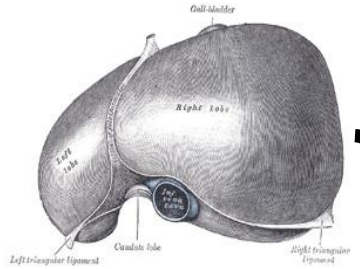
DECODING THE SIGNALING NETWORK IN MALARIA PARASITES

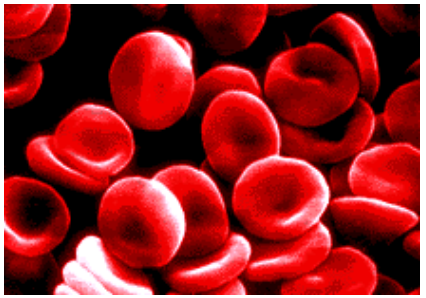
BIOTA-FAPESP International Workshop on Metabolomics in the Context of Systems Biology

February 25 - 26/2/2010

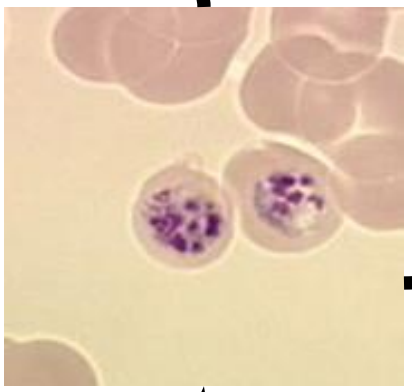
Célia R. S. Garcia – University of São Paulo

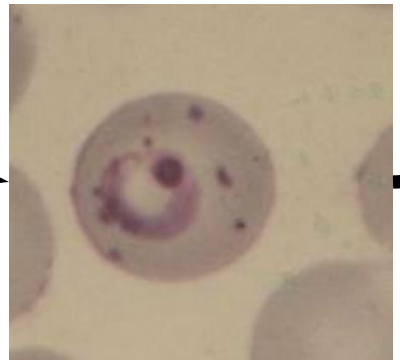
Plasmodium intraerithrocytic cycle



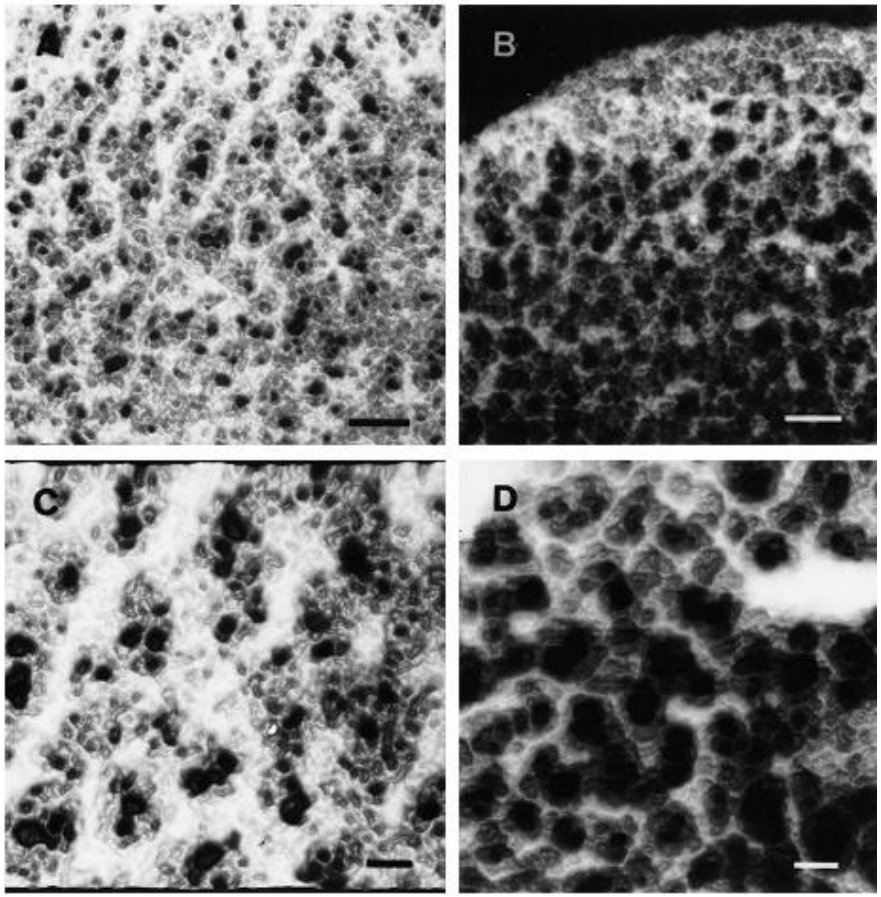


invasion

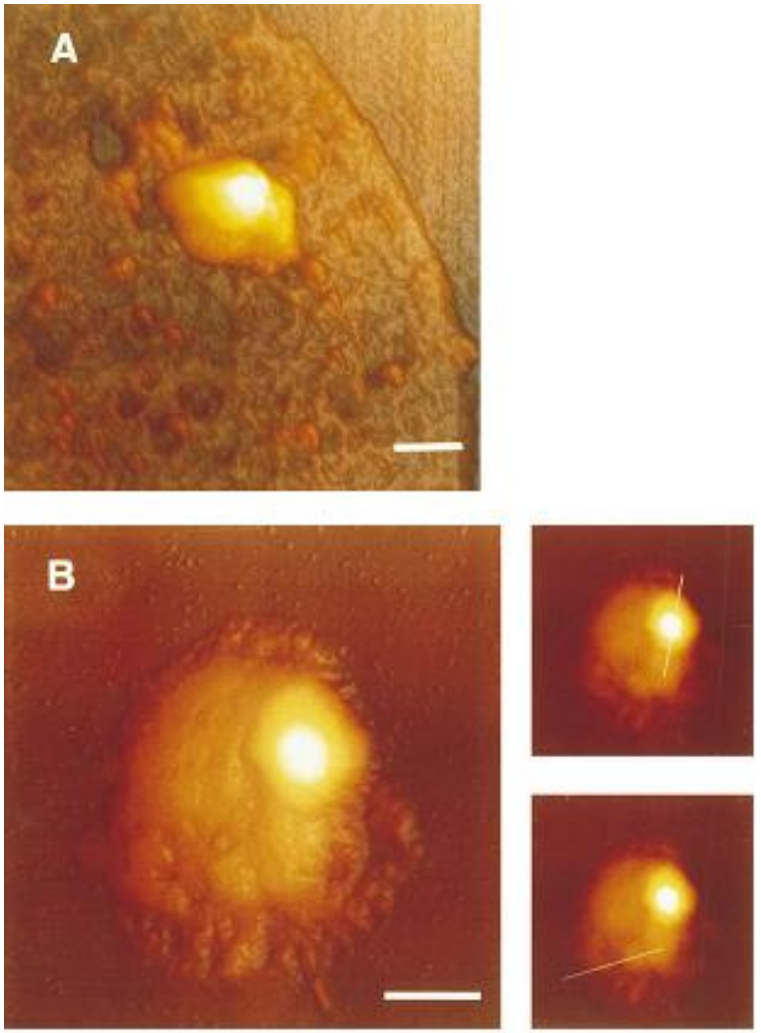




Atomic force microscopy imaging of malaria parasites



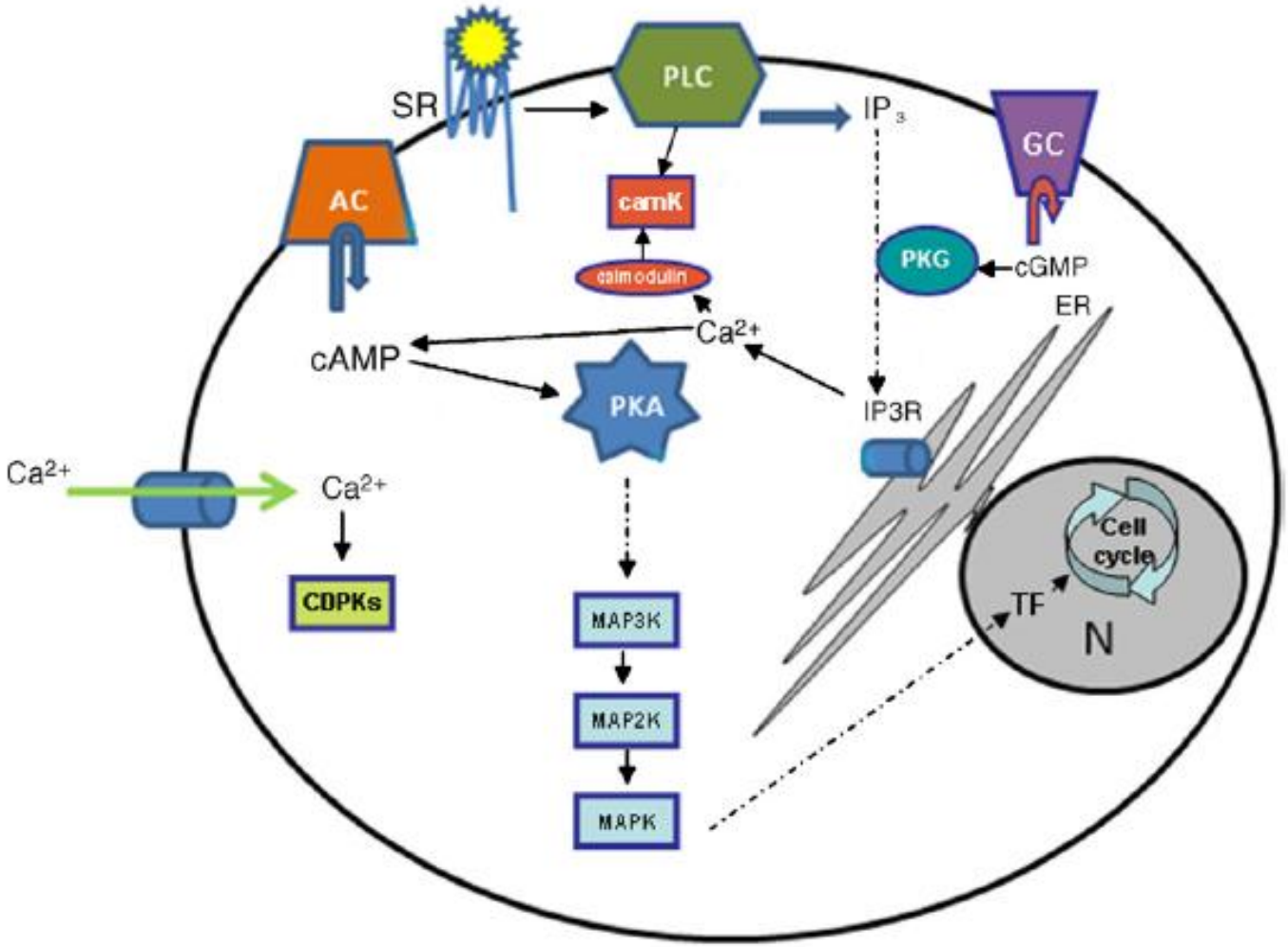
AFM image of a cytoskeletal network (A,C) and a P. falciparum infected membrane ghost (B,D) at higher resolution. Bars, 0.5 µm (A,B) and 0.2 µm (C,D).



