

# Histidine-rich proteins as tools for the diagnosis and management of falciparum malaria

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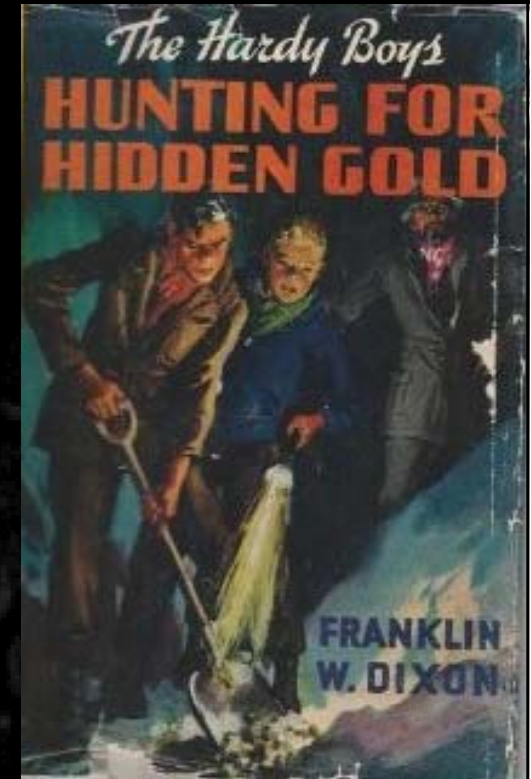
13<sup>th</sup> December 2013

# Histidine-rich proteins of *P. falciparum*

- Detection of HRPs via antibody forms the basis of an ever-expanding range of applications
  - lateral flow-based rapid diagnostic test
  - quantification of parasite growth in ex vivo drug susceptibility assays.
  - measurement in plasma to assess severity

# Value in reviewing subject

- Original discovery 1980s
- Applications developed a decade or more ago
- Subsequent developments
  - Complete malaria genomes
  - Transcriptome
- Bring together different aspects of biology



