Manipulation of membrane fusion by *Toxoplasma gondii*-secreted invasion factors

Stephen L. Denton

Presentation Overview

1. **Background**
    a. ***Toxoplasma gondii***
    b. **Tachyzoite Active Invasion**
    c. **Intracellular Growth**
2. Specific Aims
3. The Membrane Fusion Assay
4. Results

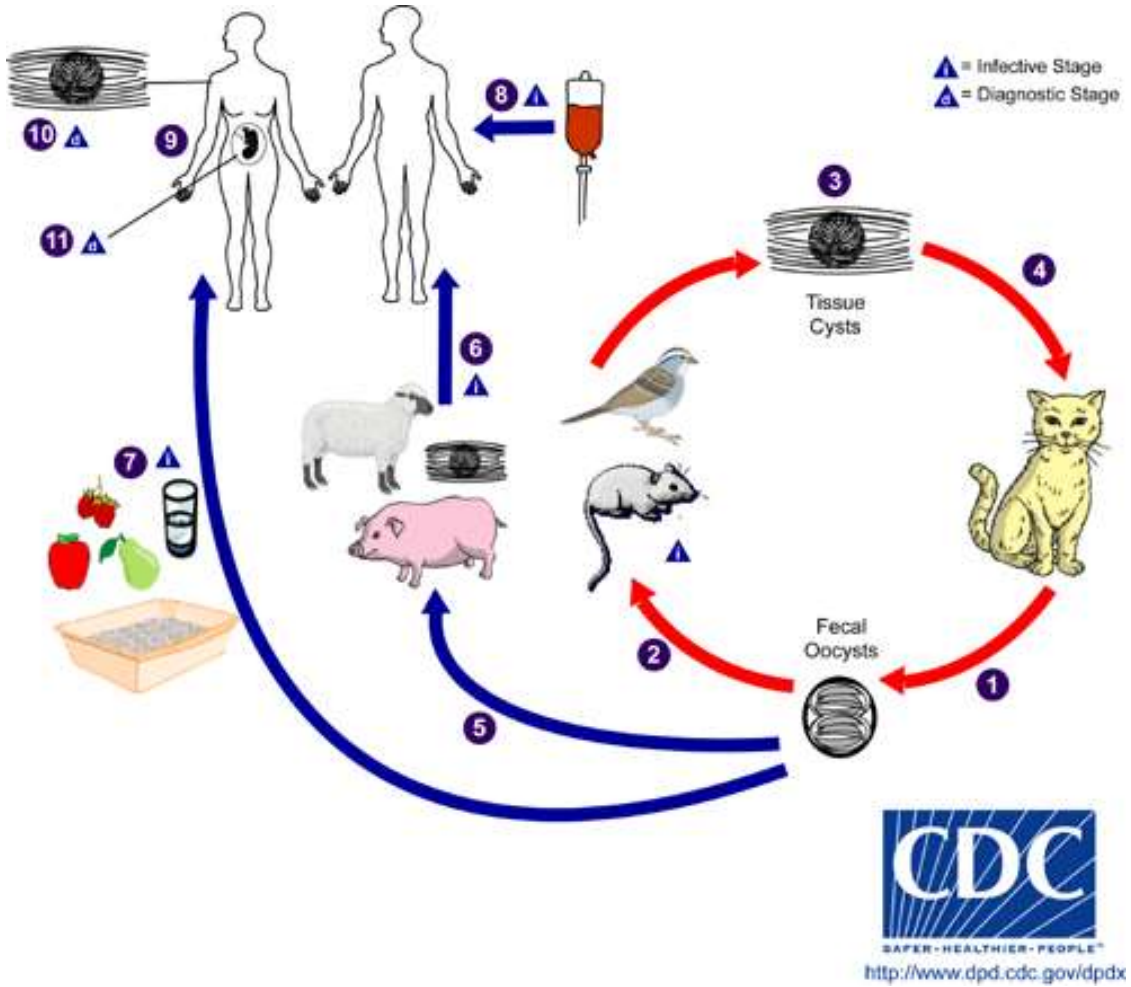
1a. *Toxoplasma gondii*

Life cycle:

Sexual reproduction in felines; oocyst transmission in feces

Asexual reproduction in mammals; *tachyzoite* or *bradyzoite* transmission

Four modes of transmission into humans



Infection Stages:

Acute stage: Clincal presentation of actively growing *Tachyzoites*. Low immune response

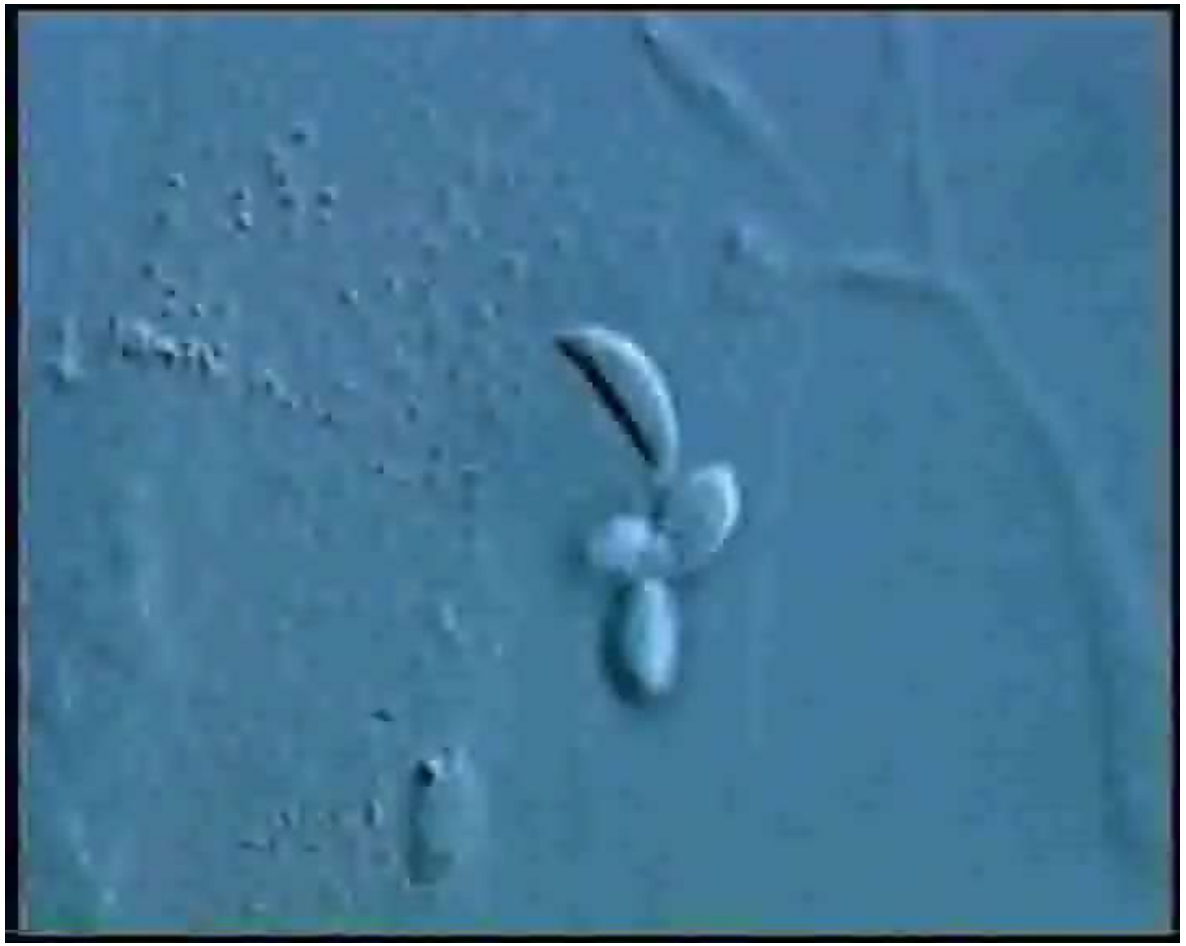
Chronic stage: Dormant *Bradyzoite* cyst formation. Infection held in check by immune system

Photo credit: CDC http://www.cdc.gov/dpdx/toxoplasmosis/index.html

1b. Tachyzoite Active Invasion

Parasitophorous vacuole (PV)- Parasite containing vacuole composed of host plasma membrane

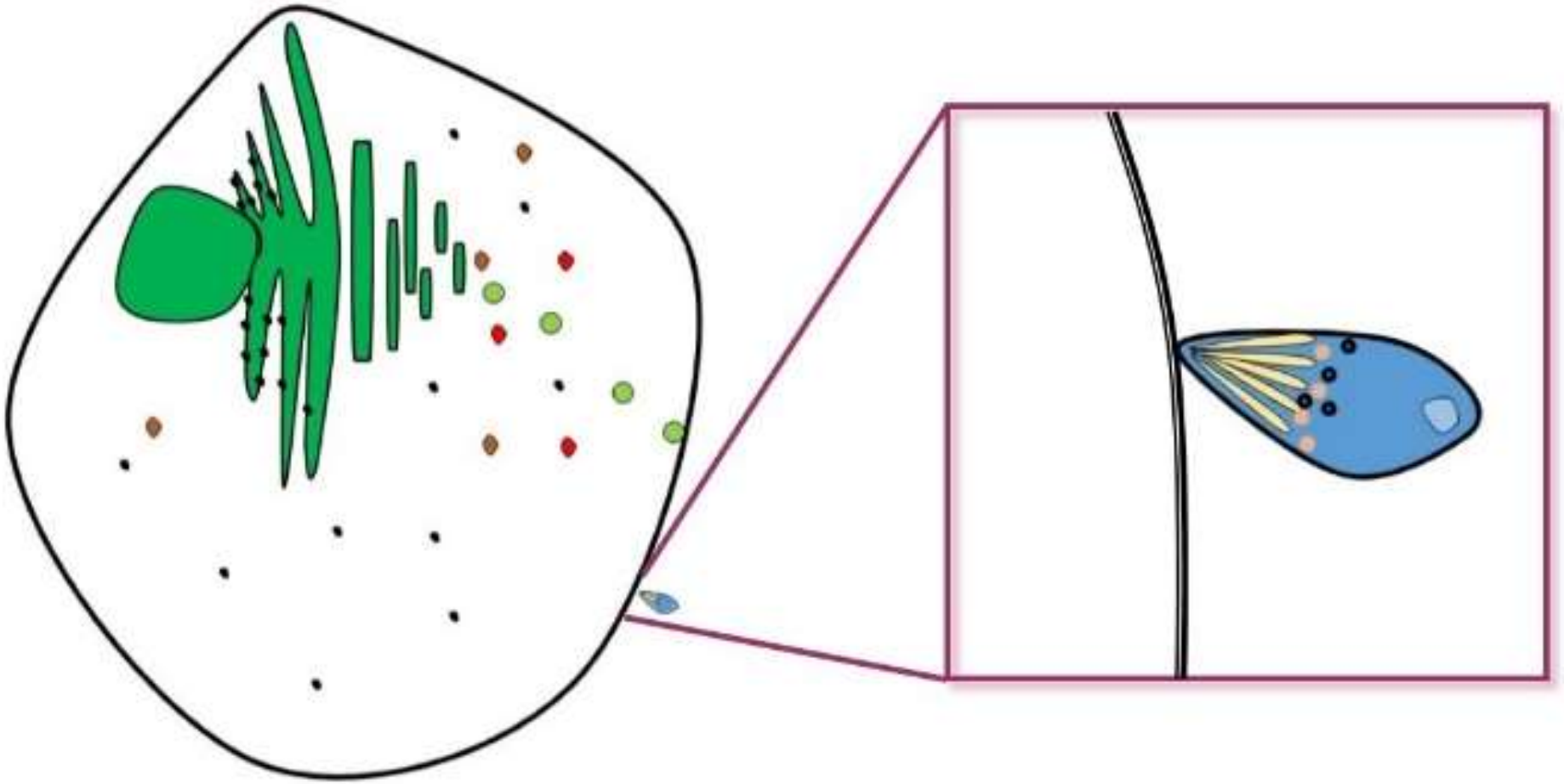
Moving junction- Parasitic actin myosin motor complex that drives PV formation and acts like a sieve to remove unwanted proteins.



