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Peer Review Information

Journal: Nature Microbiology
Manuscript Title: Structure of Trypanosome Coat Protein VSGsur and Function in Suramin Resistance
Corresponding author name(s): Erec Stebbins

Reviewer Comments & Decisions:

Decision Letter, initial version:

Dear Erec,

Thank you for your patience while your manuscript "A Parasite Coat Protein Binds Suramin to Confer Drug Resistance" was under peer-review at Nature Microbiology. It has now been seen by 4 referees, whose expertise and comments you will find at the end of this email. You will see from their comments below that while they find your work of interest, some important points are raised. We are very interested in the possibility of publishing your study in Nature Microbiology, but would like to consider your response to these concerns in the form of a revised manuscript before we make a final decision on publication.

We are committed to providing a fair and constructive peer-review process. Do not hesitate to contact us if there are specific requests from the reviewers that you believe are technically impossible or unlikely to yield a meaningful outcome.

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We hope to receive your revised paper within three weeks. If you cannot send it within this time, please let us know.

We look forward to hearing from you soon.

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Reviewer Expertise:

Referee #1: VSG antigenic variation, trypanosomes
Referee #2: VSG, African trypanosomes
Referee #3: -
Referee #4: X-ray crystallography, suramin structure

Reviewers Comments:

Reviewer #1 (Remarks to the Author):

African trypanosomes are protected by a very dense layer of Variant Surface Glycoprotein (VSG), which shields the parasite from the immune system, and is antigenically varied during the course of an infection. This manuscript by Zeelen et al presents a detailed structural analysis of an unusual VSG variant (VSGsur) which binds the trypanosome drug suramin, thereby conferring drug resistance. This unusual finding is investigated in detail in this study, and the experiments appear to be robust. I think that this study would be found very interesting by the broader microbiology community interested in host pathogen interactions as well as mechanisms of microbial drug resistance.

I think that determining the structures of a large range of different VSGs is a very exciting enterprise, as it helps us understand the interface between the pathogen and the host. I think that probing the structures of these VSGs, and investigating how they can bind different ligands is an exciting area of research. One could hypothesise that VSGs originally evolved from surface receptors involved in nutrient uptake. It is therefore conceivable that at least some of these VSGs can still bind different ligands. VSG binding to suramin is of course an artificial situation, and VSGs would not have evolved to bind it. However, this system still allows probing of different VSG structures, and can give insight into different biological processes operating within trypanosomes. In addition, this represents an unusual, and very interesting drug resistance mechanism. It is striking that selection at higher suramin concentrations produced VSGsur supermutants, as well as some other yet unidentified changes in the selected cells.

I have a few queries rather than criticisms.
Suramin appears to accumulate in the lysosome in VSGsur expressing cells. Is this because the VSGsur-

suramin complex has failed VSG quality control, and the whole complex is targeted for degradation? Alternatively, do the authors think that VSGsur has suramin stripped off of it when it is internalised during recycling and that suramin ends up in the lysosome separate from VSG?

In the first scenario, the VSG half-life of VSGsur should be shorter in the presence of suramin compared with in its absence. Have the authors investigated VSG half-life in these different scenarios? One could speculate that the binding of VSGsur to suramin might affect how it is treated by the VSG recycling machinery and its rate of degradation.

I think that these studies looking at how different ligands bind VSGs could give facinating insight into exactly what is or is not recognised by the VSG quality control mechanism operating during VSG recycling.

The authors mention that there is no defect in rate of trafficking in the VSGsur expressing strains, but I think that rate of trafficking is a separate point from VSG half-life.
A minor point is that I think that the title would be improved if it was a bit more detailed as it is a bit cryptic at the moment. Maybe say what the coat protein is? And the parasite?

Reviewer #2 (Remarks to the Author):

The nice thing about structural biology manuscripts is that the data usually leave little room for interpretation. A crystal is a crystal. In this case it is a co-crystal of a variable surface protein of trypanosomes and suramin, a substance used against the sleeping sickness caused by trypanosomes. In a previous work, it was reported that the expression of a certain VSG is associated with a 90-fold increase in suramin resistance of the parasites. However, the mechanistic basis for this resistance remained unclear. Here, the authors show that VSGsur directly binds suramin. They show that this happens between the monomers and not, as one might assume, in the region of the N-terminal variable loops. Mutations in the area of the contact points of drug and protein lead to loss of binding and resistance. This work also examines and rejects the possibility that an altered endocytosis rate causes suramin resistance. VSGsur, like another VSG reported here, has an unusual structure, which makes it clear that not all VSGs have the same blueprint. Overall, the manuscript describes a fascinating observation. All experiments are logical and very well documented. But of course, there are a few points and questions that the authors might consider.

