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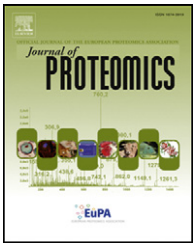

Reevaluating the *Trypanosoma cruzi* proteomic map: The shotgun description of bloodstream trypomastigotes

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ABSTRACT

Chagas disease is a neglected disease, caused by the protozoan *Trypanosoma cruzi*. This kinetoplastid presents a cycle involving different forms and hosts, being trypomastigotes the main infective form. Despite various *T. cruzi* proteomic studies, the assessment of bloodstream trypomastigote profile remains unexplored. The aim of this work is *T. cruzi* bloodstream form proteomic description. Employing shotgun approach, 17,394 peptides were identified, corresponding to 7514 proteins of which 5901 belong to *T. cruzi*. Cytoskeletal proteins, chaperones, bioenergetics-related enzymes, and trans-sialidases are among the top-scoring. GO analysis revealed that all *T. cruzi* compartments were assessed; and majority of proteins are involved in metabolic processes and/or presented catalytic activity. The comparative analysis between the bloodstream trypomastigotes and cultured-derived or metacyclic trypomastigote proteomic profiles pointed to 2202 proteins exclusively detected in the bloodstream form. These exclusive proteins are related to: (a) surface proteins; (b) non-classical secretion pathway; (c) cytoskeletal dynamics; (d) cell cycle and transcription; (e) proteolysis; (f) redox metabolism; (g) biosynthetic pathways; (h) bioenergetics; (i) protein folding; (j) cell signaling; (k) vesicular traffic; (l) DNA repair; and (m) cell death. This large-scale evaluation of bloodstream trypomastigotes, responsible for the parasite dissemination in the patient, marks a step forward in the comprehension of Chagas disease pathogenesis.

Biological significance
The hemoflagellate protozoan *T. cruzi* is the etiological agent of Chagas disease and affects people by the millions in Latin America and other non-endemic countries. The absence of efficient drugs, especially for treatment during the chronic phase of the disease, stimulates the continuous search for novel molecular targets. The identification of essential molecules, particularly those found in clinically relevant forms of the parasite, could be

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crucial. Inside the vertebrate host, trypomastigotes circulate in the bloodstream before infecting various tissues. The exposure of bloodstream forms of the parasite to the host immune system likely leads to differential protein expression in the parasite. In this context, an extensive characterization of the proteomic profile of bloodstream trypomastigotes could help to find not only promising drug targets but also antigens for vaccines or diagnostics. This work is a large-scale proteomic assessment of bloodstream trypomastigotes that show a considerable number of proteins belonging to different metabolic pathways and functions exclusive to this parasitic form, and provides a valuable dataset for the biological understanding of this clinically relevant form of *T. cruzi*.

1. Introduction

Chagas disease, caused by the hemoflagellate protozoan *Trypanosoma cruzi*, affects approximately eight million people in Latin America and is an emergent illness in non-endemic countries due to the triatomine-independent transmission routes and immigration globalization [1–3]. The pathogenesis of the disease involves a high parasitemia in the acute phase and cardiac and/or digestive injury in chronic patients [1,4,5]. In the chronic phase, a lower number of parasites are observed in the affected tissues, and the clinical manifestations are mainly associated to the host immune response [6]. Until now, benznidazole and nifurtimox are the commercial drugs available for Chagas disease therapy; however, their side effects together with controversial efficacy in chronic patients make these drugs far from ideal as trypanocidal compounds, justifying the continuous search for novel molecular drug targets in the parasite [7,8].

The *T. cruzi* biological cycle can be considered complex, involving different cell forms and hosts. In the invertebrate midgut, epimastigote forms proliferate and differentiate into infective metacyclic trypomastigotes in the triatomine posterior rectum. These forms are eliminated with the insect feces and may reach the vertebrate tissues. In the intracellular environment, two steps of differentiation occur: the replicative amastigotes and, subsequently, the bloodstream non-dividing trypomastigotes. The last form is responsible for cell lysis and for the dissemination of the infection inside of the host. The cycle is completed when a triatomine ingests trypomastigotes during foraging [9].

