Glycolysis and protein translocation in glycosome as targets for trypanosomatid drug design (Review)

GASHAW AZANAW AMARE[1], ABEBAW SETEGN[2*], YENESEW MIHRET WONDMAGEGN[2*], MAMARU GETINET[3*] and ADANE ADUGNA[1]

[1]Department of Medical Laboratory Sciences, Debre Markos University, Debre Markos 269, Ethiopia; [2]Department of Medical Parasitology, University of Gondar, Gondar 196, Ethiopia; [3]Department of Biomedical Science, School of Medicine, Debre Markos University, Debre Markos 269, Ethiopia

Received July 11, 2024; Accepted November 5, 2024

DOI: 10.3892/wasj.2024.305

Abstract. Trypanosomatids cause neglected tropical diseases and the present review discussed the potential of targeting glycolysis and protein translocation within glycosomes as therapeutic strategies against these infections. Different studies show that glycolysis serves as the primary energy source for parasites such as *Trypanosoma cruzi*, *T. brucei* and *Leishmania*, with their glycolytic enzymes showing substantial divergence from human glycolytic enzymes, offering opportunities for selective drug development. Inhibiting glycolysis can lead to significant mortality among parasites, as even partial blockage of this pathway disrupts adenosine triphosphate production, which is essential for survival of the parasites. The present review also investigated the mechanisms of protein translocation across the glycosomal membrane, especially the critical role of peroxins; mislocalization of glycosomal proteins adversely affects parasite viability. Understanding the mechanisms of protein import and the unique characteristics of glycosomal enzymes can facilitate rational drug design aimed at these specific targets. Overall, the present review emphasized the need for innovative therapeutic approaches to effectively address the challenges posed by trypanosomatid diseases, advocating for further investigation into the metabolic vulnerabilities of these parasites to develop targeted and effective treatments.

Correspondence to: Mr. Gashaw Azanaw Amare, Department of Medical Laboratory Sciences, Debre Markos University, k08 Bole Street, Debre Markos 269, Ethiopia
E-mail: gashaw1azanaw@gmail.com

*Contributed equally

Key words: glycolysis, glycosome, glycosomal enzymes, protein translocation, drug design

Contents

1. Introduction

Trypanosomatids are protozoans that belong to the order Kinetoplastidae, which includes *Trypanosoma cruzi*, *T. brucei* and different species of *Leishmania*. These parasites are transmitted by bite from tsetse fly, bed bug and sand fly, respectively (1). Trypanosomatids cause neglected tropical diseases, such as Chagas' disease, African sleeping sickness and leishmaniasis, which are debilitating and often fatal (2,3). These diseases affect >20 million individuals worldwide, primarily in rural and underdeveloped areas, resulting in significant suffering in these regions (4,5). For example, >60 million individuals in sub-Saharan African countries are at risk of sleeping sickness presenting with fever, headaches and neurological symptoms, with an annual infection rate of 300,000-500,000 (6). Furthermore, ~350 million individuals in 92 countries are at risk of leishmaniasis which causes skin lesions and organ damage, significantly affecting social structures by exacerbating poverty, limiting economic productivity and straining healthcare systems in affected communities, with ~1.2 million new infections each year (7,8).

Current therapies for trypanosomatids, such as antimonials, Melarsoprol and pentamidine, have limited efficacy due to extreme toxicity, resistance, lack of cost efficiency and the need for parenteral delivery in some cases (6,9-11). Untreated trypanosomatid infections can be fatal (10). Furthermore, there are no effective vaccines for the prevention of trypanosomatid diseases, making rational drug design and the search for excellent therapeutic targets crucial (4,12). Extensive research has been conducted on the biochemical characteristics of the life stages of these parasites, leading to the identification of several potential targets for anti-trypanosomatid treatment (13,14). Several potential targets are proposed for anti-trypanosomatid treatment, such as trypanothione redox, sterol biosynthesis, purine uptake, folate metabolism, glycosomal enzymes and

protein translocation to the glycosome (15-19). The present review explored rational drug design strategies targeting glycosomal enzymes and protein translocation in trypanosomatids. These targets are crucial because they play critical roles in the unique metabolic pathways of trypanosomatids, which are not found in the host organisms. Glycosomal glycolysis is the only source of energy production, so targeting these pathways is essential for developing effective and selective therapeutics against trypanosomatids.

2. Glycosomes

Glycosomes, which are membrane-bound cytoplasmic peroxisome-like organelles found in the protozoan parasites, Kinetoplastidae, play a vital role in the metabolism of trypanosomatids (8). These organelles have the unique feature of containing numerous enzymes from the glycolytic and gluconeogenic pathways (20).

Investigating energy metabolism in the amastigote stage of *T. cruzi* is challenging due to the complications arising from host metabolic activity. Nevertheless, studies have demonstrated that glycolysis serves as the primary energy source for the amastigote stage (21,22). Essential proteins and enzymes for parasite survival are synthesized in the cytosol and must be transported across the glycosomal membrane into the glycosome. Thus, inhibiting the transport and activity of these proteins and enzymes can adversely affect the parasites (23). The glycosome is a crucial organelle for trypanosomatid metabolism, containing enzymes essential for glycolysis and researching the energy metabolism of these parasites and their reliance on glycosomes is a promising potential therapeutic target.