AFM image of na infected RBC (A) and an isolated P. falciparum parasite. Bars, 0.5µm (A) and 1µm (B).

Garcia et al, 1997 J. Struct. Biol.

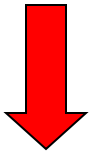
Molecular machinery of signal transduction



Koyama *et al* , 2009

Circadian rhythm in malarial infection

Sinchronicity of billions of parasites

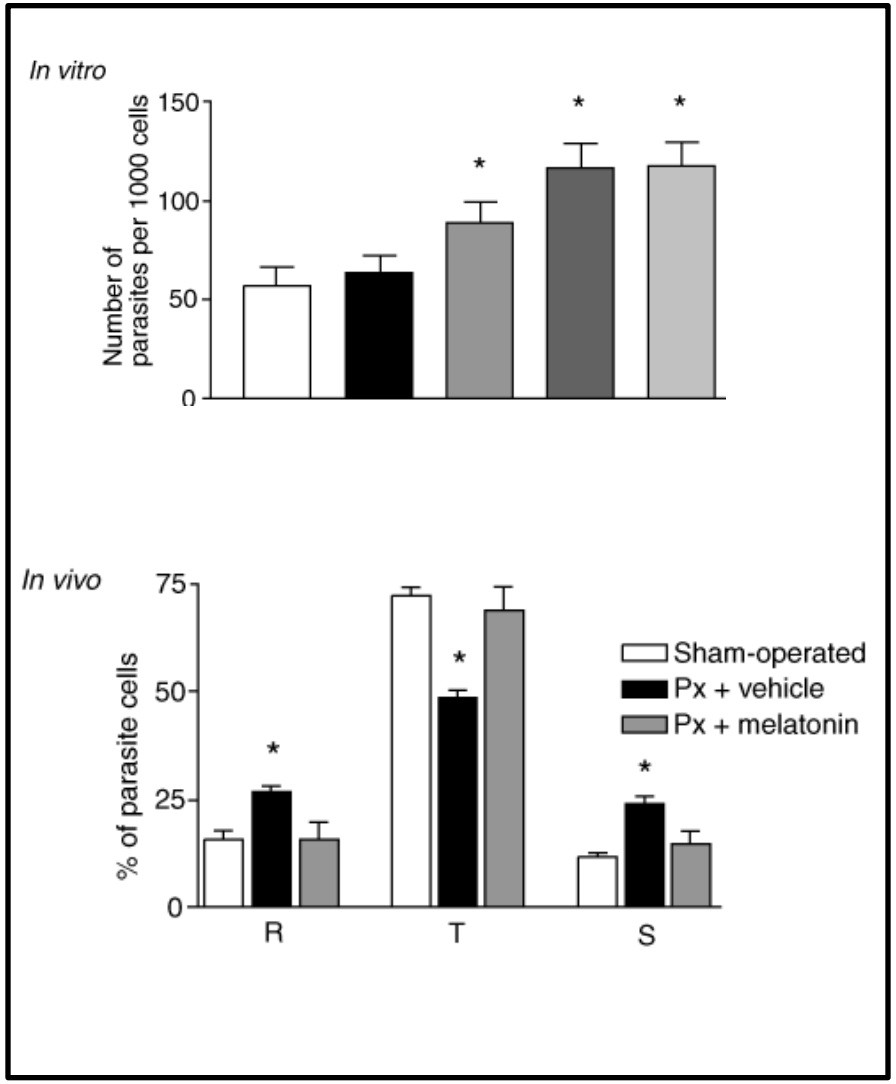


lost *in vitro*

Host is responsible for sinchronicity

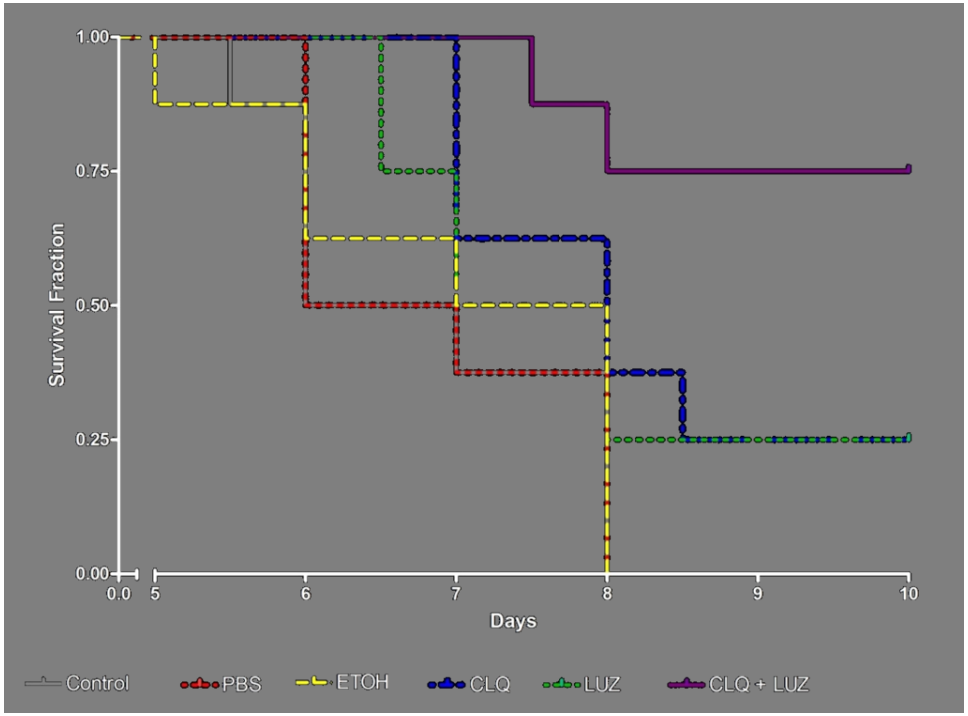
Unknown signal

Syncronization and Melatonin transduction pathway

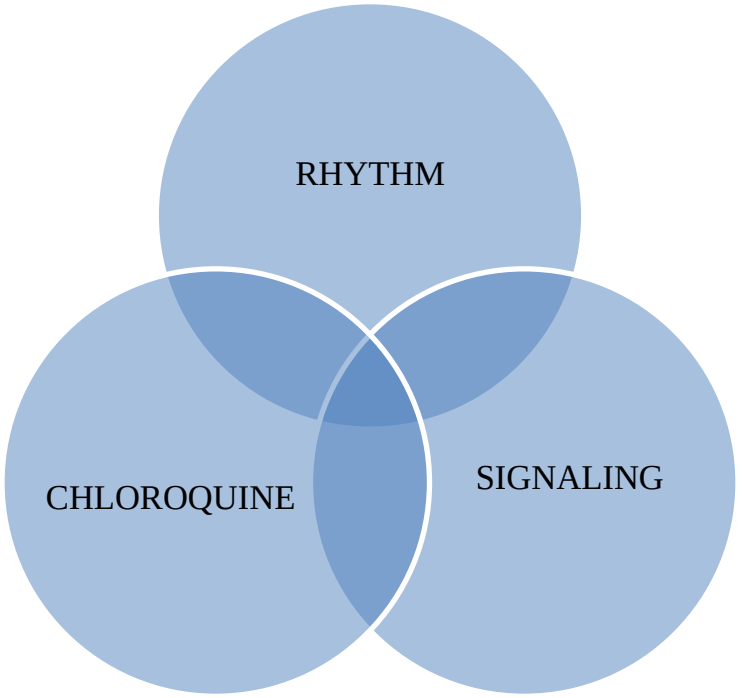


Hotta *et al*., (2000), *Nat. Cell Biol*. 7:466-469

Improvement of chloroquine action

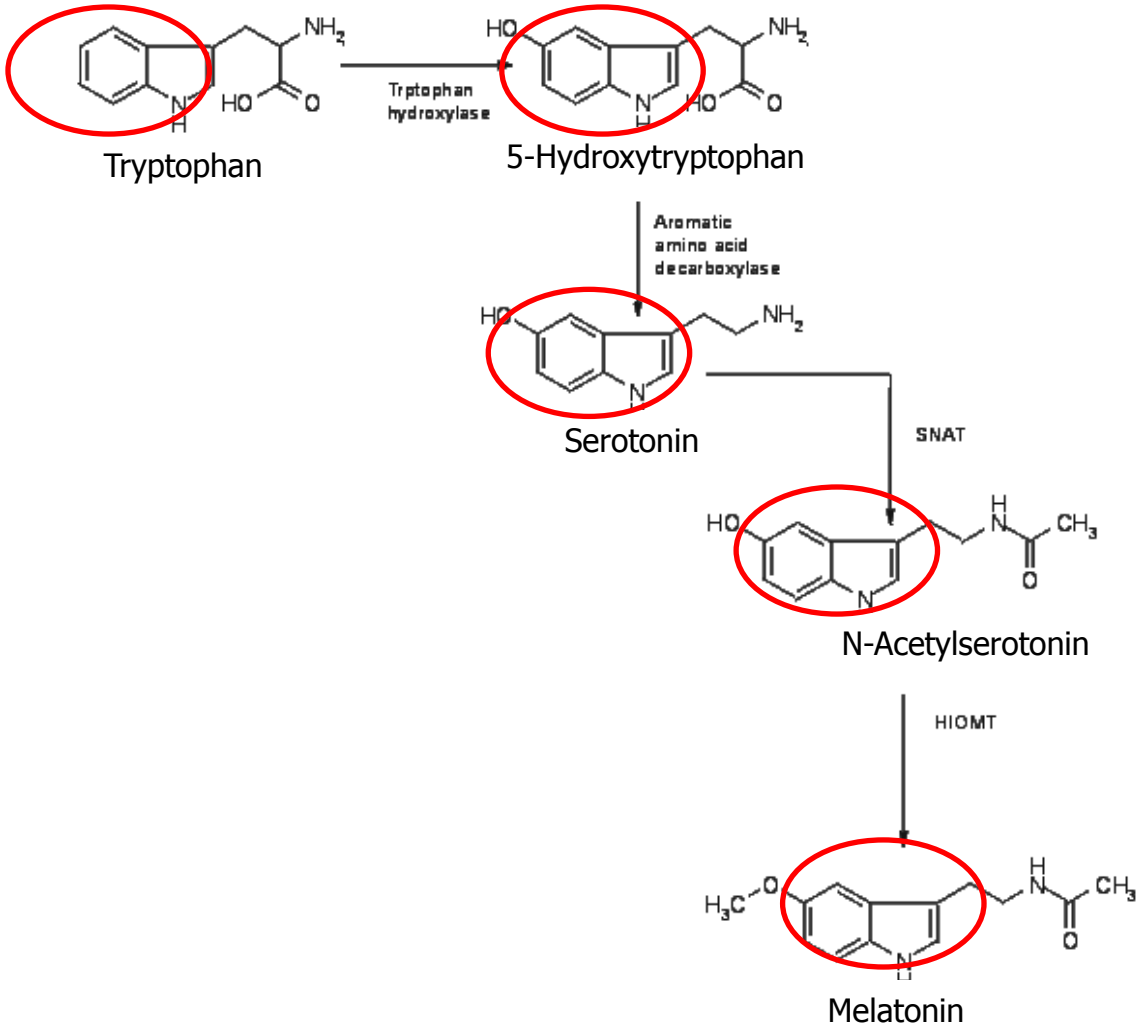


Survival of Balc/C mice after infection with *P. chabaudi.* Where indicated the animals were also injected with 1.5mg/kg Chloroquine (CLQ) and/or 15 mg/kg Luzindole (LUZ), solvent alone (PBS or ethanol) or no addition (control), as described in the Method section. 8 animals per group. Typical experiment of three independent trials.

