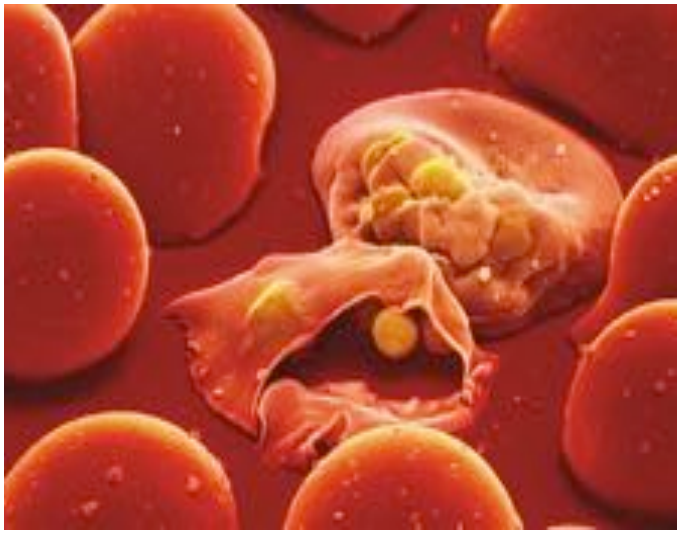
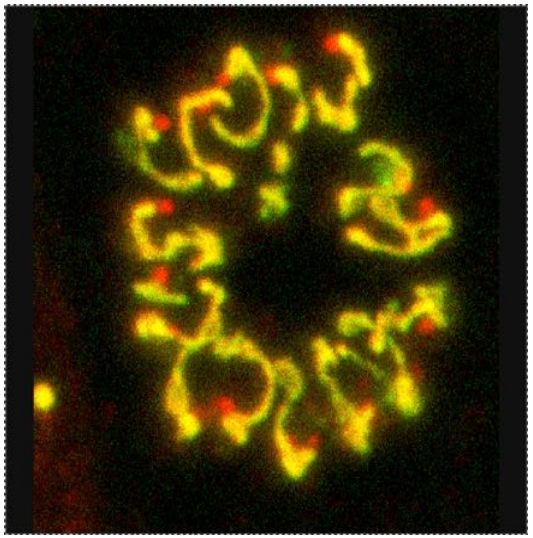
MeBOP Module 2
Introduction





UNIVERSITÉ
DE GENÈVE

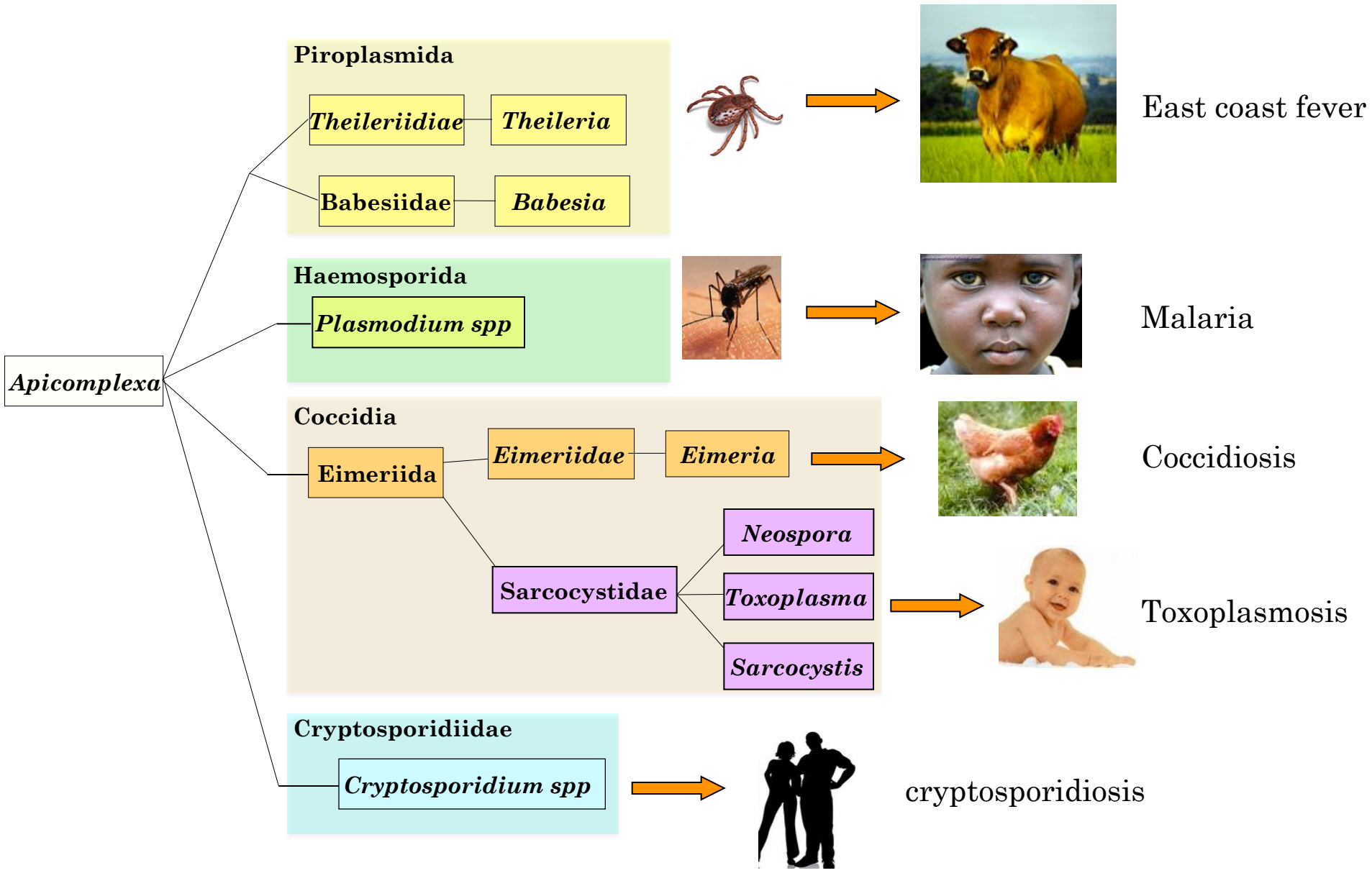
Karine Frenal:
The expert of Toxo glideosome and IMC

Damien Jacot:
Expert in … about everything…

Sunil Kumar Dogga:
TgAsp3

Budhaditya Mukherjee:
Biochemistry

Apicomplexans are human and animal pathogens

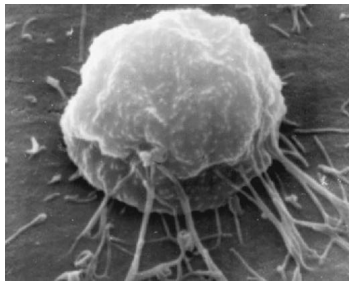


Malaria

- Caused by
  - *Plasmodium falciparum*
  - *P. vivax*
  - *P. malariae*
  - *P. ovale*
  - *P. knowlesi*

