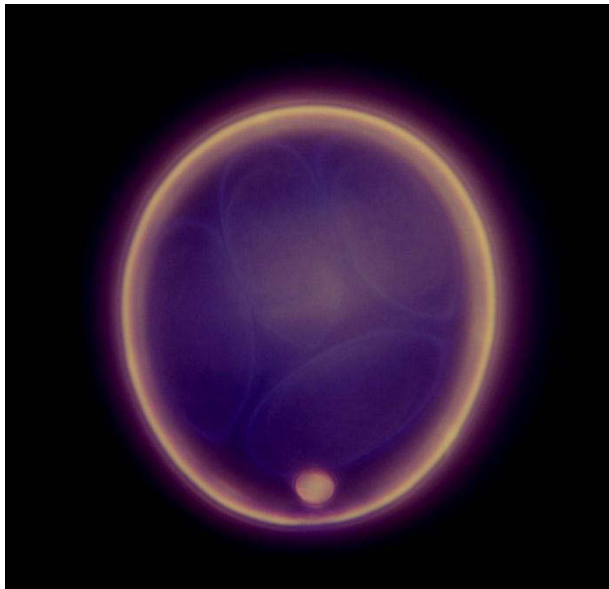


Thesis for doctoral degree (PhD)

2014

**Development of the oocyst wall in *Eimeria maxima* and  
biochemical analysis of gametocyte wall forming bodies**



**Sonja Frölich**

*To my family*

*CERTIFICATE OF ORIGINAL AUTHORSHIP*

*I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.*

*I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.*

*Signature of Student:*

*Date:*

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For any errors or inadequacies that may remain in this work, the responsibility is entirely my own.

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## Abstract

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*Eimeria* is a cyst-forming intracellular parasite that causes the economically important disease, coccidiosis, in intensely reared broiler chickens worldwide. The ability of the *Eimeria* parasite to replicate very rapidly and to synthesize an impenetrable, highly resistant oocyst wall, allows it to build up to very large numbers in the litter of broiler flocks. The molecular machinery involved in the assembly of the oocyst wall is housed in the two types of wall forming bodies (WFB1 and WFB2) of the sexual stage parasites (macrogametocytes).

The current project aimed to expand our understanding of the fundamental mechanisms involved in oocyst wall formation by: (1) characterising the morphological changes involved in oocyst wall assembly during parasite development; (2) developing a method to isolate gametocyte WFBs in order to characterise their molecular composition; and (3) studying the nature and characterizing the mechanisms of nutrient acquisition in developing *E. maxima* gametocytes *in vitro*.

Extracted macrogametocytes were stained using cytochemical and immune-labelling methods, and morphological changes of the developing zygote characterised by bright-field, scanning electron and 3D confocal microscopy. Additionally, the WFBs of macrogametocytes were enriched by subcellular fractionation and fractions containing these organelles were analysed by microscopy, western blot and label-free quantitative shotgun proteomics. Data from these studies has shown that gametocytes and early stage oocysts contain surface pores and are capable of actively taking up and internalizing nano beads via endocytosis. In addition, microscopic analyses shows that *E. maxima* is selective in compartmentalizing neutral lipids to the type 1, and glycoproteins to the type 2 wall forming bodies during gametocytogenesis.

Furthermore, it became possible to visualise both neutral lipids and glycoproteins during outer and inner oocyst wall formation. Thus, a model of outer oocyst wall formation was proposed and suggests that neutral lipids found in the WFB1s are translocated to the parasite's surface where they deposit their cargo via exocytosis. The

released molecules fuse with the parasite's limiting membrane for incorporation into the neutral lipid rich outer oocyst wall.

Finally, biochemical and proteomic methods were employed to identify and analyse vesicular trafficking proteins and other putative regulators of endocytosis and transport. The results reported here reveal valuable insights into the mechanisms by which the parasite is able to acquire nutrients essential for development, transport organelles and at the same time synthesise the impervious oocyst wall.

## List of papers

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- I. Frölich S., Entzeroth R., Wallach M., 2012, Comparison of protective immune responses to apicomplexan parasites. *Journal of Parasitology Research* 2012, 852591. doi:10.1155/2012/852591\*
- II. Frölich S., Johnson M., Robinson M., Entzeroth R., Wallach M., 2013, The spatial organization and extraction of the wall forming bodies of *Eimeria maxima*. *Parasitology* 140, 876 - 887. doi: 10.1017/S0031182012002247\*
- III. Frölich S., Wasinger V., Padula M., Wallach M., 2013, Endocytosis and intracellular trafficking in *Eimeria maxima* sexual stages. *Plos ONE*, manuscript number: PONE-D-13-49560.\*
- IV. Frölich S., Shahparee A., Wasinger V., Wallach M., 2013, In vivo localisation of antibodies raised against *Eimeria maxima* wall forming bodies. (accepted for publication in *Parasitology*, PAR-2014-0054.R2)
- V. Frölich S., Farhat J., Wallach M., 2013, Designing strategies for the control of coccidiosis in chickens on poultry farms using modern diagnostic tools. *Reports in Parasitology* 2013, 2013:3, 1 – 10. doi: <http://dx.doi.org/10.2147/RIP.S32811>