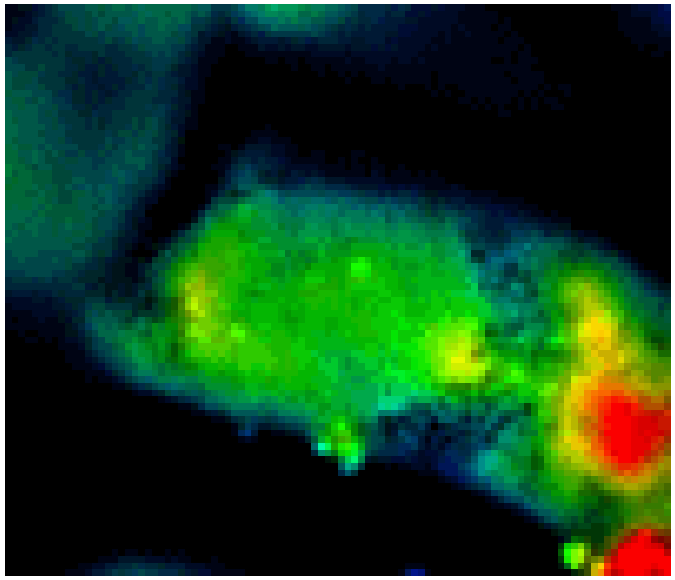


Bagnaresi *et al*., (2009), Int. J. Gen. Med 2: 47-55

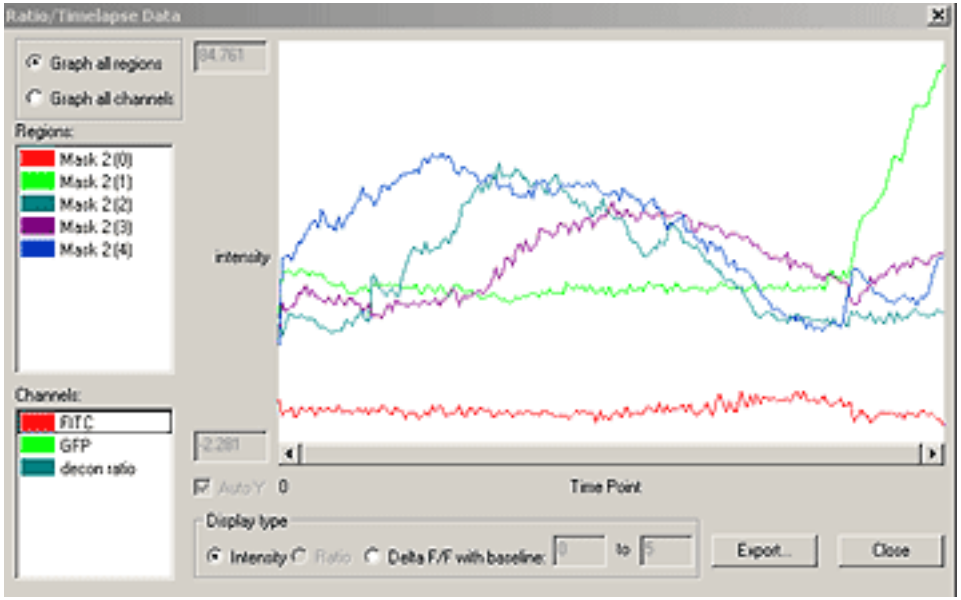
Tryptophan-related molecules



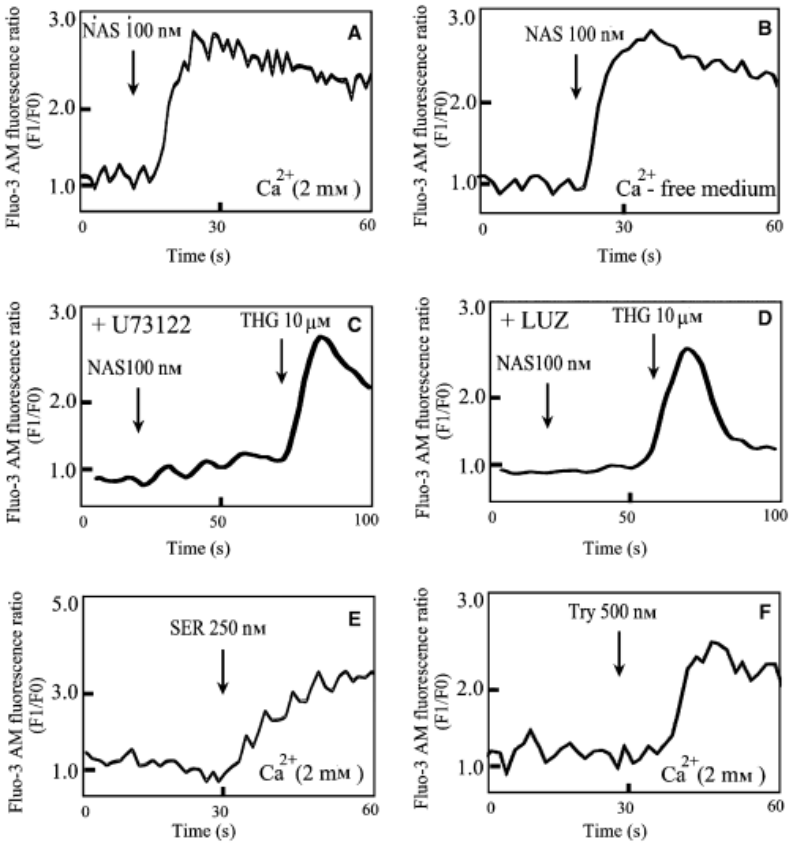
Calcium Imaging



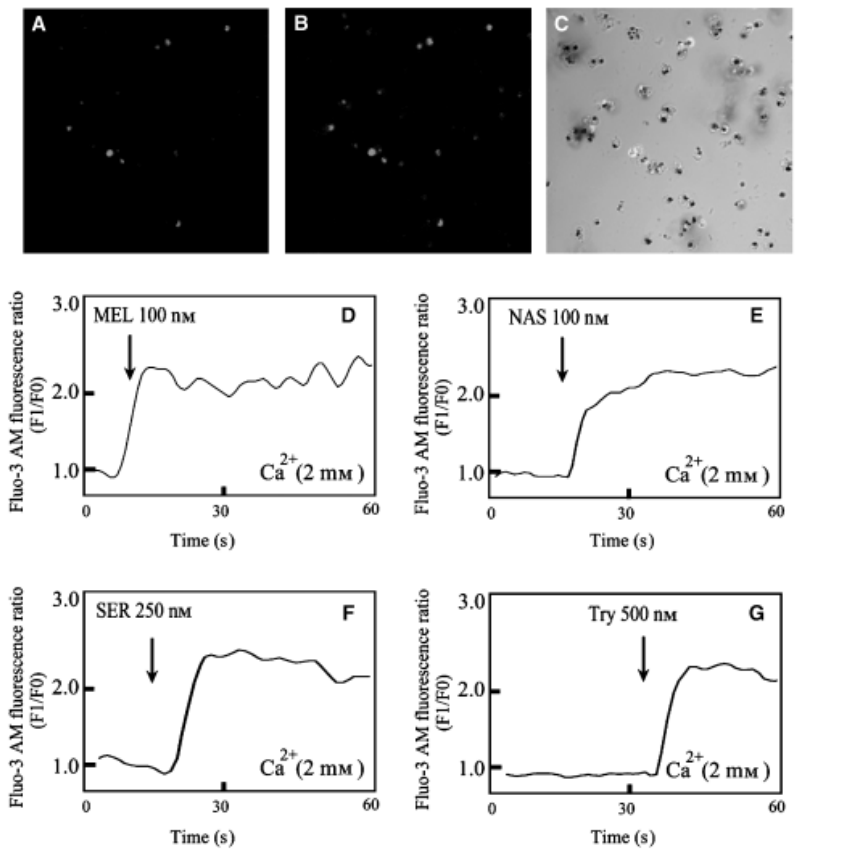
Calcium sparks in cardiac myocytes, University of British Columbia, 3D Live Cell Imaging course



Signal transduction pathway



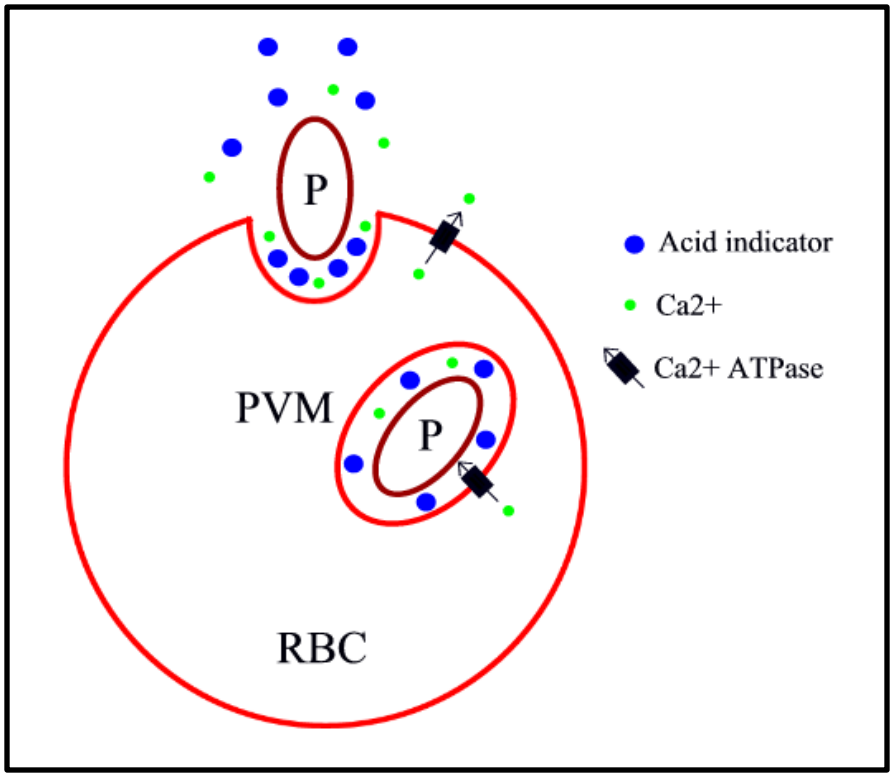
Calcium mobilization by melatonin precursors in Plasmodium falciparum infected RBCs at the trophozoite stage, analyzed by confocal microscopy.



