



All round description of the human parasite *Giardia* by mass spectrometry analysis

Serena Camerini



Protein analysis by mass spectrometry

Characterization →

PTMs (Post translational modifications)

Adducts

Structural elements

Identification →

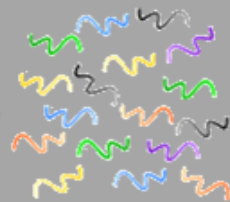
Protein-protein interactions

Differential analysis

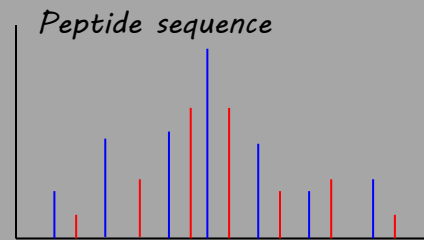
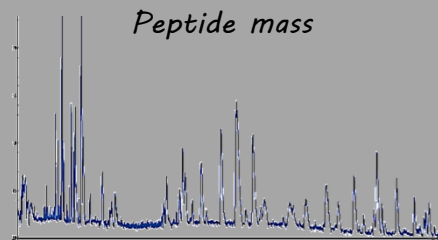
Biomarkers



Enzymatic
Digestion



LC-MS/MS



Protein analysis by mass spectrometry

Characterization →

P-e-p-t-i-d-e

Identification

P-e-p-t-i-d-e

P-e-p-t-i-d-e

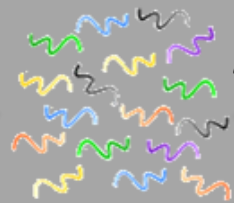
P-e-p-t-i-d-e

P-e-p-t-i-d-e

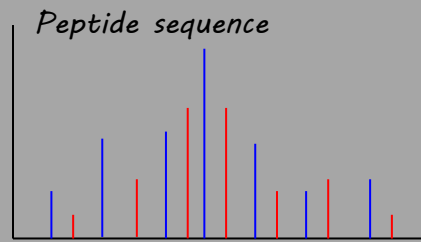
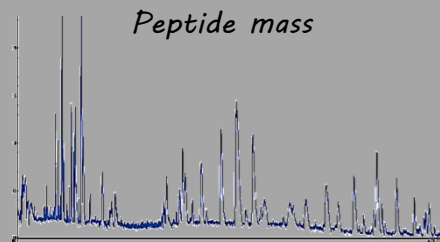
P-e-p-t-i-d-e



Enzymatic Digestion



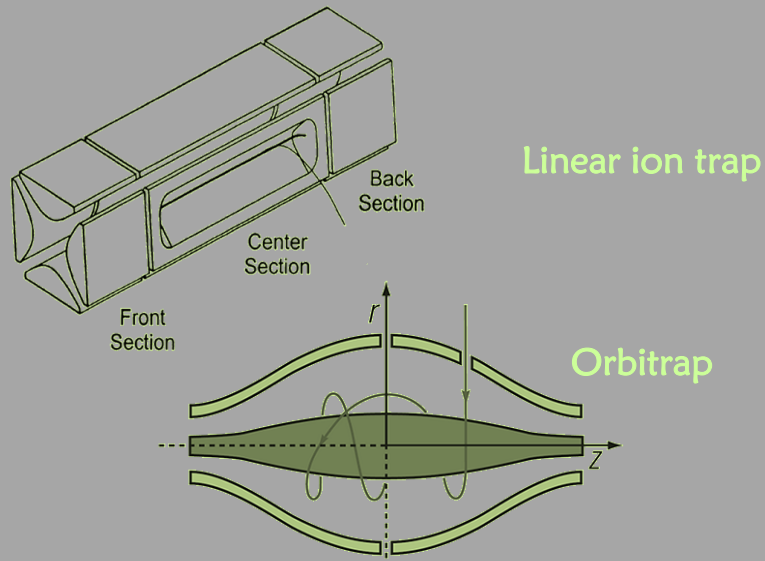
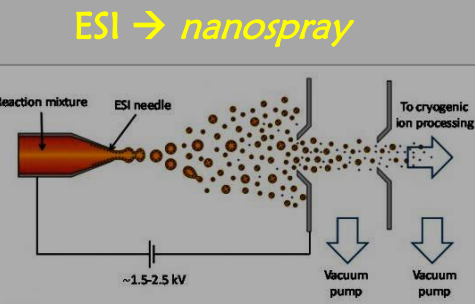
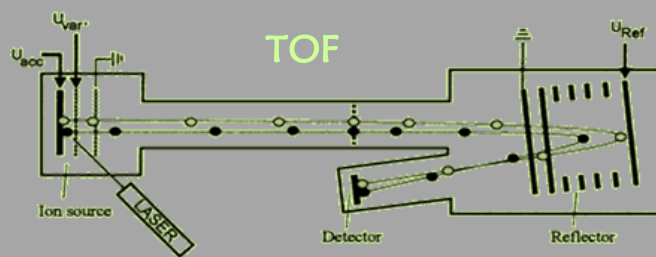
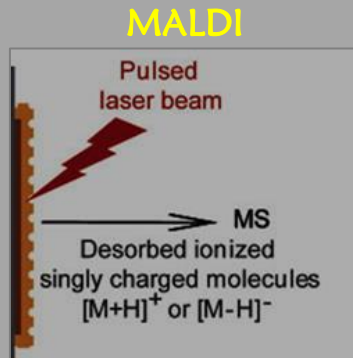
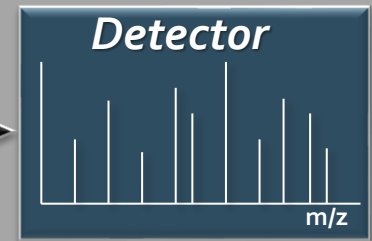
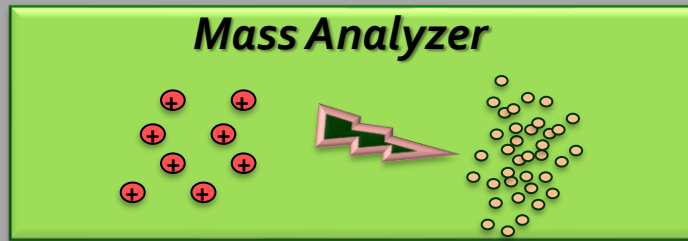
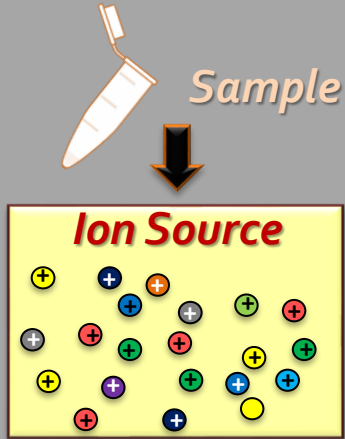
LC-MS/MS

