The Abdus Salam
International Centre for Theoretical Physics

United Nations
Educational, Scientific and
Cultural Organization

IAEA
International Atomic Energy Agency

2039-6

Conference on 2nd Drug Development for the Third World: From Computational Molecular Biology to Experimental Approaches

1 - 5 June 2009

Targeting the Malaria Parasite's Invasion Machinery

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Targeting the invasion machinery of the malaria parasite

Wim Hol
University of Washington, Seattle

-

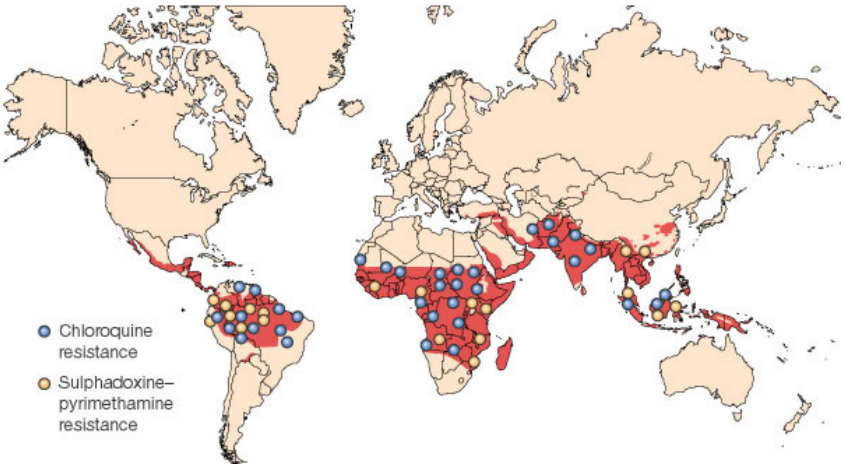
Drug Development for the Third World
International Center of Theoretical Physics (ICTP)
Trieste, Italy
June 2009

MALARIA

- *Plasmodium falciparum* and *Plasmodium vivax* cause about 500 million cases of malaria and 1 to 2 million deaths annually
- Desperate need for new antimalarials in particular due to the increase and threat of resistance against current drugs

Global status of resistance to chloroquine and sulphadoxine/ pyrimethamine, the two most widely used antimalarial drugs.

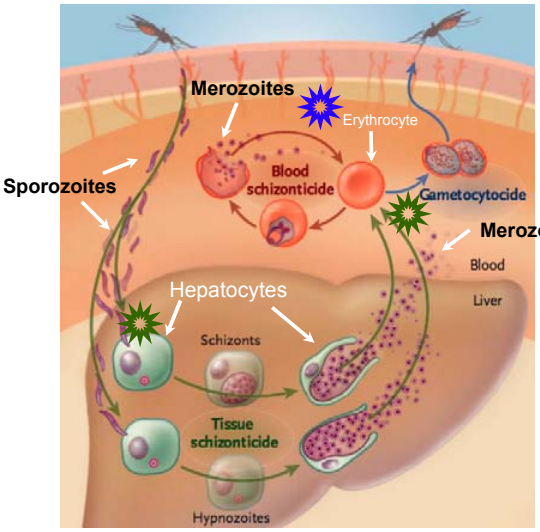
Data are from the WHO. ROBERT G. RIDLEY Nature 2002; *415*, 686 - 693



Cerebral malaria causing coma in child (Tanzania)

WHO/TDR/Crump

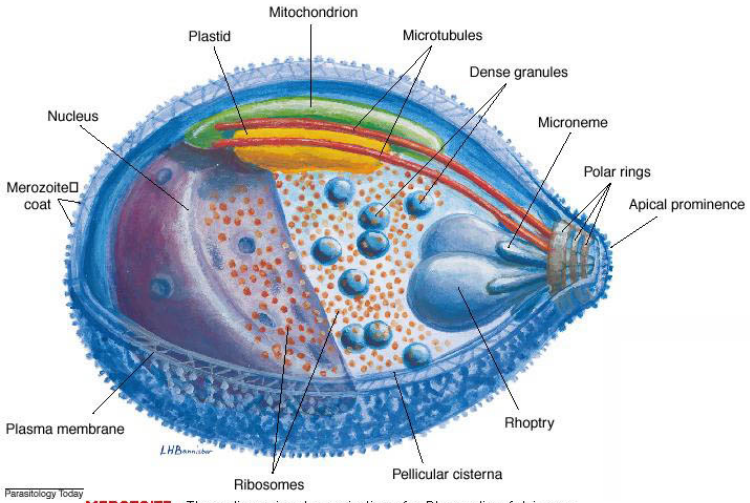
Malaria parasite host cell invasions



Invasion Inhibition has Prophylactic & Therapeutic Drug Possibilities

Plasmodium falciparum

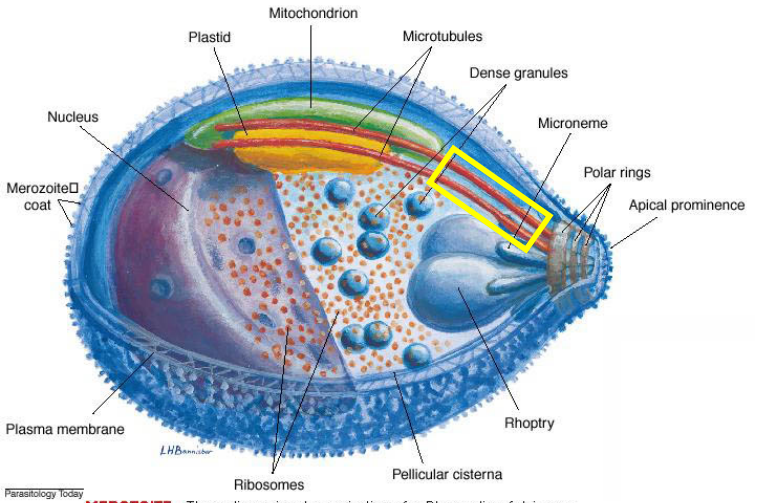
The major malaria parasite



MEROZOITE - Three-dimensional organization of a *Plasmodium falciparum* merozoite, with the pellicle partly cut away to show the internal structure.

Plasmodium falciparum

The major malaria parasite



MEROZOITE - Three-dimensional organization of a *Plasmodium falciparum* merozoite, with the pellicle partly cut away to show the internal structure.

The *P. falciparum* invasion machinery

A linked set of proteins which is essentially the same in:

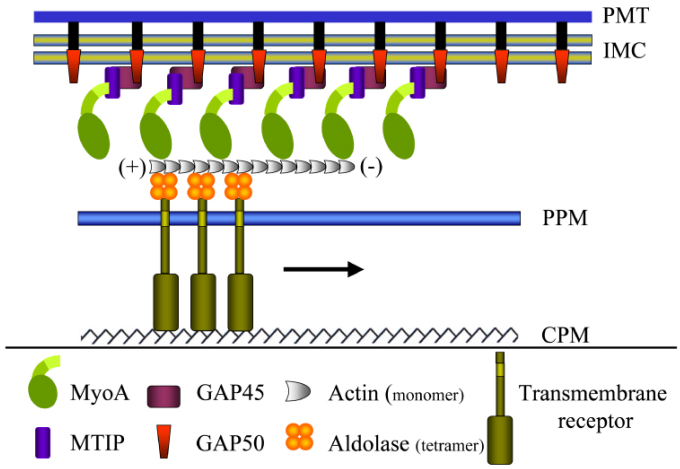
Ookinetes invading insect cells
Sporozoites invading hepatocytes
Merozoites invading erythrocytes

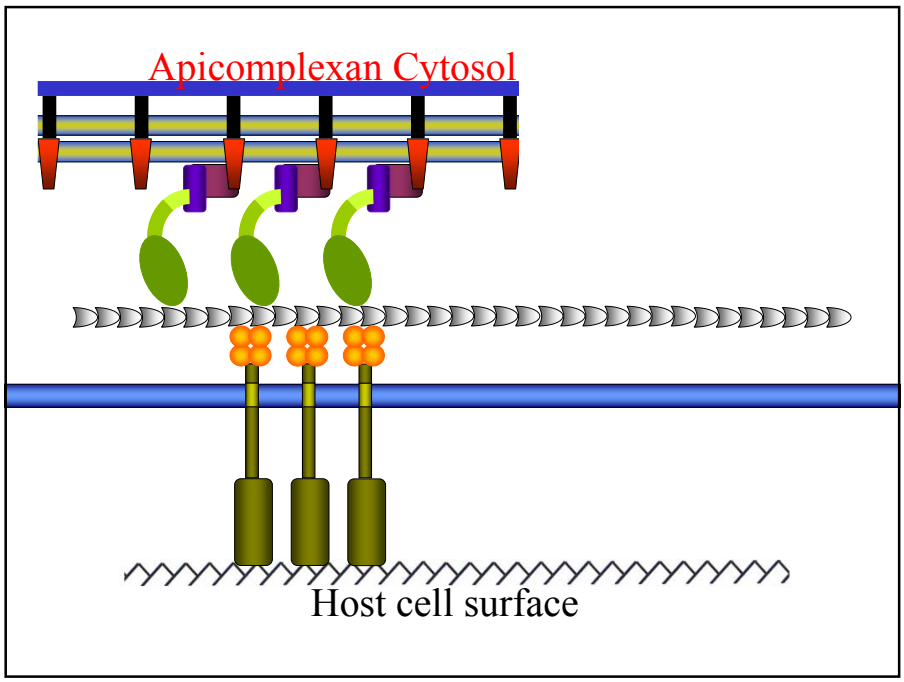
The main difference is in the protein which interacts with host cell protein(s)

