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Transporter Conference Budapest April 26-27

"Strategies to overcome resistance in malaria"

"Transporters in antimalarial drug action and resistance"

¢ Sanjeev Krishna

Special thanks to *Henry Staines*, *Cathy Moore*, *Yolanda Augustin*, *Hajnalka Kovacsevics* & *all collaborators* and *critics*

"The fight against malaria has stalled"

"We need new tools"
Pedro Alonso
Head GMP WHO
April 2018

Transporters proposed as excellent drug targets (1991)

"We studied cation motive ATPases.."

1. **"Intraerythrocytic parasites regulate their ionic composition"**
2. **"Essential and phylogenetically ubiquitous"**
3. **"Considerable divergence.."**
4. **"Experience with isoform selective ATPase inhibitors"**

Krishna & Robson in Biochemical Protozoology..

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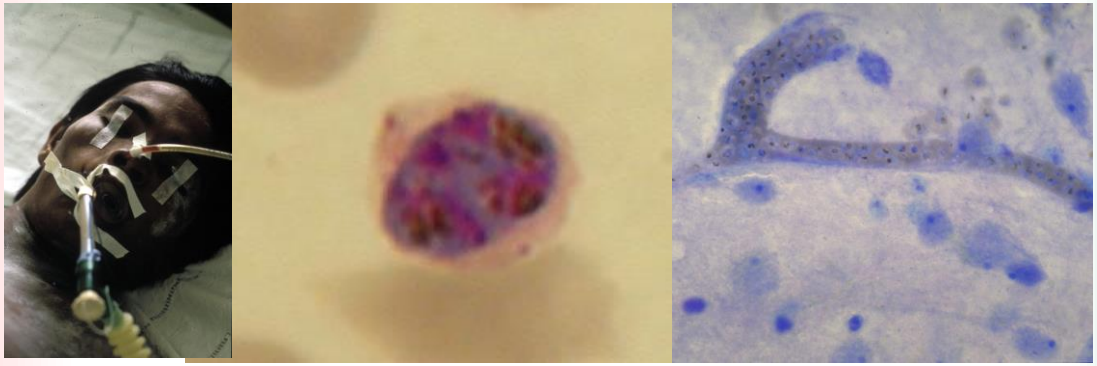
Sugar transporter PfHT - background

- Glucose essential primary energy source for parasites
- Anaerobic glycolysis, no gluconeogenesis or stores
- Glucose use increases up to 100 fold in infected cells
- PfHT only hexose transporter in genome in this class
- No known αα polymorphism in PfHT (clinical and lab isolates examined)
- Antimalarials take hours to turn off glycolysis
- Interferes with disease process (metabolic diversion)



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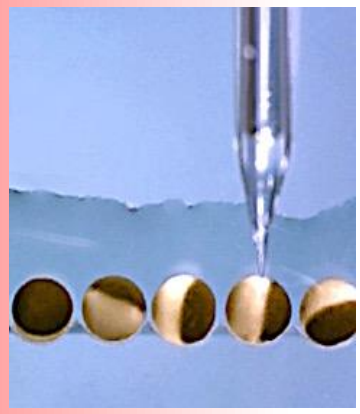
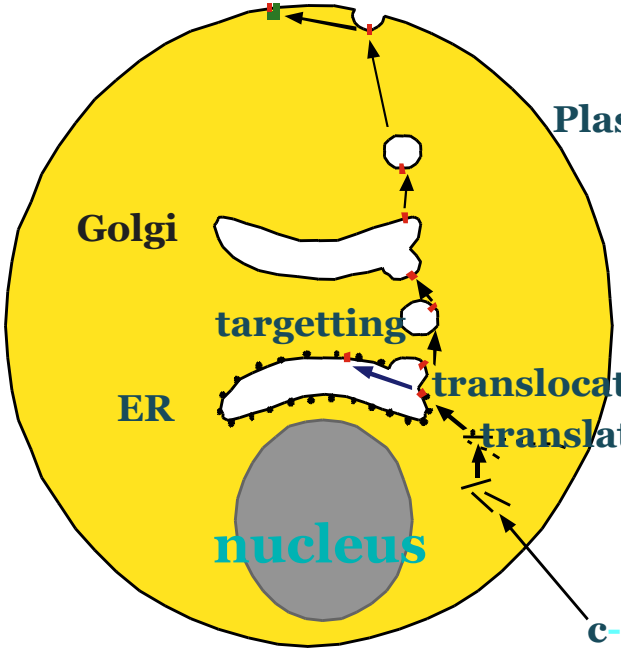
Expression in Xenopus laevis *oocytes*





48 – 72 h

Uptakes of

^{14}C-D-glucose

^{14}C-D-fructose

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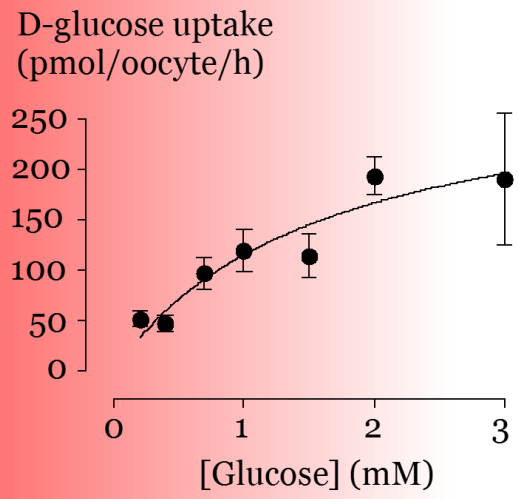
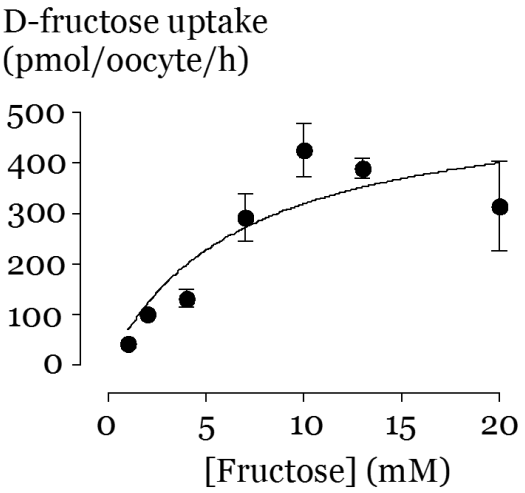
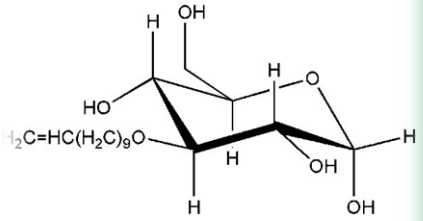
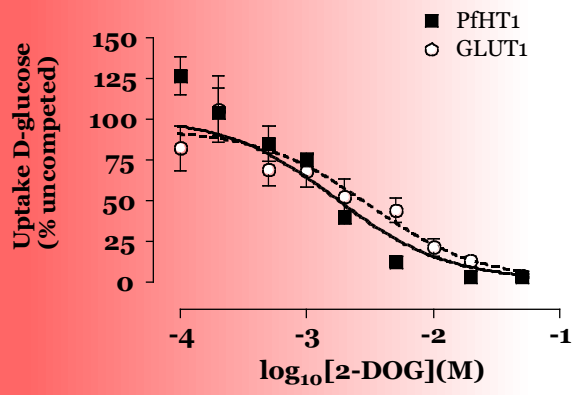
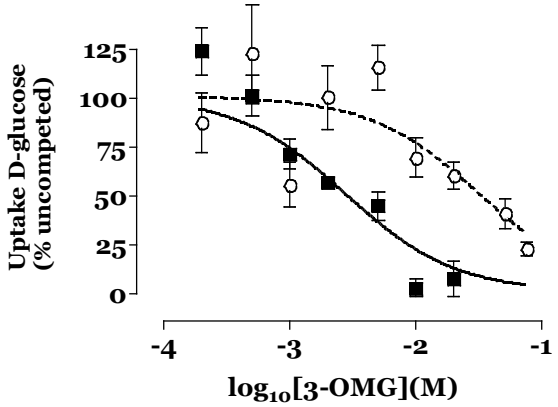
Functional studies - PfHT











