

# Can We Teach an Old Drug New Tricks?

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Head, Innovation in Graduate Studies, National University Health System

- Part 1: Chloroquine Probes for Drug Discovery
- Part 2: Novel Chloroquine Death Mechanism

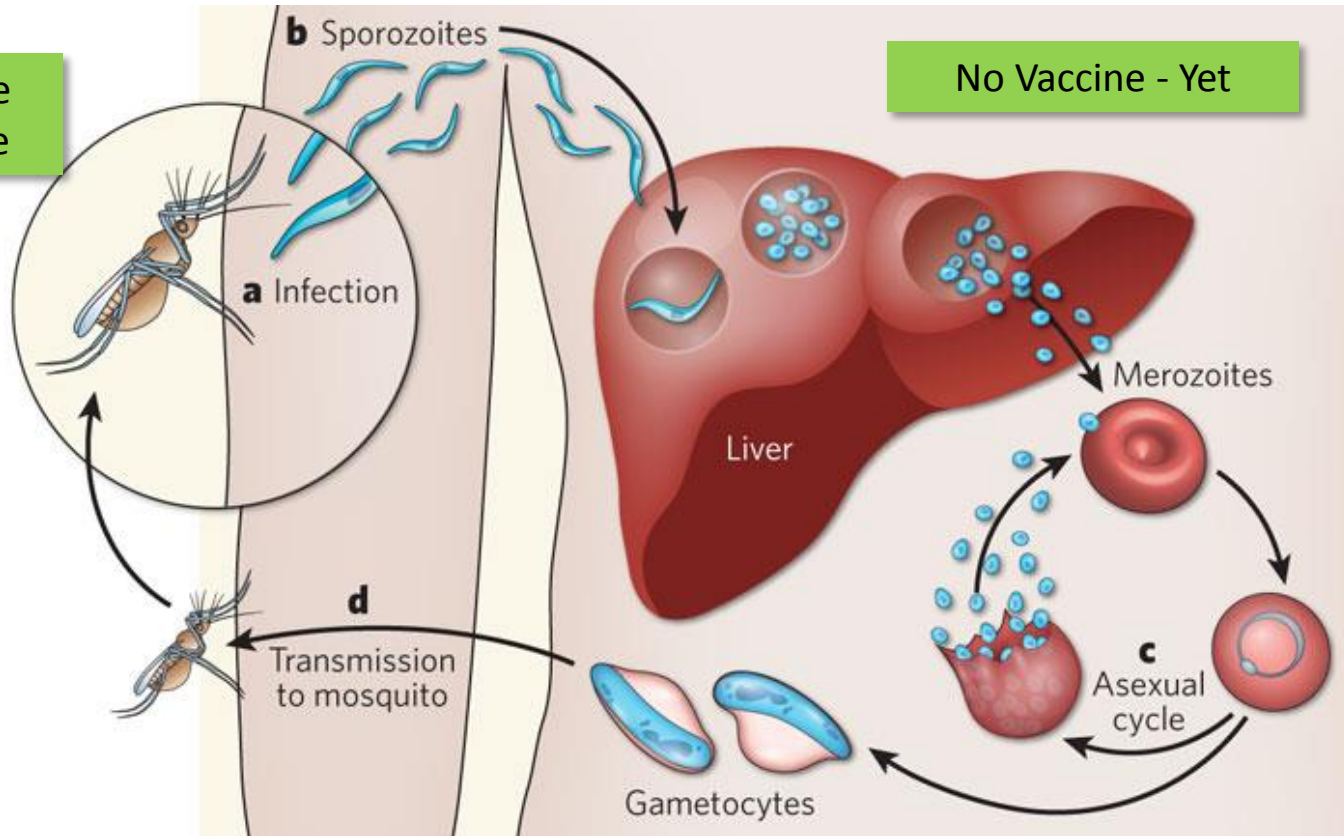


NATIONAL  
RESEARCH  
FOUNDATION



# Life Cycle of a Killer

Insecticide Resistance



No Vaccine - Yet

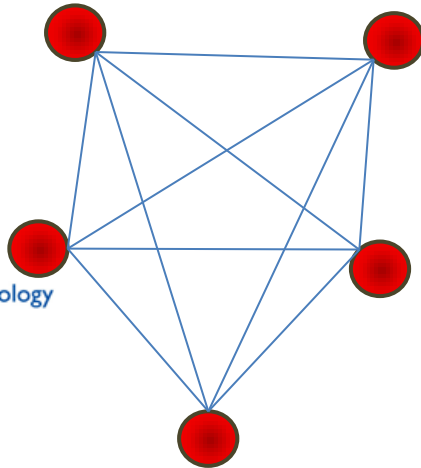
Need New Research Tools

Drug Resistance

# SingMalNet



- Molecular and cellular biology of malaria parasites
- Immunology of malaria
- Development of new tools
  - Drug discovery
  - Immunology
- Teaching
- International meetings
- Consultant to MoH

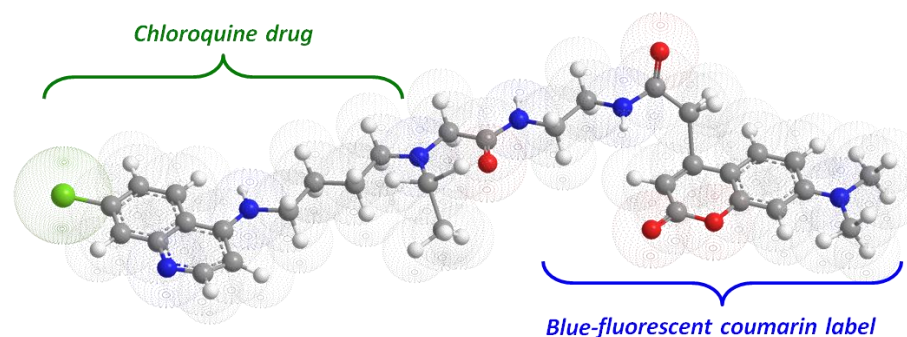
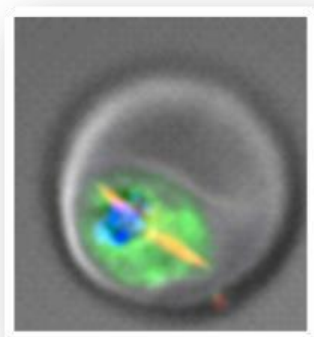


# Malaria Research @ NUS

*From Bench to Better Drugs*

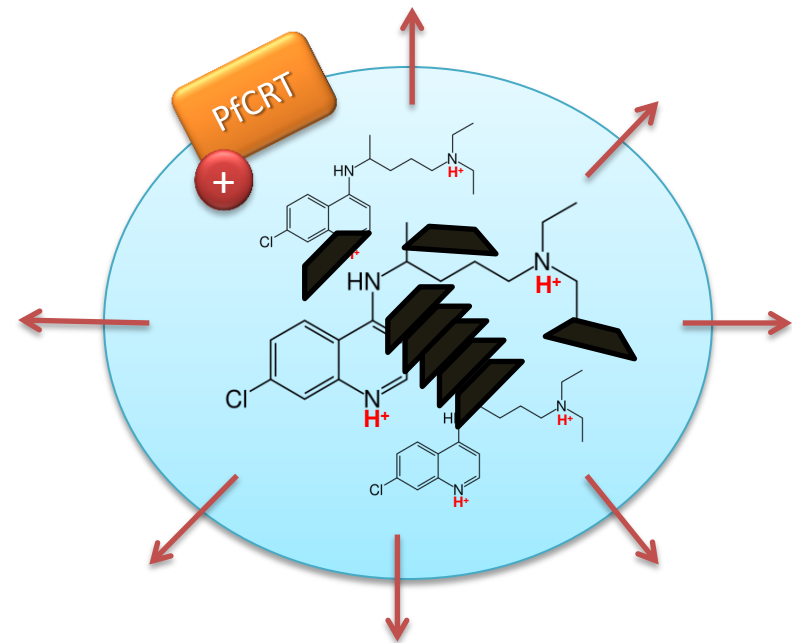
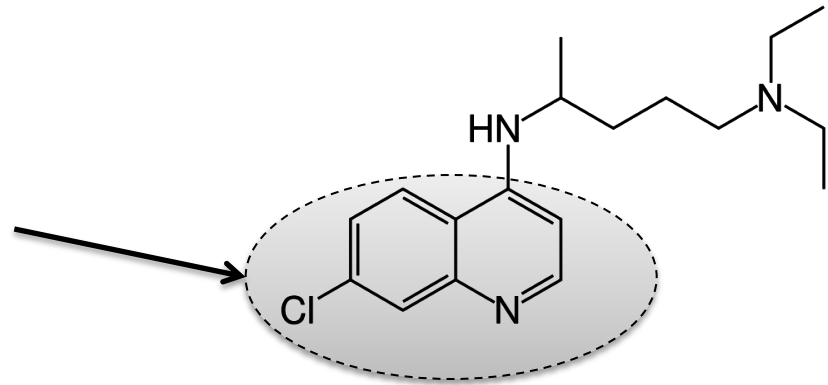


# Part 1: Chloroquine Probes for Drug Discovery



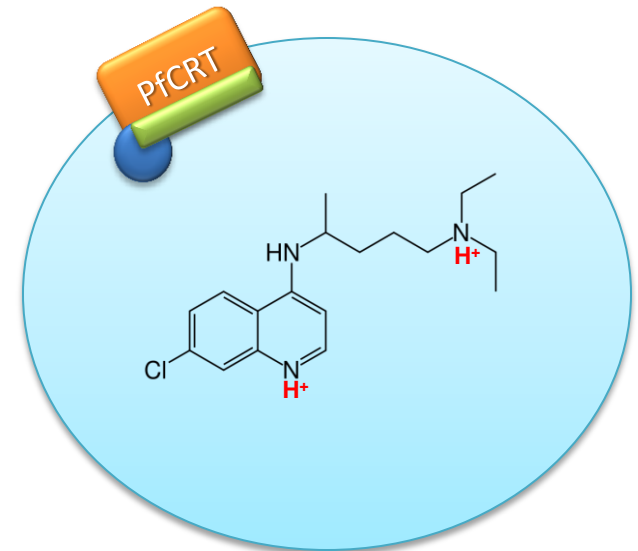
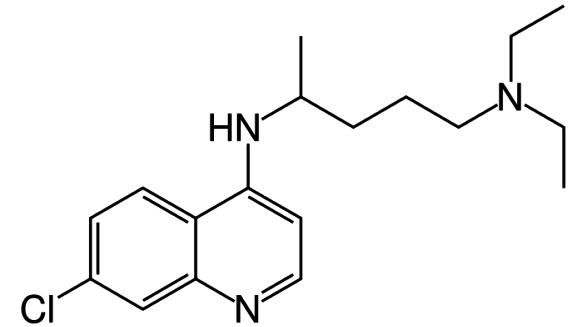
# Chloroquine

- 7-chloro-4-amino quinoline
- Weak base
- Passively diffuses into Pf DV and gets protonated and trapped
- Accumulated CQ
  - Interferes with heme detoxification
  - **Disrupts DV membrane**
- +ve charge on PfCRT K76 prevents CQ<sup>++</sup> exit

