Partial characterization of *Plasmodium falciparum* protein kinase ABCK2 (PfABCK2)

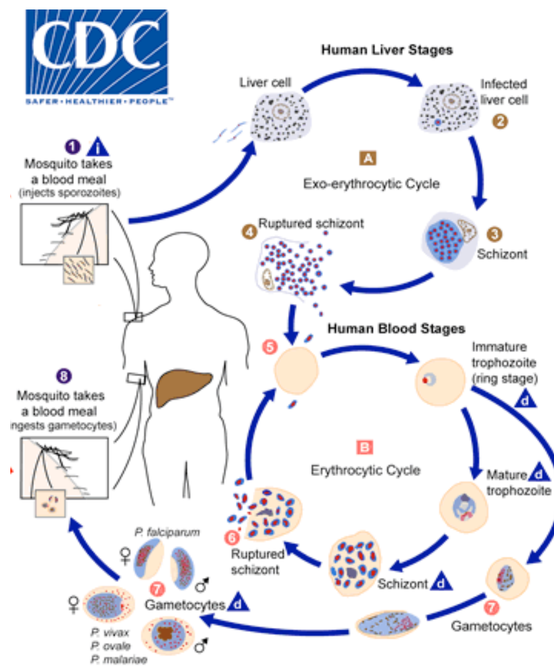
Muhammad Khalid, Francis Ntumngia, John H. Adams

Department of Global Health

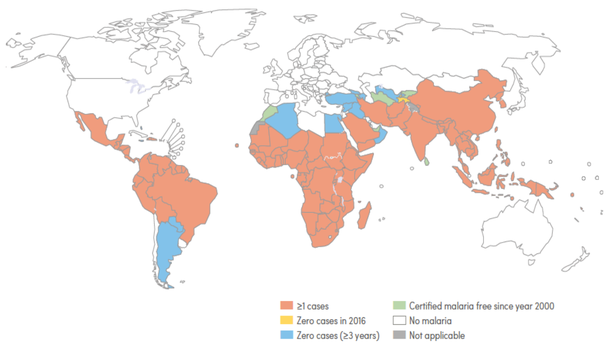
College of Public Health, University of South Florida, Tampa, USA

Introduction

- Malaria is a major threat to global public health as it effects populations in tropical and subtropical areas.
- Among the affected population are approximately 40% of pregnant women and children who are susceptible (Campbell *et al.*, 2014).
- *Plasmodium falciparum* is an aggressive agent of human malaria.
- There are approximately 100 parasite protein kinases involved in phosphorylation of asexual blood stage of the malarial parasite and have 35-60% sequence identity to mammalian orthologous (Alam *et al.*, 2015, Hallyburton *et al.*, 2017).



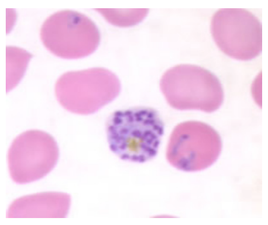
Blood stage lifecycle of *Plasmodium*



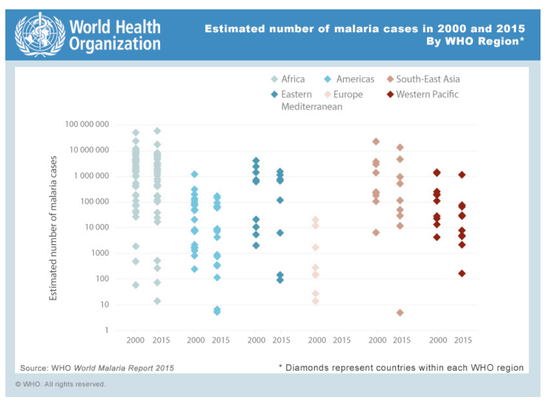
216 million malaria **cases** globally in 2016
445 000 malaria **deaths** globally in 2016