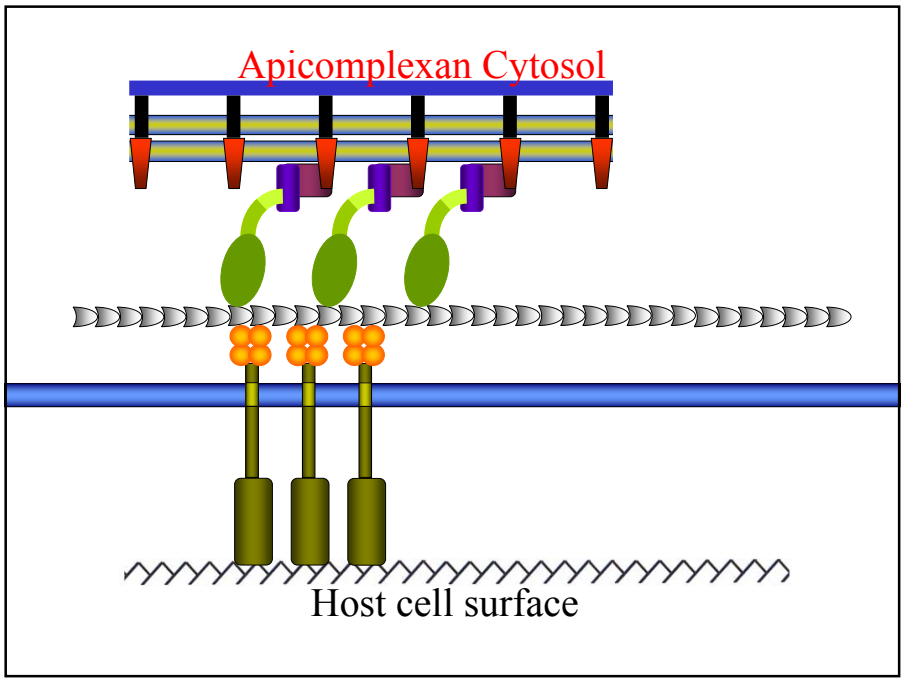
Invasion Machinery Components

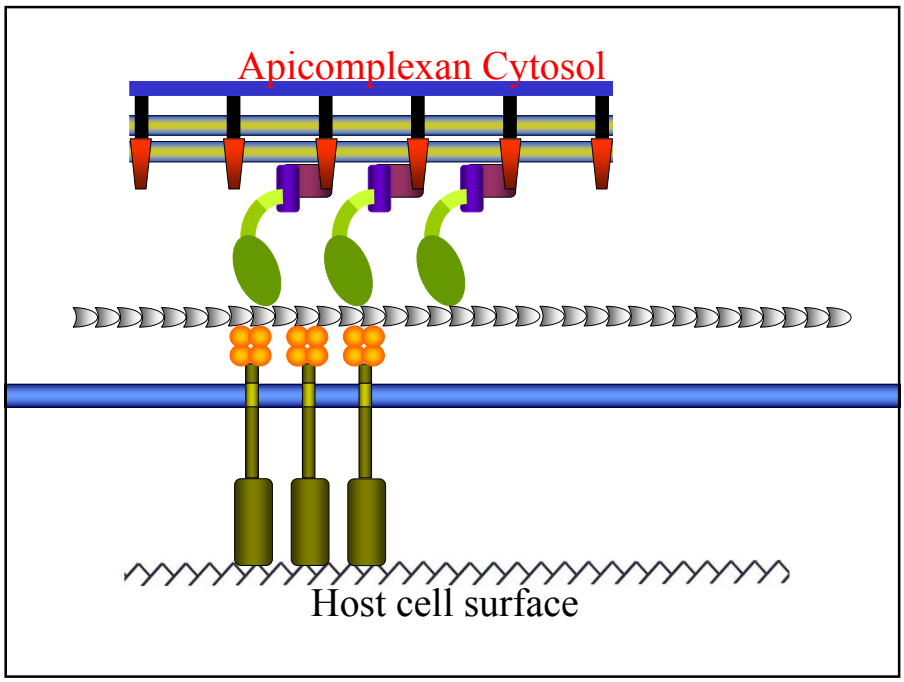
1. TRAP
2. Aldolase
3. Actin
4. Myosin A
5. MTIP
6. GAP45
7. GAP50
8. X,Y, Z
9. Microtubules

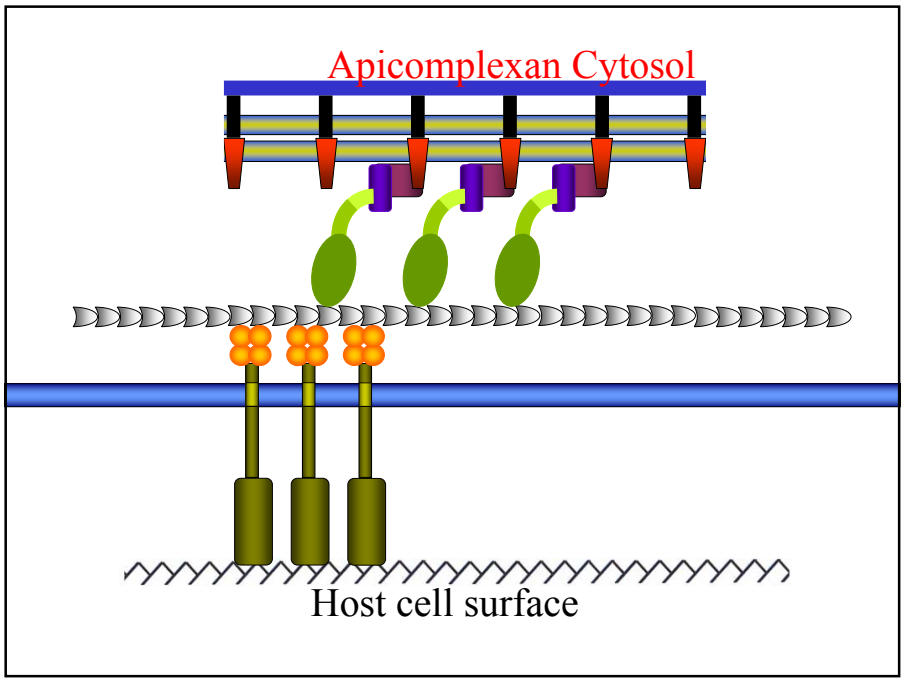
The Malaria Parasite's Cell Invasion Machinery's Motor

