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PLASMEPSIN II LABELING STRATEGIES TO STUDY CONFORMATIONAL CHANGES OF BINDING SITE WITH NMR SPECTROSCOPY

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Malaria infection in humans is caused by *Plasmodium Falciparum* parasite. *P. Falciparum* aspartic proteases (plasmepsins) are responsible for catalytic hemoglobin degradation and thus are being investigated as potential drug targets. Plasmepsins, however, share high structural similarity with human aspartic proteases, emphasizing the need for selective inhibitors. Selectivity of several known plasmepsin inhibitors is attributed to unusual binding under the flap loop [1]. One of the methods that can be used to characterize binding site dynamics upon inhibitor binding is NMR spectroscopy, however, due to large plasmepsin II size, selective protein labeling is required. Here we present two selective protein labeling strategies.

Substitution of certain amino acids with their fluorinated analogues can be exploited to study conformational changes with 19F NMR spectroscopy. In this study tryptophan was chosen as amino acid of interest due to its position near the binding site and low number of residues. Labeled protein was obtained using biosynthetic amino acid incorporation [2].

Second method used was spin labeling with a molecule containing nitroxide moiety. Paramagnetic groups such as MTSL (*S*-(1-oxyl-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-3-yl)methyl methanesulfonothioate) increase relaxation rate of nearby atoms, allowing to estimate inter-atomic distances up to 25 Å, and, thus, exact position of inhibitor in the binding site. MTSL label was attached to cysteine residue that was introduced in the flap loop using site-directed mutagenesis [3].

Obtained NMR spectra show that both labels have been successfully incorporated in plasmepsin II molecule, however, protein preparation, yields and recording conditions of NMR spectra still need to be optimized in order to apply these methods to protein dynamics studies.

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