Executive Summary

Leishmaniasis is a group of diseases caused by the protozoan parasites belonging to several different *Leishmania* species. At present there are no effective vaccines available against any of the clinical forms of leishmaniasis. Further drugs against this parasite are becoming limited in their usefulness due to inappropriate use and because of the development of drug resistance. Advanced research in genome sequence analysis has gathered exhaustive information on *Leishmania* parasites genome in terms of parasite biology in the vector and host, including host immune responses and much more. Yet, the virulence determining molecular factors involved in pathogenesis associated with any of the clinical forms of leishmaniasis remain to be explored and understood in detailed fashion, as the parasite virulence is a multi-factorial phenomenon. In India the visceral form of the disease caused by *L. donovani* is the most prevalent form that causes considerable mortality and morbidity particularly in the North eastern regions.

Post translational modification (PTM) is a diverse biological process that regulates the activity of target proteins. Ubiquitin is a classical molecule that is characteristically known for its post translational modifying activity, however modification by ubiquitin-like molecules (Ubls) is not well studied in *Leishmania* as in other eukaryotes. Research over the last decade has identified a series of Ubls in trypanosomatids like *Leishmania*. In depth studies with Ubls are warranted as they may yield a better understanding of the role of Ubls in the disease pathogenesis and may provide new leads towards identification of novel therapeutic targets.

The present piece of work is the first study that characterizes the *Leishmania* specific ubiquitin related modifier-1 (*LdUrm1*) and its conjugation pathway in *Leishmania donovani*. Based on homology modelling we depicted that *LdUrm1* possesses a β-grasp fold and a C-terminal di-glycine motif unique to Ub/Ubls, essential for its conjugation to the target proteins. *LdUrm1* also contains a pre-exposed di-glycine motif unlike other Ubls that need a C-terminal processing protease to expose the di-glycine motif that eventually lead to its conjugation to the proteinaceous targets *in vivo*. We identified *LdUba4* as the E1 enzyme for *LdUrm1* and its enzymatic activity was demonstrated *in vitro* by molybdenum blue based activity assay. The process was found to be energy dependent. Immuno-localisation studies showed that the *LdUrm1* was localized at the anterior end near flagellar reservoir, in both promastigotes and axenic amastigotes. *LdUba4* was found to be cytoplasmic. We demonstrated through Co-IP and subsequent immunoblotting that *LdUrm1* and *LdUba4* together form high molecular
Further, to functionally characterize LdUrm1 in *Leishmania*, overexpression of wild type (LdUrm1\textsuperscript{WT}) and non-conjugatable form of LdUrm1 (LdUrm1\textsuperscript{ΔG}) was performed. Expression of LdUrm1\textsuperscript{ΔG} in *L. donovani* showed depleted parasite growth suggesting its role in the pathogenesis. Mass spectrometry identified Rab-GTPase and Rab5 as LdUrm1 targets. Rab5 is known to mediate endosome-endosome fusion regulated hemoglobin endocytosis in *Leishmania*. Our identification of Rab5 as LdUrm1 target suggested that LdUrm1 is associated with early endosome and probably plays role in early endosome mediated heme internalization in *Leishmania*.

Identification of Urm1 conjugation pathways in *Leishmania*, and its set of unique specific targets may be exploited in near future for designing novel drug therapies to cure this dreadful disease.