## Supplementary materials of Rapid and quantitative antimalarial

## drug efficacy testing via the magneto-optical detection of hemozoin

Petra Molnár<sup>1,2,4\*</sup>, Ágnes Orbán<sup>1,2</sup>, Richard Izrael<sup>1,3,4</sup>, Réka Babai<sup>4</sup>, Lívia Marton<sup>1</sup>, Ádám

Butykai<sup>2</sup>, Stephan Karl<sup>5,6</sup>, Beáta G. Vértessy<sup>1,4\*</sup>, István Kézsmárki<sup>1,2,7\*</sup>

<sup>1</sup>Malaria Research Laboratory, Institute of Enzymology, Research Centre for Natural Sciences,

1117 Budapest, Hungary;

<sup>2</sup> Department of Physics, BME Budapest University of Technology and Economics, 1111

Budapest, Hungary;

<sup>3</sup> Doctoral School of Multidisciplinary Medical Sciences, University of Szeged, 6720 Szeged, Hungary;

<sup>4</sup> Department of Applied Biotechnology and Food Sciences, BME Budapest University of Technology and Economics, 1111 Budapest, Hungary;

<sup>5</sup> Australian Institute of Tropical Health and Medicine, James Cook University, 1/14-88

McGregor Road, Smithfield QLD 4870 Australia

<sup>6</sup> Vector-borne Diseases Unit, PNG Institute of Medical Research, P.O. Box 378, Madang 511,

Madang Province, Papua New Guinea

<sup>7</sup> Experimental Physics 5, Center for Electronic Correlations and Magnetism, Institute of

Physics, University of Augsburg, 86159 Augsburg, Germany;

\*corresponding authors, e-mail addresses: <u>molnar.petra@ttk.mta.hu</u>; <u>vertessy@mail.bme.hu</u>;

istvan.kezsmarki@physik.uni-augsburg.de



**Supplementary Figure S1. - An intracrythrocytic parasite maturation map.** The map follows the maturation of *P. falciparum* 3D7 parasites in laboratory cultures[1]–[5]. For details, see Materials and Methods Section.

## Supplementary Table S1. – IC50 values for chloroquine (CQ), piperaquine (PPQ) and dihydroartemisinin (DHA) obtained by different

methods.	Parasitemia	values of t	the assays	are also	included,	where applicable.
----------	-------------	-------------	------------	----------	-----------	-------------------

		RMOD	[3H]-Hypoxanthine incorporation	SYBR Green	HRP2 ELISA	pLDH DELI
IC50	CQ:	0.5%:29.15 ±2.71 nM 1%: 34.68 ±5.28 nM	0.5%: 6,5 nM [6] 0.5%: 15,7 nM [7] 0.5%: 29,6 nM [8] 0.5%: 9,73 nM [9]	0.5%: 8,1 nM [10] 1%: 15,11 nM [11] 0.5%: 16,8 nM [12] 0.5%: 11,54 nM [13]	0.5%: 7,5 nM [10] 0.5%: 21,91 nM [12] 0.5%: 9,68 nM [13] 0.5%: 22 nM [14] 1%: 18 nM [15]	0,5%: 15,0 nM [13] 1%: 15 nM [15] 0,5%: 40,5 nM [16] 15,7 nM [17]
	PPQ:	0.1%: 30.61±4.01 nM 1%: 72±1.59 nM	0.5%: 27 nM [6] 0.5%:16,9 nM [8] 1%: 36,9 nM [18]	1%: 14,88 nM [11] 0.5%: 18,5 nM [12] 46,3 nM [19]	0.5%: 21,0 nM [12] 1%: 29 nM [15]	1%: 30 nM [15] 0,1%: 15,1 nM [20]
	DHA:	1%: 2.13±0.87 nM	0.5%: 2,0 nM [6] 0.5%: 5,27 nM [7] 0.5%: 4,2 nM [8]	1%: 2,11 nM [11] 0.5%: 2,97 nM [12]	0.5%: 6,00 nM [12] 1%: 6 nM [15]	1%: 6,3 nM [15] 0,1%: 2,6 nM [20]

