Structure of trypanosome coat protein VSGsur and function in suramin resistance

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Suramin has been a primary early-stage treatment for African trypanosomiasis for nearly 100 yr. Recent studies revealed that trypanosome strains that express the variant surface glycoprotein (VSG) VSGsur possess heightened resistance to suramin. Here, we show that VSGsur binds tightly to suramin but other VSGs do not. By solving high-resolution crystal structures of VSGsur and VSG13, we also demonstrate that these VSGs define a structurally divergent subgroup of the coat proteins. The co-crystal structure of VSGsur with suramin reveals that the chemically symmetric drug binds within a large cavity in the VSG homodimer asymmetrically, primarily through contacts of its central benzene rings. Structure-based, loss-of-contact mutations in VSGsur significantly decrease the affinity to suramin and lead to a loss of the resistance phenotype. Altogether, these data show that the resistance phenotype is dependent on the binding of suramin to VSGsur, establishing that the VSG proteins can possess functionality beyond their role in antigenic variation.

Multiple species of the genus Trypanosoma cause sleeping sickness in humans and related diseases in animals1–3. A remarkable feature characterizing all such infections is the ability of the trypanosomes to thrive in the blood and tissue spaces of infected mammals despite complete exposure to the immune system of the host4. This is achieved largely through a dedicated machinery in the organism to alter its surface coat and continuously evade immune system recognition and clearance5. The trypanosome strains that were over 90-fold resistant to the drug18. The VSGs are long, rod-shaped homodimeric proteins of around 60 kDa with two subdomains: a larger, N-terminal domain (NTD) of around 350–400 amino acids and a smaller, 80–120-residue C-terminal domain (CTD) to which the GPI anchor is attached (Supplementary Fig. 1). The top lobe (facing away from the parasite) is hypothesized to harbour the majority of immune epitopes, presenting the ‘antigenic face’ of the VSG, although little epitope mapping and no antibody–VSG co-crystal structures have been published to date6–10.

While the host immune system develops rapid and effective responses to the VSGs, the trypanosomes access a genetic repository of over 2,000 VSG genes and pseudo-VSG genes with distinct antigenic properties, switching coat proteins and thereby rendering the host response naive to the new coat before the parasites can be cleared. This process of recognition, partial clearance, coat switching and pathogen escape is termed antigenic variation. Because of this ability to thwart immune clearance, African trypanosomiasis is usually fatal unless treated chemotherapeutically11.

Synthesized as early as 1917, the compound suramin (originally Bayer 205 and later sold as Germanin) was first used therapeutically to treat African trypanosomiasis in the 1920s11–13. It is one of only a few drugs (including pentamidine, melarsoprol, eflornithine, nifurtimox and fexinidazole)12–14 available to counter the disease and is only effective in the early stages of infection, before the parasite has entered the central nervous system (as suramin cannot effectively penetrate the blood–brain barrier15). It has also been used prophylactically16 and is on the World Health Organization’s List of Essential Medicines17. Recently, suramin has demonstrated potent antineoplastic properties18. It is part of a larger family of benzopurpurine dyes and naphthalene ureas that show trypanocidal activity12. Resistance to suramin has gradually spread through the animal-infective trypanosomal populations, but has yet to reach the species causing disease in humans19.

The specific mechanism behind the trypanolytic activity of suramin remains unresolved, although studies have implicated effects on the glycosome and impairment of cytokinesis20. A model for the internalization of suramin has been proposed in which the drug enters through receptor-mediated endocytosis involving two distinct pathways: (1) low-density lipoproteins (LDLs) with possible involvement of other serum proteins; and (2) the invariant surface glycoprotein 75 (ISG75) receptor of the pathogen21–23. However, to date no direct binding of suramin to any trypanosomal protein has been reported.

Recently, in vitro selection in the presence of suramin generated trypanosome strains that were over 90-fold resistant to the drug18. All these resistant strains were shown to express a specific VSG, termed VSGsur, that itself was sufficient to convey the resistant phenotype in trypanosomes genetically engineered to express it24. However, it has remained unclear how VSGsur is involved in this resistance phenotype, whether directly or indirectly. Hypotheses include a model in which VSGsur causes suramin resistance by decreasing specific, receptor-mediated endocytosis pathways critical to the uptake of the drug25.
We sought insight into the function of VSGsur through structural studies of the protein (clone from *Trypanosoma brucei rhodesiense*, GenBank ATI14856), determining the crystal structure of the NTD to 1.2-Å resolution (residues 30–408; Fig. 1a, Methods, Supplementary Fig. 2 and Supplementary Table 1). VSGsur differs markedly from previous VSG structures determined (VSG1, VSG2, VSG3 and ILTat1.24)24–27. While the core three-helix bundle scaffolding of the VSG family is present, VSGsur possesses several divergent features. The most striking is observed in the ‘upper lobe’ of the molecule. The insertion between the helices at the top of VSGsur folds into a bona fide structural subdomain (residues ~140–260) consisting of five β-strands and an elongated loop between 174–208 that travels half the length of the VSG molecule before returning to the upper lobe (Extended Data Fig. 1a). This subdomain forms an intermolecular β-sandwich with the pairing monomer in the homodimer to create a tightly interwoven quaternary fold with an extensive hydrophobic core. Perched on top of the three-helix bundle, this ‘head’ is strikingly different from the more flattened polypeptide arrangements of the other VSG top lobes that do not organize into any typified fold.