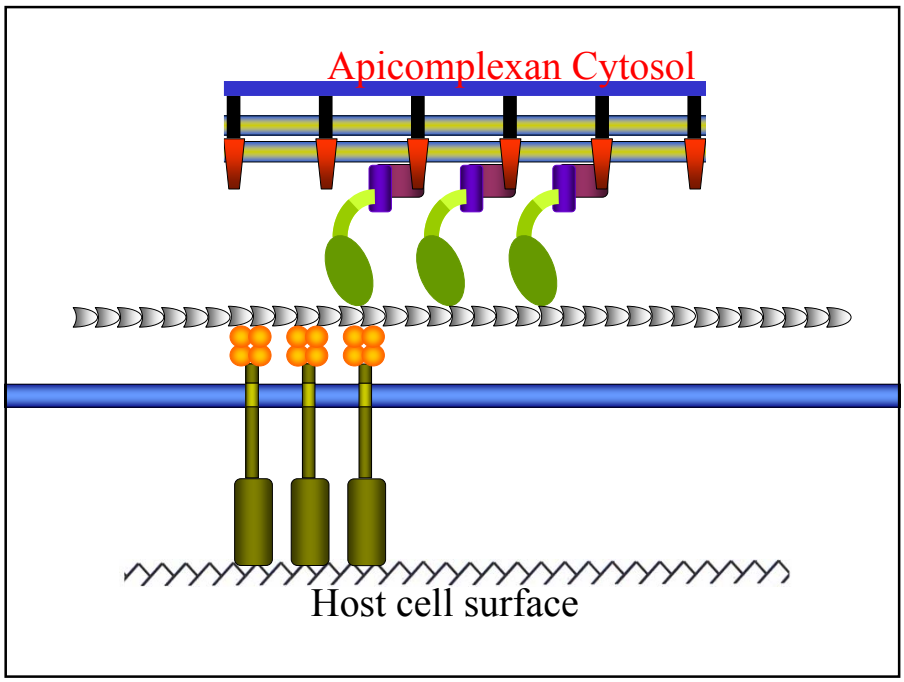


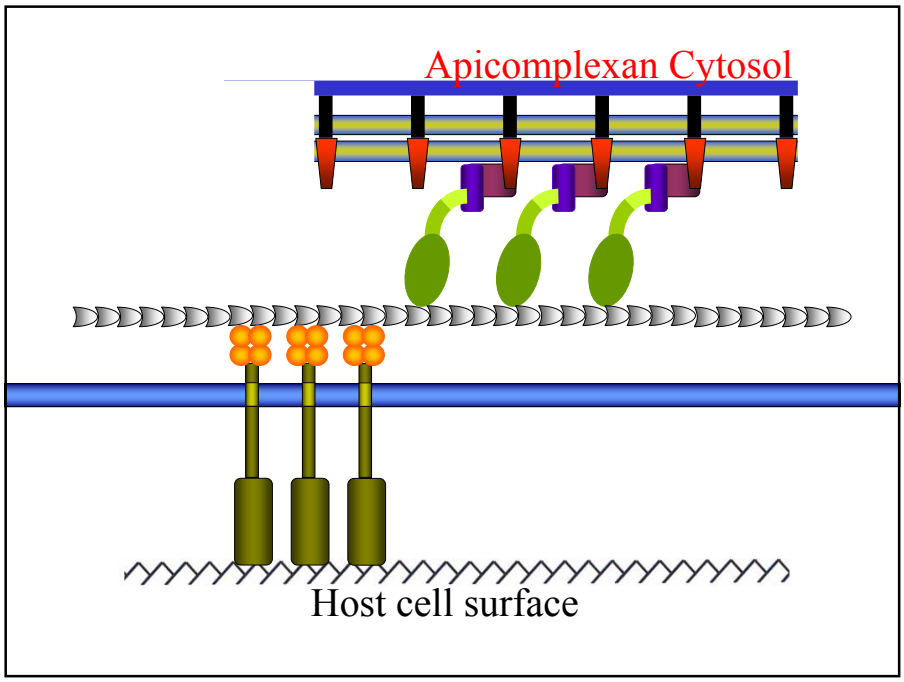

Apicomplexan Cytosol
Host cell surface

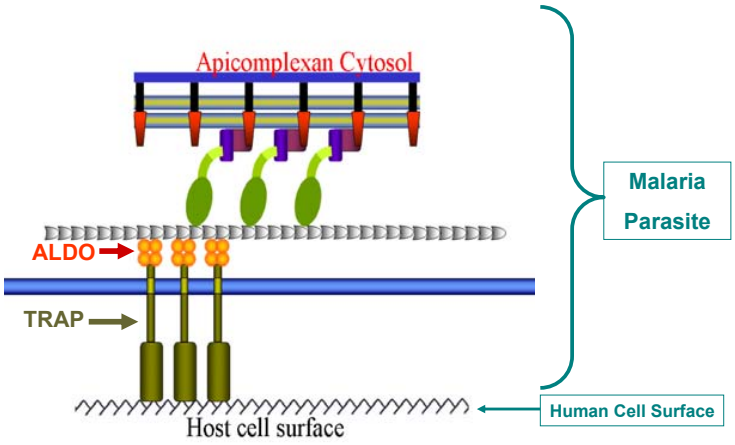

Apicomplexan Cytosol
Host cell surface


Apicomplexan Cytosol
Host cell surface


Apicomplexan Cytosol
Host cell surface


Apicomplexan Cytosol
Host cell surface


Apicomplexan Cytosol
Host cell surface



Carlos Buscaglia
Timothy Cardozo
Victor Nussenzweig

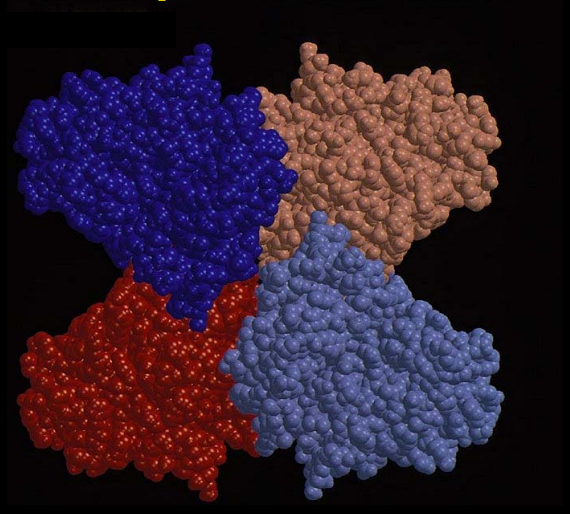
Jürgen Bosch
Stewart Turley

Aldolase interacting with the TRAP Tail in the malaria parasite

TRAP = Thrombospondin-Related Anonymous Protein

Jürgen Bosch, Brian Krumm
Carlos Buscaglia, Timothy Cardozo , Victor Nussenzweig

P. falciparum Aldolase



Homotetrameric enzyme with 363 amino acids per subunit

Hidong Kim

TRAP

(Thrombospondin-Related Anonymous Protein)

Plasmodium TRAP tails

- Highly charged cytoplasmic C-terminal tail
- Penultimate residue is ALWAYS a **W**
- **WtoF** and **WtoY** : significantly less affinity
- The penultimate **W** is critical for invasion function

Kappe et al. 1999, Journal of Cell Biology, 147,937;
Buscaglia et al, 2003, Molecular Biology of the Cell, 14,4947

P. falciparum Aldolase

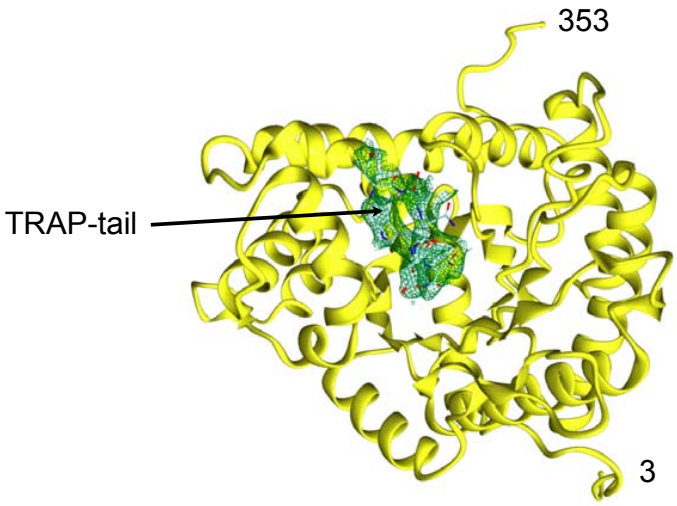
- Homotetrameric enzyme with 363 amino acids per subunit
- Co-crystallized with 5 mM *P. berghei* TRAP-tail (EDND**W**N)
- The penultimate **W** is critical for invasion function

  Kappe et al. 1999, Journal of Cell Biology, 147,937

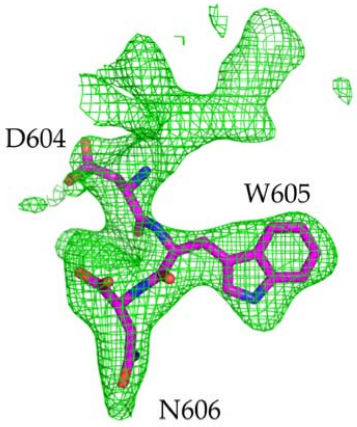
  Buscaglia et al, 2003, Molecular Biology of the Cell, 14,4947

- Structure of TRAP-bound aldolase solved by molecular replacement using uncomplexed structure of Kim et al. 1998

