Metabolomic analysis of *T. congolense* treated with isometamidium chloride and *in silico* modeling of potential drug targets

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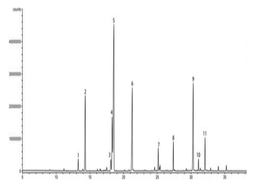
INTRODUCTION

- Animal African Trypanosomiasis (AAT) is a serious and fatal parasitic disease in Africa that is transmitted by *Glossina* species.
- AAT is caused by various *Trypanosoma* species including *T. congolense*, *T. vivax*, and *T. brucei*[1]. The disease affects about 50 million animals and causes losses of about 4.5 million USD p.a[1].
- Six compounds are licensed for treatment of AAT and the most commonly used are diminazene aceturate (DZ) – first-line drug, isometamidium chloride (**ISM**) – for prophylaxis and treatment, and homidium salts.
- Control against AAT is heavily dependent on chemotherapy with ~ 35 mil. doses/yr administered to prevent progression in endemic areas[2]. However, efforts to eliminate AAT have been hindered by resistance, toxicity, poor efficacy, and limited knowledge or lack thereof of the mechanism of action (MOA) of existing drugs.

OBJECTIVES

- To investigate how **ISM** impacts the metabolome of *T. congolense* and identification of potential drug targets.
- To infer the interaction mechanisms of **ISM** against identified drug targets through *in silico* modeling.

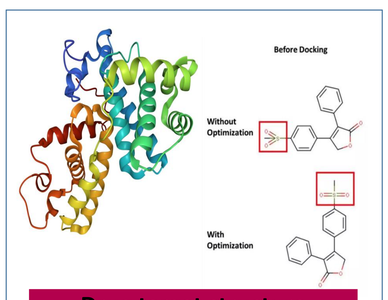
METHODS



Untargeted metabolomics profiling of *T. congolense* parasites; Treated and untreated samples

Statistical and metabolomic analysis to identify affected pathways so as to narrow down on possible targets

- Normalisation; log transformation, autoscaling
- PCA, One-way ANOVA with Tukey's HSD *post hoc*
- Volcano plot analysis
- Clustering heatmap



Drug target structure prediction using AlphaFold 2 and refinement using GalaxyRefine

Ligand retrieval from PubChem and optimization using OpenBabel

Molecular docking and visualisation

CONCLUSIONS

- Statistical and metabolomic analysis identified possible competitive inhibition of the *T. congolense* glucose transporter which was further evidenced in the molecular docking assay where isometamidium chloride interacted with the same substrate binding site as glucose.
- Owing to the lower energy of binding displayed by isometamidium chloride it is possible that isometamidium chloride has a higher competitive affinity for TcoHT1 compared to glucose.

IMPACT

- Metabolomic and molecular docking analyses show that **ISM** impacts the metabolome of *T. congolense* by interacting with the substrate binding site of the glucose transporter.
- This study identified the potential drug target for **ISM**. These findings provide new insights into the mechanism of action of **ISM** and suggest avenues for future drug development efforts.

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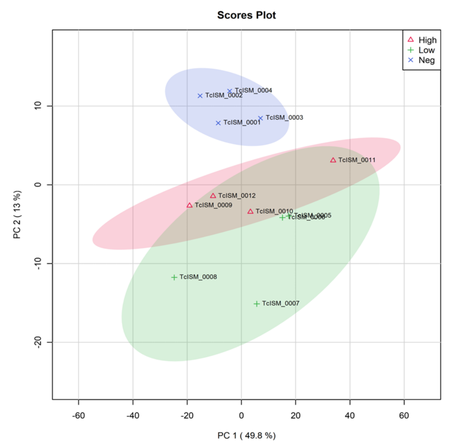
RESULTS

1. Does ISM impact the metabolome of *T. congolense*?

a.



b.

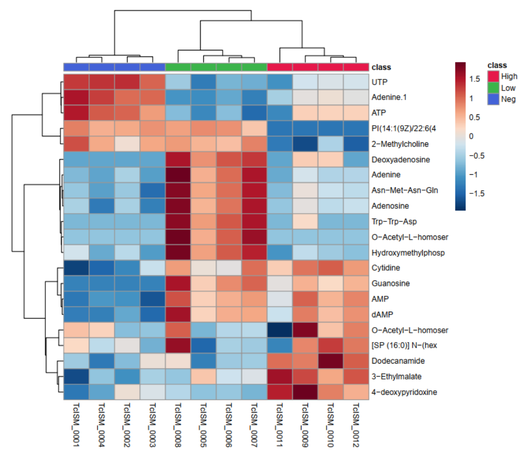


Figure 1: a. Treated samples clustered closely on the Principal Component Analysis (**PCA**) plot where 49.8% variance was observed between the first 2 principal components. **b.** Clustering heatmap of dysregulated metabolites showing distinct clustering between the control and treatment groups.

2. Can metabolomic analysis aid the identification of ISM drug targets? Why do NTPs decrease and NMPs and un-phosphorylated nucleosides increase?