## **Supplementary References**

- Kozicki, M. *et al.* The ring-stage of Plasmodium falciparum observed in RBCs of hospitalized malaria patients. *Analyst* 140, 8007–8016 (2015).
- 2. Moura, P. A., Dame, J. B. & Fidock, D. A. Role of Plasmodium falciparum digestive vacuole plasmepsins in the specificity and antimalarial mode of action of cysteine and aspartic protease inhibitors. *Antimicrob. Agents Chemother.* **53**, 4968–4978 (2009).
- 3. Grüring, C. *et al.* Development and host cell modifications of Plasmodium falciparum blood stages in four dimensions. (2011) doi:10.1038/ncomms1169.
- 4. Silamut, K. *et al.* A quantitative analysis of the microvascular sequestration of malaria parasites in the human brain. *Am. J. Pathol.* **155**, 395–410 (1999).
- White, N. J. Qinghaosu (artemisinin): The price of success. *Science* vol. 320 330–334 (2008).
- Mwai, L. *et al.* In vitro activities of piperaquine, lumefantrine, and dihydroartemisinin in Kenyan plasmodium falciparum isolates and polymorphisms in pfcrt and pfmdr1. *Antimicrob. Agents Chemother.* 53, 5069–5073 (2009).
- Duraisingh, M. T., Roper, C., Walliker, D. & Warhurst, D. C. Increased sensitivity to the antimalarials mefloquine and artemisinin is conferred by mutations in the pfmdr1 gene of Plasmodium falciparum. *Mol. Microbiol.* 36, 955–961 (2000).
- 8. Wong, R. P. M. *et al.* Desbutyl-lumefantrine is a metabolite of lumefantrine with potent in vitro antimalarial activity that may influence artemether-lumefantrine treatment outcome. *Antimicrob. Agents Chemother.* **55**, 1194–1198 (2011).
- 9. Vivas, L. *et al.* Antimalarial efficacy and drug interactions of the novel semi-synthetic endoperoxide artemisone in vitro and in vivo. *J. Antimicrob. Chemother.* **59**, 658–665

(2007).

- Bacon, D. J. *et al.* Comparison of a SYBR green I-based assay with a histidine-rich protein II enzyme-linked immunosorbent assay for in vitro antimalarial drug efficacy testing and application to clinical isolates. *Antimicrob. Agents Chemother.* 51, 1172–1178 (2007).
- Pooja, A., A. R., A., C. R., P. & Kumkum, S. In vitro susceptibility of Indian Plasmodium falciparum isolates to different antimalarial drugs & antibiotics. *Indian Journal of Medical Research* 622–628 (2017) doi:10.4103/ijmr.IJMR\_1688\_15.
- Chaorattanakawee, S. *et al.* Direct comparison of the histidine-rich protein-2 enzymelinked immunosorbent assay (HRP-2 ELISA) and malaria SYBR green i fluorescence (MSF) drug sensitivity tests in Plasmodium falciparum reference clones and fresh ex vivo field isolates from Cambodia. *Malar. J.* 12, 1–11 (2013).
- 13. Wein, S. *et al.* Reliability of antimalarial sensitivity tests depends on drug mechanisms of action. *J. Clin. Microbiol.* **48**, 1651–1660 (2010).
- Rebelo, M. *et al.* A Novel Flow Cytometric Hemozoin Detection Assay for Real-Time Sensitivity Testing of Plasmodium falciparum. *PLoS One* 8, (2013).
- 15. Chaorattanakawee, S. *et al.* Measuring ex vivo drug susceptibility in Plasmodium vivax isolates from Cambodia. *Malar. J.* **16**, 1–13 (2017).
- Kaddouri, H., Nakache, S., Houzé, S., Mentré, F. & Le Bras, J. Assessment of the drug susceptibility of Plasmodium falciparum clinical isolates from Africa by using a Plasmodium lactate dehydrogenase immunodetection assay and an inhibitory maximum effect model for precise measurement of the 50-percent inhibitory conc. *Antimicrob. Agents Chemother.* 50, 3343–3349 (2006).

- 17. Druilhe, P., Moreno, A., Blanc, C., Brasseur, P. H. & Jacquier, P. A colorimetric in vitro drug sensitivity assay for Plasmodium falciparum based on a highly sensitive double-site lactate dehydrogenase antigen-capture enzyme-linked immunosorbent assay. Am. J. Trop. Med. Hyg vol. 64 (2001).
- Fivelman, Q. L., Adagu, I. S. & Warhurst, D. C. Effects of Piperaquine, Chloroquine, and Amodiaquine on Drug Uptake and of These in Combination with Dihydroartemisinin against Drug-Sensitive and-Resistant Plasmodium falciparum Strains. *Antimicrob. Agents Chemother.* **51**, 2265–2267 (2007).
- Murithi, J. M. *et al.* Combining Stage Specificity and Metabolomic Profiling to Advance Antimalarial Drug Discovery. *Cell Chem. Biol.* 27, 158-171.e3 (2020).
- Woodrow, C. J. *et al.* Comparison between flow cytometry, microscopy, and lactate dehydrogenase-based enzyme-linked immunosorbent assay for plasmodium falciparum drug susceptibility testing under field conditions. *J. Clin. Microbiol.* 53, 3296–3303 (2015).