Also remarkable is the location of an N-linked sugar nearly two-thirds of the distance up the NTD from the bottom lobe and directly under the β-sandwich top lobe. Most VSGs possess N-linked carbohydrates, but in all studied to date they are located in the bottom lobe and have been hypothesized to function there in VSG–membrane dynamics28. Further distinguishing VSGsur from previous structures, the multiple disulfide bonds stabilizing most VSG proteins (three pairs in VSGsur) are not clustered in the upper lobe, but extend into the three-helix bundle. Finally, there is a large cavity in the homodimeric interface in VSGsur not found in other published VSG structures, located just beneath the unusually placed N-linked glycan. Altogether, VSGsur is a notable departure from previously studied VSGs while still maintaining the core scaffolding of this family.

Our additional studies show that VSGsur is not a unique fold but is in fact probably a member of a subfamily of VSGs. The 1.4-Å resolution crystal structure of VSG13 (also named MITat1.13) reveals a second VSG protein with a top ‘head’ consisting of a large β-sheet subdomain (residues 130–260) that forms an intermolecular β-sandwich in the homodimer (Fig. 1b, Methods, Extended Data Fig. 1 and Supplementary Table 2). Even more striking than VSGsur, the cysteine disulfide bonds of VSG13 (four in this case) are spread throughout the length of the VSG and not clustered in the top lobe, in fact reaching down near the bottom lobe of the NTD. As with VSGsur, VSG13 harbours an N-linked glycan approaching the top of the molecule, positioned just under the β-sheet head and not in the bottom lobe. VSG13 also possesses an internal cavity between monomers in the homodimer. While smaller than that observed.

**Fig. 1** Overall structure of VSGsur. a, VSGsur is shown as a ribbon diagram in two orientations related by a rotation of 90°, the monomers in the homodimer coloured gold and light blue. The N-linked glycans of the two VSG monomers are displayed as red space-filling atoms, disulfide bonds are shown in cyan and several key features are labelled. ‘N’ and ‘C’ indicate the N and C termini of each monomer. b, The crystal structure of VSG13 in two orientations is depicted similarly to that of VSGsur as in a. c, Molecular surfaces of published VSG structures to date. The VSGs are oriented looking ‘down’ on the top lobe of the protein, the orientation rotated 90° about a horizontal axis in comparison to upper panels. The upper row of surfaces is coloured by relative electrostatic potential (blue is basic/positively charged, red is acidic/negatively charged and white is neutral) and the lower row by the Eisenberg hydrophobicity scale51 (yellow indicates hydrophobic, white polar). Note that the NTD of VSG3 is a monomer in solution and the crystal structure, and in the absence of any structural data of an NTD dimer, we have chosen to show only the monomeric surface. Structures and molecular surfaces are illustrated with CCP4mg52 and MacPyMOL53.
in VSGsur, it is still large enough to allow solvent into the cavity from the crystallization reagents. A protein structural alignment of the VSGsur and VSG13 monomers (Extended Data Fig. 1b) shows that the core three-helix bundle aligns well and the N-linked sugars superpose very closely, whereas the β-sheet head in the upper lobes aligns poorly, evincing divergence. Comparing VSGsur and VSG13 with the ‘canonical’ VSG2 structure shows that the β-sandwich sub-domain gives more height to the NTDs of this subfamily than the other VSGs previously studied (Extended Data Fig. 1c), which could have significance for membrane dynamics and receptor-mediated endocytosis24.

Although VSG13 and VSGsur present marked divergences from previously reported VSG structures, the variability in these two VSGs is housed within more generally conserved features that make them clear members of a protein superfamily. Most obvious is the core three-helix bundle scaffolding that undergirds all the VSGs and VSG-related proteins such as serum resistance-associated protein29 and the haptoglobin–haemoglobin receptor30. The divergent structures in the top and bottom lobes of the VSGs are basically different insertions between the loops connecting the central helices of the core of these rod-like molecules. Secondly, the proteins all evince a shared functionality in antigenic variation by presenting distinct molecular surfaces to the extracellular environment with extensive variation in amino acid sequence, charge and hydrophobic distributions, and topography (Fig. 1c).

Resistance studies with suramin. To examine whether the resistance mechanism associated with the expression of VSGsur involved direct protein-to-drug binding, we tested *T. b. rhodesiense* VSGsur (expressed in *Trypanosoma brucei brucei*) and a panel of other VSGs from *T. b. brucei* Lister 427 (including VSG13) for their effect on suramin susceptibility and suramin binding (Fig. 2). Toxicity experiments demonstrated that, similarly to what has been shown for several VSGs from *T. b. rhodesiense* and *T. b. brucei* strain BS221 (refs. 18,23), suramin killed all VSG-expressing Lister 427 strains we examined (VSG2, VSG3 and VSG13), whereas those expressing VSGsur were resistant to over 20 times higher concentrations of the drug (Fig. 2a,b). In more detail, VSGsur evinced a half-maximal inhibitory concentration (IC₅₀) of 8.41 ± 0.76 µM, whereas VSG2, VSG3 and VSG13 had IC₅₀ values below 0.45 µM (Fig. 2a,b). At a concentration of 0.7 µM suramin (slightly above the IC₅₀ values of the other VSGs), VSGsur shows no significant differences in growth compared with when untreated (Extended Data Fig. 4). Isothermal titration calorimetry (ITC) showed that while suramin did not bind to VSG2, VSG3 or VSG13, it bound with nanomolar affinity to VSGsur (Fig. 2b and Extended Data Fig. 3).

