Kinetoplastid parasites have essential organelles called glycosomes that are analogous to peroxisomes present in other eukaryotes. While many of the processes that regulate glycosomes are conserved, there are several unique aspects of their biology that are divergent from other systems and may be leveraged as therapeutic targets for the treatment of kinetoplastid diseases. Glycosomes are heterogeneous organelles that likely exist as sub-populations with different protein composition and function in a given cell, between individual cells, and between species. However, the limitations posed by the small size of these organelles makes the study of this heterogeneity difficult. Recent advances in the analysis of small vesicles by flow-cytometry provide an opportunity to overcome these limitations. In this review, we describe studies that document the diverse nature of glycosomes and propose an approach to using flow cytometry and organelle sorting to study the diverse composition and function of these organelles. Because the cellular machinery that regulates glycosome protein import and biogenesis is likely to contribute, at least in part, to glycosome heterogeneity we highlight some ways in which the glycosome protein import machinery differs from that of peroxisomes in other eukaryotes.

Introduction
Kinetoplastid parasites include several pathogenic organisms such as Trypanosoma cruzi, the causative agent of Chagas disease, and Trypanosoma brucei, the causative agent of African trypanosomiasis in humans and a wasting disease called nagana in cattle. Kinetoplastids also encompass several species of Leishmania responsible for diseases that range from self-limiting cutaneous Leishmaniasis to visceral Leishmaniasis that is lethal if untreated. This parasite group is named for the presence of a single mitochondrion containing a large DNA structure named the kinetoplast. In addition to their impact on public health, studies using these parasites as model systems have advanced our understanding of numerous biological processes such as RNA editing, antigenic variation, and glycosylphosphatidylinositol (GPI) biosynthesis that also occur in other eukaryotes [1–3].

Glycosomes are specialized, kinetoplastid-specific peroxisomes that were named for their unique compartmentalization of the first several steps of the glycolytic pathway; a process that is cytosolic in other eukaryotes [4–6]. While they are named for the unique localization of this pathway, glycosomes harbor enzymes from many biochemical pathways including the pentose phosphate pathway, purine salvage, pyrimidine biosynthesis and others [7,8].

Like peroxisomes, glycosomes are membrane-bounded organelles that lack DNA. Their protein composition is dynamic [7,9], differs between species of kinetoplastids [4], and changes during development and in response to environmental changes [9]. Proteins called peroxins (Pexs) regulate glycosome and peroxisome homeostasis that is governed by multiple processes including (Figure 1): (i) organelle formation and maturation, (ii) protein import, (iii) proliferation via fission, and (iv) degradation of non-functional or unnecessary organelles via autophagy.

It is well documented that glycosome composition changes dramatically during parasite development, which occurs when the parasite alternates between a mammalian host and an insect vector [8]. In bloodstream form (BF) T. brucei, which reside in the mammalian host, glycolytic enzymes make up...
∼90% of the glycosome protein repertoire. In contrast, in glycosomes of procyclic form (PF) parasites that live in the tse
tsey fly where glucose is scarce, glycolytic enzymes make up only ∼50% of the glycosome protein [10]. In addition to these changes during development, recent studies reveal that glycosome composition between parasites within a single culture [9] is diverse and emerging data indicates that glycosome composition within a single parasite is heterogeneous [11,12]. Our knowledge regarding the basis of this heterogeneity and the biological significance is limited but recent technological advances in flow cytometry discussed here are likely to significantly advance our understanding about these organelles.