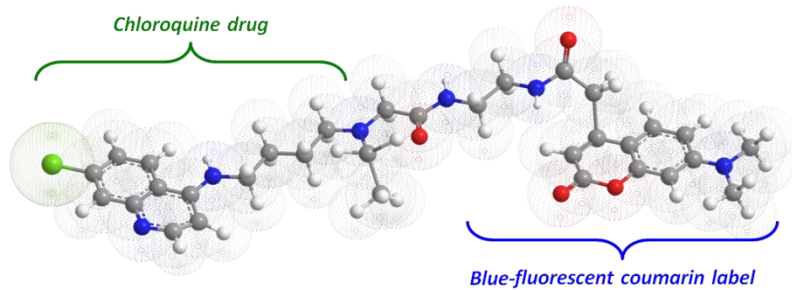


# Chloroquine Resistance

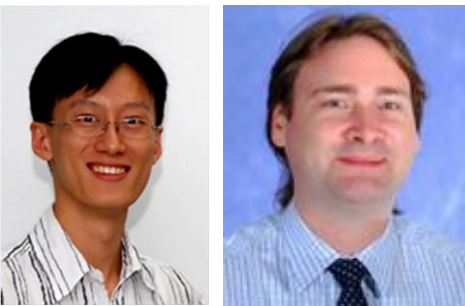
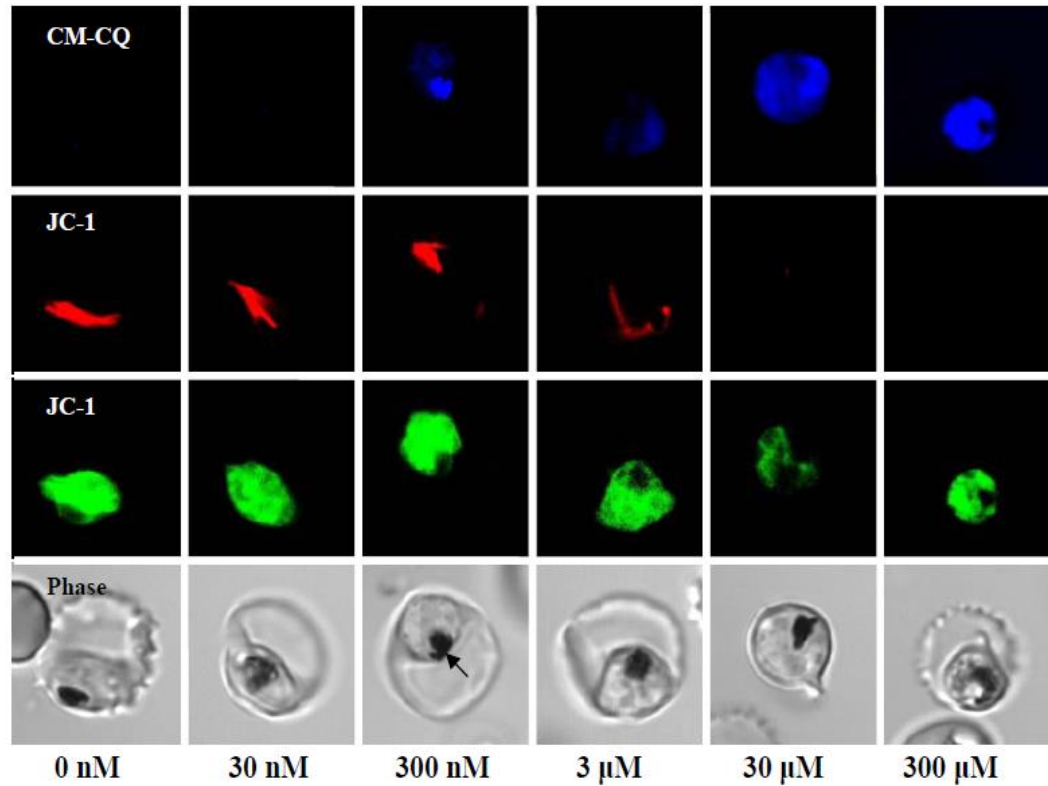
- In CQR strains of Pf
  - K76T mutation removes +ve charge
  - Allows CQ<sup>++</sup> transport out of DV
  - Chemoreversal agents inhibit CQ efflux via interaction with PfCRT



# Fluorescent Antimalarial Illuminates Drug within Parasite

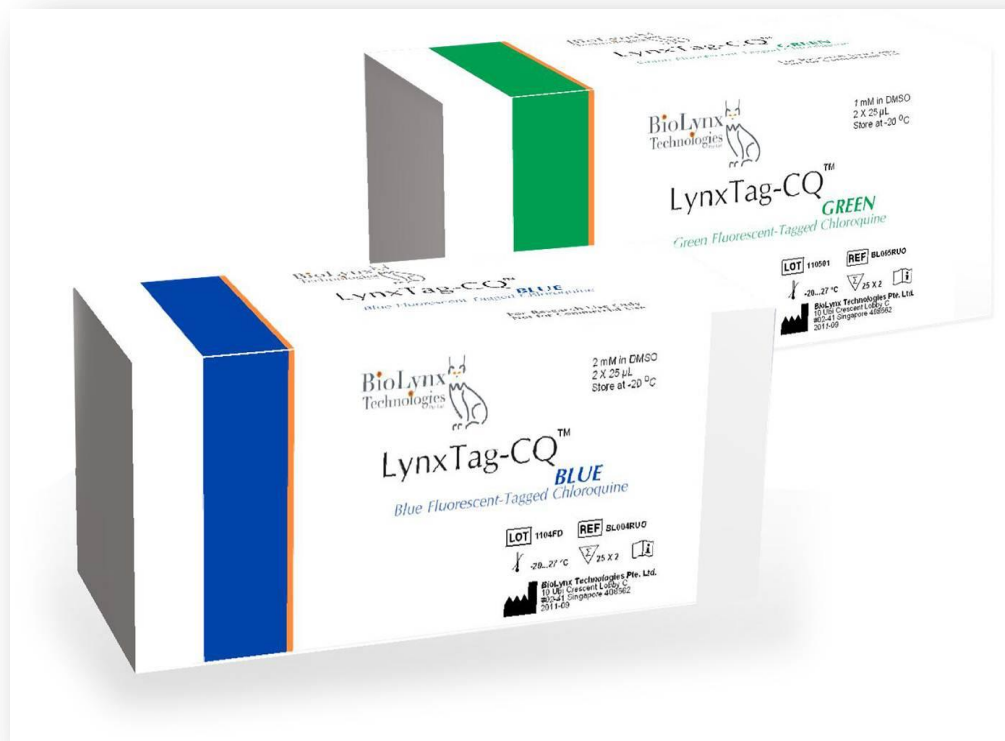


LynxTag-CQ  
BLUE





# LynxTag™ CQ RUO Kit



## Product Catalogue

Product	Catalogue No.	Description	Components	Instrumentation
LynxTag-CQ™ <sub>BLUE</sub>	BL004RUO	a proprietary green fluorophore-tagged chloroquine that allows more effective live cell imaging in critical investigative works for cell biology.	2 mM in DMSO 4 × 25 tests / kit	Confocal Microscope or Flow Cytometer
LynxTag-CQ™ <sub>GREEN</sub>	BL005RUO	a proprietary blue fluorophore-tagged chloroquine for direct visualization and detection of intracellular drug-cell interactions at low micromolar concentrations.	1 mM in DMSO 4 × 25 tests / kit	Confocal Microscope, Flow Cytometer or Plate Reader

# Validation of LynxTag Probes as Biological Tools

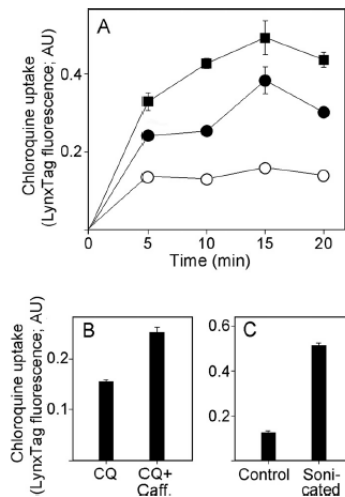


## Cell Wall Perturbation Sensitizes Fungi to the Antimalarial Drug Chloroquine

Farida Islahudin,<sup>a,b</sup> Combiz Khozoie,<sup>a</sup> Steven Bates,<sup>c</sup> Kang-Nee Ting,<sup>d</sup> Richard J. Pleass,<sup>e</sup> Simon V. Avery<sup>a</sup>

School of Biology, University of Nottingham, Nottingham, United Kingdom<sup>a</sup>; School of Pharmacy, University of Nottingham Malaysia Campus, Semenyih, Malaysia<sup>b</sup>; University of Exeter, College of Life & Environmental Sciences, Exeter, United Kingdom<sup>c</sup>; School of Biomedical Sciences, University of Nottingham Malaysia Campus, Semenyih, Malaysia<sup>d</sup>; University of Liverpool, Liverpool School of Tropical Medicine, Liverpool, United Kingdom<sup>e</sup>

AAC (2013) 57 (8): 3889-96



**FIG 6** Cells with perturbed cell walls accumulate larger amounts of chloroquine. (A) Exponential-phase cultures of *S. cerevisiae* BY4743 (○) and isogenic *bck1Δ/bck1Δ* (●) and *slt2Δ/slt2Δ* (■) strains were incubated in the presence of 0.4 mM chloroquine spiked with 20 μM LynxTag-CQ green. The fluorescence of cellular LynxTag-CQ green was determined at intervals by flow cytometry. (B) LynxTag-CQ green uptake was determined in BY4743 cells after incubation for 3 h with 1 mM CQ or 1 mM CQ plus 1 mM caffeine. (C) LynxTag-CQ green uptake was determined in BY4743 cells during incubation with 4 mM CQ, before or after sonication for 1 min. All values are means ± SEM for three independent determinations. AU, arbitrary units.

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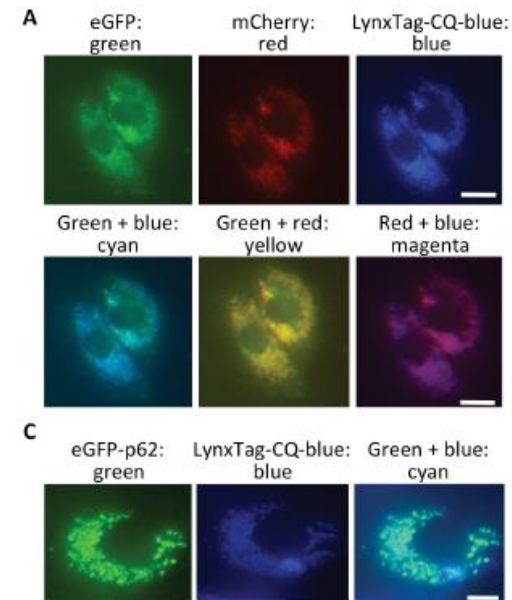