- At risk
  - More than 40% of the world population

- Deaths
  - Around 0.7 million per year

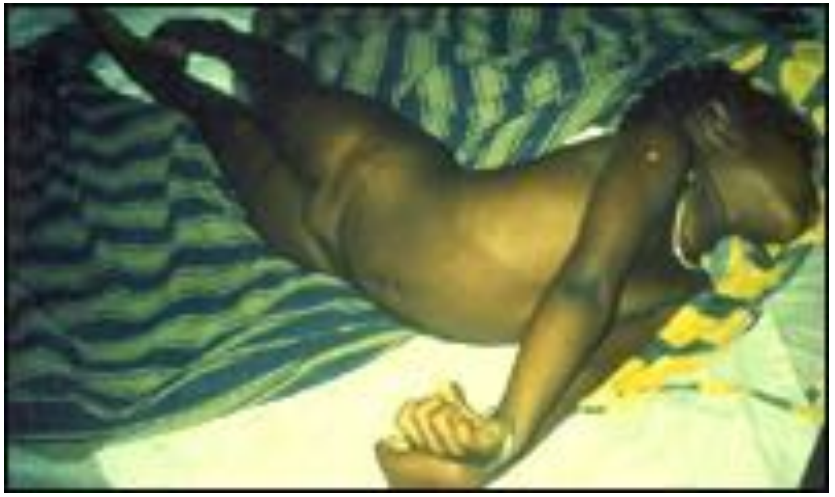


- Malaria

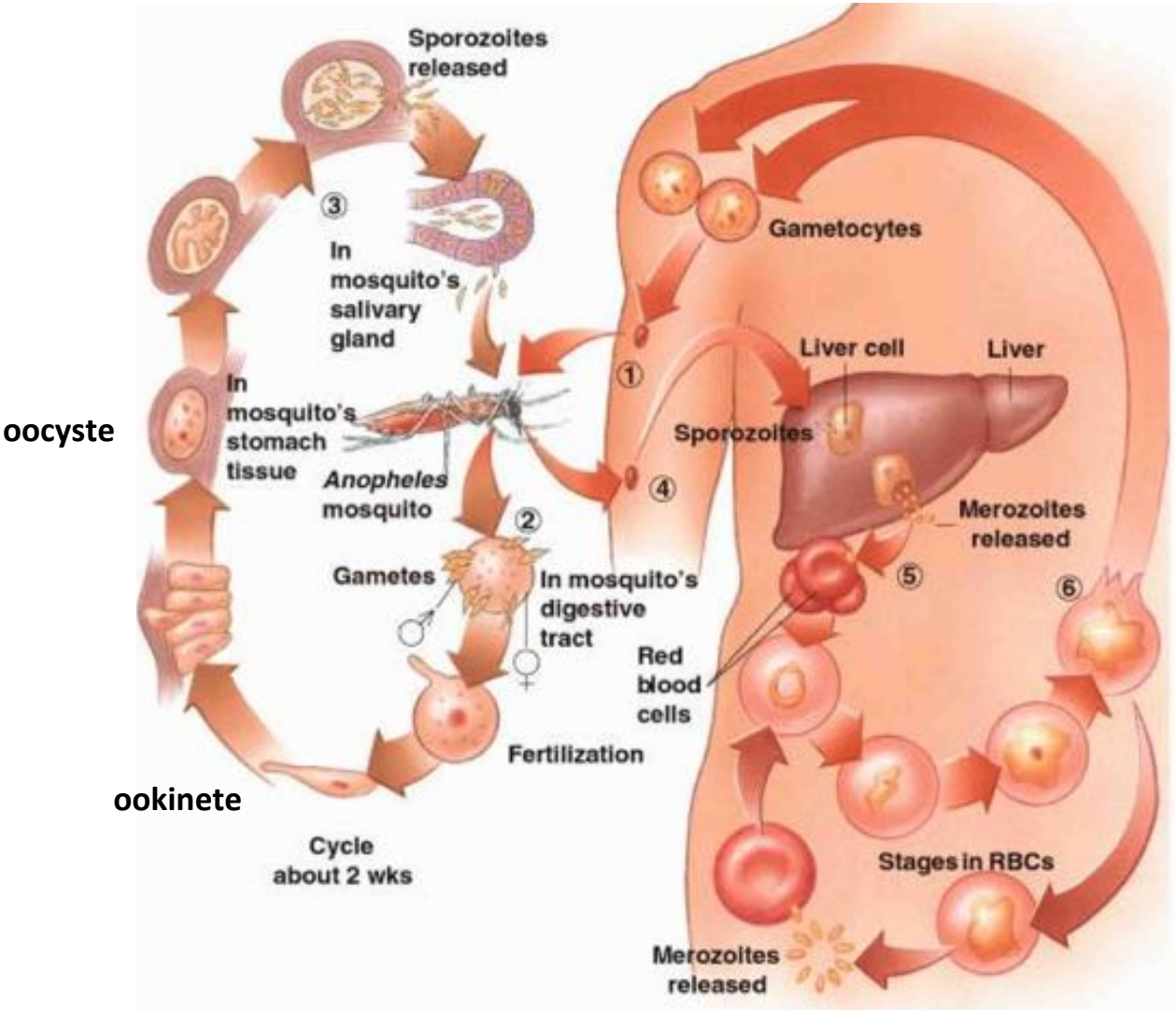
  - Fever

  - Anaemia

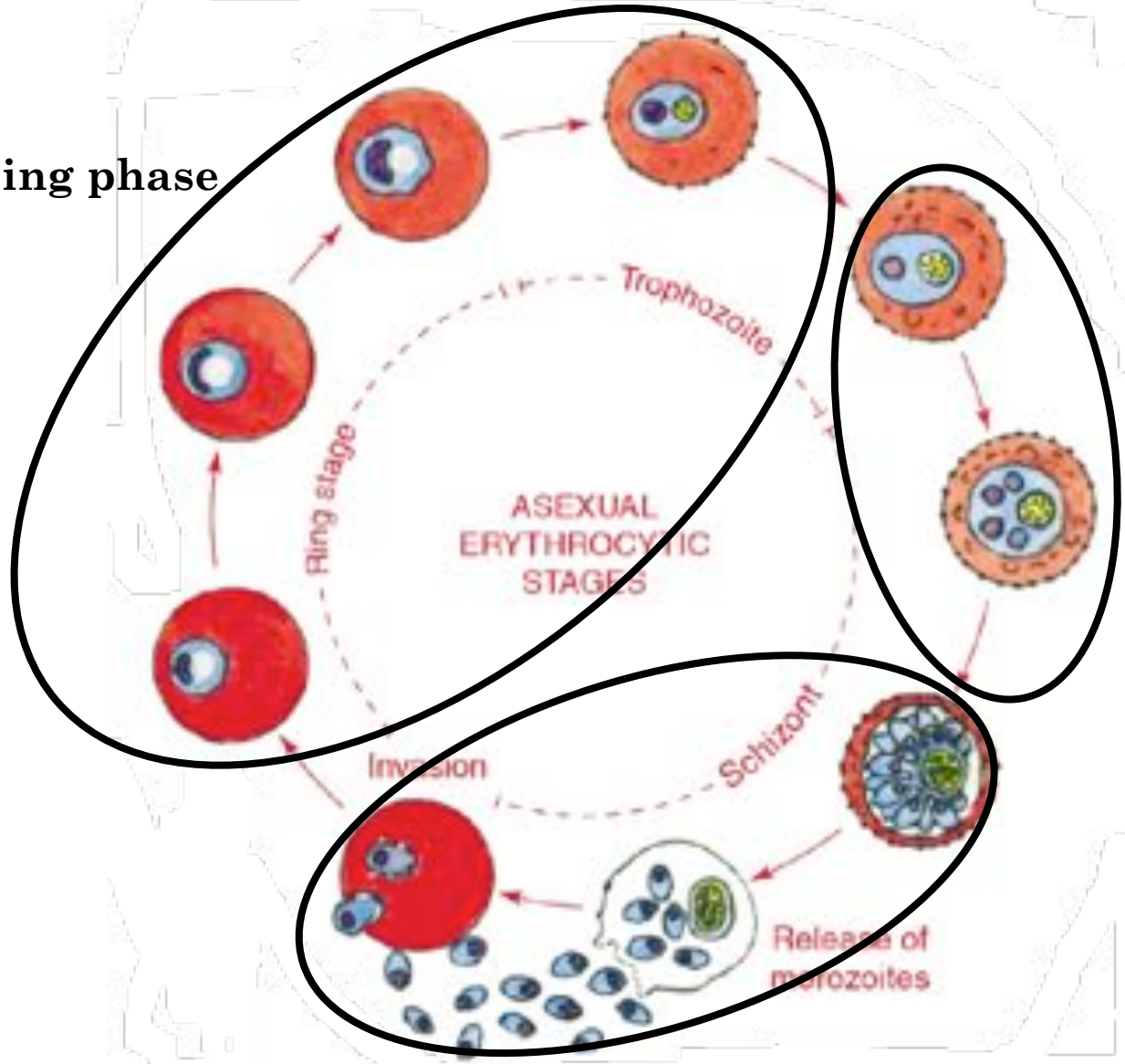
  - Metabolic dysfunctions : acidosis, hypoglycemia …