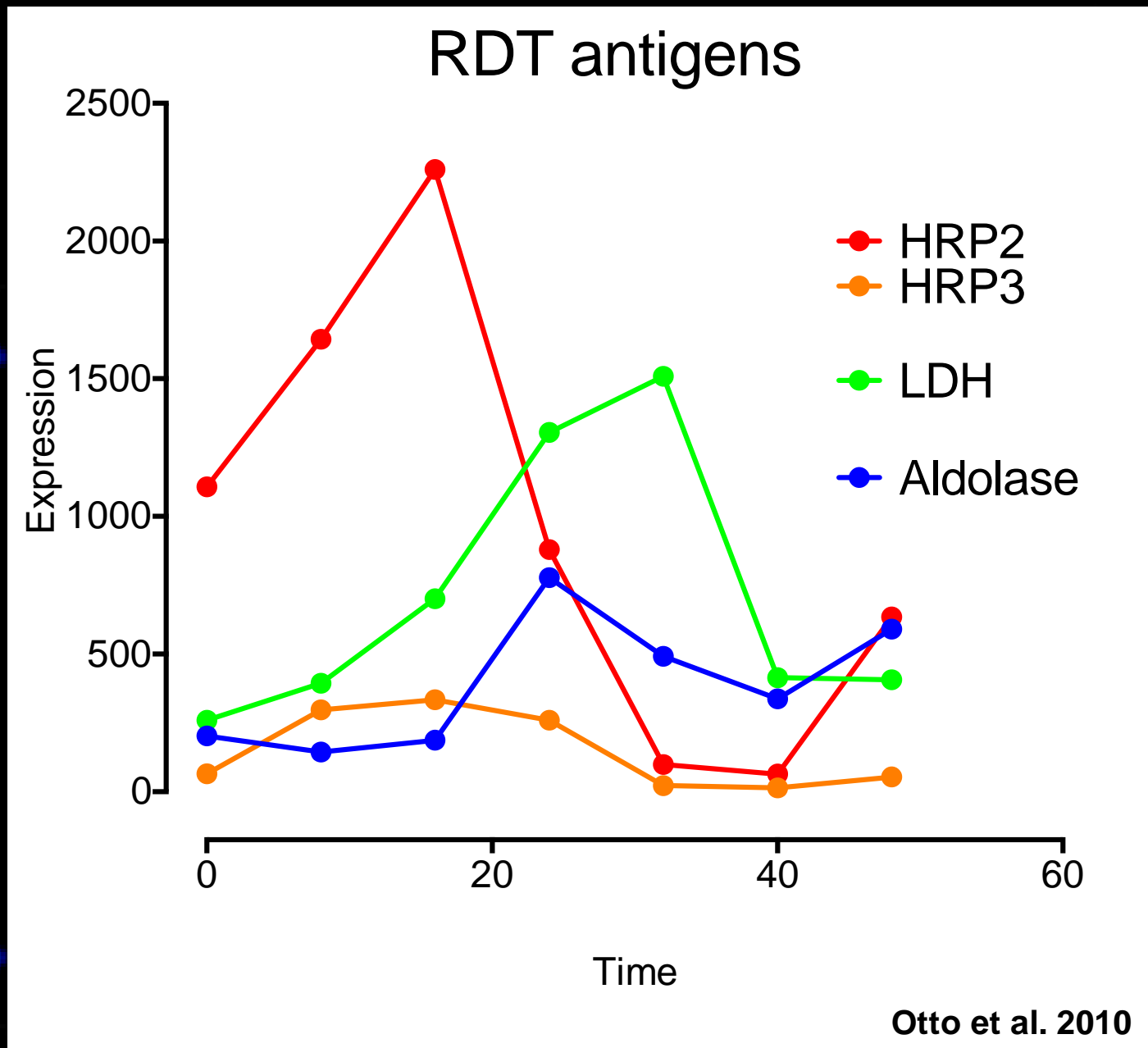
# History

- Discovery of histidine-rich proteins in *Plasmodium lophurae* (Kilejian 1974)
- *P. falciparum* 1984 Leech et al.
  - Based on  $^3\text{H}$  labelling
- DNA sequence for HRP3 isolated from library
  - Human antibody-based screening
- HRP2 DNA sequence isolated via hybridisation

