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Design of a Pyruvate Kinase II (*Pf*PKII) Construct for Antimalarial Structure-Based Drug Discovery

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Malaria elimination is threatened by the spread of artemisinin resistance, necessitating the identification of novel targets with unique mechanisms of action. Pyruvate Kinase II (*Pf*PKII) is a promising target that plays an important role generating ATP and pyruvate within the apicoplast, a unique organelle of the parasite. Notably, *Pf*PKII utilizes nucleoside diphosphates (NDPs) other than ADP, functioning as the sole source of nucleoside triphosphates (NTPs) generation within the apicoplast. This local energy production is essential for the organelle's housekeeping functions, including DNA replication and protein import. Recent studies confirmed that *Pf*PKII gene deletion is lethal, validating its essentiality. Critically, *Pf*PKII is among the few apicoplast genes that retain the transcription levels during dihydroartemisinin-induced dormant ring stages of *P. falciparum*. This suggests that *Pf*PKII inhibitors could eliminate dormant forms of the parasite, prevent recrudescence and serve as a partner drug to combat resistance. Despite this potential, a lack of structural data has limited the exploitation of *Pf*PKII for structure-based drug design. In this work, we engineered a stable and active recombinant *Pf*PKII construct to enable robust biochemical and structural analysis. We developed an optimized construct (*Pf*PKII$_{opt}$, residues 70-745) by removing a flexible N-terminal region. The recombinant protein was expressed and successfully purified through a four-step chromatography protocol, yielding a stable tetramer. The oligomeric state of *Pf*PKII$_{opt}$ was validated via size SEC-MALS analysis (279 ± 6 kDa). The predicted mass of the monomer was approximately 79 kDa. Differential Light Scattering (DLS) assays indicated a highly monodisperse sample (PDI = 0.07). *Pf*PKIIopt exhibited catalytic activity, with a K_M^{App} of 26 μM for PEP (substrate). The optimized *Pf*PKII$_{opt}$ was used for initial structural studies aiming to solve its tridimensional structure. Cryo Electron Microscopy (cryo-EM) images of the sample revealed homogenous and monodisperse particles. Preliminary data collection on a 100 kV Tundra cryo-TEM enabled the reconstruction of a 5 Å volume map of the *Pf*PKII$_{opt}$ tetramer. However, the sample exhibited severe preferred orientation at the air-water interface, limiting the views required for high-resolution reconstruction. Future efforts will focus on overcoming this orientation bias through a combination of detergent addition to tilted data collection. Resolving this structural bottleneck is essential to characterize the catalytic and allosteric sites of *Pf*PKII, positioning it as a priority target for the design of novel selective inhibitors with potent antimalarial activity.

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