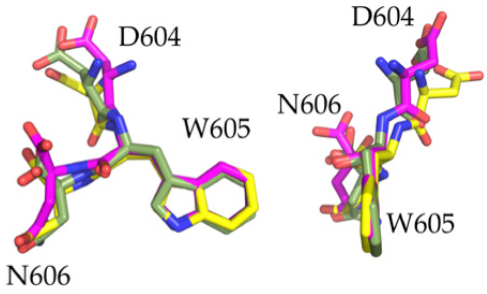
Aldolase – one subunit



TRAP-tail binding by *P. falciparum* Aldolase

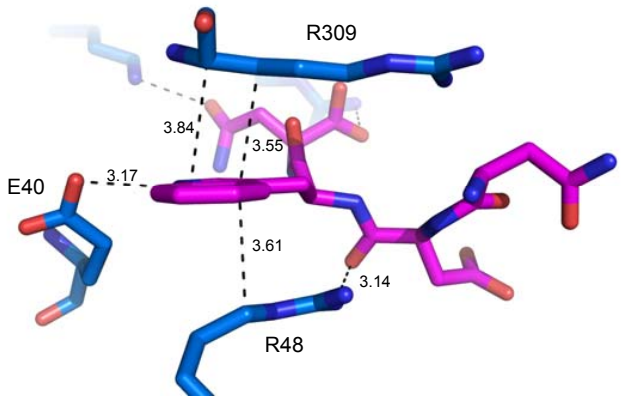


σA-weighted $2F_o$-F_c @ 1.5σ
2.4 Å resolution

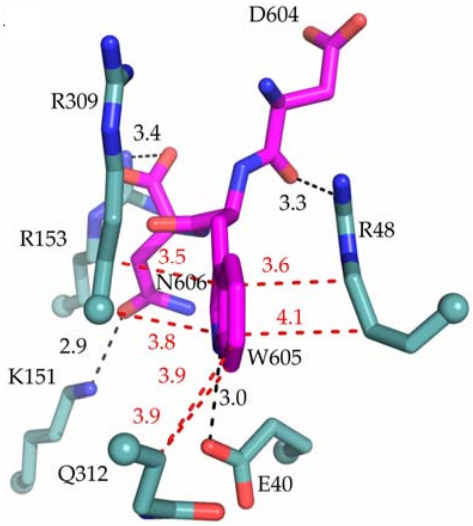


TRAP positions in three subunits
after superposition of PfAldo C_α-backbone

P. falciparum Aldolase + TRAP-tail



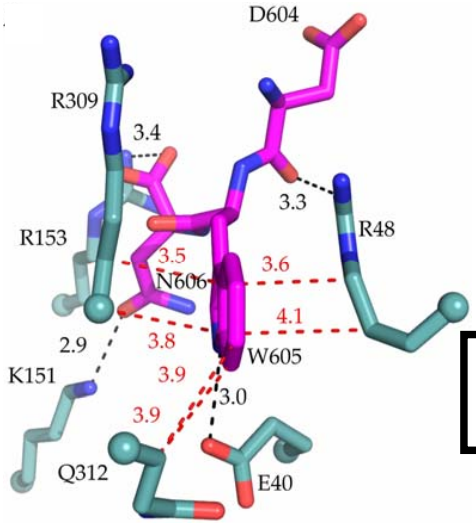
P. falciparum aldolase interactions with indole ring of *P. berghei* TRAP-tail



The hydrophobic indole ring is bound by four hydrophilic residues:
E40
R48
R309
Q312

of these three are charged

P. falciparum aldolase interactions with indole ring of *P. berghei* TRAP-tail



E40, R48, R309, Q312 100% conserved in all known aldolase structures

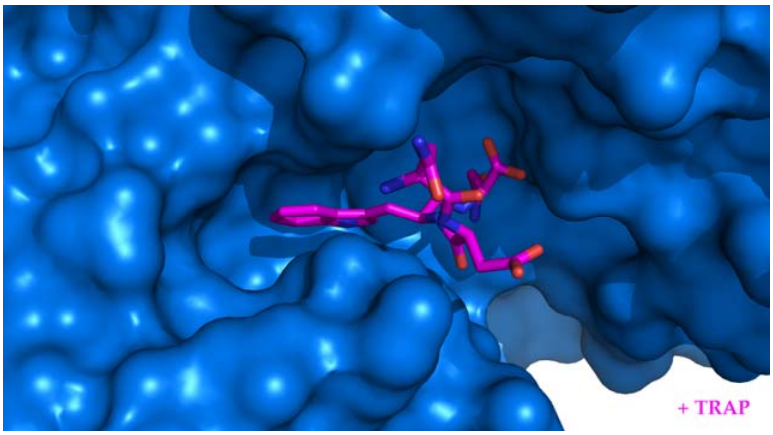
I.e. also compared to human aldolase

Conclusions so far:

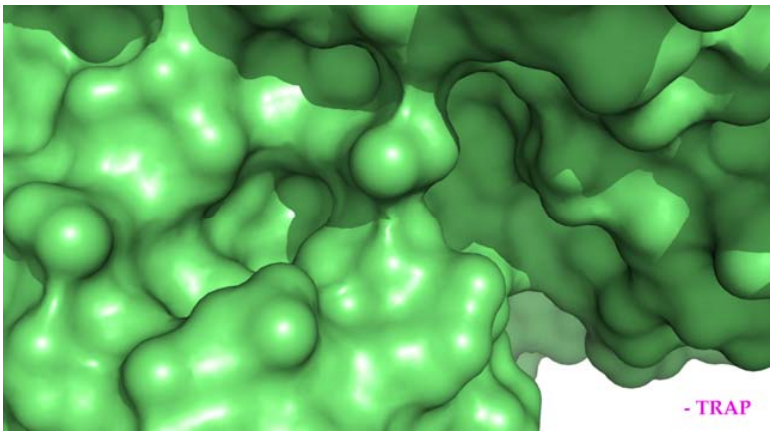
The indole ring of the penultimate and conserved Trp of the TRAP tail:

1. Creates ("digs") its own pocket in *P. falciparum* aldolase
2. Uses hydrophylic groups as walls of the binding pocket
3. These hydrophylic groups are conserved between parasite and human host.

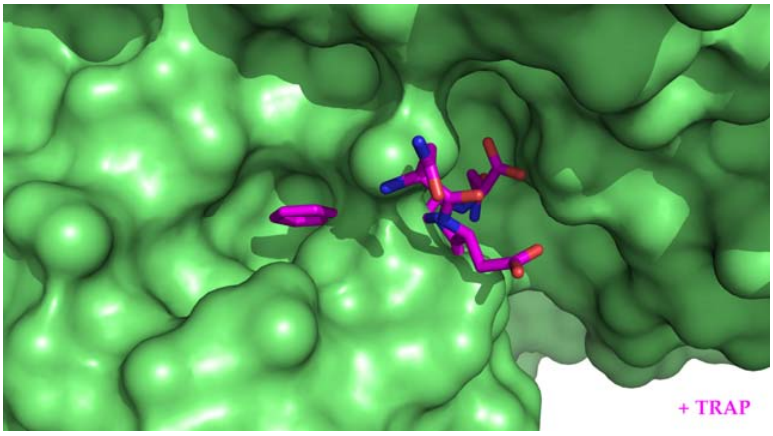
P. falciparum Aldolase + TRAP-tail



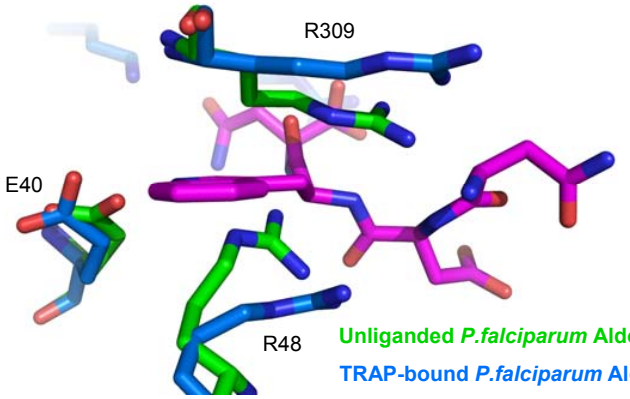
P. falciparum Aldolase - TRAP-tail



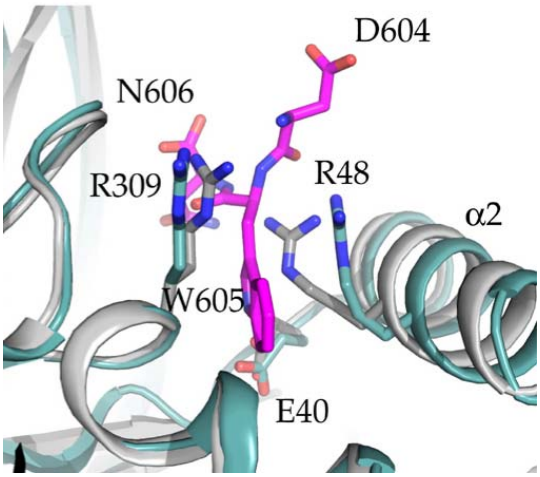
P. falciparum Aldolase +/- TRAP-tail



P. falciparum Aldolase interactions with *P. berghei* TRAP-tail



Flexibility of the TRAP binding site

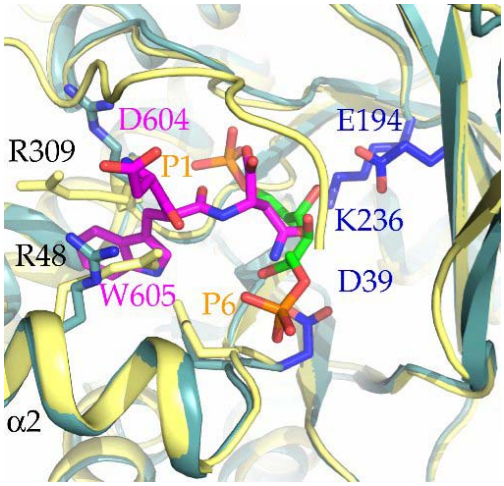


R48 shifts by 3 Å
R309 shifts by 3.1 Å
α2 moves by 1.5 Å

Classic example of "Induced Fit"