# DNA

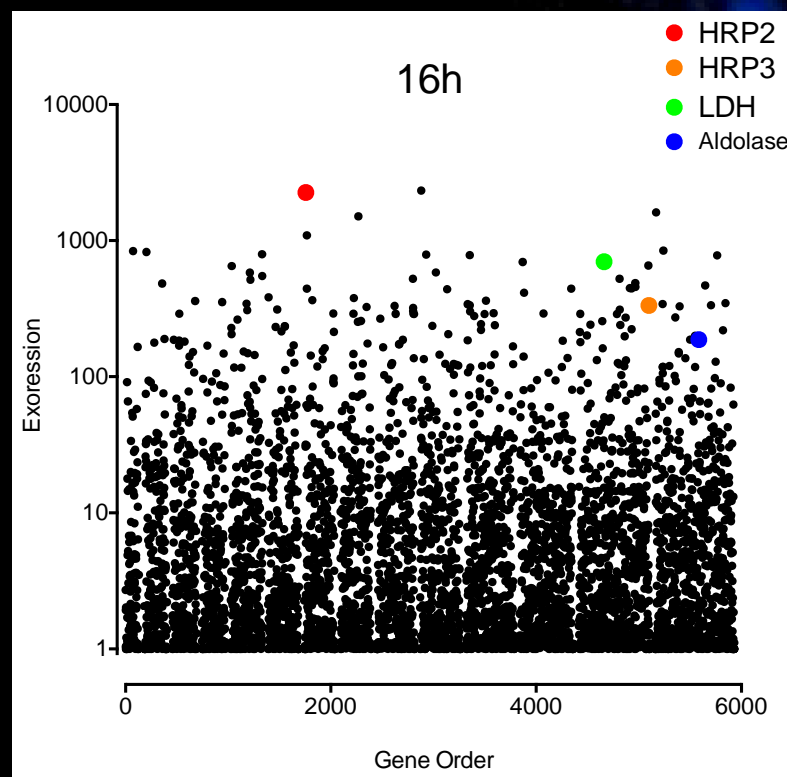
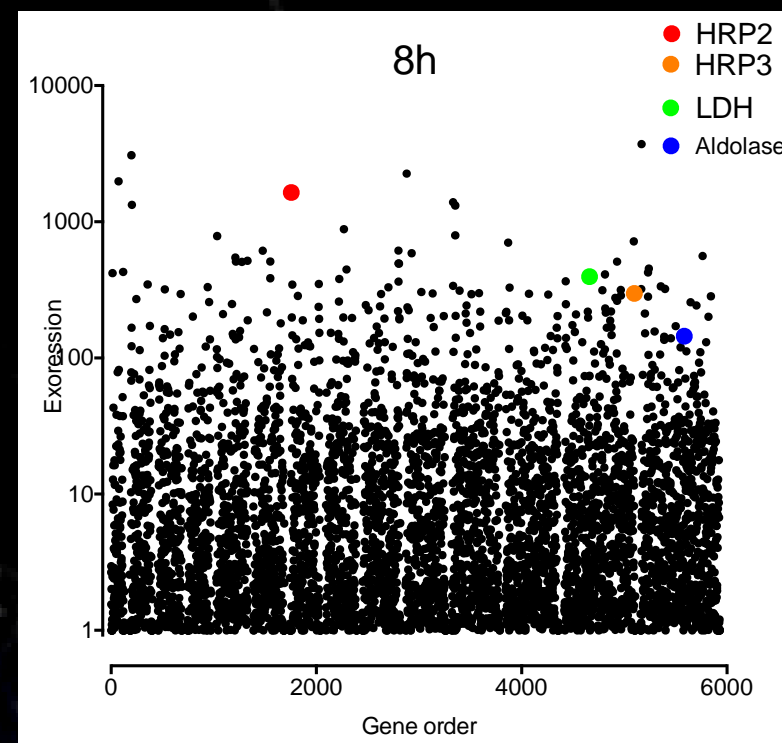
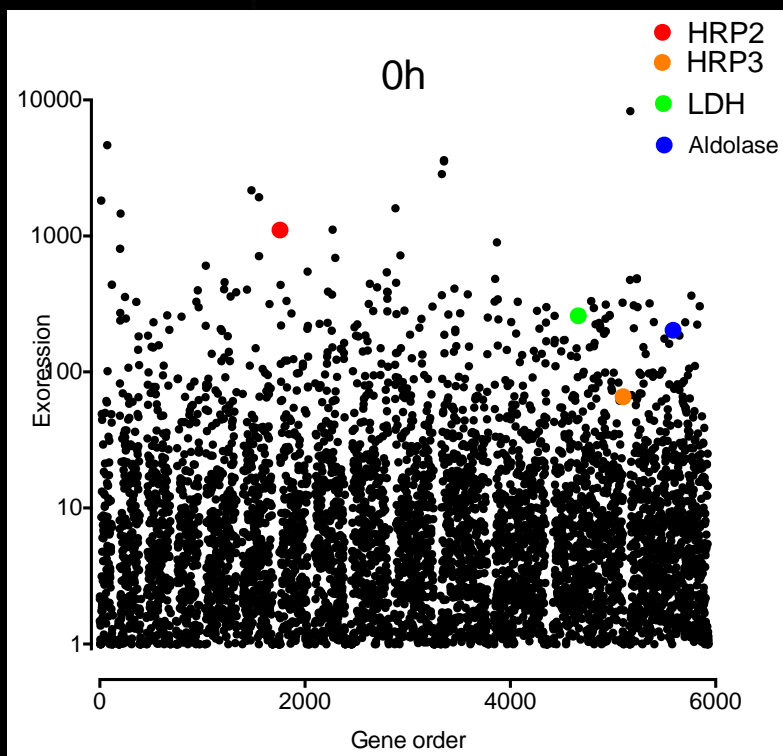
- Two genes HRP2 and HRP3
  - Chrom 8 & 13
  - ~~KAHRP, SHARP~~
- Orthologs in *P. reichenowi*
- No other sequenced *Plasmodium* species contain orthologs

