PROJECT SUMMARY FOR THE GENERAL PUBLIC

Characterization of *Trypanosoma cruzi* PEX5 as a promising target in Chagas disease.

Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a major public health burden in Latin America and a serious emerging threat to a number of countries throughout the world. Only two drugs are currently available, benznidazol and nifurtimox. They have limited efficacy in the chronic infection phase and produce severe side effects due to high toxicity. To complicate the situation, the number of drug-resistant strains is increasing, urging the development of new, more effective and safe therapeutic strategies.

In contrast to human cells, trypanosomes harbor key glycosomal enzymes in unique subcellular microbodies called glycosomes. Proper biogenesis and maintenance of the peroxisome-related organelle and correct targeting of glycolytic enzymes are essential for the parasites. A number of proteins, collectively termed "peroxins" (PEX) is responsible for the biogenesis of glycosomes by mediating the translocation of glycolytic enzymes across the glycosomal membrane. PEX5 acts as a cytosolic receptor for all proteins carrying a peroxisomal targeting signal of type 1 (PTS1). The membrane bound PEX14 is a docking protein for the cargo/PEX5 complex and together with other PEX proteins allows the cargo translocation into the glycosome. The essential roles of PEX system during the life cycle of the parasite make these proteins suitable targets for drug development. Despite the conserved nature of the mechanisms of protein import into peroxisomes and glycosomes, sequence differences among human and trypanosomal PEX indicate that specific inhibitors can be designed which will abolish essential protein–protein interactions in trypanosomes without harming the human cells.

Recent breakthrough study by our Partner in this Project demonstrated that is possible to target the docking step of the cargo/PEX5 complex at the peroxisomal membrane by blocking trypanosomal PEX14. They developed small molecule inhibitor of *Trypanosoma* PEX14/PEX5 protein-protein interaction and demonstrated that the molecule disrupted glycosomal protein import selectively killing the parasites both in cell culture and in a mouse model of infection. This achievement opens the perspectives of therapeutic targeting of the PEX system, but the optimal component of the system is yet to be identified. *T. cruzi* PEX5/PTS1 interaction is less characterized and no evidence is available concerning the utility of its inhibition in eradicating trypanosomal infection. Thus, **the aim of the project is to validate *T. cruzi* PEX5/PTS1 as a promising target for future antitrypanosomal drug design.**

The primary goal of the project will be achieved through fulfillment of the following specific goals.

(i) Structural characterization of *T. cruzi* PEX5, PEX5/PTS1 and PEX14/PEX5/PTS1 interactions by X-ray crystallography and SAXS will provide understanding of the molecular events guiding the recognition of PEX system components. Along with a broader scientific value, this data will be used within the project to guide the rational design of small molecule inhibitors. This directly relates to the second specific goal of the project.

(ii) development of probes targeting PEX5/PTS1 interface. A combination of the state-of-the-art approaches (virtual screening, X-ray and NMR fragment screening) will be used to obtain the initial hits. The interactions of the hits with PEX5 will be determined by X-ray crystallography. This will allow to rationally design the optimization of compounds for better binding affinity. Driven by structural information, fragment growth, fragment linking and rational compound optimization will be used to improve the initial hits.

Finally, (iii) the optimized probes will be tested in the *in vitro* and *in vivo* models of the trypanosomal infection. This step will provide a proof-of-concept that *T. cruzi* PEX5/PTS1 protein-protein interface is a "druggable" target for Chagas disease or it will invalidate the hypothesis.

In conclusion, **the research envisioned within this project will provide missing structural information on *T. cruzi* PEX5, PEX5/PTS1 recognition and ternary interaction with PEX14, and small molecule probes which will inhibit the cargo recognition step in the glycosomal protein translocation allowing to investigate this process *in vitro* and *in vivo*. Collectively, this will allow to validate *T. cruzi* PEX5 as a target in antitrypanosomal drug development.** In a broad perspective, the validation and developed tools will provide a valuable starting point for development of a new generation of antitrypanosomal drugs to alleviate the worldwide public health concern associated with Chagas disease.