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**Evaluation of the binding interactions between Plasmodium falciparum Kelch-13 mutant recombinant proteins with artemisinin**

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**Abstract**
Malaria, an ancient mosquito-borne illness caused by Plasmodium parasites, is mostly treated with Artemisinin Combination Therapy (ACT). However, Single Nucleotide Polymorphisms (SNPs) mutations in the P. falciparum Kelch 13 (PfK13) protein have been associated with artemisinin resistance (ART-R). Therefore, this study aims to generate PfK13 recombinant proteins incorporating of two specific SNPs mutations, PfK13-V494I and PfK13-N537I, and subsequently analyze their binding interactions with artemisinin (ART). The recombinant proteins of PfK13 mutations and the Wild Type (WT) variant were expressed utilizing a standard protein expression protocol with modifications and subsequently purified via IMAC and confirmed with SDS-PAGE analysis and Orbitrap tandem mass spectrometry. The binding interactions between PfK13-V494I and PfK13-N537I propeller domain proteins ART were assessed through Isothermal Titration Calorimetry (ITC) and subsequently validated using fluorescence spectrometry. The protein concentrations obtained were 0.3 mg/ml for PfK13-WT, 0.18 mg/ml for PfK13-V494I, and 0.28 mg/ml for PfK13-N537I. Results obtained for binding interaction revealed an increased fluorescence intensity in the mutants PfK13-N537I (83 a.u.) and PfK13-V494I (143 a.u.) compared to PfK13-WT (33 a.u.), indicating increased exposure of surface proteins because of the looser binding between PfK13 protein mutants with ART. This shows that the PfK13 mutations may induce alterations in the binding interaction with ART, potentially leading to reduced effectiveness of ART and ultimately contributing to ART-R. However, this study only elucidated one facet of the contributing factors that could serve as potential indicators for ART-R and further investigation should be pursued in the future to comprehensively explore this complex mechanism of ART-R. 

**Index Keywords**
artemisinin, Plasmodium falciparum kelch-13, recombinant protein, unclassified drug, antimalarial agent, artemisinin, artemisinin derivative, protein binding, protozoal protein; Article, cloning vector, documentation, evaluation study, gene expression, gene mutation, gene sequence, immobilized metal affinity chromatography, isothermal titration calorimetry, ligand binding, liquid chromatography-mass spectrometry, mass spectrometry, nonhuman, protein expression, protein interaction, protein purification, real time polymerase chain reaction, sequence analysis, signal detection, single nucleotide polymorphism, spectrofluorometry, chemistry, drug effect, drug resistance, genetics, metabolism, mutation, Plasmodium falciparum; Antimalarials, Artemisinins, Drug Resistance, Mutation, Plasmodium falciparum, Polymorphism, Single Nucleotide, Protein Binding, Protozoan Proteins, Recombinant Proteins

**Chemicals/CAS**
artemisinin, 63968-64-9; Antimalarials; artemisinin; Artemisinins; Protozoan Proteins; Recombinant Proteins

**Manufacturers**
Agilent; Thermo

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