Protein translocation across the glycosomal membrane. The formation of the glycosomal membrane, as well as the synthesis and importation of matrix proteins and certain membrane proteins, occurs in the cytoplasm. In some cases, membrane proteins may first be inserted into the endoplasmic reticulum before being transported via vesicles to developing organelles. This importation mechanism is conserved (24,25).

Following their synthesis during translation, glycosomal proteins are produced in the cytosol and then imported into glycosomes either as fully folded proteins or as complexes consisting of multiple subunits (26). The protein import mechanisms of glycosomes and peroxisomes share fundamental similarities and the process relies on a group of proteins called peroxins (PEX) for efficient importation of proteins into these organelles (27-29). In trypanosomatids, more than 10 PEX proteins have been described (28,30). PEX5 and PEX7, receptor proteins that recognize C- or N-terminal import signals in the polypeptide, are responsible for the importation of most glycosomal proteins (31).

Proteins designated for transport possess a peroxisomal targeting signal (PTS), which can be found either at the far end of the C-terminus (referred to as type-1 or PTS1) or in proximity to the N-terminus (known as type-2 or PTS2). While a small number of matrix proteins do not have these PTS signals, most proteins containing PTS1 are recognized by a cytosolic receptor called PEX5, while PEX7 recognizes PTS2-containing proteins. PEX5 functions as a co-receptor and also contains a binding box for PEX7 (32) (Fig. 1). The receptors that carry the cargo bind to a loading complex consisting of PEX14, PEX13.1 and PEX13.2, facilitating their attachment to the glycosomal membrane (33,34). The interaction between PEX5 and PEX14 results in the creation of a transient import pore, allowing the translocation of the cargo into the glycosomal matrix (35).

Once the cargo protein is internalized into the glycosomal matrix, the loaded PEX5 and PEX7 receptors are transported back to the cytosol through interactions with other peroxins associated with the glycosomal membrane (19,36) (Fig. 1).

In trypanosomes, the first seven enzymes involved in glycolysis possess a PTS, which guides them to the glycosomal matrix (32). These PTS sequences are specifically present in proteins intended for transport to the glycosomal matrix (Fig. 1). The glycolytic enzymes in trypanosomes are equipped with a PTS-1 at the COOH-terminus or a PTS-2 at the NH2-terminus. Studies conducted on *T. brucei* have shown that the cytosolic receptor proteins, TbPEX5p and TbPEX7p, recognize these newly synthesized enzymes (32). Subsequently, each complex, consisting of TbPEX5p with the PTS-1 protein or TbPEX7p with the PTS-2 protein, interacts with TbPEX14p, a component of the glycosomal membrane protein import machinery. This interaction facilitates the translocation of the enzyme complex into the glycosome. Research has shown that depleting PEX5p, PEX7p, or PEX14p levels using RNAi leads to mislocalization of the enzymes into the cytosol in *T. brucei*. This mislocalization significantly affects the growth of the parasite and often results in its death (32,37).

In trypanosomes, the primary pathway for energy generation is glycolysis, which takes place within the glycosomes instead of within the cytosol. As a result, the glycolytic enzymes produced in the cytoplasm exhibit a limited degree of sequence similarity to their human counterparts, which creates an opportunity for targeted drug development. The accurate localization of glycolytic enzymes is of utmost importance, as misplacement has been demonstrated to lead to the death of the parasite or hindered growth (32,37,38). However, this potential of being a target is tested in animal models (39). Understanding the mechanisms by which proteins are imported into glycosomes, especially the role of peroxins, is vital for designing drugs that disrupt protein importation.

Glycolysis in glycosomes. Glycolysis is vital for trypanosomes, as it is their main energy source. Any compound that effectively blocks glycolysis can be considered a potential antiparasitic lead. The damage caused by glycolysis inhibition will be much greater in trypanosomes than in the host. Furthermore, trypanosomal glycolysis differs from mammalian glycolysis in several ways (40,41). When comparing trypanosomatid glycolytic enzymes with their human counterparts, trypanosome enzymes exhibit changes in amino acid sequence, with only 40-50% sequence identity (42).

