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Mitochondrial plasticity in trypanosomatids as a stress adaptation mechanism

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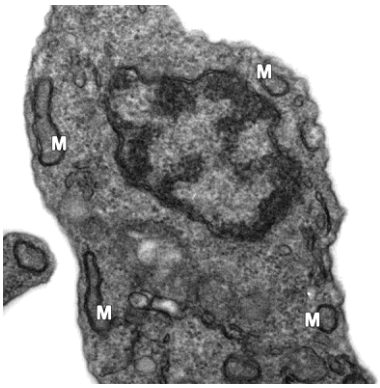
Abstract



Neglected tropical diseases impact more than a billion people globally, with millions of them at risk of infection by parasites of the Trypanosomatidae family. The need to colonize different environments in their hosts means that trypanosomatids are constantly subjected to stress situations, among which the presence of reactive oxygen (ROS) and nitrogen (RNS) species, requiring intense metabolic remodeling to ensure the parasites survival in hostile environments. Additionaly to the classical role in bioenergetics, mitochondrion has a decisive contribution to the oxidative stress, due to the electron leakage from the electron transfer system (ETS). The presence of several functional peculiarities made the mitochondrion of trypanosomatids an unique organelle, considered an excellent target for drug intervention. Some trypanosomatids such as *Leishmania* spp. can avoid the microbicidal mechanisms of the host cells, exhibiting a profile of natural resistance to oxidative and nitrosative stresses. Here, we discussed data about mitochondrial susceptibility and adaptative processes obtained by our group in the last 17 years. Mechanistic proposals

of preclinical drugs was reviewed, as well as different pathways associated with metabolic and mitochondrial remodeling during the life cycle of trypanosomatids, including the possible biological role of ROS and RNS resistance and its impact on the interaction with vertebrate and invertebrate hosts.

1. Background

Trypanosomatidae family includes the genera *Trypanosoma* and *Leishmania*, causative agents of important neglected tropical diseases, such as Chagas disease, sleeping sickness and leishmaniasis. These illnesses are directly related to povertry, affecting especially low-income populations in the developing countries (WHO 2020). This scenario worsens due to the absence of vaccines and the unsatisfactory available chemotherapy (Nussbaum et al 2010; Field et al 2017). Trypanosomatidae family also shelter many non-pathogenic species of protozoa that complete their life cycle in a single invertebrate host (Maslov et al 2013). As parasites of insects, monoxenos trypanosomatids can be as diverse and successful as their hosts, showing exceptional metabolic plasticity (Frolov et al 2021). Interestingly, species from the subfamily Strigomonadinae present a β-proteobacteria in their cytosol, in a well-stablished mutualistic dependence where the endosymbiont cell cycle is controlled by the host and the bacteria is unable to grow out of the protozoan (Roitman & Camargo 1985; Catta-Preta et al 2015). Biochemical studies revealed that the endosymbiont completes essential metabolic pathways of the host parasite, such as amino acid production and heme biosynthesis (Chang et al 1975; Alves et al 2011, 2013).

Trypanosomatids' mitochondrion emerges as a promising drug target due to several morphological and metabolic peculiarities (Fidalgo & Gille 2011). Ultrastructurally, remarkable differences can be easily observed in comparison to the mammalian organelle; the protozoan organelle shows a single, ramified and elongated morphological aspect, close to the plasma membrane (De Souza et al 2009). Additionaly, there is a specialized region named kinetoplast, in which all mitochondrial DNA are accumulated in a complex network of maxicircles and minicircles (Shapiro & Englund 1995; Liu et al 2005). Similar to mammalian cells, electron transfer system (ETS) in trypanosomatids is formed by four integral enzyme complexes in the mitochondrial cristae. Curiously, NADH:ubiquinone oxidoreductase (Complex I) in these parasites presents partial functionality, being the protozoa respiration exclusively dependent of succinate:ubiquinone oxidoreductase (Complex II) and, consequently, by the succinate availability (Denicola-Seoane et al 1992; Opperdoes & Michels 2008; Carranza et al 2009; Tielens & van Hellemond 2009). Previous studies showed a succinate-dependent respiration in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania* spp., very similar to that present in vertebrates (Vercesi et al 1991; Santhamma & Bhaduri 1995; Verner et al 2011). On the other hand, an oxidase, alternative to cytochrome *c* oxidase (Complex IV), has also been reported. Shiba et al (2013) characterized this alternative oxidase (AOX) in *T. brucei*, demonstrating its activity in the reduction of oxygen to water by ubiquinol (Shiba et al 2013). AOX was not clear described in other trypanosomatids, and its existence has been only proposed by indirect approaches (Santhamma & Bhaduri 1995; Chaudhuri et al 2006; Gonçalves et al 2011).

In parallel to its bioenergetic role, trypanosomatids' mitochondrion also takes a crucial role during oxidative stress. It is well-known that ETS electron leakage triggers the partial reduction of oxygen, culminating in the production of reactive oxygen species (ROS) (Venditti et al 2013). Ubiquinol: cytochrome *c* oxidoreductase (Complex III) and ubiquinone Q cycle are the main ROS producers in these parasites (Murphy 2009; Wang & Hekini 2016), and the incubation with Complex III inhibitor antimycin A is widely reported, strongly increasing mitochondrial ROS production (Mehta & Shaha 2004; Genes et al 2011). Additionally, Complexes II and IV are not common electron leakage sites, despite their inhibition can cause a commitment in electron flow (Mehta & Shaha 2004; Genes et al 2011; Gonçalves et al 2011). Fang and Beattie (2003) suggest a antioxidant character to AOX, due to increased production of reactive species in the *T. brucei* procyclic form after enzyme inhibition by salicylhydroxamic acid (SHAM). Thus, in the presence of AOX, the electrons would directly reduce oxygen, without the formation of semiquinone (Fang & Beattie 2003).

Although ROS at basal levels are crucial for signaling pathways and proliferation in trypanosomatids, at higher concentrations these molecules are toxic for the parasites (Nogueira et al 2011). The imbalance in ETS activity directly affects ROS generation and redox homeostasis (Venditti et al 2013), promoting protozoa virulence and disease progression by increased antioxidant environment (Piacenza et al 2013). Today, there are many reports describing that trypanosomatids deal with redox challenge derived from host response, as well as with the variation of nutrients and/or energetic substrates available during their life cycles (Tielens & Van Hellemond 1998; Gonçalves et al 2011; Bombaça et al 2017, 2020, 2021b; Pedra-Rezende et al 2021; Pinho et al 2020; Pinho & Bombaça et al 2022). In this review, an overall discussion is provided on the main advances about trypanosomatids' mitochondrion plasticity and redox metabolism made over the last 17 years from *in vitro* studies of our group.