Co-crystal structure of VSGsur with suramin. The above data motivated efforts to co-crystallize suramin together with VSGsur. Soaking native crystals in high concentrations of suramin led to well-diffracting crystals although the binding of suramin changed the space group, resulting in a dimer in the asymmetric unit into which the drug could be modelled (Methods, Supplementary Table 1 and Supplementary methods). Interestingly, the chemically symmetric drug suramin bound at the dimerization interface asymmetrically (Fig. 3a). Suramin binds tightly in the large cavity between the two monomers of VSGsur, burying a surface area of approximately 700 Å² with more than 100 atomic contacts characterized by both hydrogen bonding and non-bonding interactions. Suramin (C₅₁H₄₀N₆O₂₃S₆) is a symmetric, polysulphonated naphthaleneurea (Extended Data Fig. 4a). The compound is centred by the cavity results in fewer contacts from VSGsur to stabilize the dimer rotation axis. Three of the four benzene groups in the structure, with clear electron density and B-factors lower than the average of the overall structure (protein, ligands and solvent). The naphthalene rings, however, show weaker electron density and high B-factors, characteristic of more disorder or conformational flexibility. Their extension out of the cavity results in fewer contacts from VSGsur to stabilize their conformation.

Four amino acids from each monomer in the dimer contribute the bulk of protein contacts to the drug: H122, R125, H126 and R292 (Fig. 3b,c). These positively charged residues are centred in the cavity of the dimer interface, burying the majority of surface area upon drug binding. These major contacts are...
buttressed with a set of minor interactions from residues L49, R119, A123 and A129, as well as from the N-linked glycan, several of these moieties only making contact from one protein monomer to some of the more distally positioned elements of suramin (such as the more poorly ordered sulfonic acids connected to the naphthalene rings; Fig. 3c,d). The sidechain nitrogen atoms from two arginine residues, R125 and R292, both make hydrogen bonds with the sulfate oxygens of the most distal naphthalene moieties of suramin, as does H126. Other residues such as L49, R119, A123 and A129 primarily make close-approach, non-bonding contacts (Fig. 3d).

The interface centres on the imidazole rings of H122. In both monomers these residues make extensive contacts, stacking with two of the benzene rings of suramin (Fig. 3b). In the native protein structure, H122 has two different, partially occupied conformations in the VSGsur monomer of VSGsur (Extended Data Fig. 5a–c). One is very similar to the conformation seen in the suramin-bound co-crystal structure, whereas the other is a different rotamer, flipped away from this position. The presence of the drug locks the histidines in the stacking interaction with the suramin benzene rings, abolishing any evidence of the other possible conformation. The suramin benzene ring between the two rings contacted by H122, and which
is centred in the dimer interface, interacts with VSGsur via backbone and sidechain contacts from R125 and H126. Interestingly, the benzene group of the compound used to phase the structure (5-amino-2,4,6-triiodoisophthalic acid (I3C) or ‘Magic Triangle’; Methods) occupies the same position as the suramin benzene groups, stacking similarly with H122 (Extended Data Fig. 5d and Supplementary Table 1).

The N-linked sugar groups (the N-acetylglucosamine rings, the β-mannose and other mannose residues in the carbohydrate chain) make contacts to the sulfonic acids decorating the naphthalene rings (Fig. 3c). These contacts only occur for the sugar chain of one molecule, as the asymmetric positioning of suramin in the cavity leads the other naphthalene group to protrude too far from the carbohydrate to make effective contacts.

**Mutations in the suramin binding site abolish drug resistance.**

Altogether, the ITC and structural results show that unlike other VSGs, even the related VSG13, VSGsur binds suramin specifically and with high affinity. To examine whether the resistance phenotype of cells expressing VSGsur is tied to this binding, we created mutants (Extended Data Fig. 6 and Methods) that should not disrupt the fold of VSGsur. One mutant was a direct loss-of-contact alteration, changing H122 to alanine (H122A), thereby removing the stacking of the two histidine rings to the benzene groups in the most ordered binding mode of the ligand. The second was indirect, mutating N130 to alanine (N130A), thereby preventing the N-linked glycosylation from occurring and removing the sugar-to-drug interactions.

Figure 2 summarizes these results. Both mutants were viable and grew in the absence of the drug with doubling times of 8.9 ± 0.73 h (N130A) and 6.9 ± 0.18 h (H122A) as compared with 6.5 ± 0.35 h for the wild type (values showing the standard deviation, n = 6; Extended Data Fig. 2). The mutant VSGs behaved similarly to the wild-type protein during purification, suggesting that the changes did not destabilize or otherwise compromise the proteins. However, they both lacked the heightened resistance to suramin of the wild-type protein (Fig. 2a). This loss is correlated with a loss in binding to the drug (Fig. 2b), directly linking the suramin–VSGsur interaction to the resistance phenotype.

To verify that the mutations did not perturb the fold of VSGsur, we solved the crystal structure of the VSGsur mutant H122A (Supplementary Table 3), showing a nearly identical structure to wild-type VSGsur. We were unable to obtain well-diffracting crystals of the mutant N130A, probably as the sugar is involved in crystal packing contacts. High-concentration, long-duration soaks of suramin into the H122A mutant (Methods and Supplementary Table 3), however, revealed the presence of observable differences in density in the pocket, but the density was poorly ordered and we were unable to confidently model suramin (Extended Data Fig. 7).

