

# Elucidating antimalarial drug targets/mode-of-action by application of systems biology technologies

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## INTRODUCTION

Malaria is one of the world's most important tropical diseases, killing more than a million of an estimated 300-500 million infected people per annum. The species responsible for the most fatalities amongst the genus is *Plasmodium falciparum*. Eradication efforts are hampered by two major drawbacks - the absence of an effective vaccine coupled with the widespread occurrence of drug-resistant strains to frontline antimalarials and, of late, the emergence of resistance to current antimalarials of choice. Therefore, a pressing need has arisen for the urgent development of new frontline antimalarial drugs. Of equal importance is an understanding of their molecular targets/modes-of-action (MoA). Thus, identifying drug targets/MoA of known and novel antimalarials remains a high priority in the light of developing drugs to which no resistance amongst the parasite population exists.

In this regard, we chose the polyamine metabolic pathway in *P. falciparum* as a proof of concept project, to validate the pathway as a drug target and elucidate the MoA of cyclohexylamine in *P. falciparum* 3D7. Drug effects were examined through morphological characterisation of the parasite's life cycle, as well as transcriptomic and proteomic analyses.

## OBJECTIVE

The objective of this study was to use systems biology tools to unravel the drug target/mode-of-action (MoA) of an antimalarial drug (cyclohexylamine) with a known drug target/MoA, by analysing differential expression profiles of drug treated vs untreated cultures at the gene and protein level. If drug target/MoA prediction is successful, the approach will be applied to known as well as new drug leads in development.