The significant evolutionary divergence between trypanosomatids and their mammalian hosts, coupled with the distinct structure of the glycolytic pathway of parasites, offers unique glycolytic characteristics that can be harnessed for the development of selective inhibitor targeting structures or catalytic mechanisms that do not disrupt host glycolysis (5,9). Targeting the glycolytic pathway appears promising for intervention in

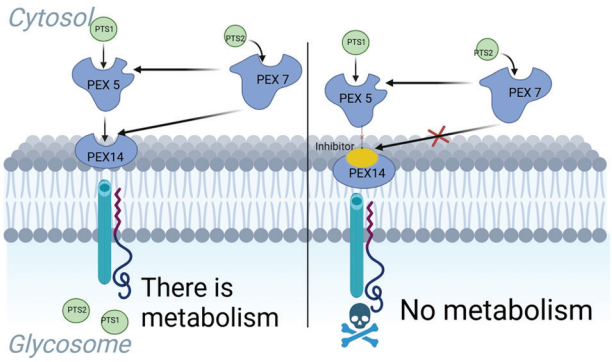

Figure 1. Translocation of glycosomal enzymes from cytosol to glycosome. Blue: Peroxin protein; Yellow: inhibitor that blocks the binding of peroxisome-targeting signal and peroxin protein; Green: peroxisome-targeting signal. PTS1, peroxisome-targeting signal type 1; PTS2, peroxisome-targeting signal type 2; PEX 5, peroxin 5; PEX 7, peroxin 7; PEX 14, peroxin 14.

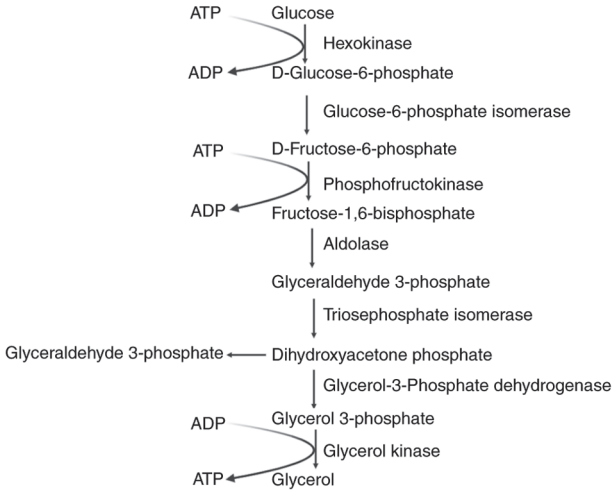

Figure 2. Glycolytic pathways of trypanosomatids inside the glycosome. ATP, adenosine triphosphate; ADP, adenosine diphosphate.

trypanosomatid. Specialized peroxisome-like organelles called glycosomes house seven glycolytic enzymes that catalyze the conversion of glucose to 3-phosphoglycerate (20,43,44).

Metabolic enzymes are attractive therapeutic targets because the growth and survival of pathogens rely on the availability of free energy and molecular building blocks provided by metabolism (45). Despite the high reliance of parasites on the oxidative degradation of fatty acids for adenosine triphosphate (ATP) production, glycolysis is an important mechanism for the stage of mammalian amastigotes of *T. cruzi* and *Leishmania* (46).

Due to the balance between ATP-consuming processes mediated by hexokinase and phosphofructokinase and the ATP-generating phase facilitated by phosphoglycerate kinase, there is no net ATP production within the glycosome. The glycosome contains a limited amount of ATP, which restricts the number of ATP-consuming processes until ATP is replenished by downstream reactions within the glycosome. Net ATP production occurs in the cytosol, where pyruvate kinase is located, with the resulting pyruvate being mostly expelled by the cells (47) (Fig. 2). Given that glycolysis is the primary source of ATP in the trypanosome lifecycle, it is critical for survival. Therefore, glycolysis represents a promising target for antitrypanosomal drugs, as even 50% inhibition can lead to trypanosome death (48). In summary, glycolysis is the primary energy source of trypanosomatides, with unique features that differs it from mammalian glycolysis. Targeting glycolysis offers a promising avenue for drug development.

Glycolytic enzymes. The bloodstream form of trypanosomatids contains ~14 enzymes involved in glycolysis. Among these enzymes, seven are located within the glycosome and catalyze the conversion of glucose to 3-phosphoglycerate, while the remaining enzymes are found in the cytosol and participate in the final steps of the process (20). The seven enzymes localized in the glycosome are hexokinase, glucose-6-phosphate isomerase, phosphofructokinase, aldolase, triosephosphate isomerase, glycerol-3-phosphate dehydrogenase and glycerol kinase. Trypanosomatids generate all their ATP by converting glucose to pyruvate, which is then released into the circulation of the host (49).

Hexokinase. Hexokinase is a carbohydrate kinase and it is only 37% identical to the human homolog, implying that selective inhibitor design is possible, so it has been targeted for the development of trypanosomatid inhibitor development (50). Hexokinase catalyzes glucose conversion to glucose-6-phosphate and various investigations have found that glucose analogs, such as glucosamine and 2-C-hydroxymethyl glucose derivatives, block the activity (51,52). Willson *et al* (53) tested numerous glucose-6-phosphate analogs against *T. brucei* hexokinase since glucose-6-phosphate possesses an affinity for the active site of *T. brucei* hexokinase. The hexokinase of *T. brucei* is inhibited by two glucose-6-phosphate derivatives, with a 75% inhibition at 3 mM for one derivative and a 60% inhibition at 0.2 mM for another derivative. The elimination of Tb HK1 RNA interference (RNAi) demonstrated that this protein was required for viability and this enzyme was blocked by lonidamine, a drug that effectively killed *T. brucei* parasites (54).