P. falciparum aldolase in complex with TRAP
P. falciparum aldolase without TRAP

TRAP and Substrate binding sites overlap

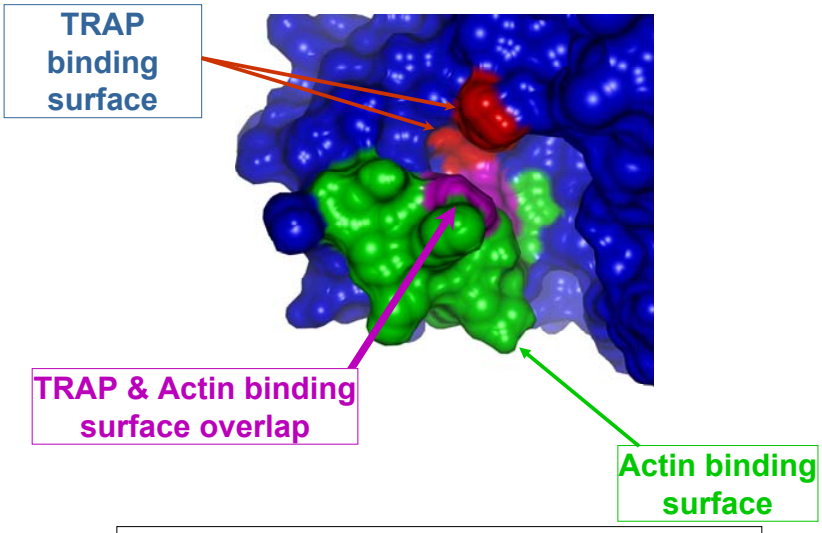


TRAP N606 clashes with C3 and C4 of substrate F1,6P

In excellent agreement with biochemical studies: TRAP inhibits enzyme activity & Substrates inhibit TRAP binding

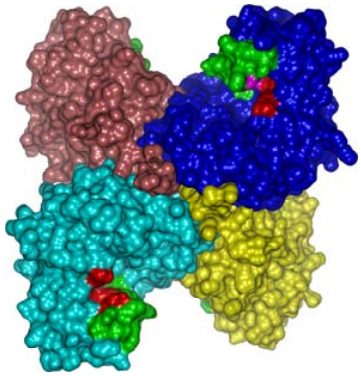
P. falciparum aldolase in complex with TRAP
H. sapiens aldolase in complex with F1,6P

TRAP and Actin binding sites overlap too



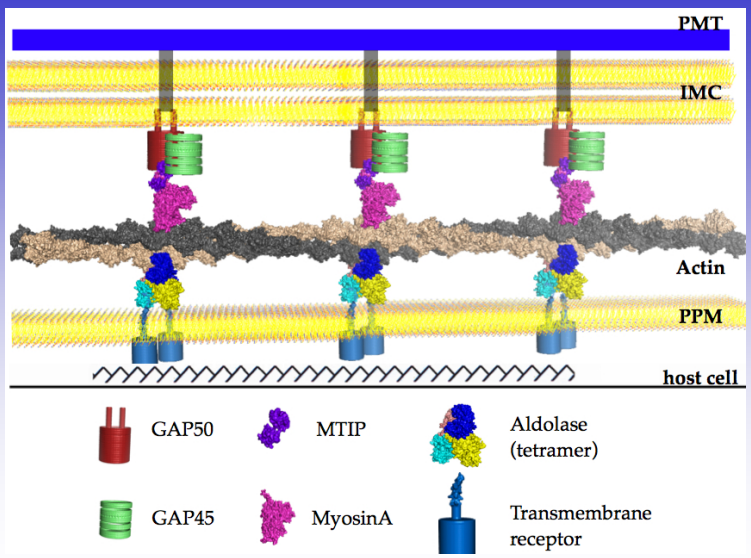
Biochemical actin binding studies - O'Reilly and Clarke (1993) FEBS Letters 321,69-72

However, aldolase is a tetramer



Therefore:
Different aldolase subunits per tetramer may have different functions in the Invasion Machinery
For instance:
two subunts bind actin,
while two other subunits bind TRAP tails

Facts and a lot of Fiction



Conclusions Part I:

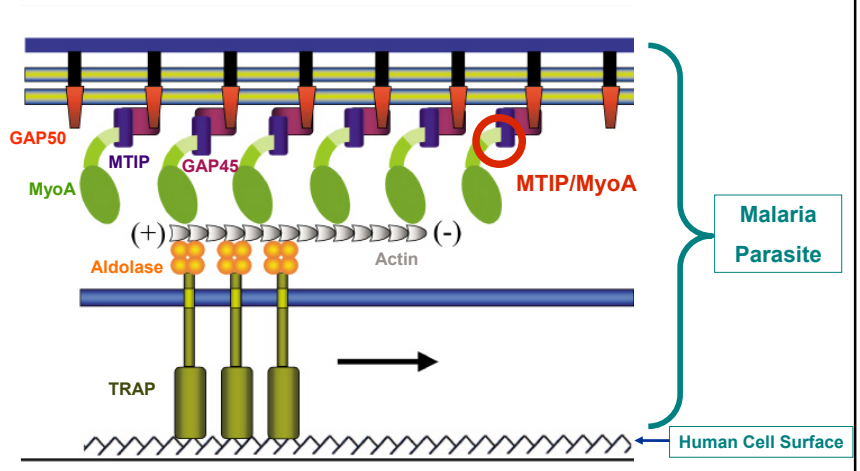
1. *P. falciparum* aldolase is interacting in surprising ways with the penultimate W of the TRAP tail

2. The W binding site in *P. falciparum* aldolase is made up of residues which are identical in the homologous enzyme from the human host

Myosin-Tail Interacting Protein (MTIP) interacting with the Myosin A Tail in the malaria parasite

Jürgen Bosch, Stewart Turley, Claudia Roach
Lawrence W. Bergman, Akhil Vaidya

Malaria Parasite's Invasion Machinery



Bill Bergman
Akhil Vaidya

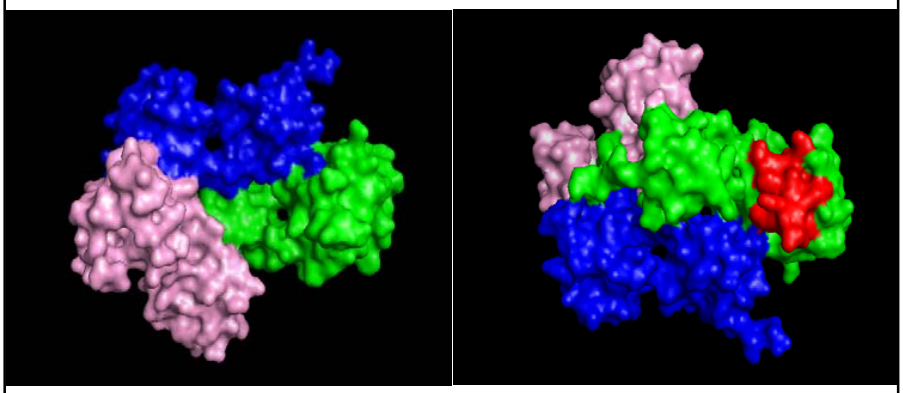
Jürgen Bosch
Stewart Turley

Crystallization of the first MTIP/MyoA complex

Construct			*P. yoelii* MyoA 15mer	Crystals
P.yoelii	79	205		
P.yoelii	60	205		
P.knowlesi	**79**	**205**	**X**	**X**
P.knowlesi	60	205	X	X
P.knowlesi	1	205		
P.falciparum	80	204		
P. falciparum	60	204	X	X
P.falciparum	1	204	X	

Stewart Turley, Claudia Roach, Bill Bergman

Myosin-Tail Interacting Protein (MTIP) plus the MyoA-tail

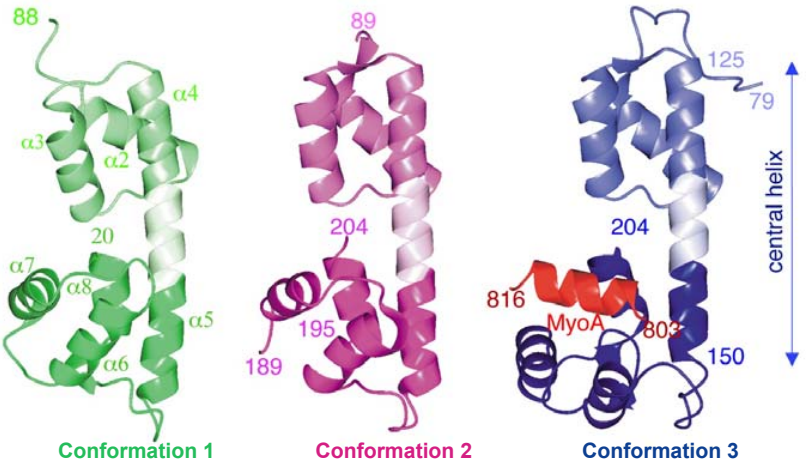


Asymmetric Unit – View 1:

Three *P. knowlesi* MTIP subunits

Asymmetric Unit – View 2:
Three *P. knowlesi* MTIP subunits
plus ONE tail from
P. yoelii MyoA

MTIP Crystal structure 1:
Three conformations of *P. knowlesi* MTIP @ pH 5.3



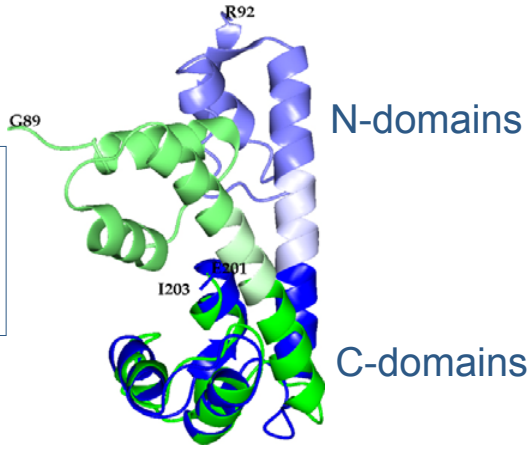
Conformation 1
Unliganded

Conformation 2
Unliganded

Conformation 3
Liganded

Myosin-Tail Interacting Protein (MTIP) plus the MyoA-tail

A major conformational change in the C-domain upon binding the MyoA- tail



Subunit 1 (without MyoA tail bound)

vs.

Subunit 3 (with MyoA tail bound)

3 helices of C-domains (α6, α7, α8) used for superposition.
Angle between α5 and α6 very different.

Interhelical angles C-domains of Subunits 1 and 3 *P. falciparum* MTIP

	Subunit 1	Subunit 3	Δ
α5 - α6	47.5	67.5	~20º
α5 - α7	83.8	68.6	~15º
α5 - α8	11.4	33.7	~22º
α6 - α7	48.5	45.3	~ 3º
α7 - α8	80.9	81.6	~ 1º
α6 - α8	128.4	136.7	~ 8º

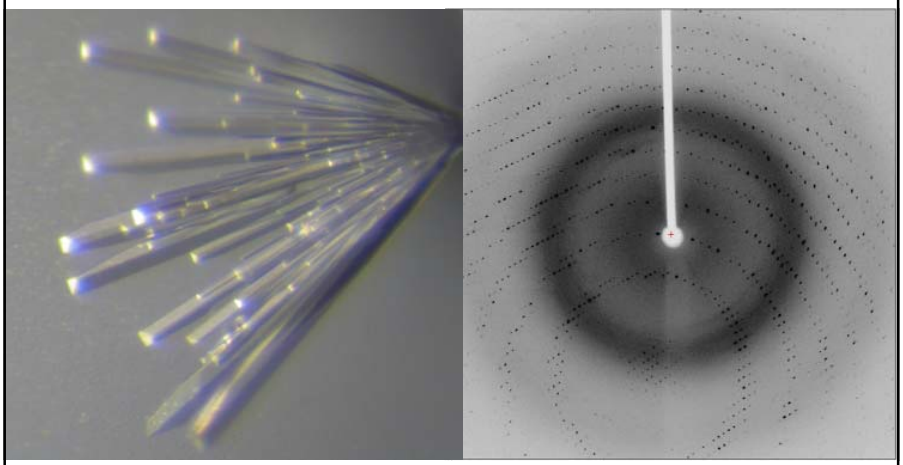
Conclusion:
Upon MyoA tail binding
helix α5 moves dramatically with respect to the other three helices

Crystallization of the second MTIP/MyoA complex

Construct	*P. yoelii* MyoA 15mer	Crystals
P.yoelii 79 — 205		
P.yoelii 60 — 205		
P.knowlesi **79 — 205**	**X**	**X**
P.knowlesi 60 — 205	X	X
P.knowlesi 1 — 205		
P.falciparum 80 — 204		
P. falciparum **60 — 204**	**X**	**X**
P.falciparum 1 — 204	X	

Claudia Roach, Bill Bergman

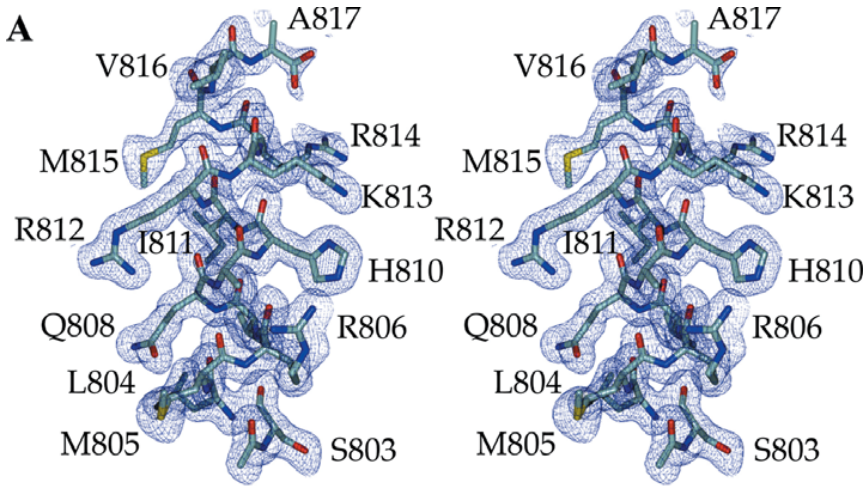
P. falciparum MTIP-Myosin A crystals @ pH 7.5



Crystal Growth Rate ~ 1 mm per 8 hrs

Stewart Turley

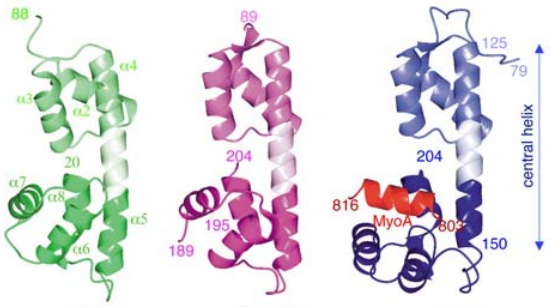
Stereo View of *P. yoelii* Myosin A-tail helix density in the *P. falciparum* MTIP-Myosin A complex at 1.7Å



Jürgen Bosch

Three conformations of Plasmodium MTIP

P. knowlesi



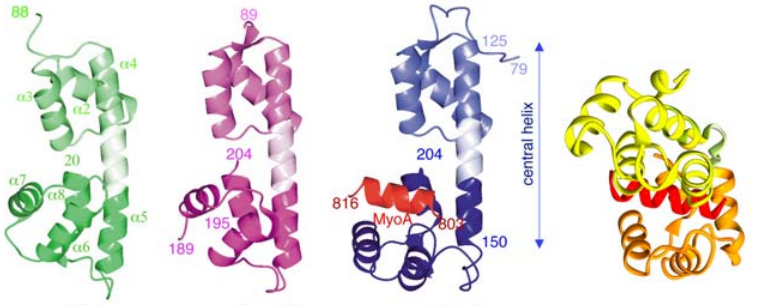
Conformation 1 Conformation 2 Conformation 3

(*P. yoelii* MyoA-tail)

Jürgen Bosch

Four conformations of Plasmodium MTIP

P. knowlesi *P. falciparum*

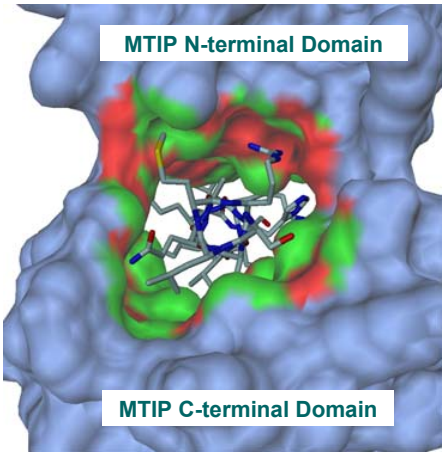


Conformation 1 Conformation 2 Conformation 3 Conformation 4

(*P. yoelii* MyoA-tail) (*P. yoelii* MyoA-tail)