## PROCEDURE

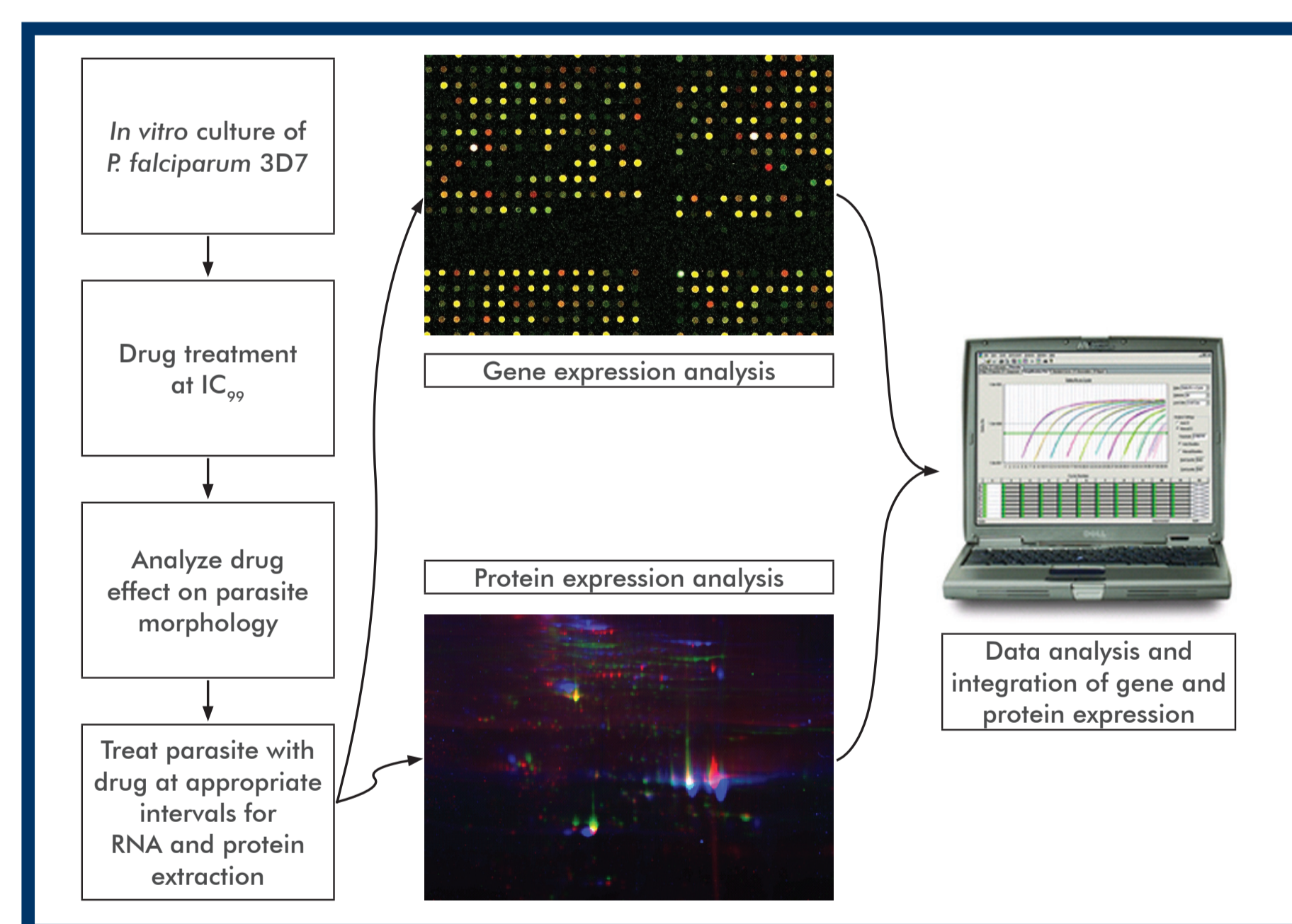


Figure 1: Experimental workflow for unravelling cyclohexylamine drug target/MoA. Briefly, parasites are treated with the drug to estimate at what life cycle stage the drug affects the parasite and estimate the resultant morphological changes the treatment causes. This data then informs drug treatments for expression analyses

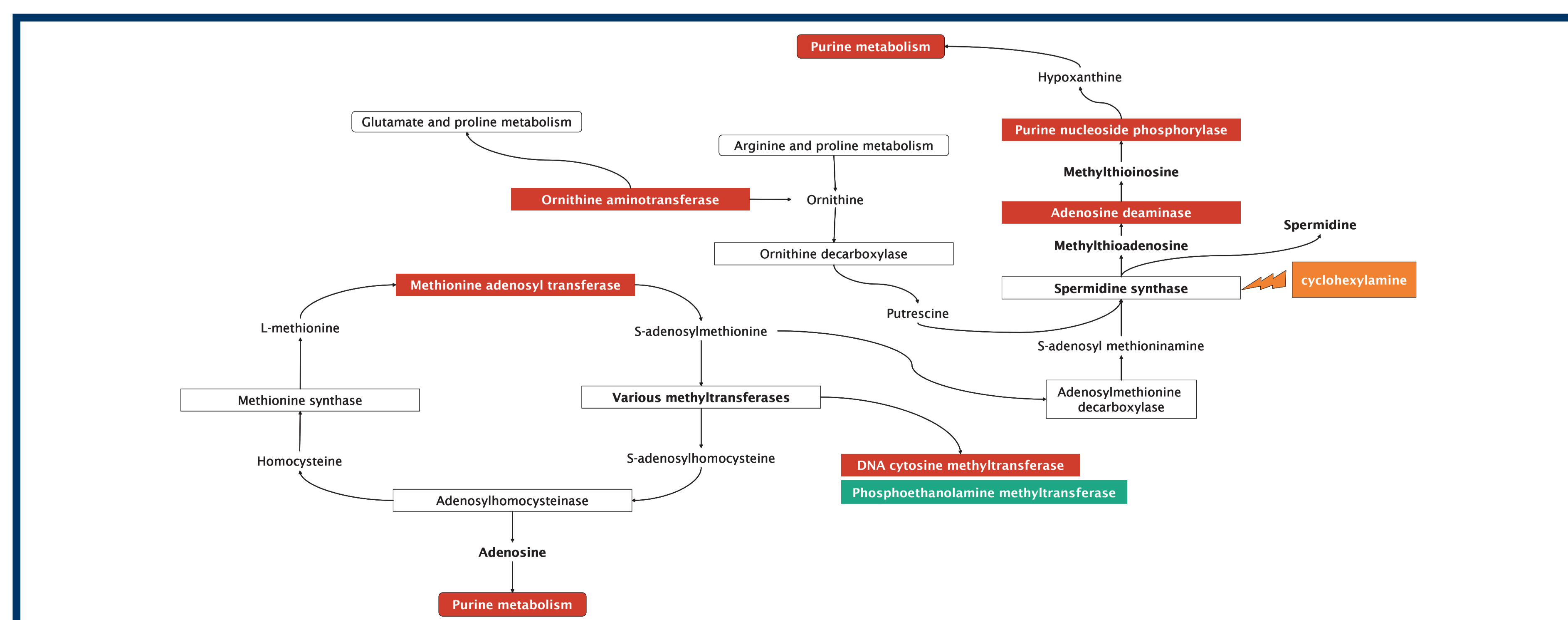


Figure 3: A representation of the polyamine pathway and the genes and proteins affected by cyclohexylamine treatment. Clearly indicated is the molecular target of cyclohexylamine, spermidine synthase. Inhibition of this enzyme by the antimalarial results in differential expression of various genes and proteins associated with the pathway. Clearly, enzymes directly following spermidine synthase, as well as associated downstream pathways, were affected by the treatment. The tools utilised may now be considered for use in predicting drug targets of known and novel antimalarials in development, in order to circumvent resistance