To date, more than 30 peroxins have been identified in eukaryotes. Homologs for 16 of these have been identified in kinetoplastids. Peroxins discussed in this review are listed in Table 1; for a comprehensive list see reviews: [4,13]. During protein import in all eukaryotic organisms, including kinetoplastids, the cytosolic soluble receptors Pex5 and Pex7 bind to peroxisome targeting sequences, (PTS1) or PTS2, respectively (reviewed in [14]). These receptors then deliver their cargo to the membrane where they dock with the import channel containing Pex13 and Pex14. Cargo is translocated into the glycosome matrix through a pore composed primarily of Pex5 and Pex14 and the receptors are recycled via monoubiquitination that is facilitated by
Pex2, 10 and 12, also called really interesting novel new gene (RING) proteins [15], and are transported back to the cytosol by the AAA ATPases Pexs 1 and 6 where they can begin the cycle again [16]. Studies in kinetoplastids indicate that the overall matrix protein import process of receptor binding, membrane targeting, and receptor recycling is conserved with other eukaryotes: kinetoplastids have Pex13, 14, 5 and 7 and the PTS1 and PTS2s are similar to sequences found in yeast and animal cells [17–20]. However, detailed protein interaction studies reveal unique associations between kinetoplastid peroxins that can be targeted for therapeutic development. Because glycosomes are essential organelles, compounds that target processes involved in organelle biogenesis or function may be lethal to parasites. For example, differences in the mammalian and *T. brucei* Pex5 binding pocket of TbPex14 leave opportunity for the development of trypanosome-specific inhibitors. As proof of principle, Dawidowski et al. [19] utilized structure-based drug design to develop small molecules that block *TbPex5*–*TbPex14* binding. Treatment of parasites with these inhibitors resulted in glycosomal protein mislocalization and parasite death and were effective at low nanomolar concentrations against *T. brucei* and low micromolar concentrations against *T. cruzi*. Similar approaches could be utilized to target additional parasite-specific peroxin interactions such as those between *Leishmania donovani* LdPex7 and LdPex14, a relationship that is

| Peroxin | Homologue identified in kinetoplastids? | Reference | Essential in kinetoplastids? | Function | Location |
|---------|----------------------------------------|-----------|-----------------------------|----------|----------|
| Pex1    | Y                                      | [68]      | ?                           | Peroxisome membrane | Membrane |
| Pex4    | Y                                      | [69]      | Y                           | Pex5/7 Transport to cytosol AAA ATPase |
| Pex6    | Y                                      | [70]      | Y                           | RING finger complex Ubiquitination of Pex5/7 |
| Pex2    | Y                                      | [71]      | Y                           | Cargo docking complex Import of peroxisome matrix proteins |
| Pex10   | Y                                      | [70]      | Y                           | Peroxisomal membrane protein import |
| Pex12   | Y                                      | [70]      | Y                           | Peroxisomal membrane protein import Peroxisomal division De novo peroxisome biogenesis |
| Pex11   | Y                                      | [40]      | Y                           | Peroxisome division processes |
| Pex14   | Y                                      | [72]      | Y                           | Cargo docking complex Import of peroxisome matrix proteins |
| Pex13.1 | Y (Ortholog of Pex13)                  | [17]      | Y                           | Peroxisomal membrane protein import Peroxisomal division De novo peroxisome biogenesis |
| Pex13.2 | Unique to Kinetoplastids              | [18]      | Y                           | Cargo docking complex Import of peroxisome matrix proteins |
| Pex16   | Y                                      | [73]      | Y                           | Peroxisomal membrane protein import Peroxisomal division De novo peroxisome biogenesis |
| Pex3    | Y                                      | [32,33]   | Y                           | Peroxisomal membrane protein import Peroxisomal division De novo peroxisome biogenesis |
| Pex5    | Y                                      | [20]      | Y                           | Cytosolic PTS1 receptor Cytosol |
| Pex7    | Y                                      | [20]      | Y                           | Cytosolic PTS2 receptor Cytosol |
| Pex18   | N                                      | -         | ?                           | PTS2 coreceptor |
| Pex21   | N                                      | -         | ?                           | PTS2 coreceptor |
| Pex19   | Y                                      | [31]      | Y                           | Peroxisomal membrane protein receptor Cytosol |
essential to glycosome protein import. Truncation experiments mapped an LdPex7 binding domain to a unique 28 residue motif on LdPex14 [21] and interaction of LdPex5 and LdPex7 was mapped to a 37 amino acid motif not present in mammalian Pex5 [21]. It is reasonable to anticipate that targeting these kinetoplastid-specific interactions may also be successful in crippling glycosome protein import and parasite growth. Further studies on the unique aspects of kinetoplastid peroxin interactions may reveal additional drug targets.