## Novel Quantitative Autophagy Analysis by Organelle Flow Cytometry after Cell Sonication

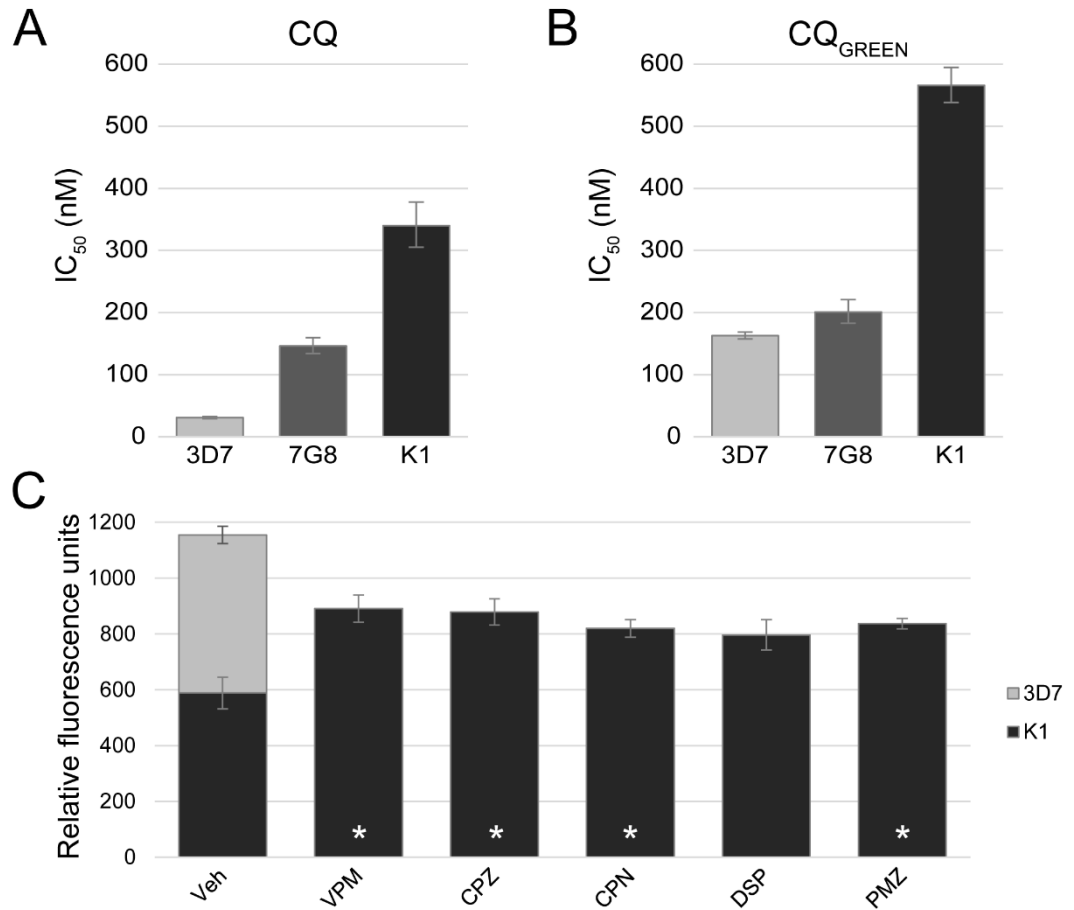
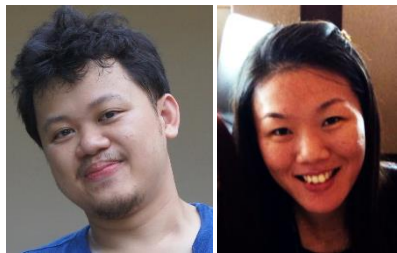
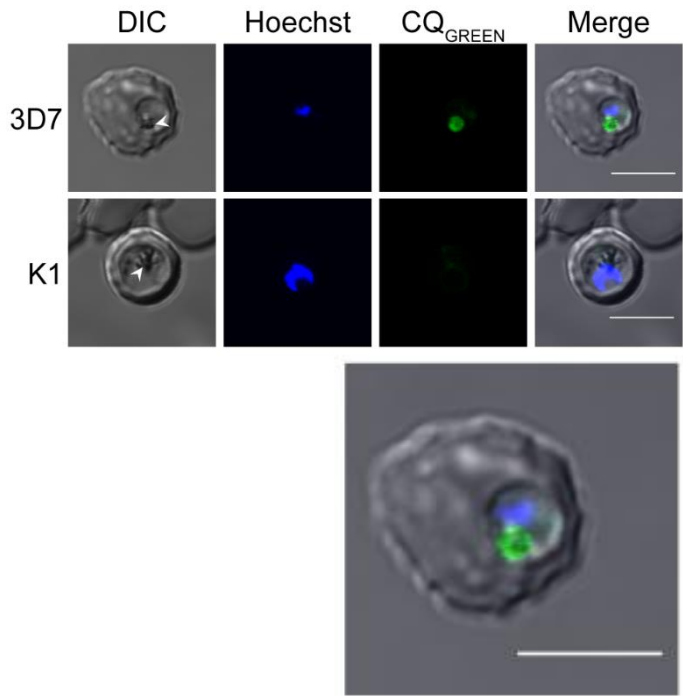
Michael Degtyarev<sup>1</sup>, Mike Reichelt<sup>2</sup>, Kui Lin<sup>1\*</sup>

<sup>1</sup>Department of Translational Oncology, Genentech, South San Francisco, California, United States of America, <sup>2</sup>Department of Pathology, Genentech, South San Francisco, California, United States of America

PLOS ONE (2014) 9 (1): e87707

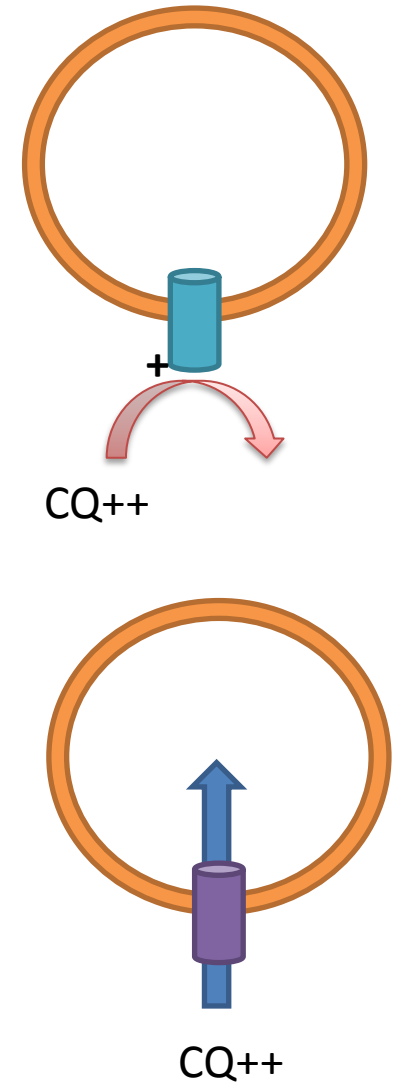
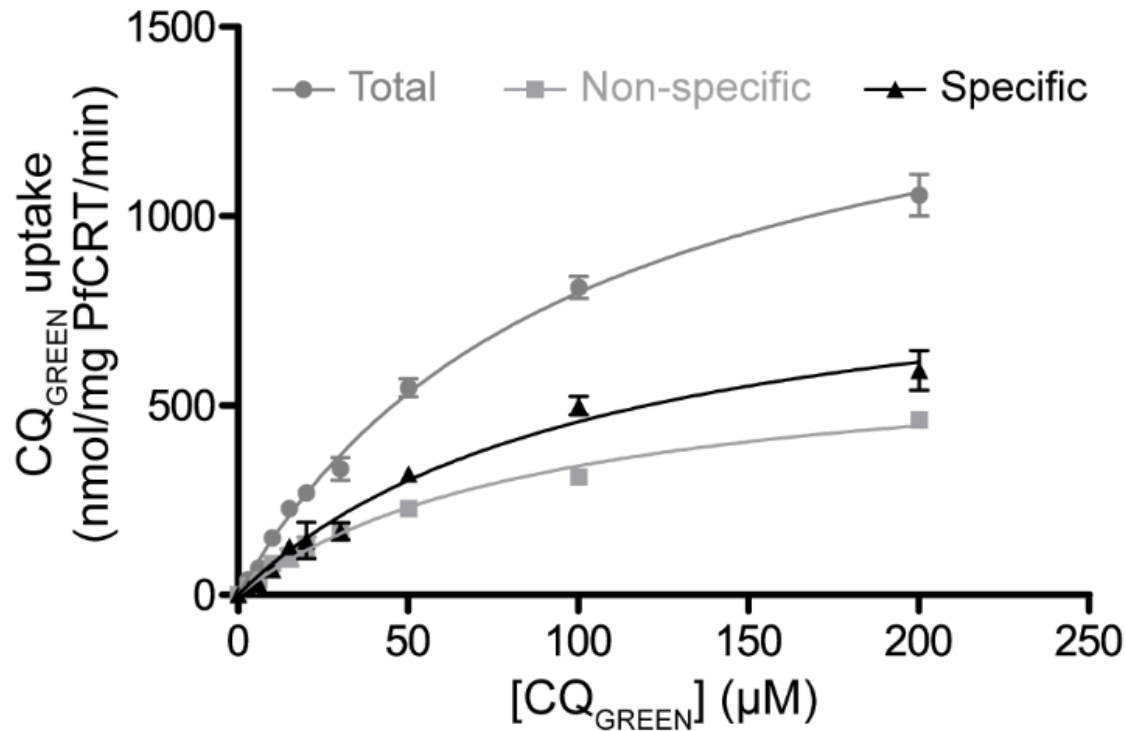


# Validation of LynxTag CQ Probes in Parasites



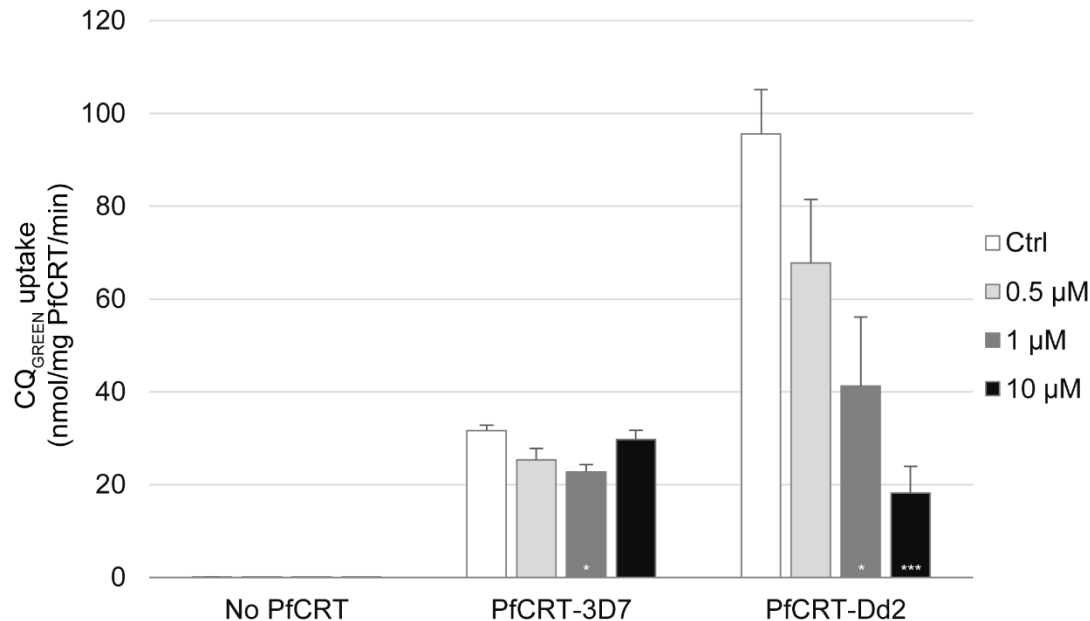
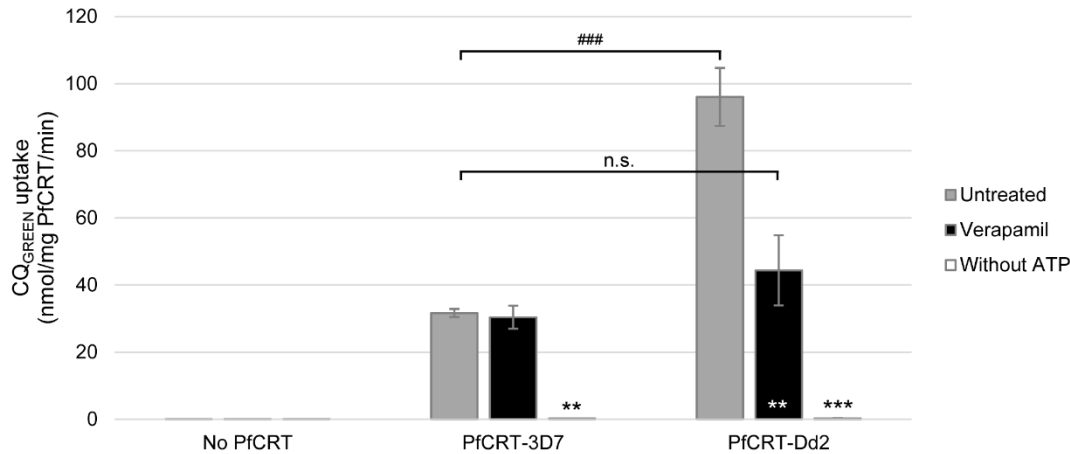
CQ<sub>Green</sub> accumulation in acidic digestive vacuole is decreased in CQR parasites and is reversible with chemoreversal agents *Loh et al (2014) PLOS ONE*