## RESULTS

### Morphology studies

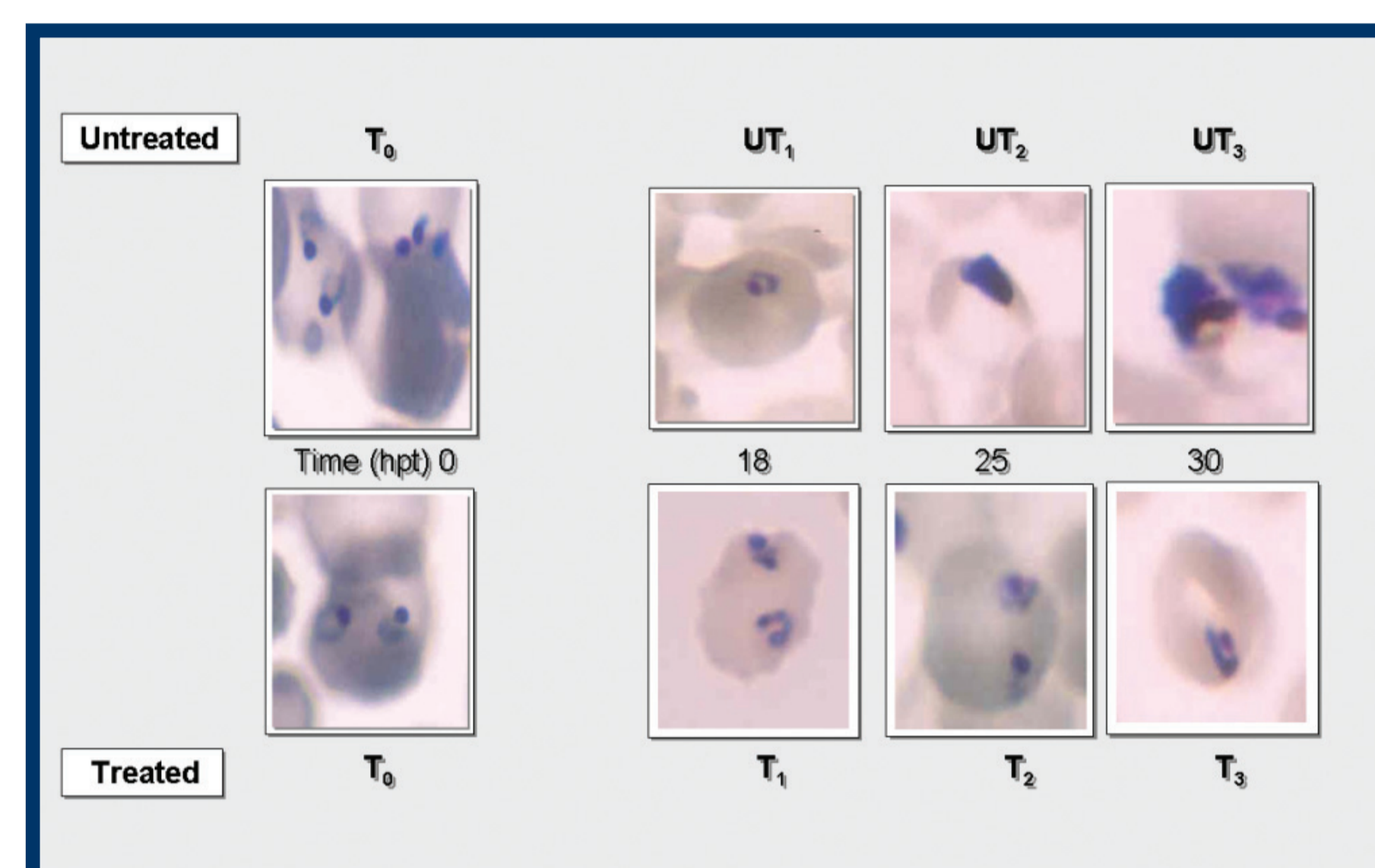


Figure 2: Young ring parasites ( $T_0$ ) were incubated in the presence (Treated, T) and absence (Untreated, UT) of 200mM cyclohexylamine at 37°C for 48 hrs with smears prepared every 6 hrs. Giemsa stained smears were viewed under the light microscope. Top and bottom panels show parasites harvested 18, 25 and 30 hrs post drug treatment (hpt). Parasites treated with the drug were clearly inhibited at the trophozoite phase. Treated parasites were therefore always compared to untreated parasites in the same life cycle stage (ie. 18 hrs post treatment)