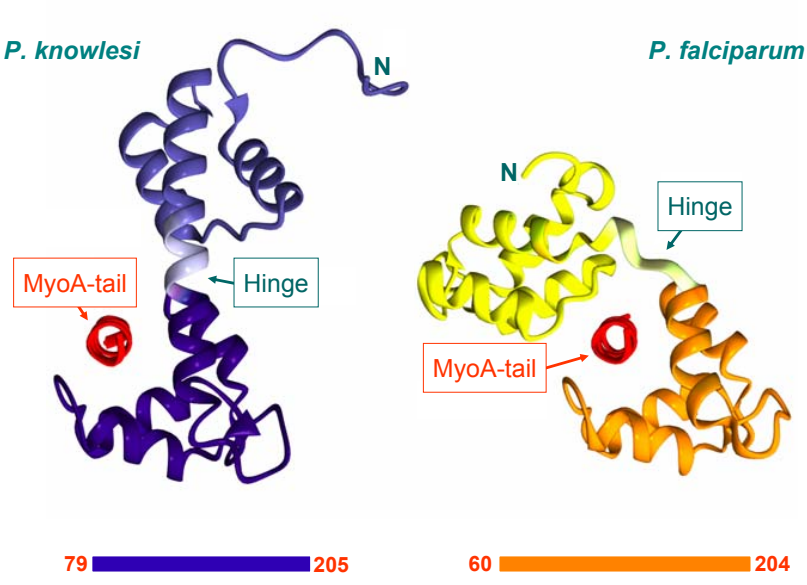
Jürgen Bosch

P. falciparum MTIP completely surrounds the Myosin A tail

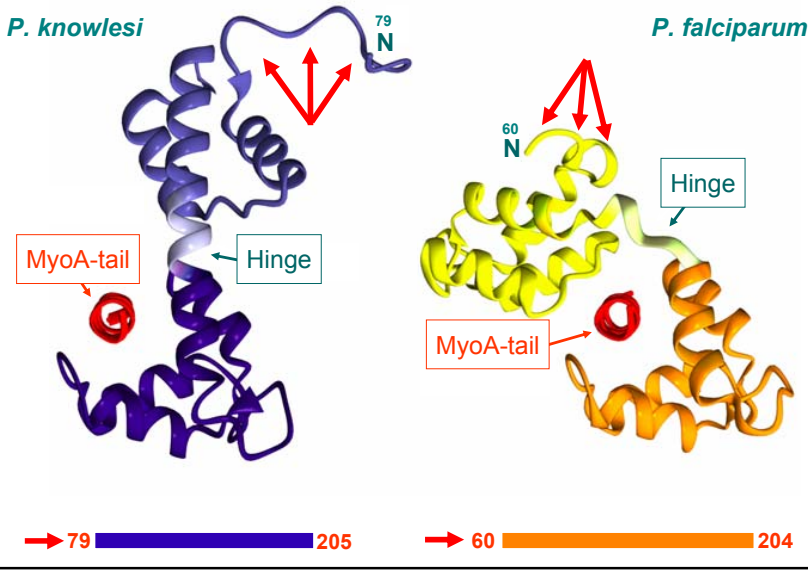


Why adopts *P. falciparum* MTIP:MyoA a *compact* conformation and *P. knowlesi* MTIP:MyoA an *extended* conformation?

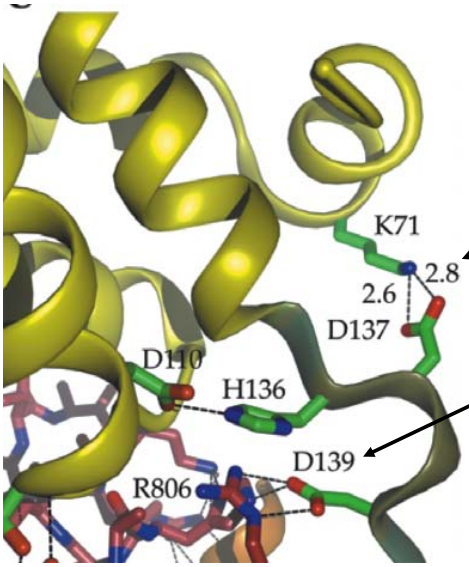
Extended and compact conformations of MTIP



Length critical for the closed conformation



Complete N-domain promotes compact MTIP conformation

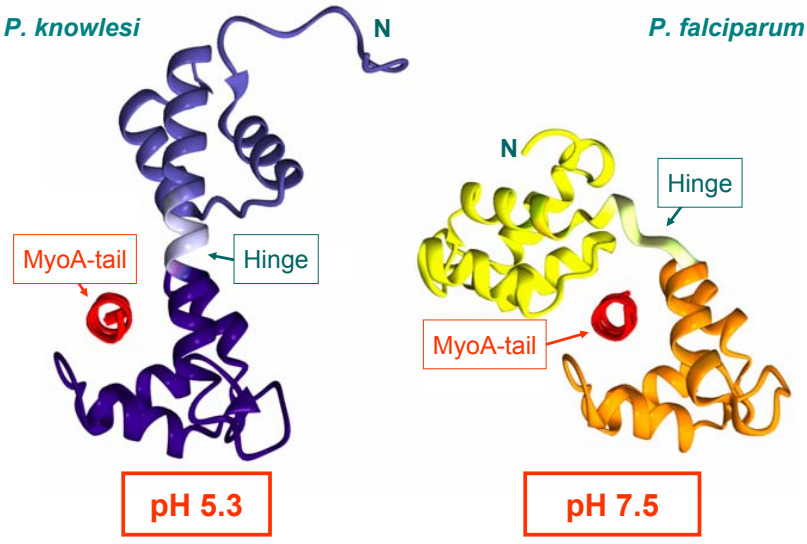


Helix "0" of N-domain interacts with "hinge": Lys71-Asp137 interaction.

This helps positioning key residues.

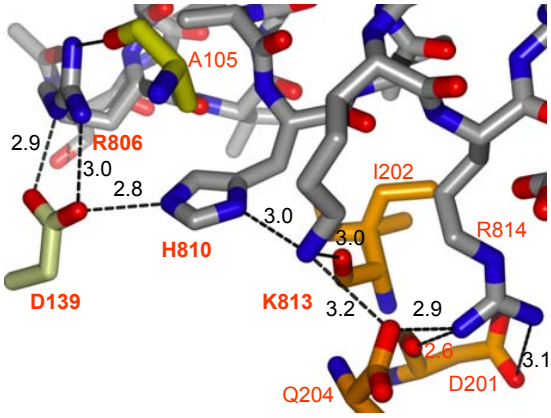
In particular Asp139 interacting with MyoAHis810.

pH critical for the closed conformation



pH critical for the closed conformation

P. falciparum

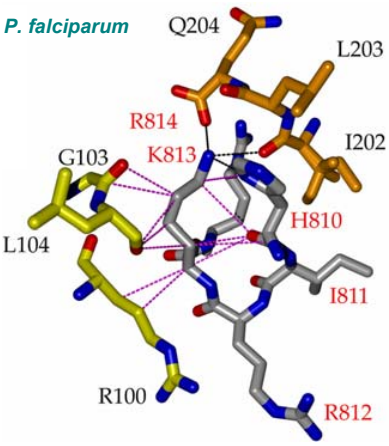


MyoA His810 makes interactions with MyoA Lys813 and with MTIP Asp 139

OK at neutral pH; not OK at low pH

K813 critical for the compact conformation

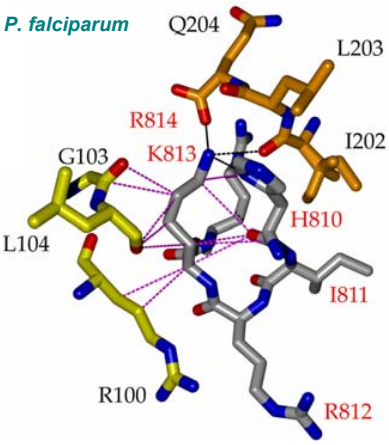
P. falciparum

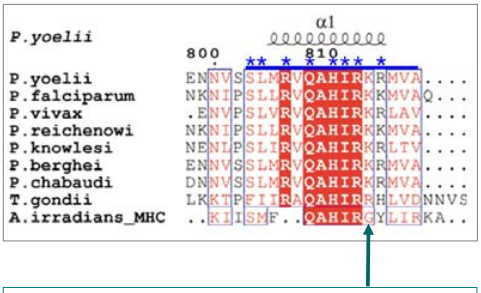


- Mutation MyoA K813A destroys affinity of Myosin A for MTIP
- Every side chain atom of MyoA K813 contacts a MTIP atom
- The N^{ζ} of MyoA K813 moves by ~5 Å going from open to compact conformation

Jürgen Bosch
Bill Bergman

K813 conserved in *Plasmodium spp.*



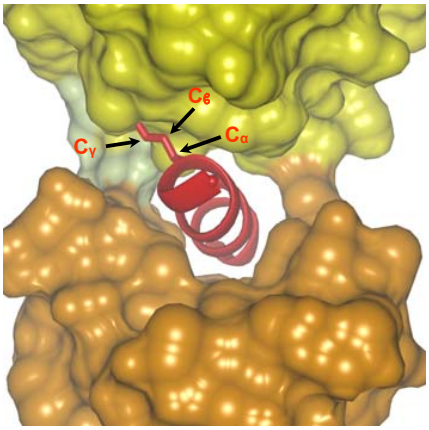


MyoA K813 is 100% conserved in *Plasmodium* species

In the so-called "IQ motif" a large residue like K813 is supposed to PREVENT compact conformation!