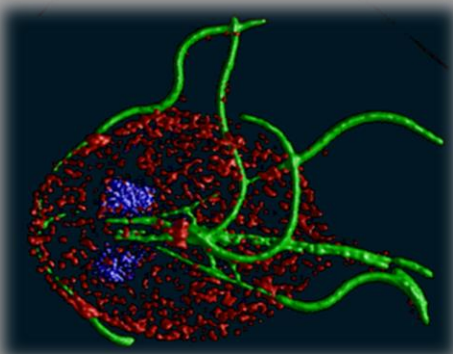


Time

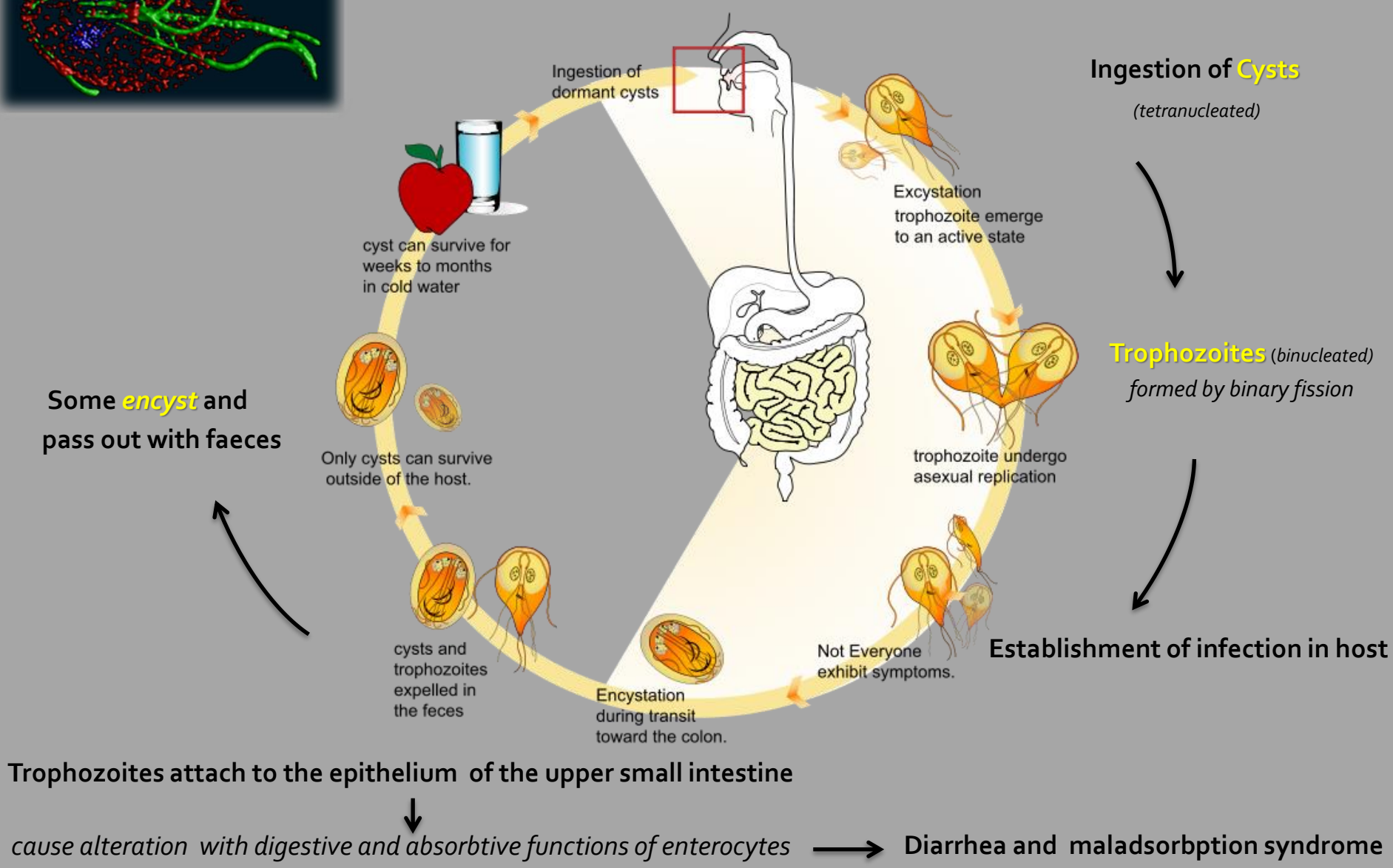
m/z

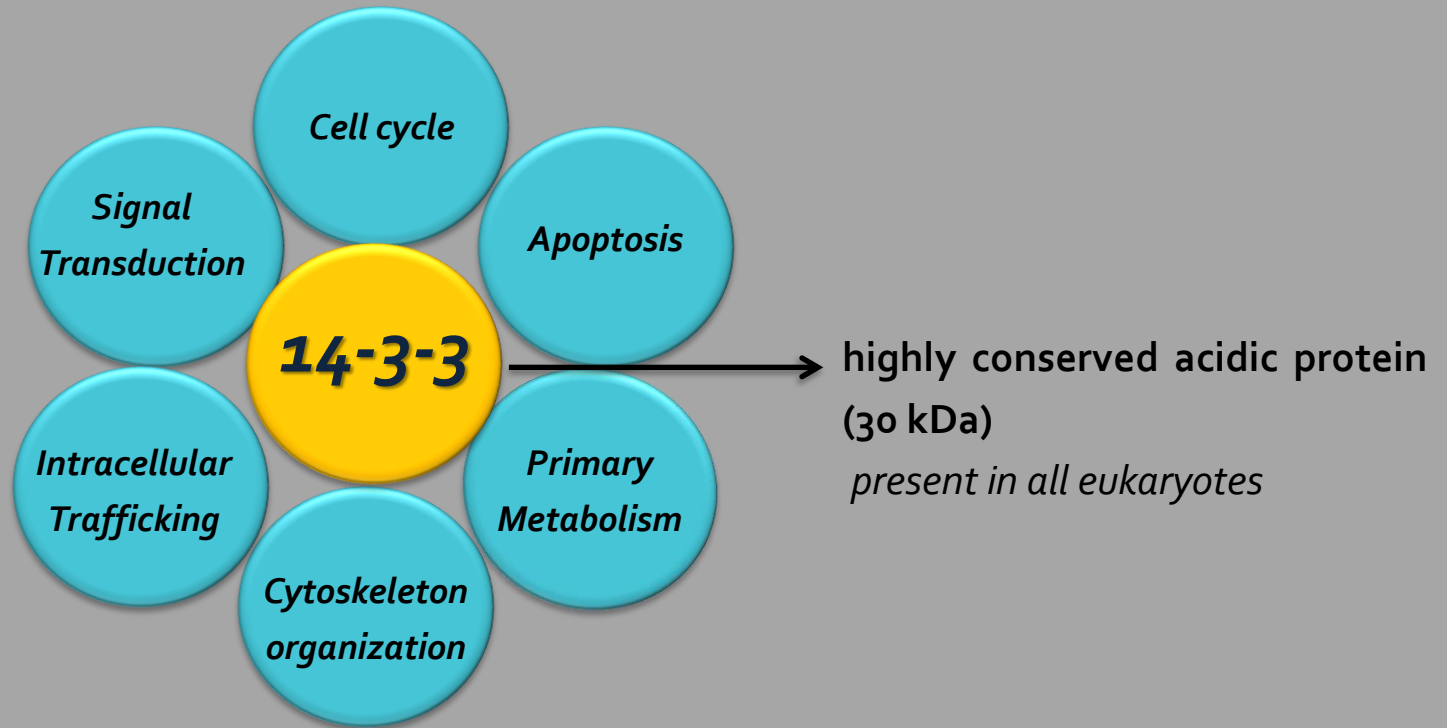
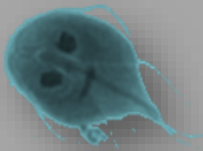
Which mass spectrometer?



