Inhibition of *Plasmodium falciparum* spermidine synthase indicates perturbation-specific effects in the transcriptome and proteome

J Becker‡, L Mtwisha‡, B Crampton‡, S Stoychev‡, A van Brummelen‡, A Louw※, L Birkholtz※ and D Mancama‡
‡CSIR Biosciences, PO Box 395, Pretoria, South Africa, 0001. ※Department of Biochemistry, University of Pretoria, Pretoria, South Africa, 0002.

Introduction: Antiplasmodial drug discovery efforts are hampered by the lack of an extensive set of novel, validated drug targets. A requirement of these targets (or the pathways in which they function) is that they prove essential for parasite survival. The polyamine biosynthetic pathway, responsible for the metabolism of highly abundant amines crucial for parasite growth, proliferation and differentiation, is currently under investigation as an antimalarial target. In order to further evaluate polyamine depletion as possible antimalarial intervention, the consequences of inhibiting *Plasmodium falciparum* spermidine synthase was examined on a transcriptomic and proteomic level.

Methods: *In vitro* cultures were treated with the spermidine synthase inhibitor cyclohexylamine (IC_{99}), following morphological analysis of drug-treated parasites. Cultures were harvested at 18, 25 and 30 hpi for RNA and protein extraction. Global gene expression was gauged by hybridizing cDNA to Operon *P. falciparum* oligonucleotide arrays employing a reference design. Arrays were analysed in limma (R) and MeV (TIGR). 2D separation of proteins was accomplished by IEF followed by SDS-PAGE. Gels were analysed by PDQuest and spots identified with a Q-STAR Elite Q TOF mass spectrometer. Differentially expressed genes and proteins were analysed with MADIBA (Law et al., 2008).

Results:

A	UT18	UT25	UT30
T18	0.59	0.40	0.20
T25	0.61	0.50	0.30
T30	0.63	0.55	0.34

B	T18	T25	T30
T18	*	0.82	0.79
T25	*	*	0.83

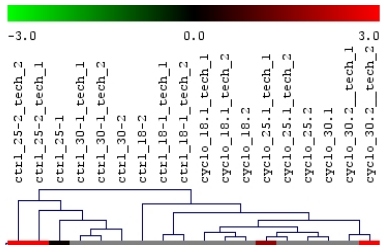

Figure 1: **Pearson correlations and hierarchical clustering of microarray data indicate a transcriptional arrest in the parasite following cyclohexylamine treatment.** Transcriptional arrest was confirmed by the following observations: (A) treated parasites showed the highest correlations with untreated parasites at 18 hpi; (B) global expression of drug treated parasites were highly correlated and (C) clustering analyses indicated drug-treated parasites clustered with untreated ones at 18 hpi. All gene and protein analyses were subsequently performed relative to 18 hpi; as confirmed and described by van Brummelen et al., (in press) for polyamine cytostatic drugs.

Transcripts	T18	T25	T30
Polyamine biosynthetic	0.214	0.102	0.088
Glycolysis	0.322	0.416	n/a
Oxidative phosphorylation	0.106	0.012	0.035

Proteins	T18	T25	T30
Polyamine biosynthetic	0.028	0.019	0.016
Glycolysis	0.054	n/a	0.054

Table 1: **The polyamine biosynthetic pathway is differentially regulated by cyclohexylamine treatment.** This pathway ranks among those most affected by the perturbation as calculated by Fisher's exact test in the software MADIBA (www.bi.up.ac.za/MADIBA/index.php; Law et al., 2008). Pathways not indicated have high p-values. Glycolysis and oxidative phosphorylation are generally regarded as pathways upregulated in response to multiple unrelated perturbations (data not shown).

Figure 2 (below): **Several differentially affected transcripts and proteins map to the polyamine biosynthetic pathway following spermidine synthase inhibition by cyclohexylamine.** Four polyamine transcripts and their encoded proteins were downregulated and a further two transcripts affected following drug treatment. Notably, lysine decarboxylase was upregulated in response to the perturbation, presumably to compensate for depletion of spermidine (by producing the putrescine analog cadaverine). Additionally, several genes active in pathways directly associated with polyamine synthesis were affected (purine metabolism and various methyltransferases). Figure legend shows gene and protein regulation at each timepoint following treatment.

