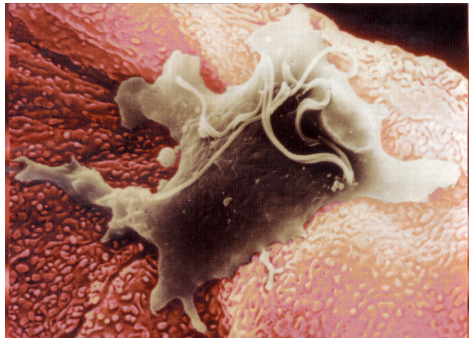
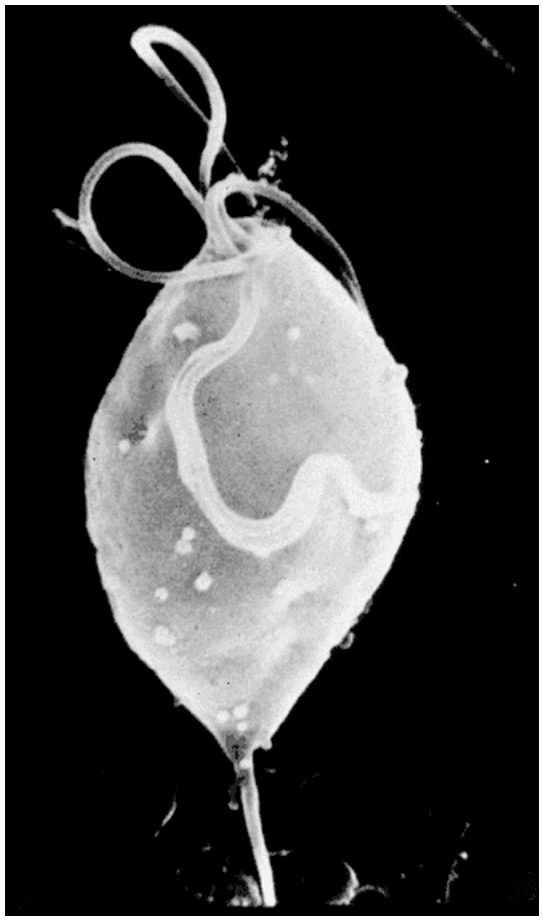


Cysteine Proteases of *Trichomonas vaginalis*

Trichomonas vaginalis



- Facultative anaerobe

- Flagellated protozoan with 5 flagella: 4 located at the anterior end and 5th is incorporated within the undulating membrane of the parasite