In contrast with the relatively well-studied pathway of glycosome protein import, very little is known about glycosome formation. The peroxin Pex3 plays a fundamental role in the targeting of peroxisome membrane proteins (PMP) and de novo biogenesis of peroxisomes in yeast and mammalian cells [22–24]. Much of what we know about peroxisome formation from the ER has been learned through studies with this protein [25–27]. PMPs bind the cytosolic receptor Pex19, which then docks with Pex3 at the peroxisome membrane [28]. Disruption of Pex3 or Pex19 in S. cerevisiae resulted in cells lacking peroxisomal structures [29] pointing to a role of these proteins in de novo biogenesis and Pex3 localization to the endoplasmic reticulum (ER) was critical for the formation and budding of preperoxisomal vesicles [30]. Until recently, although a Pex19 homolog was found in kinetoplastids [31], Pex3 had not been identified. In 2019, two laboratories independently identified a Pex3 homolog in trypanosomes that has only 7% sequence identity with human Pex3 but does maintain a conserved Pex19 binding sequence [32,33]. Although trypanosome Pex3 possesses Pex19 binding domains, they are flanked by trypanosome-specific regions, making bioinformatic identification difficult via sequence analysis [33]. Both groups reported a physical interaction of Pex3 with Pex19 and demonstrated that depletion of Pex3 resulted in mislocalization of glycosome proteins prior to cell death. While the low level of sequence similarity makes these peroxins attractive therapeutic targets, it hinders the identification of peroxins in kinetoplastids via bioinformatic approaches. As such, the search for additional peroxins will require an alternative, less biased approach such as communoprecipitation experiments and or proximity labeling to identify novel proteins that bind known peroxins and genetic screens to identify genes involved in establishing glycosome structure and function.

### Glycosomes, like peroxisomes, are heterogeneous but the basis of this heterogeneity is unclear

Studies have revealed the heterogeneous nature of glycosomes. In L. donovani, biochemical separation of intracellular organelles followed by mass spectrometry revealed the presence of at least two glycosome populations of different densities and peroxin composition [11]. High-density glycosomes contained Pex16,12,11,13 and high levels of the metabolic enzyme dihydroxyacetone phosphate acyltransferase (DHAPAT), while low-density glycosomes contained Pex7,1,6 and undetectable levels of DHAPAT. In addition to these biochemical experiments, immunofluorescence assays [12] demonstrated that two Leishmania glycosomal matrix proteins, arginosuccinate (ASS) and hypoxanthine guanine phosphoribosyl transferase (HGPR), localize to different vesicles. In T. cruzi, localization of polyphosphate (polyP) molecules to glycosomes exhibited limited overlap with the glycosomal marker phosphate pyruvate dikinase (PPDK), suggesting a heterologous distribution of polyP in individual organelles [34]. Additionally, unequal distribution of a fluorescent glycosome marker has been observed in T. brucei cultured under low glucose environmental conditions [9] and our lab has observed localization differences between certain glycosomal enzymes, discussed in the next section. Together, these data suggest that glycosomes may exist as heterologous populations within a single parasite in many kinetoplastid species.

Glycosome heterogeneity is likely a result of two processes: (i) functional specialization and (ii) multiple pathways of glycosome formation and proliferation. Functional specialization would allow for the separation of different metabolic pathways into vesicles with distinct metabolite composition and the presence of multiple biogenesis pathways would allow the parasites flexibility in modulating glycososome composition during development or in response to changing environmental conditions.

### Glycosome specialization provides a mechanism to regulate opposing pathways, which may be antagonistic if localized together

Two pathways that reside in glycosomes are the catabolic pathway glycolysis, which breaks down glucose to generate ATP, and the anabolic pathway, gluconeogenesis, which functions to produce the glucose-6-phosphate.
necesary to fuel metabolic pathways when glucose is not available [5]. Having both pathways active in the same place at the same time would lead to a futile cycle in which ATP would constantly be generated and depleted. Other eukaryotes regulate these cytosolic pathways through the allosteric regulation of enzymes such as hexokinase (HK) and phosphofructokinase (PFK) that catalyze the rate limiting steps [35]. However, in *T. brucei* and *T. cruzi*, these enzymes are not allosterically regulated by their products [36,37] (or only moderately inhibited by glucose-6-phosphate in *L. mexicana* [38]) making coordinated regulation of these pathways through such mechanisms unlikely. Localizing these pathways to different compartments, glycosomes that house glycolysis and ‘gluconeogenosomes’ that function in gluconeogenesis (Figure 1B), would provide one mechanism of regulating these processes.