To find potential biomarkers related to disease progression, antigens for vaccines or diagnosis, and/or drug targets, high-throughput proteomics can play a pivotal role in the identification of proteins and the analysis of metabolic pathway variance that could be involved in disease development [10,11]. Particularly for trypanosomatids, proteomic approaches are essential due to the special molecular characteristics of these protozoa. Trypanosomatids present an uncommon organization of their open reading frames in large polycistronic clusters and peculiar gene expression regulation at the transcriptional level directly depending on mRNA stability [12,13]. The presence of non-translated mRNA described in *T. cruzi* [14] provides strong evidence for the requirement of protein content analysis in differential gene expression studies in trypanosomatids models; therefore, large-scale proteomic technology could be an attractive alternative.

The *T. cruzi* proteomic map was first assessed in 2004 [15], and in the last ten years, descriptive analyses were performed in a variety of evolutive stages and strains, and differential expression studies were also conducted to evaluate the parasite's susceptibility/resistance to drugs and its adaptation to stress conditions [10–12,15–24]. Specifically for trypomastigote samples, despite two studies that were performed employing two-dimensional electrophoresis (2-DE) followed by matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF/TOF MS) [15,25], most of the proteomic analysis of this parasite form involved a liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC–ESI-MS/MS) approach. Until now, all studies of this infective form evaluated metacyclic or culture-derived trypomastigotes obtained as in vitro models for different purposes [17,26–29]. However, a detailed description of the bloodstream forms exposed to the host immune system had not been performed yet. It is well known that pathogenic protozoa, including *T. cruzi*, evade mammalian immune defenses by expressing key molecules such as calreticulin, trans-sialidase and mucins, among others [30–32]. In this scenario, a large-scale evaluation of the protein content could represent an interesting strategy for the comprehension of the pathogenesis of Chagas disease. Here, we disclose the large-scale shotgun proteomic analysis of bloodstream trypomastigotes.

2. Materials and methods

2.1. Parasite purification and sample preparation

T. cruzi bloodstream trypomastigotes (Y strain) were obtained by heart puncture of infected albino Swiss mice (*Mus musculus*) at the peak of parasitemia. Red and white blood cells were removed by differential centrifugation, and the yield of parasite purification was improved by repeating the centrifugation steps twice. This procedure reaches the yield of approximately 1.7×10^7 trypomastigotes/mouse. Finally, the parasites were resuspended in Dulbecco's modified Eagle's medium (DMES, Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (Cultilab, Campinas, Brazil) and kept on ice until use. The parasites were washed three times with phosphate buffered saline (PBS, pH 7.4), the washing solutions were discarded, and the washed parasites were incubated in sample lysis solution (7 M urea, 2 M thiourea, 4% CHAPS, 40 mM Tris, 60 mM dithiothreitol, 1% ampholytes) containing Complete Mini protease inhibitor cocktail (Roche Applied Science, Indianapolis, USA). Subsequently, 10 freezing-thawing cycles were performed, and the parasite homogenate was submitted to centrifugation to separate only the soluble protein fraction

as previously described for epimastigotes [11]. The protein quantification was determined by 2D Quant kit (GE Healthcare, Buckinghamshire, England). Four independent biological replicates were processed.

2.2. Protein precipitation and trypsinization

To each volume of sample (200 μg of protein), 4 volumes of ice-cold ethanol and 4 volumes of iced-cold acetone were added followed by incubation for 12 h at −20 °C. The samples were centrifuged at 20,000×*g* for 30 min at 4 °C, and the supernatant was removed. Each pellet was washed 3 times with an ice-cold solution containing 40% ethanol/40% acetone/20% water (followed by centrifugation for supernatant removal) and dried at room temperature. Each pellet was dissolved in 80.8 μL of 400 mM ammonium bicarbonate, 8 M urea and 0.1% SDS, and the protein concentration was determined again. One hundred micrograms of protein from each sample was reduced by incubating with 10 μL of 100 mM dithiothreitol for 3 h at 37 °C. After reaching room temperature, the samples were alkylated with 10 μL of 400 mM iodoacetamide for 15 min in the dark. Water was added to dilute the urea concentration to 1 M. Trypsin (Promega, Madison, USA) was added at a 1:50 (m/m) enzyme to substrate ratio. The digestion was performed for 16 h at 37 °C; the reaction was stopped by drying in a vacuum centrifuge. The samples were stored at −20 °C until use.