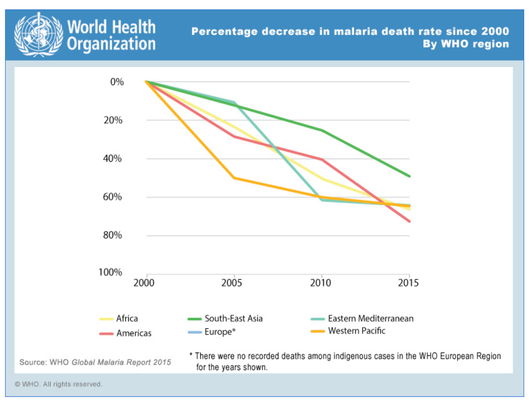
Source: World Malaria Report, 2017, W.H.O

Anopheles mosquito



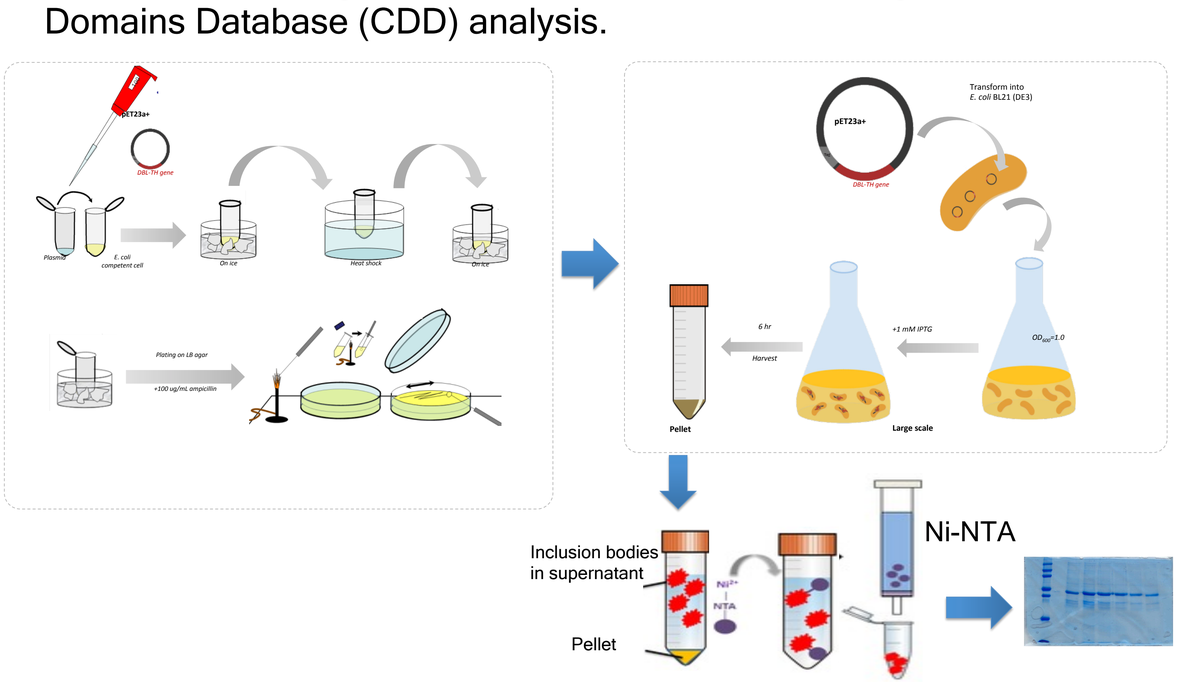
Schizont of *P. falciparum* in a thin blood smear. Source : CDC & DPDx





Experimental methods

- PfABCk2 gene and protein sequence is obtained from PlasmoDB.
- The protein multiple sequence alignment is analyzed utilizing BLASTP to select for orthologs and CLUSTAL 2.1.
- The conserved region of PfABCK2 is selected using the Conserved Domains Database (CDD) analysis.