# Validation of LynxTag CQ Probes in PfCRT Microsomes

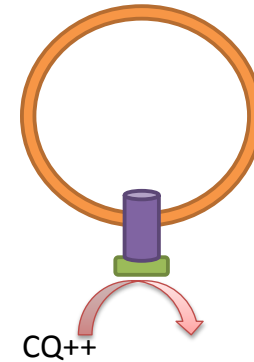


CQ<sub>Green</sub> is transported via mutant PfCRT into microsomes with typical Michaelis-Menten kinetics.  $V_{max} = 938.5$  nmol/mg PfCRT/min;  $K_m = 105.1$  μM

# Validation of LynxTag CQ Probes in PfCRT Microsomes



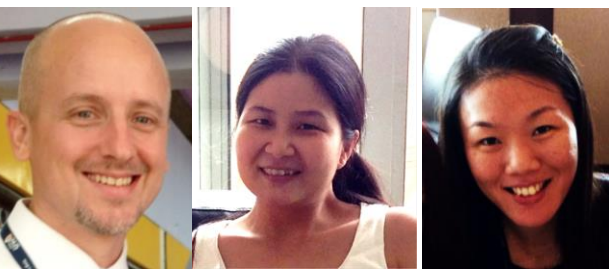
CQ<sub>Green</sub> is transported to greater extent in CQR vs CQS PfCRT and is ATP dependent



CQ<sub>Green</sub> into CQR PfCRT can be inhibited by chemoreversal agent mibefradil

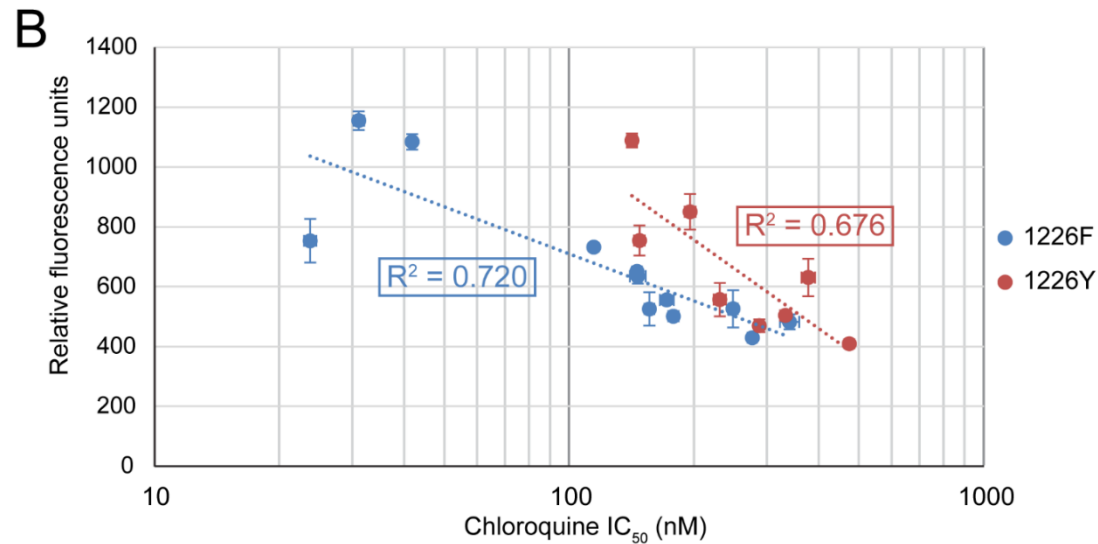
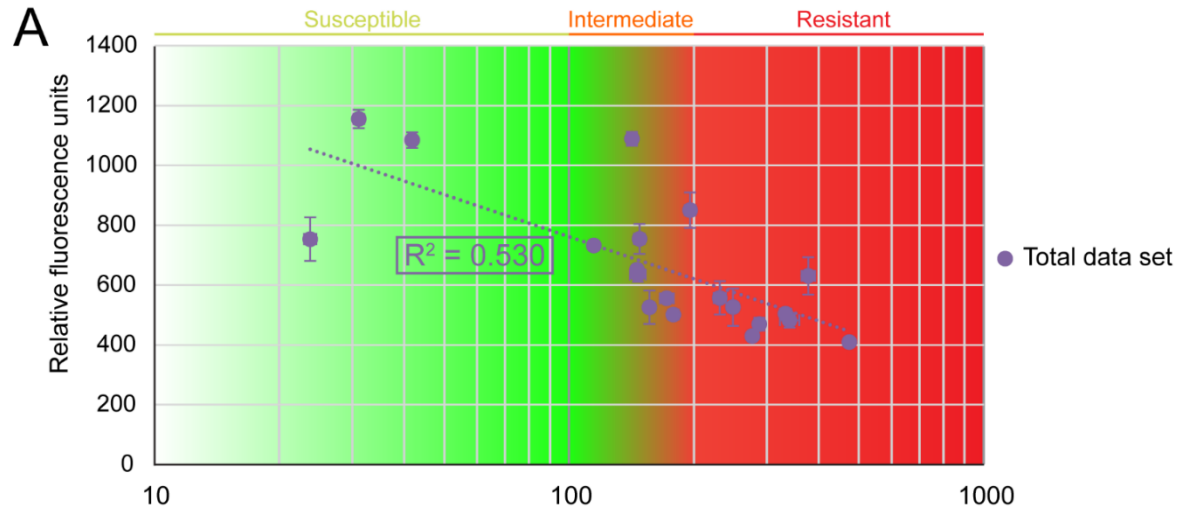
# Validation of LynxTag CQ Probes

Laboratory strains	CQ IC <sub>50</sub> (nM)	PfCRT residue no.									Pgh1 residue no.						pfmdr1 copy number
		72	74	75	76	220	271	326	356	371	86	184	1034	1042	1226	1246	
T9/96	24	C	M	N	K	A	Q	N	I	R	N	Y	S	N	F	D	1
3D7	31	C	M	N	K	A	Q	N	I	R	N	Y	S	N	F	D	1
HB3	42	C	M	N	K	A	Q	N	I	R	N	F	S	D	F	D	1
CS2	115	C	I	E	T	S	E	S	I	I	Y	Y	S	N	F	D	3
T9-94	146	C	I	E	T	S	E	S	I	I	Y	Y	S	N	F	D	3
7G8	146	S	M	N	T	S	Q	D	L	R	N	F	C	D	F	Y	1
Dd2	276	C	I	E	T	S	E	S	T	I	Y	Y	S	N	F	D	3
K1	340	C	I	E	T	S	E	S	I	I	Y	Y	S	N	F	D	1
Clinical isolates	CQ IC <sub>50</sub> (nM)	72	74	75	76	220	271	326	356	371	86	184	1034	1042	1226	1246	pfmdr1 copy number
SMRU0233	142	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	2
SMRU0116	147	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	2
SMRU0101	156	C	I	E	T	S	E	S	T	I	N	Y	S	N	F	D	2
SMRU1116	172	C	I	E	T	S	E	S	T	I	N	Y	S	N	F	D	1
SMRU0270	178	C	I	E	T	S	E	S	T	I	N	Y	S	N	F	D	2
SMRU0402	196	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	2
SMRU1093	231	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	2
SMRU0201	249	C	I	E	T	S	E	S	T	I	N	Y	S	N	F	D	1
SMRU0501	287	C	I	E	T	S	E	S	T	I	N	F	S	N	Y	D	1
SMRU0272	332	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	3
SMRU0002	377	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	1
SMRU0279	473	C	I	E	T	S	E	S	T	I	N	Y	S	N	Y	D	1



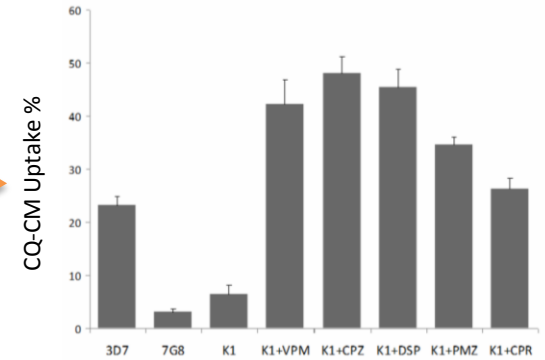
CQ<sub>Green</sub> uptake was measured against IC<sub>50</sub>s across 20 genotyped malaria isolates


# Validation of LynxTag CQ Probes

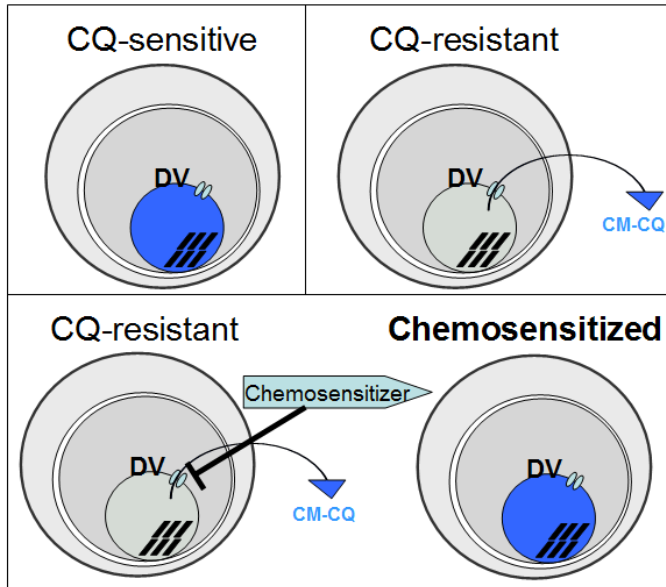


CQ<sub>Green</sub> uptake was moderately correlated with IC<sub>50</sub>s but is associated with F1226Y mutation in Pgh1

# Rapid Identification of Compounds that Reverse Drug Resistance




 LOPAC – Library of 1280 Pharmacologically Active Compounds



SCIENTIFIC REPORTS



 A Whole Cell Pathway Screen Reveals Seven Novel Chemosensitizers to Combat Chloroquine Resistant Malaria

