MALARIA TARGETS ID

Malaria is a devastating parasitic infectious disease killing around half a million person every year and affecting close to 250 million worldwide. The recent emergence of drug resistance against the newest antimalarial therapies has made the identification of novel drug targets extremely urgent. In an effort to synergise basic research with malarial drug development the pharmaceutical industry identified thousands of new compounds with antimalarial activity. These hits represent a treasure-throve of chemical tools to study parasite biology and identify new targets. However, in order to harness the potential benefits of these compounds it is crucial to understand how these small molecules kill the malaria parasite.

Our group has been using chemical biology approaches to identify the targets of some of these bioactive compounds with the goal of validating new antimalarial targets. In particular, we use small molecule tools called activity-based probes (ABPs) that allow us to simultaneously monitor the activity of dozens of enzymes in the malaria parasites. We have used this method to determine whether any small molecule from a collection of 400 compounds with anti-parasitic activity, known as the Malaria Box, was able to inhibit enzymes belonging to two families that are essential for parasite development, namely metabolic serine hydrolases and cysteine proteases (Fig. 1A-B). While none of the compounds was able to robustly inhibit any cysteine protease, three of them consistently targeted unknown serine hydrolases (Fig. 1C). We then used a quantitative chemical proteomics approach to identify these potential targets by measuring whether increasing concentrations of compound decrease the level of ABP labeling of any serine hydrolase in the malaria proteome. Interestingly, two serine hydrolases (abH112 and abH114) are inhibited in a dose-dependent manner. We are currently generating genetically modified parasites to determine the biological function of these two enzymes and to confirm that they are the targets responsible for the antimalarial activity of the selected compounds. Importantly, these two serine hydrolases have been recently annotated as essential genes in the malaria parasites based on a genetic screen, and might therefore be potential antimalarial targets.

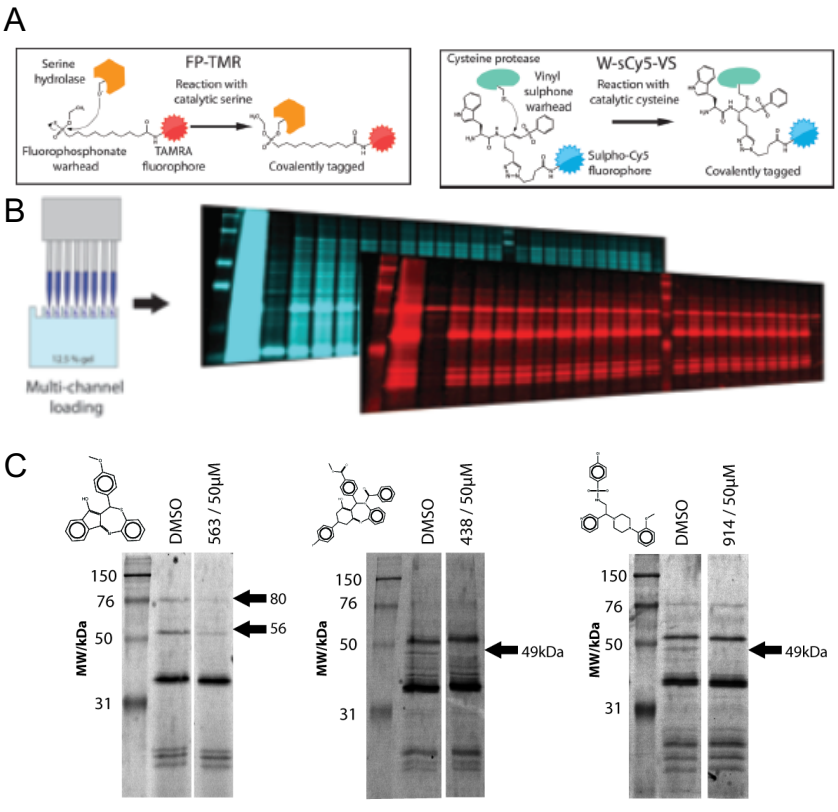

Figure 1. Identification of targets using ABPs. (**A**) Mechanism of action of FP-TMR and W-sCy5-VS to chemically label the active sites of serine hydrolases and cysteine proteases with a red and blue fluorophore, respectively. (**B**) The 400 compounds were screened in a gel-based assay. Each red and blue band correspond to a distinct serine hydrolase or cysteine protease enzyme, respectively. Each lane corresponds to a single compound. Inhibition results in a loss of fluorescent signal. (**C**) Loss of signal of specific serine hydrolases upon treatment of parasite lysates with the different compound hits is indicated by black arrows.