John C. Boothroyd and Jean-Francois Dubremetz. 2007. Kiss and spit: the dual roles of Toxoplasma rhoptries. Supplemental Video 1. Nat Reviews Microbiology. 6: 79-88

A PV (host lipids) mimics damaged internal structures.

1b. Tachyzoite Active Invasion



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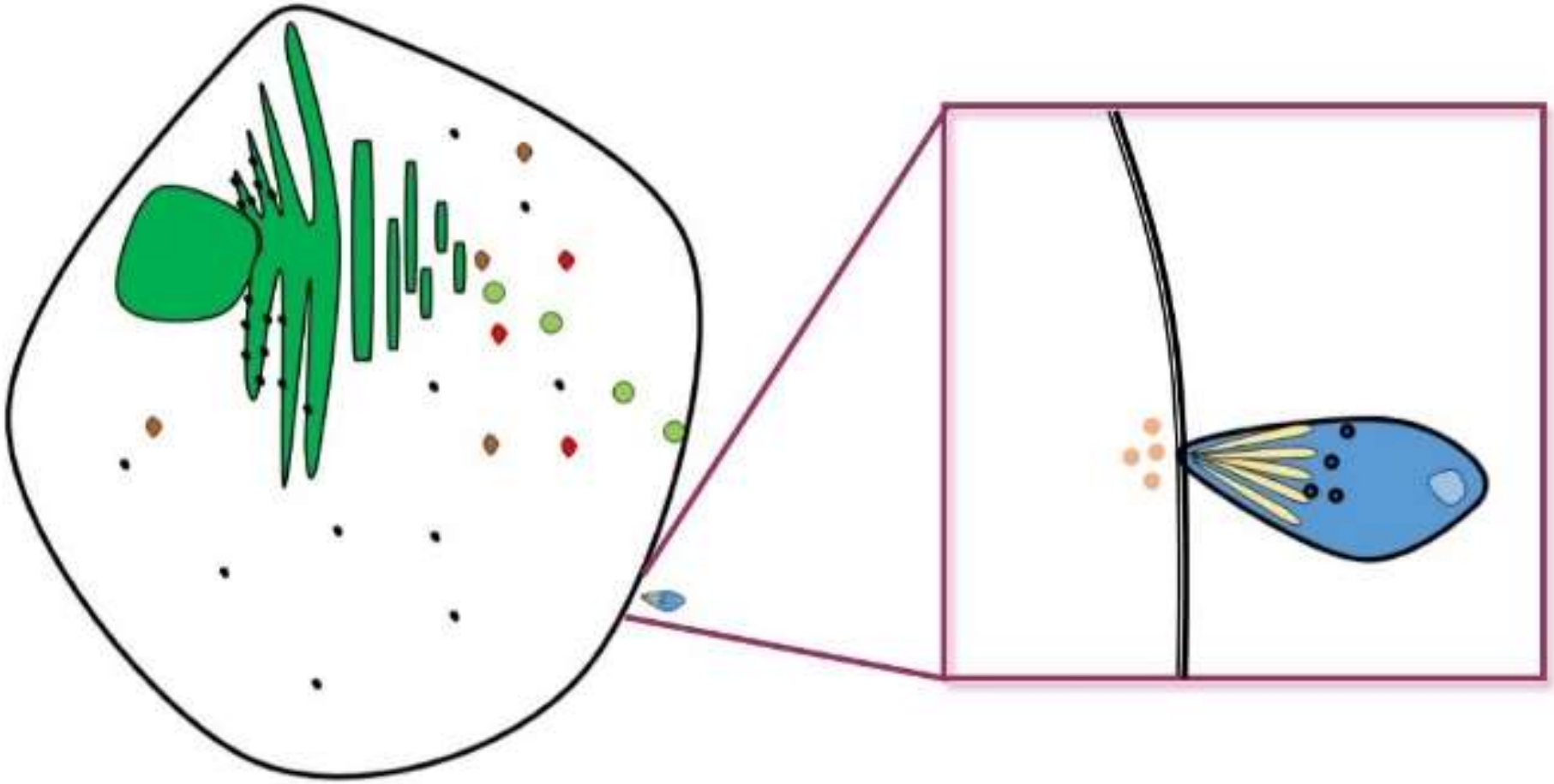

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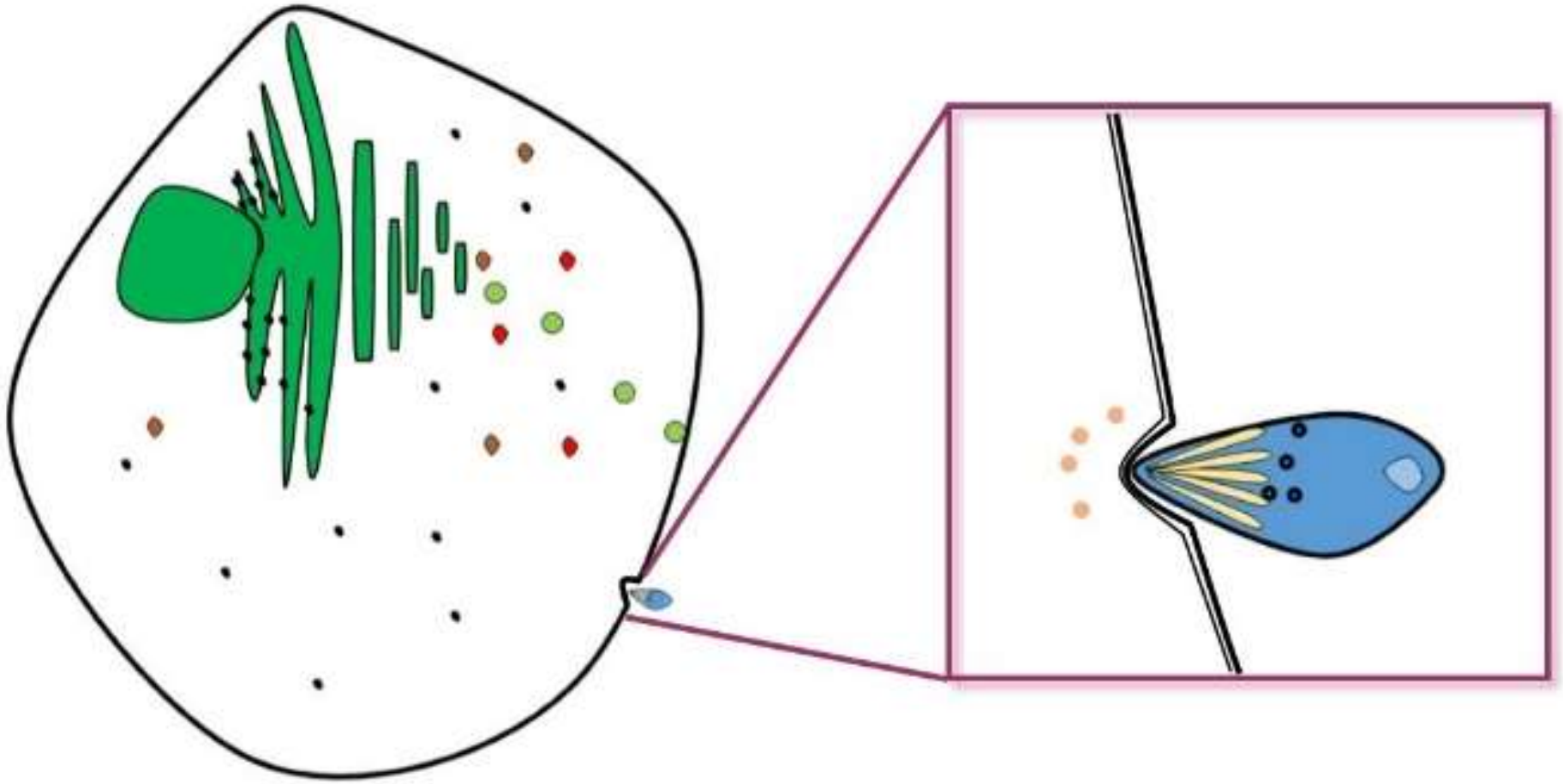

