The isolation of *plasmodium falciparum* haem species for detection by HPLC-UV-vis

Keletso Maepa, Lubbe Wiesner, Timothy Egan

University of Cape Town, South Africa

Mpxmak002@myuct.ac.za

Malaria is a mosquito-borne disease spread by the *Anopheles* vector. The deadliest parasite of all the human-infecting species is *Plasmodium falciparum* (*P. falciparum*), which accounts for majority of the reported mortalities.[1] The use of antimalarial drugs remains the mainstay of combating this parasitic disease. However, the mechanism of action of several clinically relevant antimalarials against the *Plasmodium falciparum* parasite remains poorly understood. This presents a hurdle to our understanding of newly active compounds developed in response to emerging resistance to antimalarial drugs. It is well known that haemozoin formation serves as a detoxification route for unsequestered haem released from the degradation of red blood cell haemoglobin by *Plasmodium falciparum* parasites during the intraerythocytic blood stage (Figure 1). Several antimalarials, including chloroquine, act by inhibiting haemozoin formation. Our current method[2] for measuring haemoglobin, unsequestered haem and haemozoin levels in the *Plasmodium* parasite is not readily transferable to other malaria drug discovery laboratories because of the need for specific knowledge in the area of haem chemistry, highly specialized training and the labour intensive nature of the assay. Here we report on a novel HPLC-UV-Vis fractionation method for quantifying haem species in the parasite cell. This method allows for the testing of four compounds simultaneously and has resulted in the elimination of pyridine, whilst also reducing parasite starting material and increasing efficiency by four-fold. The data will be crucial for establishing whether haemozoin inhibition is the primary intracellular mechanism of experimental antimalarial compounds. When novel targets are sought, these approaches will also prove essential in avoiding compounds that inhibit haemozoin formation, by excluding this as the mode of action.

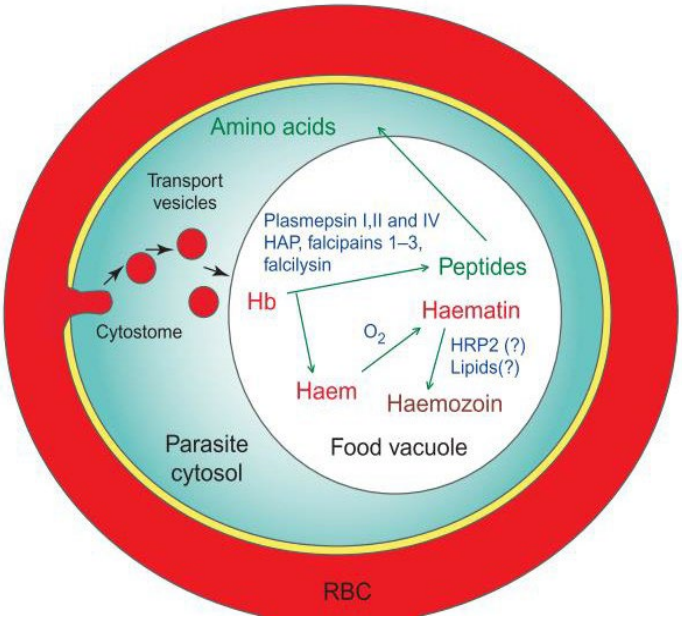

Figure 1: An illustration of a *Plasmodium falciparum* infected red blood cell. Haemoglobin (Hb) gets trafficked into the parasite cell food vacuole, then degraded into peptides (globin) and haem (reduced; +2 charge). Haem is oxidised to haematin, which is toxic to the parasite and subsequently assembles to form the inert dimeric haemozoin crystal. Antimalarials like chloroquine disrupt the formation of haemozoin, thus resulting in the accumulation of toxic haematin and inhibiting further parasite growth. [3]

References:

1 World Health Organization (WHO).,*World Malaria Report 2020* .

2 J. M. Combrinck, K. Y. Fong, L. Gibhard, P. J. Smith, D. W. Wright and T. J. Egan, *Malaria Journal.*,2015, **14**, 253.

3 T. J. Egan, *Molecular Biochemical Parasitology.*, 2008, **157**, 127–136.