The hypothesis that VSGsur functions as a suramin sponge is interesting. However, I would invite the authors to provide some simple calculations to estimate if this is theoretically possible. Each cell has about 5x10E06 VSG-dimers, so each cell can bind at most the same amount of suramin. If in theory the order of magnitude were feasible, then it should be possible to titrate the resistance to a threshold value that could be calculated from the cell concentration (ignoring binding constants etc.).

The discussion would benefit from being more precise and less speculative. Speculation is fine if it has a factual basis. Interestingly, there are also alternative binding sites in co-crystals, as well as mutations outside the main binding site, which increased resistance to suramine. The authors say that other "factors" are probably involved here. They speak of "supermutants", which is striking but not really scientific. If a replacement mutation does not yield the hoped-for success, it should not be called a "pseudo-clone", because that too is not scientifically useful.

The kinetic experiments on endocytosis were carried out over a longer period of time, although it is known that the linear measuring range is quite short. The first measurement point of the dextran intake is 10 minutes. Earlier differences in the kinetics are not measured in this way, but this is important for the statement. The method used does not appear to have the resolution required for making sound statements. Therefore, the validity of the data at this point would be questionable.

Reviewer #3 (Remarks to the Author):

The variant surface glycoprotein coat of Trypanosoma brucei has a variety of functions. Key among them are protection of the parasite from attack by the host's immune system and the ability to accommodate other surface proteins such as transporters. In addition to the many VSG genes encoded by trypanosomes, other members of this family have evolved as receptors or as a means of neutralizing host lytic factors.

A number of VSG structures have been determined previously, including by the authors of this paper. Although these share salient features, increasing the number of solved structures has revealed interesting differences that shed useful light on trypanosome biology. In this context, this is not "just one more"; it highlights the flexibility that trypanosomes can achieve in the structure and function of this protein.

In this manuscript the authors determine the structure of a particular VSG, VSGsur, which correlates with increased resistance to the trypanocidal drug suramin. They elegantly show that VSGsur is one member of a VSG sub-family with new features, but it is unique among them in its ability to bind suramin. They also dissect the interactions between the drug and the protein by creating mutants and isolating supermutants, as well as showing (indirectly) that the high level of resistance is not solely due to the VSG, but to additional, as yet unidentified, changes in the original cell line and cell lines that were further selected.

I enjoyed reading this manuscript – as much for its honesty as for the quality of the data. The authors clearly show that suramin binding is required for resistance, but cannot say with certainty how this is achieved. This does not detract from this work, rather it sets the scene for the next chapter.

There are only minor points that need to be addressed:

1.Supplementary figure 1: The schematic drawing is a bit confusing for readers unfamiliar with VSG primary structure. The signal peptide looks huge; the CTD/GPI addition sites are actually separate entities – there is a C-terminal peptide that is cleaved when the GPI is added.

2. p12: Growth of mutants. The sentence beginning "both mutants were viable...." is confusing. I assume that the growth rate of 8.5h refers to the N130A mutant. The growth of H122A is also supposedly slower. There are standard deviations on the graph. Please include these in the population doubling time to show that there is a significant difference.

3. Supplementary figure 4. 130A and 122A. It would be preferable to name them according to the mutation e.g.H122A. The figure legend refers to D130A, text to N130A. I assume the latter is correct,

since it was designed to destroy the glycosylation site.

4. Supplementary figure 8. The new VSG is flanked by 5'UTR sequences on both sides. Is this really the case or should one of them be 3'UTR?

5. Supplementary figure 11. Can the authors explain the 2-3x variation in IC50 for replicates of the same cell line?

Reviewer #4 (Remarks to the Author):

"A Parasite Coat Protein Binds Suramin to Confer Drug Resistance" by Dr Stebbins and colleagues.

Details presented in the main-body of the manuscript, the figure legends, supplementary materials section and in the PDB validation reports clearly indicate that all aspects of the X-ray diffraction data collection/reduction, structure determination and refinement procedures have been conducted correctly. The stereochemistry of the three-dimensional models and are very good and are based on careful interpretation of the electron denisty maps.
The conclusions arrrived at are valid.

Author Rebuttal to Initial comments

Reviewer #1 (Remarks to the Author):

We were heartened by the reviewer's words that our "robust" and "exciting" study "would be found very interesting by the broader microbiology community interested in host pathogen interactions as well as mechanisms of microbial drug resistance."

The reviewer also has several well thought out queries, which we attempt to answer below.