Glucose-6-phosphate isomerase. In glycolysis and gluconeogenesis, glucose-6-phosphate isomerase catalyzes the reversible aldose-ketose isomerization of D-glucose-6-phosphate to D-fructose-6-phosphate, as well as the recycling of hexose-6-phosphate in the pentose phosphate pathway, so this enzyme links glycolysis and the pentose phosphate pathway. When the crystallographic structure of the parasite glucose-6-phosphate isomerase is compared to the atomic structure of humans and other mammalian show that there are unique features of the enzyme of parasites (55).

Aldolase. The crystal structures of aldolases in trypanosomatids and mammals have been compared and it is found that the active site was largely conserved. However, by identifying minor differences and gaining detailed knowledge of the catalytic mechanism, the researchers were able to develop a specific inhibitor that binds quasi-irreversibly to the parasite enzyme. One such inhibitor is the compound 5-formyl6-hydroxy-2-naphthyl di-sodium phosphate, which can completely deactivate trypanosomatid enzymes with a Ki value of 23 μM. By contrast, this compound only has a weak effect on mammalian aldolase, even at concentrations that are 1,000 times higher than that used for parasites (56). When formulated as prodrugs to conceal the charged group, inhibitors derived from

5-formyl6-hydroxy-2-naphthyl disodium phosphate were able to effectively eliminate cultured trypanosomes with ED50 values in the low micromolar range. Importantly, these inhibitors did not have any impact on mammalian cell growth of mammalian cells (57).

Glycerol kinase. Glycerol kinase is responsible for the ATP-dependent conversion of glycerol into G3P, which is used for lipid synthesis. Furthermore, G3P can enter the glycolytic/gluconeogenic pathway by undergoing NAD+-dependent oxidation to dihydroxyacetone phosphate (DHAP) through the action of G3P dehydrogenase (G3PDH). However, the reverse reaction, which converts DHAP back to G3P, would only occur under thermodynamically favorable conditions characterized by high levels of adenosine diphosphate (ADP)/ATP and G3P/glycerol ratios. Achieving such conditions throughout the entire cell would be challenging, but they can potentially occur within a specialized compartment such as the glycosome (58). Therefore, this pathway that consumes glycerol is also important to maintain the balance of ATP/ADP and NAD+/NADH within the glycosome. In the bloodstream form of African trypanosomes, the localization of glycerol kinase (GK) within the glycosome serves as an additional mechanism to cope with periods of anaerobiosis when G3P cannot be oxidized through the shuttle mediated by mitochondrial GPO. During such periods, the inability to further metabolize G3P leads to the stoppage of glycolysis since glycosomal NADH cannot be oxidized. However, in trypanosomes where glycerol-3-phosphatase is absent, this problem is resolved by reversing the GK reaction, despite the fact that the equilibrium strongly favors phosphorylation (20).

Triosephosphate isomerase (TPI). TPI plays a crucial role in glycolysis by facilitating the conversion between dihydroxyacetone phosphate and glyceraldehyde 3-phosphate. This enzymatic activity ensures complete breakdown of the entire hexose unit, rather than just half of it. Notably, TPI in glycosomes lacks the PTS1 and PTS2 motifs, which are established peroxisome-targeting signals (59). Galland *et al* (60) conducted a study to identify the specific region within the TPI polypeptide responsible for its glycosome targeting. Surprisingly, the glycosomal TPI enzyme does not contain the recognized peroxisome-targeting signals. However, the researchers discovered a 22-amino acid fragment located internally within the polypeptide that can direct a reporter protein to glycosomes in transfected trypanosomes. This finding was confirmed by cell fractionation experiments and immunofluorescence studies. Notably, this internal routing information appears to be unique to the sequence of the trypanosome enzyme. When a reporter protein fused with a *Saccharomyces cerevisiae* peptide containing the corresponding sequence of the 22 residue fragment of the enzyme *T. brucei* was tested, it did not target glycosomes. In yeast and most other organisms, TPI is found exclusively found in the cytosol (60).

Phosphofructokinase (PFK). PFK is an enzyme that exists as a tetramer and uses ATP as a phosphate donor to facilitate the phosphorylation of fructose 6-phosphate, converting it into fructose 1,6-bisphosphate. This reaction is considered practically irreversible under normal physiological conditions. Notably, trypanosomatid PFKs share 20-30% of their genetic sequences with humans (61). However, the structure of PFK in trypanosomatids reveals the presence of two significant insertions that are unique to these parasites but absent in mammalian enzymes. These insertions are situated within and in close proximity to the active site of the enzyme. Consequently, this structural insight paves the way for conducting drug discovery experiments based on the structure of the enzyme, aiming to identify new lead compounds that specifically target the parasites responsible for diseases caused by the trypanosomatid family of protozoan parasites (62).