2. The mitochondrial plasticity among *T. cruzi* stages

In comparison to epimastigotes, the parasite form present in triatomine insect midgut, morphological and functional remodeling are observed in the mitochondrion of trypomastigotes. Despite succinate oxidation supports ETS and ROS production in both parasite stages, striking differences could be detected between them. Bloodstream trypomastigotes present reduced oxygen uptake, increased electron leakage and ROS formation, suggestive of mitochondrial remodeling. In this parasite form, the increased Complex II activity facilitates the entry of electrons in ETS; however, the low Complex III functionality limits the electrons flow to the late ETS steps and the complete reduction of oxygen. The consequent "electron bottleneck" effect results in high ROS production. On the other hand, epimastigotes has an extremely active ETS, accompanied by high mitochondrial membrane potential ($\Delta\Psi_{mt}$) and oxygen consumption. Our data using inhibitors of glycolytic pathway and ETS suggest that the bloodstream form is more fermentative than the insect stage, a phenotype consistent with the availability of glucose in the vertebrate´s blood and L-proline in the triatomine's midgut, respectively (Gonçalves et al 2011).

During its life cycle, *T. cruzi* is submitted to different stress conditions that are used as signaling for differentiation. In metacyclogenesis, low nutrient availability and an acid environment are pivotal features to the transformation of epimastigotes into metacyclic

trypomastigotes. Once mitochondrial remodeling and nutrient availability are interconnected processes, often mediated by autophagic pathway; our group studied the influence relationship between both in epimastigotes subjected to nutritional and acid stress. In the two conditions, epimastigotes' mitochondrion showed strong morphological damage and ETS activity impairment. Additionaly, epimastigotes submitted to stress situation had high ROS production, which was accompanied by increased expression and activity of trypanothione reductase and tryparedoxin peroxidase. Nutritional and acid stresses also promoted an exacerbation of autophagy, evidenced by the high number of autophagosomes and the overexpression of distinct autophagy-related genes. We also showed a direct correlation between autophagy and mitochondrial dysfunction at 24 h, which the parasite treatment with an antioxidant decreased the number of Atg8+ puncta after both stress conditions (Pedra-Rezende et al 2021). In summary, *T. cruzi* mitochondrion shows remarkable functional differences during its life cycle, crucial for the parasite survival in its distinct hosts.

3. The mitochondrial plasticity in *Leishmania* spp.

Similar remodelling can be observed also in the organelle of *Leishmania* spp. A comparative study analyzing the proteome of *Leishmania braziliensis*, *Leishmania panamensis* and *Leishmania guyanensis* species allowed us to determine the abundances of proteins involved in the main metabolic processes. The dataset pointed that proteins involved in glycolitic pathway, ETS and oxidative phosphorylation (OXPHOS) represent 3.7 % of all identified proteins, and other processes involved in ATP production comprising about 7.4 % detected. The evaluation of cumulative concentration of components of glycolitic pathway revealed a significantly higher abundance of these molecules in *L. braziliensis* than in *L. panamensis* and *L. guyanensis*, suggesting that this species relies more on glycolysis. In contrast, proteomic data pointed significant differences in the abundance of enzymes of ETS and OXPHOS in *L. panamensis* and *L. guyanensis*, reinforcing that these species are more dependent of other bioenergetic pathways such as amino acid oxidation and β-oxidation. Biochemical assays corroborated these findings, showing higher levels of 2-NBDG uptake, a fluorescent glucose analogue, in *L. braziliensis*; and, conversely, an intense mitochondrial oxygen consumption in *L. panamensis* and *L. guyanensis* (Pinho et al 2020).

The production of cytokine, ROS and nitric oxide ($NO^{\cdot}$) by mammalian cells normally lead to the control of trypanosomatid infection; however, some *L. braziliensis* strains have been highlighted due to the existence of a natural $NO^{\cdot}$ resistance. These resistant parasites are endowed with specific mechanisms of survival and persistence, causing more lesions and being frequently more resistant to pharmacological treatment (Giudice et al 2007; Souza et al 2010). Using proteomic approaches, we demonstrated that *L. braziliensis* NO-resistant parasites rapidly modulate their protein content in response to $NO^{\cdot}$ exposure, increasing significatively the total protein levels and the abundance of glutathione pathway's intermediates. It may be a mechanism to maintain the pool of NADPH and the recovery of glutathione levels, once an increase in 2-NBDG uptake and in abundance of enzymes related to pentose phosphate pathway was also detected (Pinho & Bombaça et al 2022). Other groups demonstrated that $NO^{\cdot}$ resistance in *Leishmania infantum* is accompanied by the high expression of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase (Holzmuller et al 2006; Alcolea et al 2016). Despite the higher abundance of glycolytic pathway in of NO-resistant strain, the protein

concentration of mitochondrial Complexes I, II and IV is significantly lower in comparison to NO-susceptible parasites. Curiously, after nitrasative challenge the increased abundance of molecules related to complexes III was found in NO-resistant parasites, but it is not affected in NO-susceptible promastigotes. In contrast, our functional analysis pointed to an oxygen consumption in routine state smaller in NO-susceptible strain. Interestingly, the mitochondrial-independent oxygen consumption was 3.5-fold higher in NO-susceptible parasites upon $NaNO_2$ treatment, suggesting an elevated ROS production only in this strain (Pinho & Bombaça et al 2022).

Iron is a crucial component in bioenergetics and antioxidant machinery of trypanosomatids, once it composes different complexes of ETS (heme-proteins and iron-sulfur clusters) and acts as cofactor of important antioxidant enzymes (Wilkinson et al 2002; Dufernez et al 2006). In *Leishmania* spp., iron is obtained from the extracellular environment by transferrin and heme uptake (Flannery et al 2013); or, as more recently described, by the action of two molecules: a plasma membrane-associated ferric reductase named (LFR1) and transmembrane ferrous iron transporter from the ZIP family LIT1 (Jacques et al 2010; Flannery et al 2011). This plant-like system is essential for the parasite growth and the development of cutaneous lesions (Huynh et al 2006, 2008; Flannery et al 2011; Mittra et al 2013). Our previous work showed that iron chelator 2,2-dipyridyl impaired *L. braziliensis* growth, leading to strong disorganization of mitochondrial ultrastructure, in addition with the formation of concentric membranar structures in the organelle matrix and the loss of its cristae. The mitochondrial damage was also confirmed by $\Delta\Psi_{mt}$ collapse and the negative modulation of several mitochondrial proteins expression, such as cytochrome *c* oxidase subunit V (Mesquita-Rodrigues et al 2013). Despite the recent discoveries about the iron and heme uptake in trypanosomatids, its multifactorial responses deserve further analysis.