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## **List of conferences**

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- I. Frölich S., Johnson M., Sharman P., Katrib M., Smith N. and Wallach M., 2010, Development of *Eimeria maxima* gametocytes and isolation of wall forming bodies. Oral presentation delivered at the 27<sup>th</sup> Annual Scientific Research Meeting, co-hosted by the University of Technology, Sydney, The University of Sydney, The Kolling Institute and the Royal North Shore Hospital, Sydney, NSW, November, 2010.
- II. Frölich S., Robinson M., Johnson M., Sharman P., Katrib M., Smith N., Wallach M., 2011, Development of *Eimeria maxima* gametocytes and isolation of the wall forming bodies. Oral presentation delivered at the Australian Society of Parasitology Annual Conference, Pullman Reef Casino Hotel, Cairns, Queensland, July, 2011.
- III. Frölich S., Johnson M., Wallach M., 2011, The role of neutral lipids in gametocytogenesis and oocyst wall formation of *Eimeria maxima*. Oral presentation delivered at the 28<sup>th</sup> Annual Scientific Research Meeting, co-hosted by the University of Technology, Sydney, The University of Sydney, The Kolling Institute and the Royal North Shore Hospital, Sydney, NSW, November, 2011.
- IV. Frölich S., Robinson M., Johnson M., Entzeroth R., Wallach M., 2012, The spatial organisation and extraction of the wall forming bodies of *Eimeria maxima*. Poster presentation delivered at the Biology of Host-Parasite Interactions Conference, Salve Regina University Newport, RI, USA, June, 2012.
- V. Frölich S., Robinson M., Johnson M., Entzeroth R., and Wallach M., 2012, The spatial organisation and extraction of the wall forming bodies of *Eimeria maxima*. Oral presentation delivered at the 2<sup>nd</sup> International Symposium On Protozoal Infections in Poultry, at the University of Veterinary Medicine Veterinärplatz 11210-Vienna, Austria, July, 2012.

- VI. Shahparee A., Frölich S., Wallach M., 2012, *In vitro* localization of antibodies against *Eimeria maxima* Wall Forming Bodies. Poster presentation delivered at the 29<sup>th</sup> Annual Scientific Research Meeting, co-hosted by the University of Technology, Sydney, The University of Sydney, The Kolling Institute and the Royal North Shore Hospital, Sydney, NSW, November, 2012.
- VII. Frölich S., Shahparee A., Wasinger V., Wallach M., 2013, Endocytosis and intracellular trafficking of wall forming bodies in *Eimeria maxima* gametocytes. Oral presentation delivered at the New Horizons Conference, the 30<sup>th</sup> Combined Health Science Conference, co-hosted by the University of Technology, Sydney, The University of Sydney, The Kolling Institute and the Royal North Shore Hospital, Sydney, NSW, November 2013.
- VIII. Frölich S., Wasinger V., Padula M., Wallach M., 2014, Intracellular trafficking of wall forming bodies in *Eimeria maxima* gametocytes. Oral presentation delivered at the Biology of Host-Parasite Interactions Conference, Salve Regina University Newport, RI, USA, June, 2014.
- IX. Frölich S., Wasinger V., Padula M., Wallach M., 2014, Intracellular trafficking of wall forming bodies in *Eimeria maxima* gametocytes. Oral presentation delivered at the Biology of Host-Parasite Interactions Conference, Salve Regina University Newport, RI, USA, June, 2014.
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28<sup>th</sup> RNSH-UTS-USYD-KIMR Scientific Research Meeting

### **Dean's Award for Academic Excellence, 2011**

Faculty of Science, University of Technology, Sydney

### **Vice Chancellor's Postgraduate Research Student Conference Travel Award, 2012**

Faculty of Science, University of Technology, Sydney

### **Technion Society of Australia Travel Scholarship, 2013**

Student entrepreneurship, Technion Israel Institute of Technology

## Abbreviations

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BSA	Bovine Serum Albumin
bp	Base pair
Da	Dalton
DAPI	4', 6-diamidino-2-phenylindole
DNA	Deoxyribonucleic Acid
EtOH	Ethanol
FITC	Fluoresceine isothiocyanate
h	Hour
HN	Host nucleus
IFA	Immunofluorescent microscopy
IW	Inner oocyst wall
kDa	Kilodalton
L	Litre
L	Lipid body
M	Moles
Min	Minute
Mg	Milligram
ML	Millilitre
mM	millimoles per litre
N	Nucleus
Nu	Nucleolus
OW	Outer oocyst wall
p.i.	Post infection
PG	Polysaccharide granule
PBS	Phosphate buffered saline
s	Second
SDS	Sodium-dodecyl-sulphate
TBS	Tris buffered saline
V	Veil, the outermost layer of the oocyst wall
VFBs	Veil forming bodies
WFB1	Wall forming body Type 1

WFB2	Wall forming body Type 2
μg	Microgram
μl	Microlitre