Calcium mobilization by melatonin and its precursors in isolated Plasmodium falciparum trophozoites loaded with Fluo-3 AM. Fluorescence image before (A) and after (B) melatonin addition; (C) merge of phase contrast and fluorescence

Beraldo and Garcia (2005), *J. Pineal Res*. 39:224-230

Simultaneous imaging of the [Ca^{2+}] in the PV and cytosol



A – Changes in parasite cytosolic [Ca^{2+}] – Fluo - 3 AM

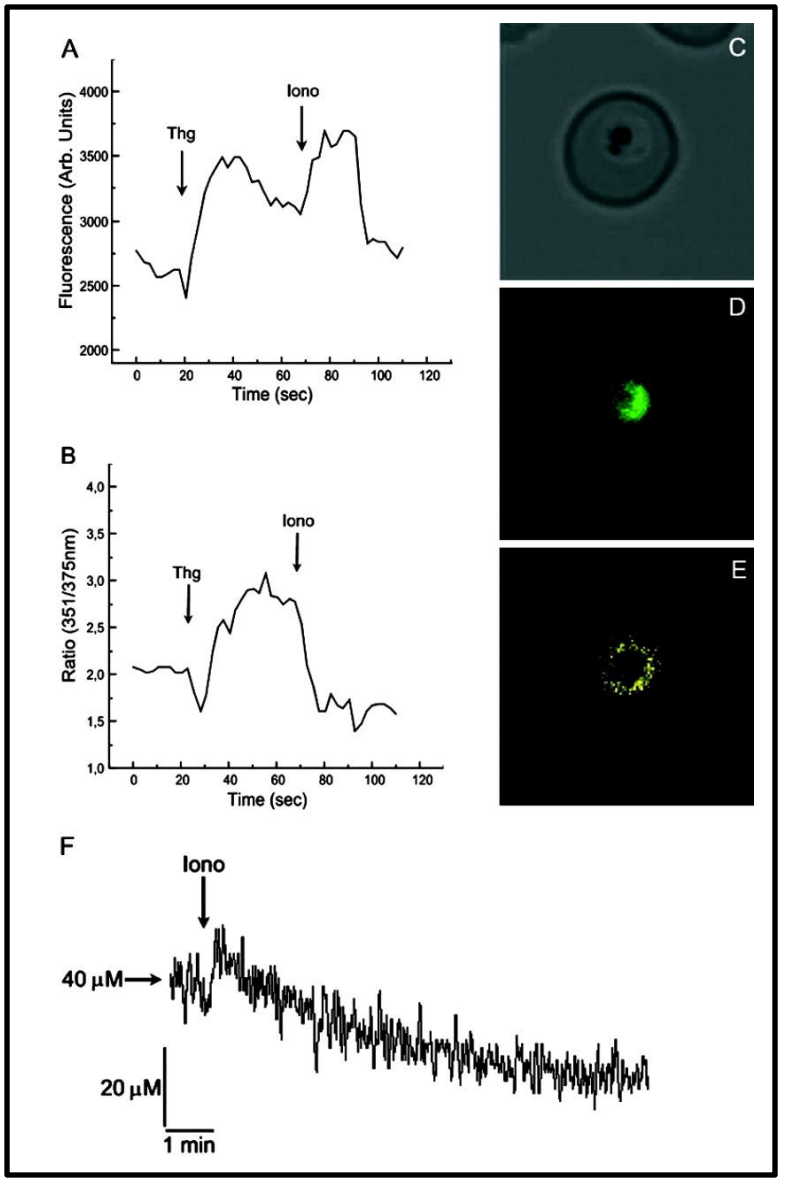
B- Changes of PV [Ca^{2+}] monitored as the ratio of Mag Fura – 2 acid

C- Phase contrast of infected RBC by *P. falciparum*

D- Fluo – 3 signal (parasite cytosol)

E- Mag Fura -2 signal (in PV)

F- RBC infected by *P. chabaudi* loaded during invasion with Mag Fura - 2 acid



Gazarini *et al*., (2003), *J. Cell Biol*. 1:103-110

Role of high Ca^{2+} concentration at PV

Molecular Microbiology (2004) 54(3), 676–691 doi:10.1111/j.1365-2958.2004.04313.x

Export of *Plasmodium falciparum* calcium-dependent protein kinase 1 to the parasitophorous vacuole is dependent on three N-terminal membrane anchor motifs

Christian Möskes,[1] Petra A. Burghaus,[2] Barbara Wernli,[1] Ursula Sauder,[4] Markus Dürrenberger[4] and Barbara Kappes[1,3]*
[1]*Parasitology Department, Institute for Hygiene, Heidelberg University, Im Neuenheimer Feld 324, D-69120 Heidelberg, Germany.*
[2]*Philipps-Universitaet Marburg, FB Biologie/Zoologie, Abt. Parasitologie, D-35032 Marburg, Germany.*
[3]*Department of Structural Biology, Biozentrum, University of Basel, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland.*
[4]*Interdisciplinary Center of Microscopy, Biozentrum, University of Basel, Klingelbergstrasse 50-70, CH-4056 Basel, Switzerland.*

cAMP in *Plasmodium*

- ***Dyer M and Day K.*** ***Mol Biochem Parasitol. 2000 Apr 30;108(1):67-78***

Expression of *Plasmodium falciparum* trimeric G proteins and their involvement in switching to sexual development

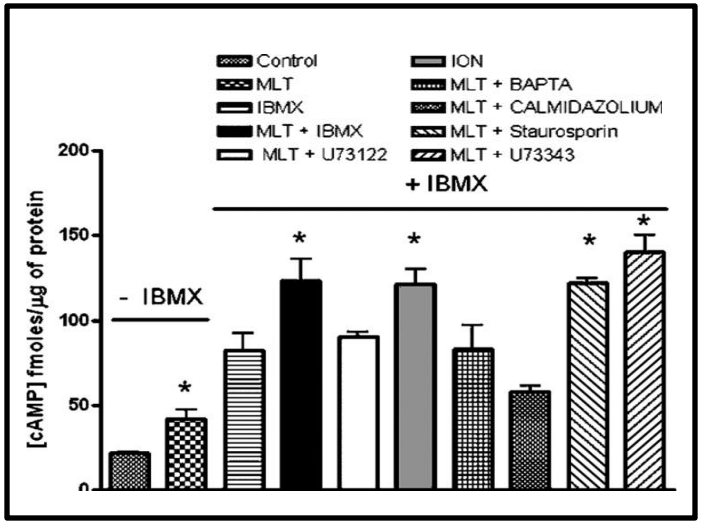
- ***Muhia DK. et al.*** ***J Biol Chem. 2003 Jun 13;278(24):22014-22.***

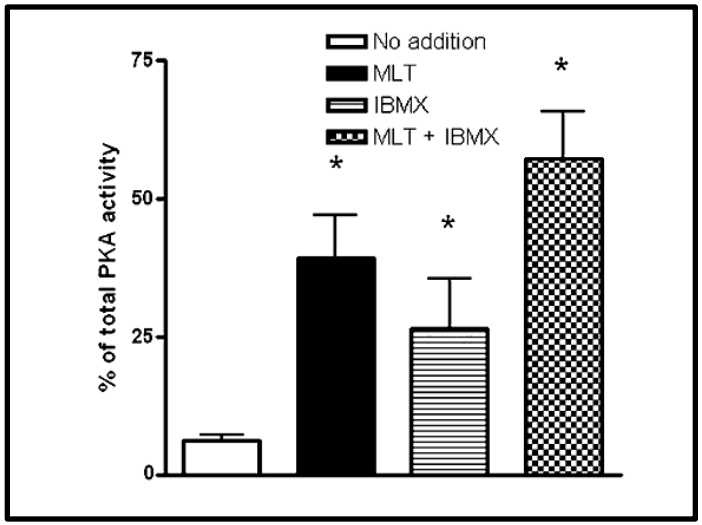
Multiple splice variants encode a novel adenylyl cyclase of possible plastid origin expressed in the sexual stage of the malaria parasite *Plasmodium falciparum.*

- ***Syin C. et al.*** ***Eur J Biochem. 2001 Sep;268(18):4842-9.***

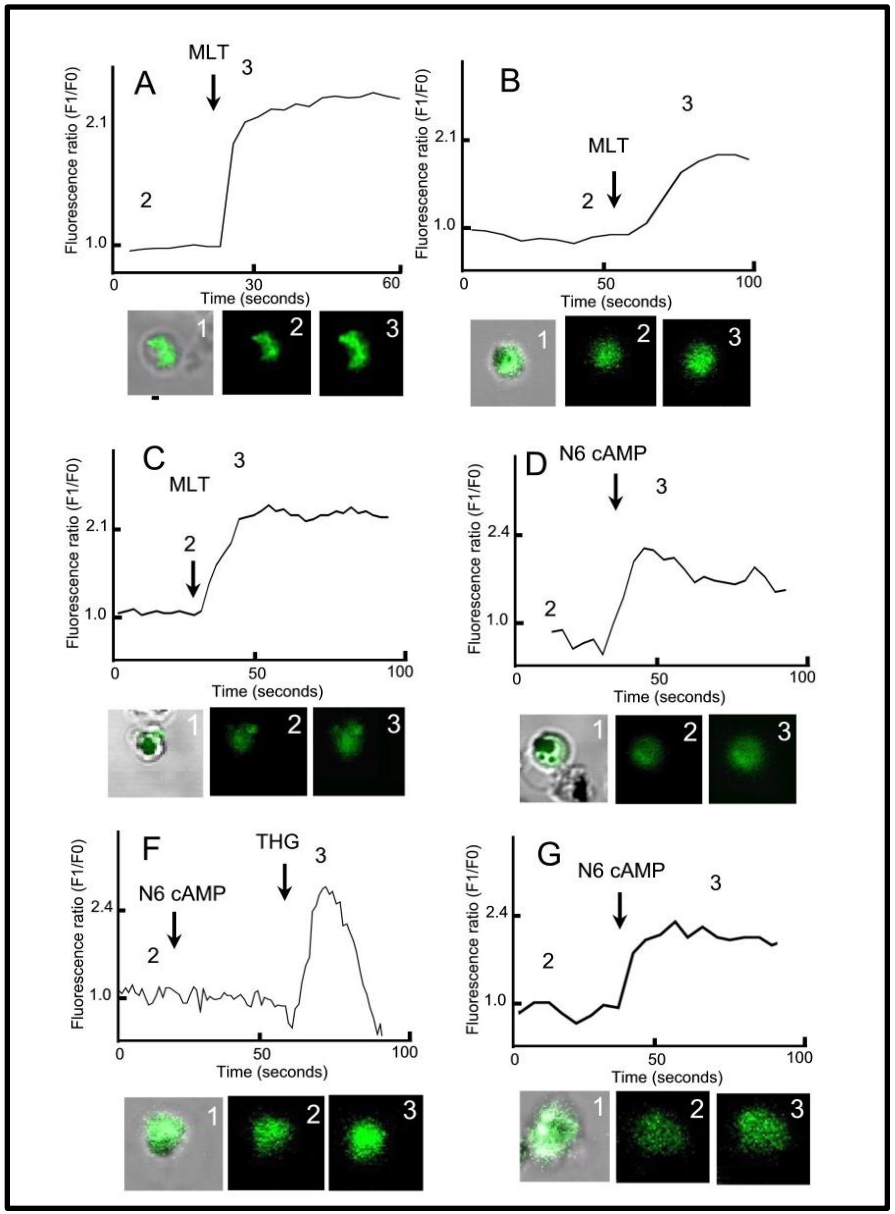
The H89 cAMP-dependent protein kinase inhibitor blocks *Plasmodium falciparum* development in infected erythrocytes.