(1) Suramin appears to accumulate in the lysosome in VSGsur expressing cells. Is this because the VSGsur-suramin complex has failed VSG quality control, and the whole complex is targeted for degradation? Alternatively, do the authors think that VSGsur has suramin stripped off of it when it is internalised during recycling and that suramin ends up in the lysosome separate from VSG?

We have reworked considerably our model for how VSGsur binding suramin contributes to resistance. We have dropped the "sponge" description (although still consider it useful) in favor of simply describing how newly synthesized VSGsur could bind internalized suramin from other pathways (e.g., ISG75 and LDL) and prevent its trafficking in the cell (to wherever toxicity is exerted – something not yet established). We show that using known measurements of VSG synthesis and suramin internalization, we can account for the resistance levels (e.g., IC50 values) we are measuring.

In this model, we hypothesize that VSGsur does not lose suramin in its recycling – a combination of the high affinity for the drug and the rapid (less than 1 minute) recycling process likely indicating that it does not contribute significant suramin to the cell (in fact, if it did, as it is 99% of the surface protein, one might expect VSGsur expressing cells to be less resistant to the drug).

We would also note, however, that there are conflicting models of where and how suramin is exerting toxic effects. As we note in the text:

Genetic studies coupled with examination of the trypanosome's single lysosome suggest that suramin accumulates in this compartment, while other evidence links suramin toxicity to effects on the glycosome and impairment of cytokinesis19,20.

And whether suramin accumulates in the lysosome in VSGsur expressing cells remains to be established. Therefore, the lysosome model may or may not have relevance. We try to be open about these uncertainties in the field (how suramin is trafficked and how it exerts its toxicity), and believe our model and observations provide paths to probing some of these unresolved issues.

(2) In the first scenario, the VSG half-life of VSGsur should be shorter in the presence of suramin compared with in its absence. Have the authors investigated VSG half-life in these different scenarios? One could speculate that the binding of VSGsur to suramin might affect how it is treated by the VSG recycling machinery and its rate of degradation.

To date, we have not made any attempts to measure the half-life of VSGsur in trypanosomes.

(3) I think that these studies looking at how different ligands bind VSGs could give facinating insight into exactly what is or is not recognised by the VSG quality control mechanism operating during VSG recycling.

We agree that this opens an entirely new line of inquiry that could prove very fruitful.

(4) The authors mention that there is no defect in rate of trafficking in the VSGsur expressing strains, but I think that rate of trafficking is a separate point from VSG half-life.

We agree that these are distinct, although possibly coupled values. Our primary focus for measuring the rate of trafficking was to address the model that this rate was altered in VSGsur expressing cells and could explain the resistance phenotype by invoking a lower rate of suramin import. Since the mutants that did not bind suramin do not alter these kinetics, we felt that we could rule this model out.

(5) A minor point is that I think that the title would be improved if it was a bit more detailed as it is a bit cryptic at the moment. Maybe say what the coat protein is? And the parasite?

We have changed the title to: “Structure of Trypanosome Coat Protein VSGsur and Function in Suramin Resistance”

Reviewer #2 (Remarks to the Author):

We were happy to note that the reviewer described our manuscript as “fascinating,” “logical,” and “very well documented."

We attempt below to address the reviewer’s points and questions.

(1) The hypothesis that VSGsur functions as a suramin sponge is interesting. However, I would invite the authors to provide some simple calculations to estimate if this is theoretically possible. Each cell has about 5x10E06 VSG-dimers, so each cell can bind at most the same amount of suramin. If in theory the order of magnitude were feasible, then it should be possible to titrate the resistance to a threshold value that could be calculated from the cell concentration (ignoring binding constants etc.).

We thank the reviewer for suggesting we bring more quantitation to our resistance model. Following this recommendation, we have reworked considerably the presentation of this model for how VSGsur binding suramin contributes to resistance (Figure 5 still unchanged, however, as the mechanism is not altered). We have dropped the “sponge” description (although still consider it useful, but potentially confusing as not well-defined) in favor of simply describing how newly synthesized VSGsur could bind internalized suramin from other pathways (e.g., ISG75 and LDL) and prevent its trafficking in the cell (to wherever toxicity is exerted – something not yet established). We show that using known rates of VSG synthesis and suramin internalization, we can account for the resistance levels (e.g., IC50 values) we are measuring. Excerpting this from the new Discussion text:

Such a model is in harmony with the known parameters of VSG production and suramin uptake. For example, cells expressing VSG2 treated with a 200 nM dose of suramin accumulate an intracellular concentration of 1.8 µM suramin within 15 minutes, finally plateauing at a concentration 2-3 fold higher. The production rate of newly-synthesized VSG has been estimated as equivalent to between 20,000 and 80,000 VSG monomers per minute. Therefore, with an estimated cell volume of ~30 cubic microns, we calculate that there is approximately a 5-20 fold excess of newly synthesized VSGsur available to export receptor-internalized suramin at an extracellular drug concentration of 200 nM (Supplementary Methods). Other considerations aside, this would allow for the cell to clear suramin in a range of extracellular concentrations from 1 µM to 4µM, very near our measured IC50 of 8.4 µM. Such “other considerations” of course 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), 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 pathway matches well with what we know about suramin import and VSG dynamics.