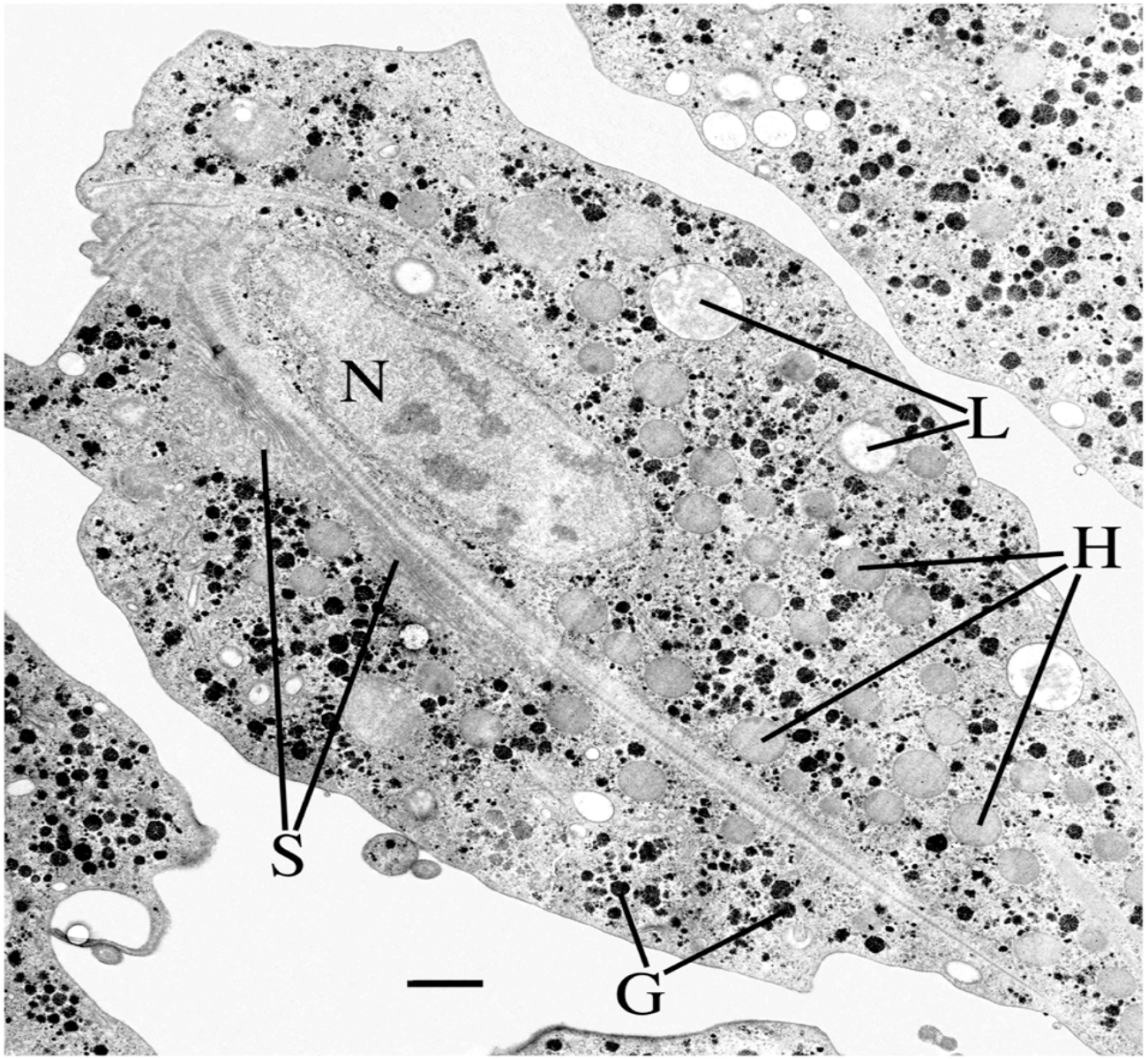
- Lacks peroxisomes and mitochondria

- Contains lysosomes

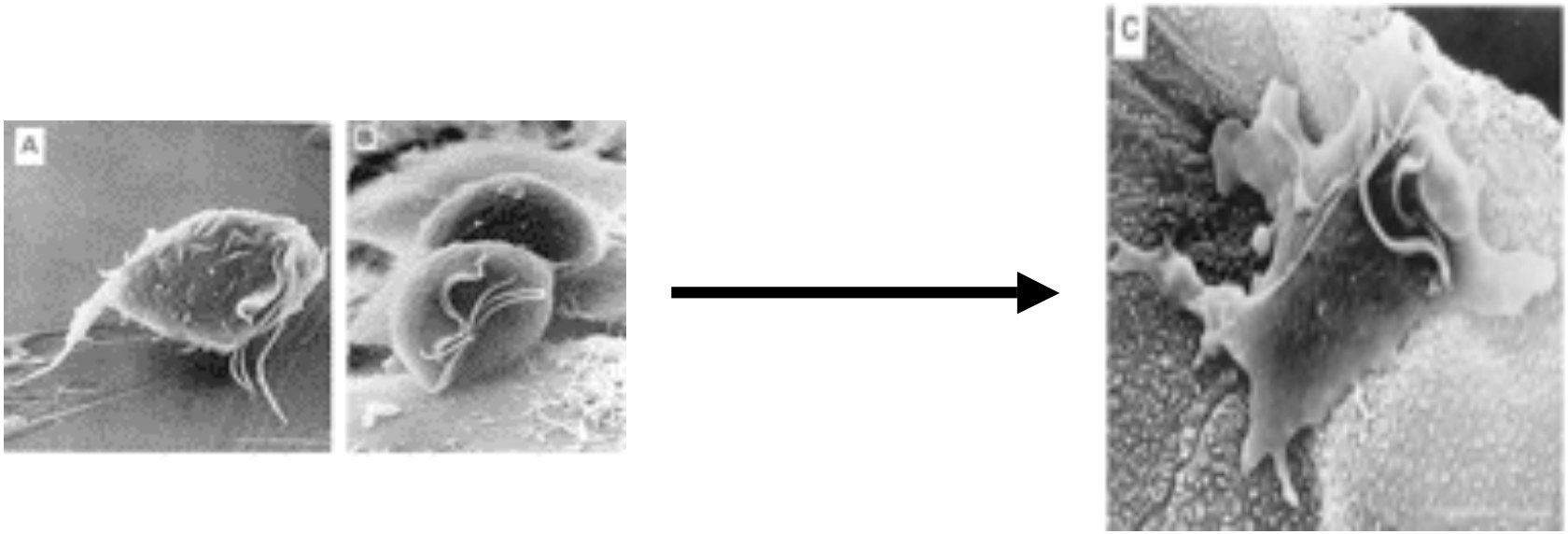
- Contains hydrogenosomes
  - -involved in carbohydrate metabolism
  - -produces ATP and H_2