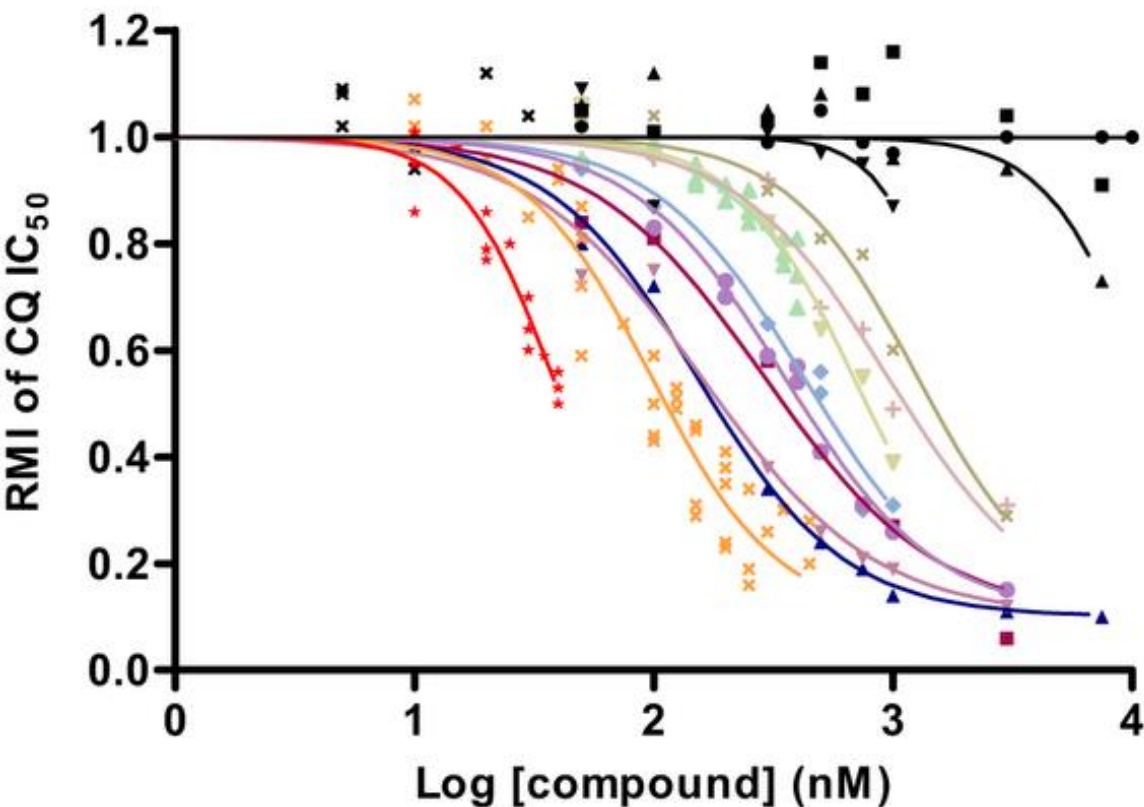
SUBJECT AREAS:  
PHENOTYPIC SCREENING  
PARASITOLOGY  
HIGH-THROUGHPUT SCREENING  
CELL DEATH

Jun-Hong Ch'ng<sup>1</sup>, Sachel Mok<sup>2</sup>, Zbynek Bozdech<sup>2</sup>, Martin James Lear<sup>3</sup>, Aicha Boudhar<sup>1</sup>, Bruce Russell<sup>1</sup>, Francois Nosten<sup>4,5</sup> & Kevin Shyong-Wei Tan<sup>1</sup>



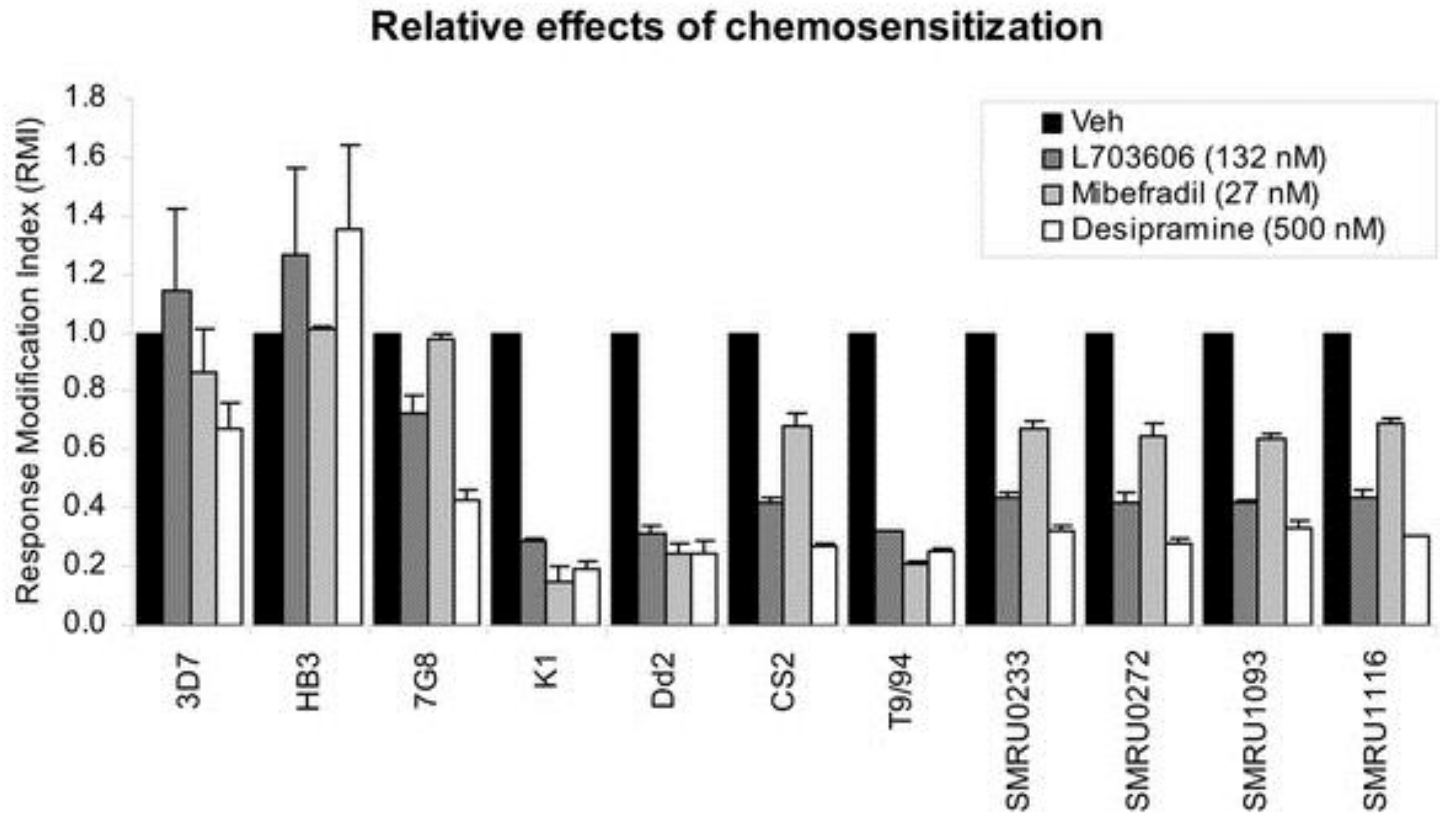
# Validation of Hit's Chemoreversal Properties via EC50

Dose-Dependent Effect of Hits



Compound	EC <sub>50</sub> (nM)
• Veh Ctrl	-
× Cyclosporin A	-
■ Xanthine amine	-
▲ Tamoxifen	>>1000
▼ L765314	>>1000
× Octoclotheptin	1267
+ Methiothepin	953.7
▼ Metergoline	720.1
▲ Loperamide	684.6
◆ Prochlorperazine	422.7
● Chlorprothixene	352.4
■ Verapamil	290.5
▼ Cyproheptidine	158.3
▲ Desipramine	152.1
× L703606	97.99
* Mibefradil	37.92

# Validation of Hit's Chemoreversal Properties on Multiple Strains



# Work Flow for Biological Validation of Hits/ Leads

## Chemoreversal Agent (CRA)

Uptake Assay – CMCQ on K1

Chose > 50%, top 3 for L703 606 and Loperamide series and 2 from Octoclothepein series

IC50 of CRA

Determine non-toxic [ ]

IC50 of CRA of chosen [ ] + CQ

Top hits from each parent series

EC50 on K1

Field isolates

## Hybrid compound

CQ

CRA

IC50

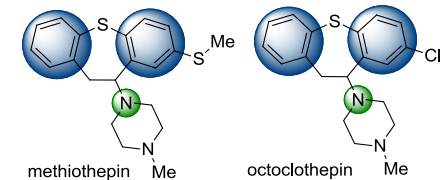
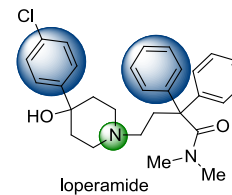
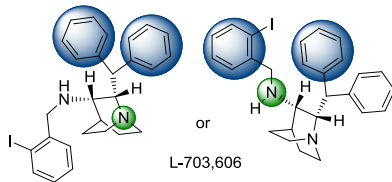
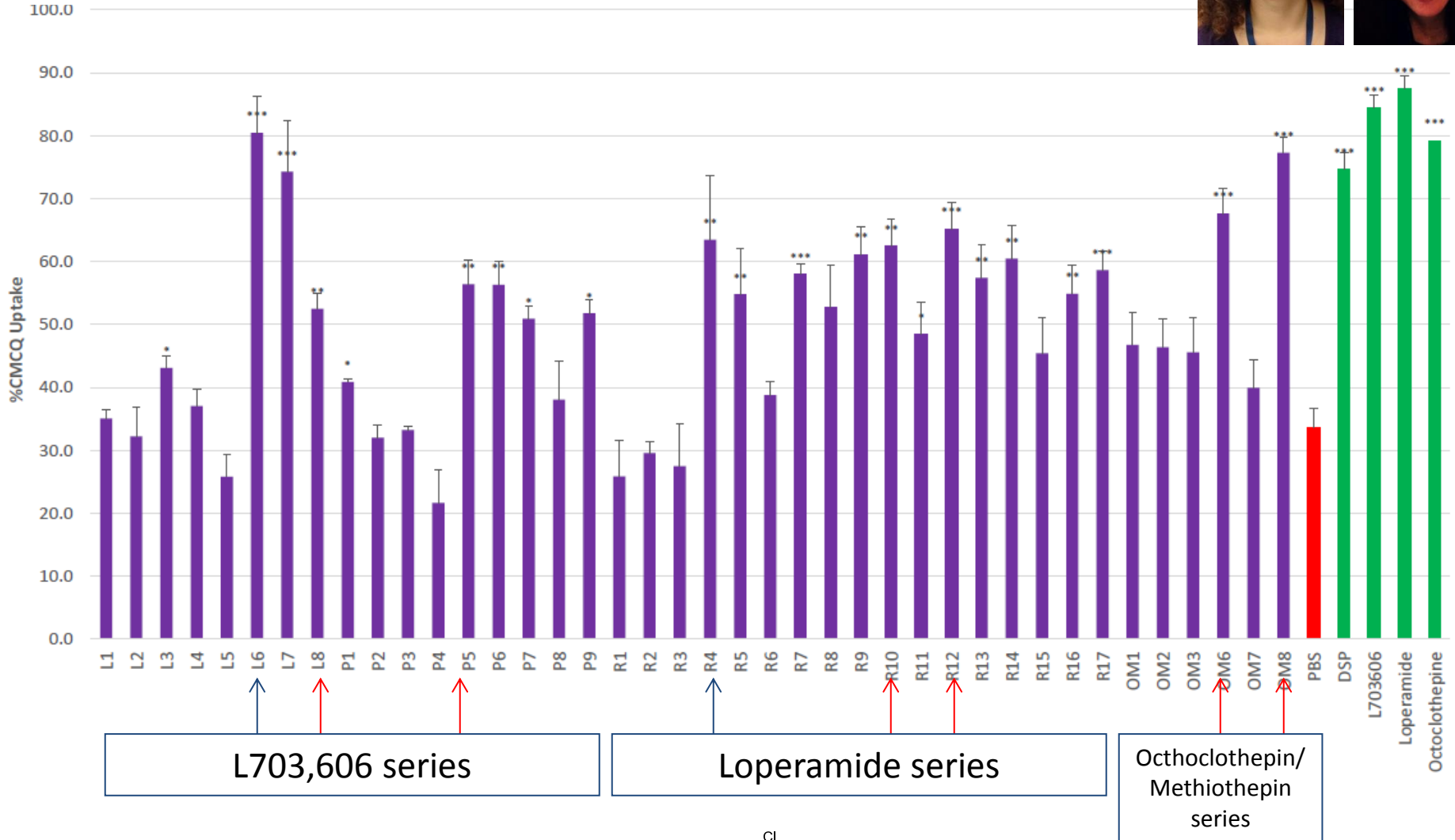
Pick the best representative based on results