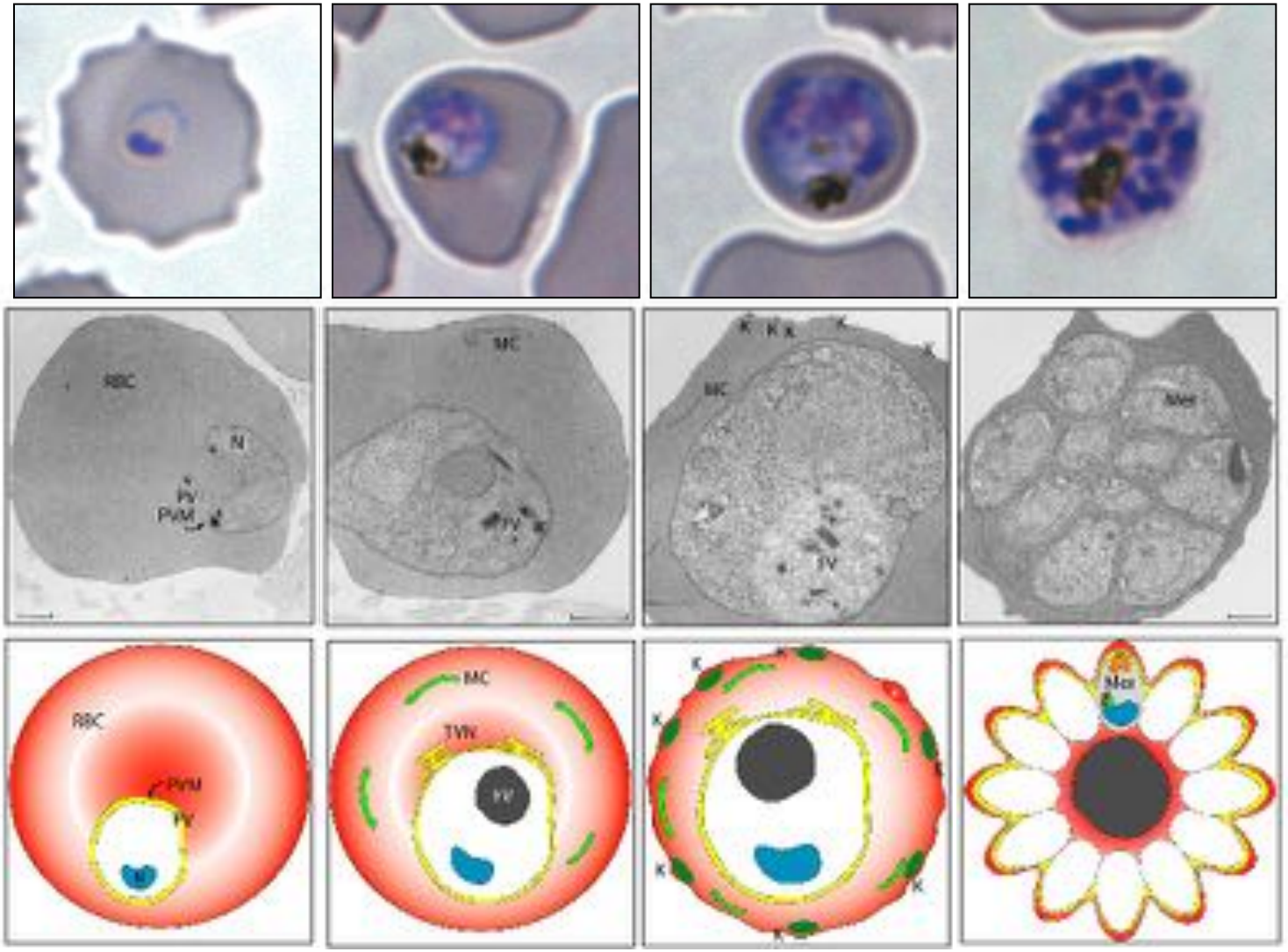
Plasmodium falciparum life cycle



Plasmodium falciparum erythrocytic stages



Plasmodium falciparum erythrocytic stages



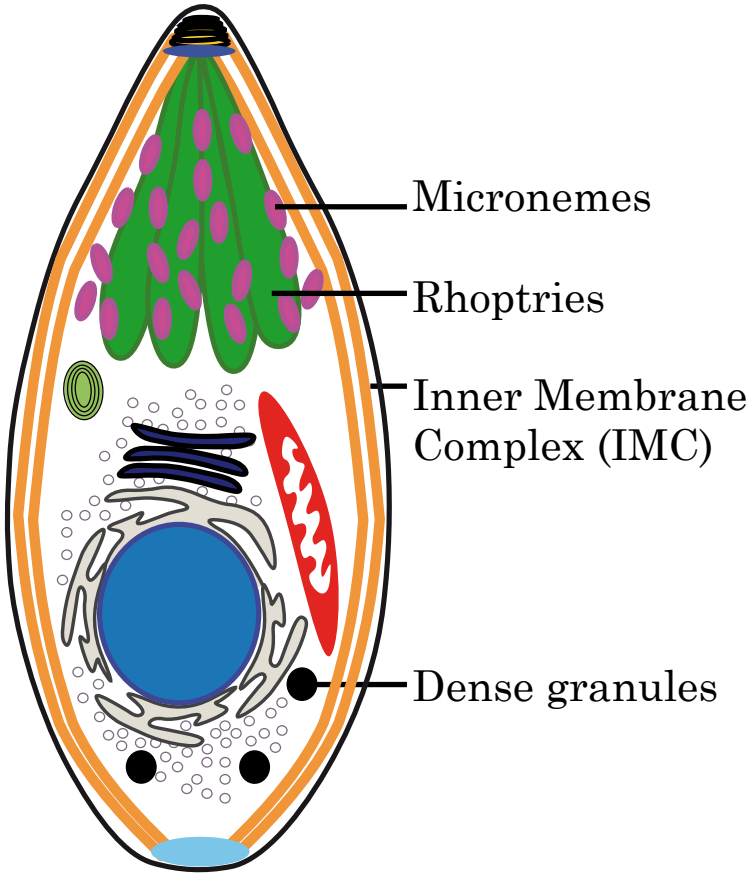
0-5h Rings

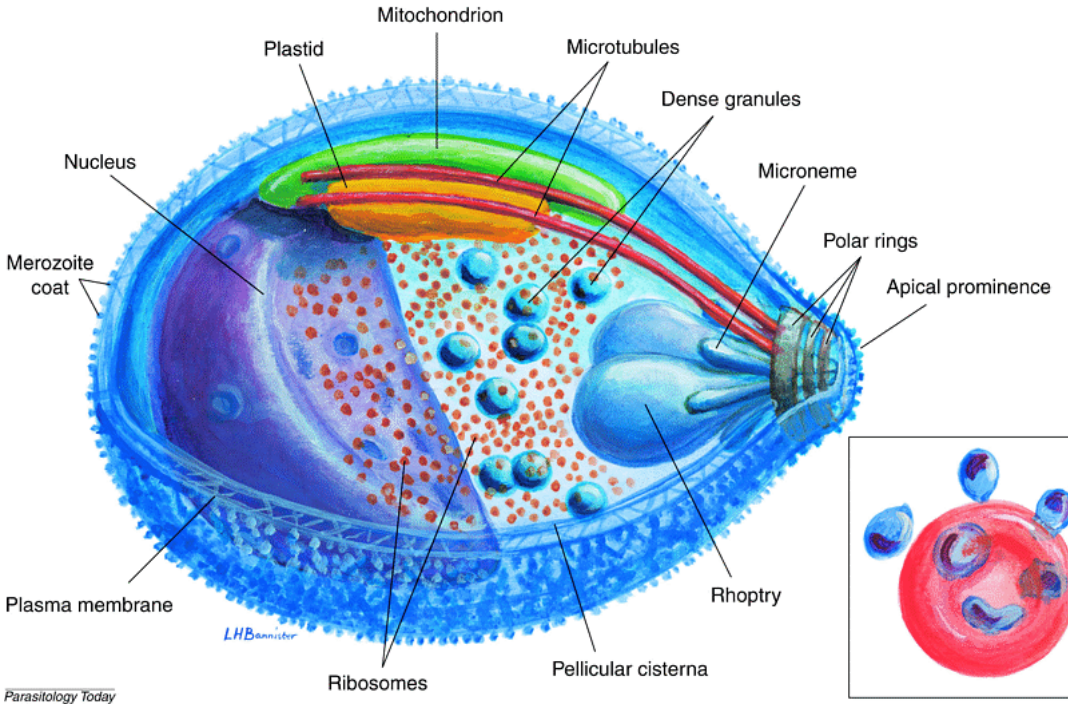
5-10h
Early trophozoite

10-20h
Mid-Late Trophozoite

>40h Schizont

Apicomplexan invasive tachyzoite and merozoite





Adapted from Frenal K *et al* (2013) Traffic

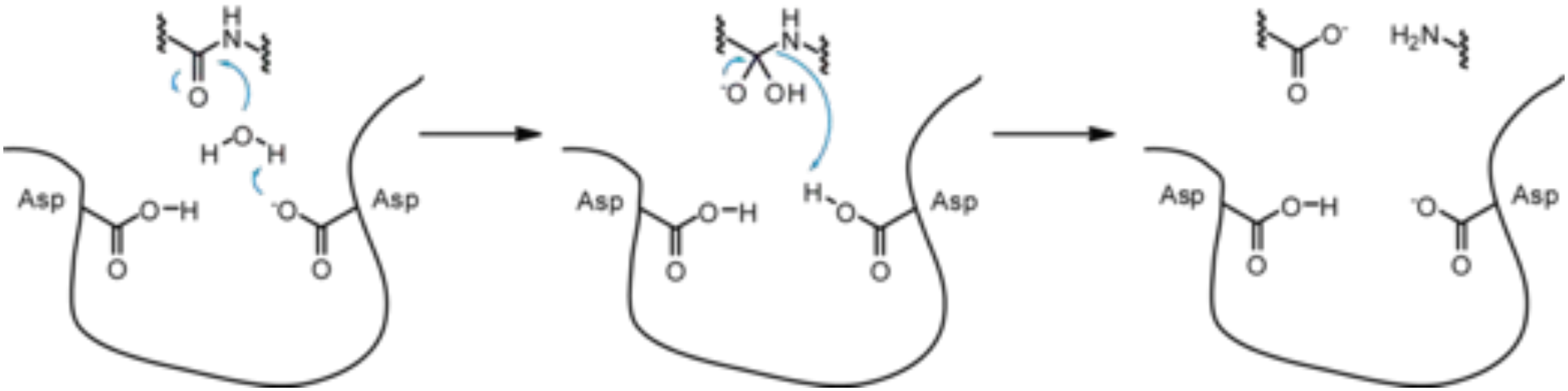
Toxoplasma gondii	***Plasmodium berghei***	***Plasmodium falciparum***
Model organism	Mouse malaria	Human malaria
Easy genetics	“Easy” genetics	Tricky genetics
“good looking”	*In vivo* only	Relevant
Easy to grow	Full life cycle accessible	

Aspartic endopeptidases (ASP/PM)

- ✓ Present in **all eukaryotes**
- ✓ **Broad range of roles** : - protein degradation
  - enzyme maturation
  - signal transduction
  - virulence factors

- ✓ **Use Asp residues in the motifs DTG or DSG**
- ✓ **Pro-region inactivates enzyme**
- ✓ **Proteolytic maturation leads to activation**

Mode of action

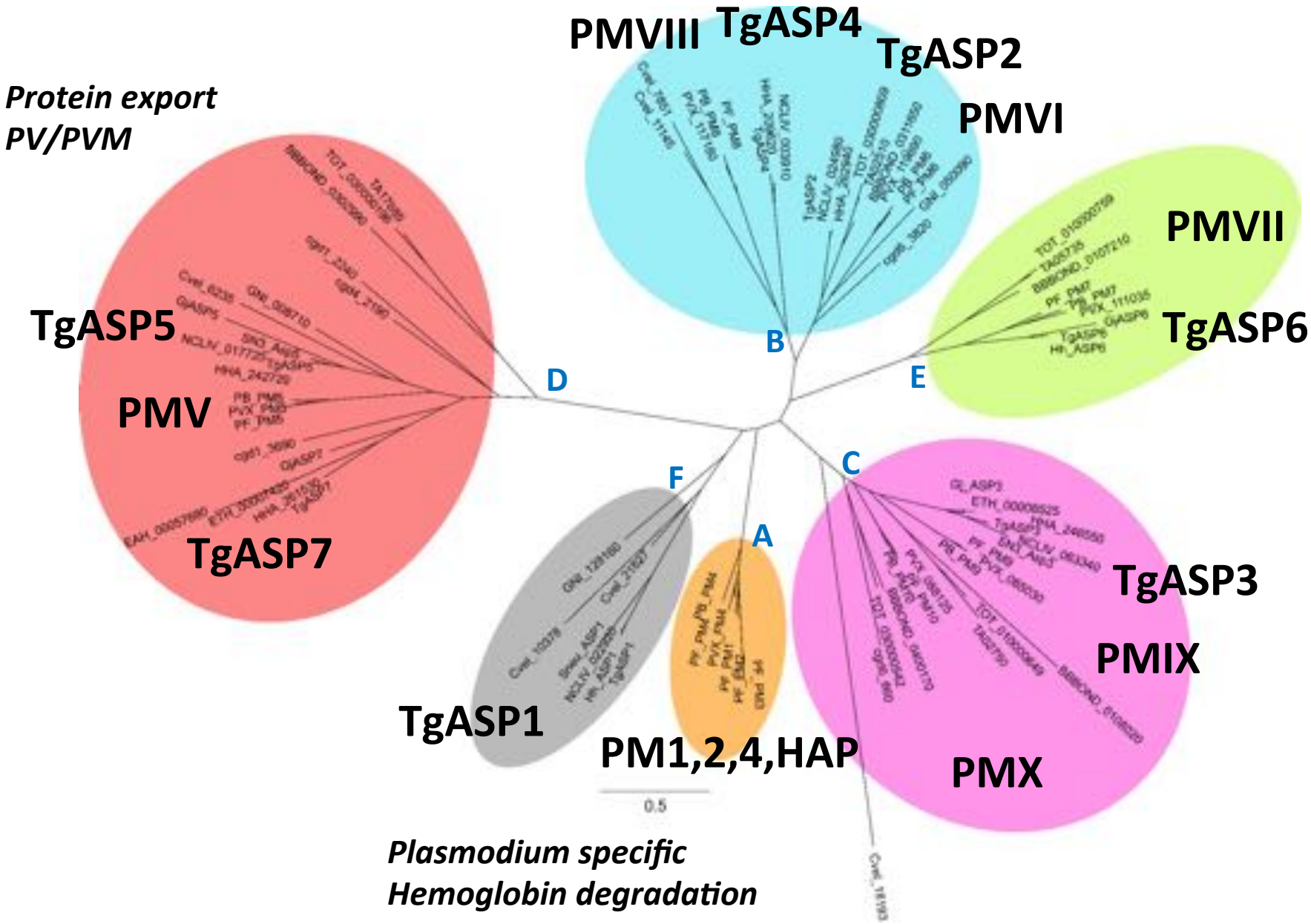


Activation with water

hydroxyl-anion formation

Peptidic bond lysis

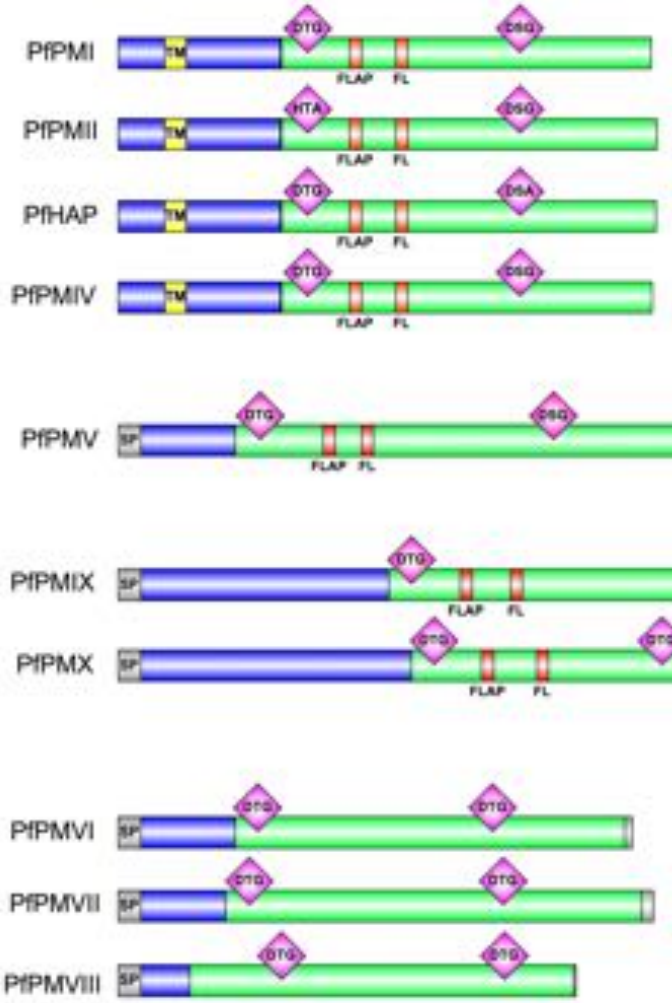
Apicomplexan ASPs follow 6 distinct groups



