Systems biology approaches to study *in vitro* produced *Plasmodium falciparum* mosquito stages

Leslie S. Itsara, PhD[1], Yaxian Zhou, MS[1], Julie Do, BS[1], Samrita Dungel, MS[1], Matthew Fishbaugher, BS[2], Ashley M. Vaughan, PhD[2], Robert Moritz, PhD[3], Stefan H. I. Kappe, PhD[2,4,#], Anil K. Ghosh, PhD[1,#]

1. MalarVx, Inc. Seattle, WA, USA 2. Center for Infectious Disease Research, Seattle, WA, USA 3. Institute for Systems Biology, Seattle, WA, USA
4. Department of Global Health, University of Washington, Seattle, WA, USA #Corresponding authors

Background

Malaria caused by *Plasmodium* infection is a devastating disease resulting in >200 million clinical cases and 440 thousand deaths in 2015 (WHO, 2015). Malaria outbreaks significantly impact military operational capabilities and readiness. Historically, malaria has caused a greater loss of manpower in tropical regions than combat-related injuries. Most deaths from malaria are caused by *Plasmodium falciparum* (*Pf*). The infectious stage of *Pf* to humans is the sporozoite stage, which develops in mosquitoes and is delivered to humans by mosquito bite. Live sporozoite vaccines are the only vaccines that provide complete sterilizing protection against *Plasmodium* infection in mice and humans. These vaccines include genetically attenuated parasites (GAP), radiation attenuated sporozoites (RAS), and wild-type sporozoites administered with chloroquine chemoprophylaxis (CPS). Current methods of vaccination with sporozoites require production using mosquitoes, are extremely labor intensive, and are not scalable. Without scalable sporozoite production enough vaccine cannot be produced to vaccinate everyone at risk of malaria infection. To solve this problem, our group is developing an *in vitro* culturing system to produce large quantities of genetically attenuated sporozoites. The *Pf* stages of mosquito development include gamete → zygote → ookinete → oocyst → sporozoite, and culturing these stages of *Pf in vitro* has proven challenging for decades. However, we can produce *Pf* mosquito equivalent stages *in vitro* and can produce and purify millions of ookinetes from a single culture set. Knowledge of ookinete proteins and surface markers, the stage before oocyst, can inform and drive our culturing system. However, identity of ookinete specific surface markers is largely unknown and antibodies against ookinete proteins are lacking. To fill in this knowledge gap, we perform and outline the first proteomics studies on *Pf* ookinetes.

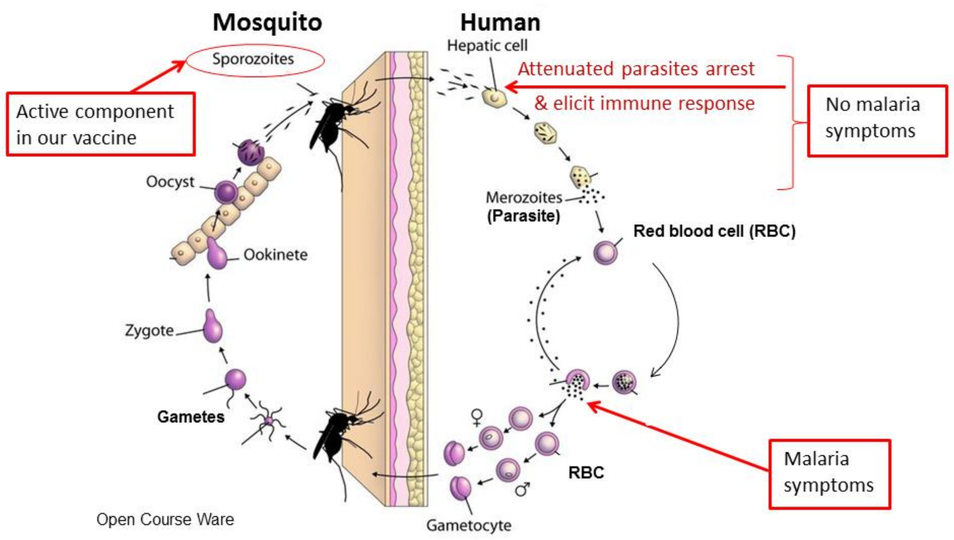


Figure 1. *Pf* life cycle in and live sporozoite vaccine strategy.