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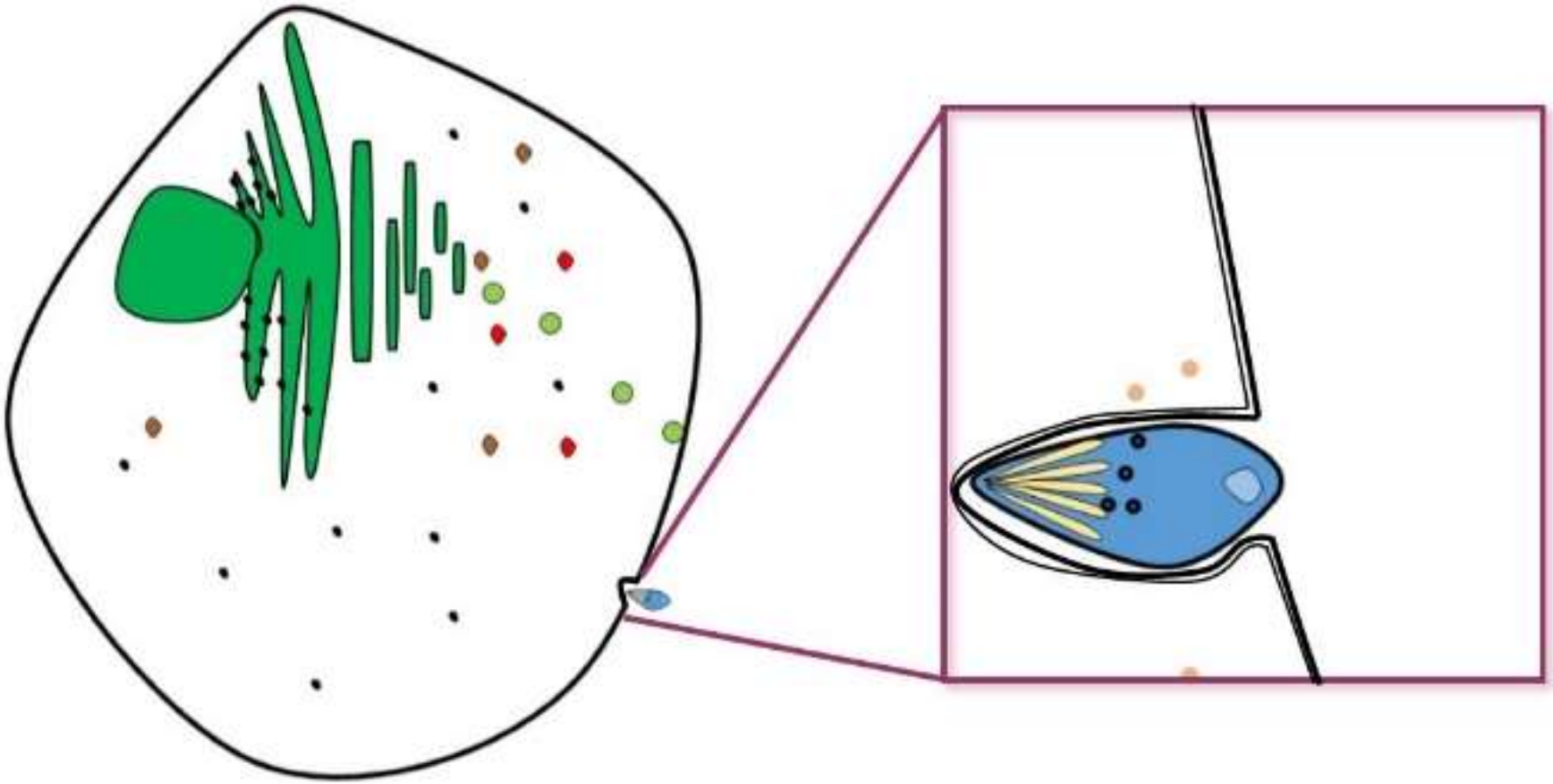

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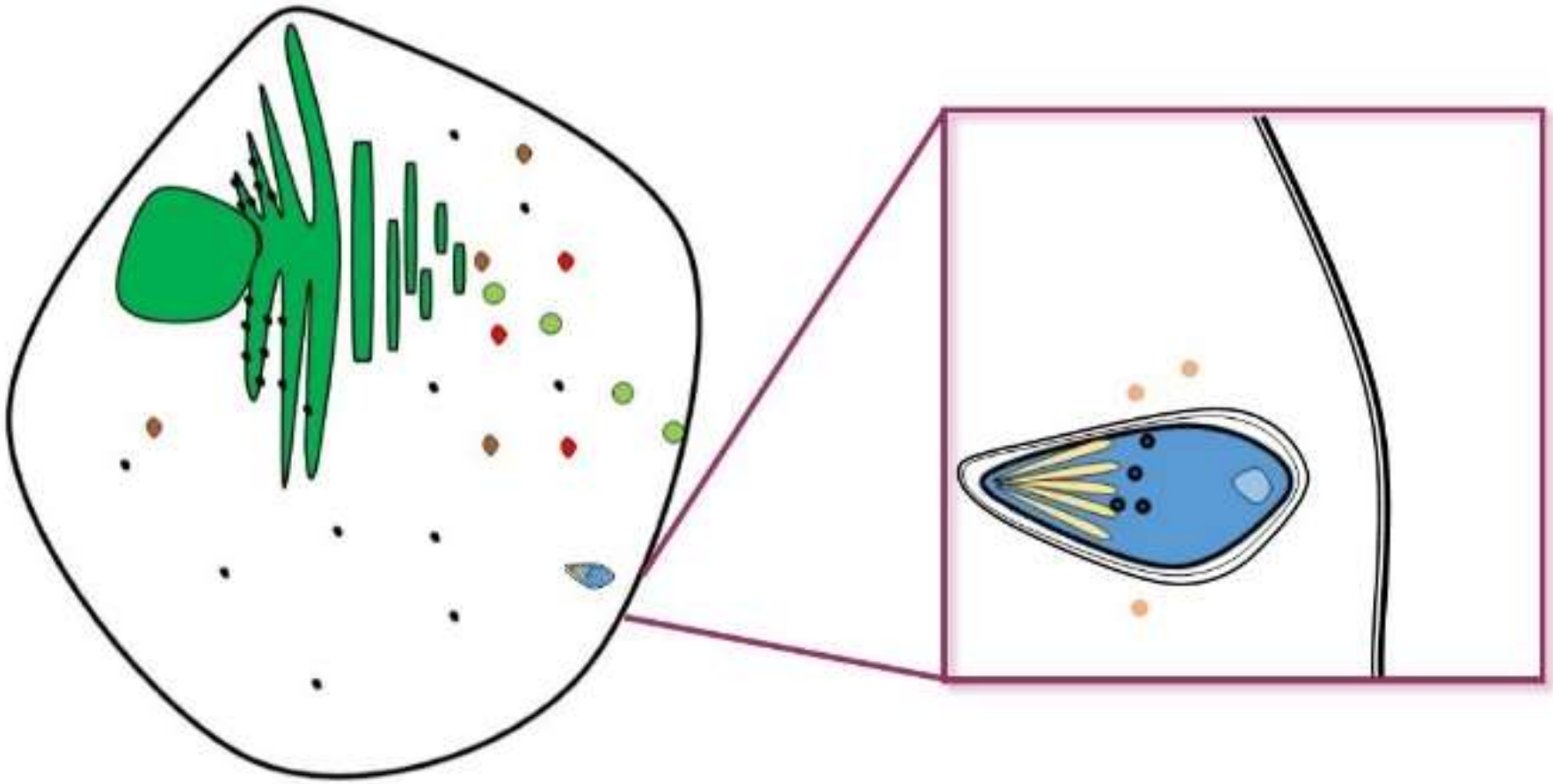

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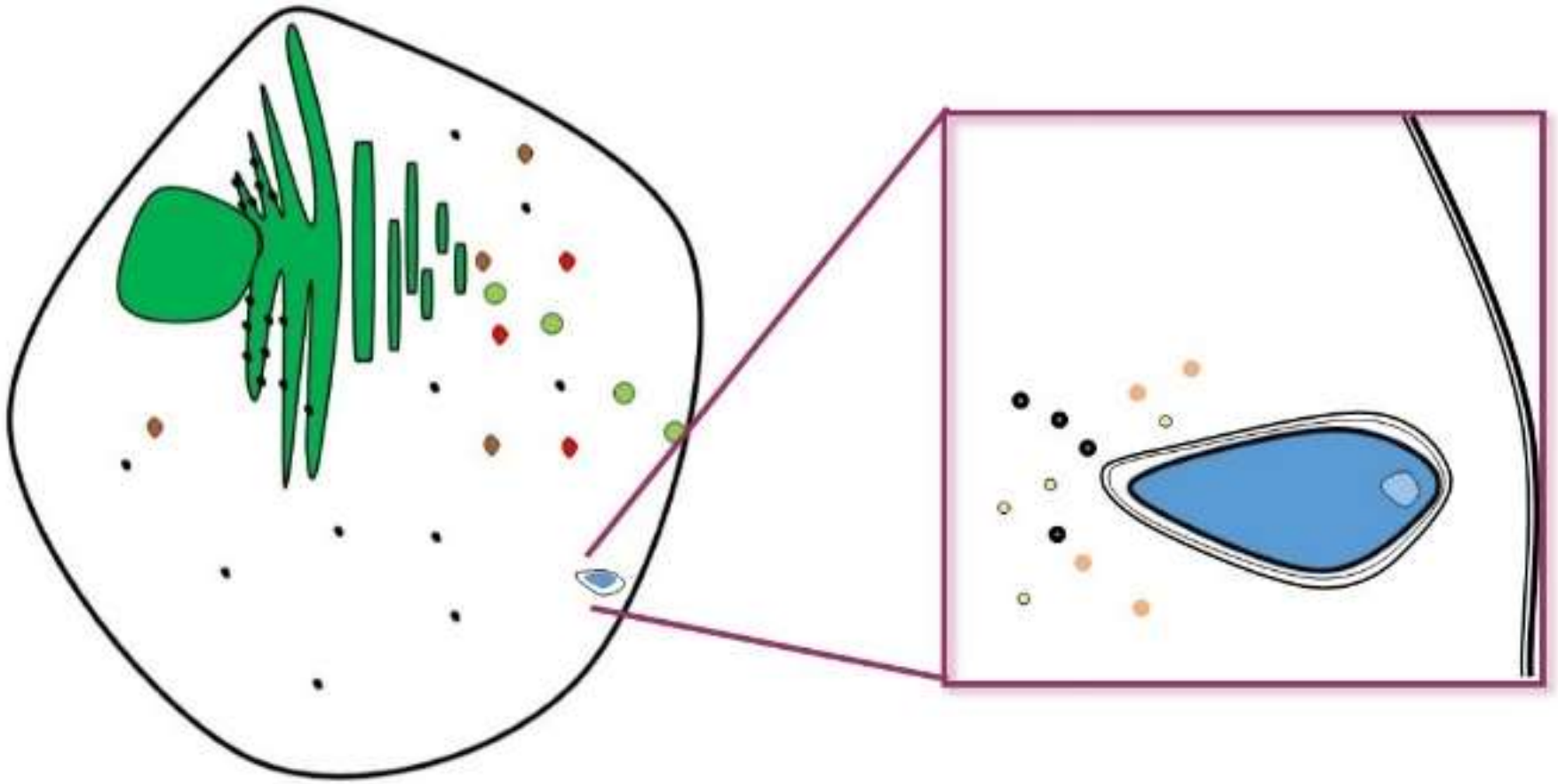