- Early-evolving g eukaryote

Generalized Cell Structure of *Trichomonas vaginalis*



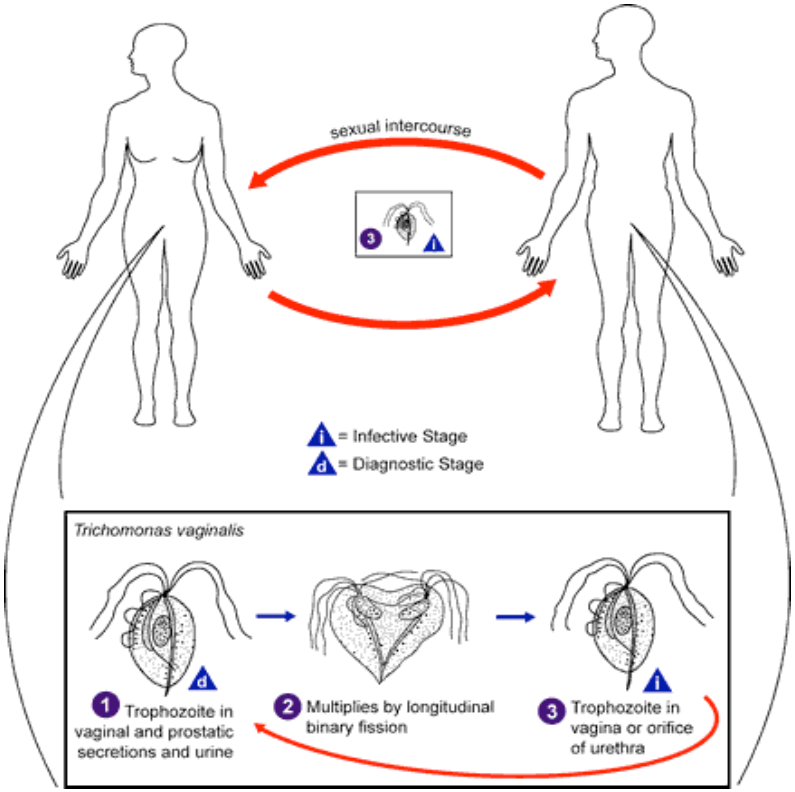
Amoeboid transformation



Free swimming form

Amoeboid form

Lifecycle of *Trichomonas vaginalis*



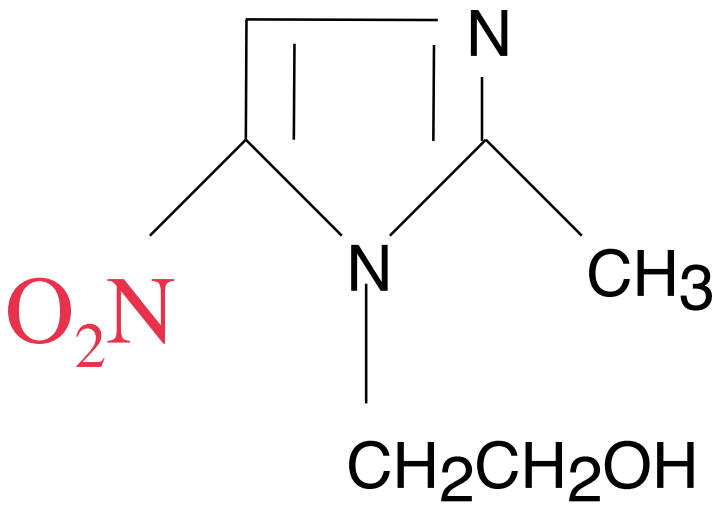
Features of Trichomoniasis

- Most common non-viral STD
- 200 million worldwide and 7-8 million in North America alone
- Clinical symptoms reported in 50% of infected females
- Infected males are often asymptomatic
- Causative agent for many complications during pregnancy
- Increasingly associated with HIV in females
- Drug resistant strains are becoming more frequent reported