Most of the glycolytic and gluconeogenic pathways share enzymes that work in the forward or reverse direction depending on which pathway is functioning. The exceptions are PFK and fructose-1,6 bisphosphatase (FBPase), which are specific to glycolysis and gluconeogenesis, respectively. The establishment of glycosomes and ‘gluconeogenosomes’ would require the differential localization of only those two enzymes. We have generated preliminary immunofluorescence data in *T. brucei* suggesting that FBPase and PFK exhibit different localization patterns (unpublished results) and work in *Leishmania* showed that PFK expression was enriched in high-density glycosomes [11]. Separation of glycolysis and gluconeogenesis may be only one example of potential glycosome sub-populations with additional subtypes of specialized organelles to be identified. Limitations in our ability to analyze the composition of individual organelles prevents us from determining whether the separation of biochemical pathways is limited to these specific pathways in *T. brucei* or if there are additional pathways in other kinetoplastids that are similarly organized. However, the development of new flow cytometry techniques described below has the potential to overcome this hurdle.

**Heterogeneity may be a reflection of glycosome biogenesis processes**

In other eukaryotes, peroxisomes multiply via a combination of *de novo* synthesis of new organelles from the ER and the fission of existing organelles [39], resulting in the presence of organelles with different protein composition (Figure 1A). The degree to which *de novo* biogenesis and proliferation of existing organelles occurs varies with organism, development, and environment. While it is clear that glycosomes divide by fission [40], the *de novo* pathway and the extent to which it contributes to glycosome proliferation is unclear. Pex3, the cornerstone of peroxisome biogenesis, has only recently been identified in kinetoplastids [32,33].

During *de novo* biogenesis in yeast, plants and animals, multiple peroxins traffic through the ER [27,41,42] from which two distinct classes of immature preperoxisomal vesicles bud and eventually fuse to form fully functional organelles [39,43]. Two labs have detected *T. brucei* Pex13.1 in the ER [7,44], indicating that at least one peroxin can transit from this organelle to glycosomes and suggests that *de novo* biogenesis of glycosomes occurs. Currently, we do not know how Pex13.1 is targeted to, and exits from, the ER or if other peroxins follow this trafficking route. The presence of two biogenesis pathways (fission and *de novo* biogenesis) is significant in that it reflects commitment by the parasite to maintain distinct biogenesis pathways that would provide flexibility in responding to environmental and developmental changes.

**Other processes may contribute to glycosome heterogeneity**

In addition to organelle biogenesis, it is likely that other processes such as organelle division and degradation contribute to the observed heterogeneity of glycosomes. Glycosome turnover has been implicated in the retooling of glycosomes for specialization between different life cycle stages with the observation that glycosomes associate with autophagic compartments during differentiation [45]. Several peroxins have been implicated in pexophagy in other systems including Pex3 and Pex14 [46]; however, it is not currently clear how these peroxins function in glycosome degradation in kinetoplastids.

Another possibility for glycosome heterogeneity is asymmetric division of existing organelles. In yeast, asymmetric division of peroxisomes with the smaller daughter organelle retaining competency for matrix protein import while the larger, ‘old’ organelle is not import competent [47]. During peroxisome division, Pex11 deforms the membrane and recruits dynamin related proteins to cause division of peroxisomes [48]. In this capacity, it is possible that Pex11 may contribute to glycosome heterogeneity. However, there is currently no evidence for asymmetrical glycosome division in kinetoplastids.
The import complex is positioned to mediate glycosome specialization and has some unique characteristics

In eukaryotes, Pex13 and Pex14 comprise the import channel. While the primary sequence of Pex13 is not well conserved among organisms, they do share several conserved functional domains; a YG-rich domain, at least two transmembrane domains, and an SH3 domain (Figure 2). The YG-rich domain is responsible for interactions with Pex7 and deletion of this domain in mammalian cells resulted in loss of Pex7 binding and disruption of Pex13 homodimerization [49,50]. The SH3 domain has been implicated in interactions with both Pex5 and Pex14 and mutations in this domain resulted in the disruption of Pex5 binding in yeast two-hybrid assays [51]. Although Pex13 interacts with Pex14 via the SH3 domain, a second intraperoxisomal binding site exists in Pex13 [52], suggesting that a Pex13 lacking the SH3 domain may still function in the import complex via the YG-rich domain.