A key assumption in this is that the surface-exposed VSGsur binds suramin tightly enough that its net contribution to free suramin in the cell is negligible (in other words, VSGsur is not a suramin *importer*). We believe this assumption is reasonable due to the high affinity measured for suramin binding to VSGsur, the higher concentration inside the cell of suramin than outside (from ISG75 import alone), the fact that the chemical interaction seen in the structure would be predicted to remain strong even if the compartment is acidified, and, perhaps most importantly, because if VSGsur did contribute to suramin uptake, the fact that it is 99% of the surface protein, its impact on suramin levels in the cell would dwarf all other known important mechanisms –

therefore, one would expect VSGsur expressing cells to enhance *susceptibility* to suramin, not resistance. Since this is not the case, but the inverse, we believe it is the unbound, newly- synthesized VSGsur trafficking outward and co-mingling with imported suramin that is the key player in resistance.

(2) The discussion would benefit from being more precise and less speculative. Speculation is fine if it has a factual basis. Interestingly, there are also alternative binding sites in co- crystals, as well as mutations outside the main binding site, which increased resistance to suramine. The authors say that other "factors" are probably involved here. They speak of "supermutants", which is striking but not really scientific. If a replacement mutation does not yield the hoped-for success, it should not be called a "pseudo-clone", because that too is not scientifically useful.

(1) We described the "alternative binding site" for suramin as a "secondary suramin binding mode." We sought to be careful not to state that there was any clear binding of suramin (and therefore a binding site), as we could not definitively interpret any of the additional, non-protein density in-between the dimers in these crystal soaks. We instead focused on what we could see – that H122 was oriented in a fashion incompatible with suramin binding as described in the only ordered structures we obtained. However, the confusion "secondary suramin binding mode" might cause has led us to remove this phase and replace it with "secondary suramin incubation state for VSGsur." We have reworded the second to make clear that we are convinced that there is a single, biologically relevant binding mode:

A secondary suramin incubation state for VSGsur was also discovered in a minority of the crystals. In this state, H122 adopts the other possible conformer seen in the native structure. This conformation we term "closed" for how it reduces the size of the cavity (Supplementary Fig. 7b). In this closed conformation soak with suramin, the drug cannot occupy the same position as in the open conformation due to steric clash. Consistent with this, suramin does not occupy the binding site. We do observe difference density of a size possibly consistent with a molecule of the size of suramin, but it is positioned differently in a more extended conformation. However, the density is too weak to model with confidence and cannot even definitively be assigned to suramin. We hypothesize that as in the native structure, H122 is able to adopt two conformations, but that the open conformation with the stacking of the histidine rings over the suramin benzene groups leads to the better ordered interaction and thus likely represents the only biological binding mode. The fact that ITC experiments show evidence for only one binding site is consistent with this. The closed conformation still leaves room for suramin perhaps to occupy the cavity, however, and the weak additional density observed in such structures may represent a transient state from solution to the stable binding mode.

Therefore, while still speculative, we felt it was important to present these observations, even if high-resolution information is not available for the drug itself in that state. However, its very weak binding and non-existence by ITC argue that it does not have a strong biological effect, which would be consistent with our hypothesis that it is a transient state on the way to the high- occupancy binding.

(2) “Other factors” for mutations outside the binding site of suramin and speculation: We also felt it was important to present these mutations and their spatial context, even if indeed the statements about them potentially being sites for binding other factors is speculative (although generally speaking, we know that such sites must occur somewhere on VSGsur). However, we

do not see any other way to present the data, as simply stating that there are localized patches of mutations in the populations that possess higher resistance to suramin would likely invite questions about why this should be so. We also cannot add more specificity for "other factors" as there is little understood about the trafficking and toxicity of suramin in the cell, but felt a discussion section afforded some leeway to consider these issues. We hope that the reviewer will agree.

(3) "Supermutants": We agree that this is not a precise term, and therefore have removed this word and describe them as "higher resistance mutants" and in similar terms.

