Using Perfringolysin O (PFO) to Create a Cell-Free *in vitro* System to Study Mouse Malaria (*Plasmodium yoelii*)

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Due to the complicated growth requirements of *Plasmodium yoelii* parasites, current *in vitro* culturing systems do not permit for the development of late liver-stages, which makes them extremely difficult to study. Our goal is to use a cholesterol-dependent cytolysin, called perfringolysin O (PFO), to selectively permeabilize the hepatocyte plasma membrane and separate the parasitophorous vacuole (PV) so that parasites can be cultured in a cell-free *in vitro* system. PFO has previously been used to separate host cytoplasmic fractions from chlamydial inclusions after infection, allowing for the isolation of secreted chlamydial proteins within the host cytosol. By separating the cytosolic fraction from the PV membrane, we hope to culture the developing *P. yoelii* liver-stage parasites within their PV using a cell-free system with the goal of observing signs of late liver-stage development. The protocol for PFO-mediated lysis of infected hepatocytes is still under development but we have successfully established that baby hamster kidney (BHK) cells can be selectively permeabilized using PFO as intracellular concentrations of a reporter molecule (Dextran-FITC) were strongest within PFO-treated cells compared to non-PFO treated cells. We have begun adapting our PFO-permeabilization protocol to a murine hepatocyte cell line (Hepa1-6 cells) but have encountered several issues stemming from differences in membrane cholesterol content. Once we have established how to selectively permeabilize Hepa1-6 cells, we will proceed with the use of infected Hepa1-6 cells. The establishment of an *in vitro* culturing system for *P. yoelii* parasites is essential to the field and the study late live-stage development *in vitro.*