2.3. Isoelectric focusing of peptides (OFFGEL)

Peptides were resolubilized in Peptide OFFGEL solution [(4% (v/v) glycerol and 1% (v/v) ampholytes pH 3–10)] and separated using a 3100 OFFGEL Fractionator with an OFFGEL High Res Kit, pH 3–10 immobilized pH gradient (IPG) DryStrip (Agilent Technologies, Santa Clara, USA). The peptides were separated according to the instructions given in the manual. Briefly, twenty-four-well fractions were focused for 50 kVh with a maximum current of 50 mA and power of 200 mW. Fractions were desalted with C18 ZipTip micropipette tips (Millipore, Bedford, USA), completely dried in a vacuum centrifuge and then resuspended in 20 μL of 1% formic acid.

2.4. Reversed phase chromatography online with mass spectrometry

The desalted peptides from each fraction of the OFFGEL separation were separately loaded on a 10-cm reversed phase (RP) column and separated on-line in the mass spectrometer by using an EASY-nLC-System (Proxeon Biosystems, West Palm Beach, USA). Four microliters was initially applied to a 2-cm (100 μm internal diameter) trap column packed with a 5-μm 200 A Magic C18 AQ matrix (Michrom Bioresources, Auburn, USA) followed by separation on a 10-cm (75 μm internal diameter) column that was packed with the same matrix, directly on a self-pack 15-μm PicoFrit empty column (New Objective, West Palm Beach, USA). Samples were loaded onto the trap column at 2000 nL/min while chromatographic separation occurred at 200 nL/min. Mobile phase A consisted of 0.1% (v/v) formic acid in water, while mobile phase B consisted of 0.1% (v/v) formic acid in acetonitrile. Peptides were eluted with a gradient of 2 to 60% of B over 32 min followed by up to 80% B in 4 min while maintaining this concentration for 2 min more before column re-equilibration. The HPLC system was coupled to the LTQ-Orbitrap XL via a nanoscale LC interface (Thermo, USA). Source voltage was set to 1.9 kV, the temperature of the heated capillary was set to 200 °C and tube lens voltage to 100 V. Ion trap full and MSn AGC target values were 30,000 and 10,000, respectively, while the FTMS full AGC target was set to 500,000. MS1 spectra were acquired on the Orbitrap analyzer (300 to 1700 *m/z*) at a 60,000 resolution (for *m/z* 445.1200). For each spectra, the 10 most intense ions were submitted to CID fragmentation (minimum signal required of 10,000; isolation width of 2.5; normalized collision energy of 35.0; activation Q of 0.25 and activation time of 30 ms) followed by MS2 acquisition on the linear trap XL analyzer. The dynamic exclusion option was enabled and set with the following values for each parameter: repeat count = 1; repeat duration = 30 s; exclusion list size = 500; exclusion duration = 45 s and exclusion mass width = 10 ppm. Data were acquired using the Xcalibur software (version 2.0.7).

2.5. Data analysis

The tandem mass spectra were extracted to the MS2 format using RawXtractor [33]. Kinetoplastida protein sequences were downloaded from the NCBInr in November 2012. *M. musculus* protein sequences were downloaded from Uniprot in December 2012 and were added to the database. Subset sequences were eliminated from the database. We then included the sequences of 127 common mass spectra contaminants (e.g., keratin), and for each sequence, a reversed version of it (i.e., a decoy) using the PatternLab's Search Database Generator [34]. Peptide sequence matching was performed using the ProLuCID search engine [35]. The search parameters were tryptic and semi-tryptic peptide candidates, the fixed modification of cysteine carbamidomethylation, a precursor mass tolerance of 50 ppm for MS1 and 500 ppm for MS/MS. The validity of the peptide–spectrum matches (PSMs) was assessed using the Search Engine Processor (SEPro) [34]. Briefly, identifications were grouped by charge state (+2 and ≥+3) and then by tryptic status (i.e., tryptic or semi-tryptic), resulting in four distinct subgroups. For each result, the ProLuCID XCorr, DeltaCN and ZScore values were used to generate a Bayesian discriminator. A cutoff score was established to accept a false-discovery rate (FDR) of 1% based on the number of decoys. This procedure was independently performed on each data subset, resulting in a false-positive rate that was independent of tryptic status or charge state. Additionally, a minimum sequence length of 6 amino acid residues was required. Similar proteins, which represent a sequence in another sequence (fragment), were eliminated. Then, only PSMs with less than 5 ppm were considered to compose a final list of proteins. All results were reported with less than 1% FDR. Table S1 lists all the identifications from all technical replicates of the four biological replicates together. A secondary list reporting the minimum number of proteins that explain all identified peptides (maximum parsimony list) according to the bi-partite graph strategy is also reported [36]. ProteinCenter software (Proxeon Bioinformatics, Odense, Denmark) was used to categorize the identified proteins by