(4) "pseudo-clones". We use this expression for clones produced by limiting dilution, as this is not a 100% guarantee for a clonal population (in contrast to "real clones" made by microdrop cloning). Therefore, the term is not referring to "yield of mutants" or the VSG-genotype, but whether the population obtained is purely clonal. Because this term is confusing, we have decided to remove it, describing the clones more precisely.

(3) The kinetic experiments on endocytosis were carried out over a longer period of time, although it is known that the linear measuring range is quite short. The first measurement point of the dextran intake is 10 minutes. Earlier differences in the kinetics are not measured in this way, but this is important for the statement. The method used does not appear to have the resolution required for making sound statements. Therefore, the validity of the data at this point would be questionable.

The reviewer is correct that the dextran measurements don't sample earlier time points. However, our intent with the dextran was more general to show no gross problems in trafficking. We have altered the main body of the text to avoid "overselling" the interpretations derived from this assay:

To interrogate this model, we performed endocytosis experiments with blue dextran and bodipy- labeled LDL (Fig. 4, Methods). Our data show that, consistent with previous results23, there does not appear to be a VSGsur-specific defect in fluid phase endocytosis. Also consistent with previous results23, we do observe that VSGsur cells have a significant decrease in LDL uptake compared to VSG2 (although there is substantial variance among different VSGs, Supplementary Fig. 10).
However, loss-of-binding suramin mutants show no significant 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 the VSG.

However, the key experiments are not the dextran, but the LDL and transferrin (especially the LDL pathway for which previous models have proposed a possible defect in suramin trafficking in VSGsur expressing strains). In those experiments, earlier time points were sampled (specifically, early time points

that are identical to the samplings taken in ref23, which is the manuscript wherein the LDL uptake-suramin resistance model was proposed), establishing that there is no such defect and therefore that the model is not likely explanatory for suramin resistance.

#3 (Remarks to the Author):

The reviewer describes our manuscript as “elegant” and that they enjoyed reading it, in part for its “honesty” in presenting uncertainty in models of suramin toxicity that “sets the scene for the next chapter.” We also strongly agree, if from a biased perspective, that “increasing the number of solved structures has revealed interesting differences that shed useful light on trypanosome biology” and that pursuing structural work on this class of antigenically distinct surface proteins “is not ‘just one more’; it highlights the flexibility that trypanosomes can achieve in the structure and function of this protein."

Here we aim to address the “minor points” raised.

(1) **Supplementary figure 1: The schematic drawing is a bit confusing for readers unfamiliar with VSG primary structure. The signal peptide looks huge; the CTD/GPI addition sites are actually separate entities – there is a C-terminal peptide that is cleaved when the GPI is added.**

These points would indeed better the figure, and we have adopted the reviewer’s suggestion. We have lengthened the NTD and CTD regions, shrunk the size of the signal sequence, and added a far C-terminal segment to indicate the portion cleaved upon GPI addition.

(2) **p12: Growth of mutants. The sentence beginning “both mutants were viable....” is confusing. I assume that the growth rate of 8.5h refers to the N130A mutant. The growth of H122A is also supposedly slower. There are standard deviations on the graph. Please include these in the population doubling time to show that there is a significant difference**.

We agree that the sentence was poorly worded and confusing. It now reads:

“Fig. 2 summarizes these results. Both mutants were viable and grew in the absence of the drug, although each evinced a lower growth rate with doubling times of 8.9h ± 0.73 (N130A) and 6.9h ± 0.18 (H122A) as compared to 6.5h ± 0.35 for the wild type (values showing the standard deviation, n=6, see also Supplementary Fig. 4).”

To compare the curves statistically, we have added the following to the supplementary figure legend:

“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 D130A (without suramin) compared to VSGsur the differences are statistically significant (*P*<.0113 and *P*<.0001, respectively).”

(3) **Supplementary figure 4. 130A and 122A. It would be preferable to name them according to the mutation e.g.H122A. The figure legend refers to D130A, text to N130A. I assume the latter is correct, since it was designed to destroy the glycosylation site.**

We apologize for this omission and have updated the mutant names of this figure to match those in the rest of the manuscript.

(4) **Supplementary figure 8. The new VSG is flanked by 5'UTR sequences on both sides. Is this really the case or should one of them be 3'UTR?**

This mistake has been corrected. (It should be 3'UTR).

(5) **Supplementary figure 11. Can the authors explain the 2-3x variation in IC50 for replicates of the same cell line?**

The original set of drug sensitivity assays was carried out using a 1:3 dilution scheme. This allowed to test a wider range of drug concentrations but resulted in a poorer resolution and high variation. We have repeated all the drug sensitivity tests with a 1:2 dilution scheme, at least five times, each in duplicate. This resulted in a lower variation and lower p-values ($p<0.001$; new Figure S11). The conclusion remains the same, i.e. the mutations in VSGsur further decrease suramin sensitivity.