# RNA in *P. falciparum* lifecycle



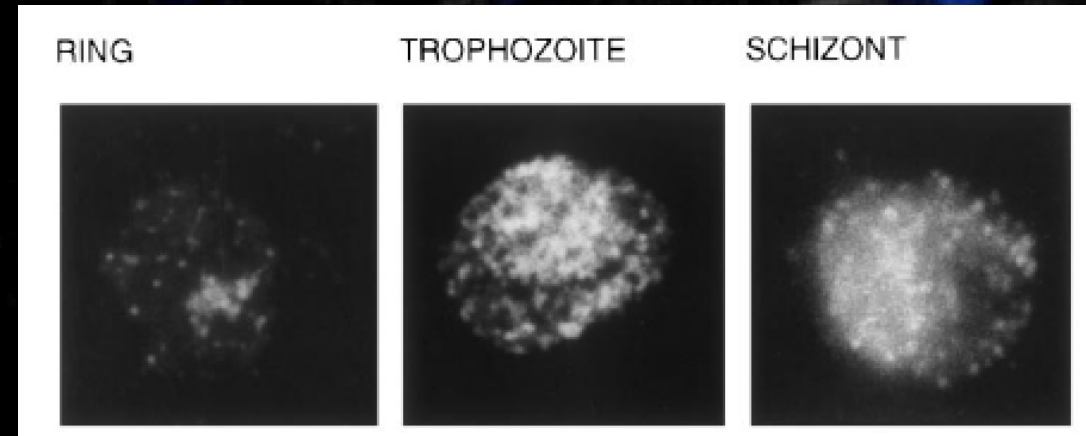
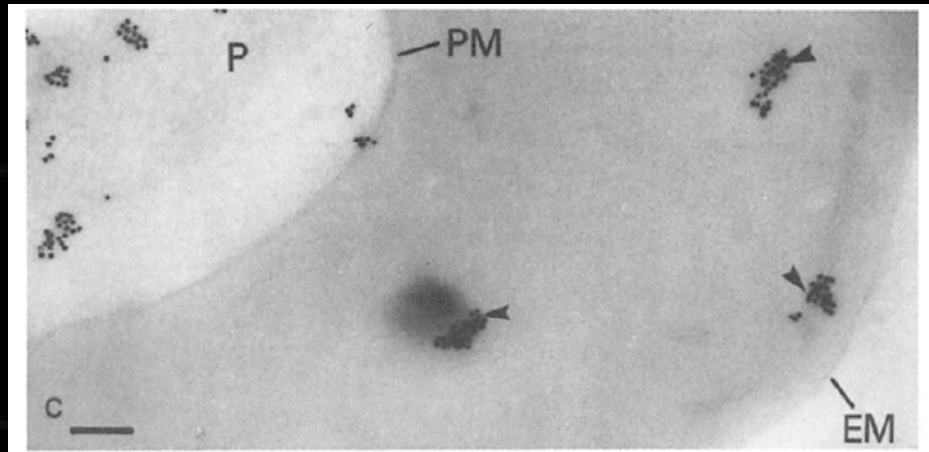
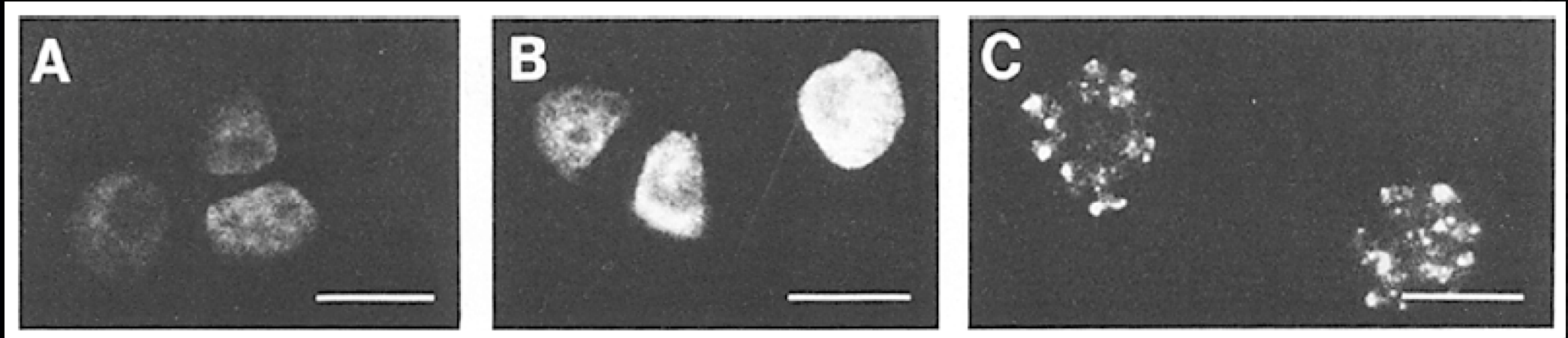
Otto et al. 2010