Glycerol-3-phosphate dehydrogenase (GPDH). NADH generated by glyceraldehyde-3-phosphate dehydrogenase during glycolysis is reoxidized by mitochondrial GPO using molecular oxygen. This electron transfer occurs via a glycosomal glycerol-3-phosphate dehydrogenase and the glycerol -3-phosphate:dihydroxyacetone phosphate (DHAP) shuttle. The resulting G3P is then transported across the glycosomal membrane and reoxidized through the G3P:DHAP shuttle, connecting with mitochondrial GPO (20). Inhibiting the activity of GPDH would hinder the ability of the trypanosome to produce ATP. Additionally, the inhibition of GPDH would result in the accumulation of DHAP within the glycosome, which could be highly detrimental to the trypanosome as DHAP is naturally converted to methylglyoxal, a toxic compound that disrupts protein function (63,64).

The unique structure of the glycosome creates an intraglycosomal environment that maintains the balance of redox and ATP. In the cytosol, ATP production occurs through the net action of pyruvate kinase. Under anaerobic conditions, G3P is converted to glycerol by the reverse action of a glycosomal glycerol kinase, accompanied by the production of ATP. This allows the glycosome to maintain the balance of ATP and NAD even in the absence of oxygen. However, instead of producing two molecules of pyruvate from one molecule of glucose, the glycosome generates one molecule each of glycerol and pyruvate, resulting in a reduced net ATP production of two to one. Due to this compartmentalization, a number of regulatory mechanisms that function in other cell types do not operate in trypanosomes. Glycosomal hexokinase and phosphofructokinase, for example, do not respond to activity-regulating substances that typically affect these enzymes in other cell types, reflecting the unique characteristics of trypanosomes (49,65-67).

In summary, the key glycolytic enzymes highlighted show promise as targets for selective inhibitors and the distinctions between trypanosomatid and human enzymes present opportunities for drug development, however, it is still on animal models only (68,69).

3. Conclusions and recommendations

Rational drug design targeting glycosomal enzymes and protein translocation in trypanosomatids offers significant promise for developing selective and effective treatments. However, current research faces limitations in elucidating the molecular interactions within these pathways. The glycosomal enzymes involved in glycolysis and the translocation of proteins across the glycosomal membrane are highly specific processes. These limitations arise mainly due to the complex structures of the enzymes and the dynamic nature of protein import mechanisms, which hinder the design of selective inhibitors that can differentiate between host and parasite proteins. This challenge is further compounded by the limited availability of high-resolution structural data, which is essential for understanding these molecular interactions in greater detail.

Future studies must expand our knowledge of the molecular mechanisms involved in glycosomal protein translocation and glycolysis to overcome these challenges. Advanced techniques such as cryo-electron microscopy, high-throughput screening and computational modeling should be employed to investigate these processes at a molecular level. By refining drug design approaches and conducting *in vivo* validation studies, the clinical translation of promising candidates can be ensured, ultimately leading to more effective treatments for neglected tropical diseases.

Acknowledgements

Not applicable.

Funding

No funding was received.

Availability of data and materials

Not applicable.