CMCQ

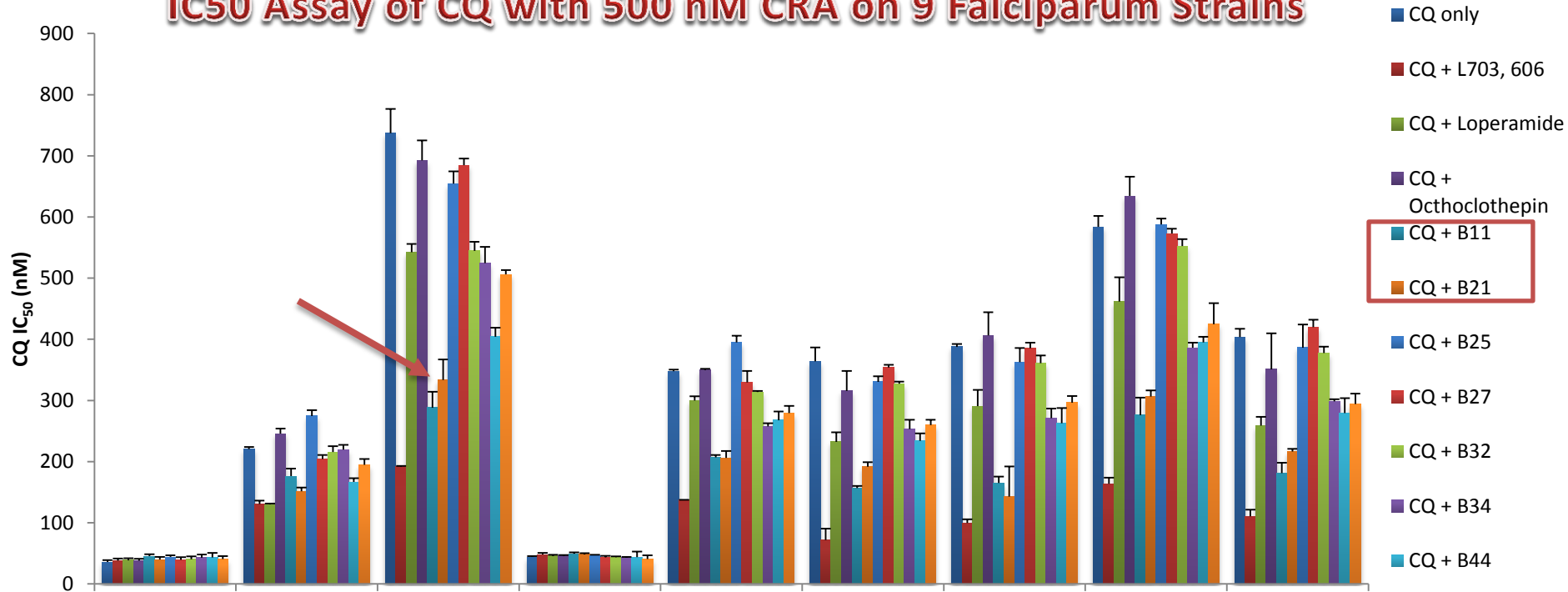
Field isolates



# CQ<sub>BLUE</sub> Uptake with CRA Analogues

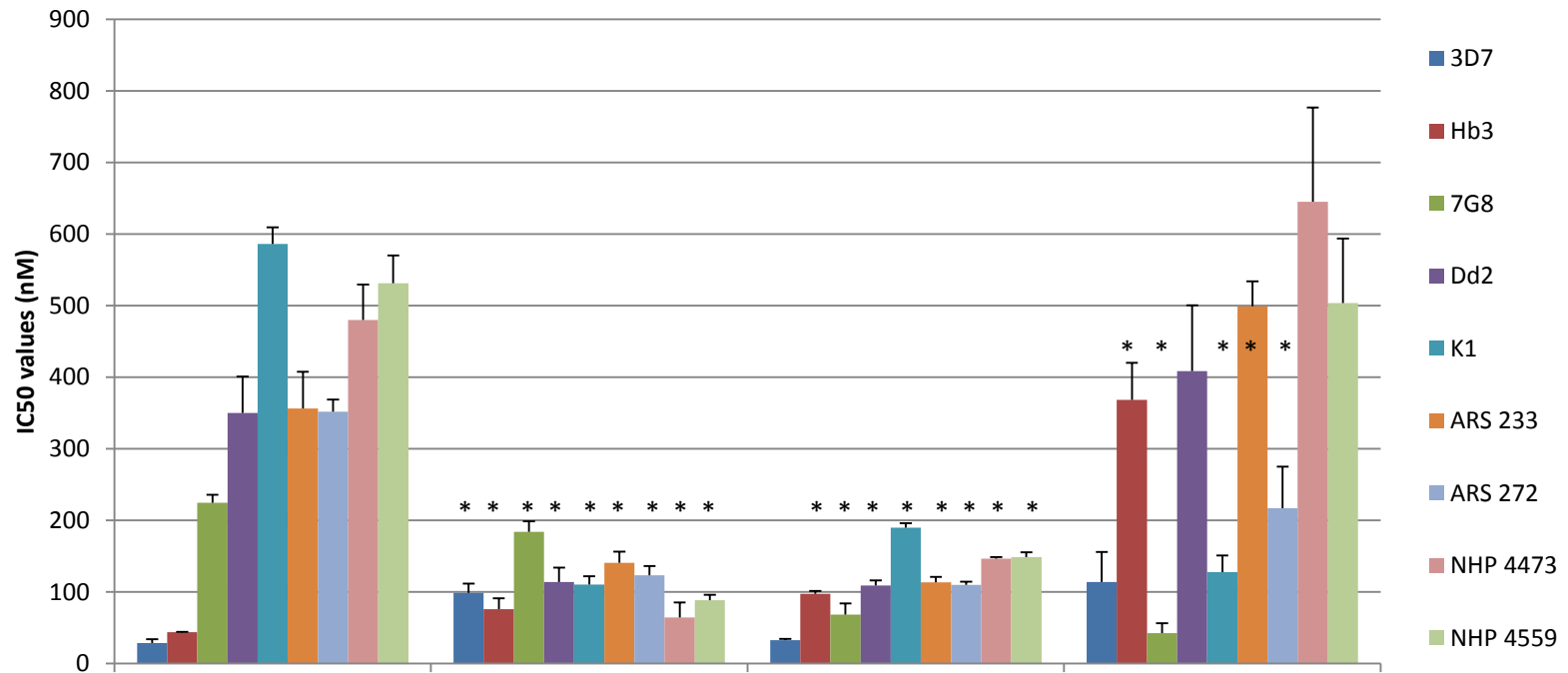


# IC50 Assay of CQ with 500 nM CRA on 9 Falciparum Strains



	3D7	7G8	K1	Hb3	Dd2	ARS233	ARS272	NHP4559	NHP4473
CQ only	35.9	220.3	737.0	44.1	347.9	364.1	387.7	583.9	403.6
CQ + L703	38.2	130.8	191.7	48.1	137.4	71.8	99.8	163.6	110.5
CQ + Lop	38.8	123.0	542.5	45.7	300.1	232.4	290.8	461.6	258.9
CQ + Octh	38.2	245.7	692.6	46.0	349.7	315.4	406.5	634.3	350.9
CQ + B11	45.4	176.3	288.2	49.5	206.9	156.6	165.0	276.0	181.0
CQ + B21	39.7	151.6	333.4	48.3	206.1	191.5	142.7	306.4	216.6
CQ + B25	44.1	274.7	654.7	46.8	395.1	330.5	362.6	587.8	386.4
CQ + B27	39.3	204.2	684.6	43.6	329.7	353.8	385.2	573.0	419.9
CQ + B32	40.5	215.5	544.7	43.6	314.0	327.6	361.1	552.3	377.5
CQ + B34	43.0	219.6	524.4	43.3	257.4	253.3	271.3	386.3	298.5
CQ + B44	43.5	166.6	404.2	44.0	267.8	234.0	262.9	395.2	279.5
CQ + B46	40.8	194.8	506.3	40.8	279.2	259.8	296.4	424.8	294.3

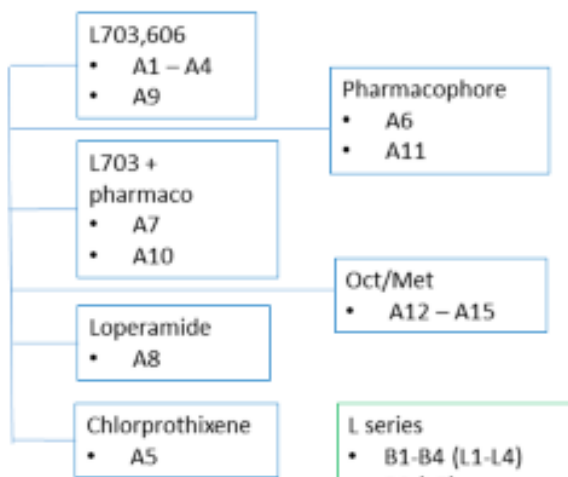
# IC50 Assay of Selected CQ-CRA Hybrid Compounds on 9 Falciparum Strains



	CQ only	A1	A9	A15
3D7	28.7	98.61	32.41333	113.6433
Hb3	43.59	75.94	97.29	368.2333
7G8	224.6667	183.8	68.19333	42.56
Dd2	350	113.767	108.7533	408.4
K1	586.0667	110.247	189.5333	127.4733
ARS 233	356.1667	140.625	113.3667	499.1667
ARS 272	351.7667	123.167	110	216.7333
NHP 4473	479.7333	64.42	146.5	644.7667
NHP 4559	531.3667	88.275	148.5333	503.3333



**Group A (Hybrids)**



Group A cmpds

IC50 reinvasion assay on K1 for all cmpds

Chosen 3 out of 15 cmpds based on their IC50 values on K1

- ✓ A1
- ✓ A9
- ✓ A15

Group B cmpds

CMCQ on K1 for all cmpds

Toxicity test on :

- B11
- B25
- B30-B50

\*to find non-tox [ ]

Chose the best :

- ✓ 3 from L703 (B11, B21, B27)
- ✓ 3 from Lop (B25, B32, B34)
- ✓ 2 from Oct/Met (B44 & B46)

IC50 reinvasion on all the strains CQ + CRA [ ]