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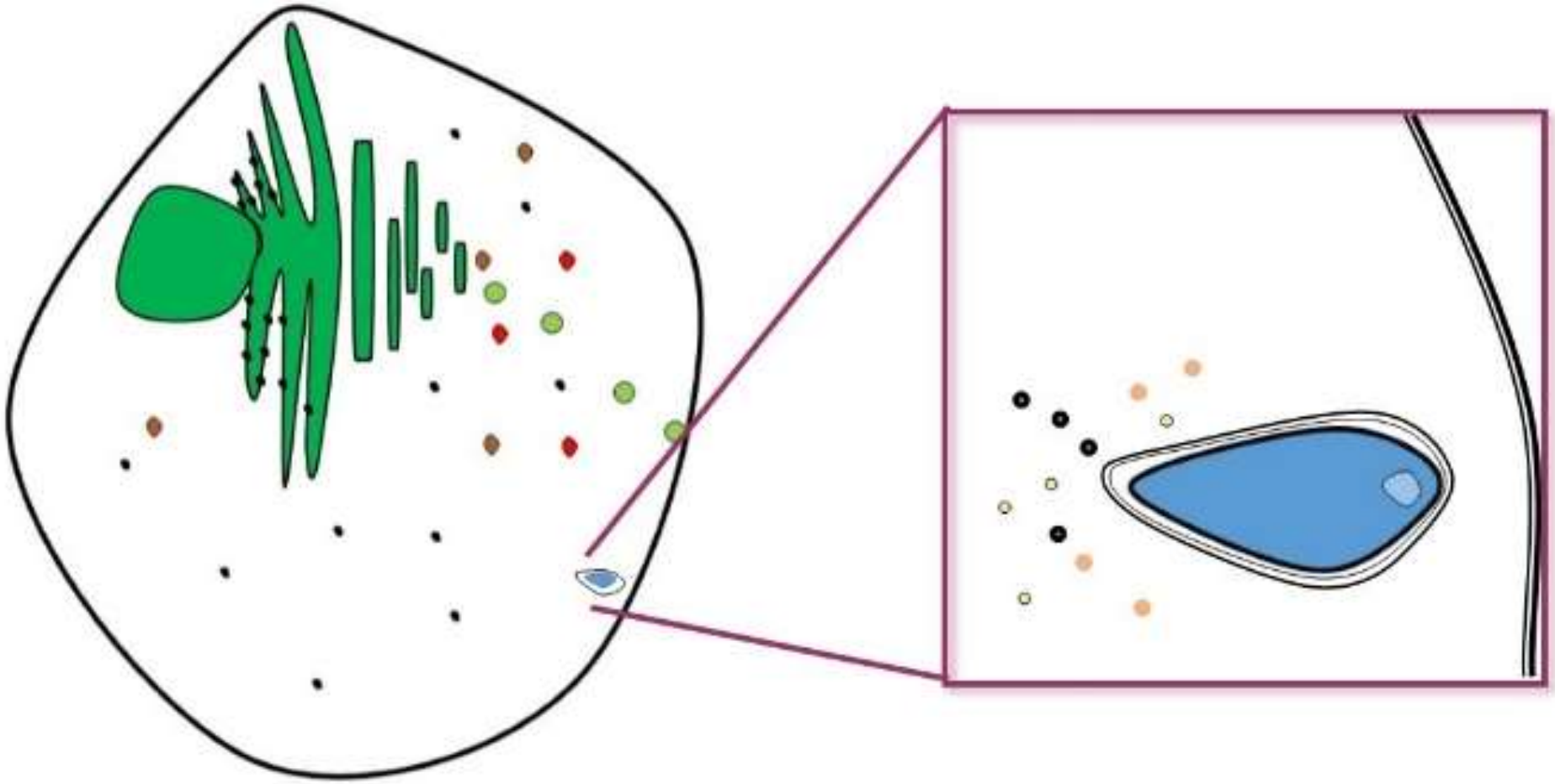

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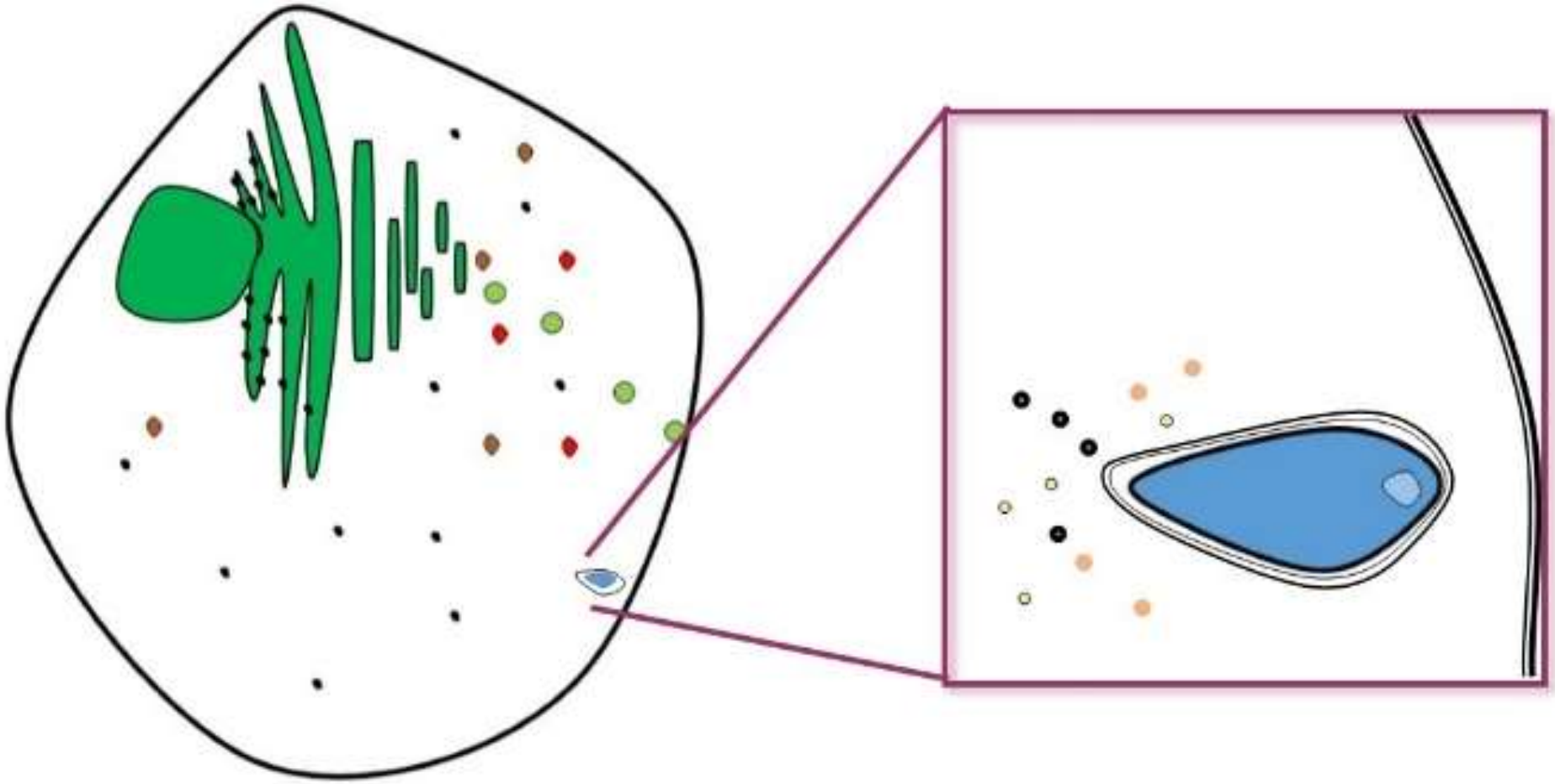
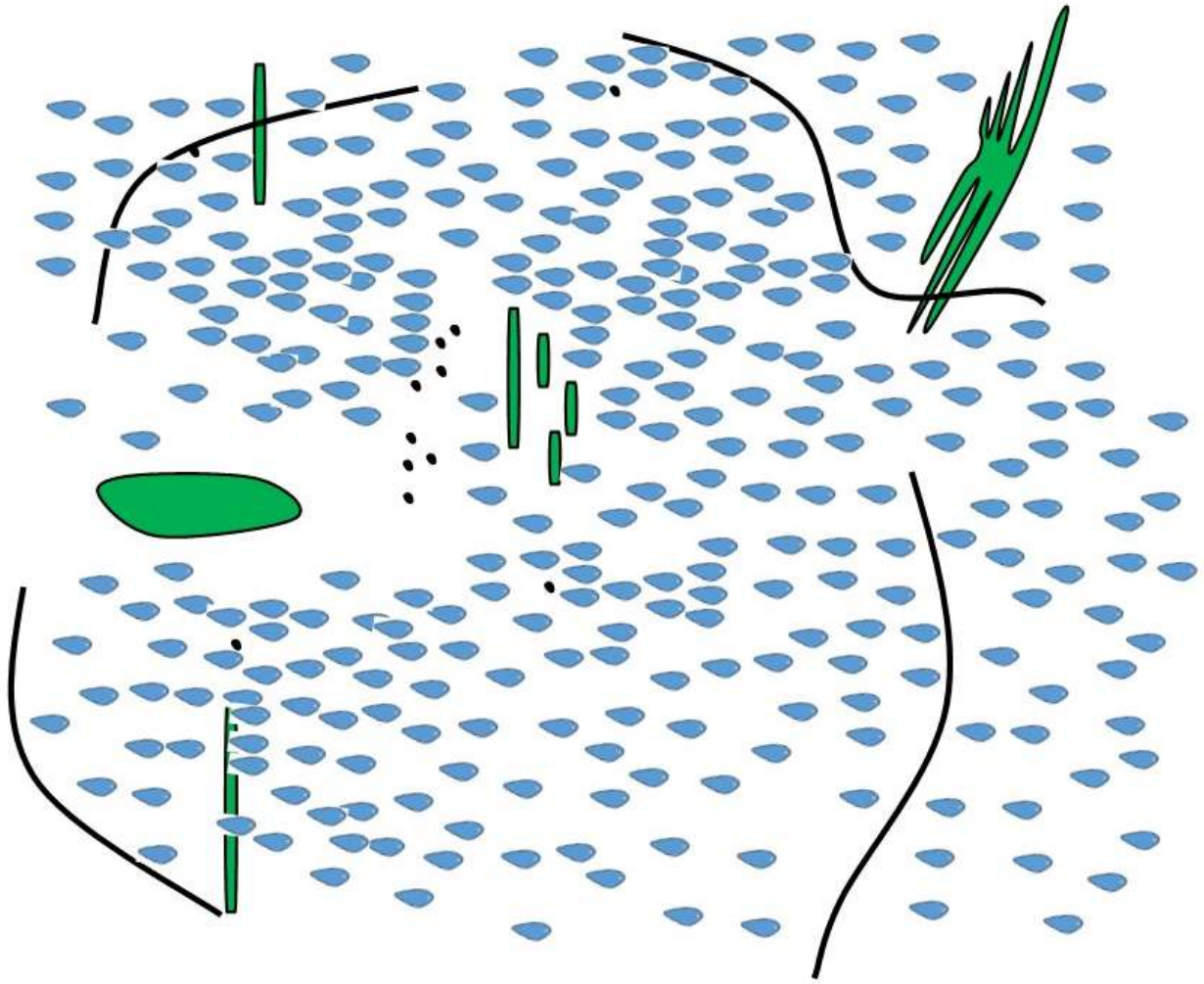
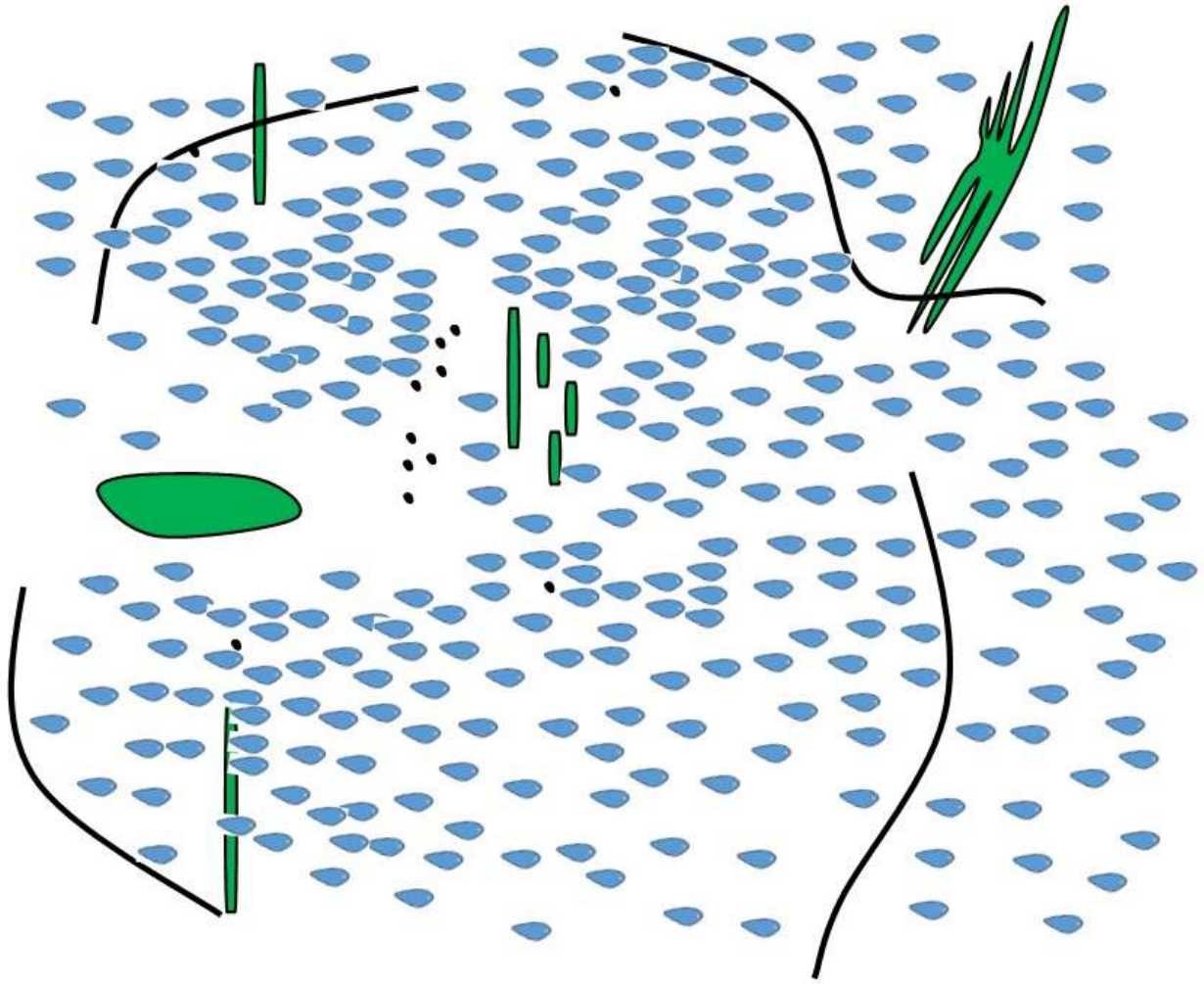


