Action number: 21111

Applicant name: Daniele Aiello

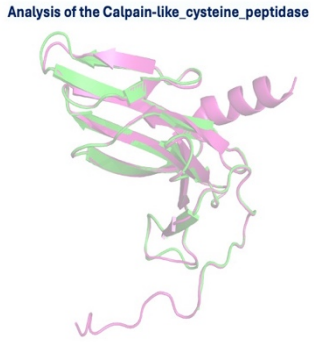
Working Group 1: Compound libraries coordination and integration of compound design: D1.1 Collection of the compound libraries; D1.3 Targets selection, structure-based drug design by advanced modelling/compound.

Details of the STSM

Title: Computational approach for calpain inhibitors discovery with potential antiparasitic activity against Leishmania Infantum. From MS label-free proteomic analysis to hits identification through virtual screening.

Start and end date: 11/01/2024 to 21/02/2024

Broader background and Summary



Background: The use of MS proteomic analysis to identify biological pathway, therefore the proteins involved in specific conditions for the identification of new druggable target together with the use of computational bioinformatic approaches, are powerful tools for the identification of molecules able to overcome drug resistance in parasitic diseases. Trypanosomatids are unicellular protozoan parasites that are responsible for the onset of several diseases, including Leishmaniasis, Chagas disease, and human African trypanosomiasis (HAT). These diseases are collectively referred to as vector-borne parasitic diseases and are characterized by a wide-ranging impact on low-income populations throughout more than 90 countries in Asia, Africa, the Middle East, and Central and South America. The existing pharmacological treatments are considered obsolete and insufficient, highlighting the urgent need for the development of innovative drug regimens that are effective, less harmful and with a lower environmental impact. The little potential for financial gain has resulted in a lack of interest from the pharmaceutical sector in the research and development of novel treatments for NTDs. Screening allows the fast evaluation of compound libraries against biological targets that are implicated in disease mechanisms, thereby identifying hit compounds that can be further optimized.

Aims: In a previous study, our group has exploited mass label-free MS proteomics to characterize the proteomes of both the host (THP-1 immortalized human macrophages) and the guest (*Leishmania infantum*) in the context of infection. From this analysis, four proteins have emerged as significant up-regulated, thus representing possible targets. Among them, calpain (EC 3.4.22.17) has exhibited limited similarity with the models employed in ecotoxicological

investigations, namely Zebrafish, *Mus musculus*, *Caenorhabditis elegans*, and *Drosophila melanogaster*. Based on these premises, our group has explored the possibility to exploit this new target to perform a structure-based virtual screening (SBVS) to identify new hit compounds with potential antileishmanial activity.

Methods: Currently, the lack of x-ray crystal structure of calpain requires the construction of a homology model of the protein. Afterwards, validation of the model will be performed, and the active site will be analysed using software like SiteMap from Maestro suite. Subsequently, a structure-based virtual screening approach will be employed to find new hit compounds. Finally, the new identified compounds will be obtained (purchased, or synthetized) and subjected to *on-target* and *in-vitro* testing against leishmania spp.

Objectives within OneHealthdrugs CA21111: The aim of this STSM is the validation of the new target and identification of novel compounds which will be tested for their antiparasitic potential (e.g., Trypanosoma and Leshmania spp.). This STSM should then contribute to deliverable D1.1 "Collection of the compounds libraries" and D1.3 "Targets selection, structure-based drug design by advanced modelling/compound docking".

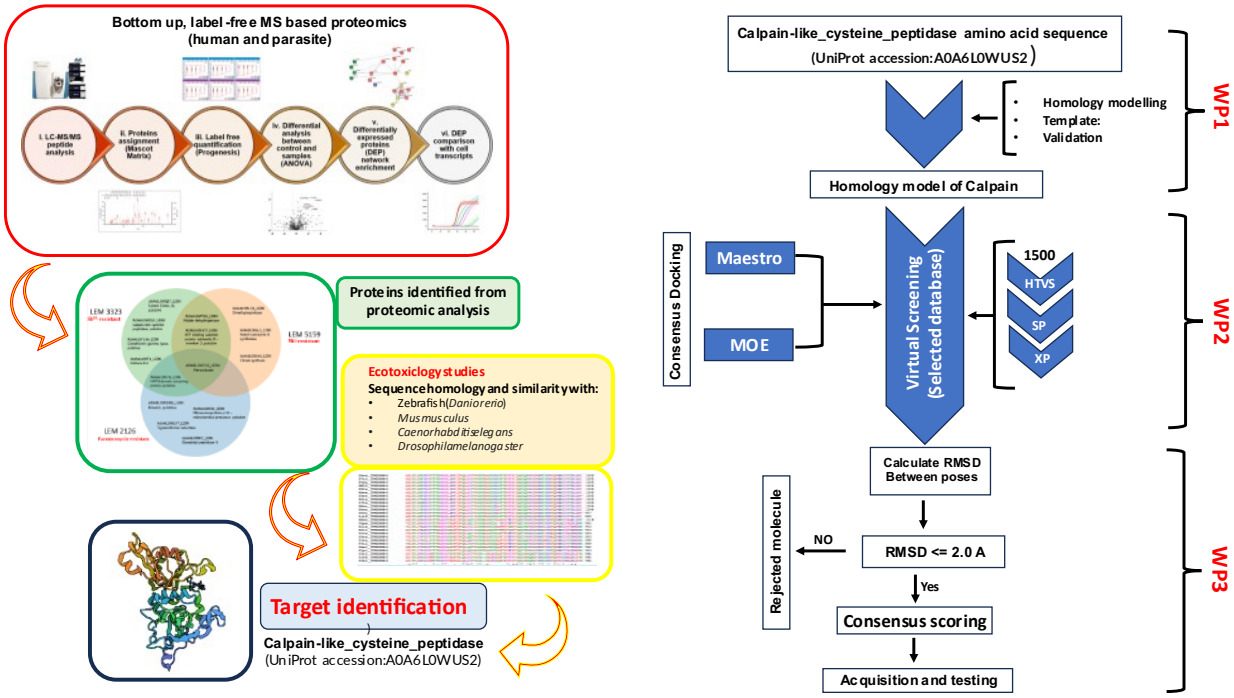


Figure 1: (a) Preliminary studies which led to target identification; (b) Experimental workflow of the proposed project.