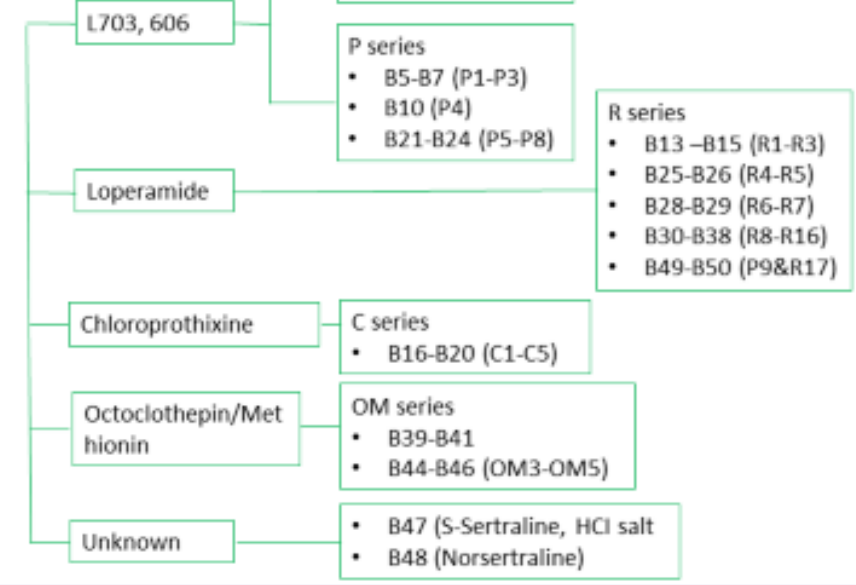
- ✓ on the chosen compounds

Top 1 hit from each parent series

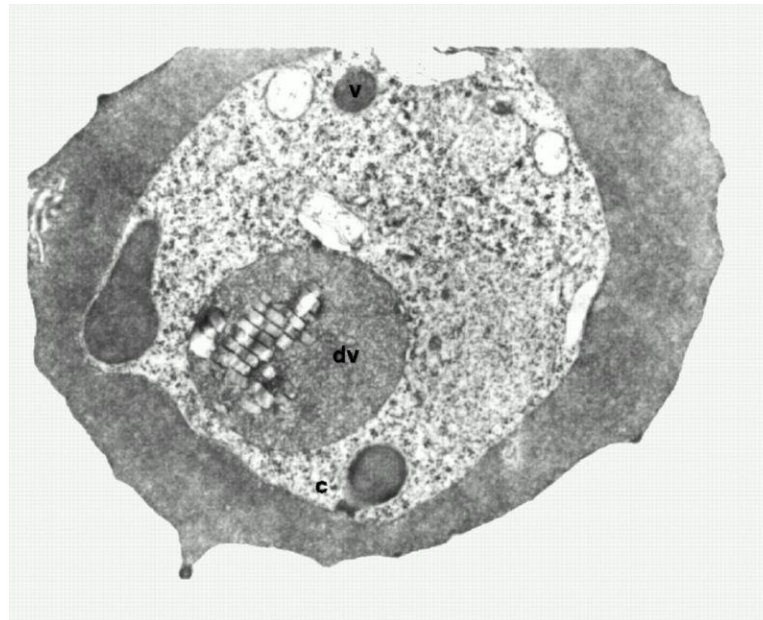
- ✓ B11, B21, B25, B34, B44

For EC50 on K1

**Group B (CRA)**



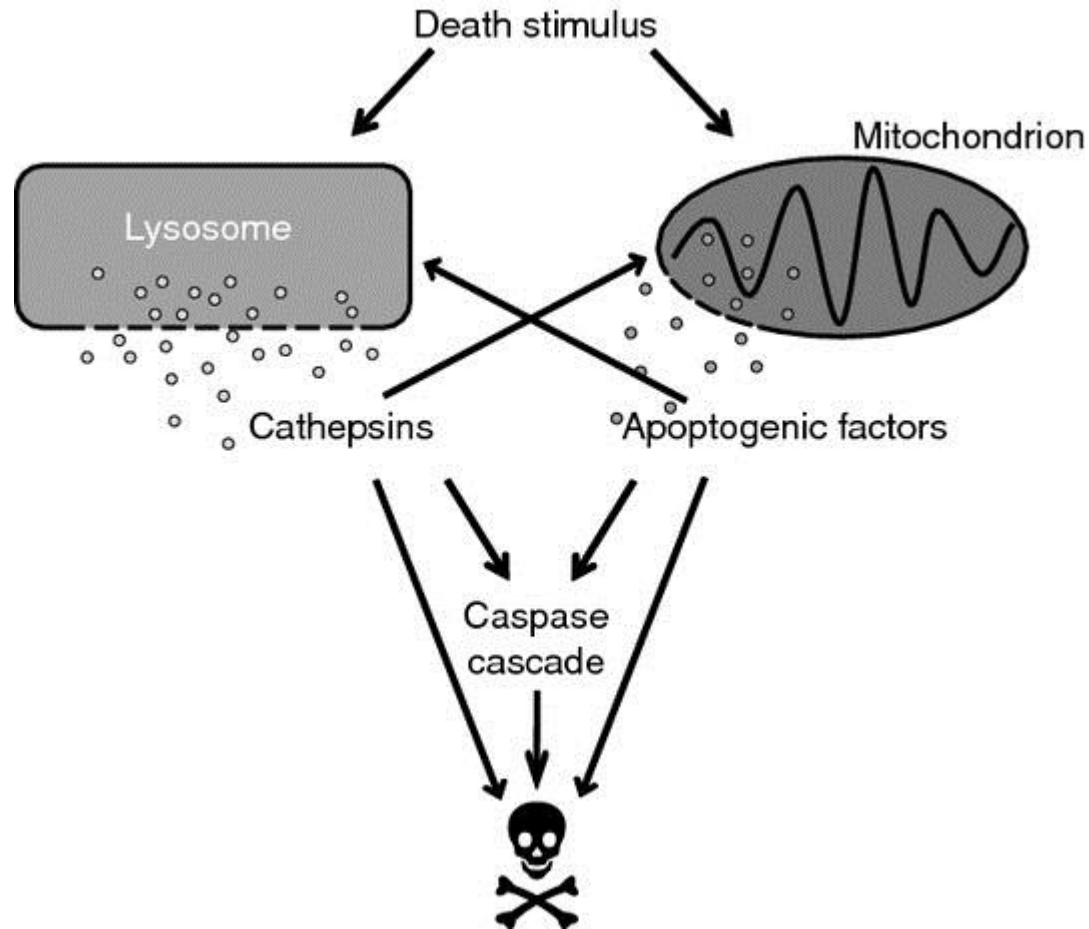
# Part 2: Novel Chloroquine Death Mechanism *In Vitro* and *In Vivo*



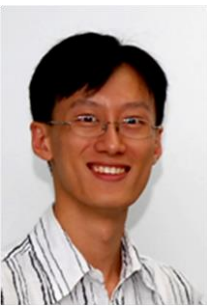
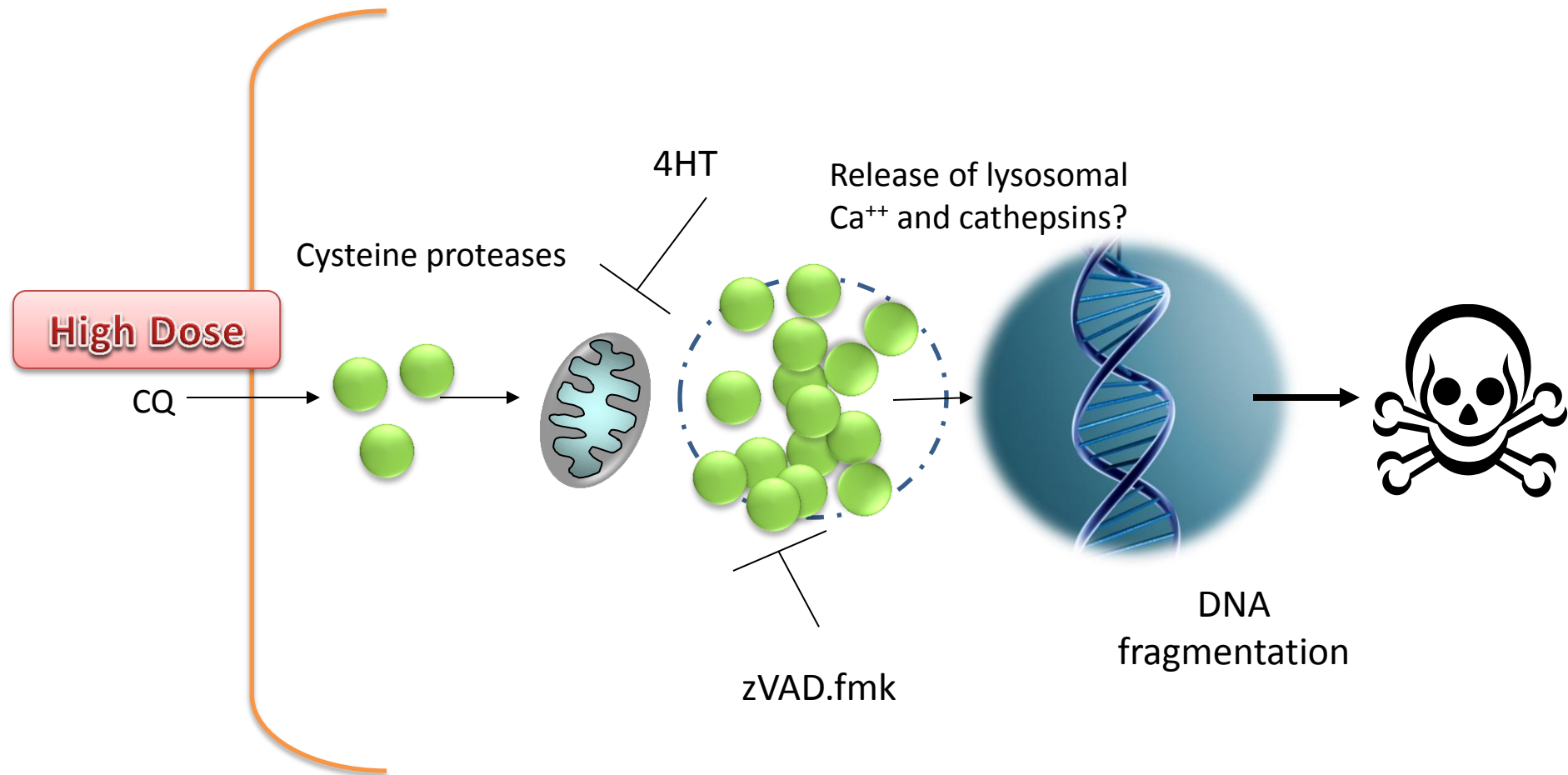
*Francis et al. (1997) Annual Rev Microbiol*



# Lysosomal Mediated PCD



# Model for Lysosomal-Mediated PCD in *Plasmodium*



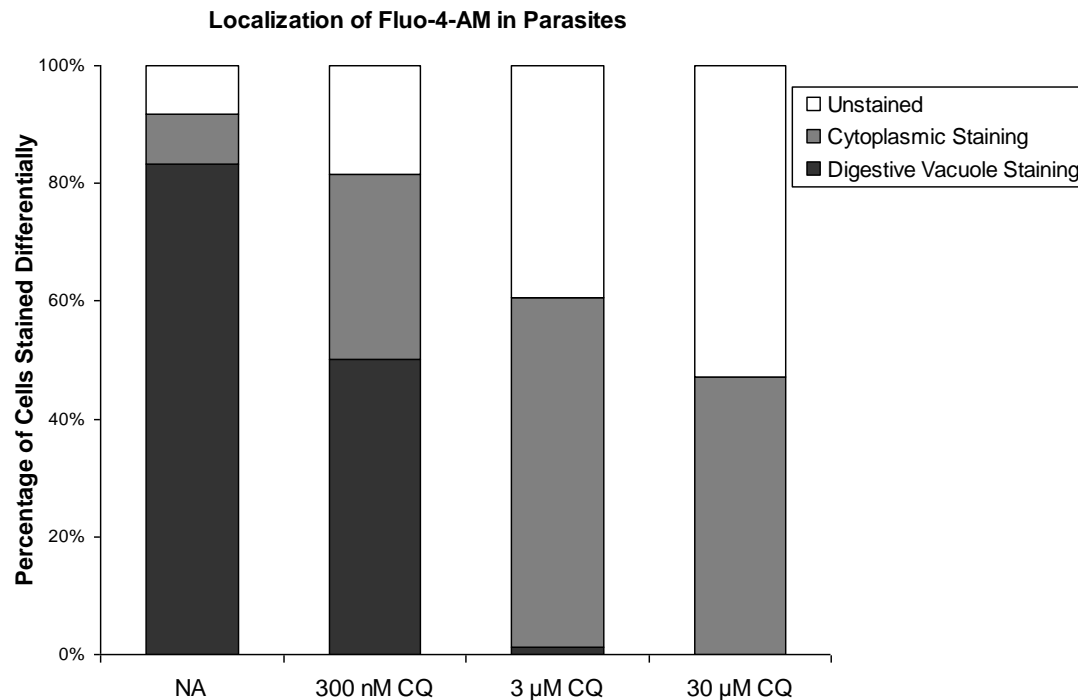
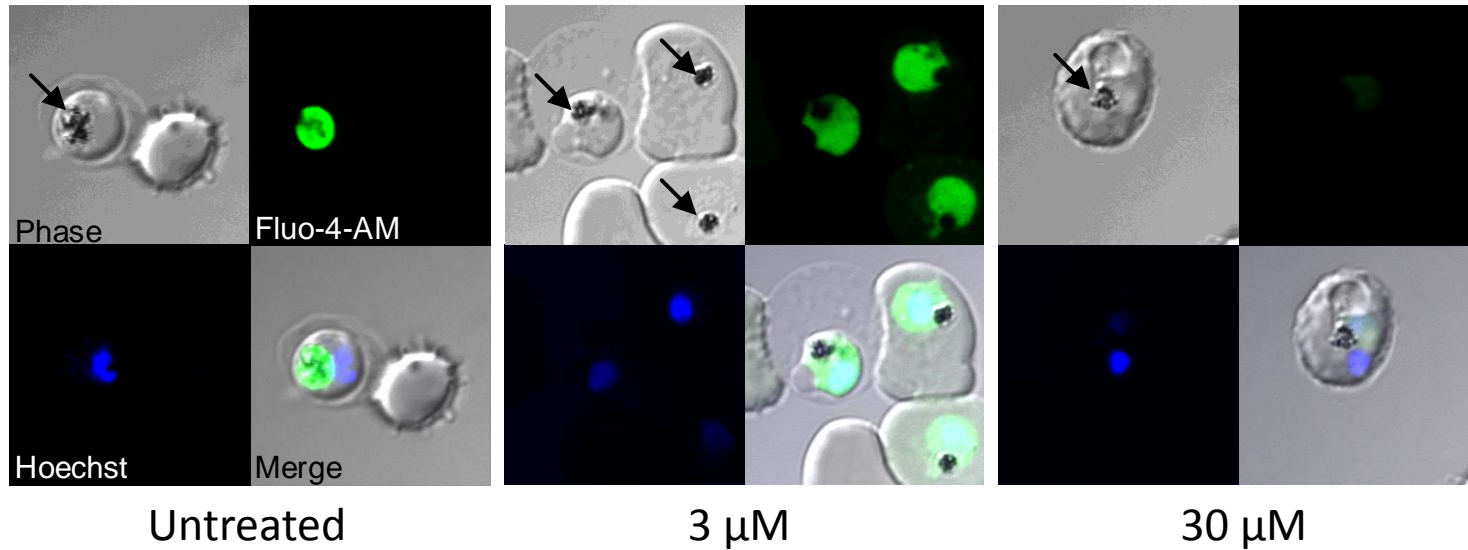
Citation: *Cell Death and Disease* (2011) 2, e216; doi:10.1038/cddis.2011.97  
© 2011 Macmillan Publishers Limited All rights reserved 2041-4889/11  
www.nature.com/cddis