1c. Intracellular Growth



1. Parasite grows rapidly
2. Nutrients redirected to parasitophorous vacuole
3. Necrotic cell lysis

1c. Intracellular Growth



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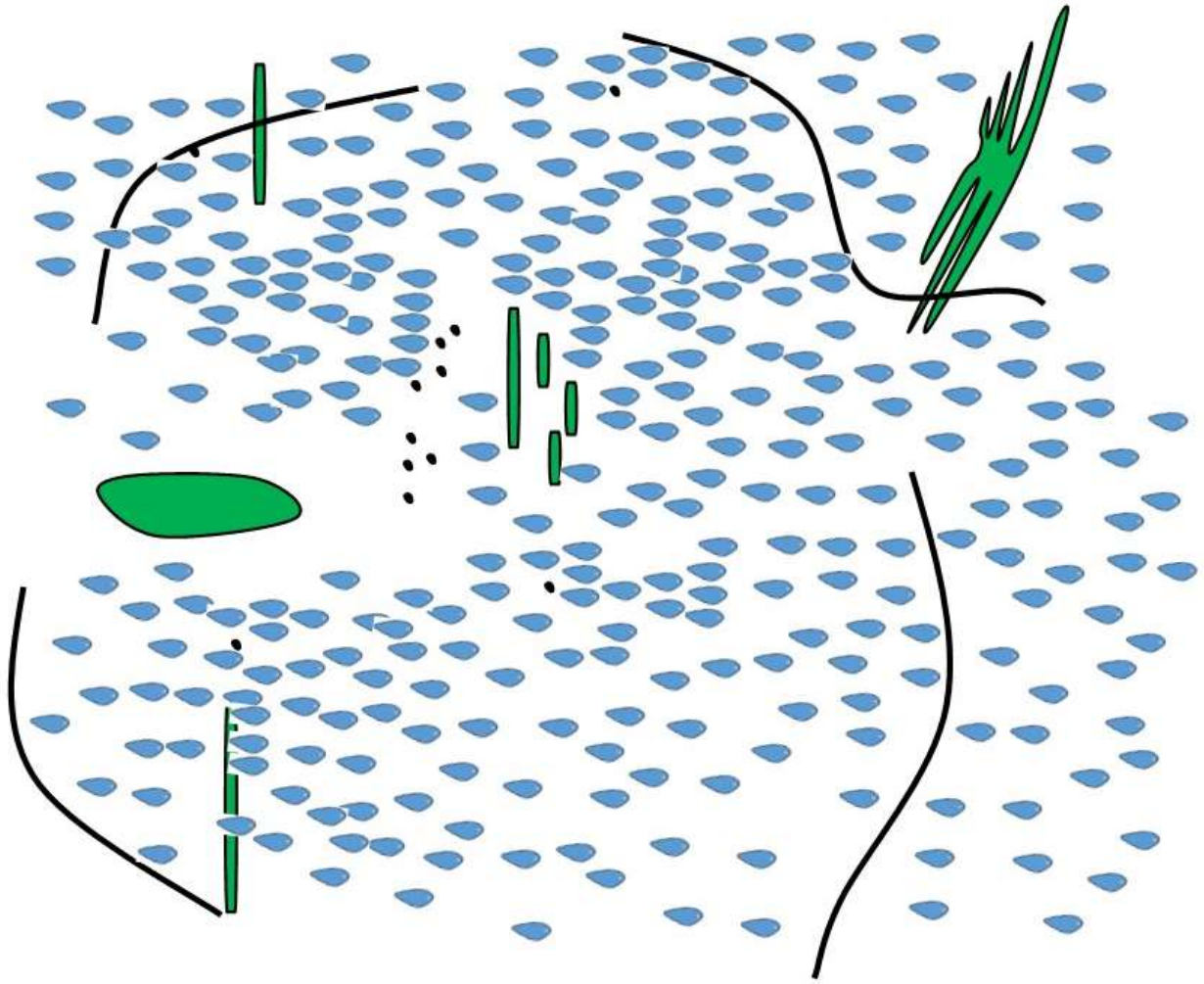