Authors' contributions

GA contributed to the conception and design of the present study, searching, analysis and writing the first draft of the manuscript. AA contributed to writing the first draft of the manuscript and YMW, AS and MG contributed to revising the manuscript. Data authentication is not applicable. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Schuster FL and Sullivan JJ: Cultivation of clinically significant hemoflagellates. Clin Microbiol Rev 15: 374-389, 2002.
2. Moreno J and Alvar J: Canine leishmaniasis: Epidemiological risk and the experimental model. Trends Parasitol 18: 399-405, 2002.
3. Nussbaum K, Honek J, Cadmus CMCVC and Efferth T: Trypanosomatid parasites causing neglected diseases. Curr Med Chem 17: 1594-1617, 2010.
4. Robays J, Nyamowala G, Sese C, Betu Ku Mesu Kande V, Lutumba P, Van der Veken W and Boelaert M: High failure rates of melarsoprol for sleeping sickness, Democratic Republic of Congo. Emerg Infect Dis 14: 966-967, 2008.
5. World Health Organization: Tropical disease research, progress 1995-1996. WHO Tech Rep Ser 1: 108-139, 1997.
6. World Health Organization (WHO): Tropical disease research. WHO, Geneva, 1997.
7. World Health Organization. Weekly epidemiological record, 2016, vol. 91, 38 [full issue]. Wkly Epidemiol Rec 91: 432-440, 2016.
8. Rodrigues JCF, Godinho JLP and de Souza W: Biology of human pathogenic trypanosomatids: Epidemiology, lifecycle and ultrastructure. Subcell Biochem 74: 1-42, 2014.
9. Atouguia J and Costa J: Therapy of human African trypanosomiasis: Current situation. Mem Inst Oswaldo Cruz 94: 221-224, 1999.
10. Pépin J and Milord F: The treatment of human African trypanosomiasis. Adv Parasitol 33: 1-47, 1994.
11. UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases: Operational guidelines for the establishment and functioning of data and safety monitoring boards: special programme for research and training in tropical diseases. Int J Pharm Med 20: 25-36, 2006.
12. Legros D, Olilvier G, Gastellu-Etchegorry M, Paquet C, Burri C, Jannin J and Büscher P: Treatment of human African trypanosomiasis-present situation and needs for research and development. Lancet Infect Dis 2: 437-440, 2002.
13. Wang CC: Molecular mechanisms and therapeutic approaches to the treatment of African trypanosomiasis. Annu Rev Pharmacol Toxicol 35: 93-127, 1995.
14. Barrett MP, Coombs GH and Mottram JC: Recent advances in identifying and validating drug targets in trypanosomes and leishmanias. Trends Microbiol 7: 82-88, 1999.
15. Patterson S, Alphey MS, Jones DC, Shanks EJ, Street IP, Frearson JA, Wyatt PG, Gilbert IH and Fairlamb AH: Dihydroquinazolines as a novel class of *Trypanosoma* brucei trypanothione reductase inhibitors: Discovery, synthesis, and characterization of their binding mode by protein crystallography. J Med Chem 54: 6514-6530, 2011.
16. Urbina JA, Concepcion JL, Rangel S, Visbal G and Lira R: Squalene synthase as a chemotherapeutic target in *Trypanosoma cruzi* and *Leishmania* mexicana. Mol Biochem Parasitol 125: 35-45, 2002.
17. Looker DL, Marr JJ and Berens RL: Mechanisms of action of pyrazolopyrimidines in *Leishmania* donovani. J Biol Chem 261: 9412-9415, 1986.
18. Sienkiewicz N, Jarosławski S, Wyllie S and Fairlamb AH: Chemical and genetic validation of dihydrofolate reductase-thymidylate synthase as a drug target in African trypanosomes. Mol Microbiol 69: 520-533, 2008.
19. Kalel VC, Emmanouilidis L, Dawidowski M, Schliebs W, Sattler M, Popowicz GM and Erdmann R: Inhibitors of glycosomal protein import provide new leads against trypanosomiasis. Microb Cell 4: 229-232, 2017.
20. Opperdoes FR and Borst P: Localization of nine glycolytic enzymes in a microbody-like organelle in *Trypanosoma* brucei: The glycosome. FEBS Lett 80: 360-364, 1977.
21. Clayton CE and Michels P: Metabolic compartmentation in African trypanosomes. Parasitol Today 12: 465-471, 1996.
22. Engel JC, de Cazzulo BMF, Stoppani AO, Cannata JJ and Cazzulo JJ: Aerobic glucose fermentation by *Trypanosoma cruzi* axenic culture amastigote-like forms during growth and differentiation to epimastigotes. Mol Biochem Parasitol 26: 1-10, 1987.
23. Moyersoen J, Choe J, Fan E, Hol WGJ and Michels PAM: Biogenesis of peroxisomes and glycosomes: Trypanosomatid glycosome assembly is a promising new drug target. FEMS Microbiol Rev 28: 603-643, 2004.
24. Gualdrón-López M, Brennand A, Avilán L and Michels PAM: Translocation of solutes and proteins across the glycosomal membrane of trypanosomes; possibilities and limitations for targeting with trypanocidal drugs. Parasitology 140: 1-20, 2013.
25. Sommer JM, Bradley PJ, Wang C and Johnson PJ: Biogenesis of specialized organelles: Glycosomes and hydrogenosomes. In: Smith DF and Parsons M (eds), Molecular biology of parasitic protozoa, Vol. 13. Oxford Press, New York, N.Y, pp159-180, 1996.
26. Titorenko VI, Nicaud JM, Wang H, Chan H and Rachubinski RA: Acyl-CoA oxidase is imported as a heteropentameric, cofactor-containing complex into peroxisomes of Yarrowia lipolytica. J Cell Biol 156: 481-494, 2002.
27. Heiland I and Erdmann R: Biogenesis of peroxisomes. Topogenesis of the peroxisomal membrane and matrix proteins. FEBS J 272: 2362-2372, 2005.
28. Verplaetse E, Rigden DJ and Michels PAM: Identification, characterization and essentiality of the unusual peroxin 13 from *Trypanosoma* brucei. Biochim Biophys Acta 1793: 516-527, 2009.
29. Lametschwandtner G, Brocard C, Fransen M, Van Veldhoven P, Berger J and Hartig A: The difference in recognition of terminal tripeptides as peroxisomal targeting signal 1 between yeast and human is due to different affinities of their receptor Pex5p to the cognate signal and to residues adjacent to it. J Biol Chem 273: 33635-33643, 1998.
30. Cyr N, Madrid KP, Strasser R, Aurousseau M, Finn R, Ausio J and Jardim A: *Leishmania* donovani peroxin 14 undergoes a marked conformational change following association with peroxin 5. J Biol Chem 283: 31488-31499, 2008.