Results

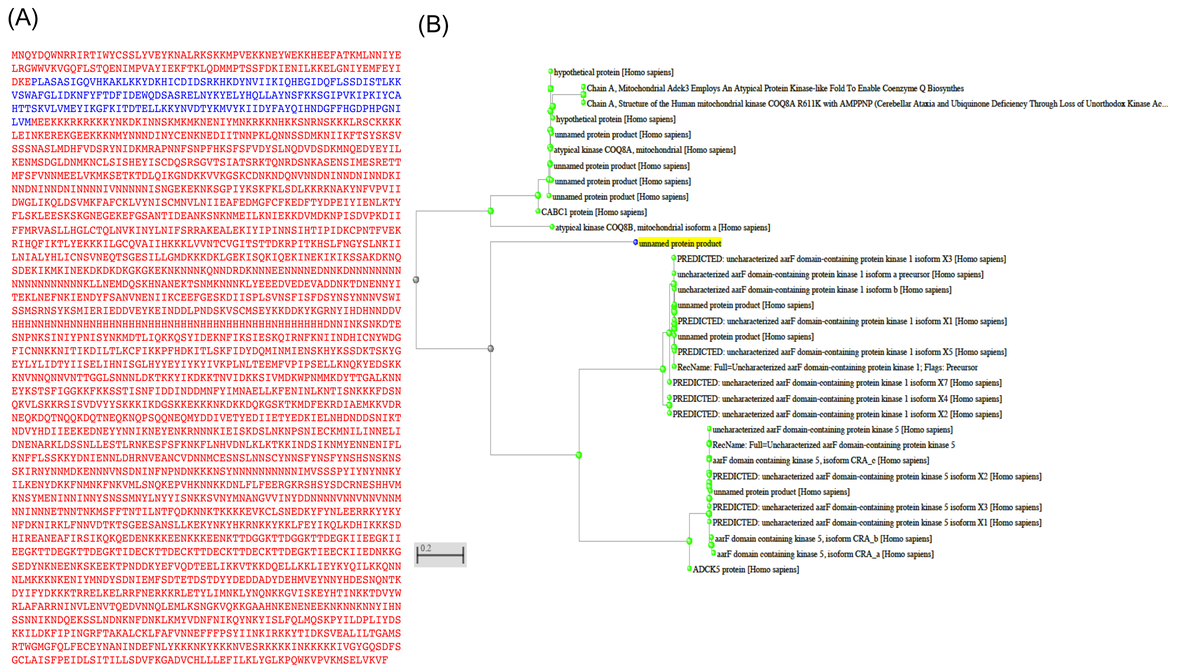
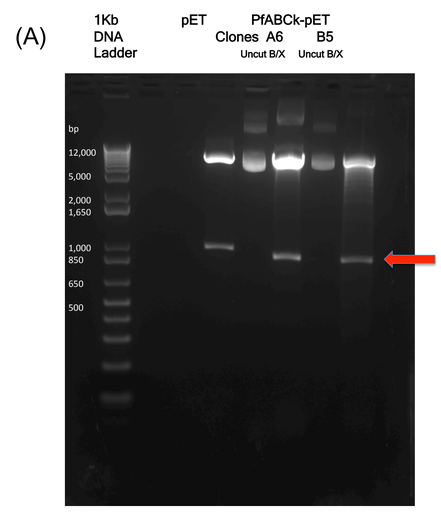


Figure 1: (A) Protein kinase conserved region from 124 to 302 amino acid (highlighted blue) in the protein sequence of PfABCk2 . **(B)** Phylogenetic tree of PfABCk2 in relation to human protein kinases with less than 30% identity.



