Targeting protein aggregates: a novel strategy for malaria treatment

In collaboration with: "la Caixa" Foundation

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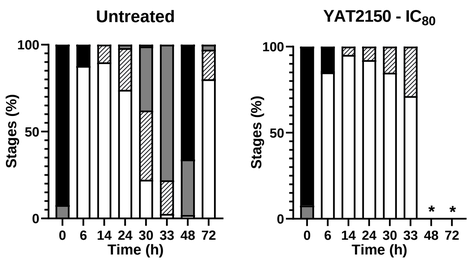
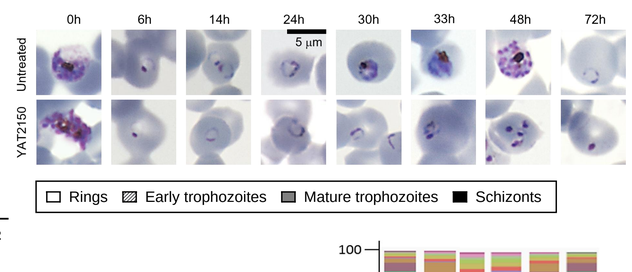
INTRODUCTION

The rapid evolution of **drug resistance** in *Plasmodium* is rendering current antimalarial treatments ineffective [1]. Consequently, new drugs that exploit previously **untargeted weaknesses** in the pathogen are urgently required.

Protein aggregation is a prominent feature across all life stages of the malaria parasite, and its disruption has been observed to impair the viability of *P. falciparum* [2], suggesting a critical, yet poorly understood, role in the parasite's biology.

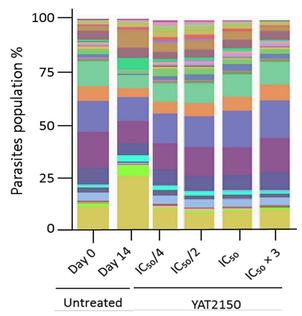
The recently developed **bis(styrylpyridinium) salt YAT2150**, which targets protein aggregates [2], exhibits potent antiplasmodial activity in the two-digit nanomolar range, even demonstrating efficacy against drug-resistant strains.





As **no *in vitro* resistance** has emerged against the compound [3], a combination of pull-down assays coupled with proteomic analysis and transcriptomic analysis are being employed to identify specific proteins affected by YAT2150.

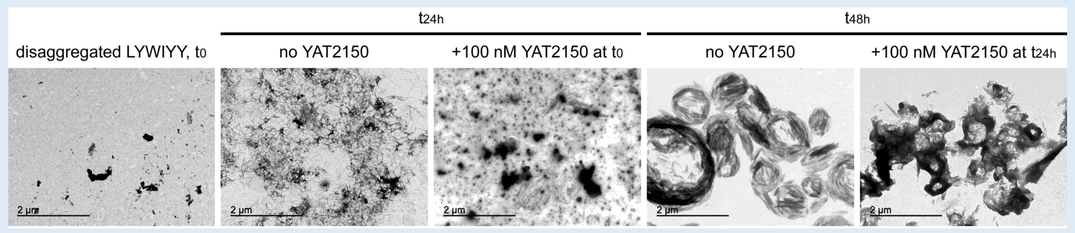
Parasite population at day 14 of treatment with YAT2150 [3].



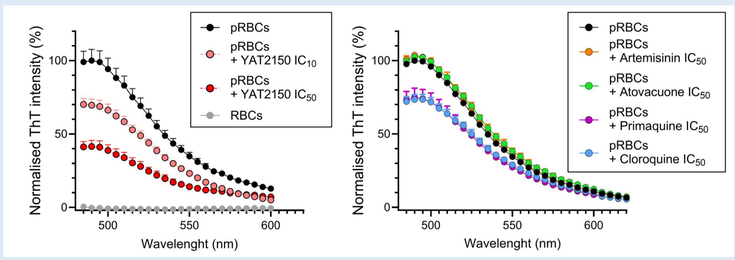
PROTEIN AGGREGATION AS A TARGET

- **Alteration of protein homeostasis**

YAT2150 impairs protein aggregation both in live cultures and in isolated amyloid-like peptides of *P. falciparum*, inhibiting the formation of structured aggregates.