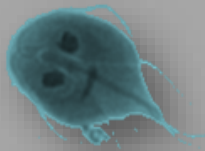


→ protozoan ***Giardia duodenalis***
↓
parasite → *Giardiasis*





Protein and the mRNA g14-3-3 are expressed both in the trophozoite and during the encystation.



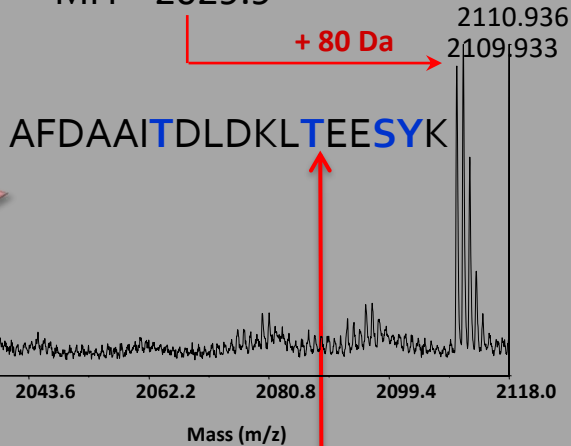
14-3-3

1- MAEAFTREDYVFMAQLNENAERYDEMVTMRKISGMEGELSDKERNLLSVAYKNVIGPRRAA
63- WRIVSSIEAKEKGRQKPNAKRIEQIRVYRQKIEKELSDICNDILKLLQEQFVPRSTNADAKV
125- FYYKMQGDYYRYLAEYSSGEDKEKIAGSALNAYNSAFEISQQLPPTHPIRLGLALNFSVFYY
187- EILASPRACELARKA₂₀₂FDAAITDLDKLTEESYK₂₁₉DSTLIMQLLRD₂₃₀NLNLWVTD SAGD
243- DNAEEK₂₄₈

