

The *Cryptosporidium* oocyst wall is essential in transmission

- **Waterborne** apicomplexan zoonotic parasite
- Diarrhea is a leading cause of death in **children under 5 years old** worldwide
- Causes the **diarrhoeal disease** cryptosporidiosis
- **Faecal-oral** transmission
- **Hardy oocyst wall** gives environmental resilience

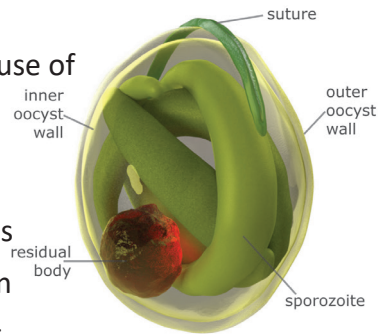
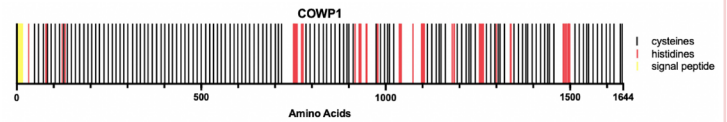


Image Credit: Konstantinos Alexandrou

Cryptosporidium Oocyst Wall Proteins (COWPs) are cysteine rich

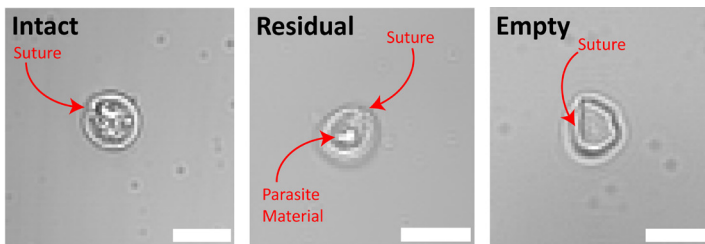
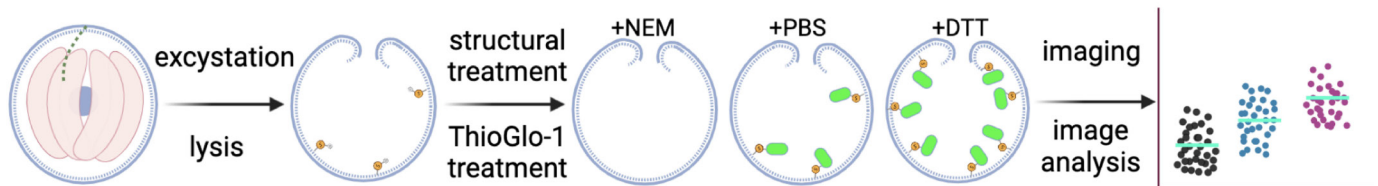
- COWPs are **enriched** in the inner layer of the oocyst wall and have a **high number of cysteines**
- Predicted that tertiary and **quaternary structure** are dependent on these residues



I hypothesise that oxidoreductase machinery crosslinks wall proteins through disulfide bonds

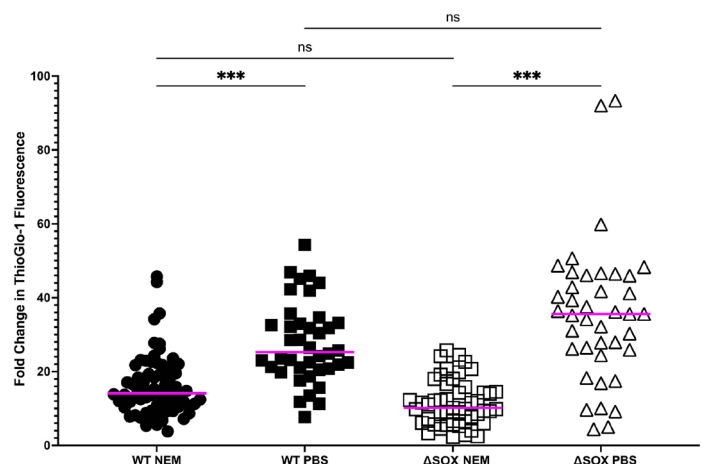
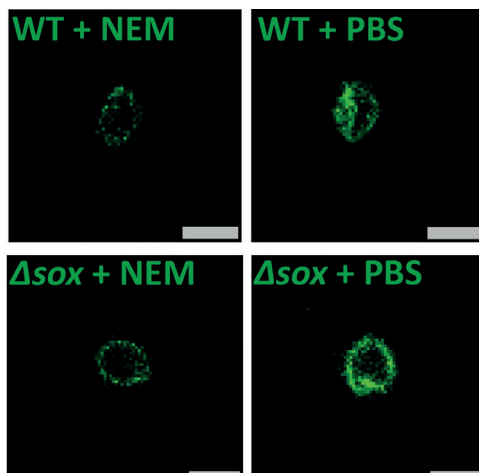
AIMS: To make an assay that can quantify disulfide bond abundance. To use this assay to determine the effect of Sulphydryl Oxidase Knockout (Δsox) in *Cryptosporidium* oocyst walls

ASSAY DEVELOPMENT



1. Expose inner wall by excystation and remove surplus parasite material by detergent based lysis
2. Treat with control compounds (DTT & NEM)
3. Use fluorescent compound ThioGlo-1 to bind free thiols
4. Image on the Zeiss 710 Confocal
5. Select appropriate 'shells' via brightfield (left) and quantify ThioGlo-1 fluorescence in Fiji

Δsox ASSAY RESULTS



Δsox has no significant effect overall on disulfide bonds in the *Cryptosporidium* oocyst wall.

FUTURE WORK - Knockout other oxidoreductases and subject them to this assay