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Drug resistances of the malaria parasite *Plasmodium falciparum* monitored with the fluorescent substrate Fluo-4 of the multi-drug resistance transporter PfMDR1

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Malaria tropica is contracted via *Plasmodium falciparum* and represents the most severe infection of malaria worldwide. Treatment options have become complicated within the last decade through spontaneous mutations in the genome of the parasite involving genes encoding for drug resistance transporters. The digestive vacuole (DV) within the malaria parasite represents an organelle where the host hemoglobin of the infected red blood cell (iRBC) is digested and biomineralized to inert hemozoin, a biocrystal inert to the parasite. Several antimalarial drugs have been suggested to inhibit this biomineralization, resulting in heme accumulation to levels that are toxic to the parasite. However, mutations in membrane transporters localized to the DV have resulted in drug efflux activity, thus protecting the parasite. One of the crucial transporters, the *Plasmodium falciparum* multi-drug resistance transporter type 1 (PfMDR1), is a *P. falciparum* analog of ABC-transporters found in many cells, cancer cells for instance. Although this transporter is well characterized regarding gene sequence and point mutations, its biophysical properties and transport kinetics is largely unknown, in particular in resistant mutation-carrying strains of *Plasmodium*. This is largely due to limitations in isolating PfMDR1 or the unavailability of assays to determine its pump activity *in situ*. We have recently developed a `*reverse Fluo-4* Ca^{2+}*-imaging*' approach based on the fact that the fluorochrome Fluo-4 represents a substrate for this ATP-dependent transporter. We were able to provide first ever information about overall PfMDR1 pump rates in the *P. falciparum*-infected RBCs. However, the iRBC represents a multi-compartment system and direct observations of isolated DV PfMDR1 activity have never been published. We have developed isolation protocols for functional DV and parasites and will characterize PfMDR1 activity and pump rates at each complexity level of the malaria parasite, i.e. isolated DVs and iRBCs. We used several *Plasmodium* strains with different copy numbers of the mutated *pfmdr1* allele and combine pump rate assays with immuno-fluorescence and quantitative Western blotting assays to determine PfMDR1 vacuolar membrane densities for normalization of transport activities from a specific C-terminal PfMDR1 antibody and recombinant protein. As a result, we aim to provide a first estimate for molecular transport rates of this crucial drug resistance transporter.

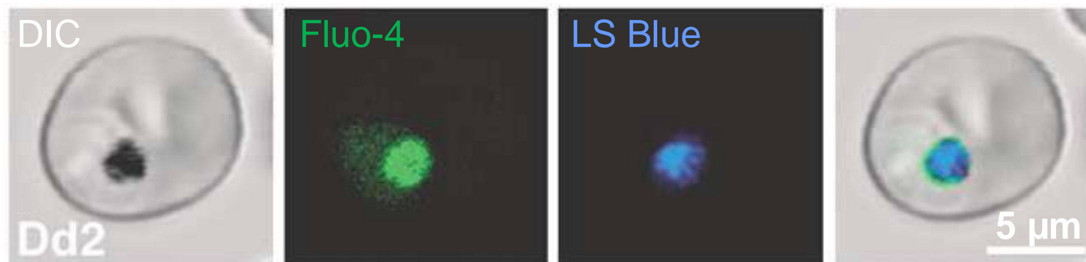

Fig. 1. *Plasmodium falciparum* multi-resistant parasite of the strain Dd2 with Fluo-4 fluorescence in the digestive vacuole, which is labeled with LysoSensor Blue [1].

References

[1] P. Rohrbach, C. P. Sanchez, K. Hayton, O. Friedrich, et al. Genetic linkage of *pfmdr1* with food vacuolar solute import in *Plasmodium falciparum*. *EMBO J.* **25**, 3000–3011 (2006)