✓Selective inhibition possible - CM3361 inhibits PfHT and kills malarial parasites without an effect on mammalian GLUTs

Woodrow *et al.* 1999. JBC 274: 7272-7

Joët *et al.* 2003. PNAS 100:7476-9

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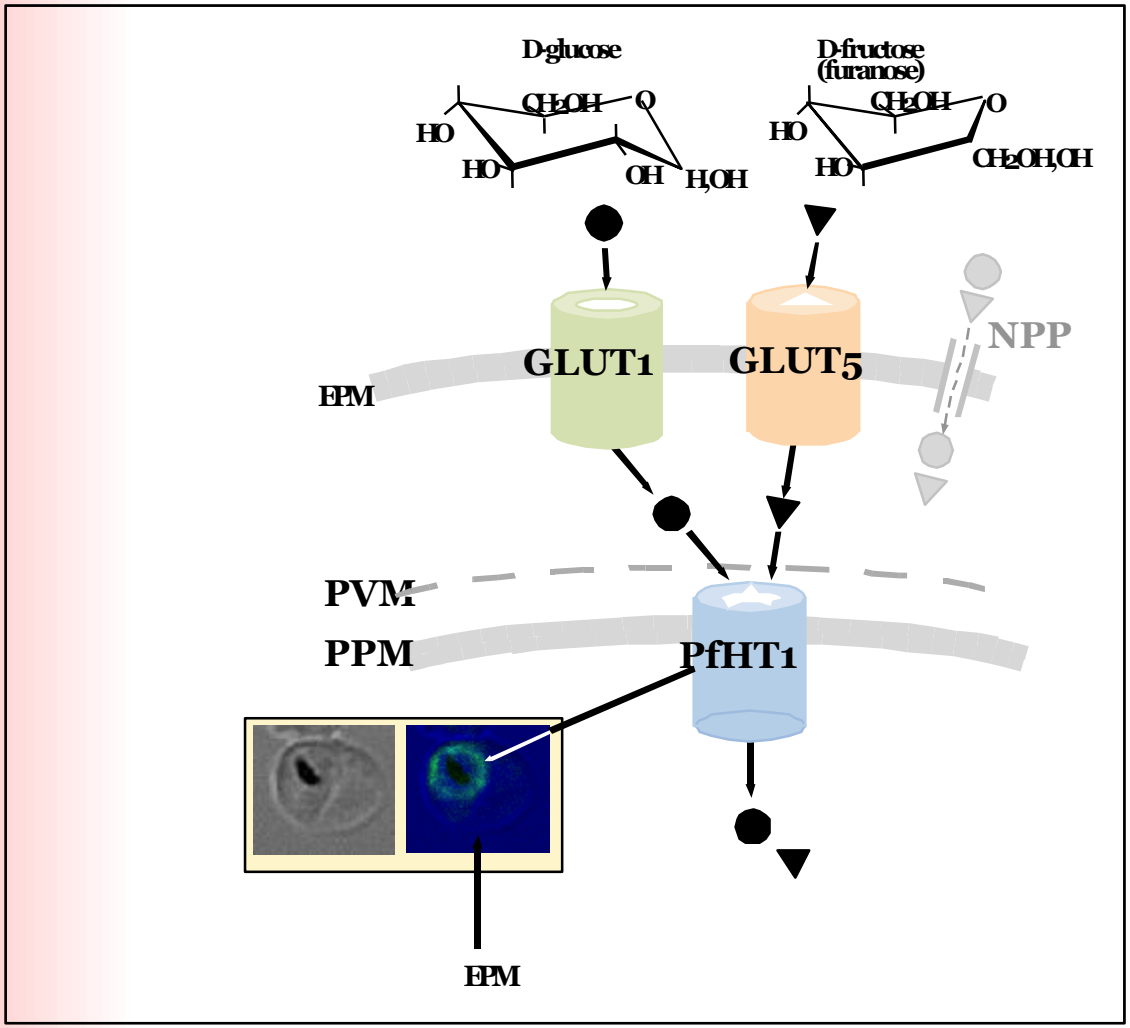
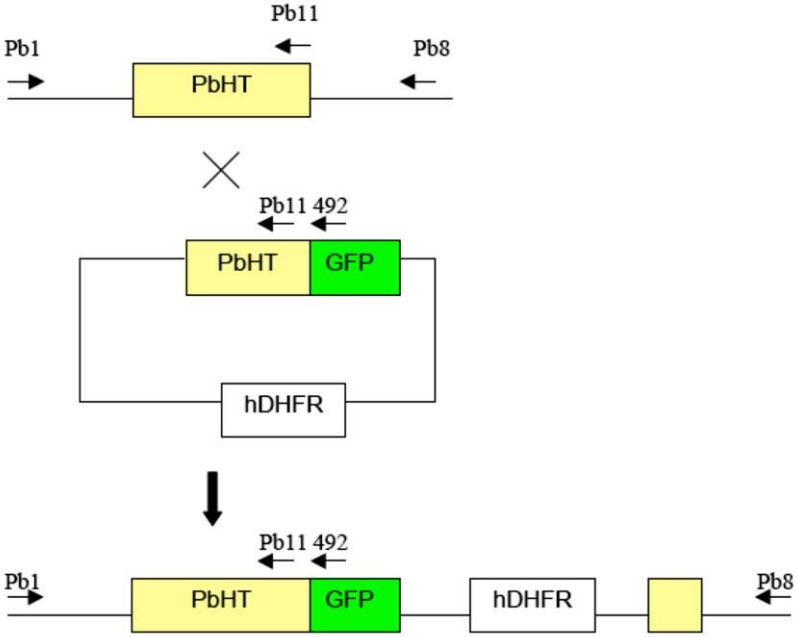
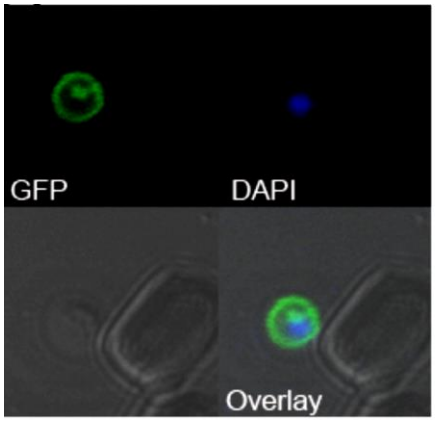