4. Mitochondrial metabolism & ROS resistance in *Strigomonas culicis*

Among the huge group of monoxenous trypanosomatids, we focused our efforts in *S. culicis* a symbiont-harboring trypanosomatid that colonizes the midgut of several insects (Novy et al 1907). In order to analysis the contribution of endosymbiont to different metabolic processes of the parasite, the proteomic profiles of wild type and aposymbiotic strains were assessed by shotgun approaches. Among the pathways most affected by elimination of the endosymbiont are amino acids synthesis and proteins folding; in addition, several molecules involved in glycolysis, gluconeogenesis, pentose phosphate pathway and glutathione metabolism were more abundant in aposymbiotic strain than in wild type. Functional data corroborate the differences concerning protein abundance, which increased activity of glucose 6-phosphate dehydrogenase and 2-NBDG uptake in the parasites without the bacteria (Brunoro et al 2019; Bombaça et al 2020). The symbiont elimination also impaired mitochondrial function in relation to wild type strain, a phenotype observed through the lower activity of Complexes II-III and IV, oxygen consumption and ATP content. In contrast, higher ROS production and remarkable antioxidant response were detected, suggesting the participation of endosymbiotic bacteria in the energy metabolism and in the mantainance of a reducing environment (Bombaça et al 2017).

Our group also induced an artificial ROS resistance in *S. culicis* wild type strain by parasite incubation with increasing concentrations of hydrogen peroxide (H_2O_2). ROS

resistance in *S. culicis* triggered the antioxidant system, increasing thiol-dependent peroxidase activity and decreasing H_2O_2 production and lipid peroxidation. Interestingly, H_2O_2-resistant strain also showed an increased expression and activity of ascorbate peroxidase in comparison to wild type. H_2O_2 resistance was also accompanied by an increase in parasites' mitochondrial functionality, which was observed through higher resistance of organelle inhibitors, elevated oxygen consumption, complexes activity and ATP content (Bombaça et al 2017, 2020). Curiously, our unpublished data point that H_2O_2-resistant strain has higher concentrations of intracellular iron and heme, suggesting that ROS resistance induction modulates heme biosynthesis once symbiont-harboring trypanosomatids are able to synthesize this porphyrin. In addition, the pretreatment of H_2O_2-resistant parasites with 2,2-dipyridyl decreases mitochondrial and ascorbate peroxidase activities at the same levels detected in wild type, demonstrating the importance of iron and heme to mantainance of energy metabolism and antioxidant environment during ROS resistance induction (Bombaça et al unpublished data). These data have been experimentally confirmed, and it will be compiled in a publication soon.

S. culicis is part of *Aedes aegypti* microbiota and is submitted to different stress conditions derived from host's metabolism, including ROS production by dual oxidases (DUOXs). Our investigation about the influence of *S. culicis* on mosquitoes' midgut oxidative environment showed the activation of different response mechanisms in the infection by both wild type and H_2O_2-resistant strains. While wild type parasites stimulated the host's mitochondrial metabolism and the consequente production of superoxide radical, H_2O_2-resistant strain exacerbated DUOX activity and caused a remarkable ROS production. In addition, the infection by both strains compromised mosquitoes' reproductive fitness, decreasing fecundity and fertility of females. These phenotypes can be related to parasite load, once ROS resistance increase the ability of *S. culicis* to infect and persist in pro-oxidant environments (Bombaça et al 2017, 2021b).

5. Preclinical drugs & the mitochondrion

In vitro analysis of parasites treated with different classes of drugs frequently report the mitochondrion as the main target during pharmacological treatment. Among the most recurrent phenotypes observed in morphological assays, mitochondrial swelling, cristae disorganization and matrix electrondensity impairment are the most usual (Figure 1); however, this phenotype varies with the drug used, concentration and time of treatment (Sem & Majumder 2008; Fidalgo & Gille 2011; Silva et al 2011). Indeed, there are many studies in the literature describing mitochondrial damage in treated parasites essentially based on ultrastructural evaluation (Menna-Barreto & de Castro 2014; Vannier-Santos et al 2019).

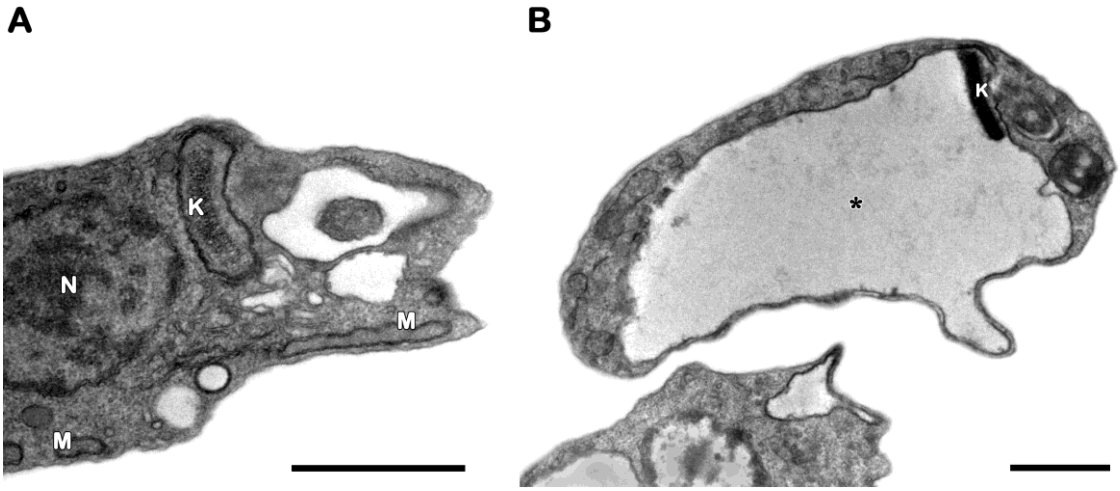


Figure 1. The mitochondrion of trypanosomatids as the most recurrent drug target. (**A**) control parasite, showing classical morphology of nucleus (N), mitochondrion (M) and kinetoplast (K). (**B**) Treated parasite presenting a strong mitochondrial swelling (asterisk), with a washed out aspect of the matrix and loss of cristae organization. Bars: 1 μm.