**Drug-induced permeabilization of parasite's digestive vacuole is a key trigger of programmed cell death in *Plasmodium falciparum***

J-H Ch'ng<sup>1</sup>, K Liew<sup>2</sup>, AS-P Goh<sup>1</sup>, E Sidhartha<sup>1</sup> and KS-W Tan<sup>1,2,3</sup>

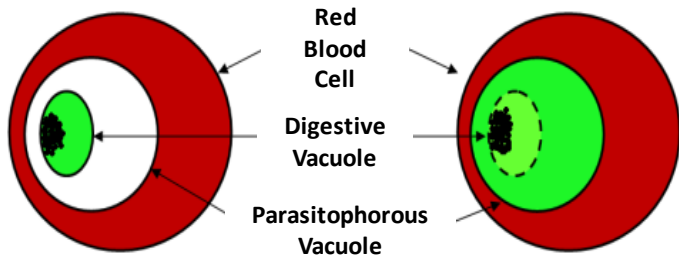


# Fluo-4-AM Localization Studies



**Concentration-dependent Relocalization of DV marker Fluo-4-AM supports DV breach model**

# Parasite Digestive Vacuole (DV) Fluorescent Reporter to Detect Drug-Mediated DV Disruption



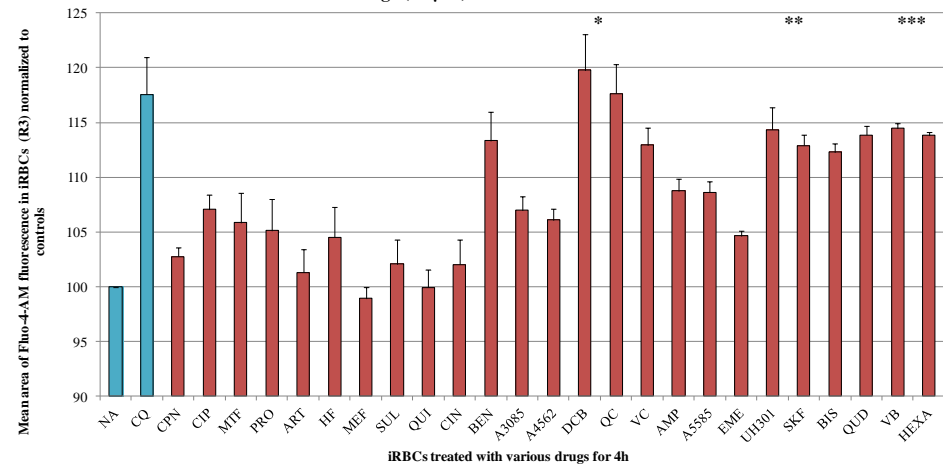
DV-Green redistribution and resulting increase in area of fluorescence upon DV permeabilization



Phenotypic screen using AMNIS Imagestream



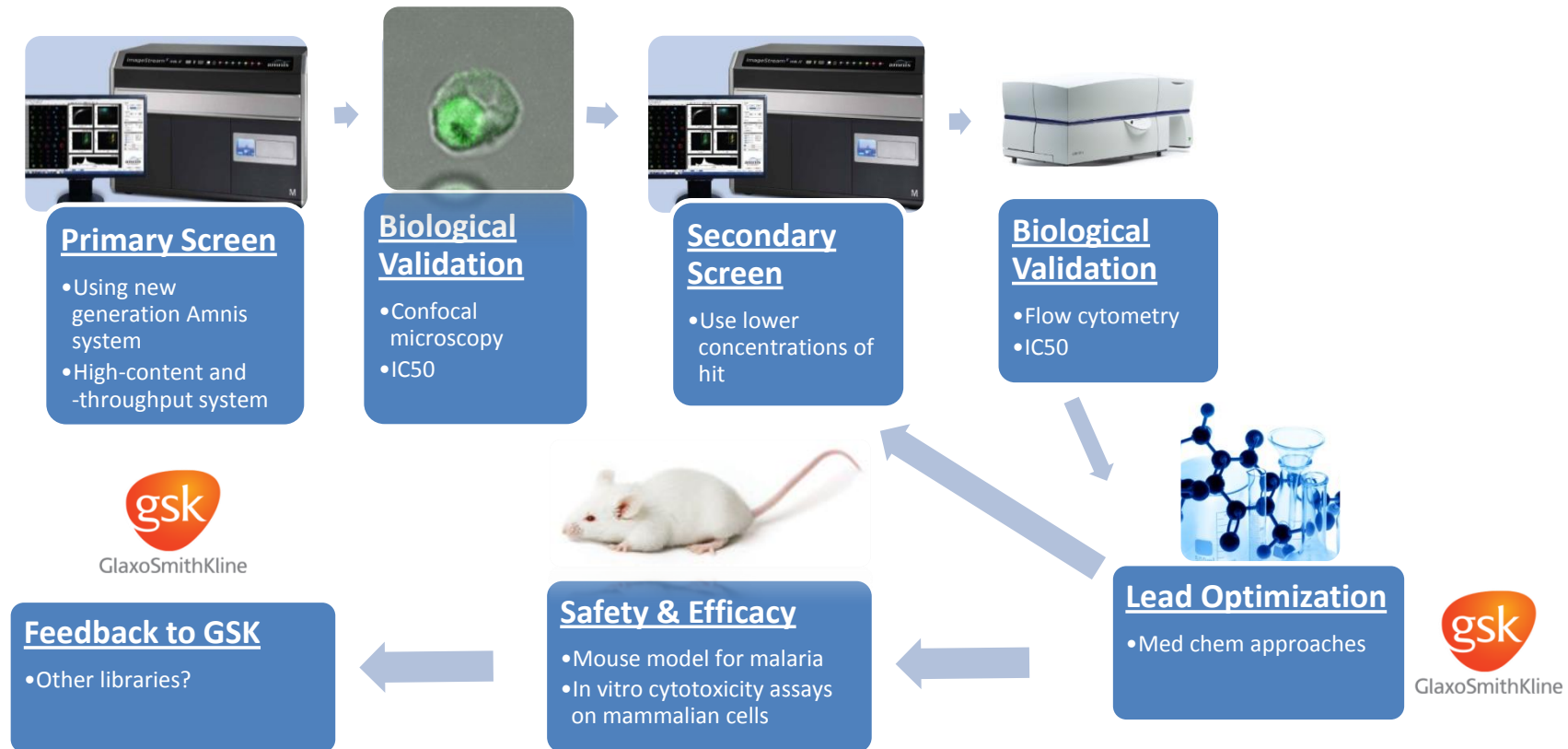
Effects of Drugs (10  $\mu$ M) on DV Permeabilization in 3D7 Parasites



A High-Content Phenotypic Screen Reveals the Disruptive Potency of Quinacrine and 3',4'-Dichlorobenzamil on the Digestive Vacuole of *Plasmodium falciparum*

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# Work Flow – GSK 5,000 Antimalarial Compound Library



Deliverable: Data on DV-disruption as a mechanism for antimalarial property + new leads

Project Duration: 6 months (pilot)

Results: 4,440 compounds → 244 hits (5.5%) → 25 high potency (0.6%)

# Can We Teach an Old Drug New Tricks?

New Mode of Action

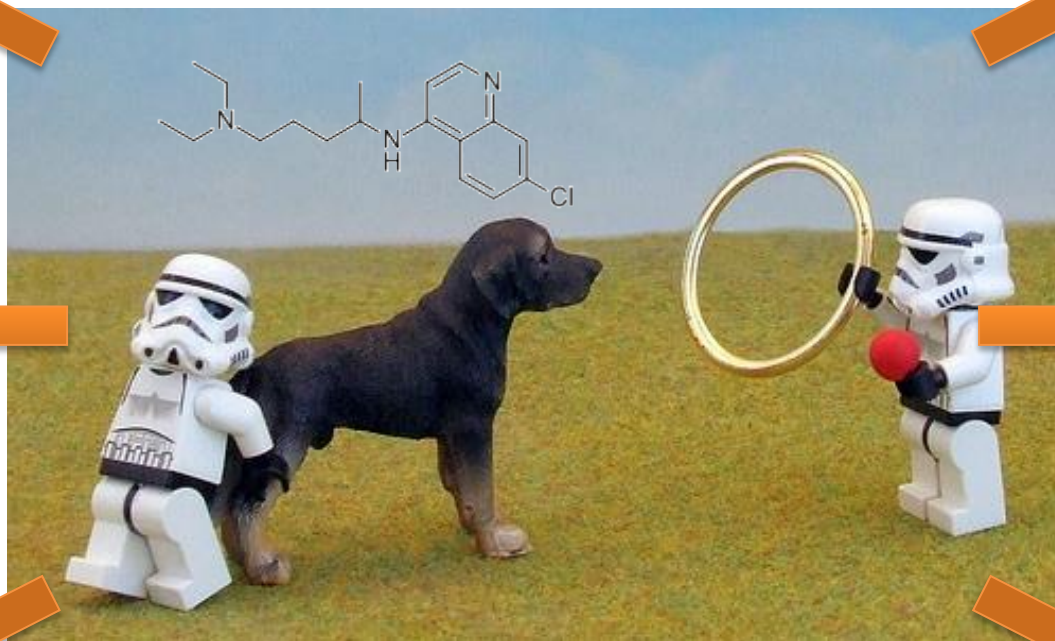
Fluorescent Probe

DV Screens

New Reversal Agents

Industry Collaboration

Hybrid Compounds



# Acknowledgements



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