biological process, molecular function and cellular localization, according to GO Slim (http://www.geneontology.org/GO.slims.shtml). The non-exclusive classification corresponds to the proteins localized in more than one cellular compartment or that participate in more than one biological process or molecular function. In order to compare the proteomic profile of bloodstream trypomastigotes, and the proteins previously identified for culture-derived and metacyclic trypomastigotes [17,28], all identified proteins in each study were converted to the same gene set (RefSeq Release 58). The comparisons were performed using the same filtering conditions (without the maximum parsimony filter).

3. Results

Each of the four independent biological replicates were fractionated and analyzed by a shotgun approach in technical triplicate in a high-resolution mass spectrometer. This experimental design allowed for the identification of 17,394 peptides [where 13% (2304 of them) were semi-tryptic in nature], which correspond to 7514 proteins or 4598 non-redundant proteins according to the maximum parsimony criterion with false-positive rates of 0.35% for peptides and 1% for proteins (Table S1). From these 4598 non-redundant proteins identified, 3716 were assigned to *T. cruzi* and 882 to *M. musculus* (5901 and 1613 proteins without the maximum parsimony criterion, respectively). All identified proteins are listed in Table S1, separated by organism and organized in a non-increasing order according to their spectral counts. Since this descriptive work generated an extensive identification list, the biological functions of the proteins identified with more than 500 spectral counts were further discussed. For *T. cruzi*, among the 62 proteins that presented more than 500 spectral counts, we identified trans-sialidases, which are surface proteins necessary for successful invasion of the host cell; protein disulfide isomerase, which is involved in cellular homeostasis; pyruvate phosphate dikinase and elongation factors, which are involved in catalysis and nucleotide binding; enolase, aconitase, NADH-dependent fumarate reductase, fructose-bisphosphate aldolase, glyceraldehyde 3-phosphate dehydrogenase, glycosomal phosphoenolpyruvate carboxykinase and hexokinase, which are energetic metabolism enzymes; and clathrin, paraflagellar rod protein, cytoskeleton-associated protein CAP5.5, dynein and microtubule-associated proteins, which provide structural functions. For *M. musculus*, 38 proteins (presenting more than 500 spectral counts) were further discussed and all were related to blood components and grouped as band 3 anion transport protein, ankyrin, hemoglobin subunits, actin, protein spnb1, tubulin, spectrin, and talin.

For *T. cruzi*, the list of identified proteins without the maximum parsimony criterion added to 5901 identifications. From these, 2627 were annotated in 15 cellular component categories (Fig. 1A). The most common cellular localizations were membranes (51.7%) and cytoplasm (23.1%) followed by nucleus (6.6%) and ribosome (6.2%). All other cellular compartments presented less than 5% of the whole identifications. As for biological processes, 2723 were annotated proteins and therefore categorized into 15 groups, with metabolic processes being the most recurrent (61.9%) (Fig. 1B). Three additional classifications presented percentages greater than 5%: cell transport, regulation and stimulus response (Fig. 1B). Regarding molecular function, 4147 annotated proteins were grouped into 14 categories: catalysis (39.9%), nucleotide binding (18.3%), protein binding (15.9%), and metal ion binding (9.7%) were the most prominent classifications (Fig. 1C). Similar localization and functional analysis were also performed for the identified proteins of *M. musculus*. The 1613 proteins identified (without the maximum parsimony criterion) were distributed among 17 cellular component categories and biological processes: cytoplasm (27.6%) and metabolic process (20.5%) were the most represented (Fig. S1A and B). Additionally, the mammalian annotated proteins identified were allocated into 14 molecular functions, and protein binding (30.9%) was the most common (Fig. S1C). All of these data are discriminated in Tables S2 and S3.

To date, proteomic profiles of trypomastigotes were assessed only from culture-derived or metacyclic forms. From these profiles, we have chosen to compare our data to the ones that represent the largest trypomastigote descriptive proteomic maps [17,28].