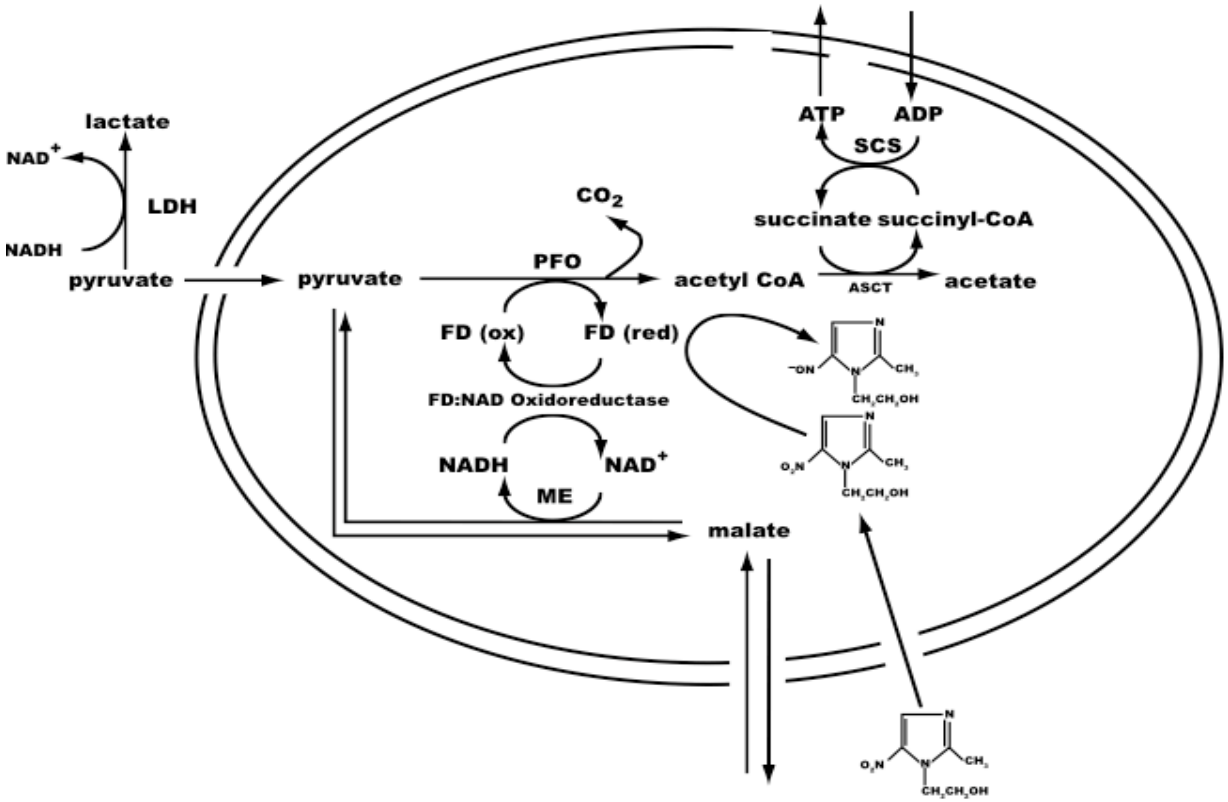
Mode of Action of Flagyl

- Metronidazole is administered in an inactive form
- Activation occurs in the parasite in a unique organelle called the hydrogenosome
- It is the activated form of the drug that confers its cytotoxic effects