31. Brocard C and Hartig A: Peroxisome targeting signal 1: Is it really a simple tripeptide? Biochim Biophys Acta 1763: 1565-1573, 2006.
32. Galland N, Demeure F, Hannaert V, Verplaetse E, Vertommen D, Van der Smissen P, Courtoy PJ and Michels PA: Characterization of the role of the receptors PEX5 and PEX7 in the import of proteins into glycosomes of *Trypanosoma* brucei. Biochim Biophys Acta 1773: 521-535, 2007.
33. Verplaetse E, Gualdrón-López M, Chevalier N and Michels PAM: Studies on the organization of the docking complex involved in matrix protein import into glycosomes of *Trypanosoma* brucei. Biochem Biophys Res Commun 424: 781-785, 2012.
34. Brennand A, Rigden DJ and Michels PAM: Trypanosomes contain two highly different isoforms of peroxin PEX13 involved in glycosome biogenesis. FEBS Lett 586: 1765-1771, 2012.
35. Meinecke M, Cizmowski C, Schliebs W, Krüger V, Beck S, Wagner R and Erdmann R: The peroxisomal importomer constitutes a large and highly dynamic pore. Nat Cell Boil 12: 273-277, 2010.
36. Francisco T, Rodrigues TA, Freitas MO, Grou CP, Carvalho AF, Sá-Miranda C, Pinto MP and Azevedo JE: A cargo-centered perspective on the PEX5 receptor-mediated peroxisomal protein import pathway. J Biol Chem 288: 29151-29159, 2013.
37. Moyersoen J, Choe J, Kumar A, Voncken FG, Hol WG and Michels PA: Characterization of *Trypanosoma* brucei PEX14 and its role in the import of glycosomal matrix proteins. Eur J Biochem 270: 2059-2067, 2003.
38. Krazy H and Michels PAM: Identification and characterization of three peroxins-PEX6, PEX10 and PEX12-involved in glycosome biogenesis in *Trypanosoma* brucei. Biochim Biophys Acta 1763: 6-17, 2006.
39. Dawidowski M, Emmanouilidis L, Kalel VC, Tripsianes K, Schorpp K, Hadian K, Kaiser M, Mäser P, Kolonko M, Tanghe S, *et al*: Inhibitors of PEX14 disrupt protein import into glycosomes and kill *Trypanosoma* parasites. Science 355: 1416-1420, 2017.
40. Opperdoes FR, Baudhuin P, Coppens I, De Roe C, Edwards SW, Weijers PJ and Misset O: Purification, morphometric analysis, and characterization of the glycosomes (microbodies) of the protozoan hemoflagellate *Trypanosoma* brucei. J Cell Biol 98: 1178-1184, 1984.
41. Visser N and Opperdoes FR: Glycolysis in *Trypanosoma* brucei. Eur J Biochem 103: 623-632, 1980.
42. Michels PA: Evolutionary aspects of trypanosomes: Analysis of genes. J Mol Evol 24: 45-52, 1986.
43. Fernandes AP, Nelson K and Beverley SM: Evolution of nuclear ribosomal RNAs in kinetoplastid protozoa: perspectives on the age and origins of parasitism. Proc Natl Acad Sci USA 90: 11608-11612, 1993.
44. Opperdoes FR: Compartmentation of carbohydrate metabolism in trypanosomes. Annu Rev Microbiol 41: 127-151, 1987.
45. Ruppin E, Papin JA, de Figueiredo LF and Schuster S: Metabolic reconstruction, constraint-based analysis and game theory to probe genome-scale metabolic networks. Curr Opin Biotechnol 21: 502-510, 2010.
46. Hart D and Coombs GH: *Leishmania* mexicana: Energy metabolism of amastigotes and promastigotes. Exp Parasitol 54: 397-409, 1982.
47. Creek DJ, Mazet M, Achcar F, Anderson J, Kim DH, Kamour R, Morand P, Millerioux Y, Biran M, Kerkhoven EJ, *et al*: Probing the metabolic network in bloodstream-form *Trypanosoma* brucei using untargeted metabolomics with stable isotope labelled glucose. PLoS Pathog 11: e1004689, 2015.
48. Haanstra JR, Kerkhoven EJ, van Tuijl A, Blits M, Wurst M, van Nuland R, Albert MA, Michels PA, Bouwman J, Clayton C, *et al*: A domino effect in drug action: From metabolic assault towards parasite differentiation. Mol Microbiol 79: 94-108, 2011.
49. Michels P, Hannaert V and Bringaud F: Metabolic aspects of glycosomes in trypanosomatidae-new data and views. Parasitol Today 16: 482-489, 2000.
50. Verlinde CL, Hannaert V, Blonski C, Willson M, Périé JJ, Fothergill-Gilmore LA, Opperdoes FR, Gelb MH, Hol WG and Michels PA: Glycolysis as a target for the design of new anti-trypanosome drugs. Drug Resist Updat 4: 50-65, 2001.
51. D'Antonio EL, Deinema MS, Kearns SP, Frey TA, Tanghe S, Perry K, Roy TA, Gracz HS, Rodriguez A and D'Antonio J: Structure-based approach to the identification of a novel group of selective glucosamine analogue inhibitors of *Trypanosoma cruzi* glucokinase. Mol Biochem Parasitol 204: 64-76, 2015.