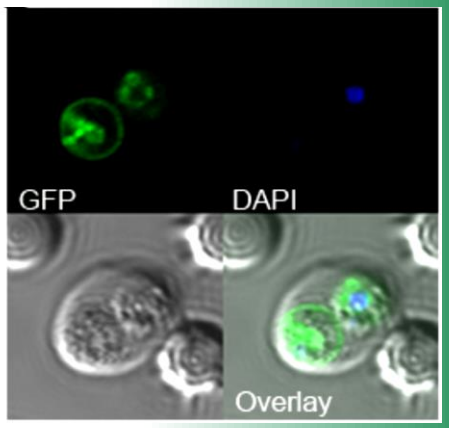
PCR analysis of PbHT-GFP transfectants



Wild-type locus



Erythrocytic stages



PbHT can not be knocked-out but can be GFP-tagged

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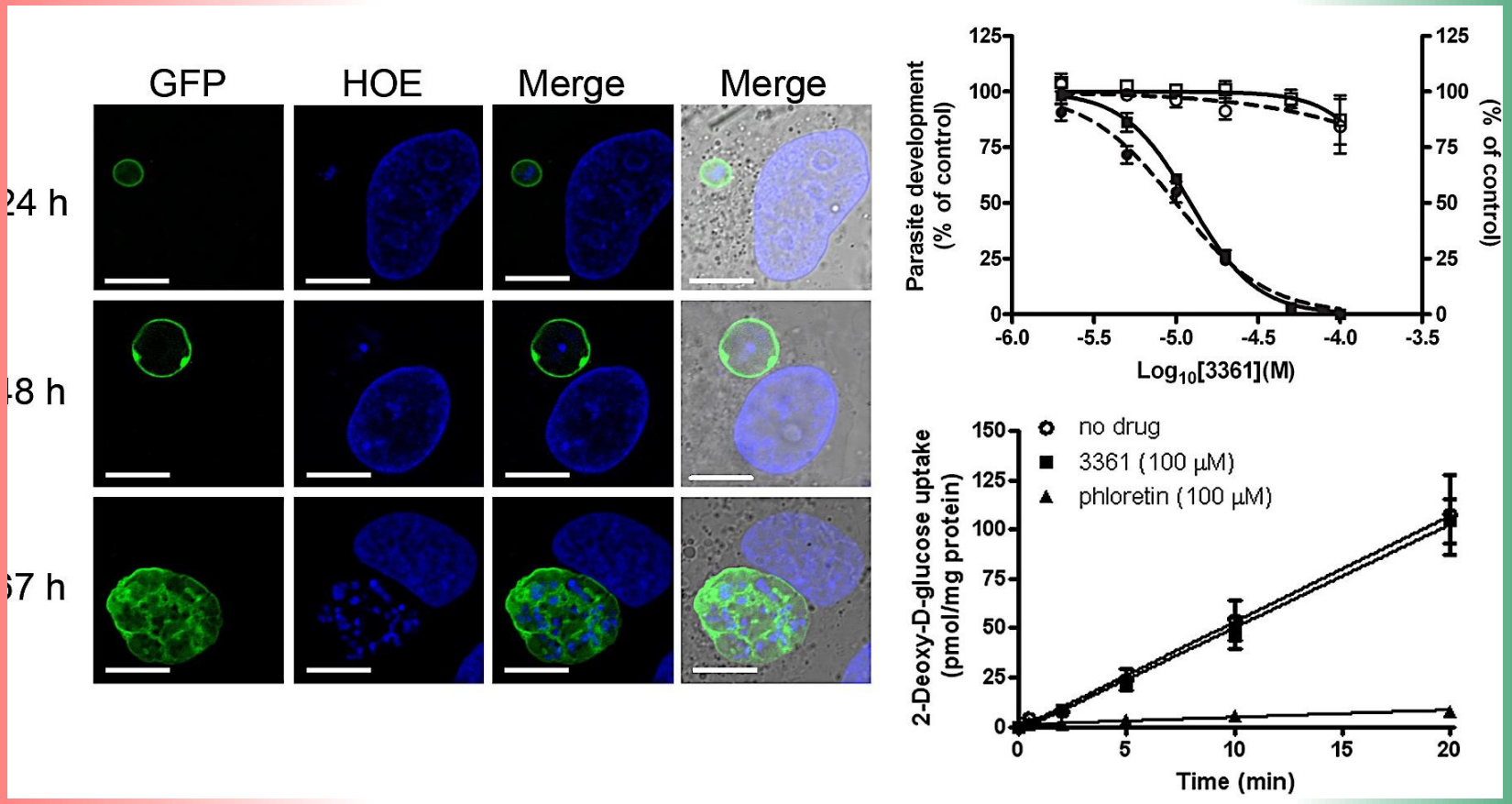
Liver stages: growth inhibition by CM3361



Slavic K, et al (2010). Molecular Microbiology 75: 1402-1413.

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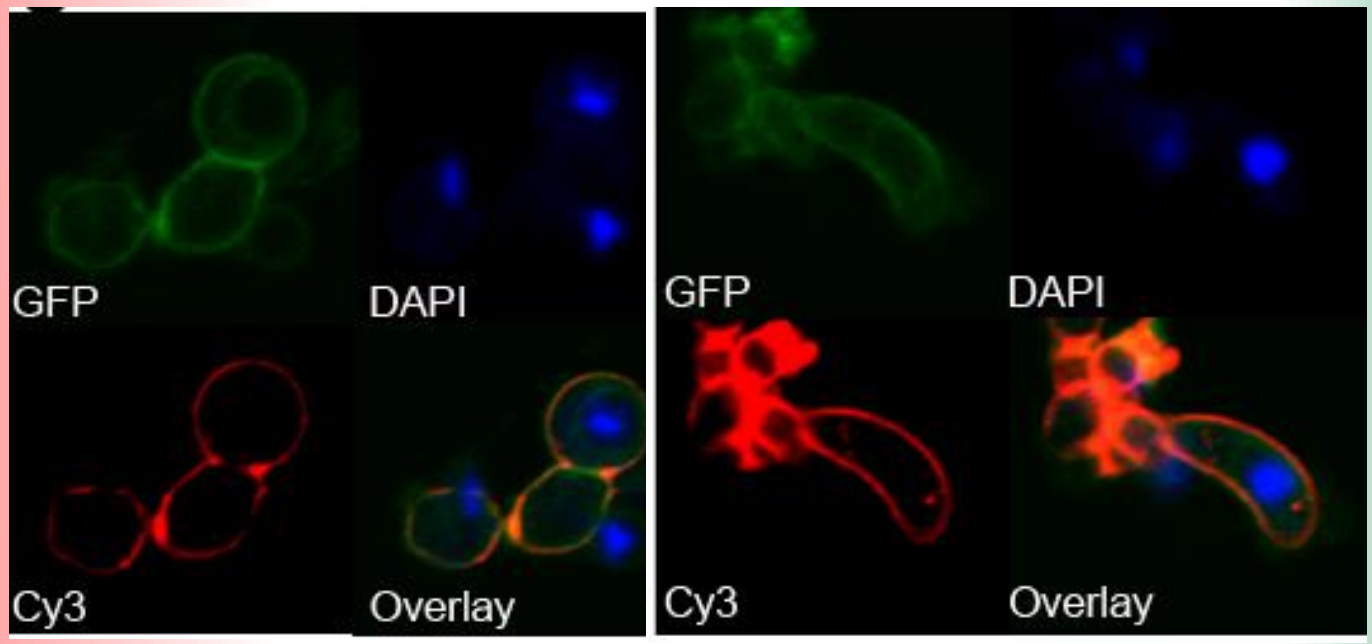
PbHT-GFP in sexual stages

Female gametocyte / zygote

Ookinete



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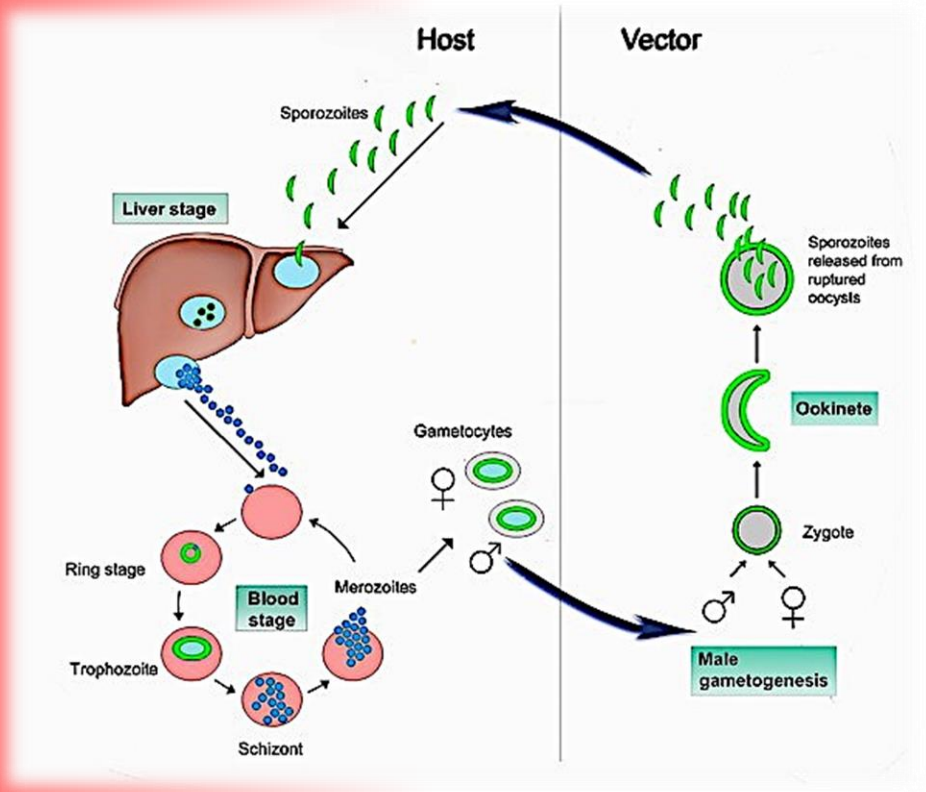

