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FLUORESCENT LABELLING OF WILD TYPE *PLASMODIUM* SPECIES WITHIN THE MOSQUITO HOST: A NOVEL METHOD TO TARGET SPOROZOITES

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Mutant *Plasmodium* parasites expressing fluorescent proteins allow preclinical imaging of malaria development and distribution in both cell lines and animal models. However, the widespread application of genetically altered organisms is limited, due to regulatory constraints and the inability to culture some *Plasmodium* species, such as *P. vivax*. As such, reporter lines are not available for all *Plasmodium* species. This calls for more generic approaches that allow for the targeting and (molecular) imaging of sporozoites. Here we present a novel method to fluorescently label the sporozoite stage of wild-type *Plasmodium* species *in vivo* and without the need for genetic modification or the extraction of the parasite from its mosquito host. *In vitro* studies demonstrated a tailored fluorescent

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cyanine-5 (Cy5) dye could efficiently stain sporozoites *in vitro*. By membrane-feeding infected *Anopheles* mosquitoes on glucose using the exact same dye we were even able to specifically label sporozoites within the mosquito's salivary glands *in vivo*. The Cy5-dye was preferentially taken up by the mitochondrion of sporozoites and the uptake therein was higher compared to native mosquito tissue such as salivary gland cells or cells of the midgut. This specificity indicates that the mitochondrial activity of sporozoites provides a valuable (*in vivo*) targeting mechanism. To demonstrate cross-species utility of this technology, it was successfully applied in *Plasmodium yoelii*, *berghei* as well as *falciparum*. Viability of the fluorescently labelled sporozoites was confirmed in a hepatocyte cell line. Targeting plasmodium sporozoites through the feed removes the need for genetic modification with imaging vectors, thereby allowing more detailed studies for species such as *Plasmodium vivax*. In addition, the specificity of the mitochondrial uptake that we observed, suggests this to be a possible molecular targeting route for sporozoites residing in the mosquito host.

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