Activity-based Protein Profiling to investigate the interactome of the antimalarial early lead Plasmodione

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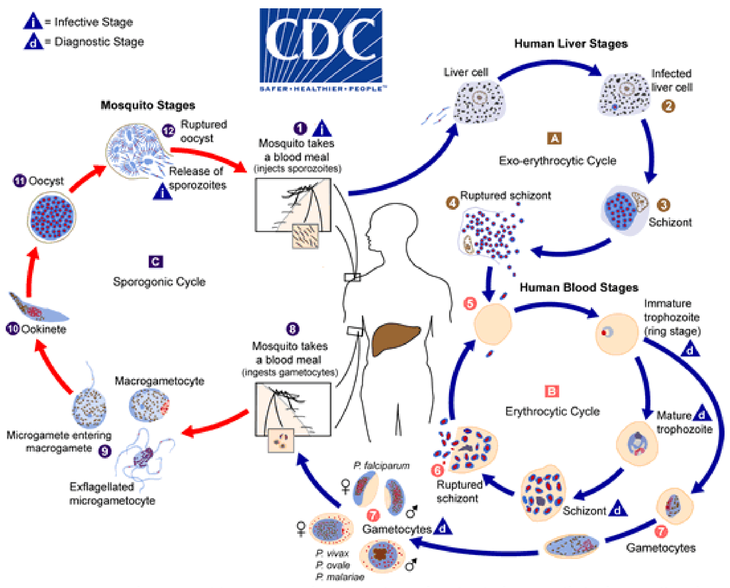


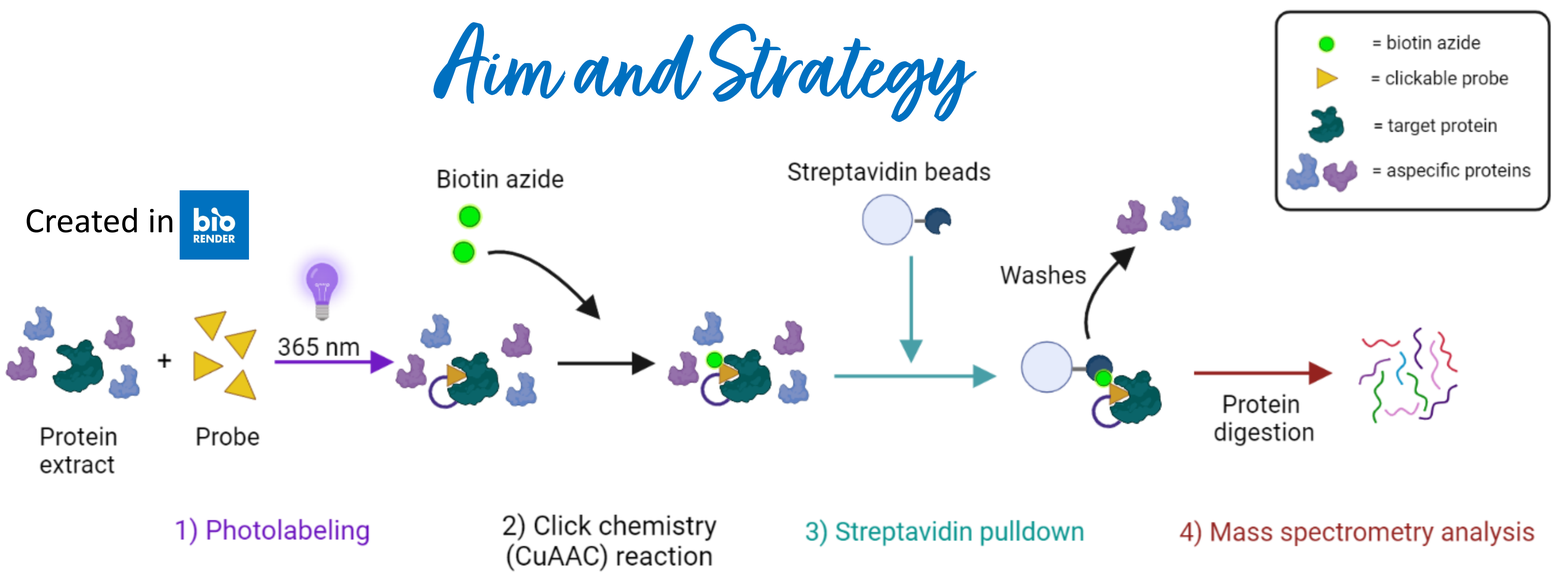
Content source: https://www.cdc.gov/malaria/about/biology/index.html

Introduction

Malaria (in 2021) caused an estimated 247 million clinical episodes and 619,000 deaths. Parasites of the Plasmodium genus are the causative agents of malaria. Several antimalarial drugs have been developed but the parasite quickly produces drug-resistances to all of them. Plasmodione is a novel antimalarial early lead drug that is highly effective in limiting the proliferation of malaria parasites in vitro in the nM range with very moderate toxicity in the host cells.

Aim and Strategy

The aim is to identify putative Plasmodione targets. The methodologies used will be those of the affinity-based protein profiling (ABPP) strategy. ABPP strategy consists of the following steps: 1) Photolabeling of the probe-protein complexes 2) biotin conjugation by Click chemistry (CuAAC), 3) Streptavidin pulldown, and 4) LC-MS/MS analysis.