Howard et al. 1986

# Location



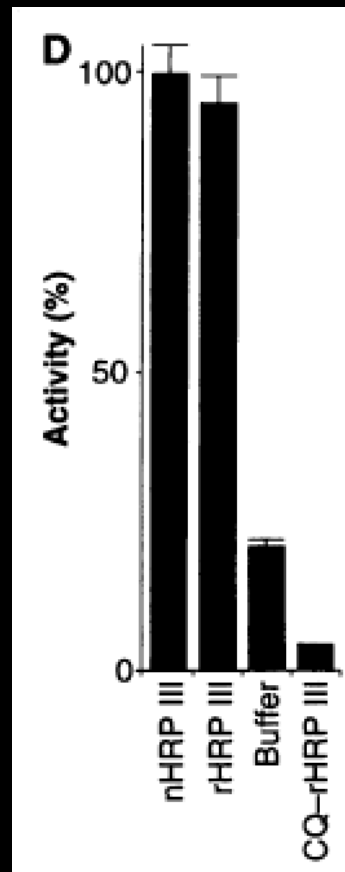
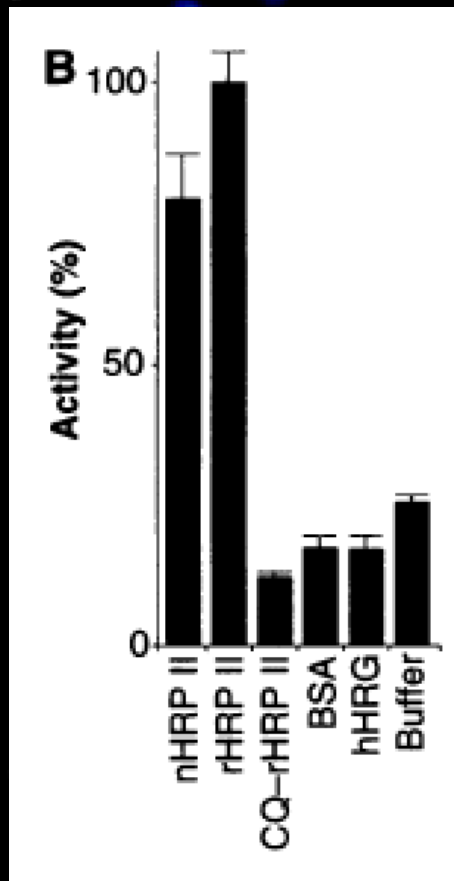
Bozdech et al. 1998



# Function of HRP2

## His-His-Ala x 51

- immunofluorescence over digestive vacuole
- In vitro haem polymerization assay



Sullivan et al. 1996

# Genetic cross

## A Histidine-Rich Protein Gene Marks a Linkage Group Favored Strongly in a Genetic Cross of *Plasmodium falciparum*

- 3D7 x HB3 cross
- HB3 strain has **HRP3 deletion**
- Progeny of cross all contained HRP3 derived from 3D7
  - (other genes potentially involved)

Wellems et al. 1987

# Implications

- Rapid Diagnostic Test
  - More sensitive than LDH
  - **Deletion of genes**
  - **Persistent positivity after treatment**
- In vitro drug assay
  - **Artemisinin resistance**

# Persistence after treatment

- Known since 1990s

**Table. Admission clinical and laboratory data of 92 patients with acute falciparum malaria (Thailand, 1998–2000)**

Parameter	Patient group	
	Severe malaria	Uncomplicated malaria
No. of patients	38	54
No. of recrudescence cases	13	27
PfHRP-2 persistence >2 wks*	34 (89%)	31 (61%)
PfHRP-2 intensity score*	8 [5–8]	7 [4–8]

