Biochemical basis of processive DNA synthesis by *Plasmodium falciparum* apicoplast DNA polymerase

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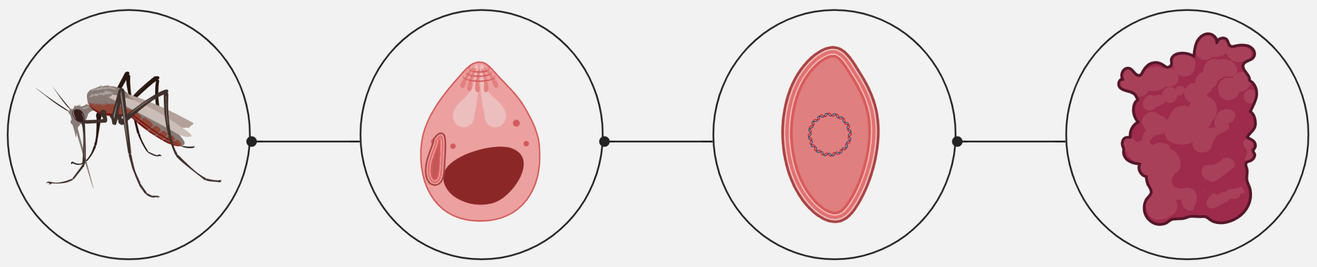
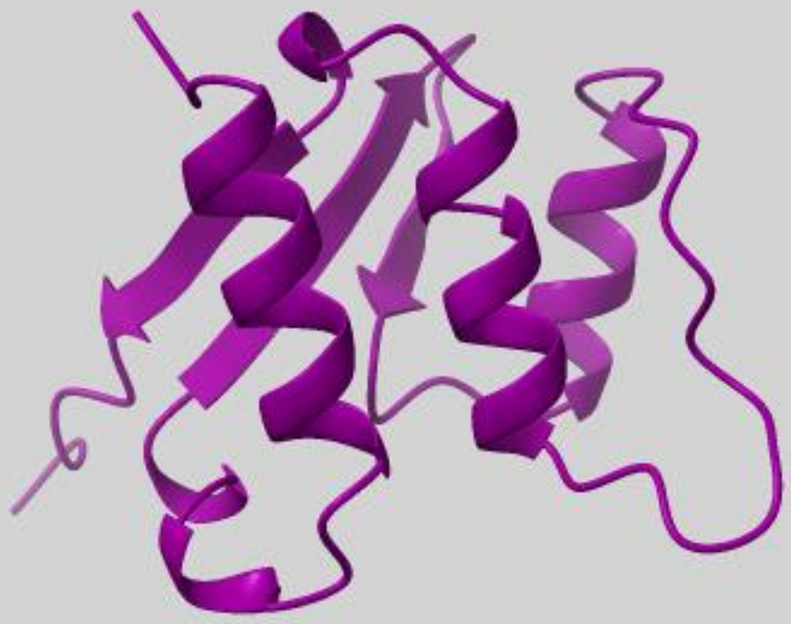
Background
Malaria: a disease which inflicts devastation around the world, having claimed 619,000 lives in 2021; 77% of these were in children under 5 (UNICEF data, 2023). Whilst anti-malarial drugs exist, their effectiveness is reducing, making it imperative that we learn more about the biochemistry of this pathogen, to save as many lives as possible.



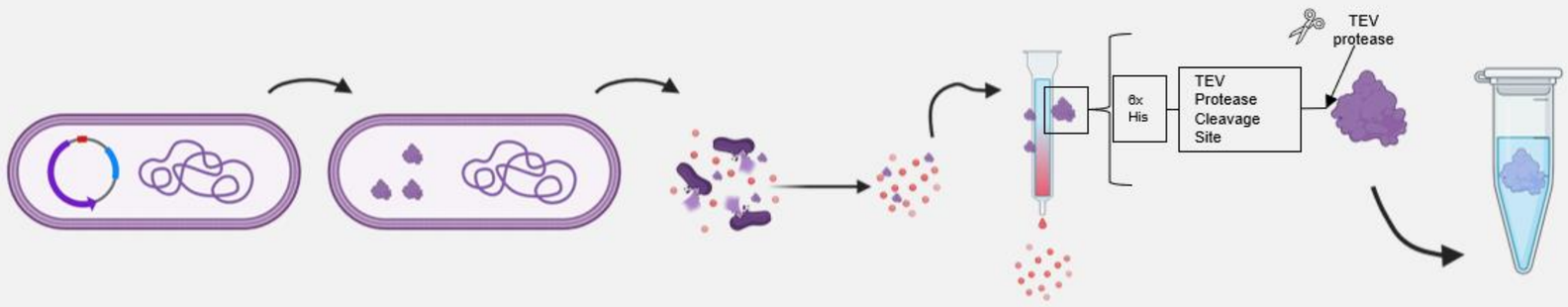
Project Focus
Malaria is primarily associated with mosquitos infected with *Plasmodium*. The most lethal malarial infection is *P. falciparum;* this parasite contains an apicoplast; responsible for essential metabolism including lipid biosynthesis (McFadden and Yeh, 2017). The apicoplast has its own ~35 kb genome replicated by a large multimeric protein called Plastid Replication-Repair Enzyme complex (PREX) (Milton and Nelson, 2016). PREX is made up of domains; sections of protein carrying out individual functions (Wang et al, 2021).