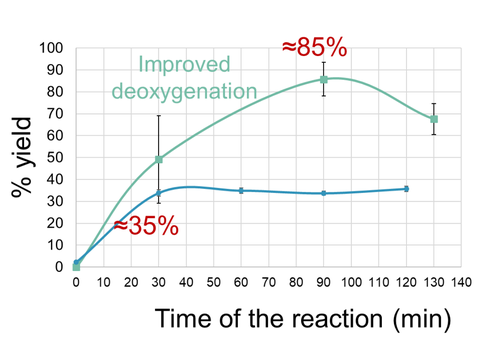


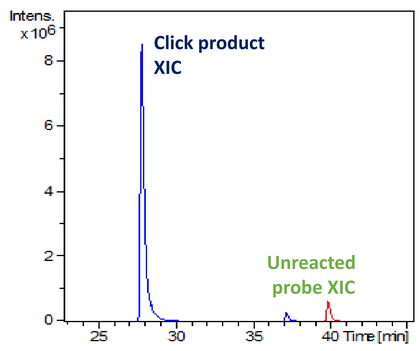
Results and Discussion

Each step of the ABPP strategy was optimized. The optimization involved various conditions to screen: from UV-irradiation and CuAAC reaction under oxygen-free conditions and then pulldown of the adducts through biotin-streptavidin beads.

CuAAC reaction optimization

Condition tested are the temperature (56 or 30°C), deoxygenation (yes or no) and time of reaction (0 to 140 min)



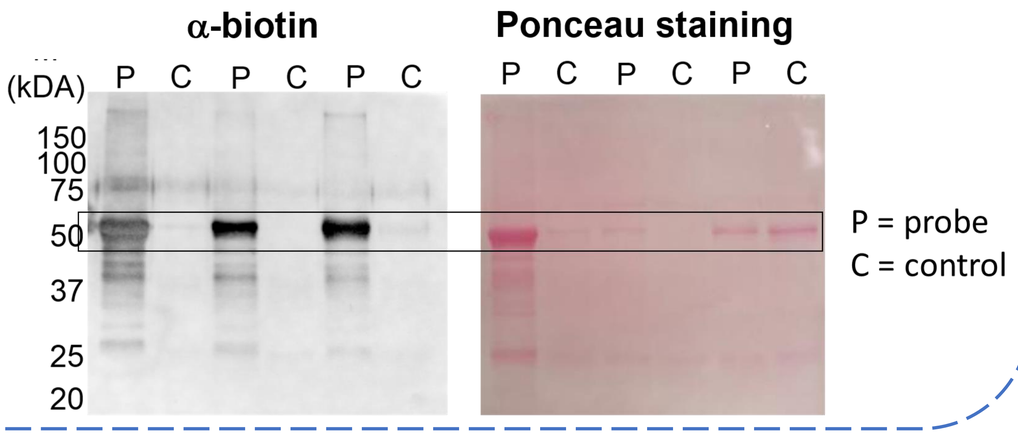


Streptavidin pulldown optimization

Different binding and washing buffers were tested

Binding: 150 mM NaCl, 0.1% NP-40 in 1x PBS;
3M Urea, 1M NaCl;
3M Urea, 1M NaCl, 0.25% SDS.

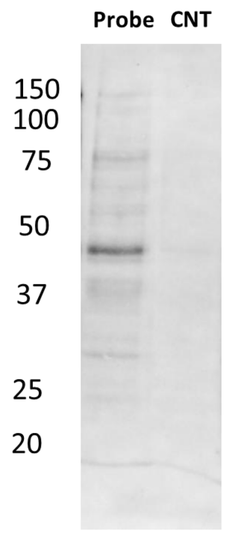
Washing: 300mM NaCl (1x), 0.1% SDS (2x), 1x PBS (1x);
RIPA buffer (4x);
8M Urea, 0.25% SDS (4x).



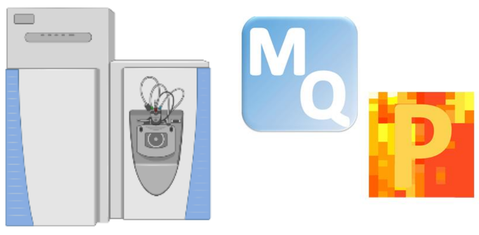
Yeast proteome pulldown

The whole procedure is applied to S. cerevisiae cell systems (WT and a strain NDE1 k.o. gene) to validate the experimental workflow. The cell system consists of the wild-type strains and S. cerevisiae mutated ones in which a potential plasmodione target was been silenced.

1) α- biotin WB check



2) LC-MS/MS analysis



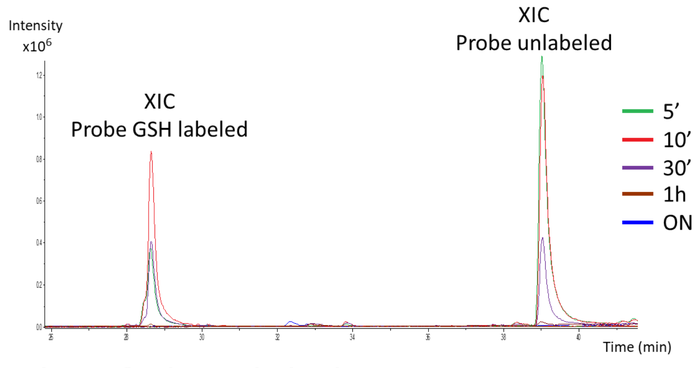
Entry code	Gene name	FC (PD/CNT)	t-test FDR
P00958	MES1	16.45	0.03
P07244	ADE5,7	33.96	0.001
P53839	GOR1	58.91	0.0016

Preliminary results, are to be validated!

Conclusions

ABPP strategy is not optimized, in particular, we want to increase the yield of the photo-labelling. For this reason, we are testing new probes. In the future, we plan to apply the ABPP procedure to the *P. falciparum* cell extracts.

WORK IN PROGRESS



Preliminary results of photolabeling optimization