In contrast with other eukaryotes, T. brucei, T. cruzi and Leishmania have two Pex13s, Pex13.1 and Pex13.2 [18], and it is unclear why these parasites have evolved two of these proteins. Like Pex13.1, Pex13.2 contains a YG-rich domain and two transmembrane domains; however, it lacks an SH3 domain (Figure 2). These Pex13s are not redundant as silencing either gene alone is lethal [17,18]. Yeast two-hybrid experiments [18] and coimmunoprecipitation experiments done with T. brucei lysates [53] reveal that Pex13.1 and Pex13.2 interact with each other and with Pex14 to form multiple complexes containing different amounts of Pex13.1 and Pex13.2. Silencing of Pex13.1 resulted in mislocalization of both PTS1 and PTS2 proteins [17] while silencing of Pex13.2 resulted in the mislocalization of two proteins, hexokinase and aldolase, both containing PTS2 sequences [53]. The YG-rich domain, which binds the PTS2 receptor Pex7, was shown by protease protection assays to face the cytoplasmic side of the glycosome where it can provide additional Pex7 binding sites that may increase the efficiency of PTS2 protein import [53]. These observations suggest Pex13.2 may serve as an additional receptor for Pex7 via the YG-rich region but may lack the capacity to interact with Pex5. Another possibility is that Pex13.2 serves as a coreceptor for PTS2-dependent import. In yeast, Pex18/Pex21 function as co-receptors in the Pex7 import pathway [49] and Pex5pL is required in mammalian cells [54], suggesting a requirement for additional receptors in the PTS2 pathway. It is possible Pex13.2 fulfills this role in kinetoplastids. Unlike peroxisome protein localization in eukaryotes, mislocalization of glycosome proteins involved in glycolysis is lethal in
T. brucei [6,55,56]. Furthermore, the silencing of many Pexs, including Pex14, Pex13.1 and/or Pex13.2 [18,56] is toxic to T. brucei. This strict requirement for efficient protein import may have necessitated the evolution of accessory proteins to increase import efficiency.

It has been proposed that Pex13.1 and Pex13.2 arose from a duplication event followed by extreme divergence [18]. Gene duplication increases genetic diversity and creates redundancy allowing for the evolution of gene sequences and function [57]. Once a gene is duplicated, there are several outcomes. Accumulation of mutations may lead to a loss of function of one gene copy. Another outcome of gene duplication is functional divergence in which an advantageous mutation changes the function of one copy. This results in the retention of both gene copies that have different functions. In neofunctionalization, one copy acquires a new function. In subfunctionalization, multiple functions of the ancestral gene are split between the duplicated genes. While the impact of gene duplication on the evolution of Pex13.1 and Pex13.2 function is unknown, it is possible that Pex13.1 and Pex13.2 have evolved different functions over time.

**Potential functions of Pex13.1 and Pex13.2 in processes other than glycosome protein import**

Eukaryotic Pex13s have multiple functions in peroxisome biology and it is possible that these multiple functions have been divided between kinetoplastid Pex13.1 and Pex13.2. A genome-wide genetic screen in mammalian cells identified Pex13 as a selective autophagy factor [58] and later experiments demonstrated that silencing Pex13 reduced the virophagy and mitophagy without impacting general autophagy [59]. Because Pex13 did not colocalize to the autophagosome it is unlikely to function as an autophagy receptor linking cargo to the autophagosome membrane but was proposed to be a member of a signaling complex that triggers downstream post-translational modifications or activation of other autophagy factors. Studies resolving the function of Pex13.1 or Pex13.2 in autophagy will significantly advance our understanding of how these Pex13s regulate glycosome biogenesis aside from their role in glycosome protein import.

In addition to a potential role in autophagy, Pex13.1 and Pex13.2 may play a role in the formation of membrane contact sites with other organelles. Membrane contact sites between peroxisomes, mitochondria, lipid droplets, and the ER have been observed in yeast and mammalian cells [60–62]. While the precise protein composition of peroxisome membrane sites is not well resolved, transmembrane proteins such as Pex13 are positioned to serve as membrane tethers that form the scaffold of such regions. While glycosome membrane contact sites have yet to be defined in trypanosomes, they likely exist and Pex13.1 or Pex13.2 may play a role in the establishment or maintenance of such structures.

**T. brucei contains at least two glycosome import complexes**

The presence of two Pex13s is evidence that the glycosome import machinery is unique and biochemical experiments support this hypothesis. At least two import complexes are required for the import of eukaryotic peroxisome proteins. In yeast there is one complex that facilitates import of PTS1 proteins and another for PTS2 proteins [63]. The PTS1 complex contains two membrane proteins, Pex13 and Pex14, as well as the receptor protein Pex5. The PTS2 complex includes Pex13, Pex14, Pex5 and Pex7, but also requires species-specific, auxiliary proteins Pex18 and 21 in yeast and Pex5L in mammals [49,64]. The presence of two Pex13s indicate that the glycosome protein import machinery is unique and the failure to identify auxiliary peroxins such as Pex18 and Pex21 suggests there may be additional kinetoplastid Pexs to be discovered.

