

Title: Proteomics-based investigation of substrates for the *Leishmania* deubiquitinase DUB2

The deubiquitinating enzyme (DUB)-mediated cleavage of ubiquitin plays a critical role in balancing protein synthesis and degradation. Twenty DUBs exist in the *Leishmania mexicana* parasite, of which four, including DUB2, are essential for the viability of *L. mexicana* promastigotes. DUB2 has a broad ubiquitin linkage specificity, and it is known to be crucial in establishing infection in mice. However, the functional role of DUB2 is not clear. Thus, we aim to identify the substrates of DUB2 through a comprehensive proteome, ubiquitinome and interactome analysis using mass-spectrometry-based quantitative proteomics, affinity-based ubiquitinated peptide enrichment and proximity dependent biotinylation. For the latter approach, 84 proximal proteins to DUB2 were identified as being significantly enriched. Gene ontology enrichment analysis categorised these proximal proteins to 17 biological processes, with protein translation being the most significant, followed by RNA binding/processing, and microtubule-associated functions, suggesting that DUB2 might have a pleiotropic function. Furthermore, initial investigation of the total ubiquitinome in *L. mexicana* using a Data Dependent Acquisition (DDA) mass-spectrometry workflow revealed that 28 of the DUB2 proximal proteins are ubiquitinated, suggesting that these might be substrates of DUB2. Currently, we are investigating whether some of these proximal proteins are interacting partners of DUB2 via co-immunoprecipitation and we are characterising the total ubiquitinome of *L. mexicana* via an improved proteomics Data Independent Acquisition (DIA) methodology.