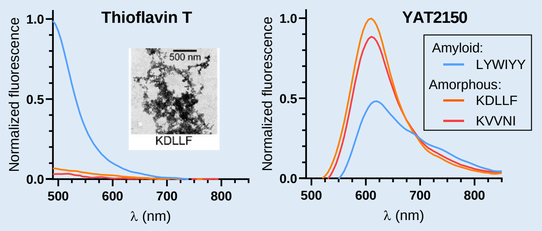


This effect has also been observed with **other antimalarials** of aminoquinoline nature, despite having a widely different chemical structure.



- **Non amyloid-like aggregates.**

YAT2150 also shows **binding affinity** towards disordered forms of aggregation.



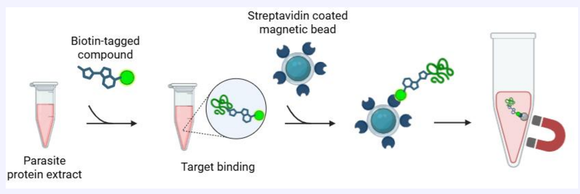
UNDERSTANDING THE MODE OF ACTION OF YAT2150

1 Pull-down assays

Biochemical method in which a small molecule is immobilized on a solid matrix (e.g. magnetic bead), incubated with a protein lysate, and **specifically interacting proteins are captured**, eluted, and subsequently identified by mass spectrometry-based proteomic analysis.

1st. Design of the chemical probes ➡ 2 approaches (*Ongoing*)

a



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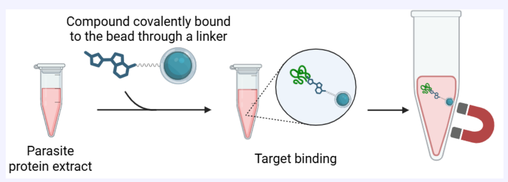
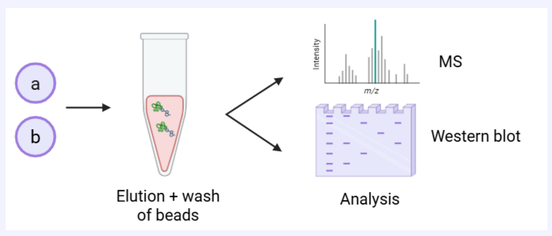
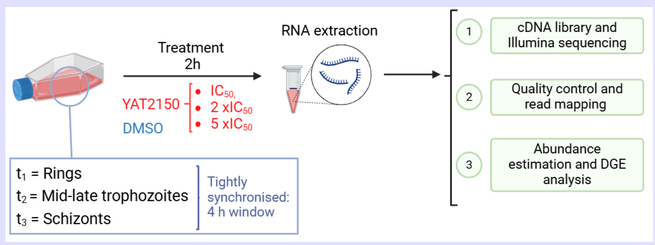


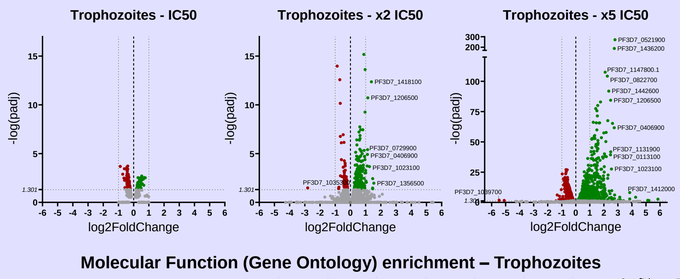
2nd. Pull down analysis after elution of the beads.