The role of the "forbidden" MyoA K813 is most surprising since a Gly was supposed to be "needed" to prevent a clash!

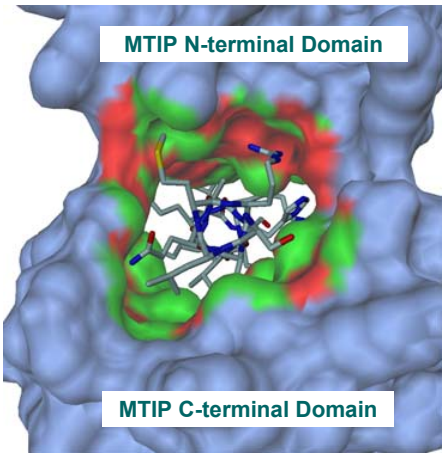
- "Forbidden" according to IQ-motif described for non-calcium dependent Calmodulin binding partners
- All known structures have a conserved glycine at this position, therefore avoiding steric problems
- K813 pointed out into solution in the PkMTIP structure
- Modeling a closed conformation resulted in a clash of K813 with the N-terminal domain



IQ-motif IQxxxRGxxxR

P. yoelii MyoA VQAHIRKRMVA

P. falciparum MTIP completely surrounds the Myosin A tail

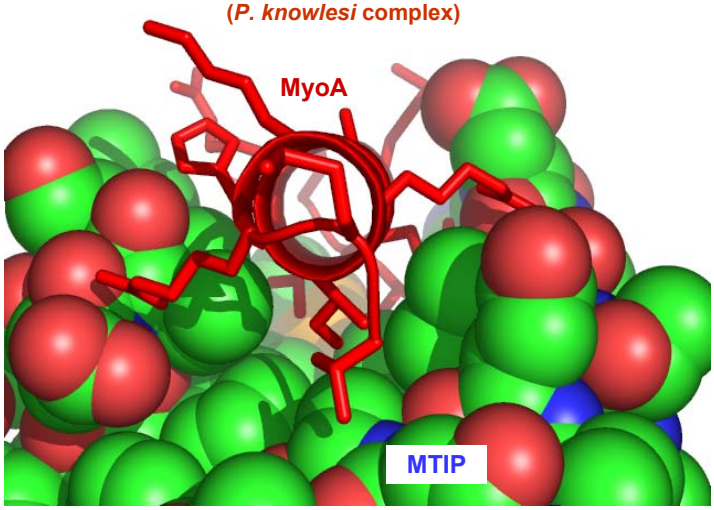


The contacts of the MyoA-tail with the MTIP N-domain are less extensive and more hydrophylic than with the MTIP C-domain

Hence for inhibitor development we focus mainly on the C-domain

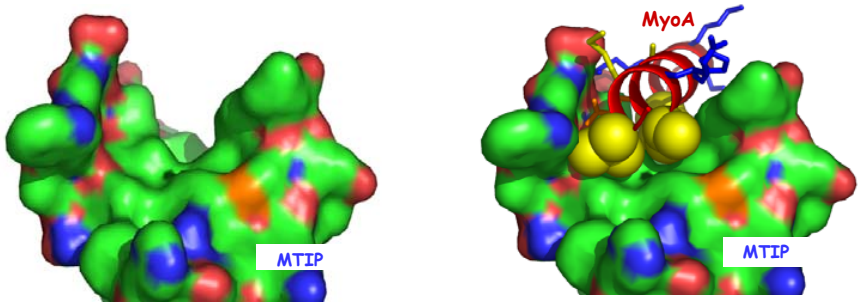
C-domain of MTIP plus the MyoA-tail

(*P. knowlesi* complex)



Note the "green" i.e. hydrophobic character of the MTIP contact surface

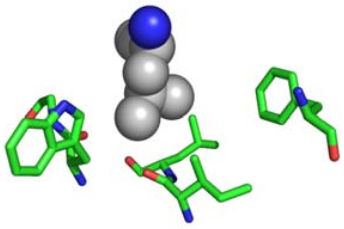
Myosin-Tail Interacting Protein (MTIP) plus the MyoA-tail



The hydrophobic pockets of MTIP contact surface are in contact with hydrophobic side chains of the MyoA-tail helix.

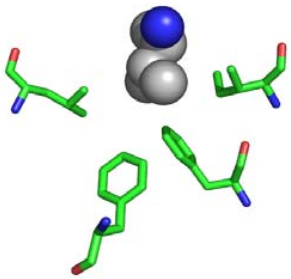
Two Myosin of the A-Tail Side Chains Interacting with MTIP

PyMyoA Leu 804



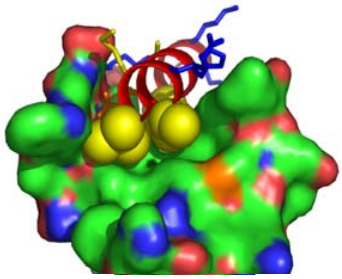
Hydrophobic "Pocket 1" provided by PkMTIP residues

PyMyoA Val 807



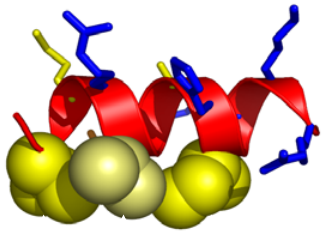
Hydrophobic "Pocket 2" provided by PkMTIP residues

The MTIP-MyoA interaction



Oxygen Carbon Nitrogen

1389 Å^2 buried surface

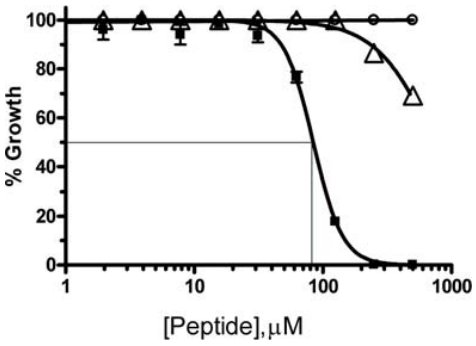


Leu804 Val807 Ile811

Linking Leu & Val & Ile side chains → → → New Antimalarials?

P. falciparum inhibition assay

Using the MyoA-tail



15mer *P. yoelii* MyoA-tail peptide

randomized MyoA-tail peptide

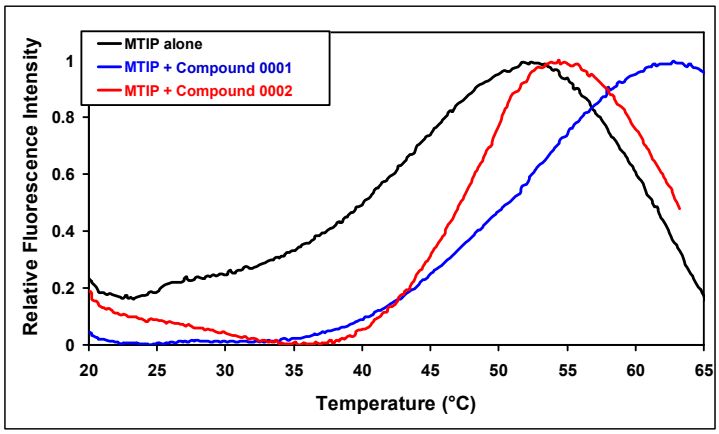
MyoA-tail peptide R812A/K813A

MyoA-tail peptide: 50% growth inhibition at 84 μM

Billl Bergman & Akhil Vaidya, Drexel University, Philadelphia

MTIP Thermal Melt Screens

Using Sypro Orange as Reporter of Unfolding



Zhongsheng Zhang, Erkang Fan, Jürgen Bosch @ UW, Seattle & Bill Bergman, Akhil Vaidya @ Drexel, Philadelphia

Myosin-Tail Interacting Protein (MTIP)

In *P. falciparum* and *P. vivax* MTIP:

11 out of 17

MyoA-contacting C-domain residues

are different in

the closest human homolog

(Myosin alkali light chain 1)

Conclusions Part II:

1. *P. falciparum* MTIP is interacting in surprising ways with the MyoA-tail helix
2. The MyoA-tail helix binding site in *P. falciparum* MTIP is made up of residues which often different from those in the closest human homolog.

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Bosch, J. et al. (2007) *J Mol Biol.*, 372, 77-88