Plasmodium falciparum Plasmepsins

10 aspartic proteases: PfPMI-PfPMX

7 expressed during the erythrocytic stages



Food vacuole

↓

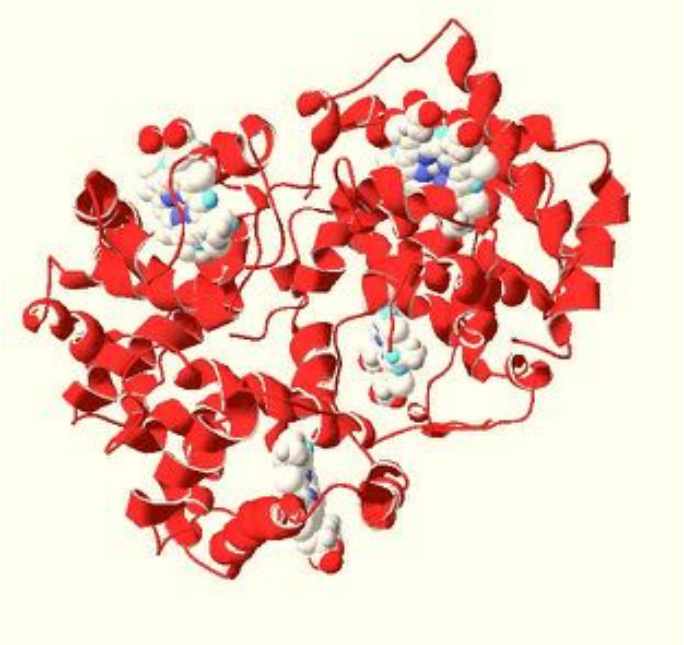
Hemoglobin degradation

ER → Export

?

Expressed during sexual stages

Hemoglobin degradation

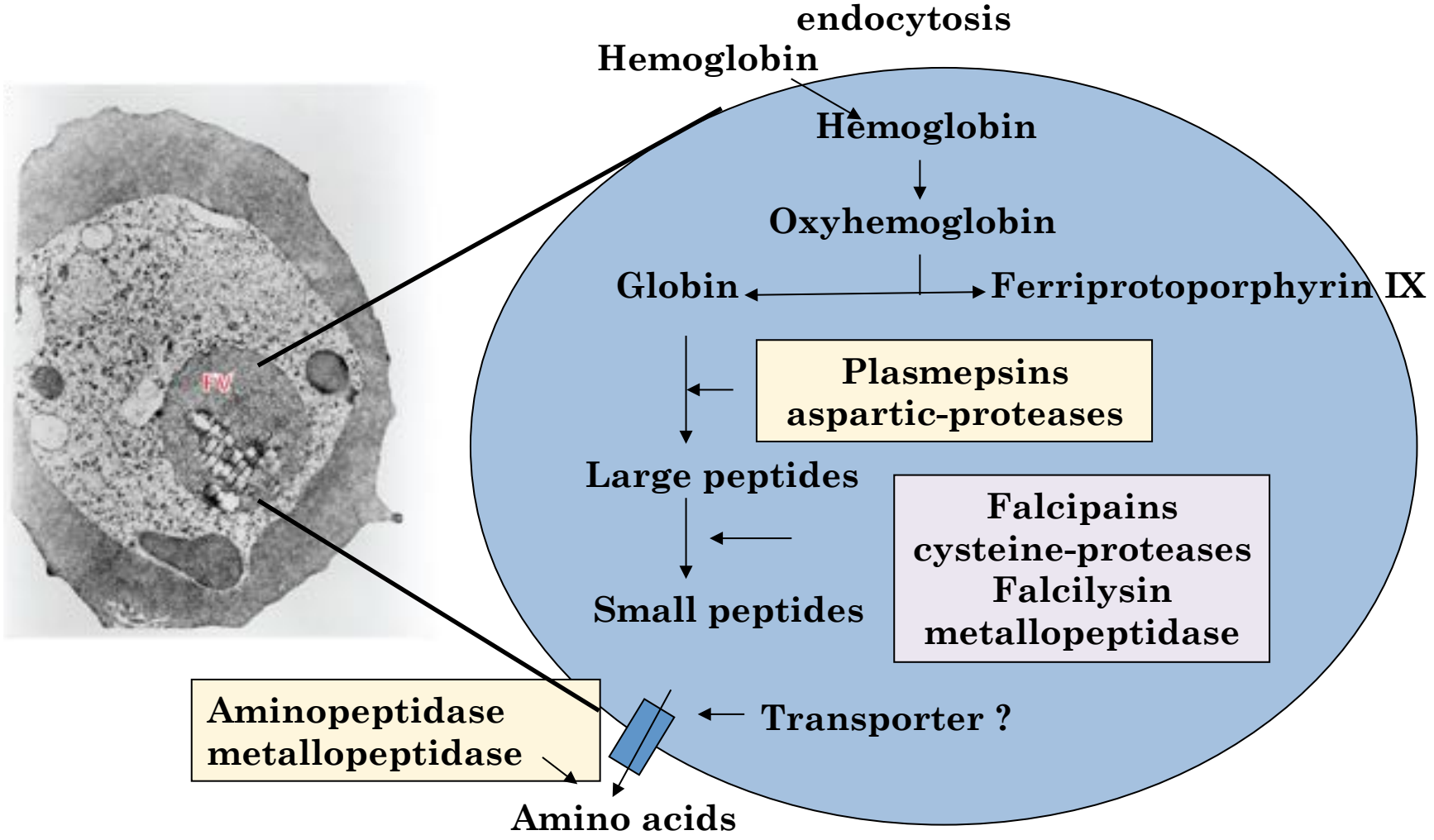


- A massive catabolic process.
- Consumes ≈75% of the infected cell Hb, which provides an important source of amino acids for the parasite growth and maturation
- In an acidic food vacuole
- Catalyzed by four aspartic proteases (plasmepsins), three cysteine proteases (falcipains) and one metalloprotease (falcilicin)

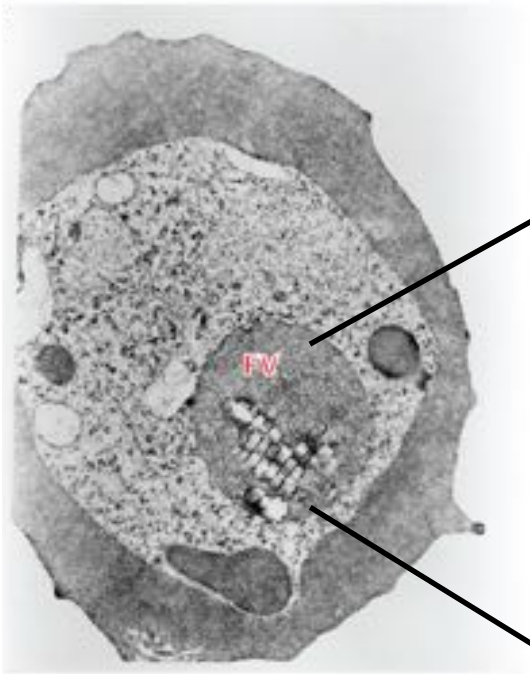
➔ **Drug target?**

Goldberg, PNAS 2006

“The way you cut your meat reflects the way you live” – Confucius



"The way you cut your meat reflects the way you live"
– Confucius



endocytosis

Hemoglobin

Hemoglobin

Oxyhemoglobin

Globin ⟷ Ferriprotoporphyrin IX

Polymerization

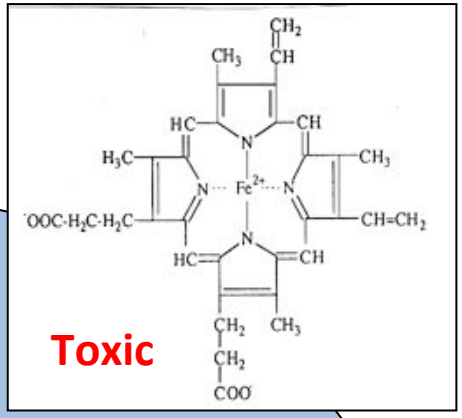
Large peptides

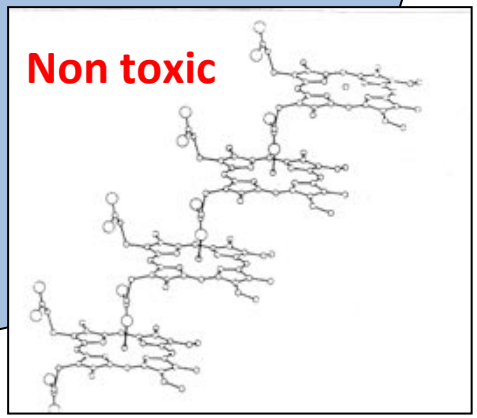
Small peptides

Hemozoin

Transporter ?