cAMP and Ca^{2+} interplay in *P. falciparum*

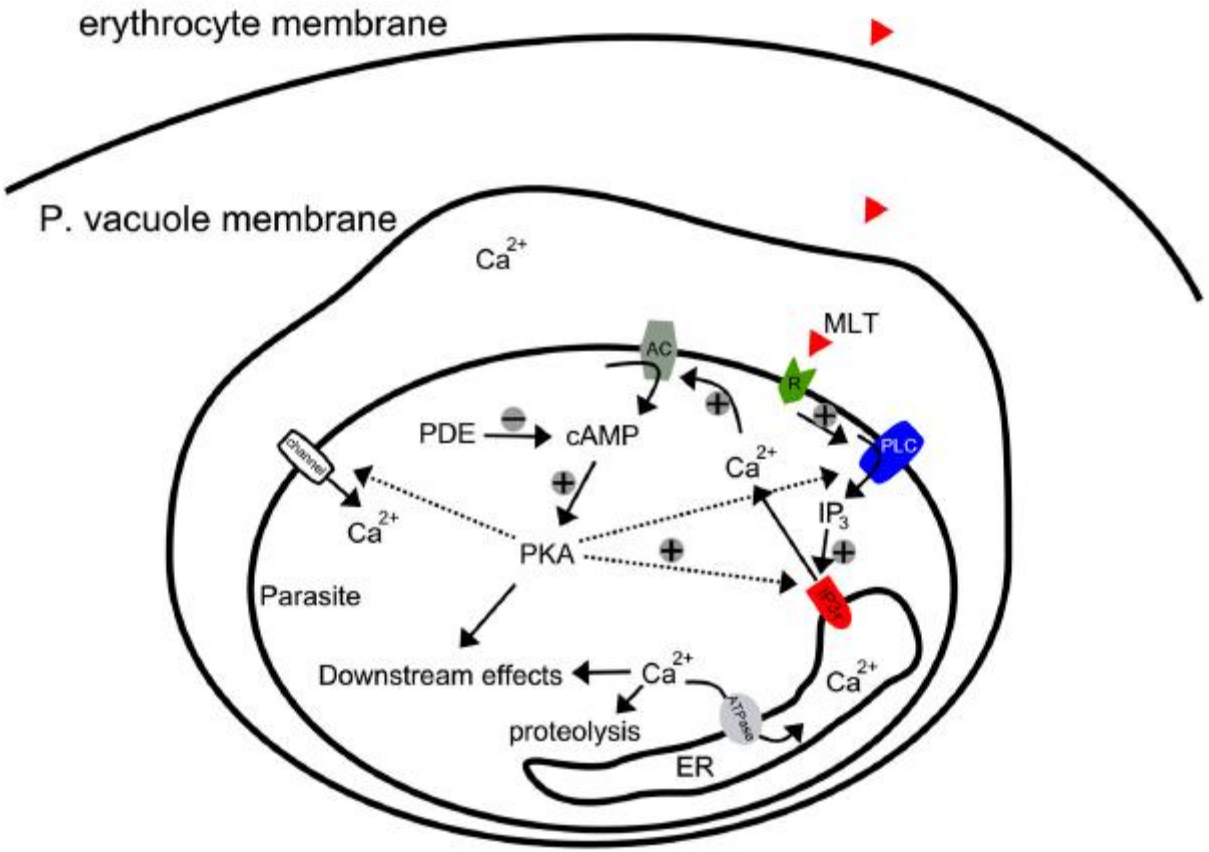




Beraldo *et al*., (2005), *J. Cell Biol*. 170:551-557



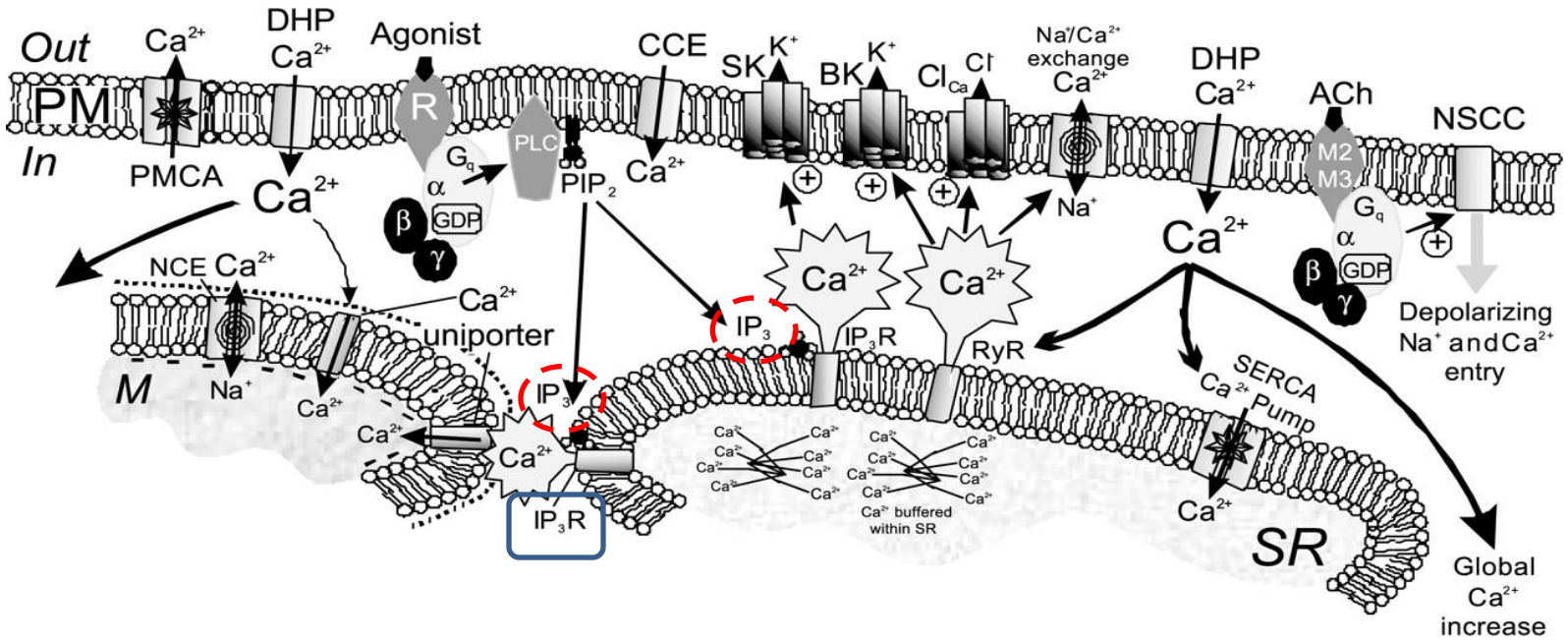
Signal transduction pathway



Schematic model of signaling events in Plasmodium with data provided from literature and our results. AC, adenylyl cyclase, PLC, phospholipase C; PDE, phosphodiesterase, PKA, protein kinase A, ER, endoplasmic reticulum, R, hypothetical melatonin receptor

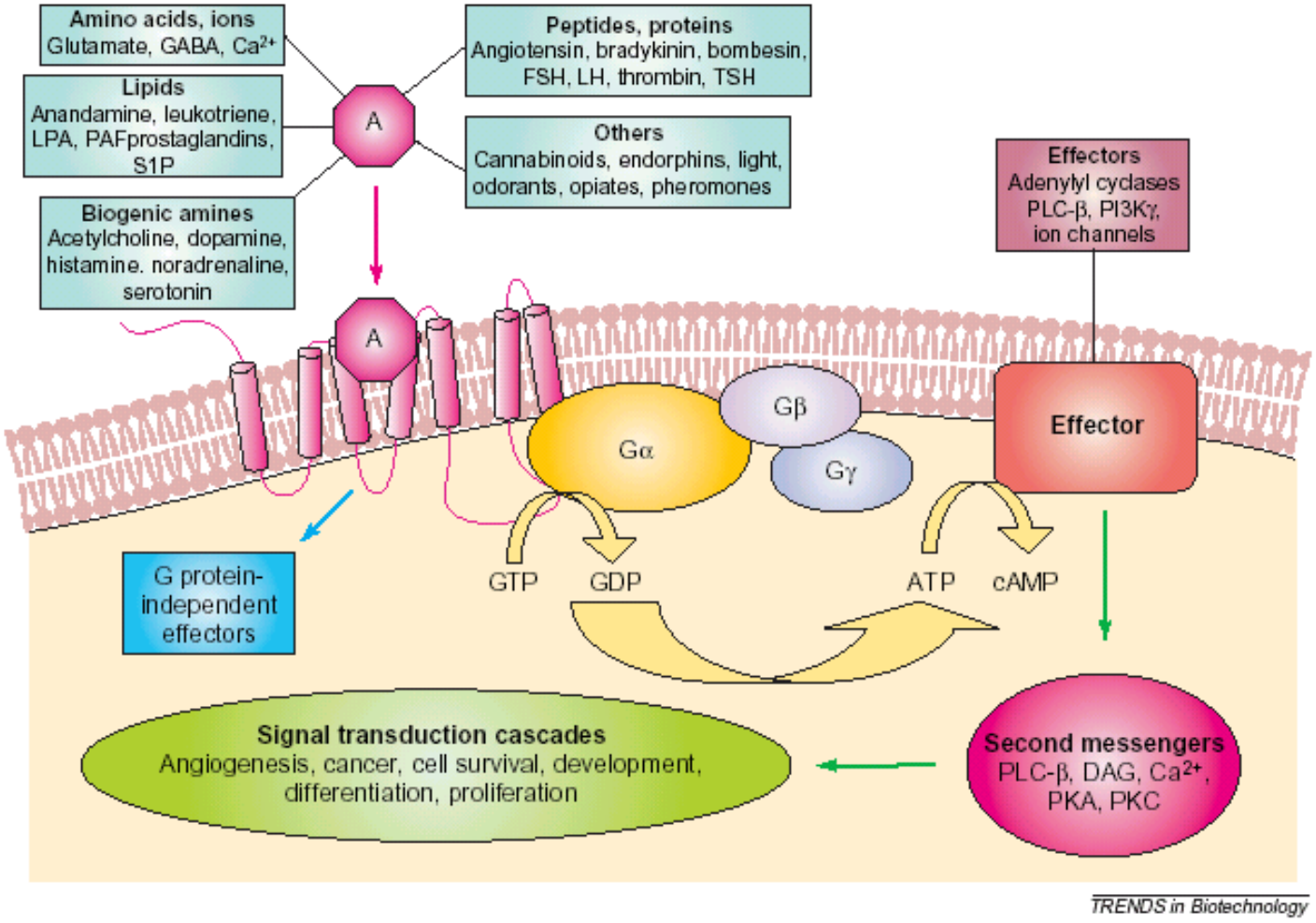
Beraldo *et al.*, (2005), *J. Cell Biol*. 170:551-557