PTMs

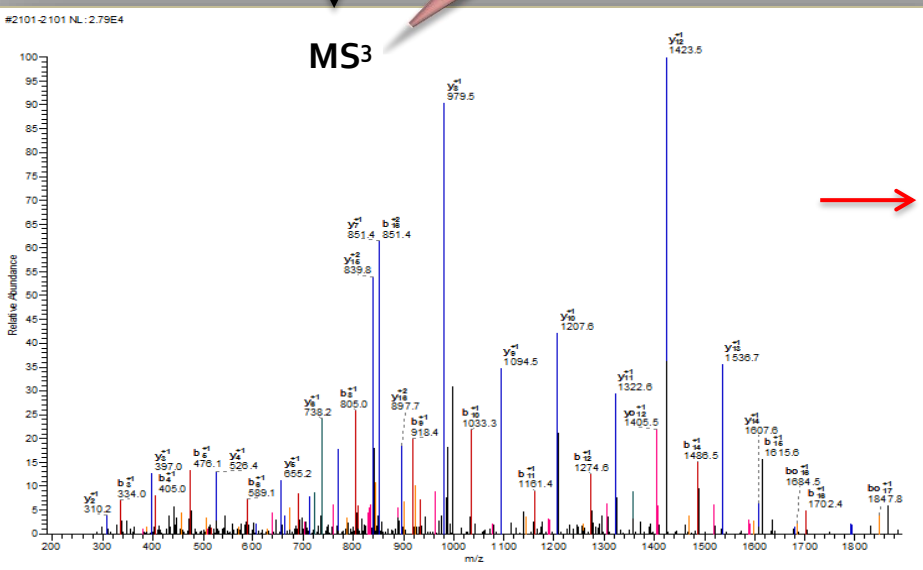


MH⁺=2029.9

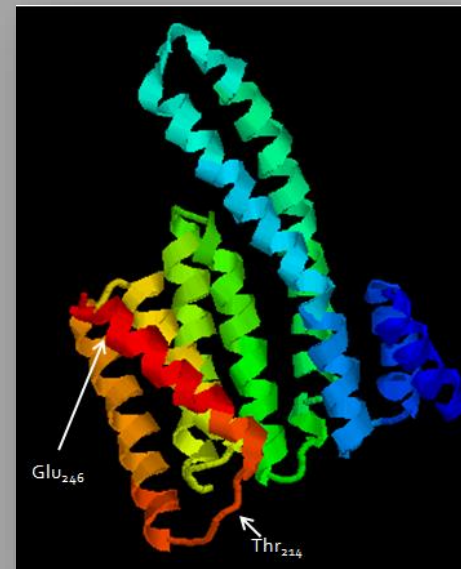


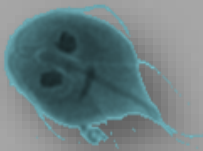
Phosphoric acid neutral loss

MS³



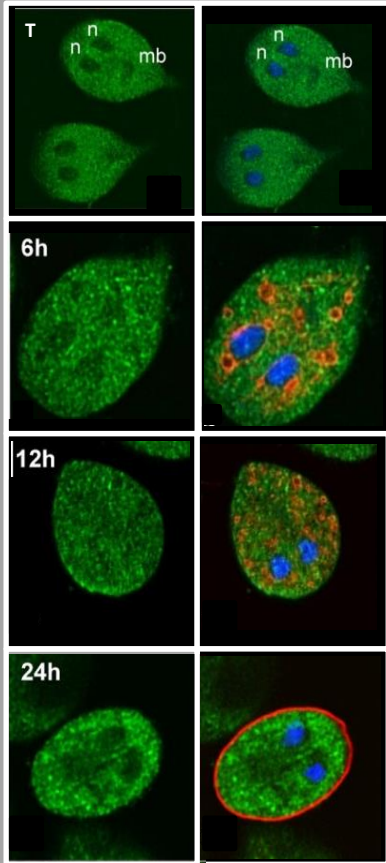
AFDAAITDLDKLT EESYK



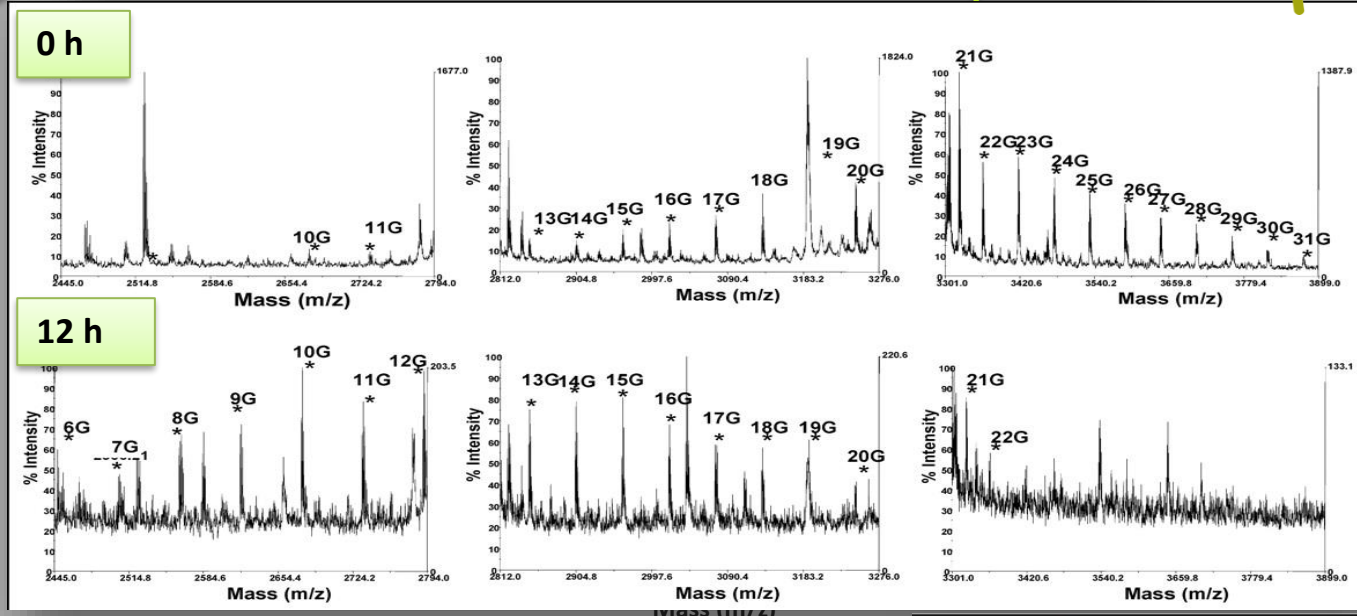


14-3-3

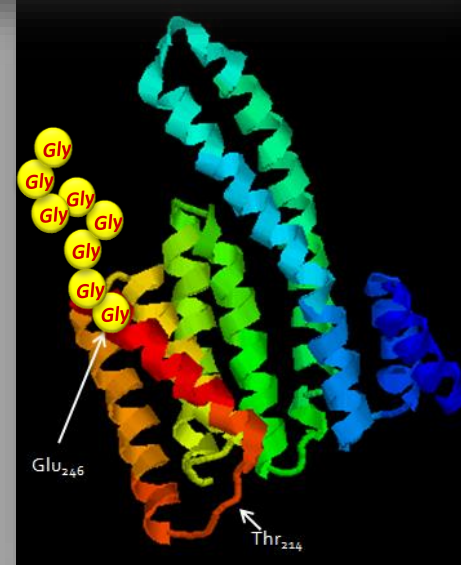
1- MAEAFTREDYV FMAQLNENAERYDEM VETMRKISGM EGELSDKERNLLSVAYKNVIGPRRAA
63- WRIVSSIEAKEKGRQKPNAKRIEQIRVYRQKIEKELSDICNDILKLLQE QFVPRSTNADAKV
125- FYYKMQGDYRYLAEYSSGEDKEKIAGSALNAYNSAF EISQQLPPTHP IRLGLALNFSVFYY
187- EILASPDRACELARK^A₂₀₂ F^AAAIT^DDLK^LTEESYK^S₂₁₉ DSTLIMQLLF^D₂₃₀ NLNLWV^TSDAGD
243- DN^AE^EK^K₂₄₈

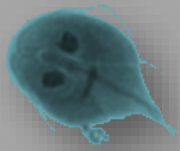


Immunofluorescence:
g14-3-3 in green



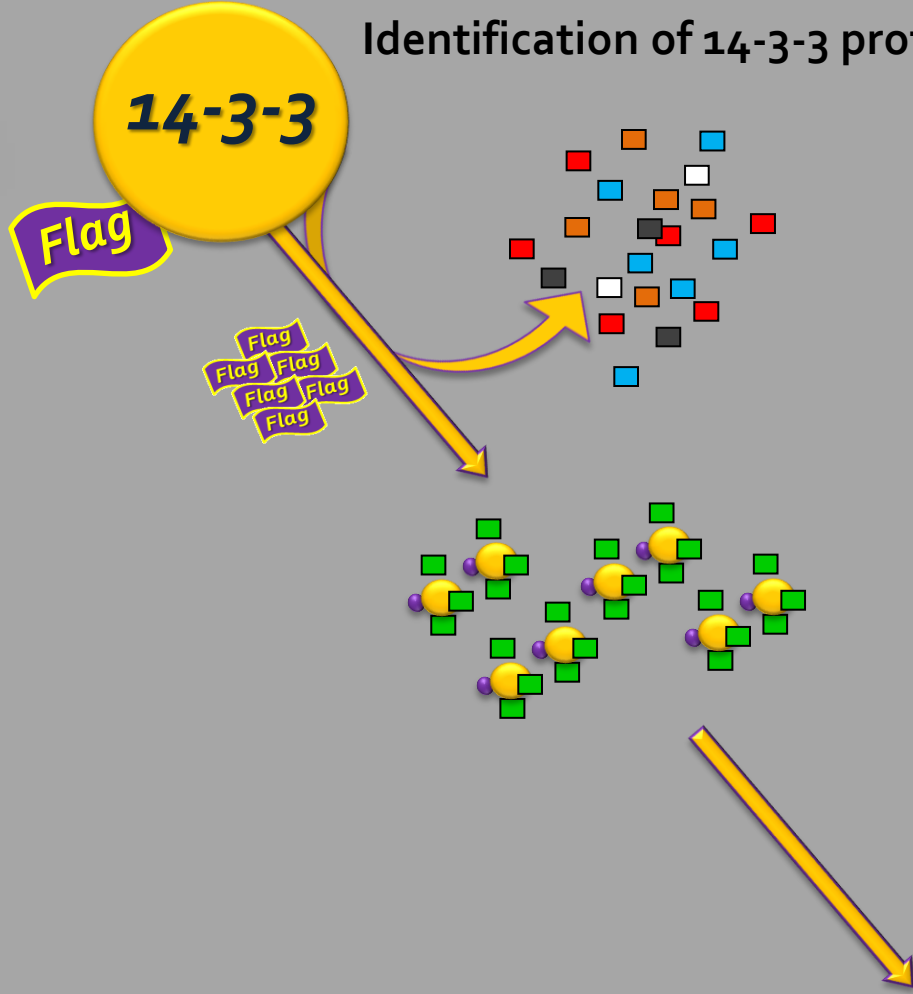
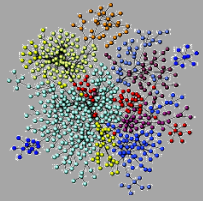
The length of polyglycine chain decreases during encystation
and allows the nuclear localization of g14-3-3