Reviewer #4 (Remarks to the Author):

The reviewer found that "details presented in the main-body of the manuscript, the figure legends, supplementary materials section and in the PDB validation reports clearly indicate that all aspects of the X-ray diffraction data collection/reduction, structure determination and refinement procedures have been conducted correctly. The stereochemistry of the three- dimensional models and are very good and are based on careful interpretation of the electron denisty maps. The conclusions arrived at are valid."

We thank the reviewer for assessing the quality of the structural work and do not see any further points we need to address.

Decision Letter, first revision:

Dear Erec,

Thank you for your patience while your manuscript "Structure of Trypanosome Coat Protein VSGsur

and Function in Suramin Resistance" was under peer review at Nature Microbiology. It has now been seen by our referees, and in the light of their advice I am delighted to say that we can in principle offer to publish it. First, however, we would like you to revise your paper to address the minor points made by reviewer #3, and to ensure that it is in Nature Microbiology format.

Editorially, we will need you to make some changes so that the paper complies with our Guide to Authors at http://www.nature.com/nmicrobiol/info/gta.

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I appreciate this email is long and recommend that you print it and use it as a checklist, reading it carefully to the end, in order to avoid delays to publication down the line.

Please note that we will be considering your paper for publication as an ARTICLE in our pages.

Specific points:
In particular, while checking through the manuscript and associated files, we noticed the following specific points which we will need you to address:

1. Main text display items and supplementary information. Please note that we have recently started publishing additional figures as "Extended Data". These figures appear online in the html version of the manuscript in the place they are referred to and greatly increase discoverability of the data that is presented in them.
All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories: EXTENDED DATA (ED); SUPPLEMENTARY INFORMATION (SI); and SOURCE DATA. Below are detailed instructions on how to format each category. For your paper, we suggest that you do the following:

a. Main figures: please maintain the current 5 main figures that illustrate the main findings of the paper.

b. Extended data (ED): please convert 10 SI figures into ED figures. These are an integral part of the paper (presented online in the online version) and are meant to be multipanel A4 size figures. More information on file formats and how the legends should be supplied can be found below and in the attached Inventory of Supporting Information.

c. Supplementary information (SI): your study will have the 'Supplementary online data' as SI. Please submit all SI as a separate pdf file. All supplementary materials need to be assembled into a single file, including all tables (excluding those that are excessively large). In the Supplementary Information file, figure legends should be immediately below each figure and the pages should be numbered.

d. Source data: this format should be used to display source data linked to the main figures and ED figures.
We strongly encourage you include as much additional raw data underlying the graphs in the main and ED figures as possible. These data should be supplied as Excel tables, one file per main or ED figure, and should be clearly labeled and presented in a way that individual experiments are identifiable (for example, across a time course if applicable).

2. Data Availability statement. The data availability statement should clearly refer to all of the source data provided in the manuscript (more instructions on how to write this section can be found in the general formatting guidelines below). Please ensure that datasets deposited in public repositories are now publicly accessible, and that accession codes or DOI are provided in the "Data Availability" section. As long as these datasets are not public, we cannot proceed with the acceptance of your paper.

3. Reporting checklist. Please revise this document according to the instructions found in the annotated PDF attached to this message and send in a final version with your article. The final reporting checklist will be published with your manuscript.

4. Competing interest statement. The competing interest statement needs to be included in the manuscript text (before or after the Acknowledgements).

5. ORCID. We now require corresponding authors to provide an ORCID identifier, and would ask that you please provide one with your final submission (please also see below). There is a step during the upload of the information to our online system in which the number can be introduced.

6. Replicates and statistics. While carefully checking the figures, we noted a few things that need to be revised so that they comply with our style guidelines and accurately report on the number of replicates, statistical testing, etc. As general rules, please note that:

General comments:

Wherever statistics have been derived (e.g., error bars, box plots, statistical significance), the legend needs to provide and define the n number (i.e., the sample size used to derive statistics) as a precise value (not a range), using the wording "n=X biologically independent samples/animals/independent experiments," etc. as applicable.

All error bars need to be defined in the legends (e.g., SD, SEM) together with a measure of centre (e.g. mean, median), and should be accompanied by their precise n number defined as noted above.

All violin plots need to be defined in the legends in terms of minima, maxima, centre, and percentiles, and should be accompanied by their precise n number defined as noted above.

The figure legends must indicate the statistical test used and if applicable, whether the test was one- or two-sided. A description of any assumptions or corrections such as tests of normality and adjustment for multiple comparisons must also be included.