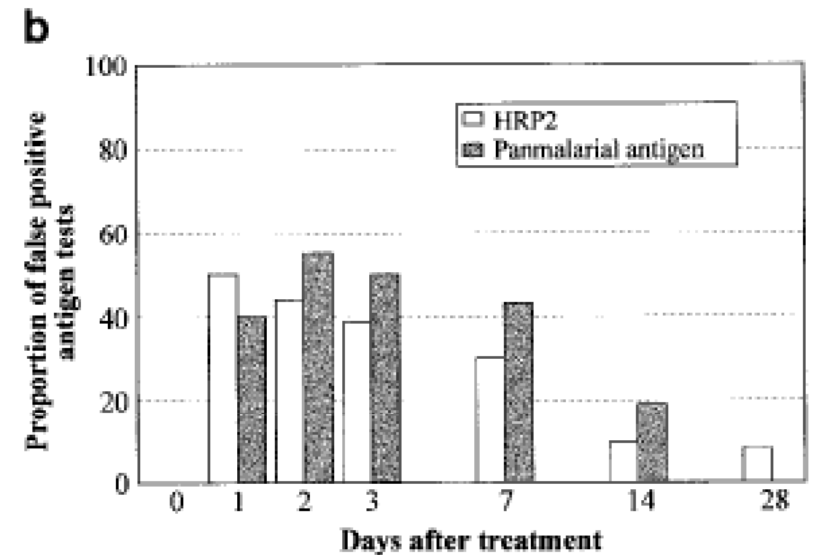
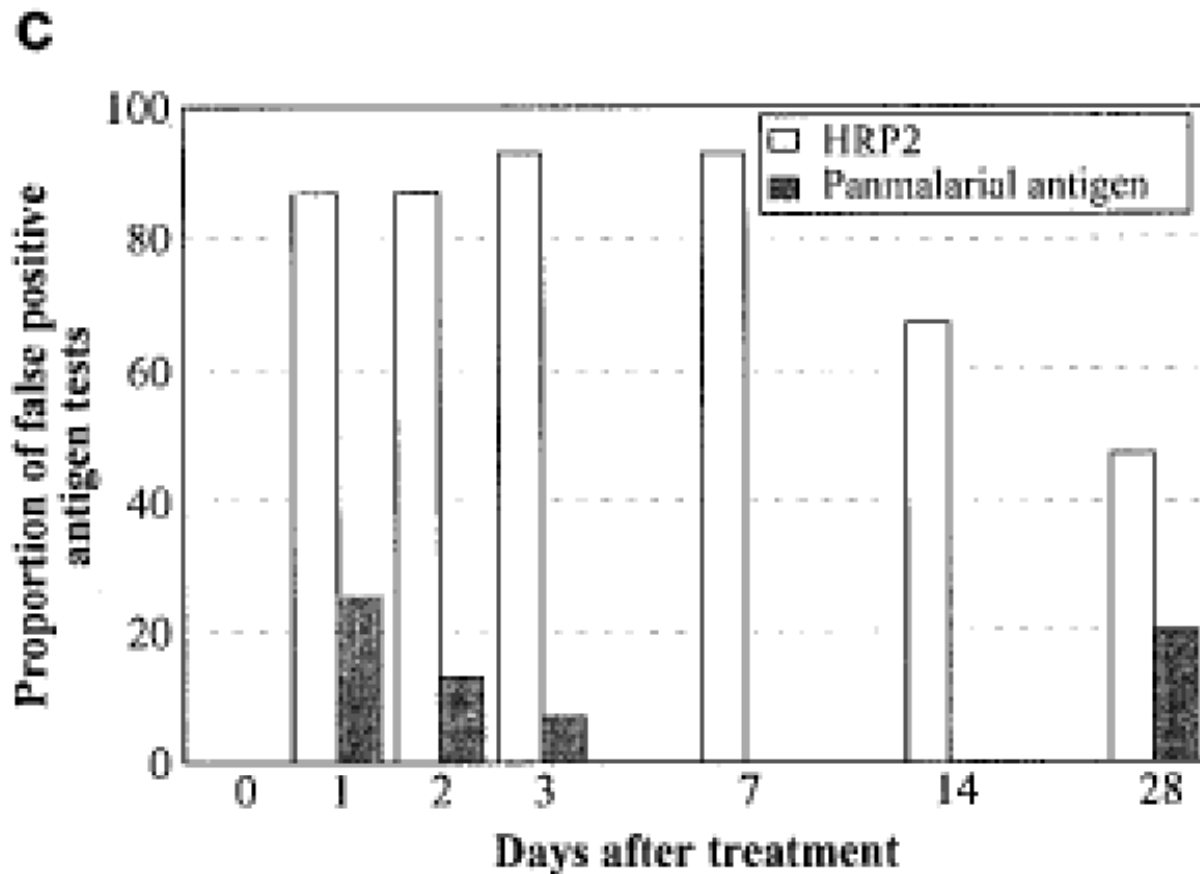
Mayxay et al.2001

# Persistence after treatment – why?

- Gametocytes?
- High starting parasitaemia?
- ‘Circulating HRP2’