1. PfHT identified – first possible target from the genome initiative
2. Expression system set up to study functionally – now used widely
3. Validated chemically and genetically – and susceptible in all species
4. Life cycle importance established

Slavic *et al.* 2011 Malaria J

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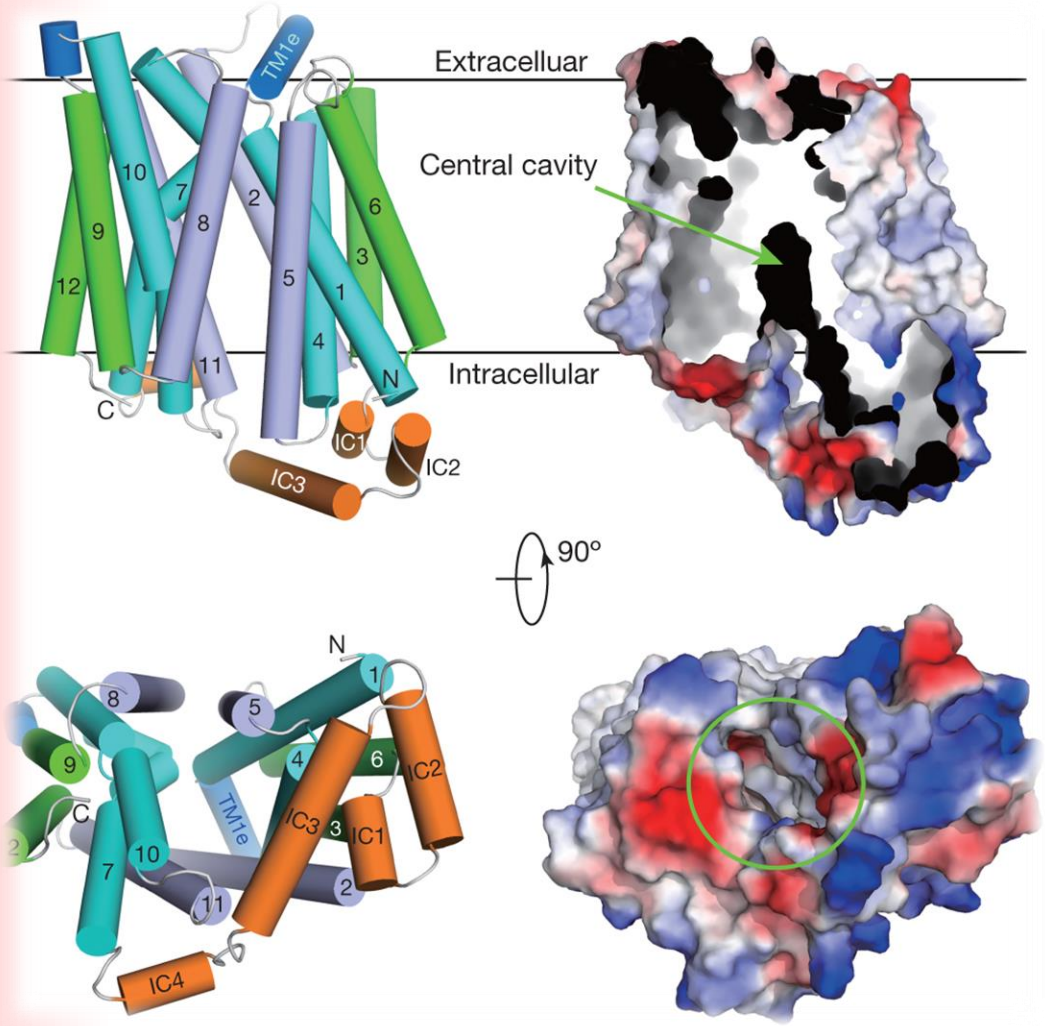
Overall structure of the human glucose transporter GLUT1.



D Deng *et al. Nature* 000, 1-5 (2014) doi:10.1038/nature13306

nature

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Target – P type ATPases

A Family of Cation ATPase-like Molecules from *Plasmodium falciparum*

Sanjeev Krishna, Gill Cowan, John C. Meade,* Richard A. Wells, James R. Stringer,‡ and Kathryn J. Robson

1993

with published ATPase sequences. It extends significantly the diversity within the family of cation-motive ATPases and has important implications for understanding the cell biology of host-parasite interactions. Furthermore, it provides a potentially novel chemotherapeutic target.

© The Rockefeller University Press, 0021-9525/93/01/385/14 $2.00
The Journal of Cell Biology, Volume 120, Number 2, January 1993 385–398

Spiroindolones, a Potent Compound Class for the Treatment of Malaria

Matthias Rottmann,[1,2*] Case McNamara,[3*] Bryan K. S. Yeung,[4*] Marcus C. S. Lee,[5] Bin Zou,[4] Bruce Russell,[6,7] Patrick Seitz,[1,2] David M. Plouffe,[3] Neekesh V. Dharia,[8] Jocelyn Tan,[4] Steven B. Cohen,[3] Kathryn R. Spencer,[8] Gonzalo E. González-Páez,[8] Suresh B. Lakshminarayana,[4] Anne Goh,[4] Rossarin Suwanarusk,[6] Timothy Jegla,[9] Esther K. Schmitt,[10] Hans-Peter Beck,[1,2] Reto Brun,[1,2] Francois Nosten,[11,12,13] Laurent Renia,[6] Veronique Dartois,[4] Thomas H. Keller,[4] David A. Fidock,[5,14] Elizabeth A. Winzeler,[3,8†] Thierry T. Diagana[4*†]

Recent reports of increased tolerance to artemisinin derivatives—the most recently adopted class of antimalarials—have prompted a need for new treatments. The spirotetrahydro-β-carbolines, or spiroindolones, are potent drugs that kill the blood stages of *Plasmodium falciparum* and *Plasmodium vivax* clinical isolates at low nanomolar concentration. Spiroindolones rapidly inhibit protein synthesis in *P. falciparum*, an effect that is ablated in parasites bearing nonsynonymous mutations in the gene encoding the P-type cation-transporter ATPase4 (PfATP4). The optimized spiroindolone NITD609 shows pharmacokinetic properties compatible with once-daily oral dosing and has single-dose efficacy in a rodent malaria model.

2010

SCIENCE VOL 329 3 SEPTEMBER 2010

Expression and Functional Characterization of a *Plasmodium falciparum* Ca^{2+}-ATPase (PfATP4) Belonging to a Subclass Unique to Apicomplexan Organisms*

Received for publication, November 22, 2000, and in revised form, December 21, 2000
Published, JBC Papers in Press, January 5, 2001, DOI 10.1074/jbc.M010554200

Sanjeev Krishna‡§, Charles Woodrow‡¶, Richard Webb‡, Jeff Penny‡, Kunio Takeyasu‖, Masatsugu Kimura**, and J. Malcolm East‡‡

The transition from an intracellular to an extracellular environment is common to many apicomplexan organisms that encode orthologues of PfATP4 and that may therefore share similar mechanisms for $[Ca^{2+}]$ homeostasis. As P type ATPases are susceptible to highly selective inhibition, this subclass of $[Ca^{2+}]$ pump presents an enticing target for antiparasitic drug development. Identification of potential inhibitors will undoubtedly be accelerated by the recent solution of the crystal structure for SERCA 1a (38).