Calcium coupled to IP_3



Sanders, K. M. (2001) *J Appl Physiol* 91: 1438-1449

Serpentin Receptors



Lundstrom (2004), *Trends Biotech* 23: 103-108.

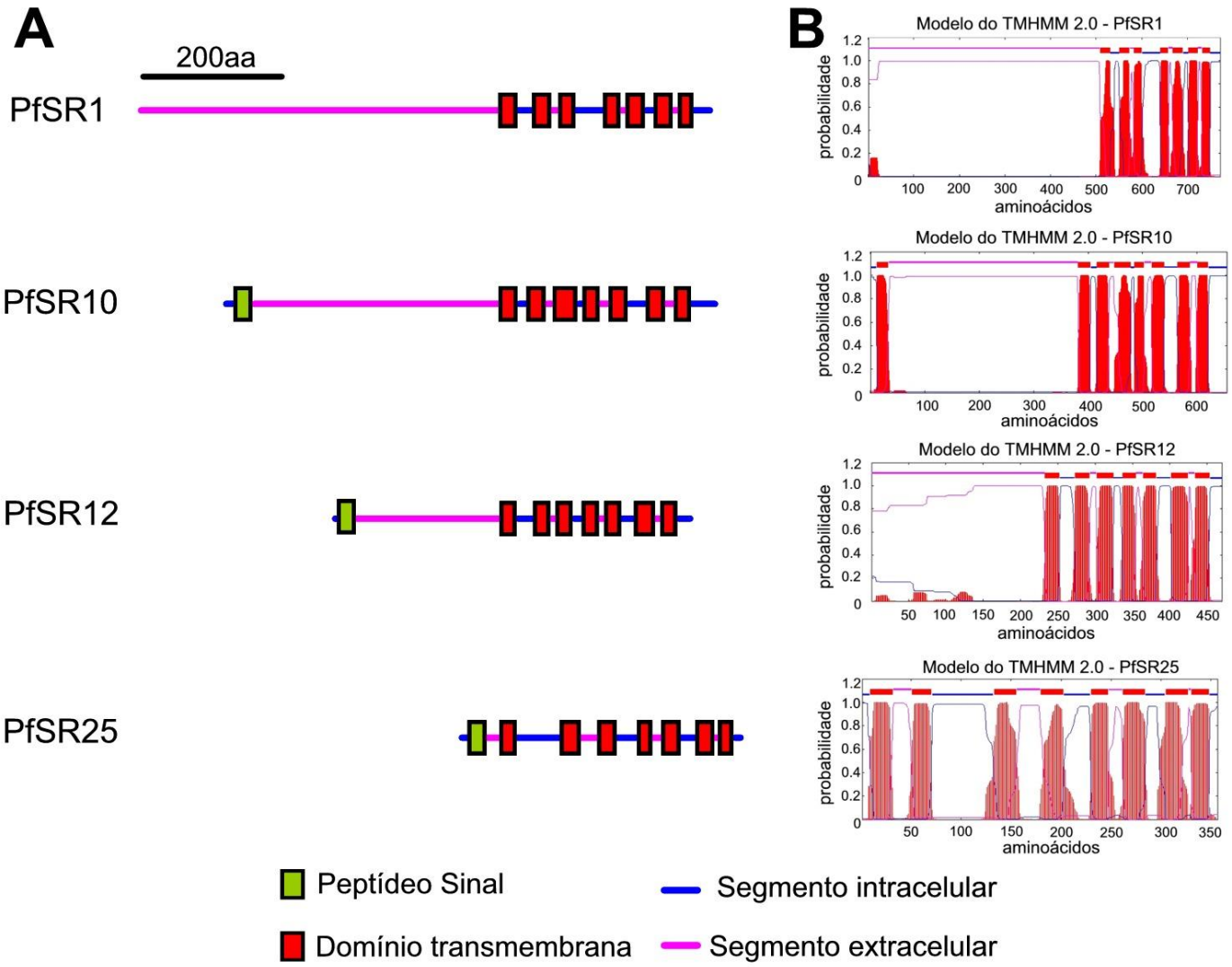
Genome-Wide Detection of Serpentine Receptor-Like Proteins in Malaria Parasites

Luciana Madeira[1☯], Pedro A. F. Galante[2,3☯], Alexandre Budu[4], Mauro F. Azevedo[4], Bettina Malnic[2], Célia R. S. Garcia[4*]

1 Departamento de Parasitologia, Instituto de Ciências Biomédicas, Universidade de São Paulo, São Paulo, Brasil, **2** Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, Brasil, **3** Ludwig Institute for Cancer Research, São Paulo, Brasil, **4** Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brasil

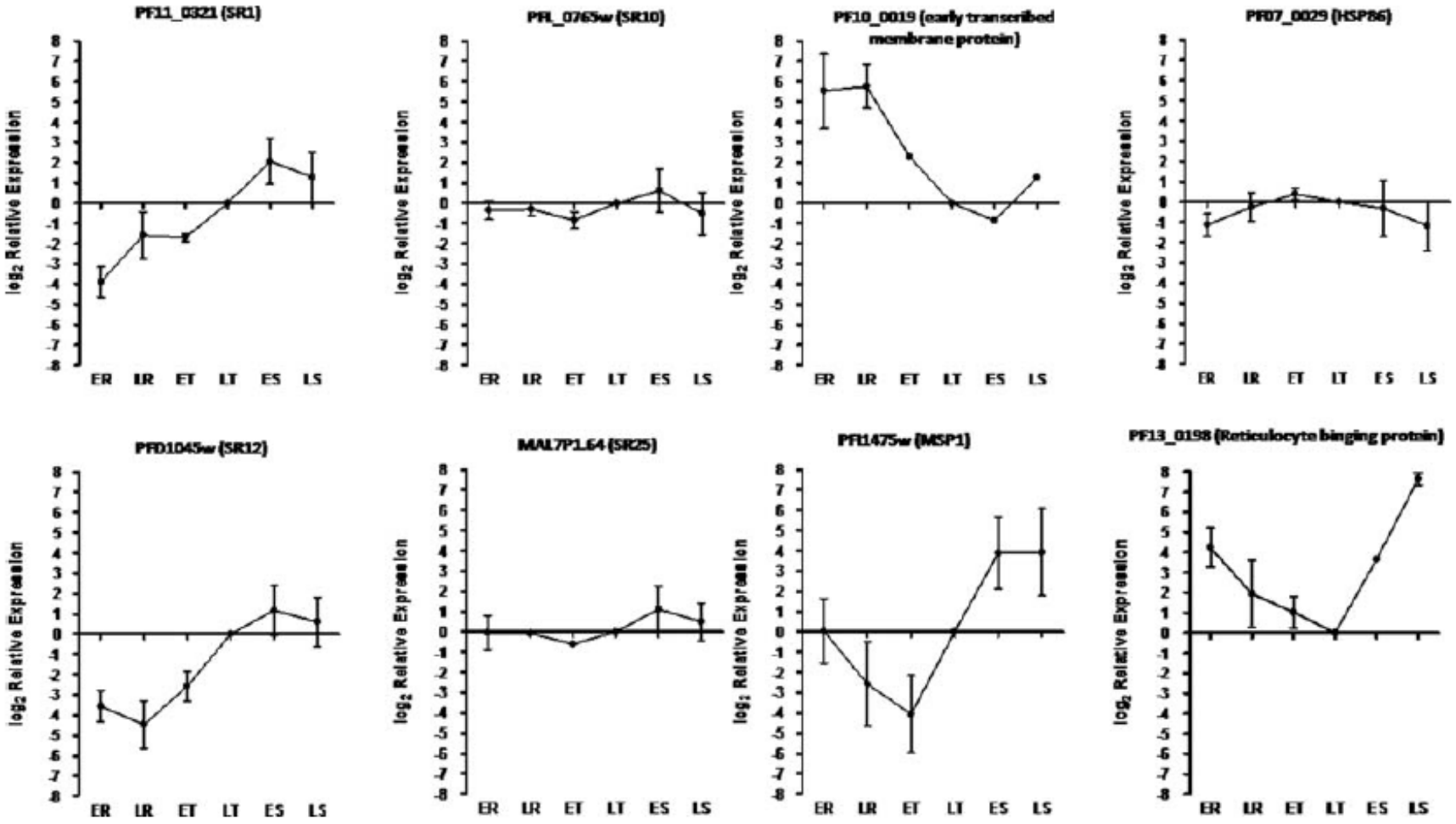
PLoS one

Membrane topologies of serpentine-receptor candidates of *P. falciparum*



Madeira et al., (2008) Plos One

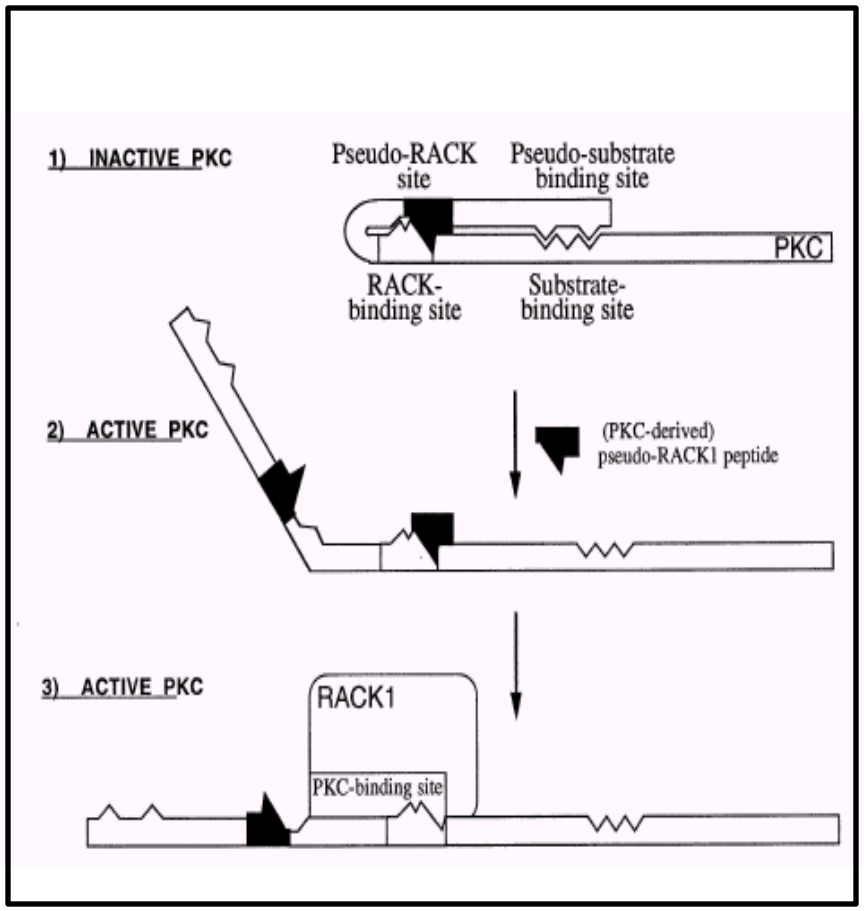
P. falciparum serpentine-receptor transcripts at RBC cycle