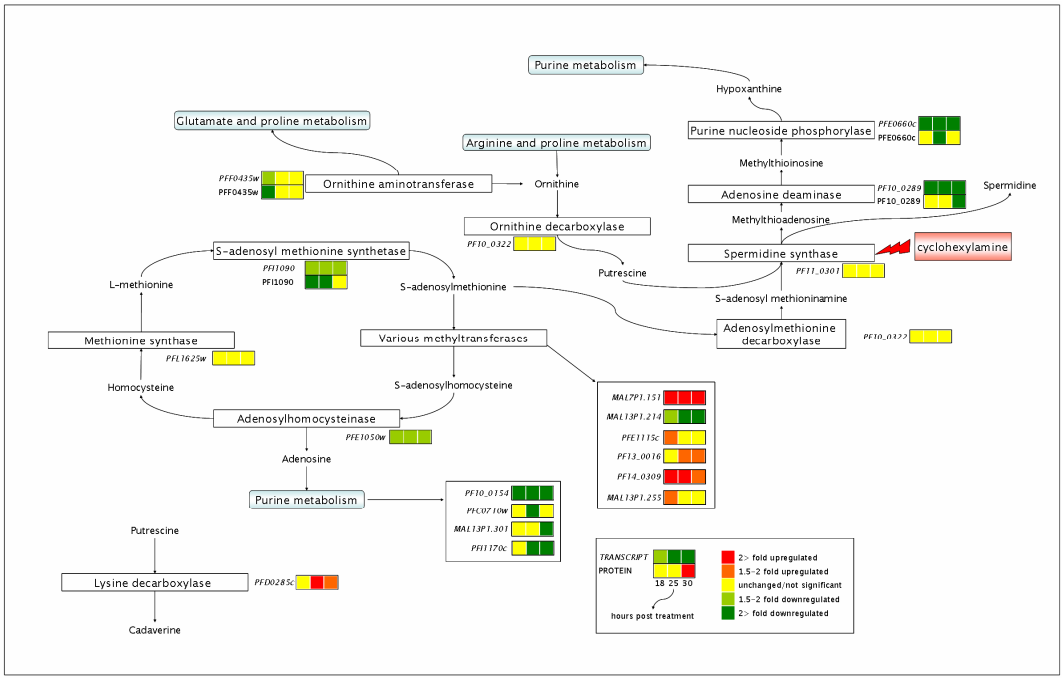

Table 2 (below): **Polyamine inhibitors show perturbation-specific effects following inhibition of their targets.** Three transcripts were differentially regulated by the polyamine inhbitors cyclohexylamine, DFMO and DFMO/MDL73811. Six transcripts of the polyamine biosynthetic pathway were similarly regulated in the transcriptional response following inhibition of spermidine synthase and SAMDC-ODC.

Annotation	PlasmoDB ID	Cyclohexylamine (This work)	DFMO/ MDL73811 (van Brummelen et al., in press)	DFMO (Clark et al., 2008)
Ornithine aminotransferase	PFF0435w	↓	↑	↑
S-adenosylmethionine decarboxylase-ornithine decarboxylase	PF10_0322	≈	↓	nd
Hypoxanthine phosphoribosyl-transferase	PF10_0121	≈	≈	↑

Impact:
- Spermidine synthase perturbation-specific effects were revealed by transcriptomic and proteomic analyses; corroborating and expanding on previous findings regarding polyamine inhibitors.
- Specific effects associated with each inhibitor revealed distinct compensatory mechanisms (OAT and LDC).
- This information will inform efforts aimed at developing novel antimalarial therapeutics targeting this vital parasite metabolic pathway.

References: •Clark K., Dhoogra M., Louw A.I. and Birkholtz L-M. (2008). Transcriptional responses of *Plasmodium falciparum* to α-difluoromethylornithine-induced polyamine depletion. Biol. Chem. **389**, 111-125. •Law PJ, Claudel-Renard C, Joubert F, Louw AI and Berger DK (2008). MADIBA: A web server toolkit for biological interpretation of *Plasmodium* and plant gene clusters. *BMC Genomics* **9**:105. •Van Brummelen A.C., Olszewski K.L., Wilinksi D., Llinás M., Louw A.I and Birkholtz L-M. Co-inhibition of *Plasmodium falciparum* S-adenosylmethionine decarboxylase/ornithine decarboxylase reveals perturbation-specific compensatory mechanisms by transcriptome, proteome and metabolome analyses J. Biol. Chem, 10.1074/jbc.M807085200, In Press.

Funding: This work was supported by a CSIR Parliamentary Grant (PG).