# Persistence after treatment

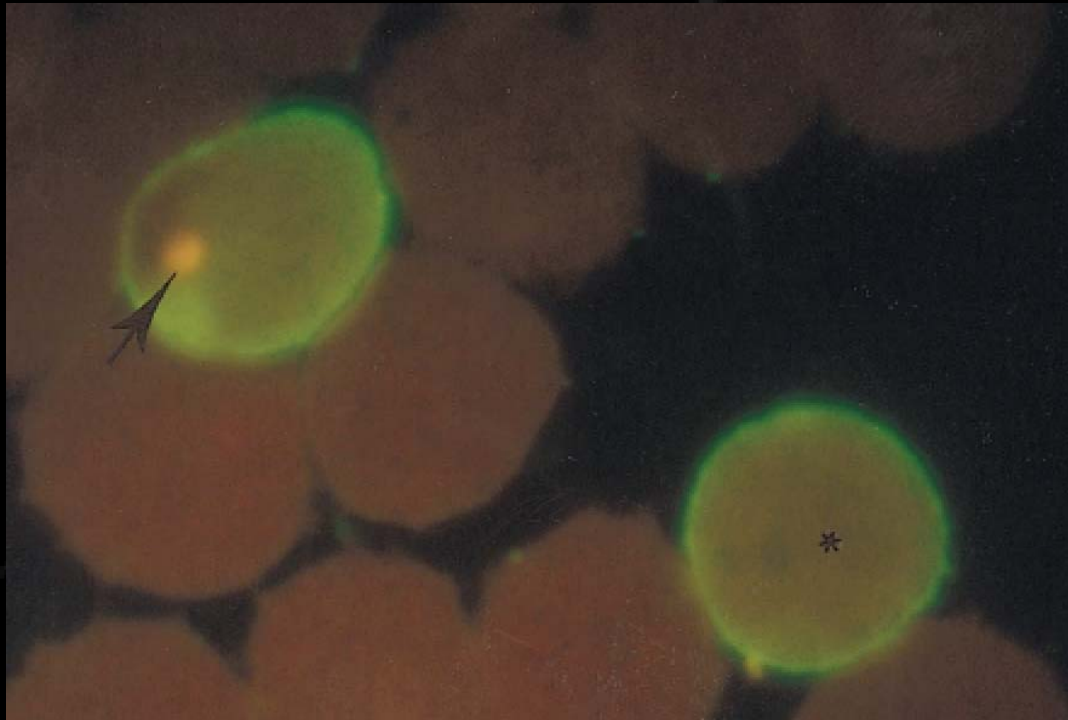


**Artesunate + SP**

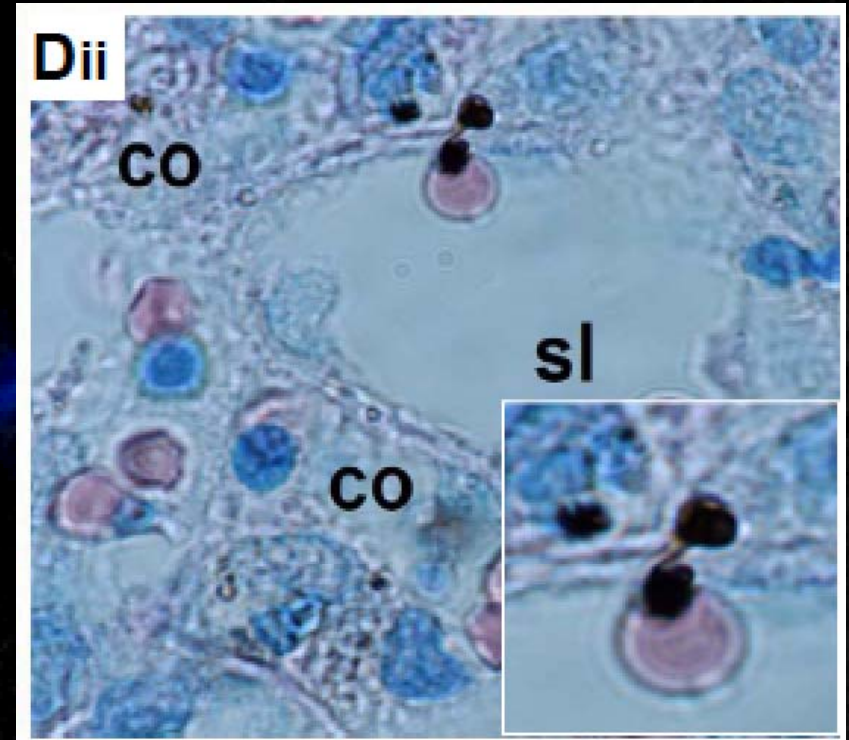
**SP only**

Tjitra et al.2001

# Parasite clearance - pitting



Chotivanich et al. 2000



Buffet et al. 2011

# Pitting appears enhanced with artesunate

- Persistence of HRP2 in blood after treatment likely related to pitting
- More pitting with artesunate
  - SP alone acts on schizonts – no pitting
  - Relevant to design and use of RDTs
- Pitting also likely to explain delayed haemolysis associated with artesunate in severe malaria

# HRP2/3 deletions

- Reported from South America (Gamboa et al., Houzé et al.)
- Are these likely to spread and compromise accuracy of RDTs?
- Fitness data from 1986 cross indicate this is unlikely
- Depends on level of transmission
- Genes present in *P. reichenowi*