For null hypothesis testing, please indicate the test statistic (e.g., F, t, r) with confidence intervals, effect sizes, degrees of freedom and P values noted.

Test results (e.g., p-values, q-values) should be given as exact values whenever possible and appropriate, and confidence intervals noted.

Please indicate how estimates of effect sizes were calculated (e.g., Cohen's d, Pearson's r).

Please state in the legends how many times each experiment was repeated independently with similar results. This is needed for all experiments but is particularly important wherever representative experiments are shown. If space in the legends is limiting, this information can be included in a section titled "Statistics and Reproducibility".

For all bar graphs, the corresponding dot plot must be overlaid.

Specific comments to address:

Please see the attached "Extended_comments" file.

General points:

Please read carefully through all of the following general formatting points when preparing the final version of your manuscript, as submitting the manuscript files in the required format will greatly speed the process to final acceptance of you work.

Titles should give an idea of the main finding of the paper and ideally not exceed 90 characters (including spaces). We discourage the use of active verbs and do not allow punctuation.

The paper's summary paragraph (about 150-200 words; no references) should serve both as a general introduction to the topic, and as a brief, non-technical summary of your main results and their implications. It should start by outlining the background to your work (why the topic is important) and the main question you have addressed (the specific problem that initiated your research), before going on to describe your new observations, main conclusions and their general implications. Because we hope that scientists across the wider microbiology community will be interested in your work, the first paragraph should be as accessible as possible, explaining essential but specialised terms concisely. We suggest you show your summary paragraph to colleagues in other fields to uncover any problematic concepts.

We strongly support public availability of data. Please place the data used in your paper into a public

data repository, if one exists, or alternatively, present the data as Source Data or Supplementary Information. If data can only be shared on request, please explain why in your Data Availability Statement, and also in the correspondence with your editor. For some data types, deposition in a public repository is mandatory - more information on our data deposition policies and available repositories can be found at https://www.nature.com/nature-research/editorial-policies/reporting-standards#availability-of-data.

Please include a data availability statement as a separate section after Methods but before references, under the heading "Data Availability". This section should inform readers about the availability of the data used to support the conclusions of your study. This information includes accession codes to public repositories (data banks for protein, DNA or RNA sequences, microarray, proteomics data etc…), references to source data published alongside the paper, unique identifiers such as URLs to data repository entries, or data set DOIs, and any other statement about data availability. At a minimum, you should include the following statement: "The data that support the findings of this study are available from the corresponding author upon request", mentioning any restrictions on availability. If DOIs are provided, we also strongly encourage including these in the Reference list (authors, title, publisher (repository name), identifier, year). For more guidance on how to write this section please see:
http://www.nature.com/authors/policies/data/data-availability-statements-data-citations.pdf

Please supply the figures as vector files - EPS, PDF, AI or postscript (PS) file formats (not raster or bitmap files), preferably generated with vector-graphics software (Adobe Illustrator for example). Try to ensure that all figures are non-flattened and fully editable. All images should be at least 300 dpi resolution (when figures are scaled to approximately the size that they are to be printed at) and in RGB colour format. Please do not submit Jpeg or flattened TIFF files. Please see also 'Guidelines for Electronic Submission of Figures' at the end of this letter for further detail.
Please view http://www.nature.com/authors/editorial_policies/image.html for more detailed guidelines.

We will edit your figures/tables electronically so they conform to Nature Microbiology style. If necessary, we will re-size figures to fit single or double column width. If your figures contain several parts, the parts should be labelled lower case a, b, and so on, and form a neat rectangle when assembled.

Please check the PDF of the whole paper and figures (on our manuscript tracking system) VERY CAREFULLY when you submit the revised manuscript. This will be used as the 'reference copy' to make sure no details (such as Greek letters or symbols) have gone missing during file-transfer/conversion and re-drawing.

All Supplementary Information must be submitted in accordance with the instructions in the attached Inventory of Supporting Information, and should fit into one of three categories:

1. EXTENDED DATA: Extended Data are an integral part of the paper and only data that directly contribute to the main message should be presented. These figures will be integrated into the full-text HTML version of your paper and will be appended to the online PDF. There is a limit of 10 Extended Data figures, and each must be referred to in the main text. Each Extended Data figure should be of the same quality as the main figures, and should be supplied at a size that will allow both the figure and legend to be presented on a single legal-sized page. Each figure should be submitted as an

individual .jpg, .tif or .eps file with a maximum size of 10 MB each. All Extended Data figure legends must be provided in the attached Inventory of Accessory Information, not in the figure files themselves.