Amino acids

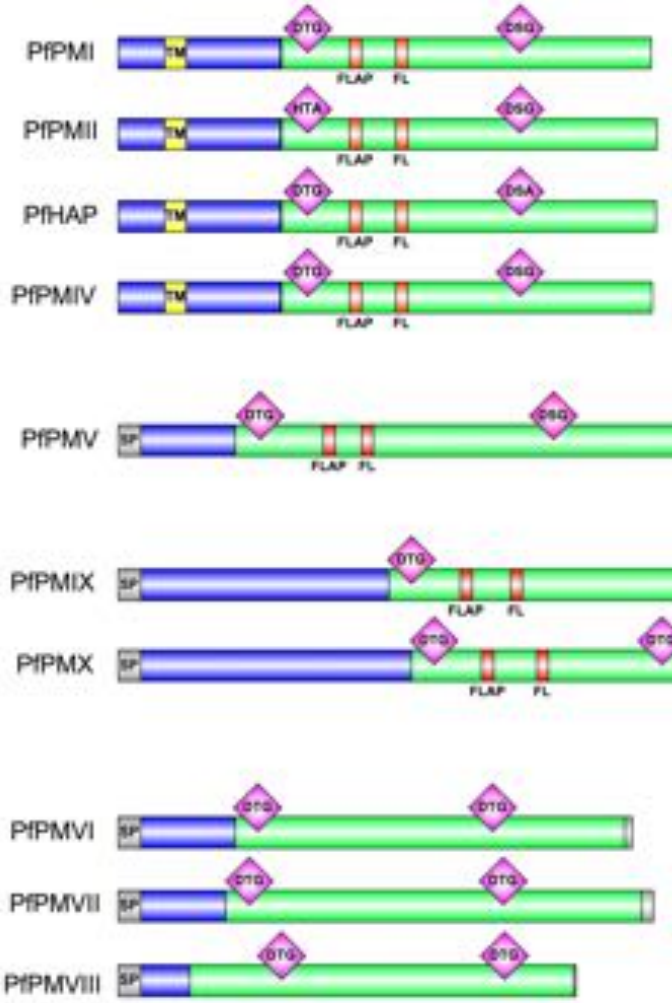




Plasmodium falciparum Plasmepsins

10 aspartic proteases: PfPMI-PfPMX

7 expressed during the erythrocytic stages



Food vacuole

↓

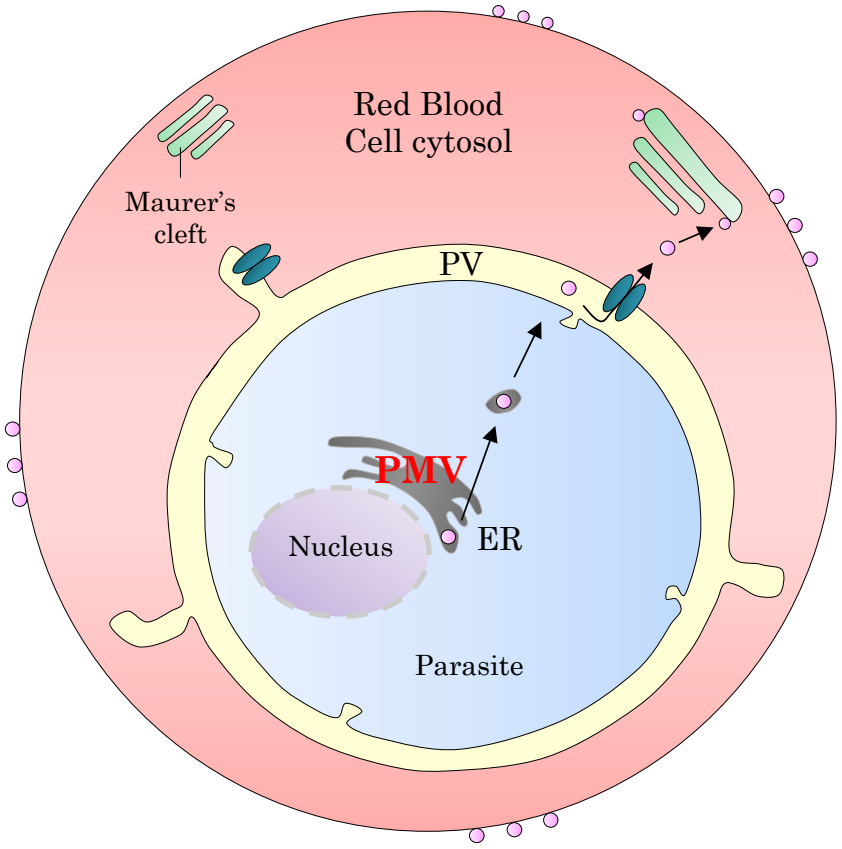
Hemoglobin degradation

ER → Export

?

Expressed during sexual stages

Aspartyl proteases implicated in protein export in *P. falciparum*



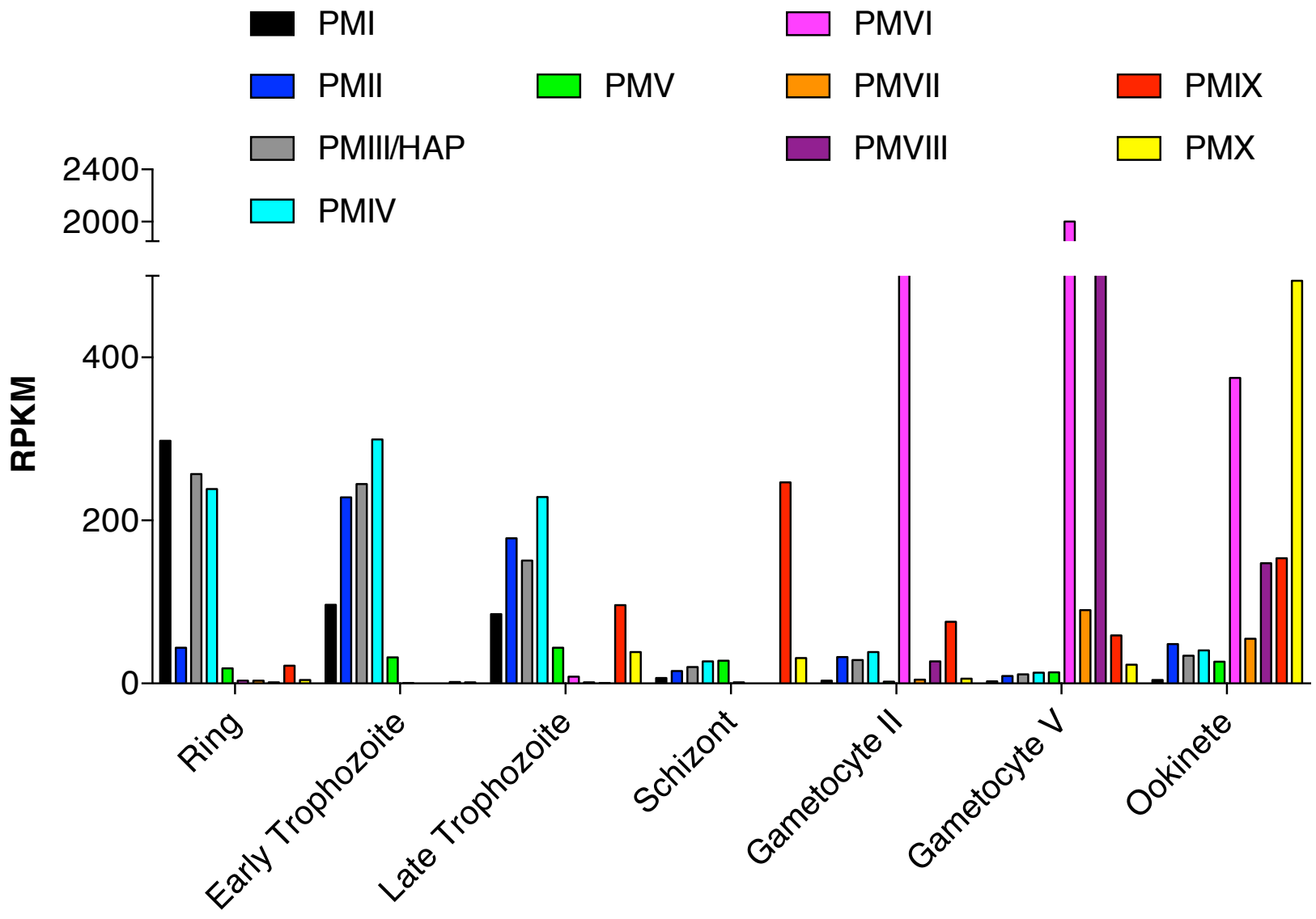
PEXEL/HT motif
R/KxLxE/Q/D
PfPMV - Plasmepsin V

Boddey *et al,* Nature, 2010
Russo *et al,* Nature, 2010

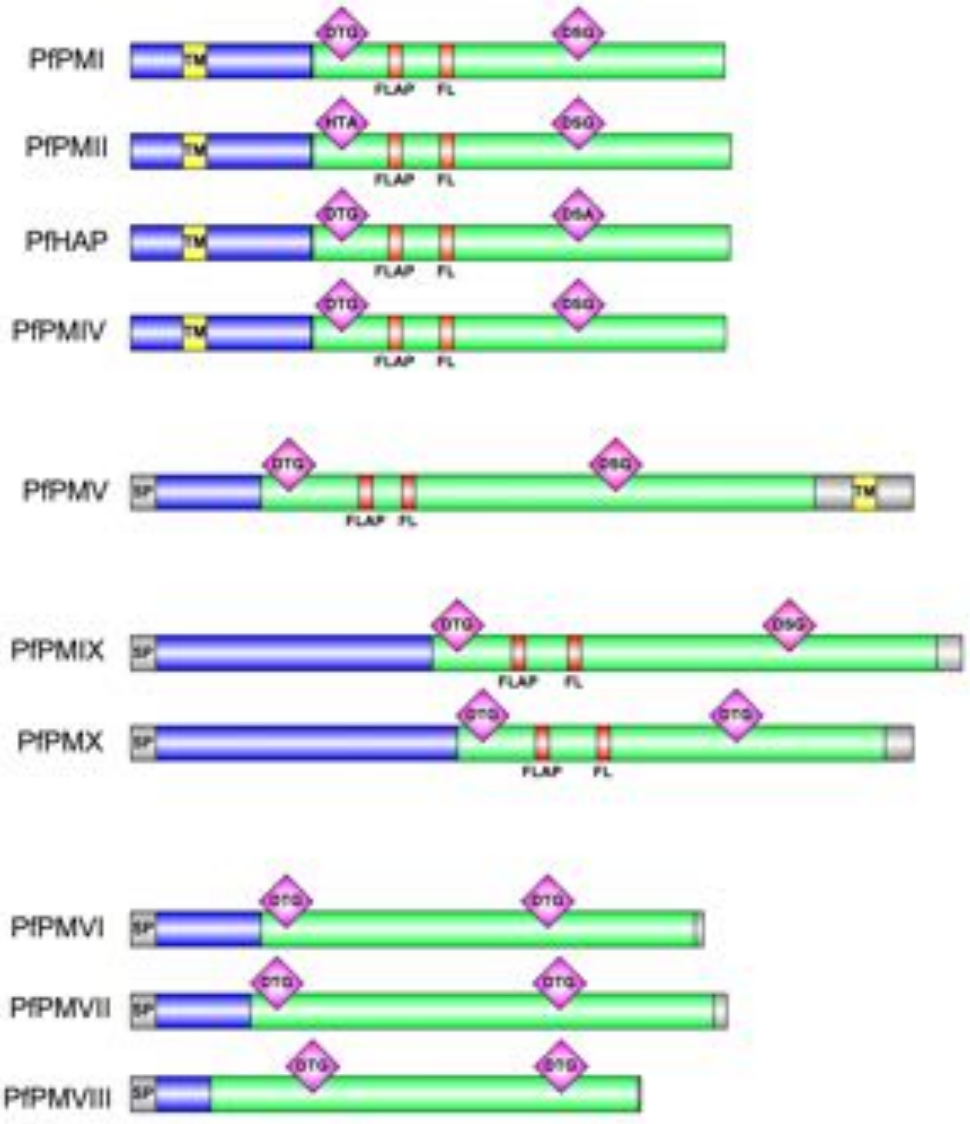
Homologue in *T. gondii*
RxLxE/D
TgASP5 - Aspartyl Protease 5