Two dimensional Blue-Native gel electrophoresis revealed the presence of three import complexes in T. brucei: one large complex of ∼1000 kDa containing Pex13.1 and Pex13.2, an intermediate size complex containing Pex13.1, Pex13.2 and Pex14 and a small complex containing Pex14 but lacking detectable amounts of Pex13.1 or Pex13.2 [53]. Studies to define the precise protein composition of each complex, their relationships to each other, and the function each play in glycosome protein import is currently underway and will provide insight into the biology of these complexes.

Currently, it is unknown whether these complexes mediate the import of different proteins or represent intermediates formed during the import complex assembly process. We anticipate that future studies focused on resolving the function and composition of each complex along with flow cytometry analysis of individual glycosomes will provide insight into whether these import complexes contribute to glycosome heterogeneity.
Technical advances open up new possibilities in glycosome research

Most analytical approaches for studying organelles utilize bulk biochemical techniques such as differential centrifugation and sucrose gradients. These isolation procedures generate mixed populations and mask heterogeneity of individual organelles. In the late 1980's, flow cytometry was used to isolate intracellular organelles that had been labeled with organelles specific dyes or probes [65]. In flow cytometry (Figure 1C), individual cells or organelles travel via a sheath column and pass through an interrogation point consisting of a laser that excites the sample and detectors to measure fluorescence emission as well as forward and side-scatter, which can be a reflection of size and sample complexity. This approach is attractive as it allows the separation of organelles on the basis of their biological properties rather than biochemical properties such as density. However, the lack of sensitivity prevented this approach from being used widely as small vesicles have low numbers of dye molecules associated with them. Recent research on extracellular vesicles has produced new methods to resolve 80 nm, 200 nm, and 500 nm particles that are well-suited to studying organelle diversity [66]. In traditional flow cytometry, the trigger channel is usually forward light scatter. When the object is much larger than the interrogation wavelength, cells are easily distinguished from other debris. However, smaller objects such as extracellular vesicles and intracellular organelles produce less light scatter and these objects are difficult to distinguish from background. This limitation can be overcome by using a short wavelength (violet, 405 nm) laser and small particle detector that are becoming more common. Cells or organelles can be labeled in a variety of ways including fluorescent-protein fusions, fluorescently labeled antibodies or small molecules that are optically active. The high-throughput format of flow cytometry enables strong statistical analysis and the detection of rare events, which are not possible via conventional imaging techniques. Flow cytometry is especially powerful as it can be coupled with sorting to isolate different glycosome populations for further analysis such as mass spectrometry to define the protein and lipid composition of different glycosome populations. Such an approach has been used to understand mitochondrial diversity [67] and has the potential to radically reshape our understanding of glycosome biology.

Perspectives

- Glycosomes are essential kinetoplastid-specific peroxisomes of kinetoplastids that compartmentalize numerous metabolic pathways. While the composition and function of these organelles is heterogeneous, the molecular basis of this heterogeneity is unclear.

- The glycosome protein import machinery is positioned to play a role in the heterogeneity of these organelles. While many of the mechanisms that regulate glycosome biogenesis are conserved with peroxisomes of other eukaryotes, there are some kinetoplastids-specific aspects of the protein import that may be exploited for treatments of diseases caused by these organisms.

- Historically, the small size of glycosomes has limited our ability to resolve the basis of this variation. Recent advances in fluorescence activated organelle sorting offer an exciting possibility for defining the molecular basis of this heterogeneity and assessing the biological significance of this diversity.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

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M.T.M and L.P.C. contributed to the conception, drafting, revising, and final approval of the manuscript. All authors attest to the accuracy and integrity of the complete manuscript.
Abbreviations
ASS, argininosuccinate; BF, bloodstream form; DHAPAT, dihydroxyacetone phosphate acyltransferase; ER, endoplasmic reticulum; FBPane, fructose-1,6 bisphosphatase; PFK, phosphofructokinase; PTS, peroxisome targeting sequences; RING, really interesting novel gene.

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