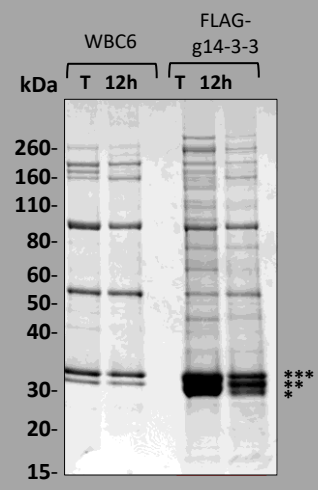


Identification of 14-3-3 protein interactors

Interactome

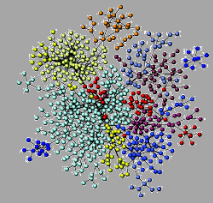
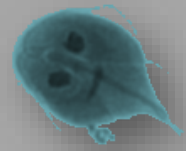


LC-MS/MS ←

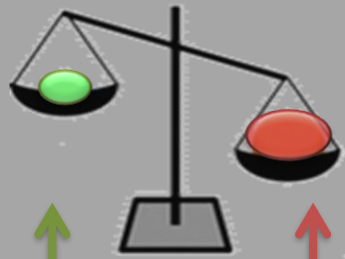


Identification of 14-3-3 protein interactors

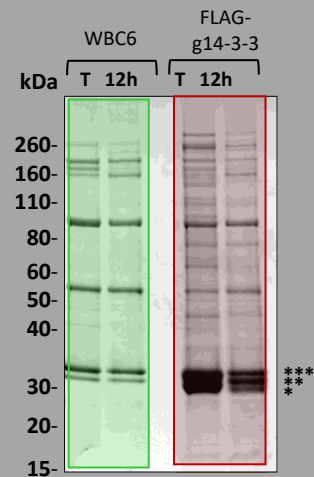
Interactome

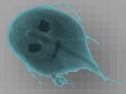


Quantitative analysis



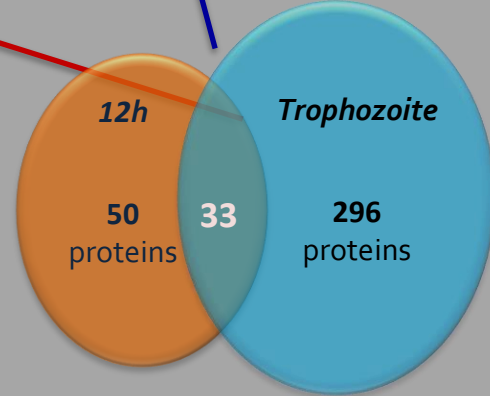
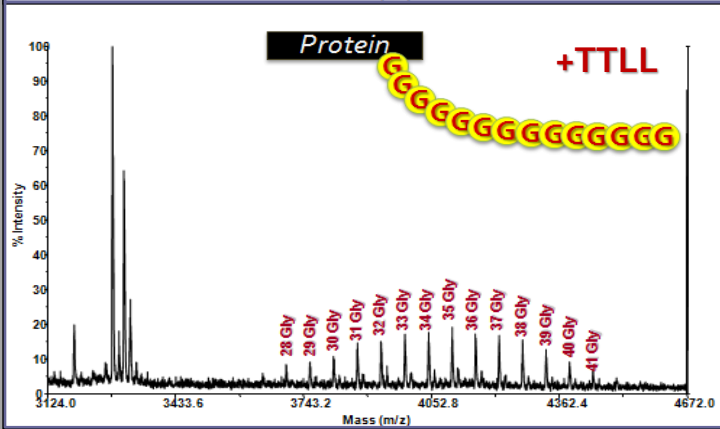
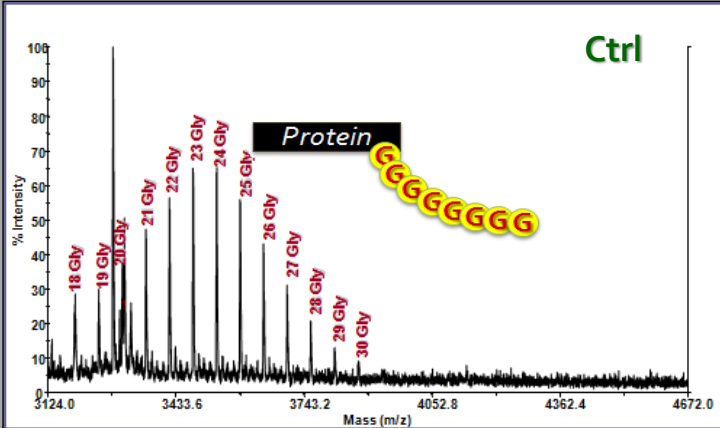
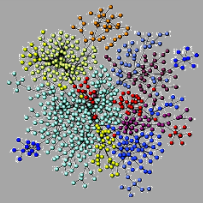
LC-MS/MS

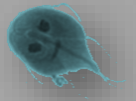




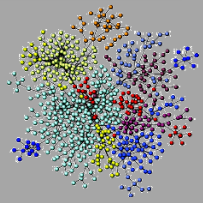
TTLL

DIP₁ & DIP₂





Interactome



Ctrl

TTLL

DIP1 & DIP2

Protein

Protein

Protein

$9 < n^{\circ}\text{Gly} < 28$

$9 < n^{\circ}\text{Gly} < 40$

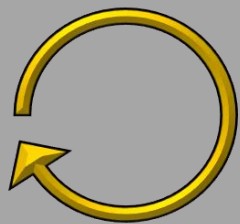
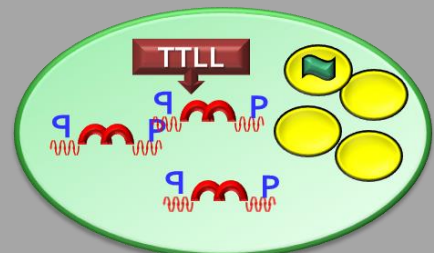
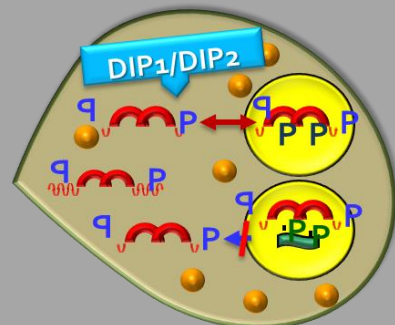
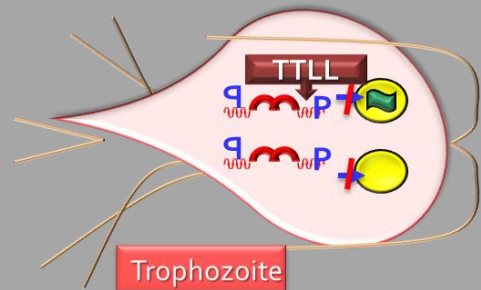
$8 < n^{\circ}\text{Gly} < 24$