In *T. cruzi*, sesquiterpenoid isolated from the Chilean flora, as well as, naphthoquinones and derivatives led to the mitochondrial swelling, with $\Delta\Psi_{mt}$ reduction and increased ROS generation (Menna-Barreto et al 2005, 2007, Salomão et al 2013, Bombaça et al 2018). Similar phenotype was detected in trypomastigotes and epimastigotes treated with geranylgeraniol obtained from Brazilian plant *Pterodon pubescens* or with HIV peptidase inhibitors (Menna-Barreto et al 2008, Sangenito et al 2014, 2018). Such ultrastructural alterations were also observed in treated *Leishmania amazonensis*. Promastigotes incubated with epigallocatechin 3-gallate, the main flavonoid in green tea, or with apigenin, a natural flavone, or even with a metallodrug (zinc complex), showed dilated aspect and decreased $\Delta\Psi_{mt}$ (Inácio et al 2012, Fonseca-Silva et al 2015, Sangenito et al 2021). Fonseca-Silva and colleagues (2015) demonstrated the protective effect of the antioxidants glutathione and N-acetyl-L-cysteine through the reduction of ROS levels in apigenin-treated parasites and reversal of leishmanicidal activity (Fonseca-Silva et al 2015). Despite morphological phenotype and $\Delta\Psi_{mt}$ loss measured by fluorescent probes are interestingly start points, they not really assess the mechanistic action of compounds in the mitochondrial physiology.

In order to further investigate the mitochondrion of trypanosomatids as a drug target, our group focused in the mechanisms involved in trypanocidal action of naphthoquinones and derivatives. In 2009, we assessed naphthofuranquinones activity on *T. cruzi* mitochondrial physiology. In both epimastigotes and bloodstream trypomastigotes, the compounds promoted a drastic effect on the organelle, leading to an impairment of Complex I–III activity and succinate-induced oxygen uptake. In our mechanistic proposal, we suggested the interference of compounds with mitochondrial electron flow, deviating electrons from the ubiquinone (Menna-Barreto et al 2009). On the other hand, proteomic approaches also corroborate our electron microscopy data, pointing to the high number of mitochondrial proteins modulated by the treatment with β-lapachone-derived naphthoimidazoles N1, N2 and N3 in both epimastigotes and bloodstream trypomastigotes of *T. cruzi* (Menna-Barreto et al 2005, 2007, 2010; Brunoro et al 2016). Reinforcing these findings, in a recent work, we described that these three

compounds strongly reduced the rates of oxygen uptake and Complexes II-III and IV activities, impairing the mitochondrial metabolism. Moreover, ROS production was related to antiparasitic activity, N2 and N3 reducing ETS electron flux and increasing the mitochondrial ROS levels. Curiously, ROS generation derived from N1 treatment did not derive from mitochondrial injury, probably resulting as a consequence of the inhibition of antioxidant enzymes (Bombaça et al 2019). In 2021, similar study was performed with a novel naphthoimidazole named N4. Its trypanocidal action is more faster than the observed for the other three naphthoimidazoles, increasing ROS levels through Complex II-III impairment in the early hours of treatment (Bombaça & Silva et al 2021a). Figure 2 shows the mechanistic proposal of naphthoimidazoles' trypanocidal activity.

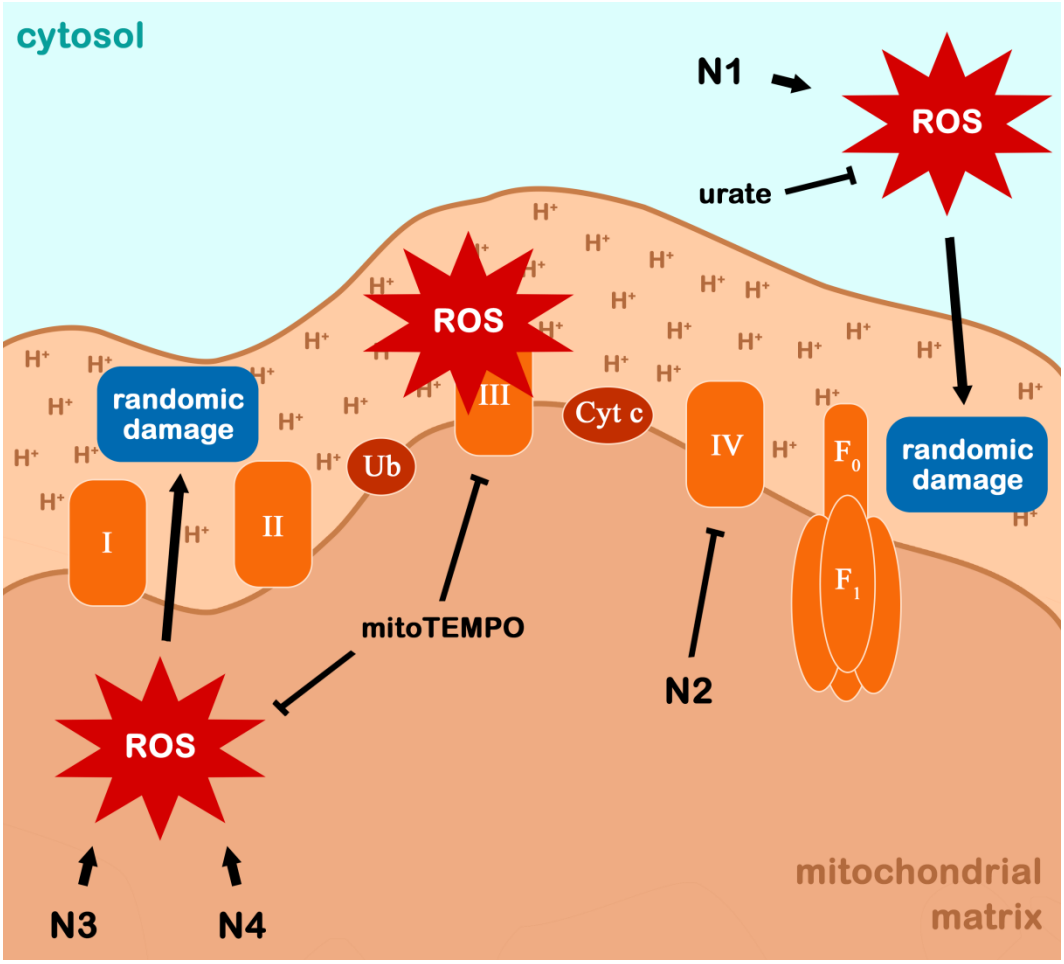


Figure 2. The mechanistic proposal of trypanocidal activity on *T. cruzi* mitochondrion. N1, N3 and N4 induced ROS production, resulting in an unspecific mitochondrial injury. Curiously, N3 and N4 showed the phenotype prevented only by the mitochondrial antioxidant mitoTEMPO. In contrast, N1 led to ROS generation in the cytosol, and the parasite can be protected from its deleterious effect by the incubation with urate. N2 inhibited cytochrome *c* oxidase (Complex IV) activity and, subsequently, induced the increase in mitochondrial ROS levels. Ub: ubiquinone; Cyt *c*: cytochrome *c*.