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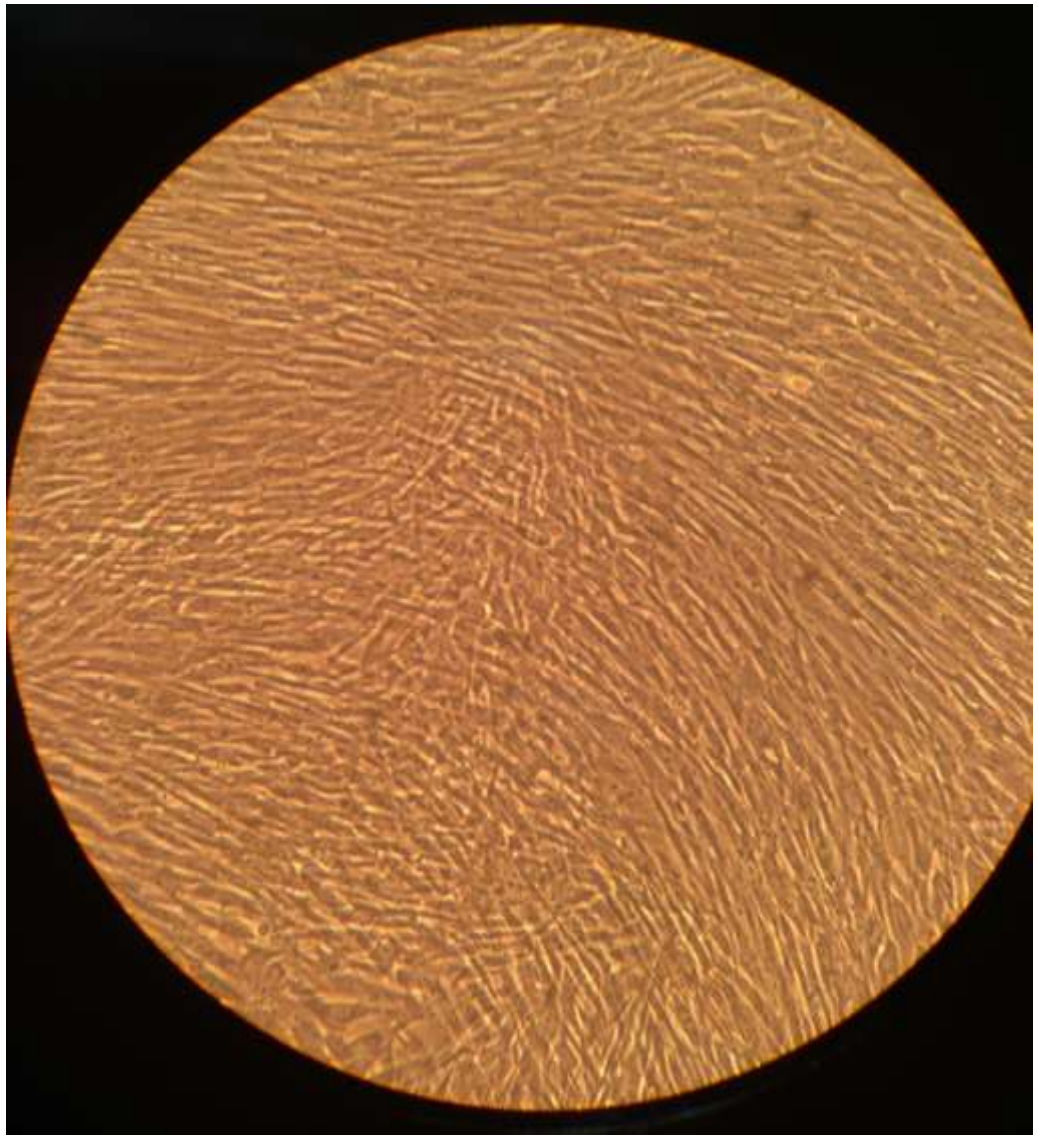
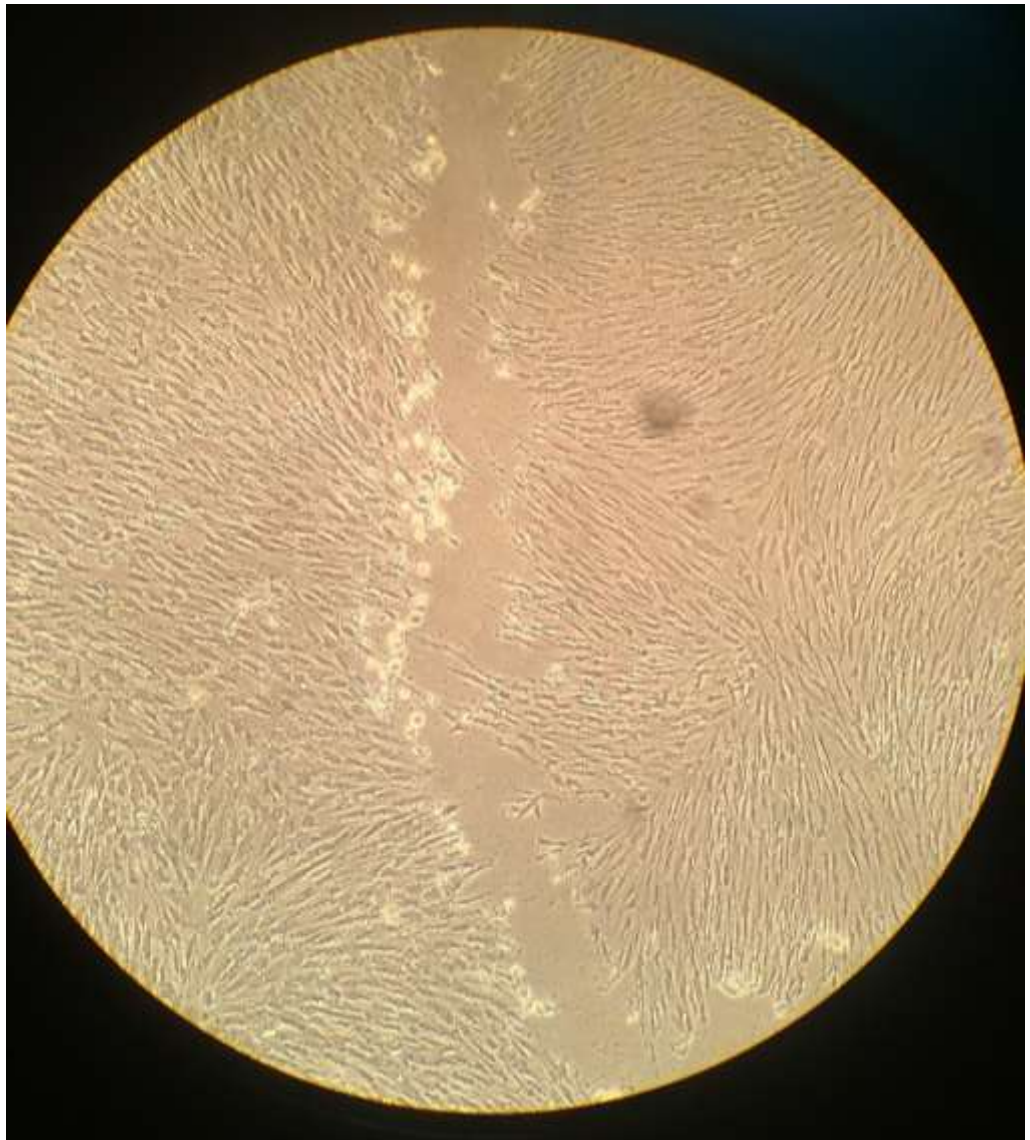


Our Infection Model:

MRC5 Human Lung Fibroblasts

Photo credit: S. L. Denton, Gigley Lab, University of Wyoming

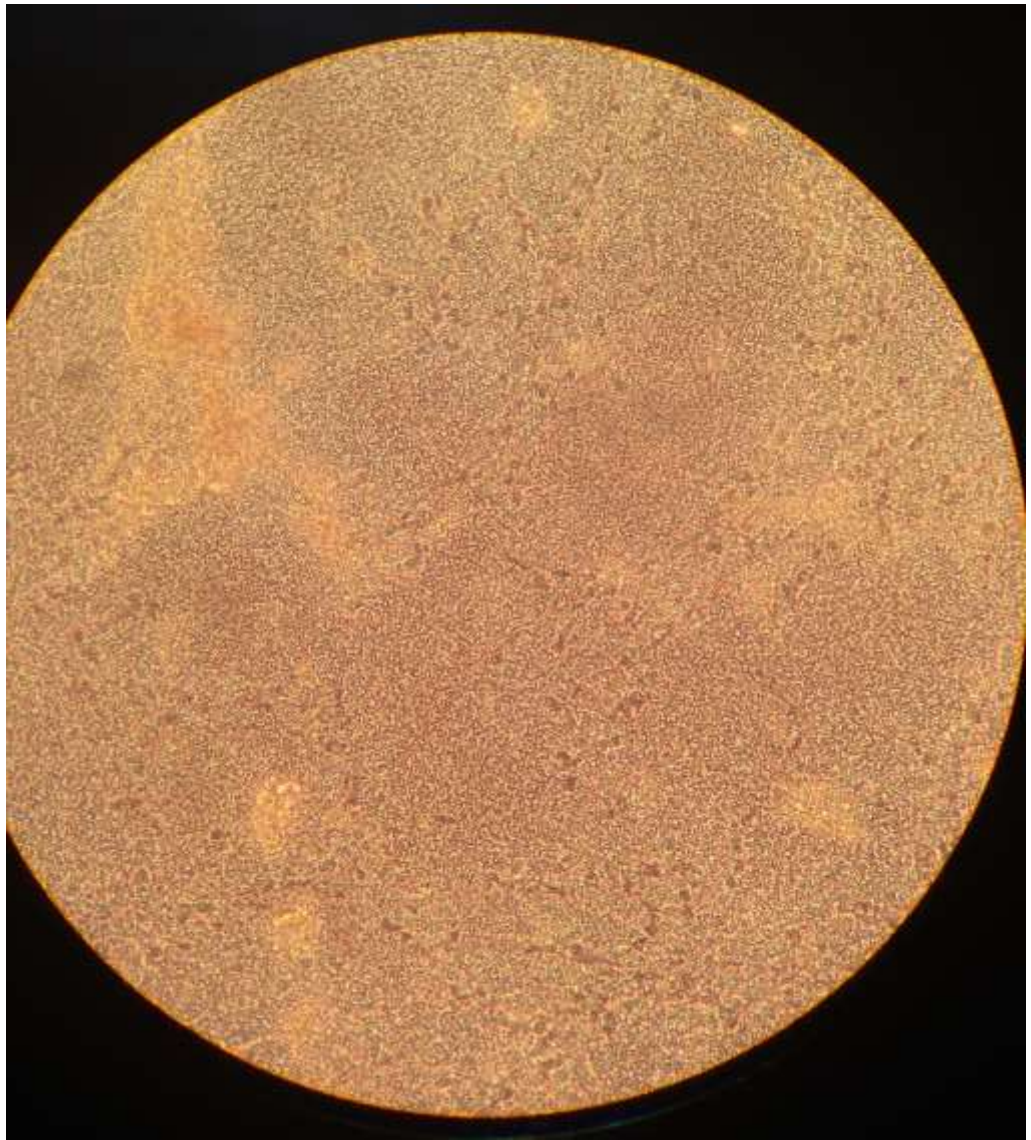
1c. Intracellular Growth



Plane of infection

Photo credit: S. L. Denton, Gigley Lab, University of Wyoming

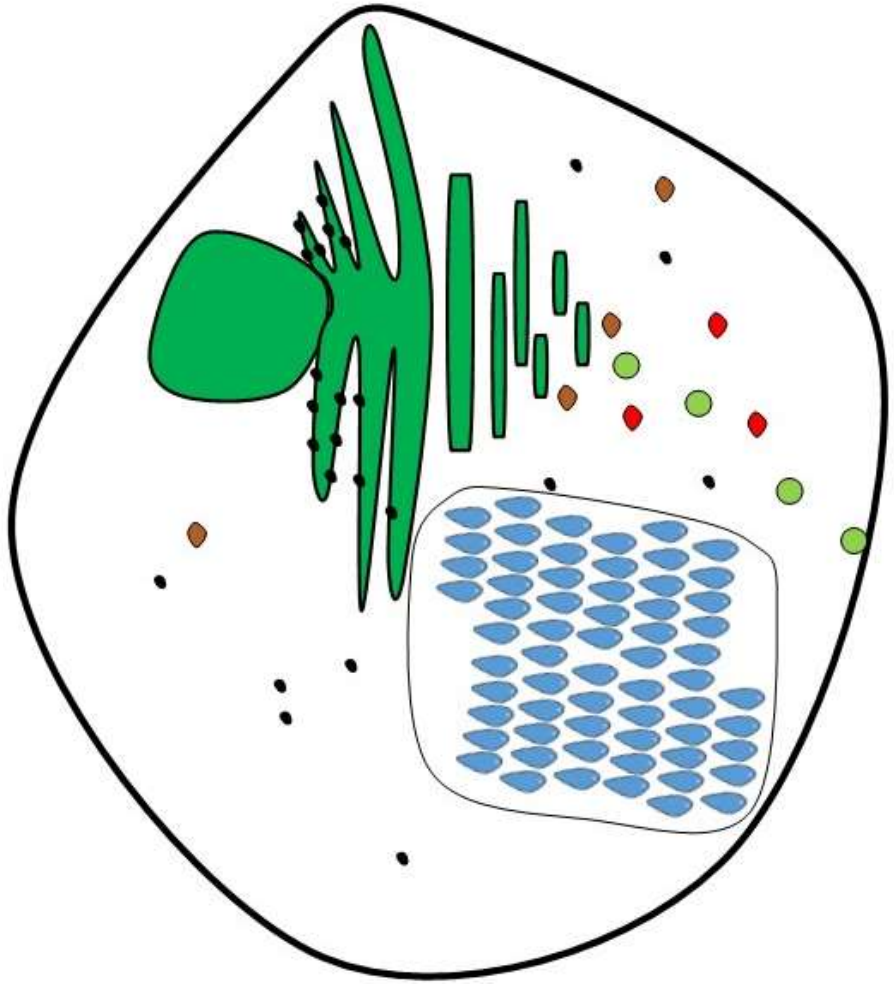
1c. Intracellular Growth



Near Complete Infection

Photo credit: S. L. Denton, Gigley Lab, University of Wyoming

1c. Intracellular Growth



<u>The parasite must accomplish three goals to survive:</u>

1. Acquire nutrients
2. Avoid host-mediated autophagic destruction[1]
3. Enhance parasitophorous vacuole to accommodate growing numbers.