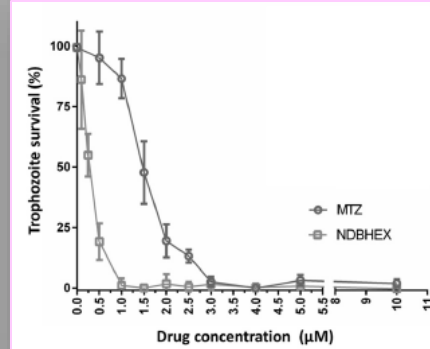
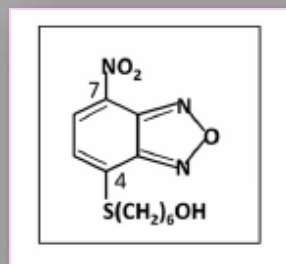
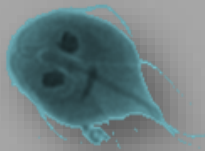
$7 < n^{\circ}\text{Gly} < 24$



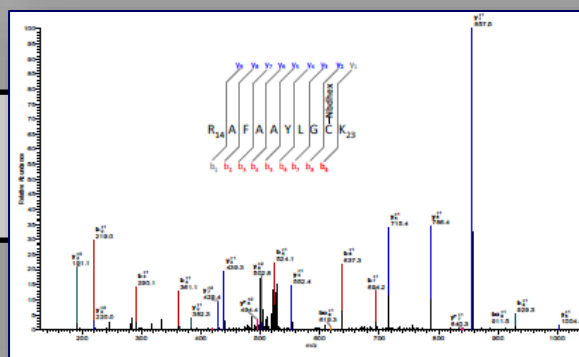
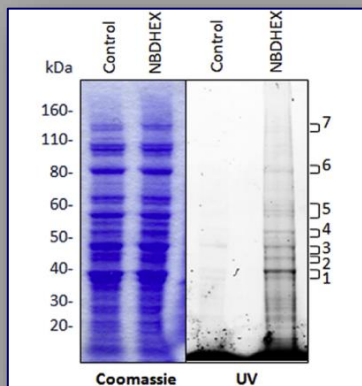
TTLL *never* localizes in the **nuclei**

DIP1 & DIP2 localize in the cytoplasm and **nuclei** at all stages

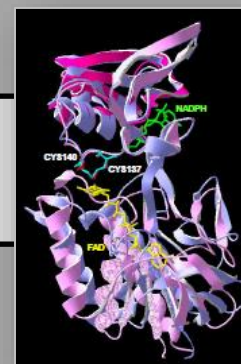




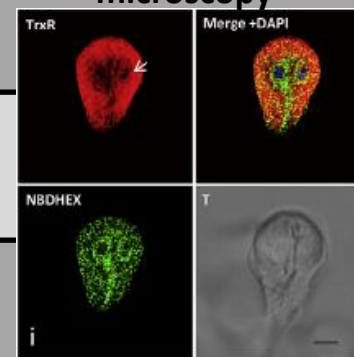
Mass spectrometry



Crystal structure



Confocal laser scanning microscopy



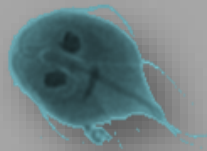
LC-MS/MS

Giardia proteins with NBDHEX adducts identified by MS/MS in UV-fluorescent bands after SDS-PAGE.

Protein name	MW (kDa)	GiardiaDB accession no (UniProt)	Adduct position
Thioredoxin Reductase (gTrxR)	33.8	GL50803_9827 (E2RU27)	C ₁₃₇ and/or C ₁₄₀
Ornithine Carbamoyl Transferase (gOCT)	36.4	GL50803_10311 (E2RTT6)	C ₁₆
Elongation Factor 1Bγ (gEF1Bγ)	45.2	GL50803_12102 (A8BFR0)	C ₃₂ and/or C ₃₄
α-Tubulin (gα-TUB)	50.5	GL50803_103676 (A8BPC0)	C ₃₄₇
Arginine deiminase (gADI)	64.1	GL50803_112103 (E2RU36)	C ₂₈₃
Pyruvate flavodoxin oxidoreductase (gPFOR-2)	131.7	GL50803_17063 (A8B852)	C ₉₆₇
Axoneme-associated protein GASP-180 (gGASP-180)	174.5	GL50803_137716 (A8B5G1)	C ₈₈₉