6. Concluding remarks

As it was previously described, the mitochondrion of trypanosomatids is commonly affected by an inummerous classes of drugs. The peculiarities in bioenergetics such as remarkable differences in ETS, the peculiar antioxidant machinery, as well as the AOX existence and the glycolysis compartmentalization into glycosomes (Tomás & Castro 2013; Michels et al 2021), make these parasites different from their hosts and justify the energetic and oxidative metabolisms as promising drug targets. Furthermore, during their life cycles, trypanosomatids were challenged to several stress conditions. As an example, the same parasite is submitted to completely different envinronments inside the invertebrate and vertebrate hosts. Temperature, pH, nutrients availability, among many other factors, can influence the protozoa metabolism and, consequently, their success in its life cycle. In this scenario, the mitochondrial plasticity plays a crucial role to guarantee their adaptation to the host (Gonçalves et al 2011; Bombaça et al 2017, 2020, 2021b; Pedra-Rezende et al 2021; Pinho et al 2020; Pinho & Bombaça et al 2022). However, despite the recently advances, further studies must be conducted to better characterize the biochemical and molecular mechanisms related to the parasite adaptative behavior.

References

Alcolea PJ, Tuñón GI, Alonso A, García-Tabares F, Ciordia S, Mena MC, Campos RN, Almeida RP, Larraga V (2016) Differential protein abundance in promastigotes of nitric oxide-sensitive and resistant *Leishmania chagasi* strains. Proteomics Clin Appl 10(11):1132-1146. https://doi.org/10.1002/prca.201600054

Alves JM, Voegtly L, Matveyev AV, Lara AM, da Silva FM, Serrano MG, Buck GA, Teixeira MM, Camargo EP (2011) Identification and phylogenetic analysis of heme synthesis genes in trypanosomatids and their bacterial endosymbionts. PLoS One 6(8):e23518. https://doi.org/10.1371/journal.pone.0023518

Alves JM, Serrano MG, Maia da Silva F, Voegtly LJ, Matveyev AV, Teixeira MM, Camargo EP, Buck GA (2013) Genome evolution and phylogenomic analysis of *Candidatus* Kinetoplastibacterium, the betaproteobacterial endosymbionts of *Strigomonas* and *Angomonas*. Genome Biol Evol 5(2):338-50. https://doi.org/10.1093/gbe/evt012

Bombaça ACS, Dias FA, Ennes-Vidal V, Garcia-Gomes ADS, Sorgine MHF, d'Avila-Levy CM, Menna-Barreto RFS (2017) Hydrogen peroxide resistance in *Strigomonas culicis*: Effects on mitochondrial functionality and *Aedes aegypti* interaction. Free Radic Biol Med 113:255-266. https://doi.org/10.1016/j.freeradbiomed.2017.10.006

Bombaça ACS, Dossow DV, Barbosa JMC, Paz C, Burgos V, Menna-Barreto RFS (2018) Trypanocidal activity of natural sesquiterpenoids involves mitochondrial dysfunction, ROS production and autophagic phenotype in *Trypanosoma cruzi*. Molecules 23(11):2800. https://doi.org/10.3390/molecules23112800

Bombaça ACS, Viana PG, Santos ACC, Silva TL, Rodrigues ABM, Guimarães ACR, Goulart MOF, da Silva Júnior EN, Menna-Barreto RFS (2019) Mitochondrial disfunction and ROS production are essential for anti-*Trypanosoma cruzi* activity of β-lapachone-derived naphthoimidazoles. Free Radic Biol Med 130:408-418. https://doi.org/10.1016/j.freeradbiomed.2018.11.012

Bombaça ACS, Brunoro GVF, Dias-Lopes G, Ennes-Vidal V, Carvalho PC, Perales J, d'Avila-Levy CM, Valente RH, Menna-Barreto RFS (2020) Glycolytic profile shift and antioxidant triggering in symbiont-free and H_2O_2-resistant *Strigomonas culicis*. Free Radic Biol Med 146:392-401. https://doi.org/10.1016/j.freeradbiomed.2019.11.025

Bombaça ACS, Silva LA, Chaves OA, da Silva LS, Barbosa JMC, da Silva AM, Ferreira ABB, Menna-Barreto RFS (2021a) Novel N,N-di-alkylnaphthoimidazolium derivative of β-lapachone

impaired *Trypanosoma cruzi* mitochondrial electron transport system. Biomed Pharmacother 135:111186. https://doi.org/10.1016/j.biopha.2020.111186

Bombaça ACS, Gandara ACP, Ennes-Vidal V, Bottino-Rojas V, Dias FA, Farnesi LC, Sorgine MH, Bahia AC, Bruno RV, Menna-Barreto RFS (2021b) *Aedes aegypti* infection with trypanosomatid *Strigomonas culicis* alters midgut redox metabolism and reduces mosquito reproductive fitness. Front Cell Infect Microbiol 11:732925. https://doi.org/10.3389/fcimb.2021.732925

Brunoro GV, Faça VM, Caminha MA, Ferreira AT, Trugilho M, de Moura KC, Perales J, Valente RH, Menna-Barreto RF (2016) Differential Gel Electrophoresis (DIGE) Evaluation of naphthoimidazoles mode of action: A study in *Trypanosoma cruzi* bloodstream trypomastigotes. PLoS Negl Trop Dis 10(8):e0004951. https://doi.org/10.1371/journal.pntd.0004951

Brunoro GVF, Menna-Barreto RFS, Garcia-Gomes AS, Boucinha C, Lima DB, Carvalho PC, Teixeira-Ferreira A, Trugilho MRO, Perales J, Schwämmle V, Catanho M, de Vasconcelos ATR, Motta MCM, d'Avila-Levy CM, Valente RH (2019) Quantitative proteomic map of the trypanosomatid *Strigomonas culicis*: The biological contribution of its endosymbiotic bacterium. Protist 170(6):125698. https://doi.org/10.1016/j.protis.2019.125698