Metronidazole

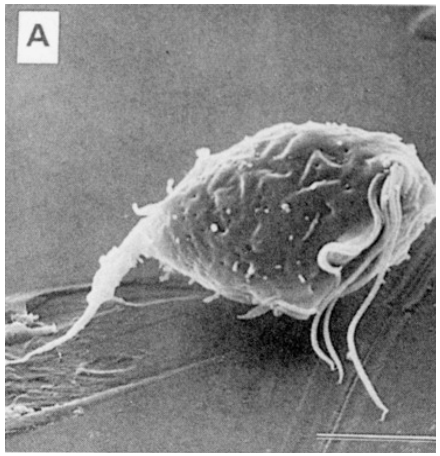


Activation of Metronidazole in the Hydrogenosome of *T.vaginalis*

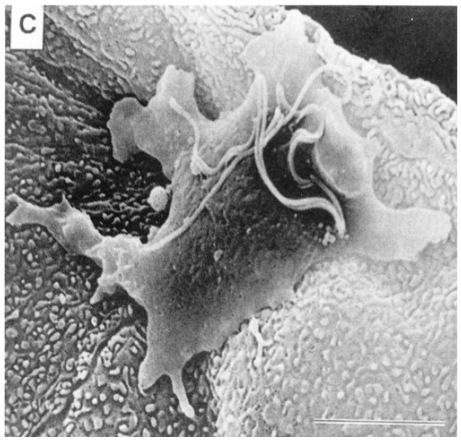


Morphological Transformation

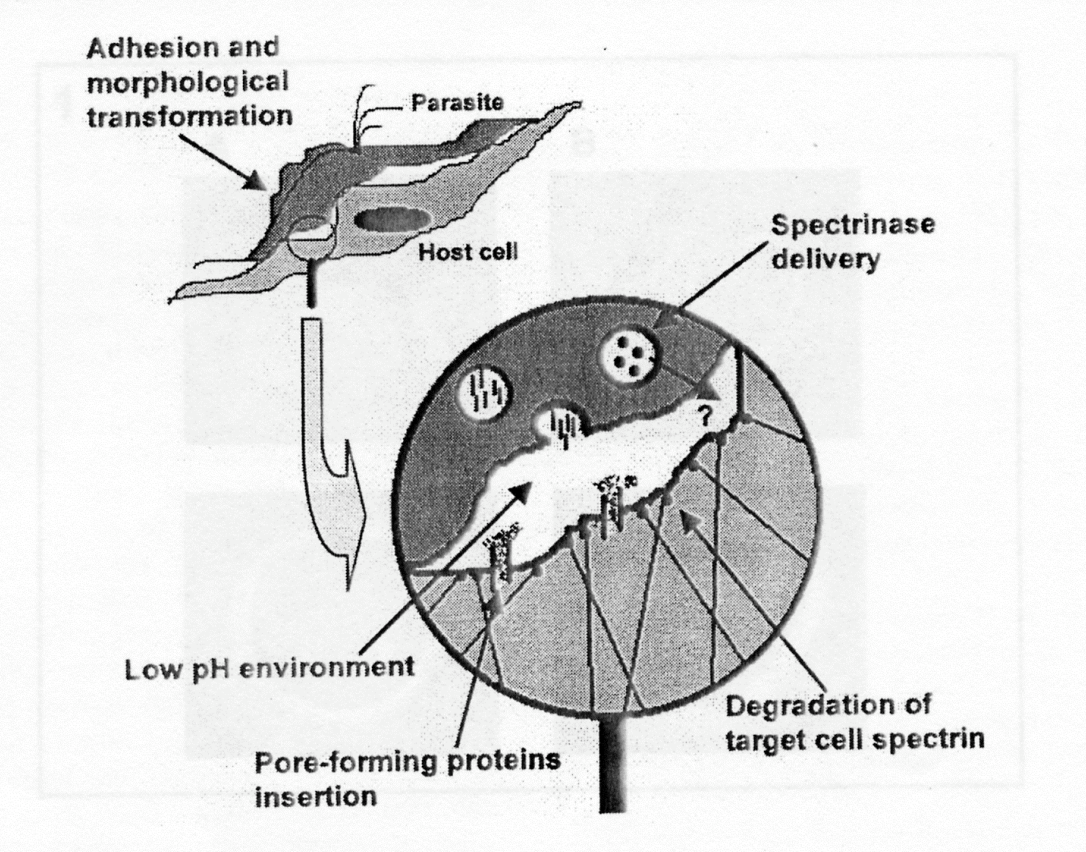
- Free swimming form



- Amoeboid form



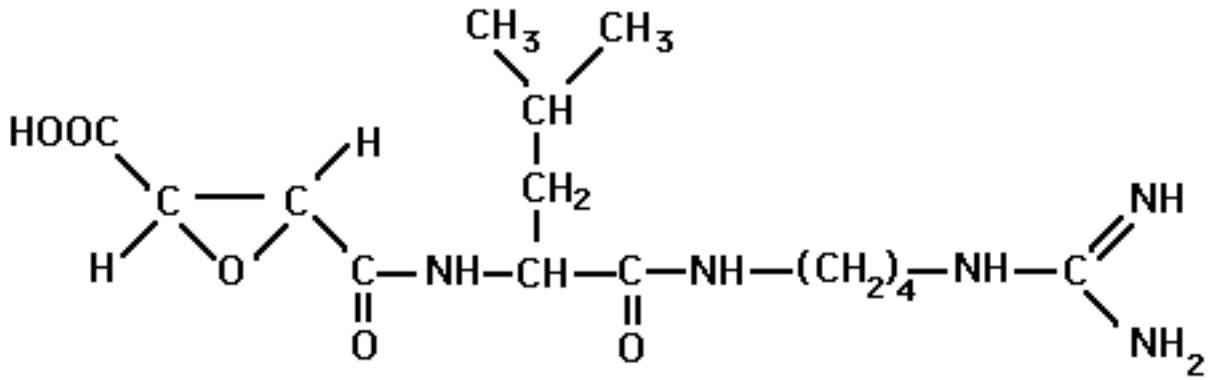
Adhesion and Host-Parasite Interactions



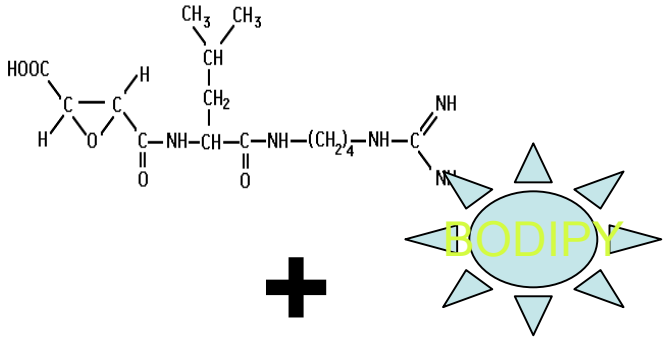
Experimental Hypothesis

- *Trichomonas vaginalis* cells contain CPs that biologically function in host cell pathogenesis.

Broad Spectrum Irreversible Cysteine Protease Inhibitor E-64

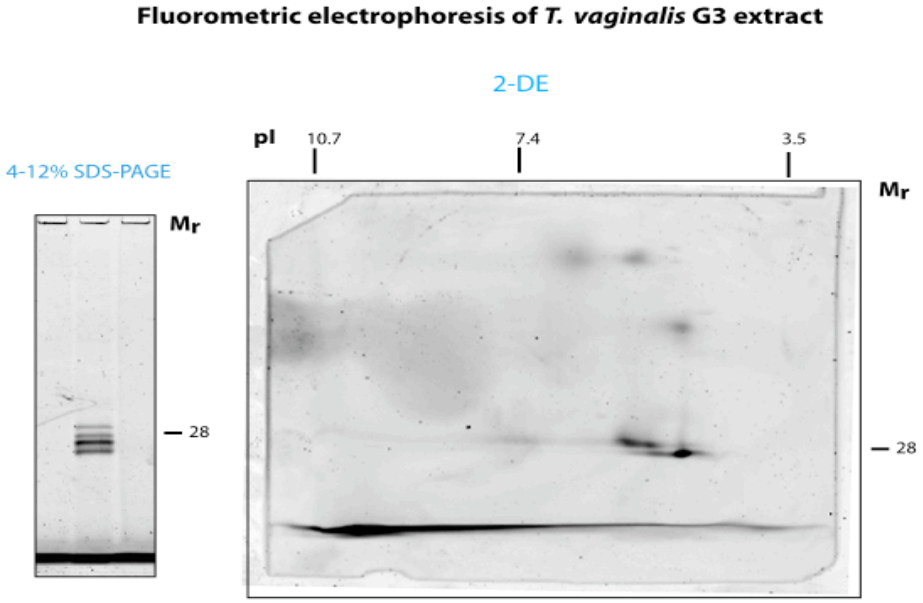


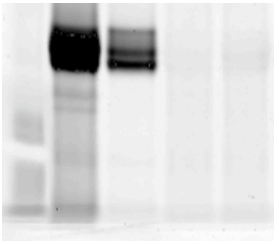
Proteomic Labeling of CPs in *T. vaginalis* G3 Strain



