Elucidating the Structure of Open Apolipoprotein-L1 Channels in Lipid Bilayers

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The eukaryotic parasite, *Trypanosoma*, is responsible for African Trypanosomiasis, a zoonoses affecting humans and livestock. Humans are resistant to infection due to innate expression of Apolipoprotein-L1 (APOL1), which forms ion channels in parasite membranes, resulting in lysis. *In vitro,* APOL1 will insert into lipid bilayers at acidic pH 6.2 ONLY if the other side is neutral pH 7.2, precluding the use of nanodiscs. The project goal is use large unilamellar vesicles (LUVs) for cryogenic electron microscopy to elucidate the structure of open APOL1 channels. Fully functional N-terminal 6x-His tagged recombinant APOL1 E328C (cysteine mutant for biotin-tagging) was expressed and purified from BL21 *E. coli* cells via nickel affinity chromatography and subsequently biotinylated with 1mM biotin. Biotinylated-APOL1 was incubated with LUVs containing pH7.2 buffer for 1hr, followed by acidification to pH6.2 to drive insertion, and finally neutralization of the surrounding buffer to create proteoliposomes containing open APOL1 channels. Anti-6x histidine antibodies were added to the proteoliposomes before freezing onto cryoEM grids to increase electron density on any LUVs containing bound APOL1 and imaged using a Talos Arctica G2 Cryo-TEM. Initial attempts to generate electron density maps of APOL1-containing showed we were able to generate consistent 100-200 nm LUVs. However, we were unable to observe any LUVs containing bound APOL1. Our primary reasoning is that insufficient concentration of APOL1 was added or was able to bind into the LUVs. We are currently optimizing our sample preparation protocols adding more initial biotinylated APOL1, and troubleshooting how to concentrate the LUVs via ultracentrifugation.