Carranza CJ, Kowaltowski AJ, Mendonça MAG, De Oliveira TC, Gadelha FR, Zingales B (2009) Mitochondrial bioenergetics and redox state are unaltered in *Trypanosoma cruzi* isolates with compromised mitochondrial complex I subunit genes. J Bioenerg Biomembr 41(3):299–308. https://doi.org/10.1007/s10863-009-9228-4

Catta-Preta CM, Brum FL, da Silva CC, Zuma AA, Elias MC, de Souza W, Schenkman S, Motta MC (2015) Endosymbiosis in trypanosomatid protozoa: The bacterium division is controlled during the host cell cycle. Front Microbiol 6:520. https://doi.org/10.3389/fmicb.2015.00520

Chang KP, Chang CS, Sassa S (1975) Heme biosynthesis in bacterium-protozoon symbioses: Enzymic defects in host hemoflagellates and complemental role of their intracellular symbiotes. Proc Natl Acad Sci U S A 72(8):2979-83. https://doi.org/10.1073/pnas.72.8.2979

Chaudhuri M, Ott RD, Hill GC (2006) Trypanosome alternative oxidase: From molecule to function. Trends Parasitol 22(10):484-91. https://doi.org/10.1073/pnas.72.8.2979

de Souza W, Attias M, Rodrigues JCF (2009) Particularities of mitochondrial structure in parasitic protists (Apicomplexa and Kinetoplastida). Int J Biochem Cell Biol 41(10):2069–80. https://doi.org/10.1016/j.biocel.2009.04.007

Denicola-Seoane A, Rubbo H, Prodanov E, Turrens JF (1992) Succinate-dependent metabolism in *Trypanosoma cruzi* epimastigotes. Mol Biochem Parasitol 54(1):43-50. https://doi.org/10.1016/0166-6851(92)90093-Y

Dufernez F, Yernaux C, Gerbod D, Noël C, Chauvenet M, Wintjens R, Edgcomb VP, Capron M, Opperdoes FR, Viscogliosi E (2006) The presence of four iron-containing superoxide dismutase isozymes in Trypanosomatidae: Characterization, subcellular localization, and phylogenetic origin in *Trypanosoma brucei*. Free Radic Biol Med 40(2):210-25. https://doi.org/10.1016/j.freeradbiomed.2005.06.021

Fang J, Beattie DS (2003) Identification of a gene encoding a 54 kDa alternative NADH dehydrogenase in *Trypanosoma brucei*. Mol Biochem Parasitol 127(1):73-7. https://doi.org/10.1016/S0166-6851(02)00305-5

Fidalgo LM, Gille L (2011) Mitochondria and trypanosomatids: Targets and drugs. Pharm Res 28(11):2758–70. https://doi.org/10.1007/s11095-011-0586-3

Field MC, Horn D, Fairlamb AH, Ferguson MAJ, Gray DW, Read KD, De Rycker M, Torrie LS, Wyatt PG, Wyllie S, Gilbert IH (2017). Anti-trypanosomatid drug discovery: An ongoing challenge and a continuing need. Nat Rev Microbiol 15(7):447. Erratum for: Nat Rev Microbiol 15(4):217-231. https://doi.org/10.1038/nrmicro.2016.193

Flannery AR, Huynh C, Mittra B, Mortara RA, Andrews NW (2011) LFR1 ferric iron reductase of *Leishmania amazonensis* is essential for the generation of infective parasite forms. J Biol

Chem 286(26):23266-79. https://doi.org/10.1074/jbc.M111.229674
Flannery AR, Renberg RL, Andrews NW (2013) Pathways of iron acquisition and utilization in *Leishmania*. Curr Opin Microbiol 16(6):716-21. https://doi.org/10.1016/j.mib.2013.07.018
Fonseca-Silva F, Canto-Cavalheiro MM, Menna-Barreto RF, Almeida-Amaral EE (2015) Effect of apigenin on *Leishmania amazonensis* is associated with reactive oxygen species production followed by mitochondrial dysfunction. J Nat Prod 78(4):880-4. https://doi.org/10.1021/acs.jnatprod.5b00011
Frolov AO, Kostygov AY, Yurchenko V (2021) Development of monoxenous trypanosomatids and phytomonads in insects. Trends Parasitol. 37(6):538-551. https://doi.org/10.1016/j.pt.2021.02.004
Genes C, Baquero E, Echeverri F, Maya JD, Triana O (2011) Mitochondrial dysfunction in *Trypanosoma cruzi*: the role of *Serratia marcescens* prodigiosin in the alternative treatment of Chagas disease. Parasit Vectors 4:66. https://doi.org/10.1186/1756-3305-4-66
Gonçalves RLS, Menna Barreto RFS, Polycarpo CR, Gadelha FR, Castro SL, Oliveira MF (2011) A comparative assessment of mitochondrial function in epimastigotes and bloodstream trypomastigotes of *Trypanosoma cruzi*. J Bioenerg Biomembr 43(6):651-61. https://doi.org/10.1007/s10863-011-9398-8
Holzmuller P, Hide M, Sereno D, Lemesre JL (2006) *Leishmania infantum* amastigotes resistant to nitric oxide cytotoxicity: Impact on *in vitro* parasite developmental cycle and metabolic enzyme activities. Infect Genet Evol 6(3):187-97. https://doi.org/10.1016/j.meegid.2005.03.003
Huynh C, Sacks DL, Andrews NW (2006) A *Leishmania amazonensis* ZIP family iron transporter is essential for parasite replication within macrophage phagolysosomes. J Exp Med 203(10):2363-75. https://doi.org/10.1084/jem.20060559
Huynh C, Andrews NW (2008) Iron acquisition within host cells and the pathogenicity of *Leishmania*. Cell Microbiol 10(2):293-300. https://doi.org/10.1111/j.1462-5822.2007.01095.x
Inacio JD, Canto-Cavalheiro MM, Menna-Barreto RF, Almeida-Amaral EE (2012) Mitochondrial damage contribute to epigallocatechin-3-gallate induced death in *Leishmania amazonensis*. Exp Parasitol 132(2):151-5. https://doi.org/10.1016/j.exppara.2012.06.008
Jacques I, Andrews NW, Huynh C (2010) Functional characterization of LIT1, the *Leishmania amazonensis* ferrous iron transporter. Mol Biochem Parasitol 170(1):28-36. https://doi.org/10.1016/j.molbiopara.2009.12.003
Liu B, Liu Y, Motyka SA, Agbo EE, Englund PT (2005) Fellowship of the rings: the replication of kinetoplast DNA. Trends Parasitol 21(8):363-9. https://doi.org/10.1016/j.pt.2005.06.008
Maslov DA, Votýpka J, Yurchenko V, Lukeš J (2013) Diversity and phylogeny of insect trypanosomatids: All that is hidden shall be revealed. Trends Parasitol 29(1):43-52. https://doi.org/10.1016/j.pt.2012.11.001
Mehta A, Shaha C (2004) Apoptotic death in *Leishmania donovani* promastigotes in response to respiratory chain inhibition: Complex II inhibition results in increased pentamidine cytotoxicity. J Biol Chem 279(12):11798–813. https://doi.org/10.1074/jbc.M309341200
Menna-Barreto RF, Henriques-Pons A, Pinto AV, Morgado-Diaz JA, Soares MJ, De Castro SL (2005) Effect of a beta-lapachone-derived naphthoimidazole on *Trypanosoma cruzi*: Identification of target organelles. J Antimicrob Chemother 56(6):1034-41. https://doi.org/10.1093/jac/dki403
Menna-Barreto RF, Corrêa JR, Pinto AV, Soares MJ, de Castro SL (2007) Mitochondrial disruption and DNA fragmentation in *Trypanosoma cruzi* induced by naphthoimidazoles synthesized from beta-lapachone. Parasitol Res 101(4):895-905. https://doi.org/10.1007/s00436-007-0556-1
Menna-Barreto RF, Laranja GA, Silva MC, Coelho MG, Paes MC, Oliveira MM, de Castro SL (2008) Anti-*Trypanosoma cruzi* activity of *Pterodon pubescens* seed oil: Geranylgeraniol as the major bioactive component. Parasitol Res 103(1):111-7. https://doi.org/10.1007/s00436-008-0937-0