4. Discussion

To reevaluate the *T. cruzi* proteome map, we compared our results with two other descriptive studies that evaluated the culture-derived [17] and metacyclic trypomastigotes [17,28]. The comparison correlated the identified proteins in (a) bloodstream trypomastigotes from this work; (b) culture-derived trypomastigotes from Atwood et al. (2005); and (c) metacyclic trypomastigotes from Atwood et al. (2005) and de Godoy et al. (2012). In this context, we found 2214 proteins exclusively present in the bloodstream form, 9 proteins common to both bloodstream and cultured-derived forms, and 3215 proteins common to both bloodstream and metacyclic trypomastigotes (Table S3).

The proteins only identified in bloodstream trypomastigotes were analyzed according to their protein description (Table S2) and function, as discussed below. Although differences in the parasite surface proteomic profile would be expected between the distinct trypomastigotes considering their environment, the identification of surface proteins is among the most interesting exclusive proteins due to their exposure to the host immune system, leading to a better understanding of its interaction with mammalian cells [37]. Among these proteins are phospholipases (A1 and C), mucins, transporters (amino acids, nucleobases, cations), cAMP-phosphodiesterase, GPI-anchor transamidase, acid phosphatase, Tb-291 membrane-associated protein, TolT and surface antigen 2. Further evaluation of the bloodstream trypomastigote surface proteomics is imperative for a deep characterization of crucial molecules that participate in parasite-host cell interactions.

Interestingly, some proteins of the parasite non-classical secretion pathway were also detected in the bloodstream form of the parasite in this study. Exosomes are extracellular small vesicles that carry a variety of secreted molecules important for cell communication, among other functions. In *T. cruzi*, exosomes (10–100 nm in diameter) and ectosomes (100–200 nm in diameter) have already been described in metacyclic trypomastigotes and epimastigotes [38]. Recently, Garcia-Silva and colleagues