Hsiao *et al,* Traffic, 2013
Curt-Varesano *et al*, Cell microbial, 2015
Hammoudi *et al*, PLoS pathogens, 2015
Coffey *et al*, eLife, 2015

Plasmepsins' expression throughout the life cycle

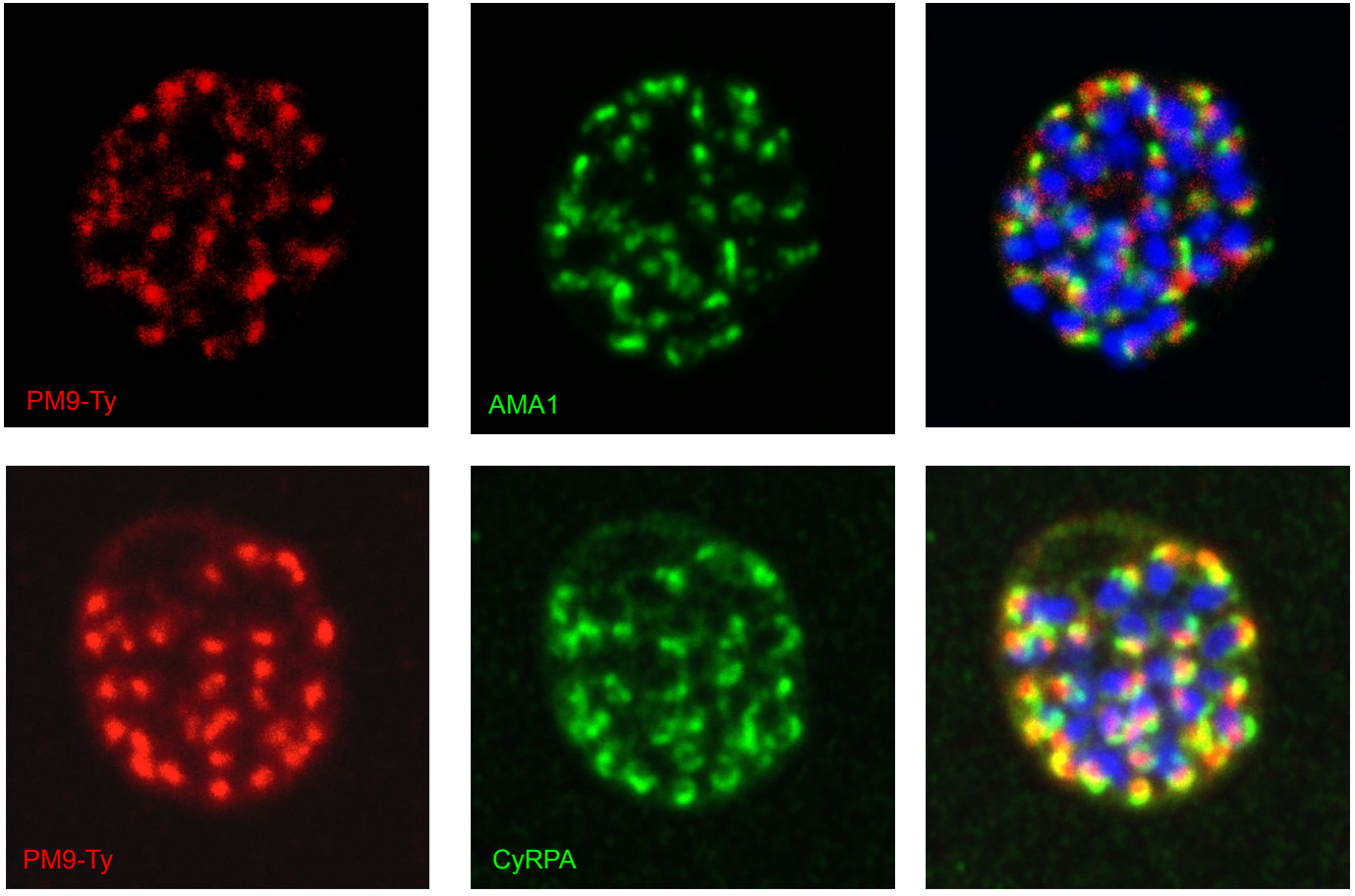


Plasmodium falciparum Plasmepsins

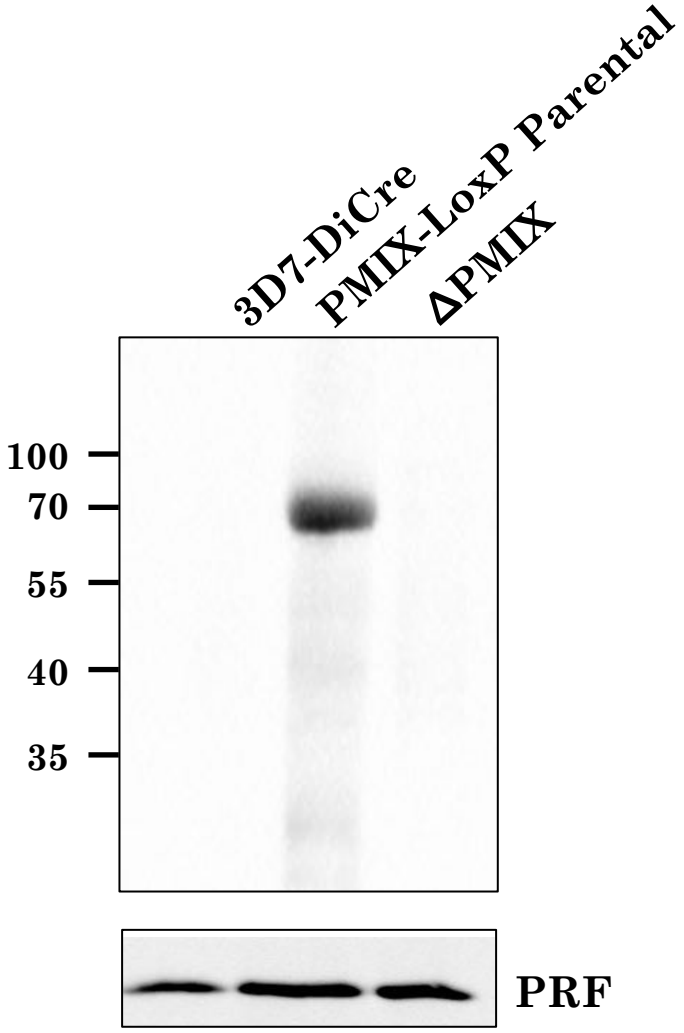


	KO viable	Phenotype
PfPMI	✔	None
PfPMII	✔	None
PfHAP	✔	None
PfPMIV	✔	None
PfPMV	✗	Asexual growth
PfPMIX	?	?
PfPMX	?	?
PfPMVI	✔	Oocyst
PfPMVII	✔	None
PfPMVIII	?	?