Menna-Barreto RF, Goncalves RL, Costa EM, Silva RS, Pinto AV, Oliveira MF, de Castro SL (2009) The effects on *Trypanosoma cruzi* of novel synthetic naphthoquinones are mediated by mitochondrial dysfunction. Free Radic Biol Med 47(5):644-53. https://doi.org/10.1016/j.freeradbiomed.2009.06.004

Menna-Barreto RF, Beghini DG, Ferreira AT, Pinto AV, De Castro SL, Perales J (2010) A proteomic analysis of the mechanism of action of naphthoimidazoles in *Trypanosoma cruzi* epimastigotes *in vitro*. J Proteomics 73(12):2306-15. https://doi.org/10.1016/j.jprot.2010.07.002

Menna-Barreto RF, de Castro SL (2014) The double-edged sword in pathogenic trypanosomatids: The pivotal role of mitochondria in oxidative stress and bioenergetics. Biomed Res Int 2014:614014. https://doi.org/10.1155/2014/614014

Mesquita-Rodrigues C, Menna-Barreto RF, Sabóia-Vahia L, Da-Silva SA, de Souza EM, Waghabi MC, Cuervo P, De Jesus JB (2013) Cellular growth and mitochondrial ultrastructure of *Leishmania (Viannia) braziliensis* promastigotes are affected by the iron chelator 2,2-dipyridyl. PLoS Negl Trop Dis 17;7(10):e2481. https://doi.org/10.1371/journal.pntd.0002481

Michels PAM, Villafraz O, Pineda E, Alencar MB, Cáceres AJ, Silber AM, Bringaud F (2021) Carbohydrate metabolism in trypanosomatids: New insights revealing novel complexity, diversity and species-unique features. Exp Parasitol 224:108102. https://doi.org/10.1016/j.exppara.2021.108102

Mittra B, Cortez M, Haydock A, Ramasamy G, Myler PJ, Andrews NW (2013) Iron uptake controls the generation of *Leishmania* infective forms through regulation of ROS levels. J Exp Med 210(2):401-16. https://doi.org/10.1084/jem.20121368

Murphy MP (2009) How mitochondria produce reactive oxygen species. Biochem J 417(1):1-13. https://doi.org/10.1042/BJ20081386

Nogueira NP, de Souza CF, Saraiva FM, Sultano PE, Dalmau SR, Bruno RE, Gonçalves Rde L, Laranja GA, Leal LH, Coelho MG, Masuda CA, Oliveira MF, Paes MC (2011) Heme-induced ROS in *Trypanosoma cruzi* activates CaMKII-like that triggers epimastigote proliferation. One helpful effect of ROS. PLoS One 6(10):e25935. https://doi.org/10.1371/journal.pone.0025935

Novy FG, MacNeal WJ, Torrey HN (1907) The trypanosomes of mosquitoes and other insects. J Infect Dis 4(2):223–76. https://doi.org/10.1093/infdis/4.2.223

Nussbaum K, Honek J, Cadmus C, Efferth T (2010) Trypanosomatid parasites causing neglected diseases. Curr Med Chem 17(15):1594–617.https://doi.org/10.2174/092986710790979953

Opperdoes FR, Michels PAM (2008) Complex I of Trypanosomatidae: Does it exist? Trends Parasitol 24(7):310–7. https://doi.org/10.1016/j.pt.2008.03.013

Pedra-Rezende Y, Fernandes MC, Mesquita-Rodrigues C, Stiebler R, Bombaça ACS, Pinho N, Cuervo P, De Castro SL, Menna-Barreto RFS (2021) Starvation and pH stress conditions induced mitochondrial dysfunction, ROS production and autophagy in *Trypanosoma cruzi* epimastigotes. Biochim Biophys Acta Mol Basis Dis 1867(2):166028. https://doi.org/10.1016/j.bbadis.2020.166028

Piacenza L, Peluffo G, Alvarez MN, Martínez A, Radi R (2013) *Trypanosoma cruzi* antioxidant enzymes as virulence factors in Chagas disease. Antioxid Redox Signal 19(7):723-34. https://doi.org/10.1089/ars.2012.4618

Pinho N, Wiśniewski JR, Dias-Lopes G, Saboia-Vahia L, Bombaça ACS, Mesquita-Rodrigues C, Menna-Barreto R, Cupolillo E, de Jesus JB, Padrón G, Cuervo P (2020) In-depth quantitative proteomics uncovers specie-specific metabolic programs in *Leishmania (Viannia)* species. PLoS Negl Trop Dis 14(8):e0008509. https://doi.org/10.1371/journal.pntd.0008509