Results and Methods

Plasmodium falciparum gametocytes were cultured to maturity and development of zygote and ookinete stages was promoted by addition of essential components to the culture and incubation as previously described (Ghosh A 2010, Ghosh A 2013). Proteomic analysis was performed on whole purified *Pf* ookinetes, and data are presented here (Figure 2). We identified eight novel *Plasmodium* conserved surface protein candidates. These and future studies are planned to identify ookinete surface proteins as antigens for antibody development. Once antibodies are generated, they will be tested on *Pf* ookinetes from mosquitoes and from *in vitro* culture using biochemical approaches and immunohistochemistry. Antibodies validated after these tests will then be used in a quality control step to study *in vitro* ookinetes for production of sporozoites for malaria vaccines.

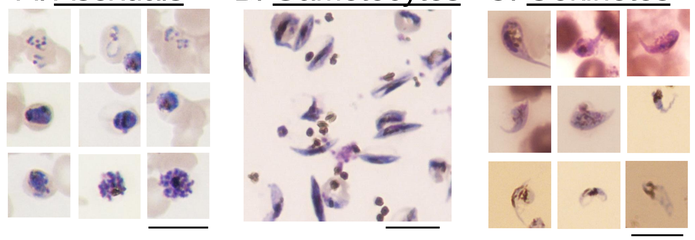
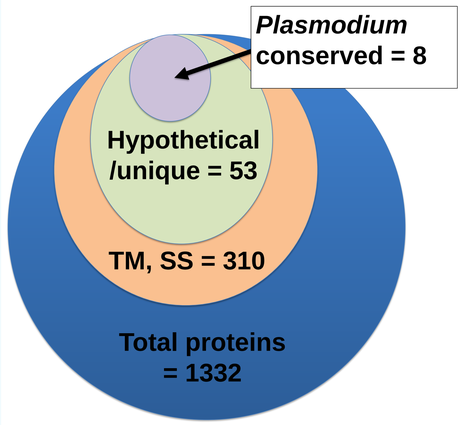


Figure 2. Giemsa stained *Pf* stages produced using *in vitro* methods. A) Asexual culture containing ring, trophozoite, and schizont stages. B) Gametocyte-enriched culture containing gametocyte stages II, III, and IV. C) Retort ookinetes. Scale bar = 10 μm.

A



B

Gene	Probable Function	Conserved domain
1. PF3D7_1024800	Signaling	LMP1 (Latent Membrane Protein 1)
2. PF3D7_0713700	Adhesion	VCBS (Vibrio, Colwellia, Bradyrhizobium, and Shewanella) repeat
3. PF3D7_1125100	Cell Binding	NBD94 (Nucleotide-Binding Domain 94)
4. PF3D7_030500	Transporter	MSF (Major Facilitator Superfamily)
5. PF3D7_1215100	Adhesion	F 5/8 discoidin
6. PF3D7_0412000	Membrane trafficking	LITAF (LPS-induced-TNF-α-transcription factor)
7. PF3D7_0630400	Adhesion	Cysteine Rich
8. PF3D7_0723200	Adhesion	Laminin G3

Figure 3. *Pf* Ookinete proteome analysis reveals potentially unique ookinete surface proteins. Panel A depicts proteins identified in proteomic analysis. Proteins were predicted to demonstrate surface localization by presence of a transmembrane domain (TM) and/or signal sequence (SS). Of these 310 proteins, 53 have undescribed (unique) functions. Eight of the 53 proteins are conserved across *Plasmodium* species, and analysis using several protein databases identified functional domains or protein signatures from other known proteins (**B**). These domains were used to predict function of potentially unique ookinete surface proteins.

Conclusions

We aim to identify ookinete proteins by using *in vitro* production of *Pf* mosquito-equivalent stages and proteomics, as well as address the profound reagent gap by producing antibodies against ookinetes. These data will drive development of our *in vitro* culturing system and inform the malaria research community on the *Pf* ookinete whole proteome and surface proteome, including those interested in transmission blocking vaccines. Future plans include extensive characterization of all *Pf* mosquito stages to further guide our *in vitro* culture system and ultimately produce GAPs for malaria vaccines.