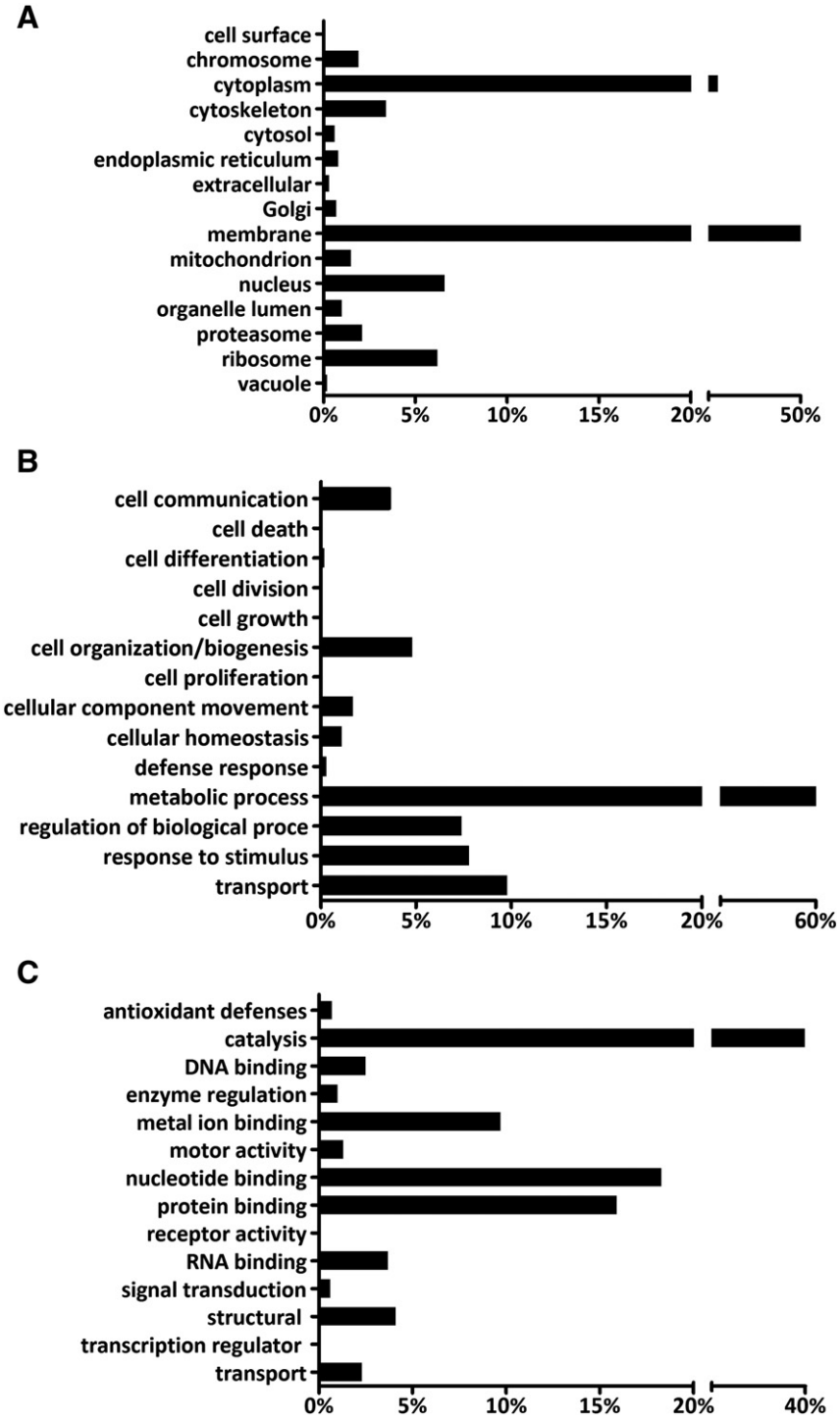

Fig. 1 – GO Slim classification of identified *T. cruzi* annotated proteins. (A) Cellular component; (B) Biological process; (C) Molecular function. Total protein number for each classification is represented as a percentage.

(2014) showed that exosomes stimulated metacyclogenesis and also increased the susceptibility of host cells to infection [39]. Here, we identified four exosome-related proteins in bloodstream trypomastigotes, suggesting the existence of exosomal pathways in vivo. Additionally, some structural proteins were originally described in this work. Proteins such as ARP2/3, which regulates actin dynamics, were identified [40]. The presence of these proteins only in bloodstream trypomastigotes suggested special motility and vesicular traffic characteristics of this parasite form; further analyses are under way.

Different cell cycle and transcriptional regulation factors (especially elongation factors) were also exclusively identified in the bloodstream forms, including three splicing-related proteins. This suggests the presence of peculiarities in post-transcriptional control in infective forms exposed to constituents of mammalian blood. The presence of various proteins

involved in proteolysis and degradation processes as well as enzymes from redox metabolism were also previously observed in trypomastigotes [17]. Nevertheless, autophagic and proteasomal pathways were only predicted in silico in trypanosomatids. Thus, their components, such as E1-like enzyme, were misannotated in databases.

The other exclusive proteins found in bloodstream trypomastigotes were distributed among the following classes of biological function: (a) biosynthetic pathways (especially fatty acids metabolism); (b) bioenergetics (polyphosphate, carbohydrate and mitochondrial metabolisms); (c) protein folding (chaperones such as T-complex protein); (d) cell signaling (diacylglycerol kinase, phosphatidylinositol kinase); (e) vesicular traffic (snare and rab proteins); (f) DNA repair and degradation; and (g) programmed cell death.

Among the 5901 identified proteins, those likely to be the most abundant due to a presentation of high spectral counts (>500 spectra) were cytoskeletal proteins, chaperones, energetic metabolism enzymes, trans-sialidase and elongation factors. Dynein, clathrin, a hypothetical protein and a 75–77-kDa antigen were also abundant. Among the most abundant structural proteins were tubulin, paraflagellar rod protein, and cytoskeletal associated proteins. These proteins were highly expressed in almost all cells and were commonly present on the most abundant lists in proteomic studies of trypanosomatid models [11,17,18,28]. Another abundant cytoskeleton associated protein identified was dynein, which is involved in active intracellular vesicular traffic and flagellar motility, which are crucial for essential processes such as organelle movement and beating of the flagellum [41].

Chaperones, such as heat shock proteins and calreticulin, were commonly overexpressed in various *T. cruzi* forms, especially in response to distinct chemical and physical stress conditions [11,17,18,23,28]. Our data are in accordance with the expression of high amounts of heat shock proteins as a consequence of the extreme temperature variation that trypomastigotes are submitted to in the bloodstream, more severe than that observed in the insect midgut [42]. The presence of the host immune response may also contribute to stress conditions in trypomastigotes, leading to the expression of chaperones. Additionally, elongation factors are GTP-dependent enzymes involved in prokaryotic and eukaryotic protein syntheses [43]. Therefore, their observed high abundance suggests an intense protein synthesis demand in bloodstream trypomastigotes.