[1]Muniz-Feliciano, L. et al. 2013. *Toxoplasma gondii*-Induced Activation of EGFRPrevents Autophagy Protein-Mediated Killing of the Parasite. PLOS Pathogens 9(12): e1003809.

OUR HYPOTHESIS

The proteins contained in the parasite secretome promote the intracellular life cycle of *Toxoplasma gondii* by modifying membrane-membrane fusion in the host cell.

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2. **Specific Aims**
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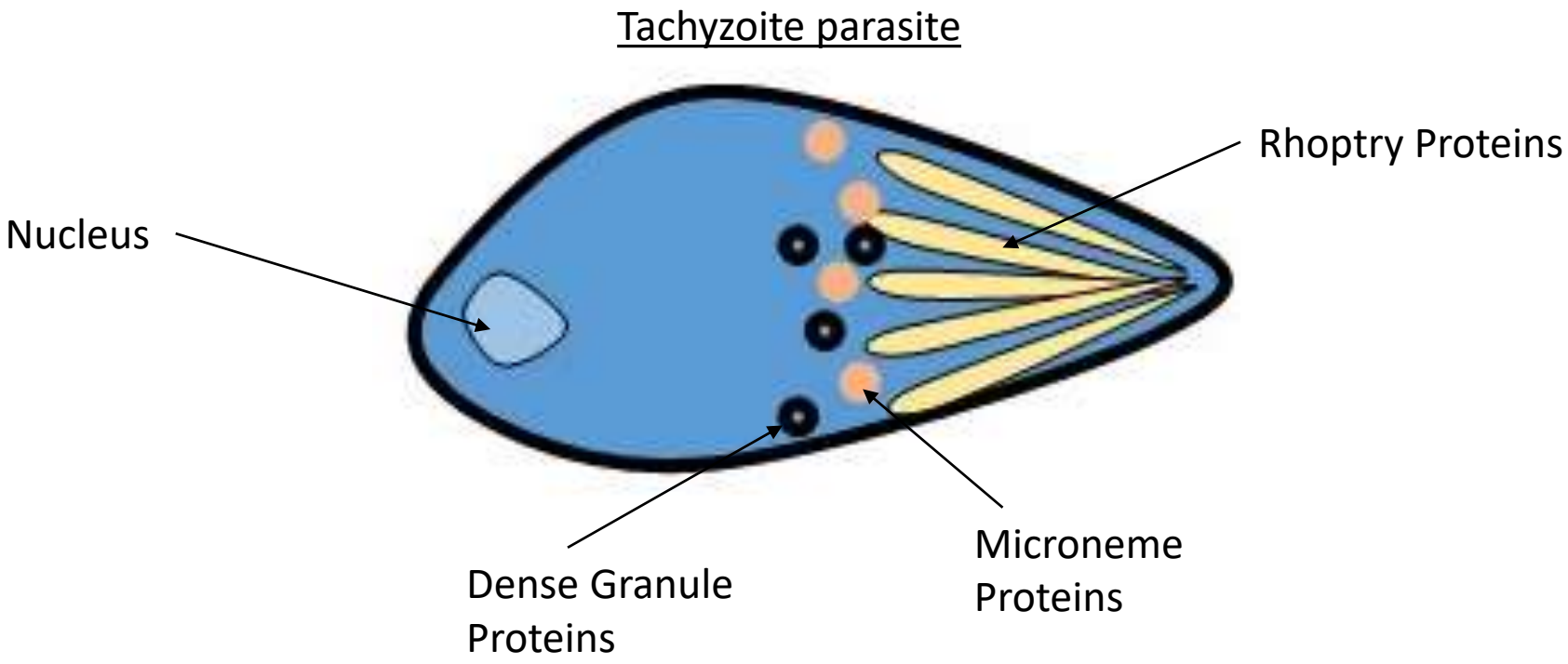
2. Specific Aim 1

To isolate and fractionate and secretome of *Toxoplasma gondii.*



2. Specific Aim 1

- Parasite grown by infection and complete destruction of tissue culture-grown MRC5 Human Lung Fibroblasts.
- Parasites filtered through 3µm pore-sized membranes.
- Parasites pressure-lysed at 1500 psi.
- (Optional) Secretome isolated via differential centrifugation.
- Protein sample treated with detergent to lyse protein organelles.
- Protein sample fractionated via size exclusion chromatography.

2. Specific Aim 2

To identify specific activity of *Toxoplasma gondii* secreted invasion factors on membrane fusion capability.

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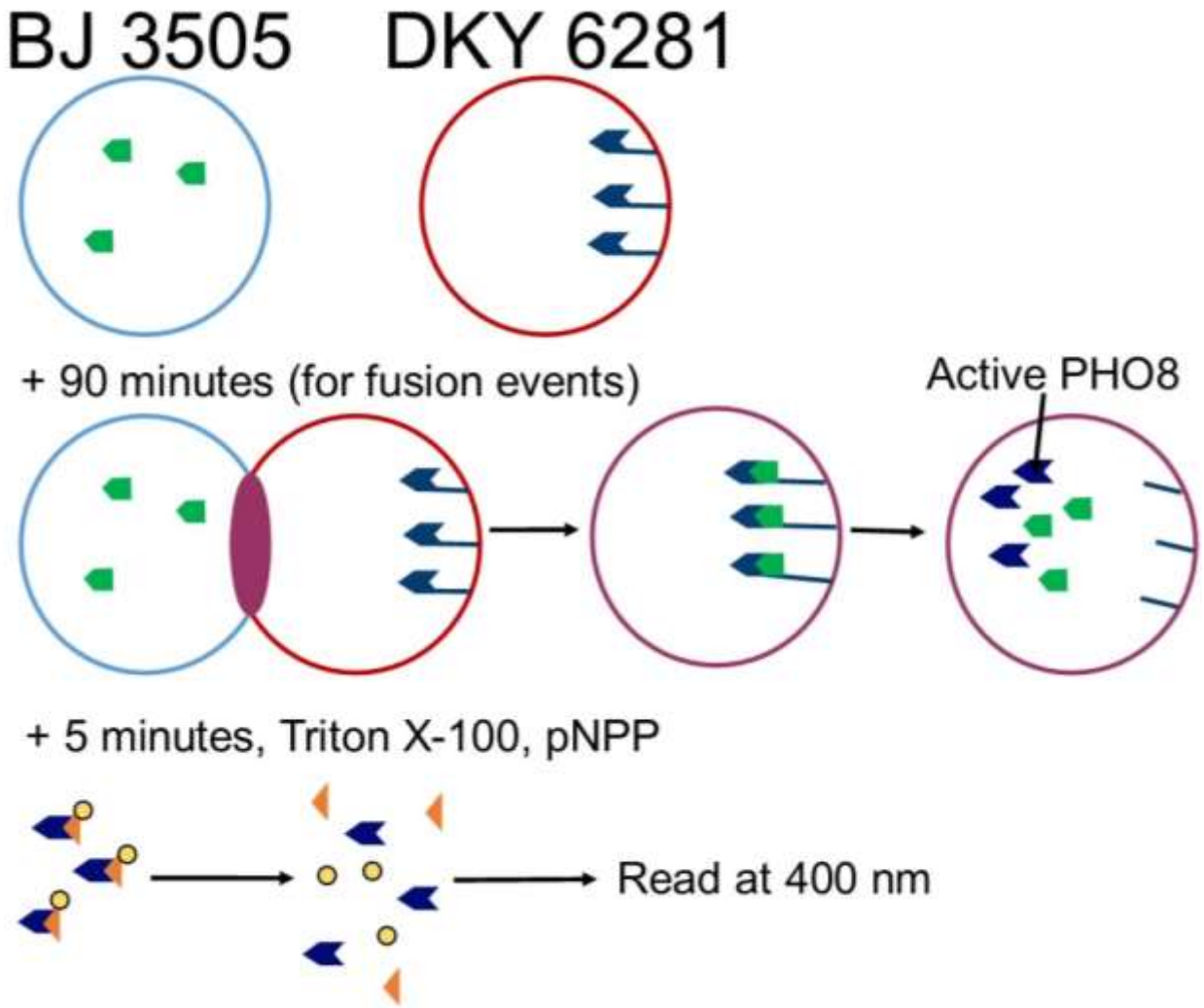
3. The Membrane Fusion Assay

- Model system to quantitatively measure fusion rates[2].
- Essential components
  - Pro-PHO8: Pro-alkaline phosphatase once active converts p-nitrophenyl phosphate (pNPP) to p-nitrophenol (pNP). Not available in *Saccharomyces cerevisiae* strain DKY6201.
  - PEP4: Protease specific to Pro-PHO8. Not available in *Saccharomyces cerevisiae* strain BJ3505.
  - pNPP: When converted to pNP, absorbs light at 400nm according to concentration, producing a yellow color.

[2]iWckner, W. 2010. Membrane Fusion: Five Lipids, Four SNAREs, Three Chaperones, Two Nucleotides, and a Rab, All Dancing in a Ring on Yeast Vacuoles. Annu. Rev. Cell. Dev. Biol. 26:115-36.