Extracts of Cells

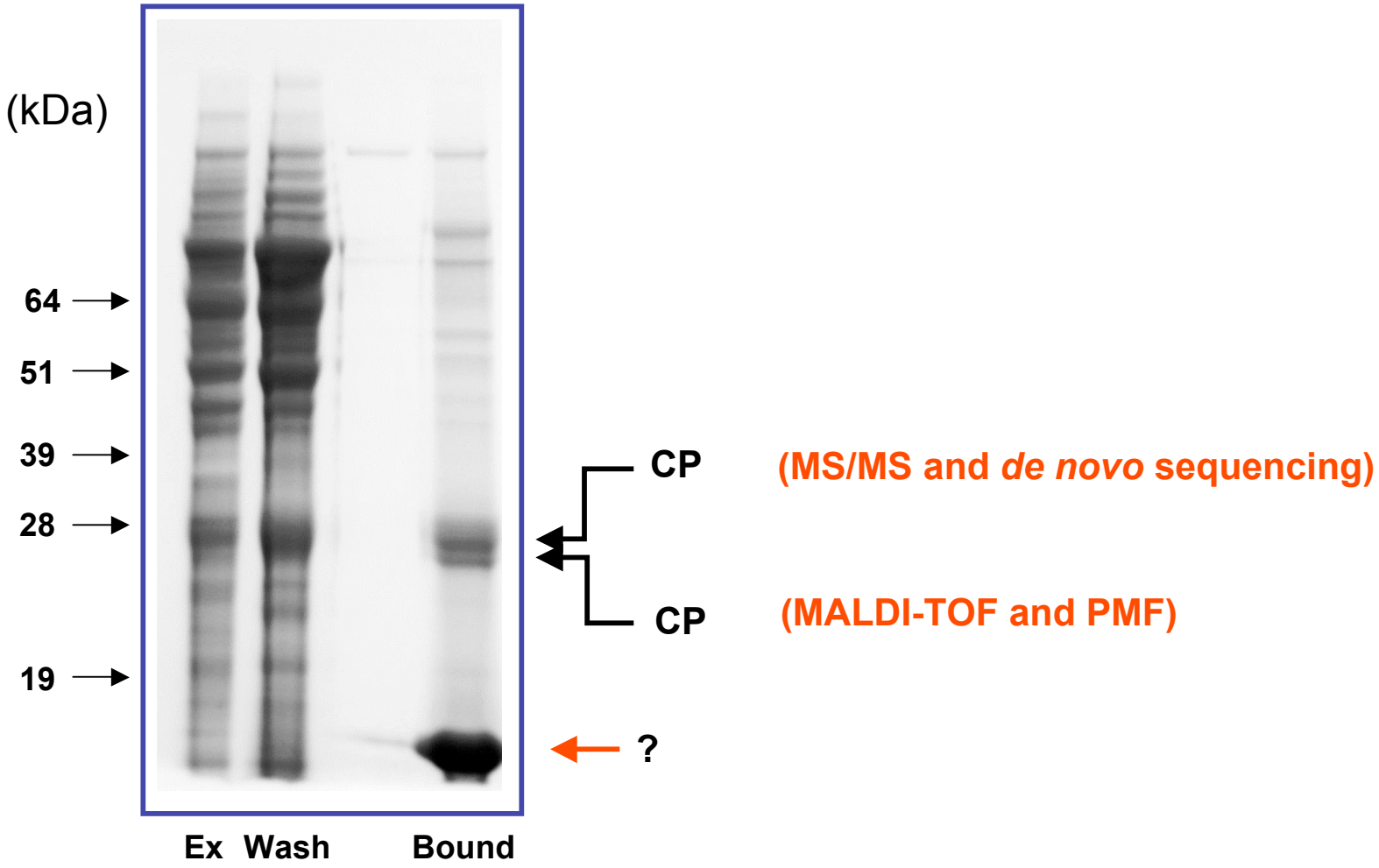
Label Cysteine Proteases
Run on 12% NuPAGE Gel
Scan at 555 nm





DTT DTT DTT
K77 E64

Purification of *T. vaginalis* CPs using DCG-04 and Strepavidin-Sepharose



Biological Function: Pathogenesis and Virulence

- Cell to cell adhesion
- Release of extra-cellular factors
- Hemolysis

Cysteine Proteinases

- *T. vaginalis* produces the greatest quantity of cysteine proteases (CPs) compared to other parasitic protozoa

- Possible roles of CPs in pathogenesis include:
  1. Adhesion to vaginal epithelial cells
  2. Lysis of erythrocytes
  3. Degradation of host immunoglobulins