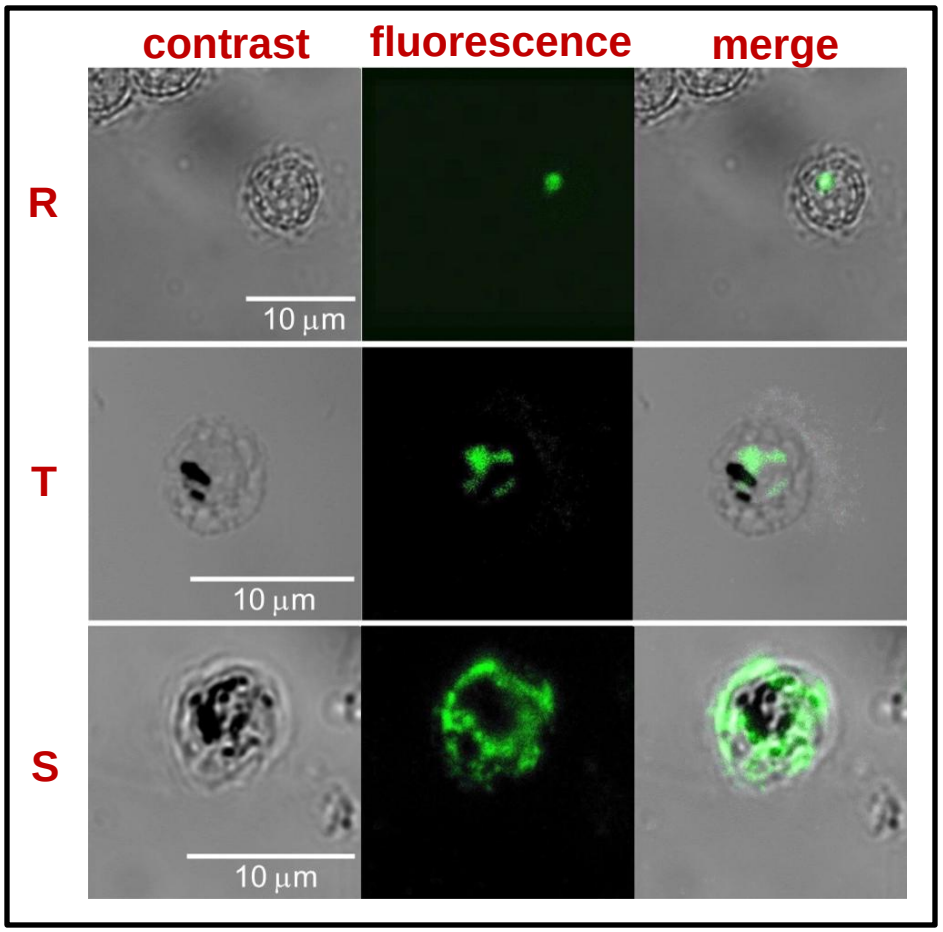


Madeira et al., (2008) Plos One

Receptor for Activated Kinase C (RACK1) ortholog in *P. falciparum*

RACK1: receptor for activated C kinase and immunolocalization of RACK in the intraerythrocytic stages of *P. falciparum*





Ron and Mochly-Rosen (1995) PNAS 92: 492-496.

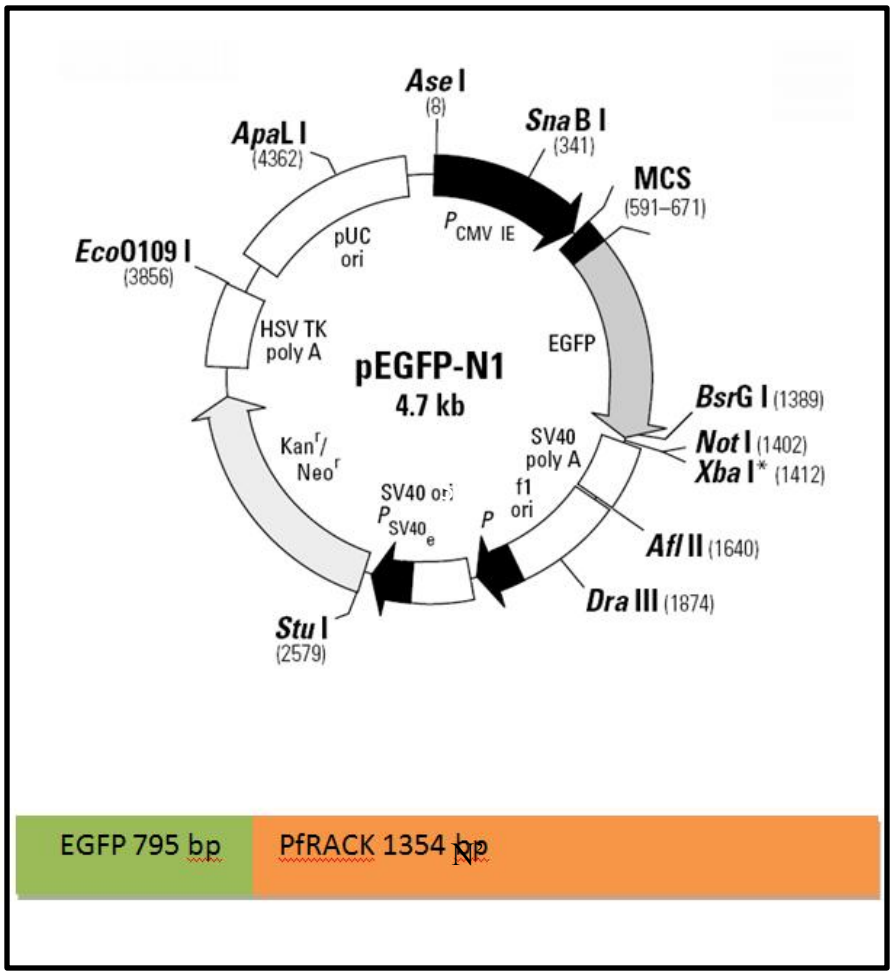
Madeira et al. (2003) Biochem Biophys Res Commun 306: 995-1001.

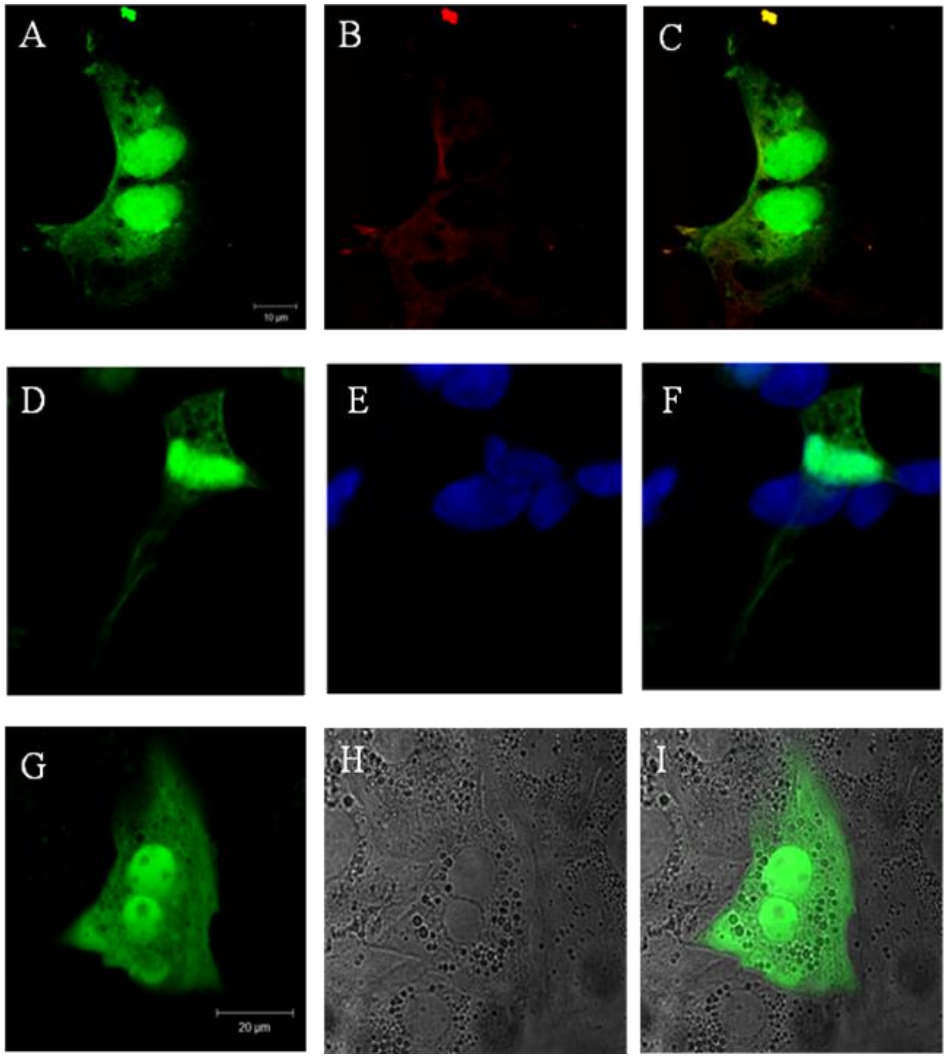
Receptor for Activated Kinase C (RACK1) ortholog in *P. falciparum*

