The extent and functional role of membrane protein interactions in *Leishmania mexicana*

Leishmaniasis is a potentially fatal disease caused by the insect vector-borne parasite *Leishmania*, prevalent in over 80 countries worldwide. Current treatments for leishmaniasis are inadequate to address the growing public health crisis, necessitating the development of novel, more effective therapeutics. During its digenetic life cycle, *Leishmania* alternates between two major developmental stages. The flagellated extracellular promastigote develops in the digestive tract of the sand fly vector and is injected together with the saliva into a mammalian host during blood feeding. Once within the vertebrate host, promastigotes are internalized by macrophages and differentiate into intracellular non-motile amastigotes that survive and replicate. This initial interaction between the host cell and *Leishmania* promastigotes is dependent on surface proteins of both organisms. Membrane proteins are fundamental molecules involved in a variety of tasks, such as recognition, adhesion and penetration in the host cell, transport of nutrients and enzymes, as well as cell signalling. *Leishmania* spp., like many other parasites, have lost the ability to synthesise certain nutrients de novo and must scavenge essential amino acids from their environment. This scavenging is mediated by transporter proteins, such as amino acid transporter proteins, located on the parasite's membranes. The analysis of the membrane proteome profile of *Leishmania* promastigotes may permit a more detailed understanding of the host- parasite interaction and aspects of *Leishmania* biology. Among the proteomic studies of *Leishmania* parasites, there are only a few reporting membrane-associated proteins. Hence, this project aims to glean insight into membrane proteins by first identifying the functional roles of two putative amino acid transporters encoded in *L. mexicana*, then, specifying membrane proteins that interact with our proteins of interest. These genes are annotated as LmxM.30.1820 and LmxM.30.1800.

CRISPR-Cas9 knockout strategy was used to generate two mutant cell lines for the selected genes in *L. mexicana*. A complete knockout was successfully generated for LmxM.30.1820, while one allele of LmxM.30.1800 was retained despite the deletion of 2 alleles. Subsequent, various assays were used to evaluate these mutants, LmxM.30.1820$^{(-/-)}$ and LmxM.30.1800$^{(-/-/+)}$.

The uptake of radiolabelled amino acids, including tryptophan, lysine, glycine, and threonine, was performed in mutant promastigotes and wild type to investigate the involvement of these genes in specific amino acid transport. While bioinformatic analysis initially suggested both genes might function as tryptophan transporters, the uptake assay shows that neither LmxM.30.1820 nor LmxM.30.1800 significantly contribute to tryptophan transport in the promastigote stage, whereas the uptake of glycine in both

mutants was reduced by 90% compared with the control cell line. No other remarkable distinction was observed in the uptake of other aromatic amino acids tested.

Furthermore, gene expression in the absence of glycine was examined, and an up-regulation in the mRNA level encoding both genes was observed in the mutants compared to the control. Considering the demonstrated role of LmxM.30.1820 in glycine uptake, metabolic changes were assessed in the mutant cell line LmxM.30.1820$^{(-/-)}$ using untargeted LC-MS metabolomics. Significant metabolic changes occurred in glycerophospholipid and fatty acid metabolism, which were significantly decreased in LmxM.30.1820$^{(-/-)}$. This decrease could be attributed to the role of glycine as a precursor for glycerophospholipid synthesis.

In order to identify potential partners that interact with our proteins of interest, tagged cell lines have been generated, and Co-IP have been performed to pull down LmxM.30.1800 & LmxM.30.1820 and their protein complexes. A different pull-down approach, TurboID, has also been established to compare outcomes.

The next step is to disrupt specific proteins in these complexes and investigate the repercussions in terms of their functions and parasite biology. Assessing phenotypes such as cell growth and drug susceptibility. Examining the hypothesis of these being a potential focus for therapeutic intervention against this parasite pathogenicity.