Among top-scoring proteins, trans-sialidase is presented on the parasite surface and, upon its secretion, catalyzes the trans-glycosylation of sialic acid to glycoconjugates. This enzyme is highly expressed in trypomastigotes, and its biological roles include participation in adhesion and invasion processes and in the evasion of the host immune system [44]. Interestingly, five enzymes involved in energetic metabolism were also observed in the top list. Pyruvate phosphate dikinase, enolase, hexokinase and fructose-bisphosphate aldolase are enzymes involved in highly active glycolysis/gluconeogenesis pathways in *Trypanosoma brucei* and *T. cruzi* bloodstream trypomastigotes, which corroborates previous suggestions that the bloodstream form is more dependent on glycolysis compared to the insect form [45,46]. However, aconitase (citric acid cycle enzyme) has been poorly studied in *T. cruzi*, and further experiments about its function in the infective parasite form must be performed.

Clathrin is a key protein in receptor-mediated endocytosis, promoting the entry of important molecules into eukaryotic cells, including *T. cruzi* epimastigotes, that present a high endocytic capacity. Indeed, the activity of this pathway has been extensively discussed in the mammalian forms of the parasite [47,48]. Although Sant'Anna et al. (2008) described at least part of the endocytic pathway in bloodstream trypomastigotes, clathrin expression had not been detected until now. The detection of this protein in this work raises once more the discussion about endocytosis in trypomastigotes. However, clathrin was also present in the Golgi apparatus, both in mammals and parasite, actively participating in exocytosis. This correlates with the presence of exosomes and secretion pathways in trypomastigotes, as already mentioned above [38,39]. Moreover, the role(s) of hypothetical protein and 75–77-kDa antigen in bloodstream trypomastigotes need to be investigated.

GO analysis of *T. cruzi*-identified proteins showed that all cellular compartments were assessed, being approximately 50% of the whole identifications from membranes, indicating that the extraction method used also works well to extract hydrophobic proteins, as previously described by our group [11,49]. Furthermore, an in silico analysis revealed that 62% of identified proteins were involved in metabolic processes, and 40% presented catalytic activity as a molecular function. Such information may correlate the high metabolic activity of trypomastigotes to the previous data of Gonçalves et al. (2011) and Clayton & Michels (1996) about the intense glycolytic pathway activity of bloodstream forms, as previously mentioned [45,46].

We note that many mammalian proteins from blood components such as erythrocytes and platelets were identified together with the parasite content. The high stringency for purifying and washing the parasites and the highly sensitive and accurate mass spectrometry performed helped to identify a possible interaction between the parasite and the host cell [50]. During this interaction, the recognition of molecules is essential prior to protozoa invasion, and the adhesion step may have contributed to the presence of the host cells proteins in the sample. As glycoconjugates play an important role as targets in the recognition process, even desialylated erythrocytes are able to interact with the parasite [51]. Although red blood cells have a higher density than trypomastigotes, we identified many proteins present in those cells, which reinforces that a possible adsorption to the parasite surface may occur. Following the same criteria employed for *T. cruzi*, thirty-eight *M. musculus* proteins were considered abundant, including hemoglobin subunits, spectrin, ankyrin, talin, tubulin and actin, as well as putative uncharacterized proteins. Interestingly, GO analysis pointed to the presence of several mice cytosolic and membrane proteins, and also to protein binding characteristics. A plausible explanation for this observation is that trypomastigotes may specifically bind to host proteins to avoid the immune response or even trigger some biochemical pathway to use the mammalian machinery to obtain nutrients and metabolites; however, further analysis is required to test this hypothesis. The proteomic evaluation of the bloodstream contributes to a better understanding of Chagas disease pathogenesis and

opens new perspectives for parasite biology studies in the near future.

Supplementary data to this article can be found online at http://www.journals.elsevier.com/journal-of-proteomics/ http://dx.doi.org/10.1016/j.jprot.2014.12.003.

Conflict of interest

The authors declare that there are no competing interests.

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