**Distal alterations to the suramin binding pocket are associated with increased resistance to suramin.** We further subjected *T. b. rhodesiense* STIB900_sur1 (ref. 19), which expresses VSGsur and is about 100-fold more resistant to suramin than other strains (IC50 of 1.1 ± 0.13 μM), to increasing suramin pressure in vitro (up to 18μM over 1 yr). This produces mutants that tolerated higher levels of the drug (IC50 of 11 ± 1.2 μM). Analysis of the expressed VSG of a highly resistant clone showed that it was VSGsur with 14 mutations in the nucleotide sequence, producing eight amino acid substitutions in the protein sequence. These mutations clustered in the structure in two locations (Extended Data Fig. 8). G174T, E175A and K288A are located around the N-linked glycans with several contacts to the sugar. They do not, however, contact the drug but could conceivably modulate contacts to suramin indirectly through its interactions with the carbohydrate. A304S, T313A, D317E, A318T and A326T cluster on one face of the bottom lobe, distal from the drug binding site, creating a patch of surface exposed residues. This distance from the binding pocket makes it very likely that these mutations do not affect drug binding and thus could represent a functional region of VSGsur that interacts with other factors critical to generating resistance.

A construct of the mutant VSGsur was targeted into the active VSG expression site of *T. b. rhodesiense* STIB900_sur1 (Extended Data Fig. 9a). Of four transfected clones, two had replaced VSGsur with the mutant version and two had retained the original VSGsur, as verified by PCR. The clones with the original VSGsur sequence showed equivalent resistance to previously characterized strains expressing VSGsur, whereas the two clones that expressed the mutant version of VSGsur showed an increase in their IC50 to suramin (Extended Data Fig. 9b), suggesting that these mutations indeed further enhance suramin resistance. However, this increased resistance was only slightly more than twofold higher than that mediated by wild-type VSGsur, indicating that there are probably other mutations in the VSGsur selected mutant strain that synergize with the VSGsur mutations to produce the full 11-fold increase in IC50.

**Alterations in endocytic trafficking are not coupled to resistance.**

Wiedemar et al. had shown that VSGsur-expressing strains have reduced levels of endocytic trafficking relative to VSG2 (also called VSG221), and, in particular, a reduced internalization of LDL, a factor hypothesized to be involved in suramin uptake into the cell21–23. Therefore, VSGsur might enhance resistance to the drug by decreasing suramin uptake through reducing endocytosis via the LDL receptor. However, as VSGsur probably comprises over 99% of the surface protein on the parasite and binds suramin with nanomolar affinity at blood pH, understanding suramin uptake in these strains is complicated by the potential for a massive influx of the drug as the VSG is internalized and then recycled back to the membrane13. To interrogate this model, we performed endocytosis experiments with blue dextran and bodipy-labelled LDL (Fig. 4, Methods and Extended Data Fig. 10). Our data show that, consistent with previous results15, there does not appear to be a VSGsur-specific defect in fluid-phase endocytosis. Also consistent with previous results15, we do observe that VSGsur cells have a decrease in LDL uptake compared with VSG2 (although there is substantial variance among different VSGs; Extended Data Fig. 10). However, loss-of-binding suramin mutants show no alteration in these kinetics (Fig. 4c). Similar results are seen with uptake via the transferrin receptor (Fig. 4d). As these mutants also lose resistance to suramin, it seems that the dynamics of LDL uptake (and thus, presumably, suramin via this pathway) are not strongly coupled to the resistance phenotype in VSGsur. In contrast, the key determinant appears to be the binding of the drug to VSGsur.

**Discussion**

Despite the wide genetic variance in the VSGs, only a handful of the membrane-distal, antigenically distinct NTD domains have been characterized at the atomic level16–27. With the structures of VSGsur and VSG13 presented here, we show that the ‘antigenic space’ of the VSGs is much broader than anticipated. Moreover, the co-crystal structure of VSGsur with the trypanocidal compound suramin directly links the binding of the drug to the resistance phenotype displayed by strains of *Trypanosoma brucei* expressing VSGsur.

This binding of suramin establishes that the VSGs can have a function beyond that of antigenic variation. This idea is buttressed by recent results that show that VSG2 is able to bind a ligand as well (the divalent metal calcium, manuscript in preparation). Also consistent with this, the recently determined crystal structure of the *Trypanosoma congolense* haptoglobin–haemoglobin receptor29 shows that this protein adopts a fold very similar and clearly related
to the VSGs, packing functionally with all possible VSGs on the surface as it is required for parasite survival in the host to scavenge iron. These observations raise the possibility of other functions for the VSGs in binding small molecules or even proteins, and to therefore function for the benefit of the pathogen in manners more diverse than mere immune evasion.

A parsimonious model to explain VSGsur-mediated resistance involves the VSG redirecting the trafficking of the drug, shunting it to some kind of disposal pathway and thereby reducing the effective concentration in the cell (Fig. 5). VSGsur is continuously produced at high levels in the trypanosome and exported toward the cell surface from the Golgi through the vesicular trafficking pathways. Therefore, suramin that is internalized through other pathways (for example, via the LDL receptor or ISG75) could comingle with VSGsur in specific endocytic compartments, the newly synthesized VSG binding the drug and preventing it from being trafficked further (see also additional discussion in the Supplementary Methods). Such a model is in harmony with what we know about suramin import and export receptor-internalized suramin at an extracellular drug concentration of ~30 cubic microns, we calculate that there is approximately a 5–20-fold excess of newly synthesized VSGsur available to export suramin within M to 4 M, very near our measured IC50 of 8.4 M. Such other considerations include issues such as the growing contribution of fluid-phase import at higher suramin concentrations, the actual toxic levels of suramin in the cell (and, more critically, in what compartments) and the binding of suramin to serum proteins, as well as other unknowns. Nevertheless, this simple model of newly produced VSGsur serving to bind the imported suramin and shunt it out of the endocytic lysosomal pathway matches well with what we know about suramin import and VSG dynamics.