In the search of this protein function we used a functional genomics approach to investigate whether PfRACK interacts with host cell $InsP_3$ receptors in affects Ca^{2+} release from mammalian cells

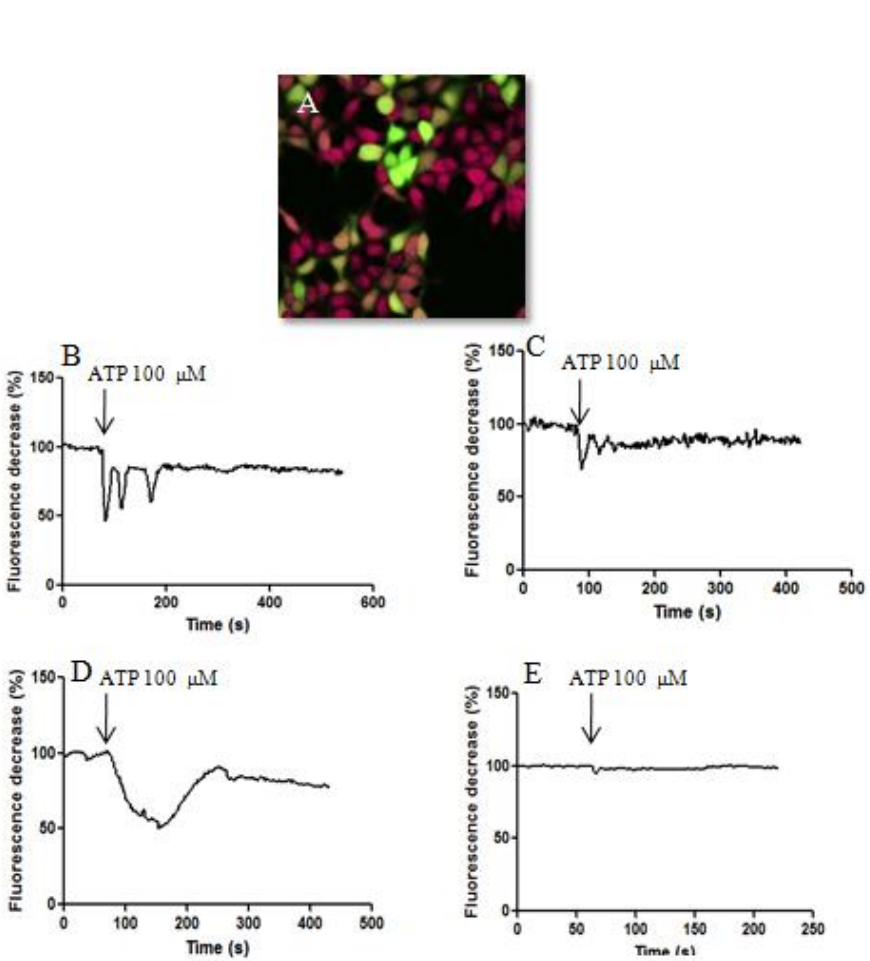
Sartorello et al, 2009

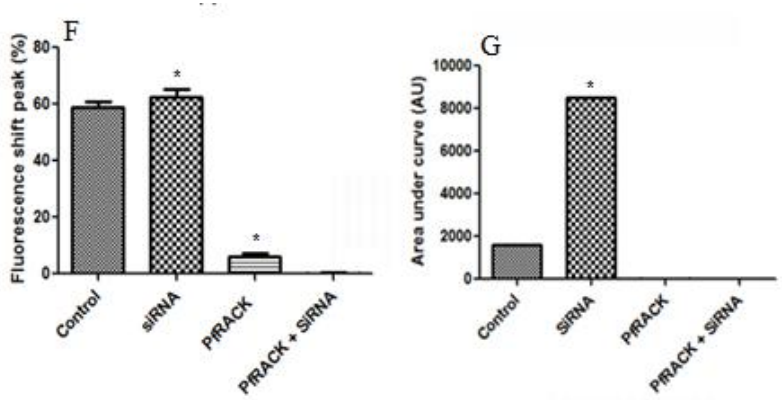
Plasmid construction and Mammalian cell expression of GFP-PfRack

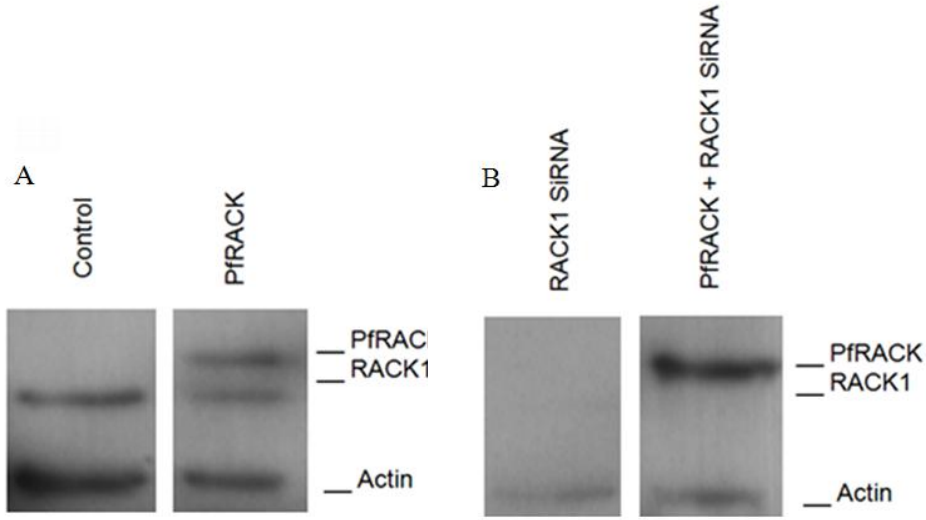




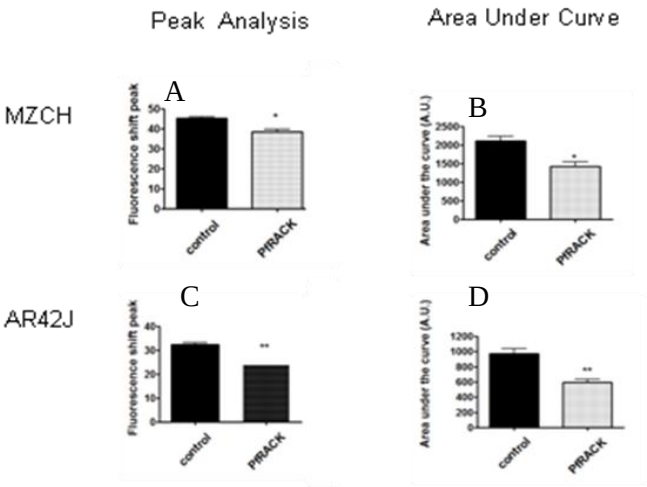
PfRACK inhibits Ca2+ signaling in mammalian cells

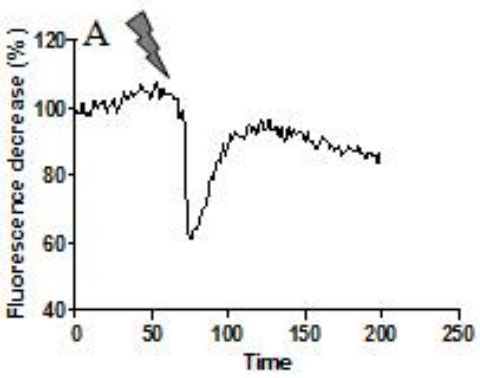


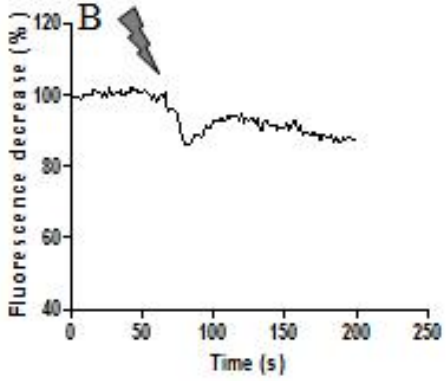


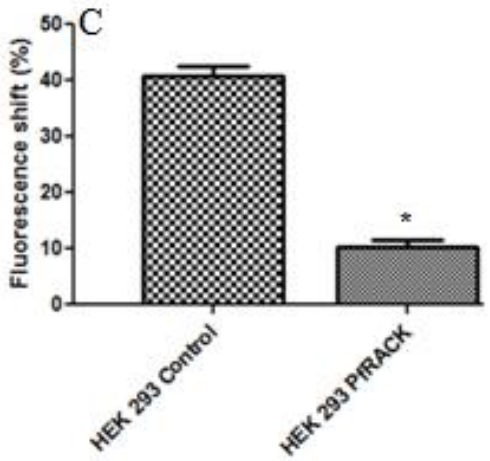


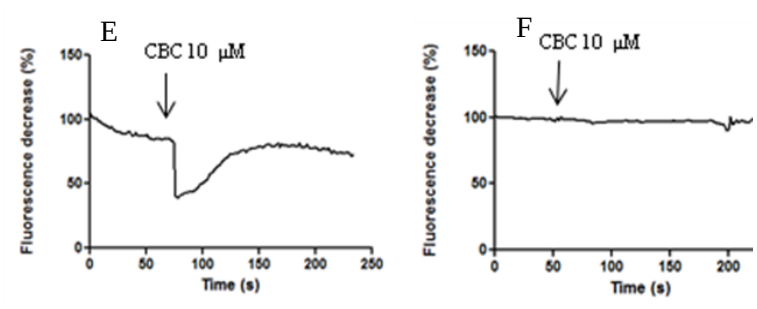
PfRACK inhibits Ca^{2+} signaling and abolishes IP_3 induced- Ca^{2+} release in mammalian cells











Conclusions

Plasmodium displays molecular handling machinery for sensing the environment.

The inhibition of IP_3-induced Ca^{2+} signals by PfRACK shows its coupling to the mammalian cell system using a synthetic codon-optimized gene.

This opens the possibility of studies of functional malaria genomics using better characterized mammalian cell systems.

Acknowlegments

Malaria group

Alexandre Budu

Desiree Shuck

Dr Dario Passos

Eduardo Alves

Fernanda Koyama

Dr Julio Garcia

Laura Nogueira

Dr Miriam Moraes

Dr Myna Nakabashi

Ramira Yuri

Dr Robson Sartorello

Dr Wânia Rezende

Collaborators

US

Dr Andrew Thomas - UMDNJ
Dr Debopam Chakrabarti - UCF
Dr Michael Nathanson – Yale

Europe

Dr Tullio Pozzan
University of Padova
Italy

Dr Christian Doerig
Global Health Institute
Switzerland

USP

Dr Aline Silva
Dr Bettina Malnic
Dr Regina Markus

Dr Flávio Beraldo
Dr Marcos Gazarini
Dr Piero Bagnaresi
Dr Luciana Madeira

FAPESP CNPQ – INCT PRONEX –MALARIA