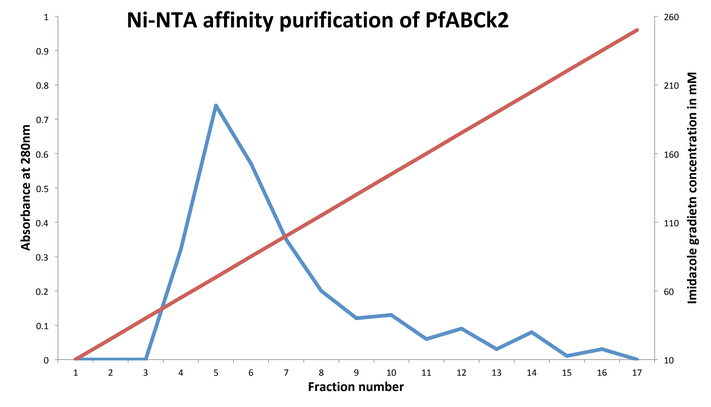


Figure 2 : (A) 1% agarose gel in Tris-Acetate EDTA. Lane 1: 1Kb DNA Ladder, Lane 3: PfABCk-pET21a+ construct, Lane 4: Clone A6 , Lane 5: Clone A6 with restriction enzymes *BamH1 and Xho1* (B/X), Lane 6: Clone B5, Lane 7: Clone B5 with B/X. **(B)** Ni-NTA affinity purification of recombinant PfABCk2 with His-tag on C-terminus using elution buffer containing from 10mM to 250mM Imidazole

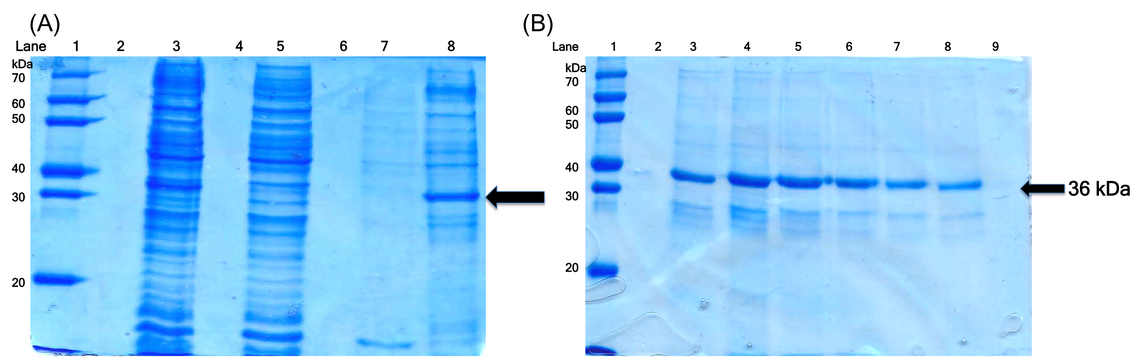


Figure 3: (A)10% Resolving gel of PfABCk heterologous expression in BL-21 (DE3) competent cells. Lane 1: Protein Marker (Bio-Rad), Lane 3: Whole cell lysate, Lane 5: supernatant, Lane 7: supernatant of cell pellet, Lane 8: solubilized cell pellet. **(B)**10% Resolving gel of fractions after nickel affinity chromatography. Lane 2: Protein Marker, Lanes 4 -9 : Fractions 4 -9.

Conclusion & Future work

- Successful transformation and orientation of construct into competent cells.
- The purified protein obtained concentration is 3.29 mg from 4L culture.
- The future work will be focused on protein kinase activity assay and inhibitor studies in order to utilize it as a potential therapeutic target.

References

Alam, *et al.* (2015). *Nature Communications*, 6, 7285. doi:10.1038/ncomms8285
Campbell, *et al.* (2014) *Chemical Biology Drug Design*, 84(2), 158-168. doi:10.1111/cbdd.12315.
Hallyburton, *et al.* (2017*) Malar J*, 16(1), 446. doi:10.1186/s12936-017-2085-4

Contact : khalidm@health.usf.edu

USF HEALTH
COLLEGE OF PUBLIC HEALTH
UNIVERSITY OF SOUTH FLORIDA

Our Practice Is Our Passion