3. The Membrane Fusion Assay



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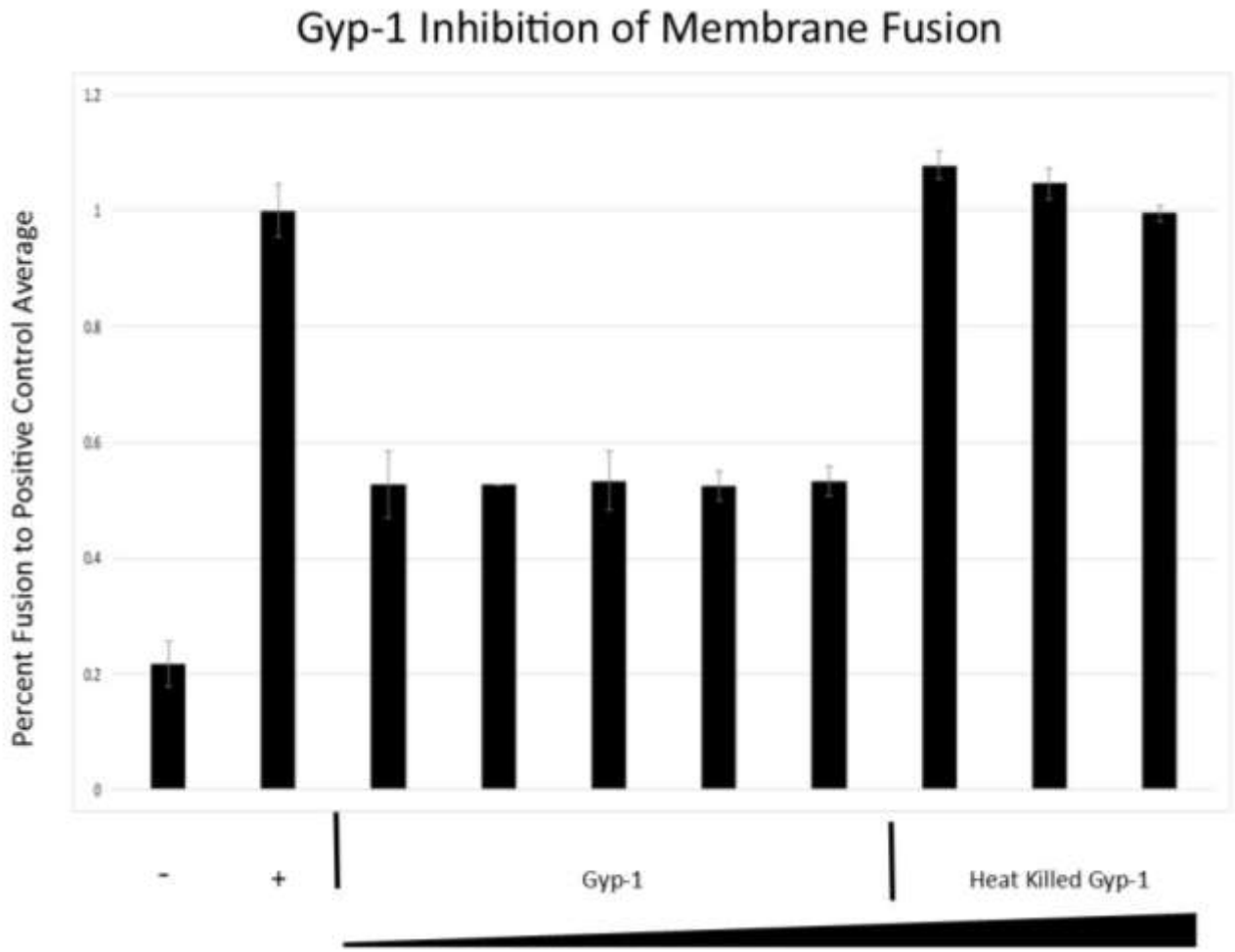

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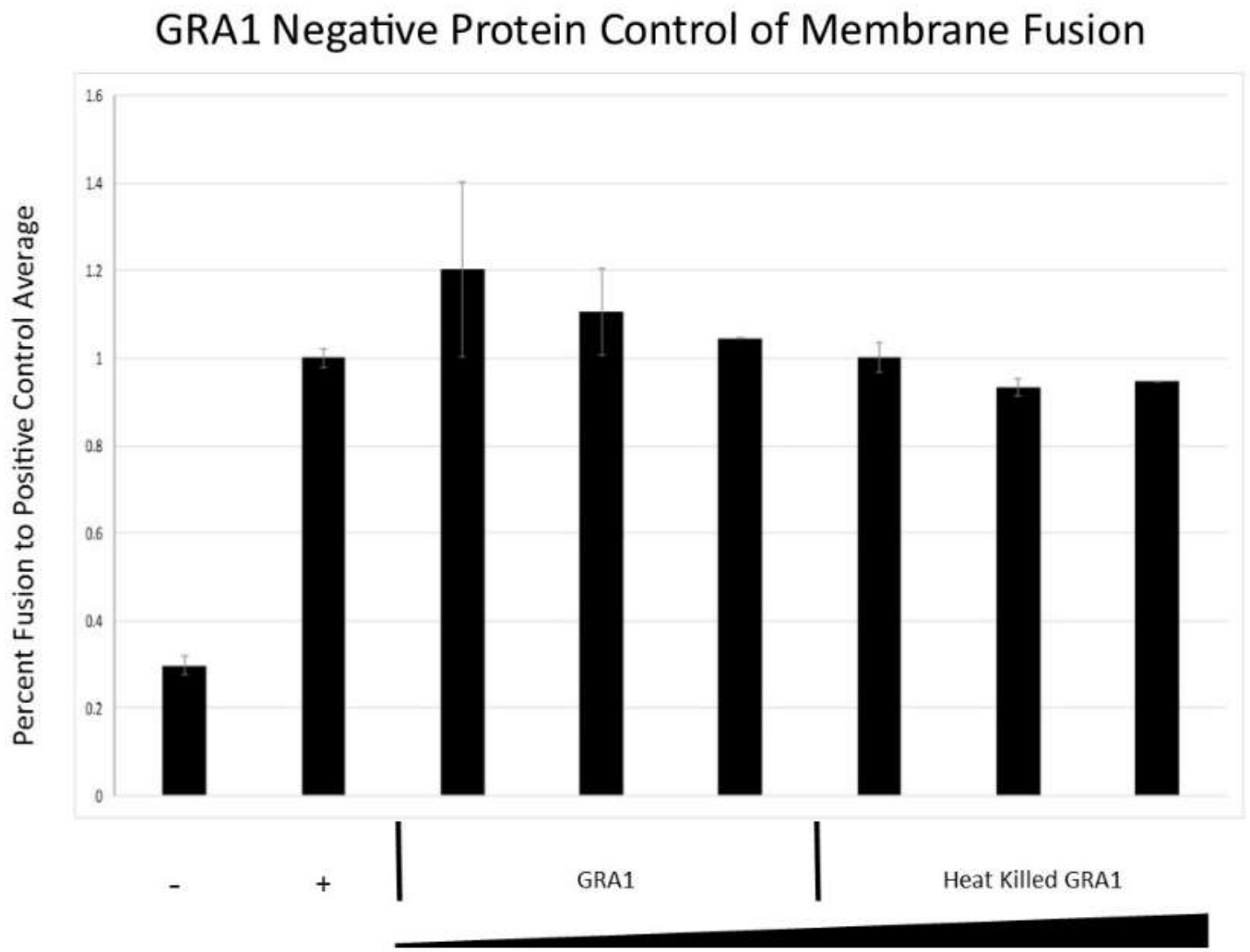
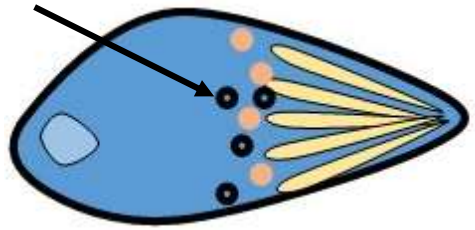
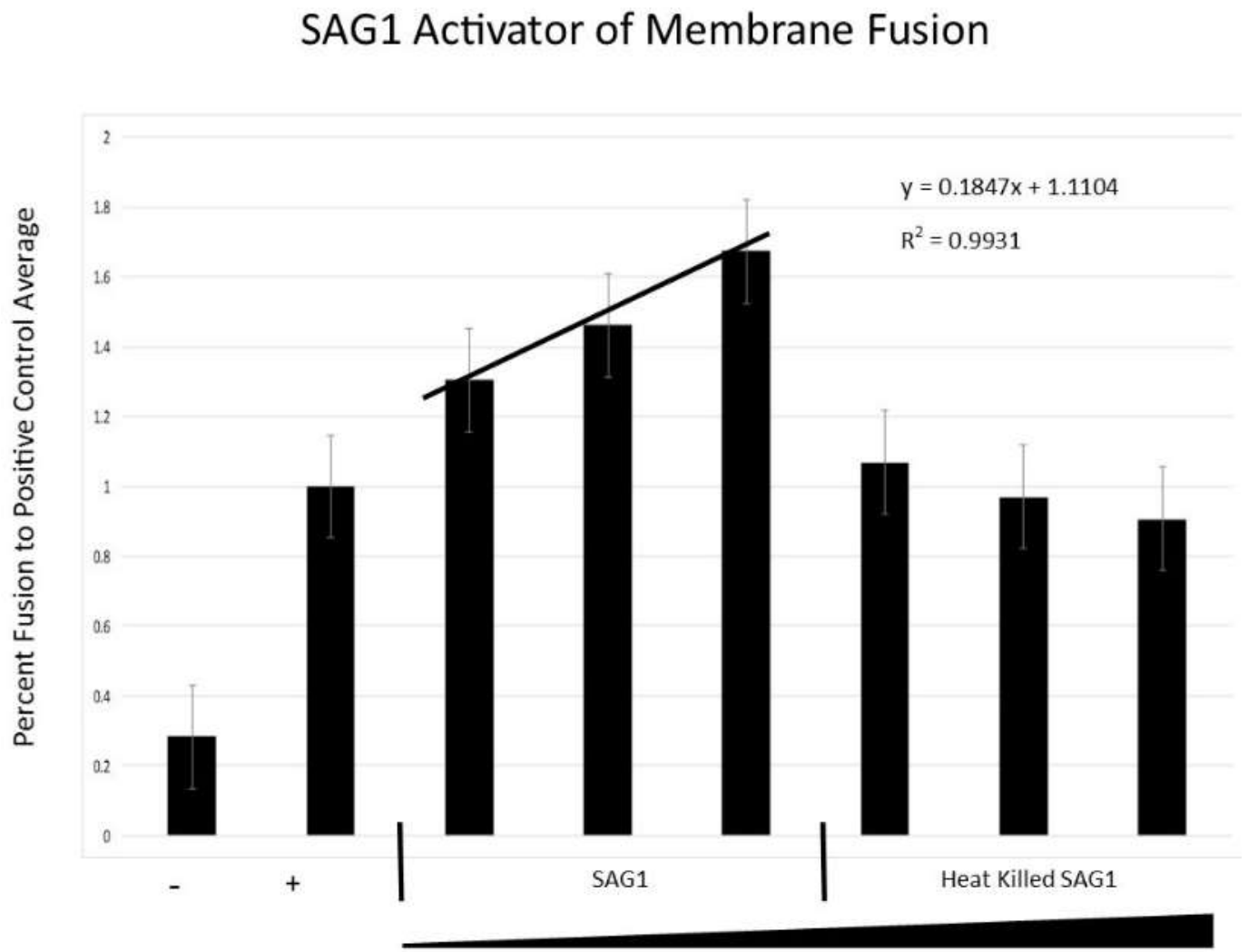
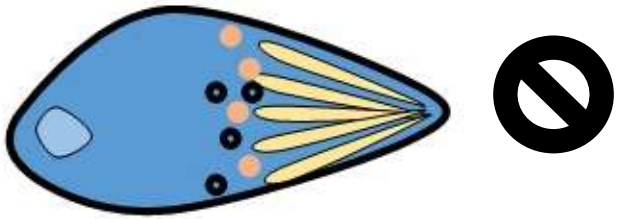

GRA1 Negative Protein Control of Membrane Fusion
Percent Fusion to Positive Control Average
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-
+
GRA1
Heat Killed GRA1



4. Results





SAG1: Gene encoding primary *T. gondii* antigen p30

Ongoing Operations

- Collection of *T. gondii* derived protein.
- Fractionation of collected protein.
  - Four distinct groups according to size have been collected in a manner that does not require denaturation or dissolving protein complexes.
- Evaluation of fractionated protein in the Membrane Fusion Assay.
- Identification of single proteins or protein complexes that modify membrane fusion.
- Identification of proteins restricted to parasitophorous vacuole localization.
- Biochemical analysis of identified proteins in vivo and in vitro.

Acknowledgements

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