The higher-resistance VSGsur mutants could offer a tantalizing clue toward investigating some of the unknowns and uncovering the mechanism of suramin toxicity. While one subset of the mutations clusters around the carbohydrate and could conceivably alter binding to the drug, another set clusters in a surface patch on the bottom lobe of the protein. This patch is suggestive of a binding site and may point the way toward future studies of the macromolecular interactions involved in suramin resistance.

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**Fig. 4 | VSGsur and the loss-of-contact mutants have indistinguishable endocytic kinetics.**

**a.** Alexa 488-dextran endocytosis by *T. b. brucei* 2T1 cells expressing a variety of different VSG genes. Due to technical limitations, each cell line could not be analysed simultaneously within each experiment for each ligand. Therefore, VSG2 was used as a control in all experiments, allowing the calculation of the relative uptake rate for each cell line as compared with the uptake rate of each ligand observed by VSG2-expressing cells within each separate experiment. Each of these graphs therefore represents the combined experimental results from two separate experiments. Cell lines with a relative fluorescence intensity below 1 at a given time point have less efficient endocytic rates compared with VSG2-expressing cells, and vice versa. All graphs share the same y axis. **b-d.** Alexa 488-dextran (b), bodipy-LDL (c) and Alexa 488-transferrin (d) endocytosis by *T. b. brucei* 2T1 cells expressing VSGsur (black triangles), VSG2 (pink circles), VSGsur H122A (blue hexagons) or VSGsur N130A (green diamonds). All graphs depict arbitrary fluorescence units, determined by flow cytometry, on the y axis. Each graph represents one of multiple biological replicates of each experiment, with n = 2 individual experimental replicates for each time point.
β-mercaptoethanol), supplemented with 10% fetal calf serum (Gibco), l-cysteine and optionally shock-frozen in liquid nitrogen and stored at −80°C.

Methods

Cloning and production of *T. brucei* strains. A VSG13-expressing *T. brucei* clone was produced as described and was kindly provided by the group of Luisa Figueredo (Universidade de Lisboa). VSGsur from *T. b. rhodesiense* (GenBank MF093647.1) was codon-optimized and synthesized as a pUC19 clone (BioCat, Germany), and introduced into *T. brucei* strain Lister 427 as described in the Supplementary methods. VSGsur mutants N130A and H122A were cultivated in vitro in HMI-9 medium formulated as described by PAN Biotech without FBS, l-cysteine or albumin. VSGsur-N130A and VSGsur-H122A mutants were cultivated in vitro in HMI-9 medium (formulated as described by PAN Biotech without FBS, l-cysteine or albumin). Lysine residues were methylated (as per ref. 41) using dimethyl-amino-borate complex (Sigma) and formaldehyde (Thermo, methanol-free). The methylated VSG13 NTD was purified by gel filtration on a Superdex 200 Increase 20/200 GL column (GE Healthcare) in 50 mM HEPES, pH 7.6, and 150 mM NaCl.

Structural determination of VSG13. Purified methylated VSG13 NTD was concentrated to 2.5 mg ml⁻¹ in 10 mM Tris.Cl, pH 8.0. Crystals were grown at 23°C by vapour diffusion using hanging drops formed from mixing a 1:1 volume ratio of the protein with an equilibration buffer consisting of 1.8–2.0 M (NH₄)₂SO₄ and 100 mM Tris.Cl, pH 8.5. For cryoprotection, crystals were transferred into the same buffer including 20% v/v glycerol and flash-cooled immediately afterward to 100 K (−173.15°C). For phase determination, crystals were soaked for 30 s in a buffer containing 0.5 M sodium bromine and 20% v/v glycerol before flash-freezing.

Native data were collected at the European Synchrotron Radiation Facility at a wavelength of 1 Å on beamline ID29 and bromine soaks at the Diamond Light Source at 0.9198 Å on beamline i03 (Supplementary Table 2). The data were phased by single-wavelength anomalous diffraction (SAD) from 33 bromine sites that were identified using the SHELEX suite. Several models were manually combined from automated model building by PHENIX and CRANK of CCP4 (refs. 41, 42). Native datasets were then used to refine the model building (PHENIX) and refinement (PHENIX) into the native dataset (Supplementary Table 2).

For crystallization of VSG13, the CTD was removed by digestion with Endoproteinase LysC (NEB) at a 1:800 LysC/substrate ratio by mass for 1–2 h at 37°C. VSG13 NTD was purified by gel filtration on a HILoad 16/600 Superdex 200 pg column (GE Healthcare) in 50 mM HEPES, pH 7.6, and 150 mM NaCl. Lysine residues were methylated (as per ref. 17) using dimethyl-amino-borate complex (Sigma) and formaldehyde (Thermo, methanol-free). The methylated VSG13 NTD was purified by gel filtration on a Superdex 200 Increase 10/300 GL column (GE Healthcare) in 10 mM Tris.Cl, pH 8.0. Fractions from the gel filtration runs were subjected to SDS−PAGE analysis for visual inspection.

For crystallization of VSGsur and VSGsur mutants, the CTD was removed by limited proteolysis with trypsin at a 1:100 trypsin/substrate ratio by mass for 1 h on ice. The VSGsur NTD was purified by gel filtration on a HILoad 16/600 Superdex 200 pg column (GE Healthcare) in 10 mM Tris.Cl, pH 8.0. The peak fractions were pooled and concentrated to 4 mg ml⁻¹.