Experimental Hypothesis

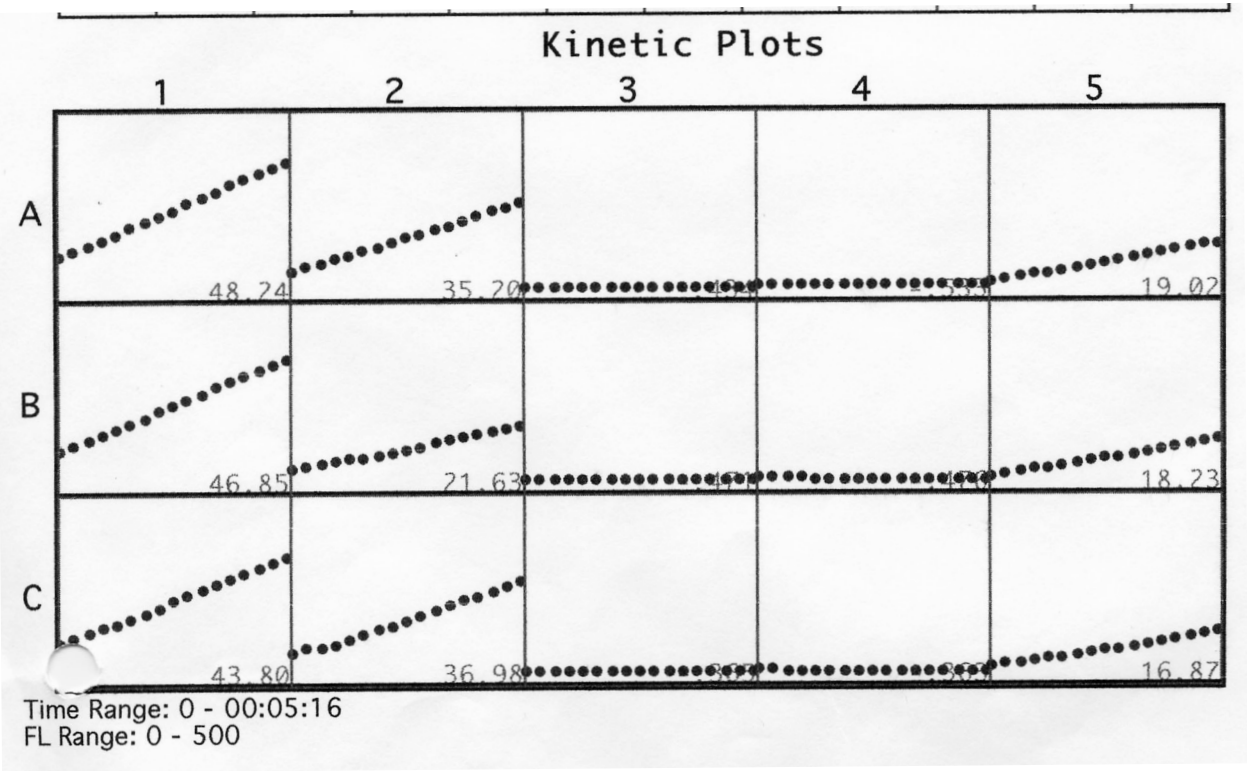
- *Trichomonas vaginalis* CPs are found within lysosome-like compartments in the cell.

How will we determine the location of these cysteine proteases?