Aims
The project focus is to identify the function of one PREX domain, named Structure Domain (pfapSD). For this, pfapSD needed to be expressed and purified, followed by experimental assays to determine its role in Plasmodium apicoplast replication.



pfapSD tertiary structure prediction by AlphaFold2 through ChimeraX (Jumper, J. et al, 2021 and Pettersen, EF. et al, 2021). The prediction is similar to the configuration of the Rossmann Fold, a characteristic pattern for binding nucleic acids (Hanukoglu, I., 2015).

Purification of pfapSD



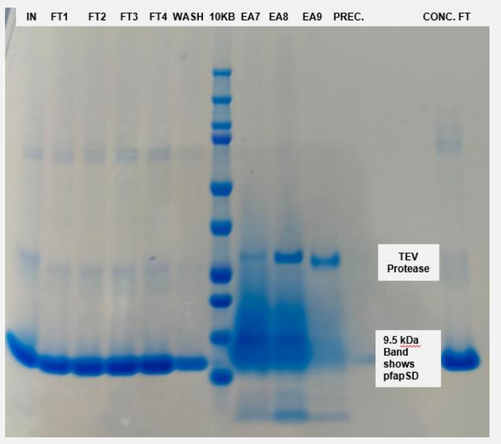
(1) Transformation of plasmid coding for pfapSD into Rosetta DE3 *Escherichia coli* cells

(2) Culturing in Auto-Induction Media to express pfapSD

(3) Lysis (bursting) by sonication, centrifugation to remove larger cell debris

(4) Purification by affinity chromatography; pfapSD separated using 6x His tag

(5) Tag removal by digestion using Tobacco Etch Virus (TEV) protease



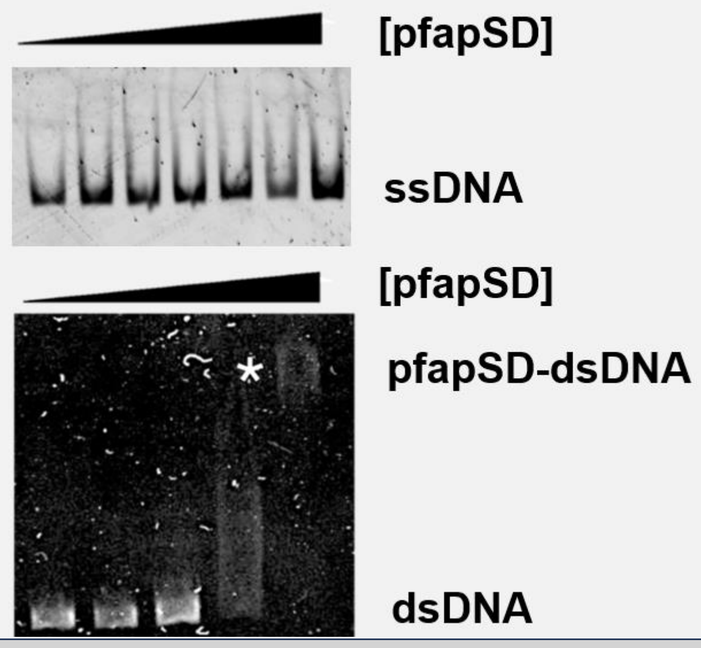
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) following TEV protease digestion – the resulting gel showing the successful purification of pfapSD; the flow-through (FT) lanes contain pure pfapSD, shown by a thick 9.5kDa band, and the elution (E) lanes show the separated tag and TEV protease.

pfapSD Smears dsDNA at high concentrations
To investigate whether pfapSD can bind to DNA, either double or single-stranded, several Electrophoretic Mobility Shift Assays (EMSAs) were run. EMSAs are a quick way to detect interactions between protein and nucleic acids – these include DNA. If a protein binds to DNA, the combined molecular weight will increase, and can be observed on a non-denaturing gel; the band will not travel as far compared to free protein or DNA, which will be smaller and travel faster, therefore further.

Testing pfapSD with single-stranded DNA (ssDNA) was completed using an 8% native polyacrylamide gel electrophoresis (native-PAGE). The template DNA was M13mp18 (the genomic DNA of λ bacteriophage), an ideal long single-strand of DNA. The reaction contained 16 μM ssDNA with a serial dilution of pfapSD with a maximum concentration of 20 μM. The gel revealed no binding interactions.

The double-stranded (dsDNA) EMSA (bottom) used a 0.7% agarose gel and 1x TAE buffer, with 3 μg/μl 3kb circular dsDNA and a serial dilution of pfapSD with a maximum concentration of 20 μM.

Smearing was observed at concentrations above 10 μM for both sets of dsDNA samples – this indicates that there is an interaction between the dsDNA and pfapSD.



Future Research
We still don't know the true role of pfapSD within the apicoplast replisome, particularly how it interacts with the other replication proteins, however we have learned important information about its behaviour. Based on information obtained from the rest of PREX, we know that the polymerase, the domain responsible for adding nucleotides to the new DNA strands during replication, requires other proteins to increase processivity that are yet to be identified (Kumari et al, 2022). To see whether pfapSD could be improving the polymerase's processivity, we are currently designing a processivity assay using M13mp18 single-stranded DNA as a template for apPol in the presence of apSD.

Future research must include pfapSD's interactions with other apicoplast replication proteins, namely the helicase, and single-stranded DNA binding protein (SSB). The more we can learn about PREX, the closer we come to designing specific anti-malarial drugs to save lives around the world.

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Diagrams created by BioRender.com
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