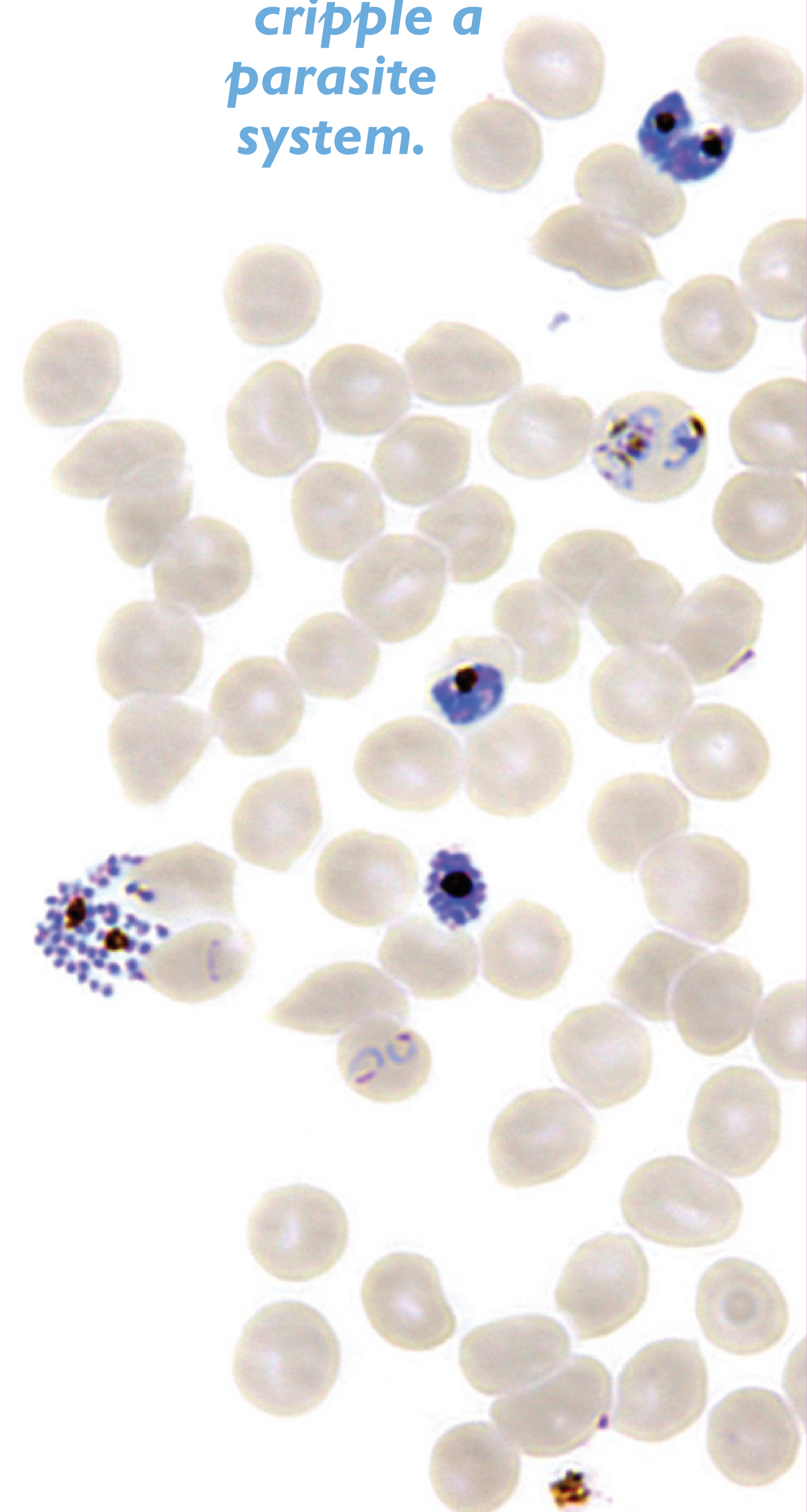
## CONCLUSION

Despite a number of limitations of the two-dimensional gel and DNA microarray technologies we have successfully identified and validated the polyamine metabolic pathway as a target of cyclohexylamine in *P. falciparum*. DNA microarray results also correlated well with proteomics data. Based on these results we have decided to apply this approach to identify drug targets/MoA of other known drugs as well as new drug leads. This work represents an important aspect of drug discovery/development since drugs with new drug targets/modes-of-action (MoA) are urgently needed.

## ACKNOWLEDGEMENTS

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Using systems biology approaches, researchers are able to pinpoint the metabolic pathway a drug will alter to cripple a parasite system.



Pictured above: Different developmental stages the malaria parasite undergoes through its intra-erythrocytic life cycle