Purification of VSGs. *T. b. brucei* strains expressing VSG2, VSG3, VSG13, VSGsur, VSGsur-N130A and VSGsur-H122A mutants were cultivated in vitro in HMI-9 medium formulated as described by PAN Biotech without FBS, l-cysteine or β-mercaptoethanol, supplemented with 10% fetal calf serum (Gibco), l-cysteine and β-mercaptoethanol. Cells were cultured at 37°C and 5% CO₂. VSGs were purified according to established protocols. Briefly, cells were pelleted and then lysed in 0.4 mM ZnCl₂. The lysis mixture was centrifuged and the pellet containing the membrane material was resuspended in prewarmed (40°C) 10 mM phosphate buffer, pH 8. Following a second centrifugation, supernatant containing VSG protein was loaded onto an anion-exchange column (Q-Sepharose Fast-Flow, GE Healthcare), which was pre-equilibrated with 10 mM phosphate buffer, pH 8. The flow-through containing highly pure VSG was concentrated in an Amicon Stirred Cell and optionally shock-frozen in liquid nitrogen and stored at −80°C.

For structural determination of VSGsur and VSGsur mutants. Crystals were grown at 4°C by vapour diffusion using hanging drops formed by mixing 2½ of the protein solution (4 mg ml⁻¹) with 2½ of the equilibration buffer (19–24°C (v/v) PEG 400, 100 mM trithionalmine/HCl, pH 7.5, and 10% (v/v) isopropanol). The crystals were transferred to a cryobuffer (40% (v/v) PEG 400 and 100 mM trithionalmine/HCl) and flash-cooled in liquid nitrogen. Native datasets were collected at a wavelength of 0.9184 Å at the Helmholtz-Zentrum Berlin at beamline MX 14.1.
For phasing, the crystals were soaked overnight in 50 mM 13C/L10H (Magic Triangle, Jena Bioscience) in cryobuffer. The 13C-soaked crystals were collected at a wavelength of 0.66 Å at the Helmholtz-Zentrum Berlin at beamline MX 14.2. The structure was solved by SAD from the anomalous signal from two 13C molecules bound per VSgur monomer using the SHELex and HKL3000 suites. Arp/warp was used for automated building of the initial model.

PHENIX and COOT were used for several cycles of model building and refinement.

Suramin complexes were obtained either by soaking VSgur crystals in the drug or by premixing and growing crystals de novo. Complexes were produced by soaking the crystals for between 1 h and 6 d in cryobuffer supplemented with 0.77–7.7 mM suramin before cryo-cooling. Co-crystals were obtained at 4°C using 3 mg ml-1 VSgur, 0.7 mM suramin and an equilibration buffer containing 16–20% (v/v) PEG 3350, 200 mM NaCl and 100 mM Hepes/NaOH, pH 7.5. Before cryo-cooling, these complexes were transferred to 16% (v/v) PEG 3350, 100 mM Hepes/NaOH, pH 7.5, 0.7 mM suramin and 25% (v/v) PEG 400. The VSgur–suramin datasets were collected at the Helmholtz-Zentrum Berlin, the Paul Scherrer Institut Villingen and the Diamond Light Source. The structures were solved by molecular replacement with PHENIX using the native structure of VSgur as the search model (Supplementary Table 1; see also the Supplementary Methods for additional information).

Uptake assays. The LDL and transferrin uptake assays were performed as described with slight modifications, while the dextran assays were performed similarly. Cells were cultured to a density of approximately 10⁵ per ml in 25-cm² cell-culture flasks in duplicate. After incubation with 0.7–7.7 mM suramin before cryo-cooling. Co-crystals were obtained at 4°C using 3 mg ml-1 VSgur, 0.7 mM suramin and an equilibration buffer containing 16–20% (v/v) PEG 3350, 200 mM NaCl and 100 mM Hepes/NaOH, pH 7.5. Before cryo-cooling, these complexes were transferred to 16% (v/v) PEG 3350, 100 mM Hepes/NaOH, pH 7.5, 0.7 mM suramin and 25% (v/v) PEG 400. The VSgur–suramin datasets were collected at the Helmholtz-Zentrum Berlin, the Paul Scherrer Institut Villingen and the Diamond Light Source. The structures were solved by molecular replacement with PHENIX using the native structure of VSgur as the search model (Supplementary Table 1; see also the Supplementary Methods for additional information).

Titration buffers contained 10 mM NaPi (pH 8.00) and 150 mM NaCl. Proteins were transferred into the titration buffer by gel filtration chromatography, followed by concentration in 10-kDa disposable ultrafiltration centrifugal devices. Protein concentrations were measured by ultraviolet absorbance at 280 nm. In each experiment, the protein concentration in the cell varied between 40 and 55 μM. Suramin was injected at concentrations between 300 and 600 μM, and all samples were degassed before each experiment. All VSgur protein–suramin experiments were performed at least in duplicate to check the reproducibility of the data. The data were baseline-corrected, integrated and analysed with the PEAQ ITTC Analysis software (Malvern), fitting with a single-site binding model.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

Coordinates and structural factors have been uploaded to the RCSB PDB (www.rcsb.org); VSgur 13C (PDB ID 62Z9), VSgur WT native (PDB ID 62Z7A), VSgur + suramin (PDB ID 62Z7B), VSgur H122A (PDB ID 62Z7C), VSgur H122A 0.7–7.7 mM suramin (PDB ID 62Z7D), VSgur H122A 7.7 μM suramin (PDB ID 62Z7E), VSgur H122A 7.7 mM suramin (PDB ID 62Z7F), VSG13 NaBel (PDB ID 62Z86G27C) and VSG13 native (PDB ID 62Z8H1). Other data supporting the findings of this study are available from the authors upon request. Source data are provided with this paper.

