Studying the digestive vacuole lumen in *Plasmodium falciparum*

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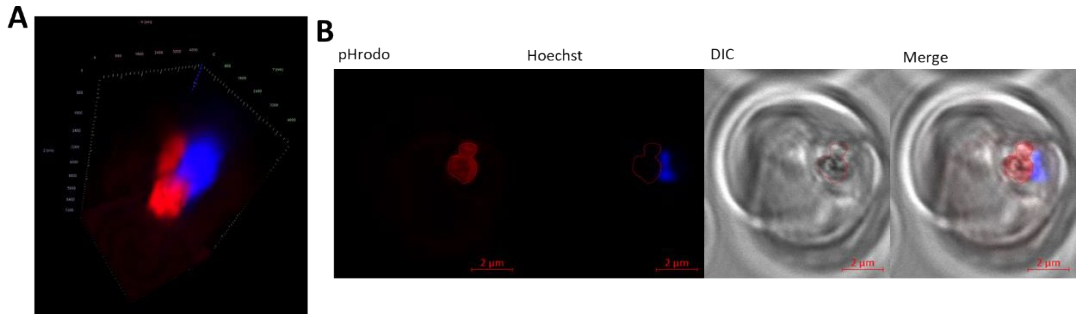

***A.** 3D rendering of confocal microscopy Z-stack images taken of a Plasmodium falciparum (Pf) parasite containing pHrodo dextran red and stained with Hoechst (nuclear stain). **B.** Single slice through Pf parasite shown in A.*

Introduction: *Plasmodium falciparum (Pf)* causes the deadliest form of malaria, the scourge of which is felt particularly in Sub-Saharan Africa. Imperative to parasite survival is the haem detoxification pathway. In this pathway, erythrocytic haemoglobin is endocytosed into the digestive vacuole (DV) anddigested to release amino acids and toxic haem, which is then incorporated into an insoluble crystal termed haemozoin. This pathway is well known, however, the intricacies need to be fully explored. Previous studies have shown that macromolecules are taken up through endocytosis, pinocytosis andthe formation of cytostomes.[1] In the current study, the volume of the digestive vacuole lumen and rate of uptake throughout the trophozoite stage was studied using confocal microscopy and flow cytometry.

Methodology: NF54 *Pf* parasites were allowed to reinvade red blood cells loaded with pHrodo™ red dextran beads and cultures were incubated at 37°C. Post incubation, parasite age was determined using flow cytometry. Samples were imaged using an AiryScan LSM 980 confocal microscope. Imageswere visualised using Zen blue software and analysed in ImageJ to determine volume.

Results: ***Flow cytometry.*** Analysis of the FL-1 channel data in combination with Giemsa smears, confirmed parasite age. ***Confocal microscopy.*** The volume of the DV lumen from 20 h to 40 h post invasion at 4-hour intervals was found to be 0.32 ± 0.03 fL (T20), 0.37 ± 0.05 fL (T24), 1.04 ± 0.18 fL (T28), 1.93 ± 0.14 fL (T32), 3.07 ± 0.25 fL (T36) and 3.92 ± 0.32 fL (T40). The growth follows an exponential pattern from T20 to T36. After 40 h post invasion the DV lumen begins to collapse until it effectively disappears. The absolute fluorescence intensity follows an exponential trend similar to the volume, however the probe concentration reveals a steady state.

Discussion and conclusion: The DV lumen grows exponentially over the trophozoite life cycle. These data contradict the “big gulp” theory at the beginning of the trophozoite cycle. Instead, these data support that haemoglobin is taken up via pinocytosis in the early trophozoite stages. The intensity datareveals that as the DV lumen grows, more material is taken up. However, the concentration of the probe remains constant. This has important implications for protein, drug and other macromolecule concentrations.

References: 1. Milani, K. J.; Schneider, T. G.; Taraschi, T. F., *Eukaryot Cell* **2015,** *14* (4), 415-26.

Keywords: Malaria, *Plasmodium falciparum*, volume, digestive vacuole, confocal microscopy, flowcytometry.