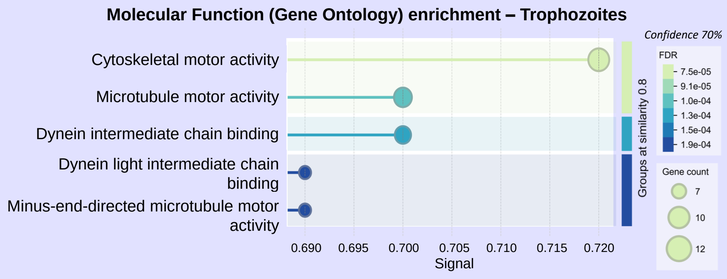


2 Transcriptomic approach

Transcriptomic profiling after treatment with a drug has been described to **successfully pinpoint the specific targets of several compound** [4, 5].



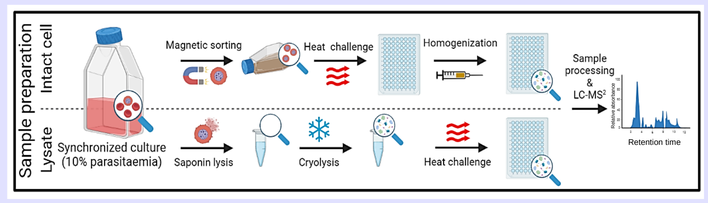


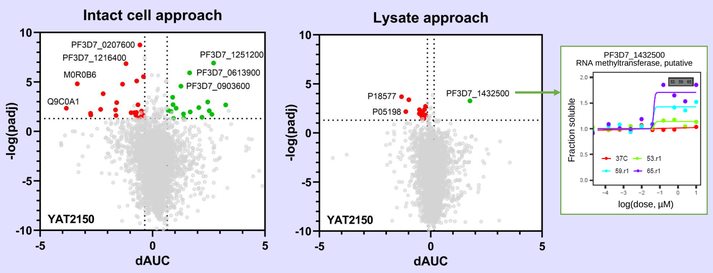


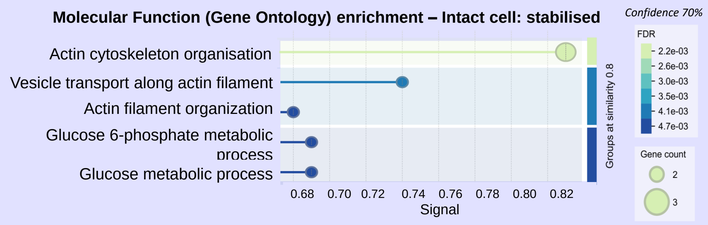
Preliminary transcriptomic results indicate that YAT2150 treatment **dysregulates multiple pathways**, however, no clear target can be singled out yet.

3 Proteomic approach: CETSA

The Cellular Thermal Shift Assay (CETSA) is a proteomic technique employed for drug target identification by detecting **compound-induced alterations in protein thermal stability** through solubility profiling across a temperature gradient.







CETSA shows preliminary results further indicating the **dysregulation of multiple pathways.** ➔ Validate the experiment with YAT2150 analogues.

CONCLUSIONS AND FUTURE WORK

- YAT2150 emerges as a promising first-in-class antimalarial drug, with a fast acting mechanism of action.
- Preliminary results show the **dysregulation of multiple targets**, consistent with the compound's presumed mode of action, and which would hamper the evolution of resistance through point mutations.
- The most immediate steps include the **experimental validation** of the preliminary results, using YAT2150 and its derivatives.
- Once potential targets are narrowed down, their **functional validation** will be carried out ➔ Knockout and truncated parasite lines.
- A **transversal analysis looking at protein structural properties and motifs**, such as the presence of **disordered regions**, will be carried out to further analyse the results obtained and try to understand YAT2150's mechanism of action.

REFERENCES

[1] B. Blasco *et al.* 2017. *Nat. Med.* 23(8):917; [2] I. Bouzón-Arnáiz *et al.* 2022. *BMC Biol.* 20(1):197; [3] I. Bouzón-Arnáiz *et al.* 2025. *Sci Rep.* 15(1):2941; [4] P.J. Shaw *et al.* 2015. *BMC Genomics*.16:830; [5] G. Hu *et al.* 2010. *Nat Biotechnol*. 28(1):91-8.

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