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Extended Data Fig. 1 | Comparison of the VSG NTDs. a, A single monomer of the VSGsur NTD is shown as a ribbon diagram. The β-sheet subdomain that forms the top lobe is colored in orange and the rest of the structure in light blue. The N-linked glycan is shown as red space-filling spheres, the cysteine disulfide atoms as space-filling cyan spheres. b, Structural alignment of monomers of VSGsur (orange) and VSG13 (grey) with corresponding glycans shown in space filling representation and colored the same as the protein to which they are linked, while disulfides are shown in cyan and green for VSGsur and VSG13, respectively. The alignment produces an overall root-mean-square deviation of 1Å for the conserved portions of the structure (calculated over 220 Ca positions that primarily encompass the three-helix bundle and elements of the bottom lobe). c, Comparison of the structures of VSGsur, VSG13, and VSG2. The N-linked glycans are displayed as red space-filling atoms and the disulfide bonds are shown in cyan. Approximate dimensions of the molecules are noted, as well as the directions toward the external environment and toward the C-terminal domain (CTD) and plasma membrane of the trypanosome.
Extended Data Fig. 2 | Growth curves of VSGsur and VSG mutants. Strains expressing VSGsur and VSGsur mutants were grown with and without suramin (incubation of 0.7µM suramin for 24 h, Methods). The cell densities were determined by cell counting using a Neubauer hemocytometer. This procedure was repeated for 3-4 days in a row. Analysis was performed with GraphPad Prism, using a nonlinear regression model for curve fitting (Exponential growth with log). For statistical comparisons, we tested whether the slopes and intercepts of the best fit curves were significantly different. For VSGsur and VSGsur + 0.7µM suramin, the differences between the slopes is not significant (P value = 0.7720). For H122A and N130A (without suramin) compared to VSGsur the differences are significant (P<.0113 and P<.0001, respectively). Two independent experiments with different cultures were made for each data point.
Extended Data Fig. 3 | Sample ITC results. ITC data for suramin binding to each VSG protein. The upper panels contain the baseline corrected raw data, and the lower panel contains the peak-integrated, concentration normalized data for the heat of reaction vs. molar ratio of suramin per VSG protein.

a, VSGsur was measured 3 times, independently: 300 µM suramin was titrated into 46 µM VSGsur, the curve fitted with a single binding site model to calculate a Kd of 234+/- 28 nM and N of 0.49 +/- 0.03. b, VSGSur H122A was measured 3 times, independently: 450 µM suramin was titrated into 51.1 µM VSGSur H122A. A Kd could not be fit to the data, although it is clear that the mutation negatively affected the binding affinity. c, VSGSur N130A was measured 2 times, independently: 300 µM suramin was titrated into 40 µM VSGSur N130A. No binding was detected. d, VSG2 was measured 2 times, independently: 200 µM Suramin was titrated into 20 µM VSG2 protein. No binding was detected. e, VSG3 was measured 2 times, independently: 300 µM suramin was titrated into 53.1 µM VSG3. No binding was detected. f, VSG13 was measured 2 times, independently: 200 µM Suramin was titrated into 20 µM VSG132 protein. No binding was detected.
Extended Data Fig. 4 | Two-fold axis of dimer symmetry for VSGsur-suranin complex. a, The top drawing illustrates the chemical structure of suramin with several of its functional groups denoted, whereas the bottom renders the drug as found in the protein structure with a transparent molecular surface shown. Oxygen atoms are shown in red, nitrogen in blue, and carbon gray. b, Ribbon diagram of VSGsur (one monomer blue and the other gold) looking down the two-fold axis of symmetry for the dimer. Suramin is shown as a ball-and-stick chemical representation as in (a). In the center of the rotational axis for the dimer, one of the suramin benzene rings is visible.
Extended Data Fig. 5 | H122 “open” and “closed” conformations. a, Two H122 conformations in the native crystals structure of VSGsur with corresponding electron density (b) Closed conformation electron density of H122 (c) Closed conformation surface of VSGsur (d) Position of H122 and the I3C group used in phasing the crystal structure with corresponding electron density.
**Extended Data Fig. 6 | Creation of VSGsur and VSGsur mutant expressing strains.** The same construct was used multiple times to generate different VSG-expressing cell lines. The top schematic shows the endogenous sub-telomeric expression site in 2T1 cells, which express VSG2 (pink) with its endogenous UTRs (dark green). The vector used to integrate new VSGs was adapted from Pinger et al.\(^2\), wherein the homology directed integration of a novel VSG ORF (black) flanked by the UTRs of VSG2 is mediated by 3’ telomere seeds (Tel-S) and 5’ homology to the upstream co-transposed region (CTR - teal). Transfected cells are identified by screening for the integration of a hygromycin selection cassette (red) that is expressed via read-through transcription driven by the subtelomeric promoter.
Extended Data Fig. 7 | Panels of WT and H122A with suramin soaks. Molecular surface of one monomer in the VSGsur dimer shown colored by electrostatic potential (white is neutral, blue is positive/basic, and red is negative/acidic in charge). Electron density in the suramin binding site is shown in green.
Extended Data Fig. 8 | Resistance enhancing mutations mapped to VSGsur. Ribbon diagrams of VSGsur/suramin co-crystal structure in two orientations. Mutations discovered in “supersur” VSGsur mutants with heightened resistance to suramin are shown as green space filling atoms. Suramin and the N-linked glycan of VSGsur are depicted as space filling atoms in purple and crimson respectively.
Extended Data Fig. 9 | Replacement of VSGsur with “supermutant” VSGsur at the active expression site of *T. b. rhodesiense* STIB900_sur1. (a) Construct for gene replacement; mutations in the VSGsur coding sequence (dark red) are shown in light red, arrows indicate primer binding sites. BSD, blasticidin resistance gene; αβ tub, αβ tubulin splice site. (b) 50% inhibitory concentrations of the transfected clones and the parent (sur1) as measured with Alamar Blue assays. The clones sur1_tr1 and sur1_tr2 still expressed VSGsur, while the clones sur1_tr3 and sur1_tr4 expressed the mutant version. The scatter plots represent independent drug assays, each carried out in duplicate. Error bars represent mean ± standard deviation. Small letters indicate significance groups as determined by one-way ANOVA (Df = 4, F-value = 31.02, p-value = 3.6x10^{-9}), followed by Tukey’s multiple pairwise comparisons test (p-values <0.001) as calculated with R 3.6.2. n = 6 for STIB900_sur1, sur1_tr1, sur1_tr2, and sur1_tr4; n = 5 for sur1_tr3.
Extended Data Fig. 10 | Endocytosis rates by different VSGs relative to VSG2. Alexa 488-dextran (a), bodipy-LDL (b), and Alexa 488-transferrin (c) endocytosis by T. b. brucei 2T1 cells expressing a variety of different VSG genes. Due to technical limitations, each cell line could not be analyzed simultaneously within each experiment for each ligand. Therefore, VSG2 was used as a control in all experiments, allowing the calculation of each cell line’s relative uptake rate of each ligand as compared to the uptake rate of each ligand observed by VSG2 expressing cells within each separate experiment. Each of these graphs therefore represent the combined experimental results from 2 separate experiments. Cell lines with a relative fluorescence intensity below 1 at a given time point have less efficient endocytic rates compared to VSG2 expressing cells, and vice versa. All graphs share the same Y axis.
Reporting Summary

