

Application of APEX2 for labeling mitochondrial proteins of *Plasmodium falciparum* associated with in silico predictions and data mining

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Malaria is caused by protozoa of the phylum Apicomplexa, belonging to the genus *Plasmodium*. In 2022, 249 million cases of malaria were reported across 85 countries. *P. falciparum* is the species responsible for the most severe symptoms of the disease and has developed resistance to reference antimalarial drugs over the years, raising public health concerns. In general, organelles of symbiotic origin are interesting targets for new therapeutic interventions because they have their own genome and machinery for transcription and translation. The mitochondrion and the apicoplast are essential organelles for the parasite, and disruption of their functions can lead to cell death. Advanced protein labeling techniques enable a comprehensive understanding of these plastids and their metabolic integration, facilitating the identification of new therapeutic targets. This study aimed to utilize APEX2 for biotinylation-based labeling of *P. falciparum* mitochondrial proteins in live cells. Protein biotinylation was performed after obtaining parasites transfected with an episomal expression plasmid containing the coding sequence for APEX2, fused to a non-consensus transit peptide from a mitochondrial protein. Western Blot and streptavidin-blot analyses confirmed the expression of HA-APEX2 and the biotinylation labeling. However, immunofluorescence indicated that HA-APEX2 was not specifically targeted to the *P. falciparum* mitochondrion. Concurrently, as the organelles of symbiotic origin in *P. falciparum* are biochemically interrelated, though this relationship lacks concrete data, in silico prediction and data mining strategies were employed to identify the predicted subproteomes of mitochondrial and apicoplast proteins. A total of 708 mitochondrial proteins and 781 apicoplast proteins of *P. falciparum* were predicted, with an overlap of 108 proteins between the databases. For the mitochondrial proteins, those related to the electron transport chain were identified, while for the apicoplast proteins, those involved in type II fatty acid synthesis (FASII) were listed. This bioinformatics analysis reinforces known evidence about the biology of *P. falciparum* organelles and identifies some proteins that are already being studied as potential antimalarial targets. Due to the non-specific mitochondrial labeling by HA-APEX2, a new sequence is currently being transfected. The main goal of these organelle predictions is to provide a detailed overview of the biochemical characteristics of the mitochondrion and the apicoplast to aid future proteomic analyses.

Agradecimentos: CAPES: (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FAPDF (Fundação de Amparo à Pesquisa do Distrito Federal) e FINEP (Financiadora de Estudos e Projetos).