The protozoan parasite *Trypanosoma cruzi*, agent of Chagas disease, uses the metacyclogenesis to acquire infective capabilities. This crucial molecular event involves protein phosphorylation that is a dynamic and reversible post-translational modification that regulates a number of biological changes. Advances in mass spectrometry and bioinformatics analysis on last decades allowed a number of large-scale identification and correct pinpoint of phosphorylation sites enabling the elucidation over biological events. The metacyclogenesis of *T. cruzi* is a heterogeneous molecular process where suspended and adhered parasites are found with morphological differences. Thus to better understand the progression of differentiation through the time, we performed phosphoproteome analysis over 72 hours on metacyclogenesis. For this, we monitored *in vitro* metacyclogenesis with high pH C18-RP fractionation, TiO2 phosphopeptide enrichment and LTQ-Orbitrap XL mass spectrometer-based proteomics. At the moment 2,806 protein groups were quantified, containing a total of 5,037 phospho-sites. However, data reveal that some protein phosphorylation sites are always found even among different adhered time point during metacyclogenesis. Gene ontology enrichment analysis identified an overrepresentation of catalytic activity, protein kinase activity and ribonucleotide binding. Due to post-transcriptional regulation of gene expression in *T. cruzi* and expressive phosphorylation of RNA binding proteins on different time points during our analysis, phosphorylation event may be important to the control of mRNA stability in this organism. Supported by CAPES, CNPq, IBMP and ICC/FIOCRUZ-PR.

Keywords: phosphoproteome, metacyclogenesis, *Trypanosoma cruzi*