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Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a  Confirmed
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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- For null hypothesis testing, the test statistic (e.g. t, f, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Software and code

Policy information about availability of computer code

Data collection
Crystallographic data collection (beamlines) is described in Supplementary Tables 1-3 and Methods. For ITC, MicroCal PEAK ITC Control Software (Malvern) was used.

Crystallographic software includes iMosflm 7.2.1, XDSAPP, XDS, CCP4 DIALS, POINTLESS, PHENIX 1.18, CCP4 7.1

Data analysis
Crystallographic data collection (software used to process, etc) is described in Supplementary Tables 1-3 and Methods. Data plotting and statistical analyses were performed using Prism (Graphpad). For ITC, MicroCal PEAK ITC Control Software (Malvern) was used.

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Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Coordinates and structure factors have been uploaded to the RCSB PDB (www.rcsb.org): VSGsur I3C (PDB ID 6Z79), VSGsur WT native (PDB ID 6Z7A), VSGsur + Suramin (PDB ID 6Z7B), VSGsur H122A (PDB ID 6Z7C), VSGsur H122A 0.77mM Suramin (PDB ID 6Z7D), VSGsur H122A 7.7 mM (PDB ID 6Z7E), VSG13 NaBr (PDB ID 6Z8G, and VSG13 native (PDB ID 6Z8H).
Field-specific reporting

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size

For internalization assays, the experiments must be performed on living cells with endocytosis inhibited by incubation of ice. It is physically impossible to analyze more than 2 samples per time point per cell line in an appropriate amount of time to conduct a biologically reliable experiment. Therefore the experiments were repeated extensively with comparable results. For cell counting experiments for IC50s and growth curves, 3-5 independent experiments were performed counting hundreds of cells each, except for the highest concentrations of suramin, when so few cells remained, 0-20 were counted per experiment.

For cell growth assays, no sample size calculation was performed. The number of cells after 24h was chosen to be about 1 million cells per ml for a healthy cell culture. For this cell density, about 300 to 400 cells are counted in a hemocytometer (in a volume of 0.3-0.4 μl). For the drug sensitivity assays, culture volumes of 2 ml were used with an initial cell density of 10E5 cells per ml. Growth experiments were performed in T25 culture flasks (Initial culture density 10E5 cells/ml, culture volume 10 ml).

Data exclusions

No data was excluded.

Replication

Experiments were repeated 2-6 times. These repeats reproduced the data as described in the text.

Randomization

N/A

Blinding

N/A

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a

Involved in the study

☐ Antibodies
☐ Eukaryotic cell lines
☐ Palaeontology and archaeology
☐ Animals and other organisms
☐ Human research participants
☐ Clinical data
☐ Dual use research of concern

Methods

n/a

Involved in the study

☐ ChIP-seq
☐ Flow cytometry
☐ MRI-based neuroimaging

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Trypanosoma brucei Lister 427 strain; George Cross Laboratory, Rockefeller University. See also Methods.

T. b. brucei 2T1 from PMID: 16182389

Authentication

RNAseq, CDNA cloning, and antibody binding.

Mycoplasma contamination

Tested and found negative.

Commonly misidentified lines

(See ICLAC register)

No commonly misidentified cell lines were used.
Flow Cytometry

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

| Sample preparation | Internalization assays: Cells are cultured in vitro and harvested in mid-log phase growth (at a cell density of approximately 1 million cells per mL). Details of the experimental set up from this point are described in the methods section. |
|--------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument         | Beckton Dickinson FACS Calibur |
| Software           | Cell Quest Pro |
| Cell population abundance | Cell populations were always comparable in terms of abundance. Cell line identity (VSG expression) was previously verified as described elsewhere. |
| Gating strategy    | FSC and SSC were used to identify the cell population and exclude dead cells and debris. No other gating was performed. Fluorescence intensity was collected and the mean fluorescence intensity of each read is depicted in the graphs. |

☑️ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.