2001

Antimicrob Agents Chemother. 2018 Mar 19. pii: AAC.00087-18. doi: 10.1128/AAC.00087-18. [Epub ahead of print]

Cell swelling induced by the antimalarial KAE609 (cipargamin) and other PfATP4-associated antimalarials.

Dennis ASM[1], Lehane AM[1], Ridgway MC[1], Holleran JP[2], Kirk K[3].

2018

Na^+ efflux transporter on the plasma membrane

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Artemisinins

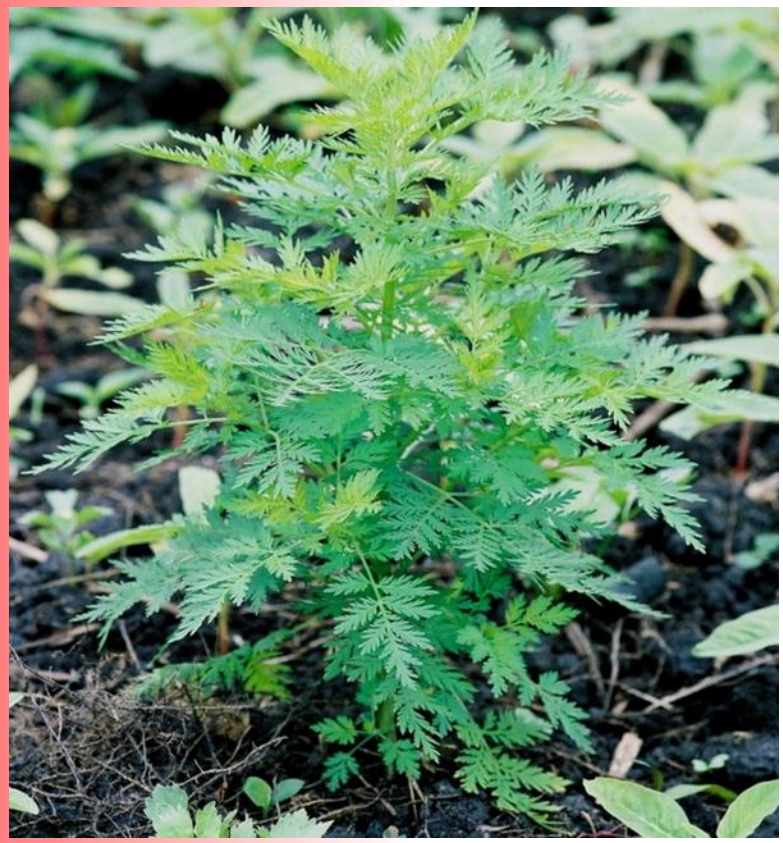

Fig 1: Artemisia Annua (Sweet Wormwood)

Fig 2: Tu Youyou, Chinese Scientist, 'Project 523' Nobel Prize Medicine 2015

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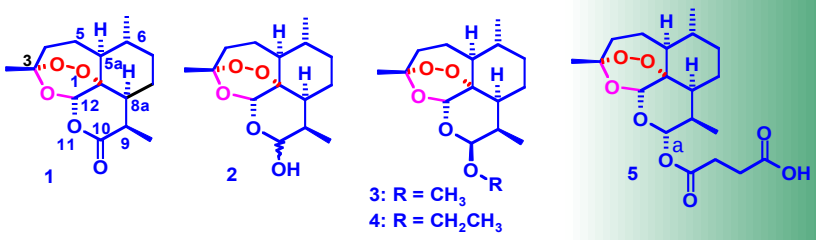
Artemisinins



Qing hao su or artemisinin **1** and derivatives dihydroartemisinin **2**, artemether **3**, arteether **4**, and artesunic acid **5**.

Artemisinin is a sesquiterpene - a natural product containing 15 carbon atoms

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Artemisinins

- **Used to treat malaria in combination therapies**
- **Effective against multidrug resistant parasites**
- **Act very rapidly, od dosing for uncomplicated disease**
- **Active in severe malaria**
- **MOA controversial**
- **Cheap**
- **Can be used po, im, iv, ir**
- **Safe in malaria (experience in millions)**

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Artemisinins – the new aspirin?

And also useful in the following infections, based on clinical trials, case reports or lab models:

- ***Schistosoma* infections**
- **Drug resistant CMV**
- ***In vitro* activity against other infections (*e.g. Toxoplasma gondii)***

•Haemorrhagic shock

Now being trialed as anticancer agents….open to recruitment at SGUL

Neoadjuvant treatment for colorectal cancer 'NeoArt' Phase II trial

NATURE | VOL 424 | 21 AUGUST 2003 |

Artemisinins target the SERCA of *Plasmodium falciparum*

U. Eckstein-Ludwig[1], R. J. Webb[1], I. D. A. van Goethem[2], J. M. East[2], A. G. Lee[2], M. Kimura[3], P. M. O'Neill[4], P. G. Bray[5], S. A. Ward[5] & S. Krishna[1]

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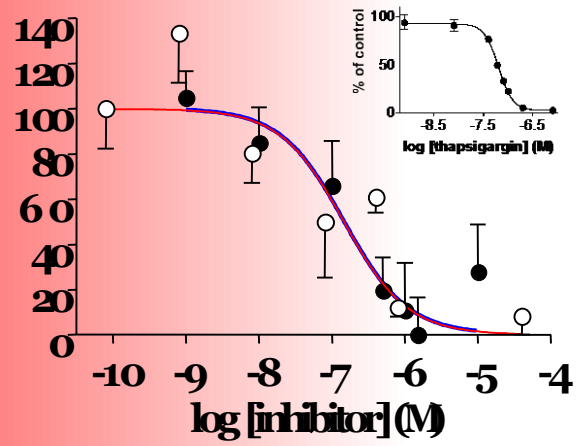
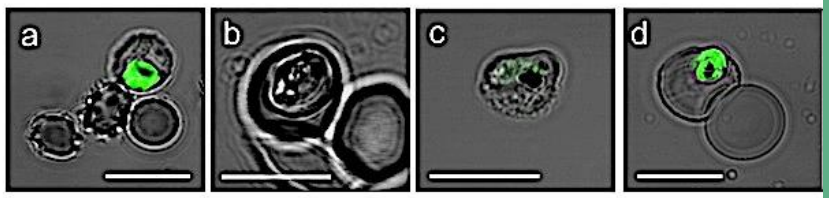
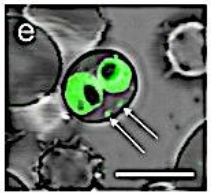
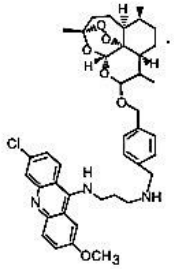


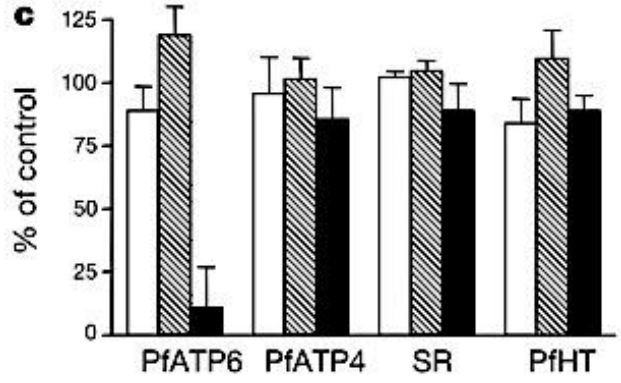
Thapsigargin







Artemisinin



These data provide compelling evidence that artemisinins act by inhibiting PfATP6 outside the food vacuole after activation by iron.