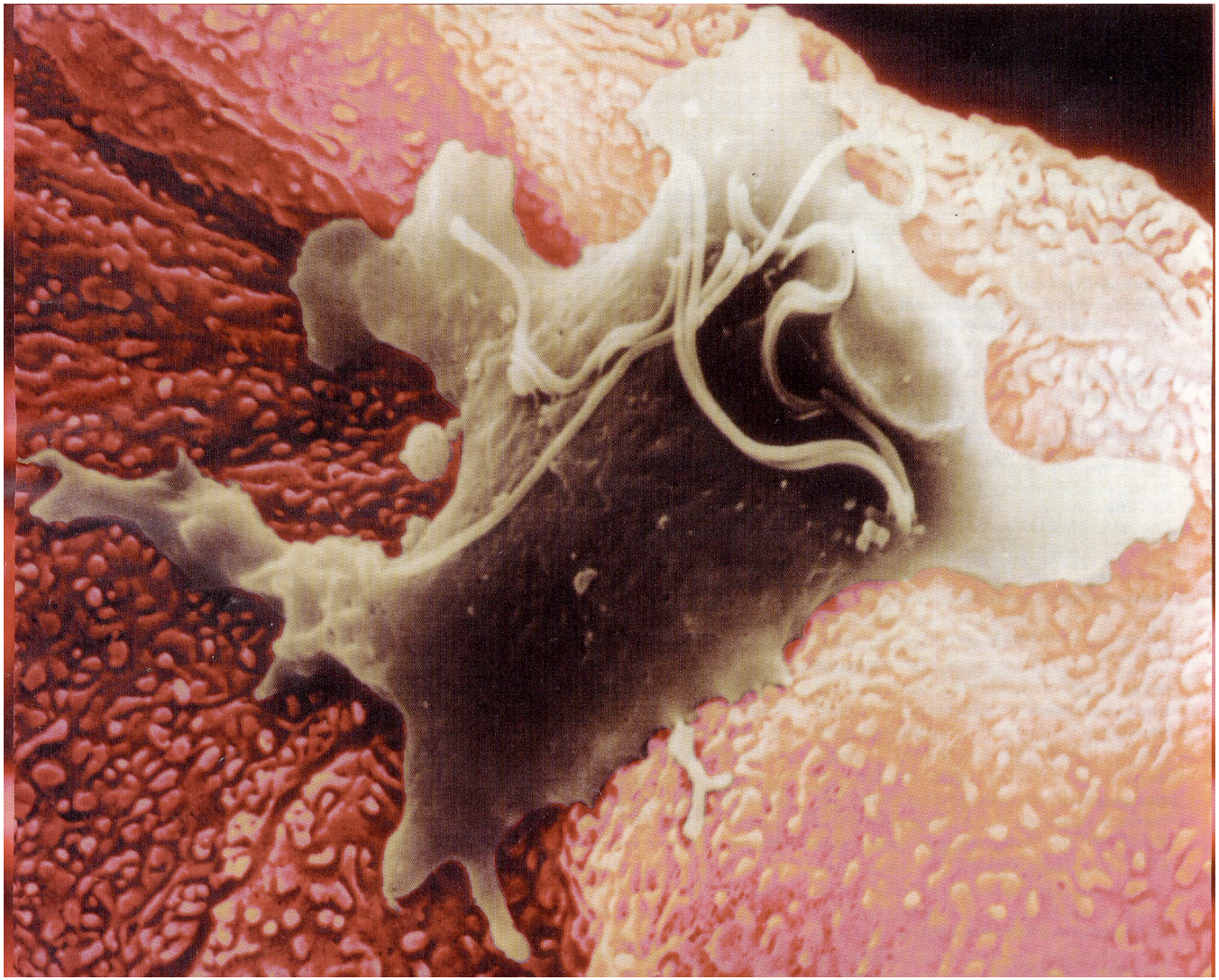
Synthesize recombinant CP

- antibody production
- inhibitor assays
- crystal structure for drug design

Determination of CP1 Activity from BTEC101
Look at how cool this is!!!

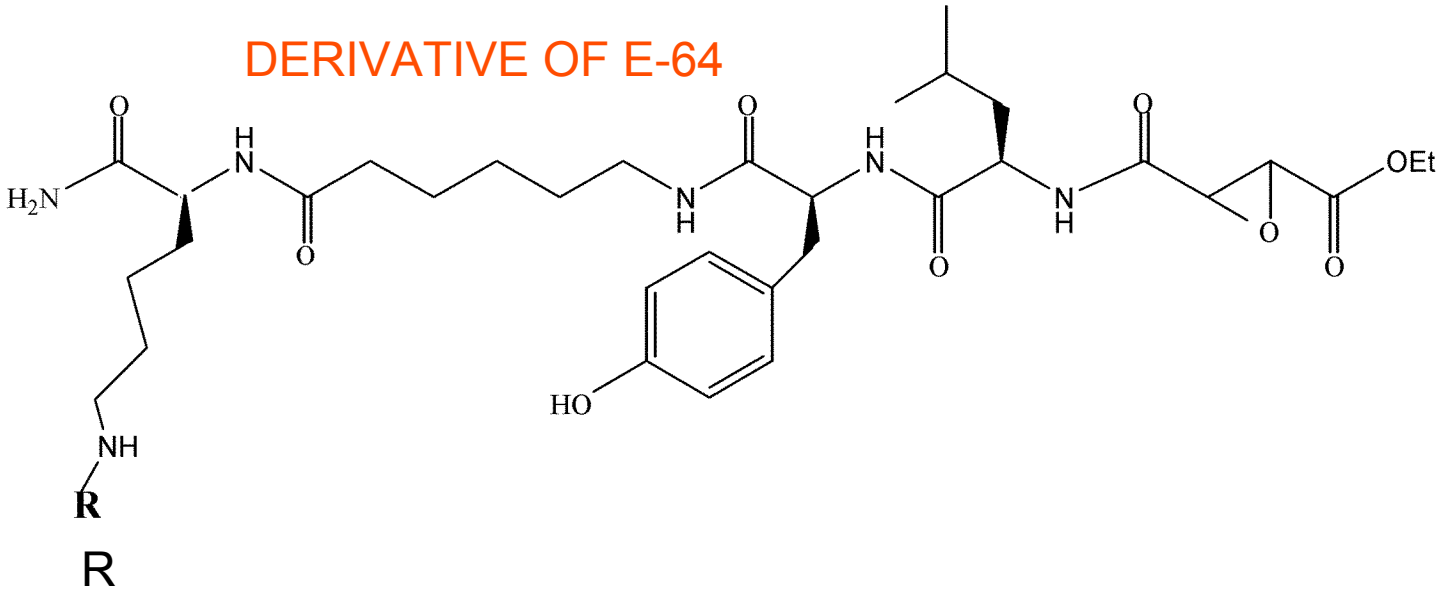


Extract DMSO **E-64** **K-777** CA-074



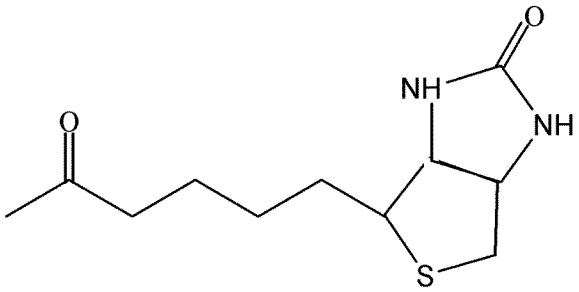
AFFINITY LABELS FOR CYSTEINE PROTEASES

DERIVATIVE OF E-64



R

DCG-04 (biotin tag)



BODIPY-GREEN 530/550 DCG-04