Project proposal for the attribution of a PhD grant within the *ParaFrap* PhD Programme

Title of the project: Role(s) of glycerol metabolism in the biology of African trypanosomes

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Website URL: https://www.mfp.cnrs.fr/wp/la-recherche/intermediate-and-energy-metabolism-of-trypanosomes-imet/

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KEYWORDS: trypanosome-adipocyte metabolic interactions; unusual glycerol-to-glucose preference; *in vivo* (fly and mouse) and *in vitro* approaches; production of trypanosome mutants; *in vitro* differentiation of parasites

ABSTRACT
Trypanosoma brucei, the agent of Human African Trypanosomiasis (HAT), undergoes a complex life cycle from the bloodstream of a mammalian host (BSF) to the alimentary tract (procyclic forms - PCF) and the salivary glands of its blood-feeding insect vector (the tsetse). BSF trypanosomes have been considered for decades to propagate exclusively in the fluids of its mammalian hosts and mainly in the blood. In a break with this dogma, the Co-PI of this project showed that most parasites actually reside in the extravascular compartment of the skin in the vicinity of hypodermal adipocytes (Capewell and Travaillé *et al*., eLife, 2016). In resonance with this major discovery, the PI of this project recently broke another strong dogma considering that *T. brucei* relies exclusively on the glucose provided by its mammalian hosts to feed its central carbon metabolism. Indeed, we established long-term conditions for growth of the parasite in glucose-free medium containing glycerol (Pineda *et al*., PLoS Pathog, 2018). ***Since adipocytes excrete large amounts of glycerol from lipolysis and glycolysis, we hypothesised that interactions between adipocytes and extravascular trypanosomes may confer a selective advantage to the parasites in the mammalian host***.
In the glucose-free environment that is the midgut of the tsetse fly vector, PCF trypanosomes primarily use proline to feed their central carbon and energy metabolism. In these conditions, the parasite needs to produce glucose 6-phosphate through gluconeogenesis from metabolism of non-glycolytic carbon source(s). The PI and the Co-PI recently characterised the gluconeogenic pathway and showed that glycerol is an excellent gluconeogenic carbon source for PCF (Wargnies *et al*., PLoS Pathog, 2018). More interestingly, the PI showed that trypanosomes perform gluconeogenesis from glycerol even in the presence of glucose, making them the only known microorganism using gluconeogenesis in the presence of glucose. The new mechanism controlling this modulation of carbon source preference was elucidated and named "metabolic contest" (Allmann *et al*., BioRxiv, in revision in PLoS Biol). ***We hypothesised that this unique glycerol-to-glucose preference developed by PCF trypanosomes may have a crucial role for the growth and/or development of the parasite in its insect vector.***
The objectives of this project are to understand the role(s) of glycerol metabolism during the entire development of *Trypanosoma brucei* (1) in the different organs of the insect vector (PCF and epimastigote trypanosomes), as well as (2) in the vascular and extravascular compartments of the mammalian host (BSF trypanosomes).
To address these questions, the PhD student will use a combination of *in vivo* (mice and tsetse flies) and *in vitro* approaches using wild-type and mutant (KO generated by CRISPR-Cas9 and/or RNAi) cell lines of the BSF and PCF trypanosomes. The *in vitro* approaches that will be used include the co-culture of BSF trypanosomes and adipocytes, RBP6-driven differentiation of PCF into epimastigotes and metacyclics, production of trypanosome mutants, metabolomics, proteomics, etc.).