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Thaperoxide

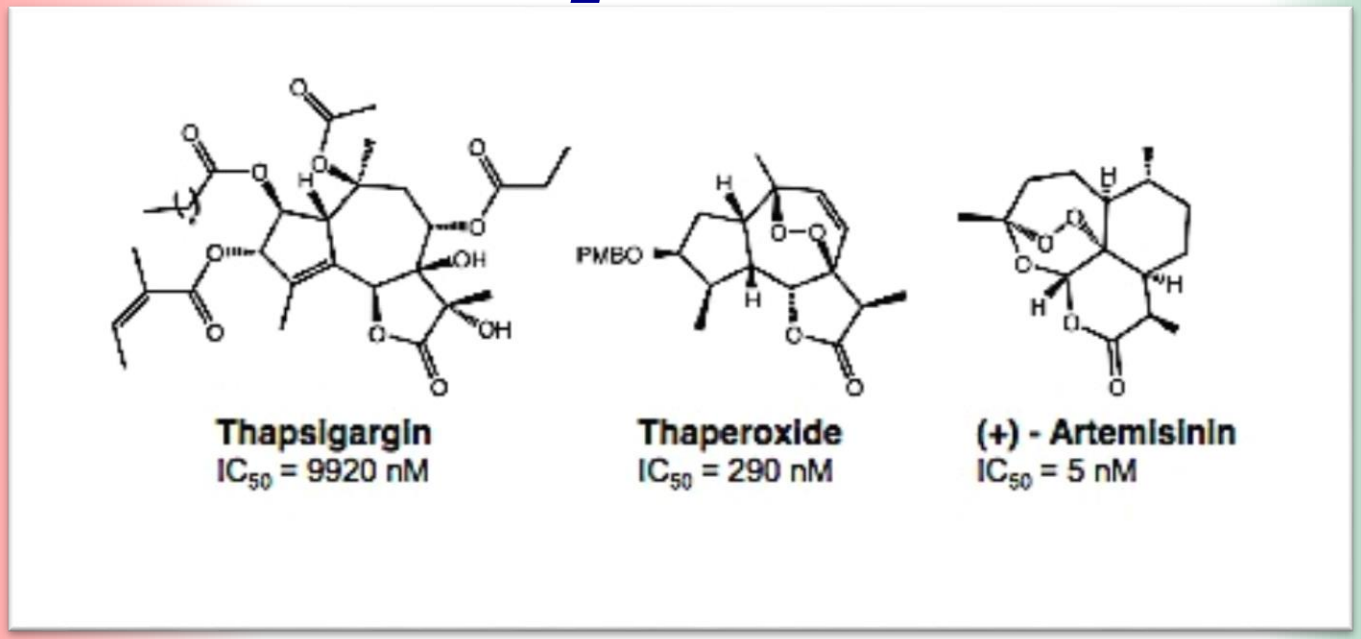

Figure 1. Structures of thapsigargin, thaperoxide and artemisinin and their IC_{50} values against the chloroquine resistant W2 strain of *Plasmodium falciaprum*. Note that an acetal derivative of thaperoxide (in a β configuration at C-12) produced an IC_{50} value against W2 of 13 nM. PMB, *p*-methoxybenzyl. Data taken from [7].

Sun *et al.* (2010) J Med Chem 53: 7864-8

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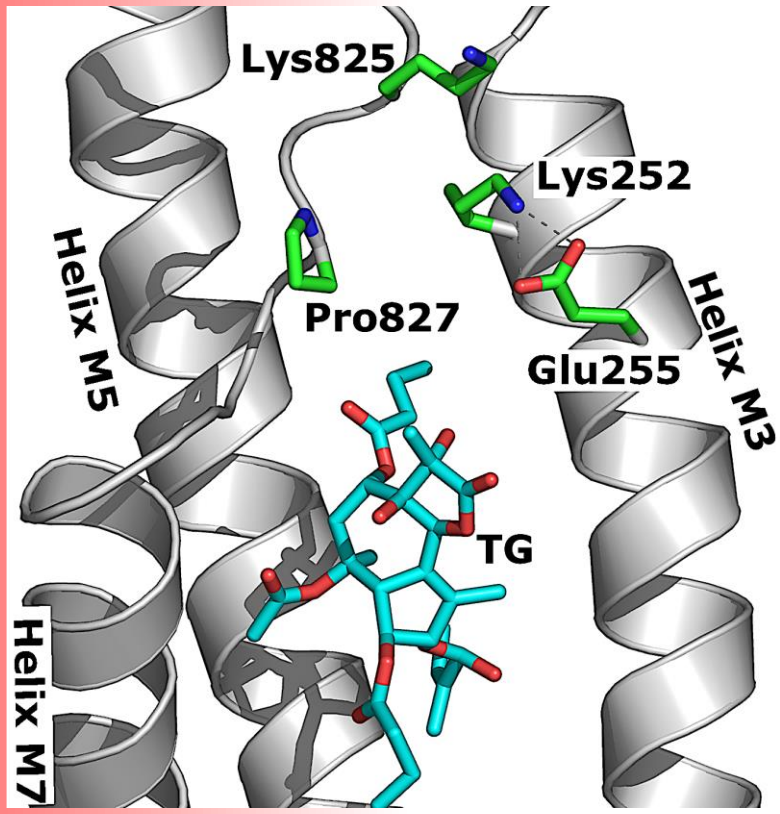
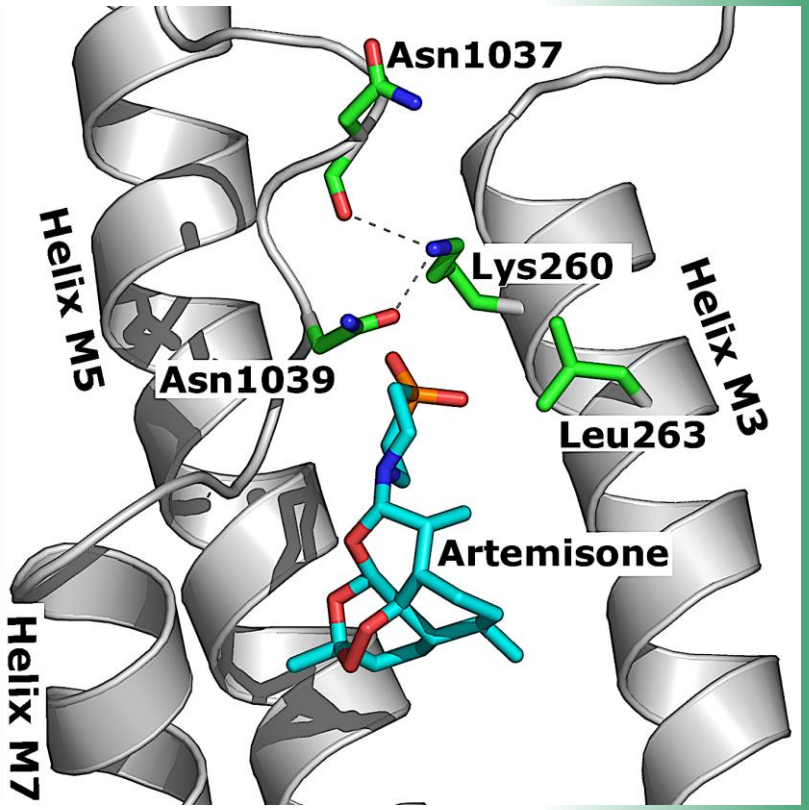
SERCA + TG



PfATP6 + artemisone



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Purified E255L Mutant SERCA1a and Purified PfATP6 Are Sensitive to SERCA-type Inhibitors but Insensitive to Artemisinins*[S]

Received for publication, November 30, 2009, and in revised form, June 2, 2010 Published, JBC Papers in Press, June 8, 2010, DOI 10.1074/jbc.M109.090340