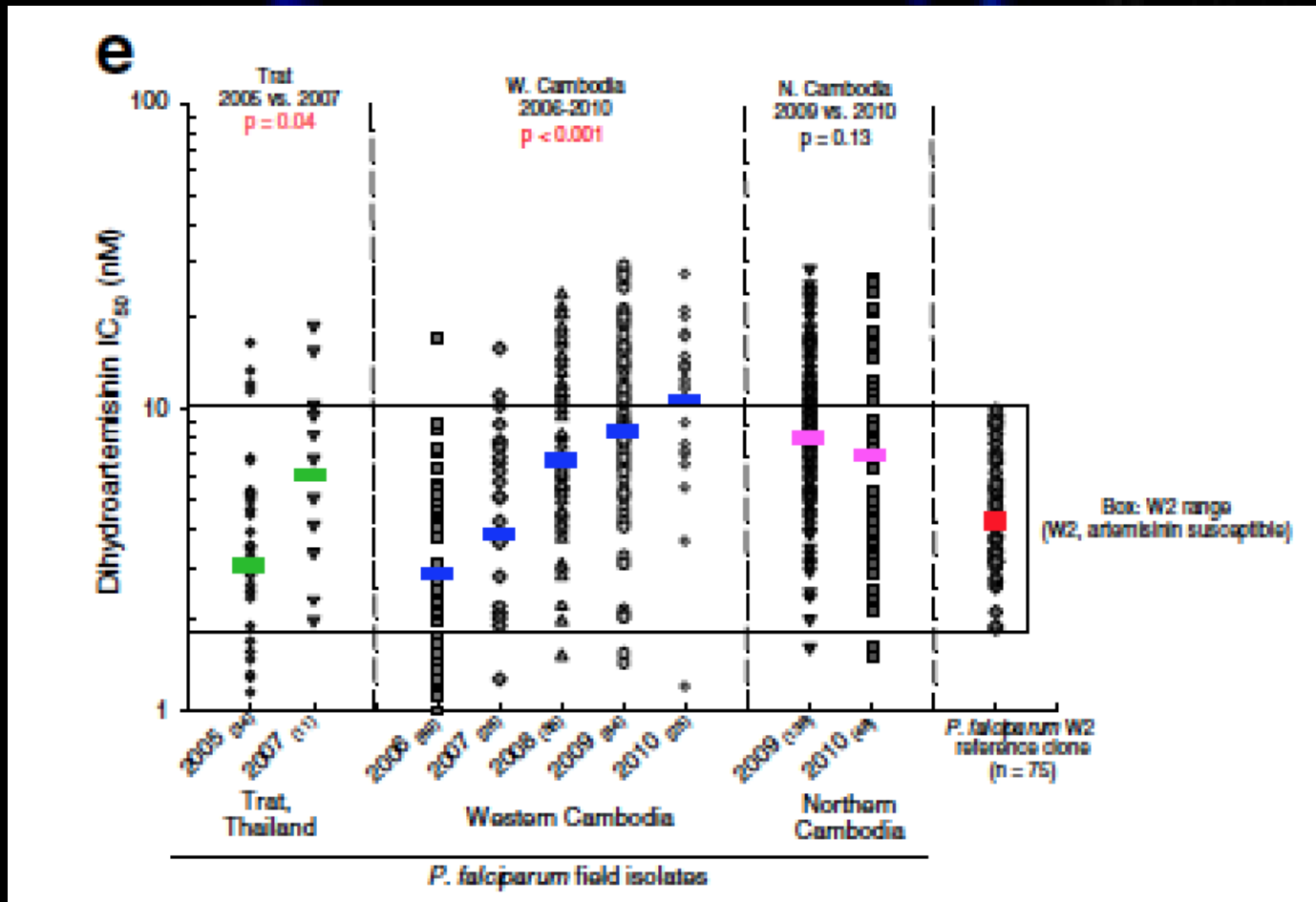
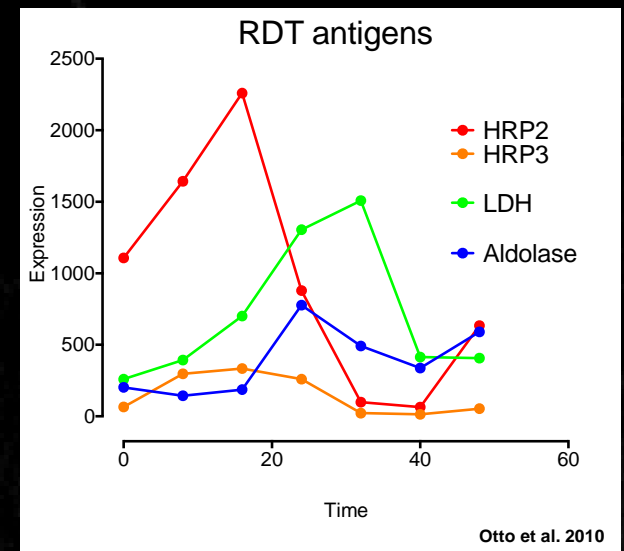
# Histidine-rich proteins





# Artemisinin resistance

- May reflect ring-stage susceptibility of artemisinins more than other readouts



Tyner et al. 2012

# Acknowledgements

- Kesinee Chotivanich
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