PMIX localizes at the apical end of merozoites

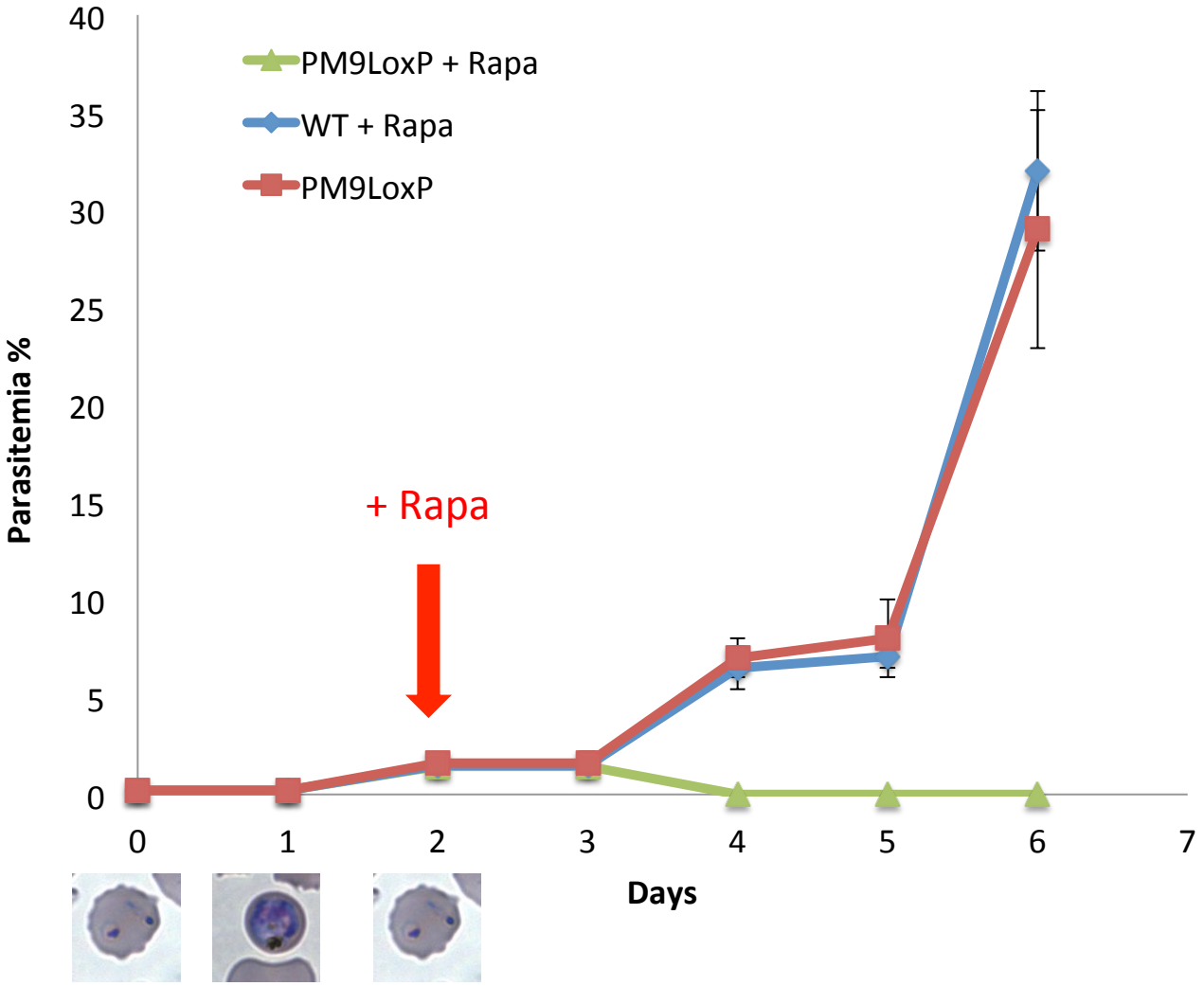


PfPMIX-Ty-Lox expression/excision

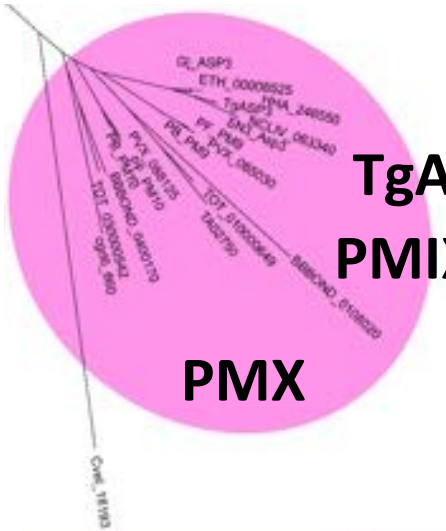


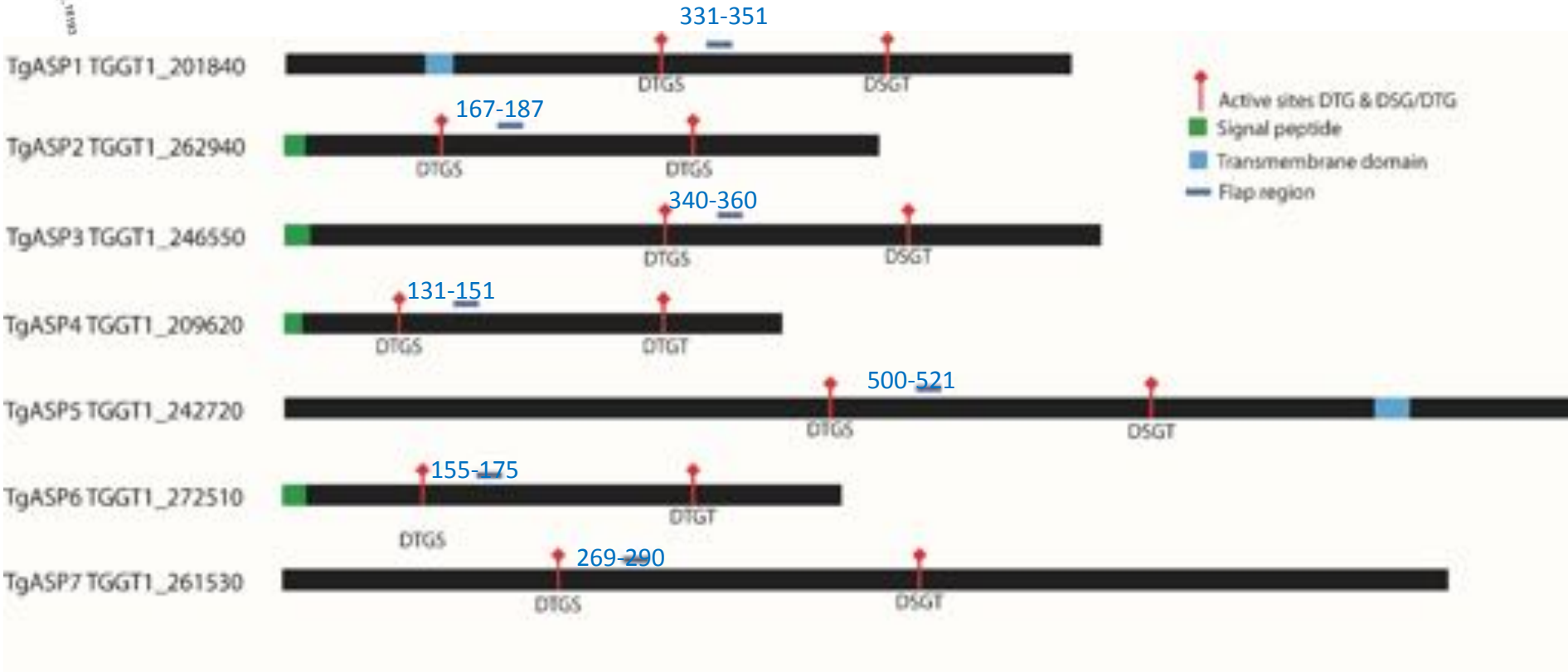
PfPMIX expected size 74 kDa

PMIX is critical for blood stages development



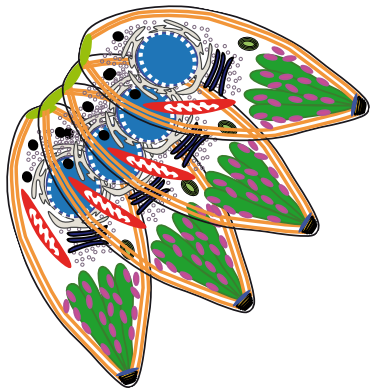
Toxoplasma gondii ASPs

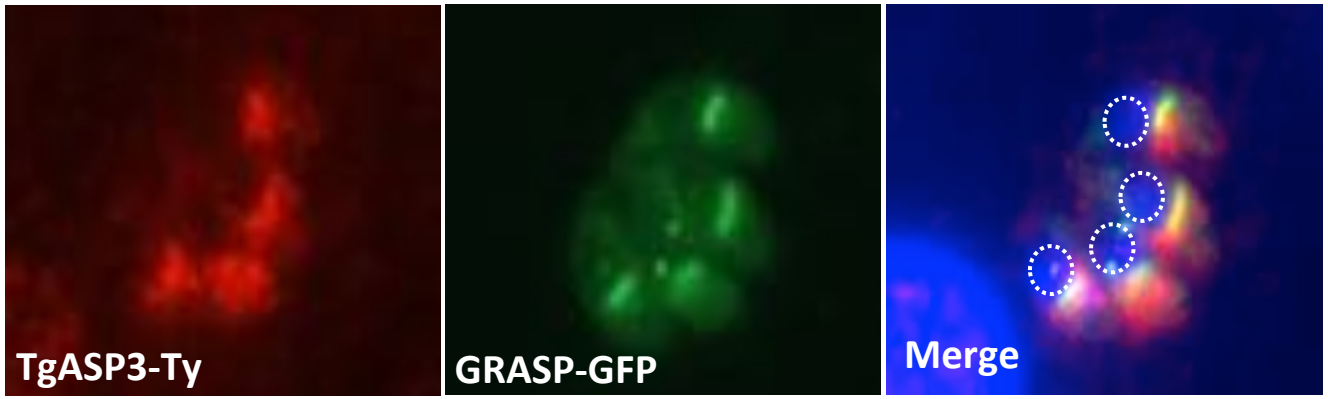




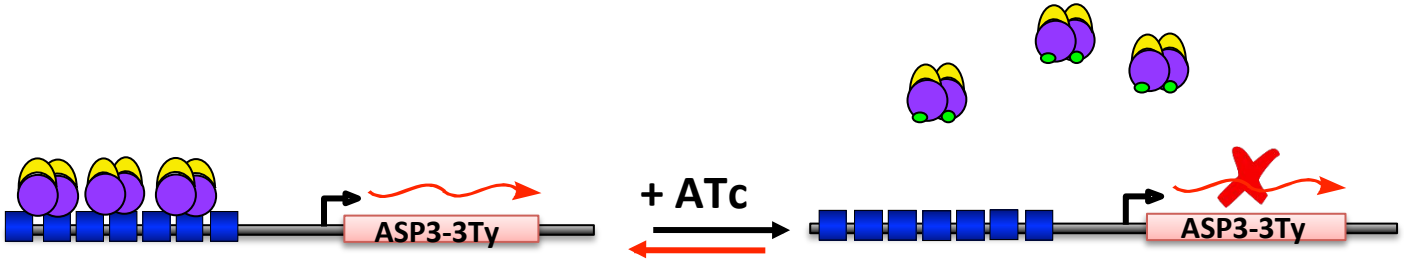
Asp3 is a ‘post-Golgi’ resident protease