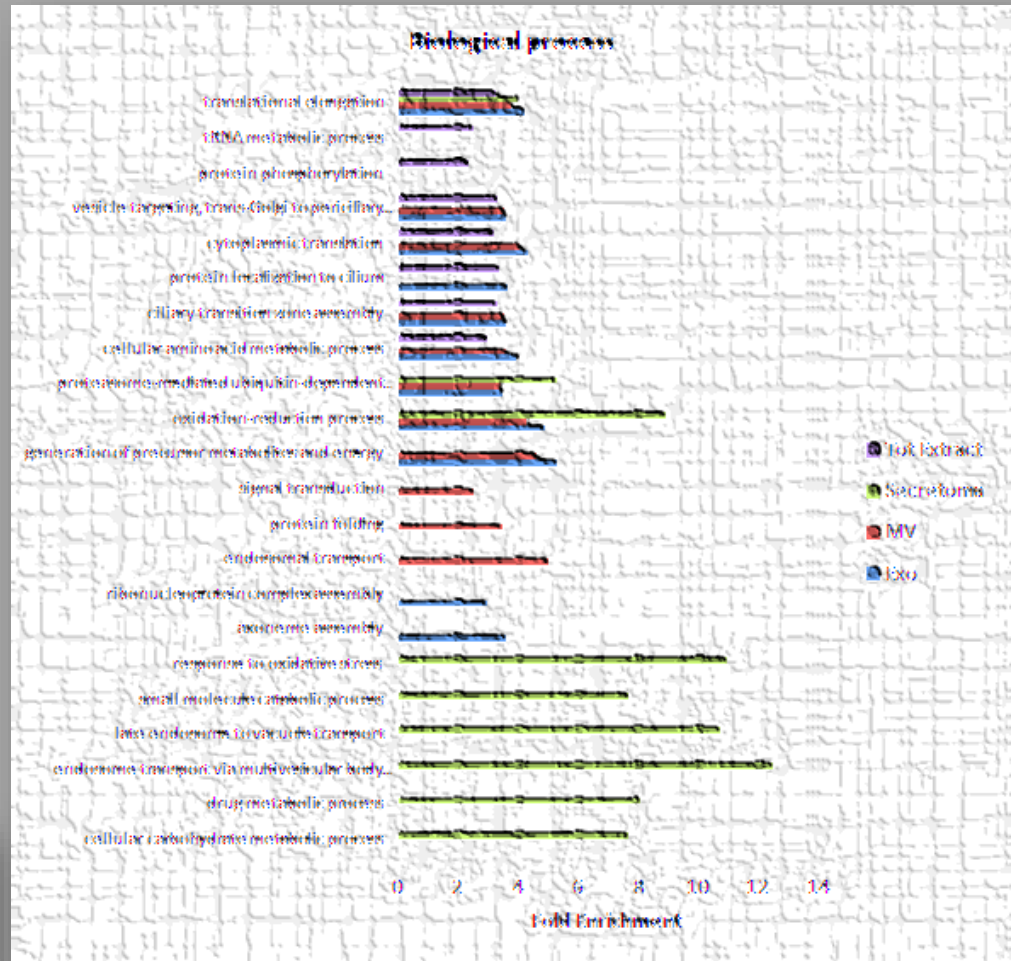
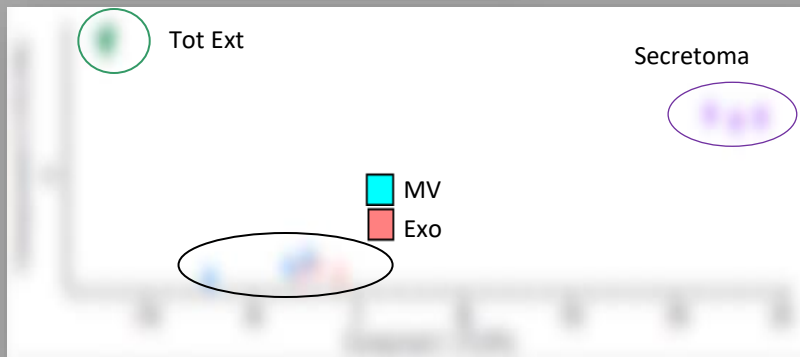
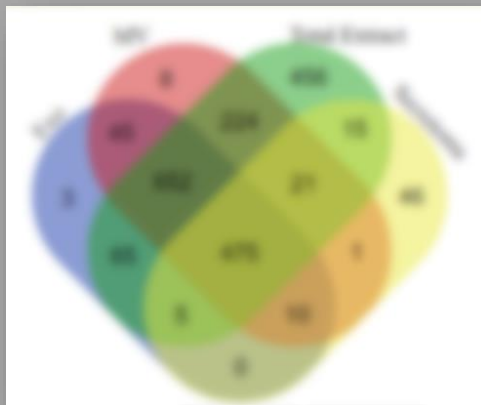
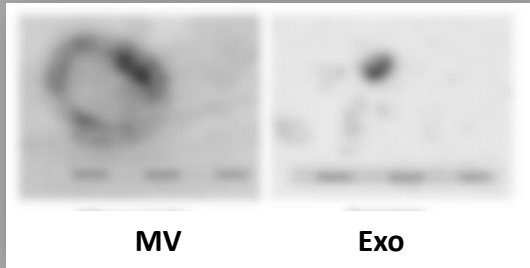


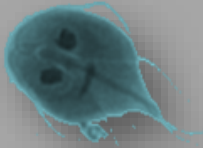
Mechanism of action



Analysis of proteins secreted by Giardia in the medium (*secretome*) or compartmentalized in particles [*exosomes* (EXO) and/or *microvesicles* (MV)]

Secretome & Vesicles



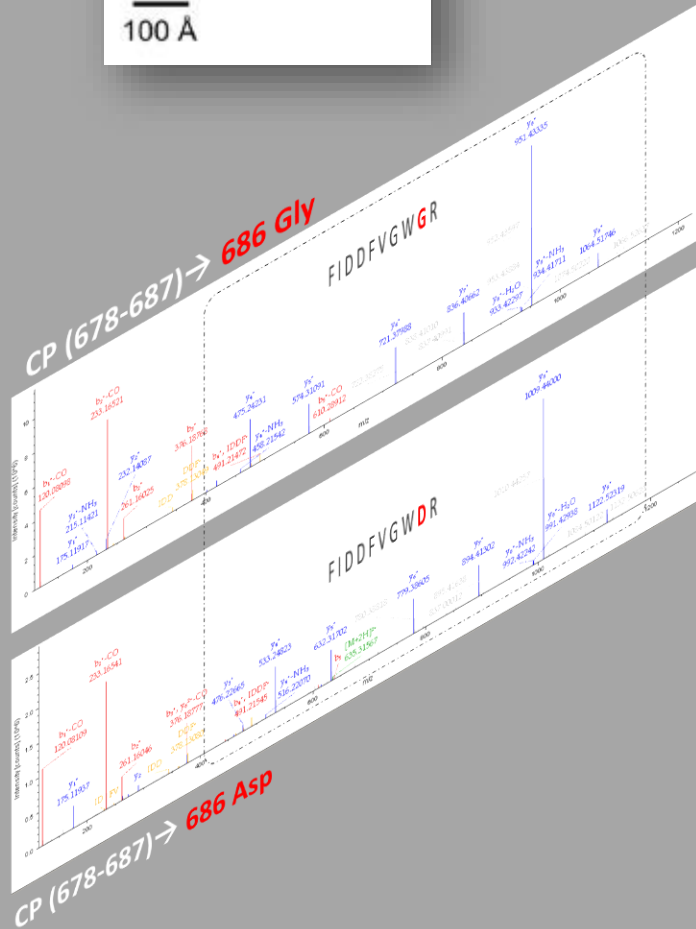
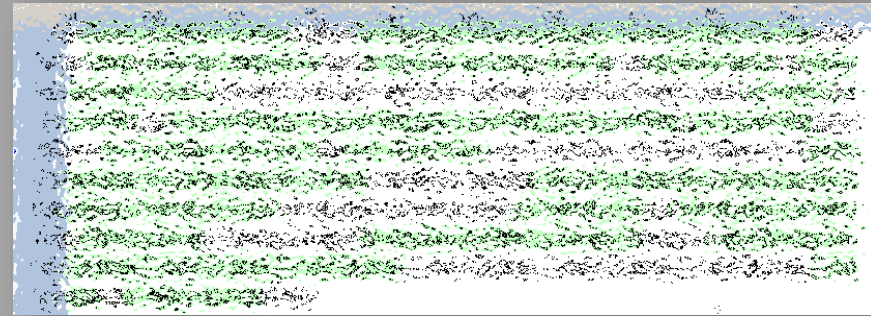
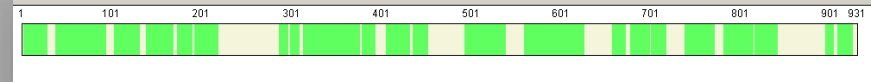
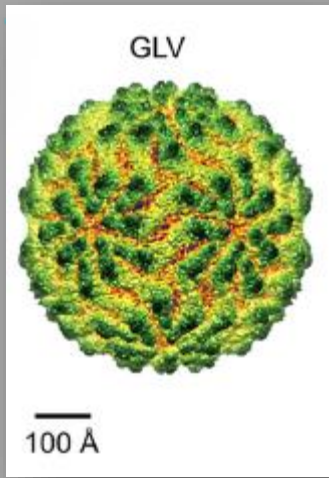