Delphine Cardi[‡§¶], Alexandre Pozza[‡§¶], Bertrand Arnou[‖], Estelle Marchal[‡§¶], Johannes D. Clausen[‖], Jens Peter Andersen[‖], Sanjeev Krishna[**1], Jesper V. Møller[‖], Marc le Maire[‡§¶2], and Christine Jaxel[‡§¶3]

NATURE STRUCTURAL & MOLECULAR BIOLOGY | CORRESPONDENCE

Reappraising the effects of artemisinin on the ATPase activity of PfATP6 and SERCA1a E255L expressed in *Xenopus laevis oocytes*

Stéphanie David-Bosne, Michael Voldsgaard Clausen, Hanne Poulsen, Jesper Vuust Møller, Poul Nissen & Marc le Maire

Affiliations | Corresponding authors

Nature Structural & Molecular Biology **23**, 1–2 (2016) | doi:10.1038/nsmb.3156
Published online 06 January 2016 | Corrected online 13 January 2016
Erratum (April, 2016)

SCIENTIFIC REPORTS | 6:23454 | DOI: 10.1038/srep23454

Ca^{2+} monitoring in *Plasmodium falciparum* using the yellow cameleon-Nano biosensor

Kishor Pandey[1,2,*], Pedro E. Ferreira[1,3,4,*], Takeshi Ishikawa[5], Takeharu Nagai[6], Osamu Kaneko[1] & Kazuhide Yahata[1,*]

Calcium (Ca^{2+})-mediated signaling is a conserved mechanism in eukaryotes, including the human malaria parasite, *Plasmodium falciparum*. Due to its small size (<10 μm) measurement of intracellular Ca^{2+} in *Plasmodium* is technically challenging, and thus Ca^{2+} regulation in this human pathogen is not well understood. Here we analyze Ca^{2+} homeostasis via a new approach using transgenic *P. falciparum* expressing the Ca^{2+} sensor yellow cameleon (YC)-Nano. We found that cytosolic Ca^{2+} concentration is maintained at low levels only during the intraerythrocytic trophozoite stage (30 nM), and is increased in the other blood stages (>300 nM). We determined that the mammalian SERCA inhibitor thapsigargin and antimalarial dihydroartemisinin did not perturb SERCA activity. The change of the cytosolic Ca^{2+} level in *P. falciparum* was additionally detectable by flow cytometry. Thus, we propose that the developed YC-Nano-based system is useful to study Ca^{2+} signaling in *P. falciparum* and is applicable for drug screening.

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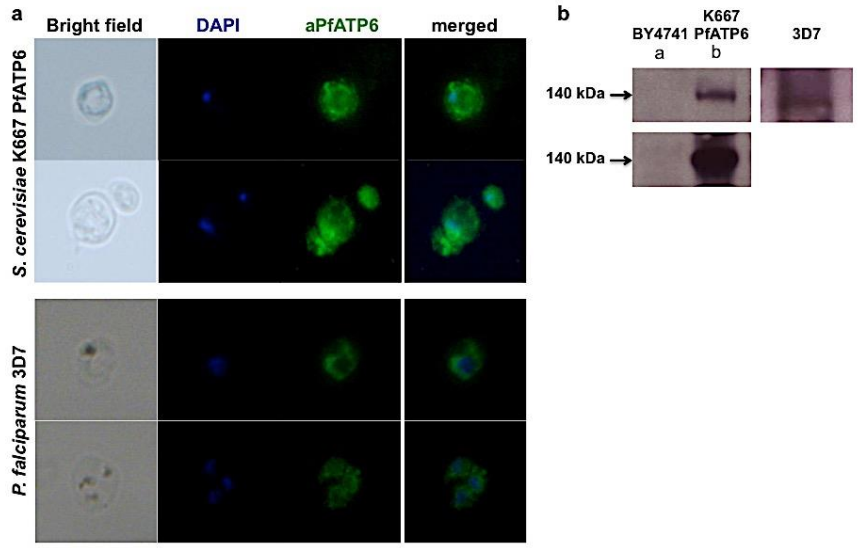
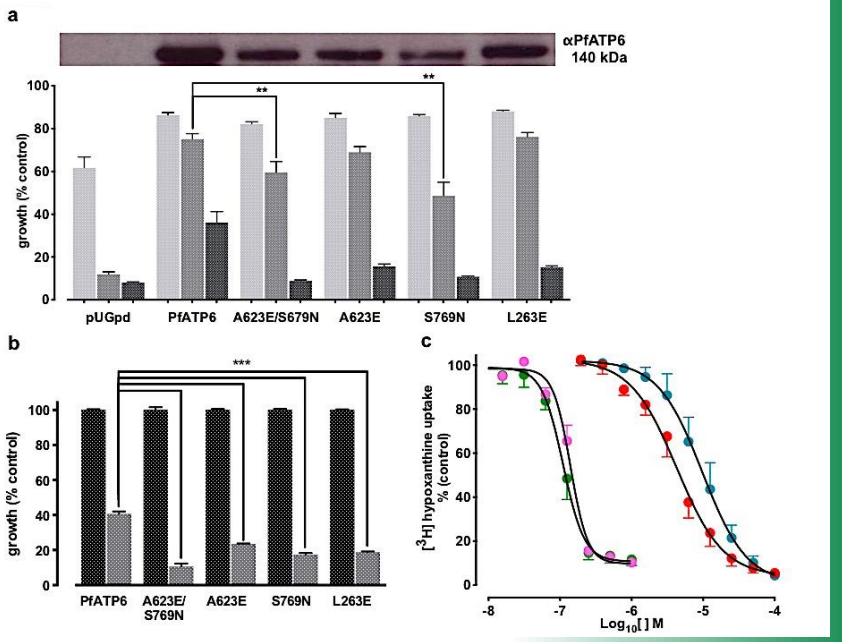
Expression in Yeast Links Field Polymorphisms in PfATP6 to in Vitro Artemisinin Resistance and Identifies New Inhibitor Classes

J Infect Dis. 2013 Aug 1;208(3):468-78. doi: 10.1093/infdis/jit171. Epub 2013 Apr 18.





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ARTICLE

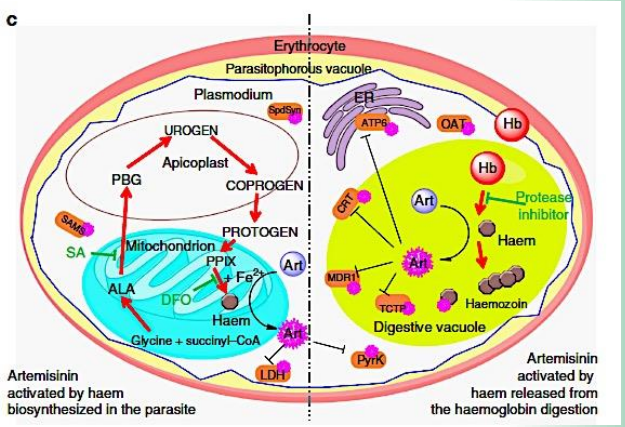
Received 10 Mar 2015 | Accepted 4 Nov 2015 | Published 22 Dec 2015