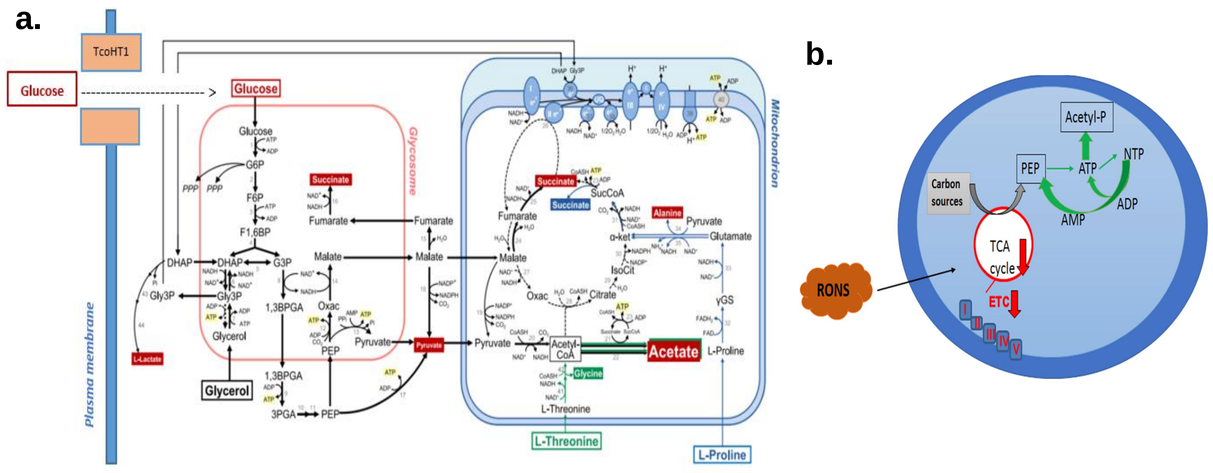


Figure 2: **a.** Classic glycolysis in PCF *T. brucei* that is hypothesized to resemble BSF *T. congolense*[3]. **b.** The nucleotide phosphorelay system in *Pseudomonas fluorescens*[4].

3. Interaction of ISM and the T. congolense glucose transporter (TcoHT1)

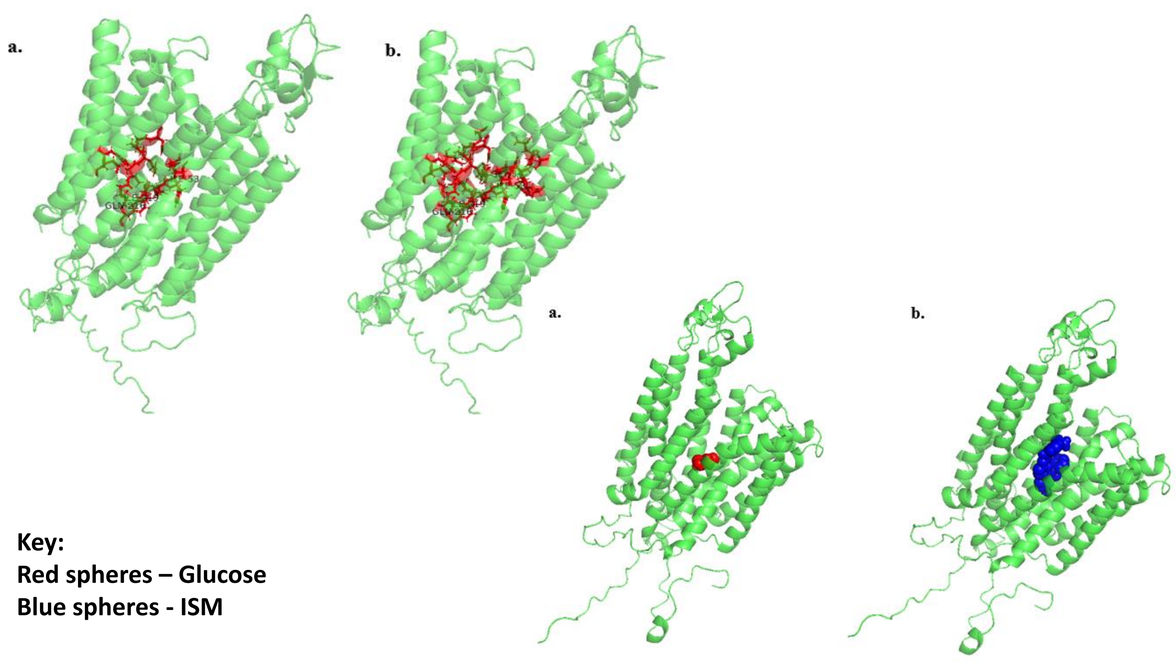


Figure 3: a. Interactions of glucose with the predicted *T. congolense* glucose transporter (TcoHT1)(-5.7 kcal/mol). **b.** Interactions of **ISM** with the predicted *T. congolense* glucose transporter (TcoHT1) (-10.1 kcal/mol).

ACKNOWLEDGEMENT

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