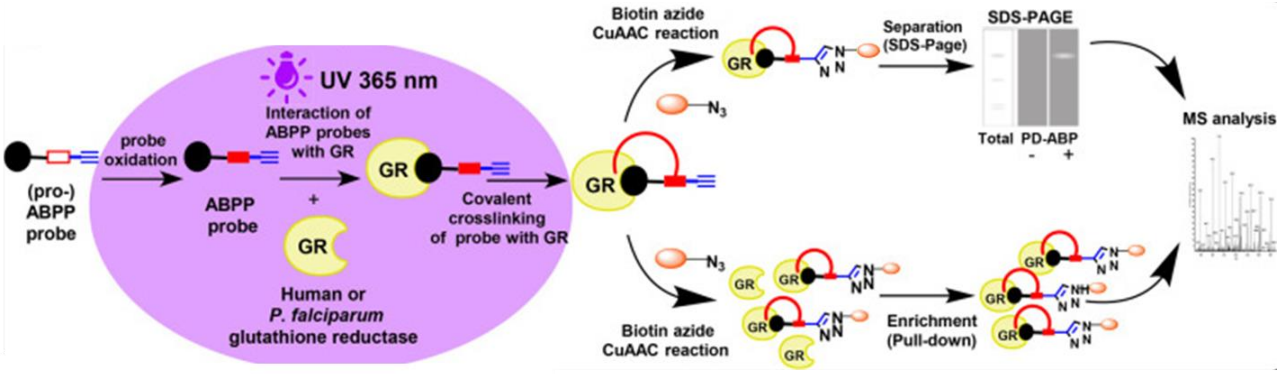
STSM grantee (1st period): **Vittoria MONACO**

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

Home institution : University of Naples "Federico II", Naples, Italy (Prof. Maria Monti)

Host institution: Bio(IN)organic & Medicinal Chemistry Team, Laboratoire d'Innovation Moléculaire et Applications (LIMA), European School of Chemistry, Polymers and Materials (ECPM), Strasbourg, France (Prof Elisabeth Davioud-Charvet)

The STMS project aims at the optimization of the activity-based protein profiling strategy for the identification of the antiplasmodial plasmodione targets. After a proof of concept (doi: 10.1021/jacsau.1c00025) with the pure protein pfGR – a known target of the key plasmodione metabolite (PDO) – we applied the strategy to the yeast proteome expressing at various levels a known target of PDO metabolite, the protein Nde1. The ABPP strategy was carried out using the MD43 probe used in the proof-of-concept experiments with pfGR protein. The preliminary results obtained showed a low yield in the isolation of drug targets. Given these results, we followed two distinct strategies. First, we designed a new probe (BD437) with improved photoreactivity to obtain a better yield in the photolabelling step. In a model reaction with BD437 and the GSH peptide, the yield of insertion, calculated by monitoring the formation of the specific adduct after UV irradiation with the LC-MS technique, was 2.4-fold higher than the yields in the reactions with AZ47 or MD43 probes. Future perspectives are to test the new probe in both yeast and *P. falciparum* proteomes. In parallel, we modified the strategy to avoid the photolabelling step by switching to a drug pulldown workflow. The strategy was applied to the purified protein pfGR using the MD43 probe, which allowed us to optimise the protocol after testing different conditions. We were also able to apply the optimised protocol to the yeast proteome, results are pending, and future perspectives are to apply the drug pulldown strategy to the *P. falciparum* proteome. The project has explored a wide range of techniques and methodologies, including different screening conditions, from click-CuAAC and photolabelling chemical reactions under oxygen-free conditions with a biotin azide, pull-down techniques of the adducts through biotin-streptavidin beads, to different detection methods (i.e. detection of biotinylated proteins using antibodies in Western blots or detection and MS analysis of peptides after digestion of protein adducts).



Workflow of ABPP strategy (doi: 10.1021/jacsau.1c00025)

Communication

The STMS project was presented in a poster entitled: ''Activity-based Protein Profiling to investigate the interactome of the antimalarial early lead Plasmodione'' at the BSPR-EUPA 2023 Conference in Newcastle upon Tyne, 17-20 July 2023.