2. SUPPLEMENTARY INFORMATION: Supplementary Information is material that is essential background to the study but which is not practical to include in the printed version of the paper (for example, video files, large data sets and calculations). Each item must be referred to in the main manuscript and detailed in the attached Inventory of Accessory Information. Tables containing large data sets should be in Excel format, with the table number and title included within the body of the table. All textual information and any additional Supplementary Figures (which should be presented with the legends directly below each figure) should be provided as a single, combined PDF. Please note that we cannot accept resupplies of Supplementary Information after the paper has been formally accepted unless there has been a critical scientific error.

All Extended Data must be called you in your manuscript and cited as Extended Data 1, Extended Data 2, etc. Additional Supplementary Figures (if permitted) and other items are not required to be called out in your manuscript text, but should be numerically numbered, starting at one, as Supplementary Figure 1, not SI1, etc.

3. SOURCE DATA: We strongly encourage you to provide source data for your figures whenever possible. Full-length, unprocessed gels and blots must be provided as source data for any relevant figures, and should be provided as individual PDF files for each figure containing all supporting blots and/or gels with the linked figure noted directly in the file. Numerical source data that underlie graphs are required for in vivo experiments and strongly encouraged generally. They should be provided in Excel format, one file for each relevant figure, with the linked figure noted directly in the file. They should be clearly labelled such that individual experiments and/or animals are labelled (for example, across a time course if applicable). For imaging source data, we encourage deposition to a relevant repository, such as figshare (https://figshare.com/) or the Image Data Resource (https://idr.openmicroscopy.org).

Please ensure that you retain unprocessed data and metadata files after publication, ideally archiving data in perpetuity, as these may be requested during the peer review and production process or after publication if any issues arise.

Please include any references for the Methods at the end of the reference list. Any citations in the Supplemental Information will need inclusion in a separate SI reference list.

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Finally, we require authors to include a statement of their individual contributions to the paper -- such as experimental work, project planning, data analysis, etc. -- immediately after the acknowledgements. The statement should be short, and refer to authors by their initials. For details please see the Authorship section of our joint Editorial policies at http://www.nature.com/authors/editorial_policies/authorship.html

We will not send your revised paper for further review if, in the editors' judgement, the referees'

comments on the present version have been addressed. If the revised paper is in Nature Microbiology format, in accessible style and of appropriate length, we shall accept it for publication immediately.

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* Extended Data & Supplementary Information, as instructed

* a point-by-point response to any issues raised by our referees and to any editorial suggestions.

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We hope that you will support this initiative and supply the required information. Should you have any query or comments, please do not hesitate to contact me.

We hope to hear from you within two weeks; please let us know if the revision process is likely to take longer.

****

Reviewer Expertise:

Referee #1: VSG antigenic variation, trypanosomes
Referee #2: VSG, African trypanosomes

Referee #3: -
Referee #4: X-ray crystallography, suramin structure

Reviewer Comments:

Reviewer #1 (Remarks to the Author):

I felt that the authors responded well to the issues that were raised. I think that it will be a nice submission for Nature Microbiology.

Reviewer #2 (Remarks to the Author):

The authors have addressed all questions raised. The paper is now ready for publications. Well done!

Reviewer #3 (Remarks to the Author):

As reviewer 3 I raised some minor points, not all of which have been addressed completely. It should be trivial to fix these.

1. The schematic diagram has been modified.

2. Growth of mutants: can it really be said that mutant H122A grows more slowly than the parental line, given the standard deviations? These, together with the growth curve in Fig S4 make me doubt this. There is, undeniably, a difference in the presence of suramin.

3. Corrected as asked.

4. Fig S8. Two out of three have been corrected to 3'UTR. One is still wrongly labelled.

5. Explained.

Reviewer #4 (Remarks to the Author):

My initial evaluation of the crystal structure determination, refinement and analysis indicated that the experiments were well conducted.
I have no additional comments regarding the crystallographic aspects presented in the manuscript.

Final Decision Letter:

Dear Erec,

I am pleased to accept your Article "Structure of Trypanosome Coat Protein VSGsur and Function in Suramin Resistance" for publication in Nature Microbiology. Thank you for having chosen to submit your work to us and many congratulations.

Before your manuscript is typeset, we will edit the text to ensure it is intelligible to our wide readership and conforms to house style. We look particularly carefully at the titles of all papers to ensure that they are relatively brief and understandable.

The subeditor may send you the edited text for your approval. Once your manuscript is typeset you will receive a link to your electronic proof via email within 20 working days, with a request to make any corrections within 48 hours. If you have queries at any point during the production process then please contact the production team at rjsproduction@springernature.com. Once your paper has been scheduled for online publication, the Nature press office will be in touch to confirm the details.

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