52. Merritt C, Silva LE, Tanner AL, Stuart K and Pollastri MP: Kinases as druggable targets in trypanosomatid protozoan parasites. Chem Rev 114: 11280-11304, 2014.
53. Willson M, Alric I, Perie J and Sanejouand YH: Yeast hexokinase inhibitors designed from the 3-D enzyme structure rebuilding. J Enzyme Inhib 12: 101-121, 1997.
54. Chambers JW, Fowler ML, Morris MT and Morris JC: The anti-trypanosomal agent lonidamine inhibits *Trypanosoma* brucei hexokinase 1. Mol Biochem Parasitol 158: 202-207, 2008.
55. Cordeiro AT, Michels PAM, Delboni LF and Thiemann OH: The crystal structure of glucose-6-phosphate isomerase from *Leishmania* mexicana reveals novel active site features. Eur J Biochem 271: 2765-2772, 2004.
56. Dax C, Duffieux F, Chabot N, Coincon M, Sygusch J, Michels PAM and Blonski C: Selective irreversible inhibition of fructose 1,6-bisphosphate aldolase from *Trypanosoma* brucei. J Med Chem 49: 1499-1502, 2006.
57. Azéma L, Lherbet C, Baudoin C and Blonski C: Cell permeation of a *Trypanosoma* brucei aldolase inhibitor: Evaluation of different enzyme-labile phosphate protecting groups. Bioorg Med Chem Lett 16: 3440-3443, 2006.
58. Bakker BM, Michels PA, Opperdoes FR and Westerhoff HV: Glycolysis in bloodstream form *Trypanosoma* brucei can be understood in terms of the kinetics of the glycolytic enzymes. J Biol Chem 272: 3207-3215, 1997.
59. Wierenga R, Noble M, Vriend G, Nauche S and Hol W: Refined 1.83 A structure of trypanosomal triosephosphate isomerase crystallized in the presence of 2.4 M-ammonium sulphate: A comparison with the structure of the trypanosomal triosephosphate isomerase-glycerol-3-phosphate complex. J Mol Biol 220: 995-1015, 1991.
60. Galland N, de Walque S, Voncken FGJ, Verlinde CLMJ and Michels PAM: An internal sequence targets *Trypanosoma* brucei triosephosphate isomerase to glycosomes. Mol Biochem Parasitol 171: 45-49, 2010.
61. López C, Chevalier N, Hannaert V, Rigden DJ, Michels PAM and Ramirez JL: *Leishmania* donovani phosphofructokinase. Gene characterization, biochemical properties and structure-modeling studies. Eur J Biochem 269: 3978-3989, 2002.
62. Martinez-Oyanedel J, McNae IW, Nowicki MW, Keillor JW, Michels PA, Fothergill-Gilmore LA and Walkinshaw MD: The first crystal structure of phosphofructokinase from a eukaryote: *Trypanosoma* brucei. J Mol Biol 366: 1185-1198, 2007.
63. Lo T, Westwood ME, McLellan AC, Selwood T and Thornalley PJ: Binding and modification of proteins by methylglyoxal under physiological conditions. A kinetic and mechanistic study with N alpha-acetylarginine, N alpha-acetylcysteine, and N alpha-acetyllysine, and bovine serum albumin. J Biol Chem 269: 32299-32305, 1994.
64. Denise H, Giroud C, Barrett MP and Baltz T: Affinity chromatography using trypanocidal arsenical drugs identifies a specific interaction between glycerol-3-phosphate dehydrogenase from *Trypanosoma* brucei and Cymelarsan. Eur J Biochem 259: 339-346, 1999.
65. Nwagwu M and Opperdoes FR: Regulation of glycolysis in *Trypanosoma* brucei: Hexokinase and phosphofructokinase activity. Acta Trop 39: 61-72, 1982.
66. Cronin CN and Tipton KF: Purification and regulatory properties of phosphofructokinase from *Trypanosoma* (Trypanozoon) brucei brucei. Biochem J 227: 113-124, 1985.
67. Bakker BM, Mensonides FI, Teusink B, van Hoek P, Michels PA and Westerhoff HV: Compartmentation protects trypanosomes from the dangerous design of glycolysis. Proc Natl Acad Sci USA 97: 2087-2092, 2000.
68. McNae IW, Kinkead J, Malik D, Yen LH, Walker MK, Swain C, Webster SP, Gray N, Fernandes PM, Myburgh E, *et al*: Fast acting allosteric phosphofructokinase inhibitors block trypanosome glycolysis and cure acute African trypanosomiasis in mice. Nat Commun 12: 1052, 2021.
69. Cortés-Figueroa AA, Pérez-Torres A, Salaiza N, Cabrera N, Escalona-Montaño A, Rondán A, Aguirre-García M, Gómez-Puyou A, Pérez-Montfort R and Becker I: A monoclonal antibody that inhibits *Trypanosoma cruzi* growth in vitro and its reaction with intracellular triosephosphate isomerase. Parasitol Res 102: 635-643, 2008.