Pinho N, Bombaça AC, Wiśniewski JR, Dias-Lopes G, Saboia-Vahia L, Cupolillo E, de Jesus JB, de Almeida RP, Padrón G, Menna-Barreto R, Cuervo P (2022) Nitric oxide resistance in *Leishmania (Viannia) braziliensis* involves regulation of glucose consumption, glutathione metabolism and abundance of pentose phosphate pathway enzymes. Antioxidants (Basel)

11(2):277. https://doi.org/10.3390/antiox11020277
Roitman I, Camargo EP (1985) Endosymbionts of Trypanosomatidae. Parasitol Today 1(5):143-4. https://doi.org/10.1016/0169-4758(85)90060-2
Salomão K, De Santana NA, Molina MT, De Castro SL, Menna-Barreto RF (2013) *Trypanosoma cruzi* mitochondrial swelling and membrane potential collapse as primary evidence of the mode of action of naphthoquinone analogues. BMC Microbiol 13:196. https://doi.org/10.1186/1471-2180-13-196
Sangenito LS, Menna-Barreto RF, D Avila-Levy CM, Santos AL, Branquinha MH (2014) Decoding the anti-*Trypanosoma cruzi* action of HIV peptidase inhibitors using epimastigotes as a model. PLoS One 9(12):e113957. https://doi.org/10.1371/journal.pone.0113957
Sangenito LS, Menna-Barreto RFS, Oliveira AC, d'Avila-Levy CM, Branquinha MH, Santos ALS (2018) Primary evidence of the mechanisms of action of HIV aspartyl peptidase inhibitors on *Trypanosoma cruzi* trypomastigote forms. Int J Antimicrob Agents 52(2):185-194. https://doi.org/10.1016/j.ijantimicag.2018.03.021
Sangenito LS, Rodrigues HD, Santiago SO, Bombaça ACS, Menna-Barreto RFS, Reddy A, Branquinha MH, Velasco-Torrijos T, Santos ALS (2021) *In vitro* effects of bis(N-[4-(hydroxyphenyl)methyl]-2-pyridinemethamine)zinc perchlorate monohydrate 4 on the physiology and interaction process of *Leishmania amazonensis*. Parasitol Int 84:102376. https://doi.org/10.1016/j.parint.2021.102376
Santhamma KR, Bhaduri A (1995) Characterization of the respiratory chain of *Leishmania donovani* promastigotes. Mol Biochem Parasitol 75(1):43-53. https://doi.org/10.1016/0166-6851(95)02510-3
Sen N, Majumder HK (2008) Mitochondrion of protozoan parasite emerges as potent therapeutic target: Exciting drugs are on the horizon. Curr Pharm Des 14(9):839-46. https://doi.org/10.2174/138161208784041024
Shapiro TA, Englund PT (1995) The structure and replication of kinetoplast DNA. Annu Rev Microbiol 49:117-43. https://doi.org/10.1146/annurev.mi.49.100195.001001
Shiba T, Kido Y, Sakamoto K, Inaoka DK, Tsuge C, Tatsumi R, Takahashi G, Balogun EO, Nara T, Aoki T, Honma T, Tanaka A, Inoue M, Matsuoka S, Saimoto H, Moore AL, Harada S, Kita K (2013) Structure of the trypanosome cyanide-insensitive alternative oxidase. Proc Natl Acad Sci U S A 110(12):4580-5. https://doi.org/10.1073/pnas.1218386110
Silva TM, Peloso EF, Vitor SC, Ribeiro LH, Gadelha FR (2011) O_2 consumption rates along the growth curve: new insights into *Trypanosoma cruzi* mitochondrial respiratory chain. J Bioenerg Biomembr 43(4):409-17. https://doi.org/10.1007/s10863-011-9369-0
Tielens AG, Van Hellemond JJ (1998) Differences in energy metabolism between Trypanosomatidae. Parasitol Today 14(7):265-72. https://doi.org/10.1016/S0169-4758(98)01263-0
Tielens AG, van Hellemond JJ (2009) Surprising variety in energy metabolism within Trypanosomatidae. Trends Parasitol 25(10):482-90. https://doi.org/10.1016/j.pt.2009.07.007
Tomás AM, Castro H (2013) Redox metabolism in mitochondria of trypanosomatids. Antioxid Redox Signal 19(7):696–707. https://doi.org/10.1089/ars.2012.4948
Vannier-Santos MA, Brunoro GF, Soeiro MNC, DeCastro SL, Menna-Barreto R (2019) Parasite, compartments and molecules: Trick vs treatment on Chagas disease. Biology of *Trypanosoma cruzi*. https://doi.org/10.5772/intechopen.84472
Venditti P, Di Stefano L, Di Meo S (2013) Mitochondrial metabolism of reactive oxygen species. Mitochondrion 13(2):71–82. https://doi.org/10.1016/j.mito.2013.01.008
Vercesi AE, Bernardes CF, Hoffmann ME, Gadelha FR, Docampo R (1991) Digitonin permeabilization does not affect mitochondrial function and allows the determination of the mitochondrial membrane potential of *Trypanosoma cruzi in situ*. J Biol Chem 266(22):14431-4. https://doi.org/10.1016/S0021-9258(18)98703-X
Verner Z, Cermáková P, Skodová I, Kriegová E, Horváth A, Lukes J (2011) Complex I (NADH:ubiquinone oxidoreductase) is active in but non-essential for procyclic

Trypanosoma brucei. Mol Biochem Parasitol 175(2):196-200. https://doi.org/10.1016/j.molbiopara.2010.11.003

Wang Y, Hekimi S (2016) Understanding ubiquinone. Trends Cell Biol 26(5):367–78. https://doi.org/10.1016/j.tcb.2015.12.007

WHO (2022) World Health Organization. Fact Sheets - Chagas disease (American trypanosomiasis). https//www.who.int/news-room/fact-sheets/detail/chagas-disease-(american-trypanosomiasis). Accessed on april 4, 2022.

Wilkinson SR, Obado SO, Mauricio IL, Kelly JM (2002) *Trypanosoma cruzi* expresses a plant-like ascorbate-dependent hemoperoxidase localized to the endoplasmic reticulum. Proc Natl Acad Sci U S A 99(21):13453-8. https://doi.org/10.1073/pnas.202422899

Wilson ME, Vorhies RW, Andersen KA, Britigan BE (1994) Acquisition of iron from transferrin and lactoferrin by the protozoan *Leishmania chagasi*. Infect Immun 62(8):3262-9. https://doi.org/10.1128/iai.62.8.3262-3269.1994