The same chemical

proteomic method we used to identify the targets of compounds can also be used to determine how the activity of all serine hydrolases in the malaria parasite change during its asexual replication cycle, which consists of red blood cell (RBC) invasion, parasite growth and replication within RBCs, and escape from the host cells for further RBC invasion (Fig. 2A). The exponential replication of parasites during this erythrocytic cycle is responsible for all the pathology associated with malaria. Our chemical proteomic approach has identified 29 different serine hydrolases that are active at different stages of parasite development (Fig. 2B). Interesting, we observed a substantial number of enzymes that seem to be specifically activated at the time of RBC invasion. We think these enzymes might be important for establishing the adequate metabolic environment within newly infected RBCs to allow initial parasite development. We have selected a few of these parasite serine hydrolases to determine whether they are essential for parasite development and to study their biological functions.

Moreover, we have shown that selective inhibitors of two of these host enzymes have antimalarial activity, suggesting that the parasite coopts the activity of human serine hydrolases to fully develop within infected RBCs. We are currently investigating whether these compounds prevent parasite development by targeting human enzymes. This result can have great implication in terms of drug development given that it would be much more difficult for the parasite to develop resistance to a drug that targets a human rather than a parasite target.

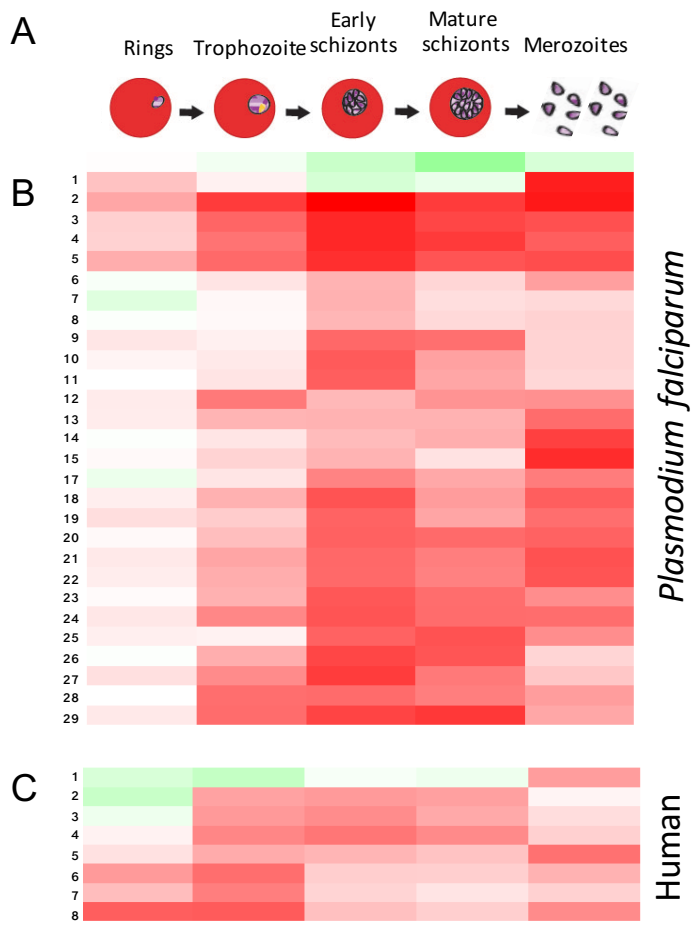

Figure 2. Chemical profiling of serine hydrolases across the parasite life cycle. (**A**) Illustration of the erythrocytic cyle of the malaria parasite. Live parasites were treated with ABP at the indicated stages, and samples were prepared for chemical proteomic analysis. (**B**) Quantification of the level of activity of the 29 parasite serine hydrolases identified in this study. Red indicates increase in activity and green a decrease. (**C**) Same as in B but for the 8 human enzymes identified to be active during parasite development.

This is the first comprehensive study on malarial serine hydro-lases. This enzyme family plays essential metabolic functions in all living organisms but very little is known about its role in parasite development. We are currently performing follow-up work on two human and ten parasite enzymes to determine whether they are essential for parasite development and potential drug targets.

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