Several pathogenic protozoa, including *Giardia lamblia*,
are persistently **infected with dsRNA viruses**

**Giardia
Virus**



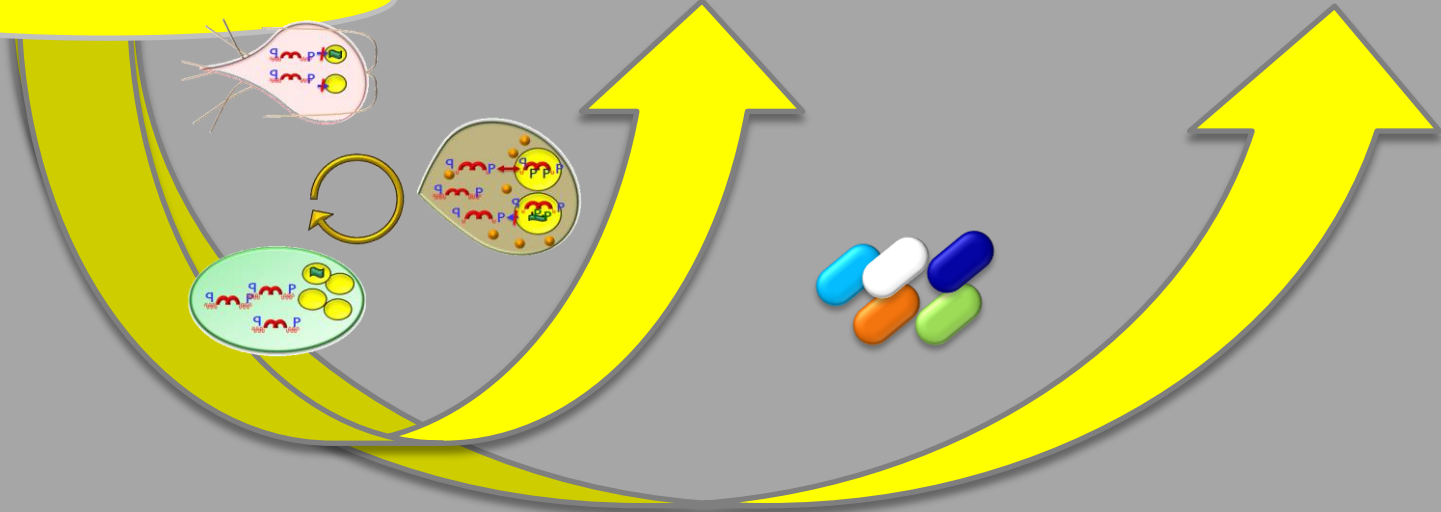
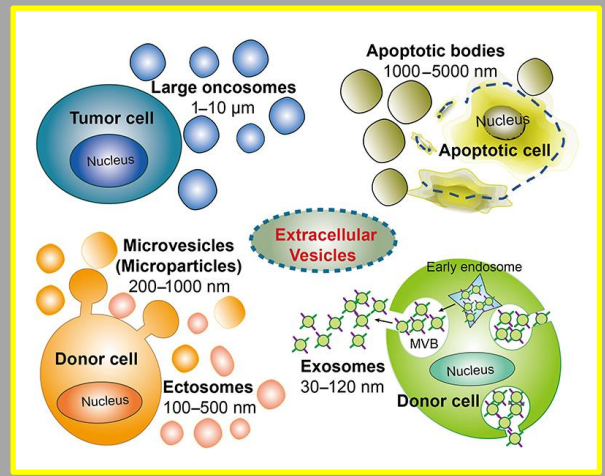
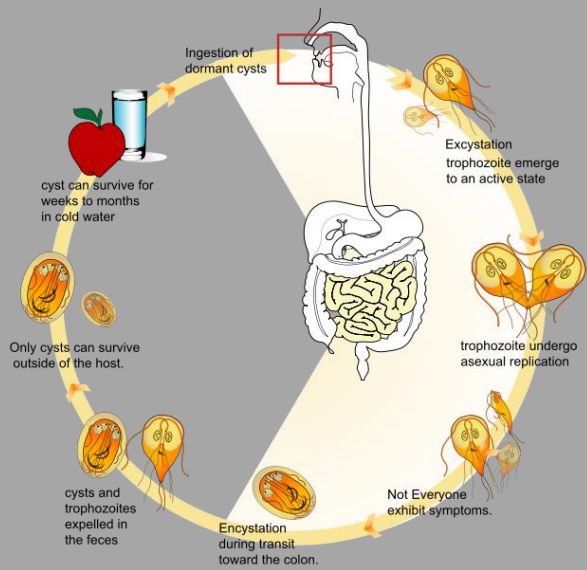
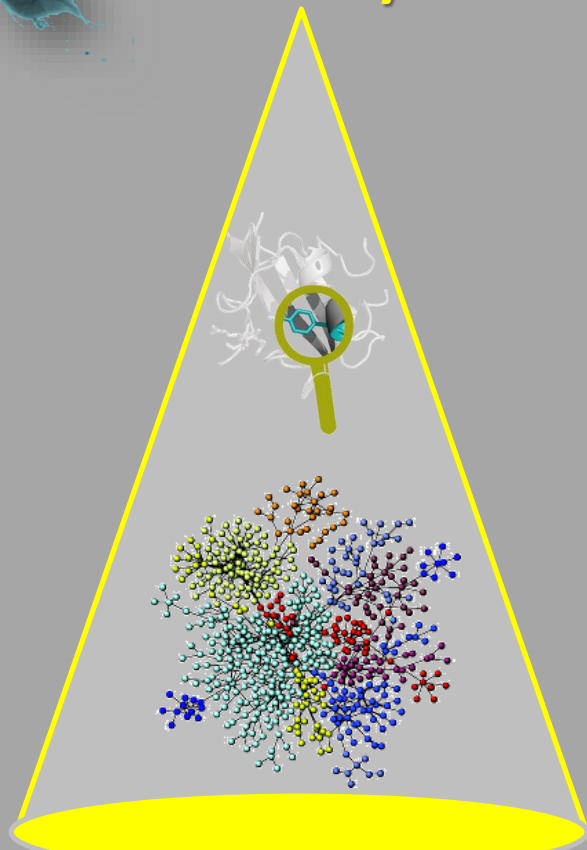
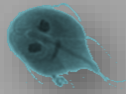
some of these protozoal viruses can likewise
enhance the pathogenicity of their hosts.



MS analysis of the protein isoforms
expressed by GLV.



MS analysis





Luisa Casella Serena Cecchetti

Marco Crescenzi

Core Facilities (FAST) Istituto Superiore di Sanità

Marco Lalle

Dept. of Infectious, Parasitic and Immunomediated Diseases

Istituto Superiore di Sanità

