enzyme can be explored as a potential drug target. We cloned, expressed and purified the ribBX protein from *H. pylori 26695*. Despite several attempts, the crystallization of full length ribBX was not successful. Therefore, to determine structure of this ribBX, we initiated the characterization of individual domains of ribBX from *H. pylori*. The three dimensional structure of N-terminal domain of ribBX (ribB) was successfully determined by X-ray crystallography however the crystallization of C-terminal domain was unsuccessful.

To facilitate the determination of ribBX structure we explored the same protein from other homologous organisms. After exploring ribBX from several organisms, we successfully cloned, expressed, purified and determined the crystal structure of ribBX from *Nautilia profundicola* (nribBX) upto 4.2 Å. The structure of nribBX shows four molecules in an asymmetric unit. Although, the ribB domain is well resolved in the crystal structure, the ribX domain was not clear at this resolution. The superposition of the nribBX with Mtb-ribA2 revealed that its N-terminal ribB domain is conserved, while the C-terminal domain showed significant conformational changes indicating it may have different function than GCHII activity. In fact, it is reported that the C-terminal domain of ribBX may affect the function of N-terminal domain and may play important role in iron reduction or acquisition through flavin production.

A future direction would be the determination of high resolution three-dimensional structure of ribBX which may shed light on the function of C-terminal domain. The co-crystallization of the ribBX enzyme with its ligand may also elucidate the structure and function of this important enzyme.