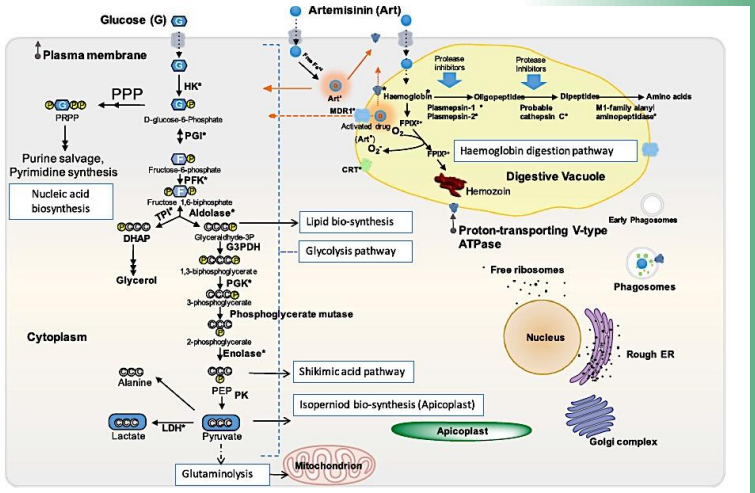
DOI: 10.1038/ncomms10111 OPEN

Haem-activated promiscuous targeting of artemisinin in *Plasmodium falciparum*

Jigang Wang[1,2,3,4,*], Chong-Jing Zhang[5,*], Wan Ni Chia[6], Cheryl C.Y. Loh[6], Zhengjun Li[7], Yew Mun Lee[1], Yingke He[8], Li-Xia Yuan[9], Teck Kwang Lim[1], Min Liu[3], Chin Xia Liew[7], Yan Quan Lee[6], Jianbin Zhang[4], Nianci Lu[10], Chwee Teck Lim[11,12], Zi-Chun Hua[2], Bin Liu[5], Han-Ming Shen[4], Kevin S.W. Tan[6] & Qingsong Lin[1,7]

The mechanism of action of artemisinin and its derivatives, the most potent of the anti-malarial drugs, is not completely understood. Here we present an unbiased chemical proteomics analysis to directly explore this mechanism in *Plasmodium falciparum*. We use an alkyne-tagged artemisinin analogue coupled with biotin to identify 124 artemisinin covalent binding protein targets, many of which are involved in the essential biological processes of the parasite. Such a broad targeting spectrum disrupts the biochemical landscape of the parasite and causes its death. Furthermore, using alkyne-tagged artemisinin coupled with a fluorescent dye to monitor protein binding, we show that haem, rather than free ferrous iron, is predominantly responsible for artemisinin activation. The haem derives primarily from the parasite's haem biosynthesis pathway at the early ring stage and from haemoglobin digestion at the latter stages. Our results support a unifying model to explain the action and specificity of artemisinin in parasite killing.





S4. Diagram of the main pathways targeted by ART-ABPPs in *P. falciparum* blood-stage trophozoites. The hemoglobin digestion pathway provides the

Artemisinin activity-based probes identify multiple molecular targets within the asexual stage of the malaria parasites *Plasmodium falciparum* 3D7

Hanafy M. Ismail[a,b], Victoria Barton[c], Matthew Phanchana[a], Sitthivut Charoensutthivarakul[c], Michael H. L. Wong[b], Janet Hemingway[b,1], Giancarlo A. Biagini[a], Paul M. O'Neill[c], and Stephen A. Ward[a,1]

[a]Research Centre for Drugs and Diagnostics, Department of Parasitology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; [b]Vector Biology, Liverpool School of Tropical Medicine, Liverpool L3 5QA, United Kingdom; and [c]Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, United Kingdom

Wang 2016	Ismail 2016	Zhou 2016
actin I (ACT1)	Actin-1	Actin, cytoplasmic 1*13164
alpha tubulin 1	Tubulin alpha chain	Tubulin alpha-1C chain*7424
Calcium-transporting ATPase (ATP6)	Calcium-transporting ATPase	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2*
heat shock protein 70 (HSP70)	Heat shock 70 kDa protein	Heat shock 70kDa protein 1A
		Heat shock 70kDa protein 4
heat shock protein 90 (HSP90)	Heat shock 90 kDa protein homolog (Fragment)	Heat shock protein HSP90-beta*
L-lactate dehydrogenase (LDH)	L-lactate dehydrogenase	L-lactate dehydrogenase A chain *54

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How artemisinins work?

- **Important to examine relevance of chemistry in *in vivo* models**

- **No single MOA is likely to account for activities against:**
  - **Apicomplexan parasites**
  - **Other protozoan parasites**
  - **Schistosomes**
  - **CMV**
  - **Cancer cells**
  - **SLE**
  - **Schizophrenia**
  - **Haemorrhagic shock**

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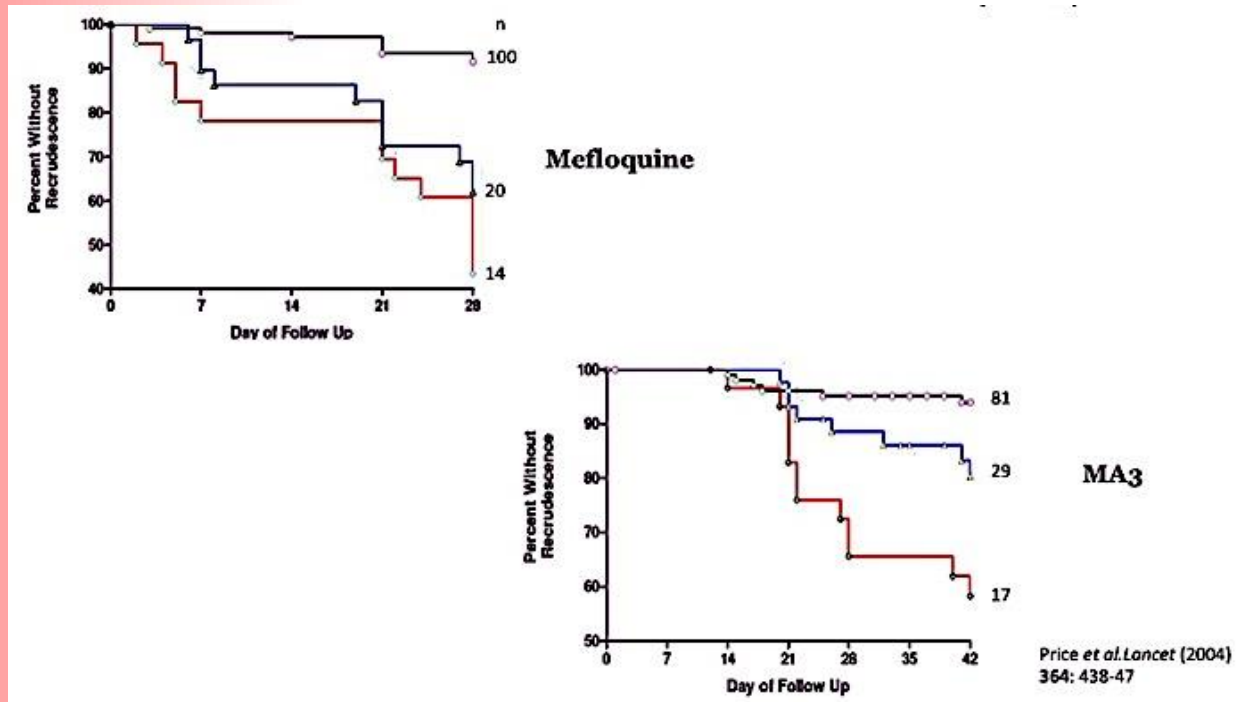
Prediction of treatment failure



Relevance to practice Multidrug resistant *P falciparum* malaria is common in southeast Asia, but difficult to identify and treat. Genes that encode parasite transport proteins maybe involved in export of drugs and so cause resistance. In this study we show that increase in copy number of *pfmdr1*, a gene encoding a parasite transport protein, is the best overall predictor of treatment failure with mefloquine. Increase in *pfmdr1* copy number predicts failure even after chemotherapy with the highly effective combination of mefloquine and 3 days' artesunate. Monitoring of

First evidence of TFACT?

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"Target Product Profile" for RDTs

- **Rapid < 15m 'bleed to read'/swab/sputum/urine**
- **PoC – power, robust, simple**
- **Accurate – precise characteristics dependent on disease prevalence**
- **Throughput**
- **eRecord - communicable**
- **Added value – 1 pathogen *vs.* many**
  Drug resistance markers

(not for environmental screening)

QuantuMDx

CoI Advisor and shareholder