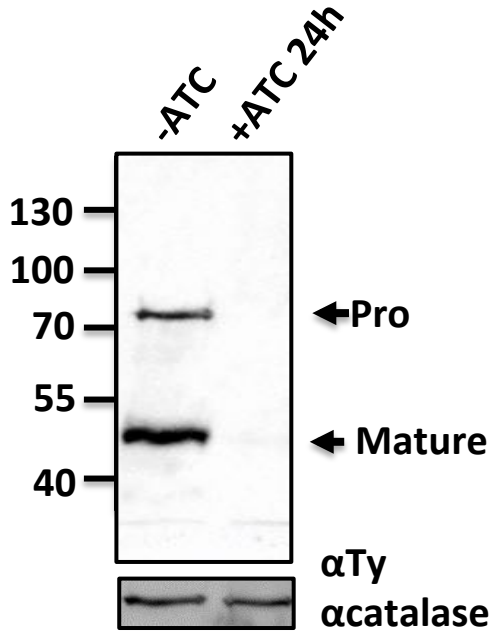
Modified locus

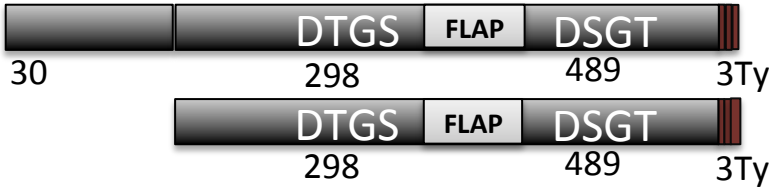


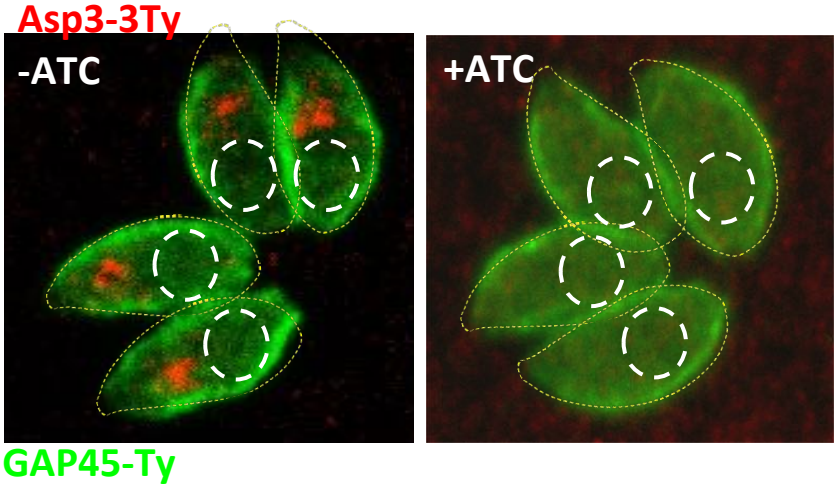


Tet-inducible knock-down of ASP3

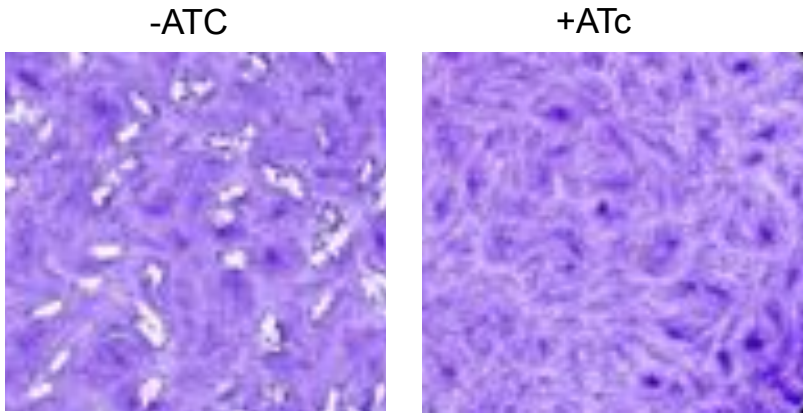


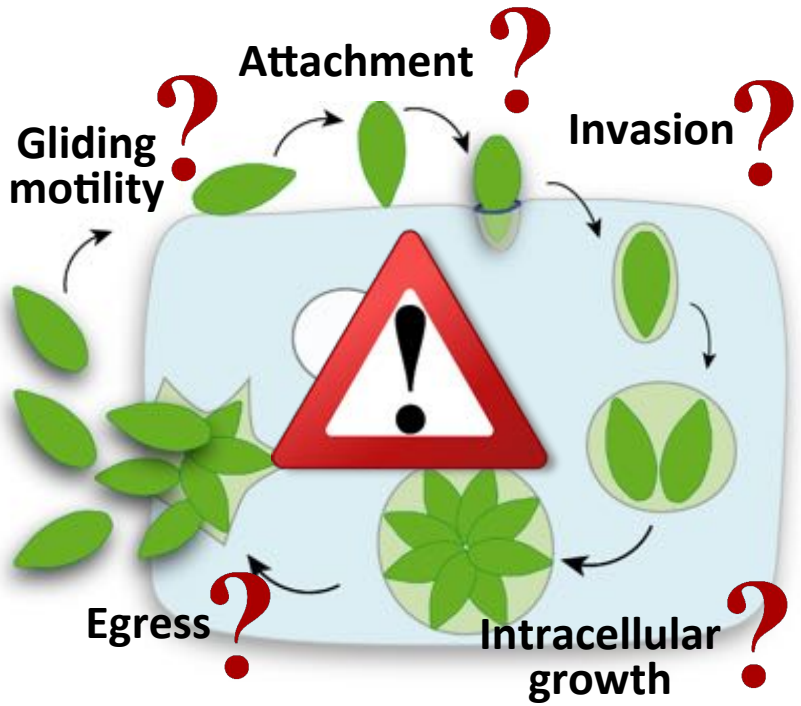




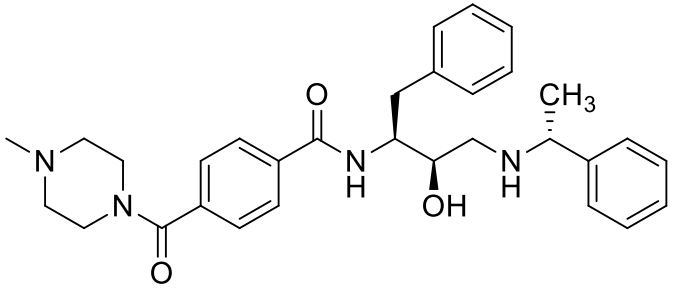


TgAsp3 is critical for Toxo lytic cycle





hydroxyethylamine scaffold-based drug 49c



Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel in vivo active anti-malarials based on a hydroxy-ethyl-amine scaffold

Claire-Lise Ciana[a], Romain Siegrist[a], Hamed Aissaoui[a], Léo Marx[a], Sophie Racine[a], Solange Meyer[a], Christoph Binkert[a], Ruben de Kanter[a], Christoph Fischli[b,c], Sergio Wittlin[b,c], Christoph Boss[a,*]

[a] Actelion Pharmaceuticals Ltd, Drug Discovery Chemistry and Biology, Hegenheimermattweg 91, CH-4123 Allschwil/BL, Switzerland
[b] Swiss Tropical and Public Health Institute, Parasite Chemotherapy, Socinstrasse 57, CH-4002 Basel, Switzerland
[c] University of Basel, CH-4003 Basel, Switzerland

Table 2
In vitro anti-malarial activity of hydroxy-ethyl-amine compounds: optimization of the acid part

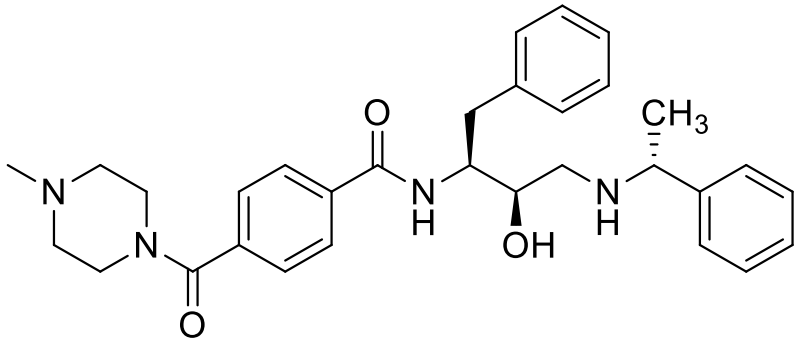
Entry	Compound	R	IC_{50} NF_{54} alb 72 h (nM)	IC_{50} NF_{54} ser 72 h (nM)	IC_{50} NF_{54} alb 24 h (nM)	IC_{50} NF_{54} alb 48 h (nM)	IC_{50} *P. berghei* 24 h (nM)	MLM (µl/(min mg))
1	**26**	3-CON^{i}Pr$_2$	2.0	10	>500	<3.1	>500	>1250
2	**49a**	4-CON^{i}Pr$_2$	1.6	6.5	>500	–	>500	>1250
3	**49b**	2-CON^{i}Pr$_2$	>500	>500	–	–	–	–
4	**49c**	4-CO-Me-piperazine	0.6	<0.6	>500	–	>500	75
5	**49d**	3-CO-Me-piperazine	98	102	–	–	–	–
6	**49e**	3-SO_2-Me-piperaz		138		–	–	908
7	**49f**	4-CO-piperidine		1.3		–	>500	860
8	**49g**	3-CO-pyrolidine	4.9	9.3	–	–	–	–
9	**49h**	3-CO-azepane	3.8	13	–	–	–	–
10	**49i**	3-CONH^{i}Pr	12	30	–	–	–	–
11	**49j**	4-Me-piperazine	8.7	8.5	>500	–	>500	80
12	**49k**	3-Me-piperazine	190	300	–	–	–	–

Low IC50 at 72 hr

High IC50 at 24hr

hydroxy-ethyl-amine scaffold-based drug 49c

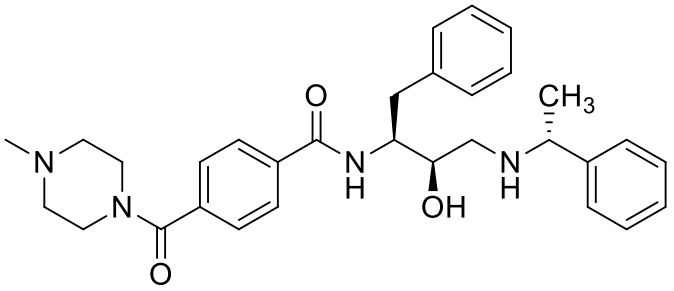
IC_{50} (24 hours) >500 nM, IC_{50} (72 hours) 0.6 nM



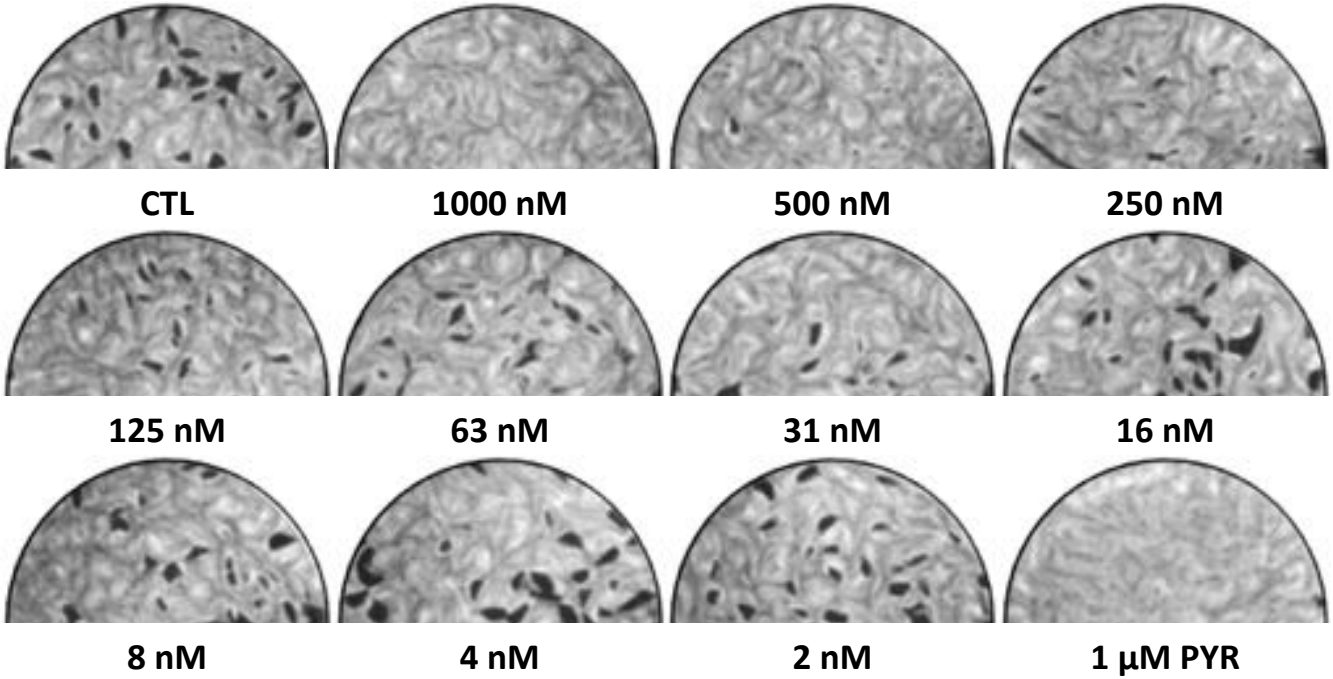
Ciana et al. 2013

- Peptidomimetic inhibitor of aspartic proteases
- Designed to target *Plasmodium* food vacuole aspartyl proteases
- "slow" acting drug and dropped…

Compound 49c efficiently blocks Toxo lytic cycle



IC_{50} 650 nM (4 days)



What we want!

- Functional characterization of PfPMIX
- Functional characterization of TgAsp3
- Molecular targets of compound 49c