2018

Expression and purification of full-length recombinant Plasmodium falciparum PfMC-2TM Maurer's cleft protein

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Expression and Purification of Full-length Recombinant *Plasmodium falciparum* PfMC-2TM Maurer's cleft Protein

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Office of Research

Abstract

Malaria caused by *Plasmodium falciparum* remains the most virulent form of malaria, resulting in 216 million cases and 445,000 deaths globally. Invasion of red blood cells by *P. falciparum* leads to the formation of membranous structures known as Maurer's clefts (MC). Virulence markers of *P. falciparum* such as PfEMP1 are transported across the MC to the surface of the infected red blood cell. Insight into the formation and function of the MC will be important for the discovery of new vaccine and drug candidates. The PfMC-2TM is encoded by a multi-gene family of 13 members. PfMC-2TM is a protein localized to the MC. We induced expression of PfMC-2TM encoded by 1 family member [PF3D7_0114100 (PFA0680c)] in BL21 DE3 strain of *Escherichia coli* following transformation with recombinant pET-28a plasmid containing a chemically synthesized gene. The purpose of this study was to determine immunogenic properties of the resulting recombinant protein using western blot analysis. The recombinant plasmid was isolated and analyzed in 1% agarose gel and an approximately 5kb band was identified. Pilot expression of transformants showed expression of recombinant PfMC-2TM by western blot. Recombinant PfMC-2TM protein will be expressed and purified for antibody production to allow subsequent domain analysis and characterization.

Introduction

❑ Malaria

- 216 million cases worldwide and 445,000 deaths worldwide (World Health Organization, www.who.int/)
- Infection transmitted by female *Anopheles* mosquito
- Infection caused by *Plasmodium* parasites (protozoans)
  - *P. falciparum*- most virulent form
  - *P. ovale*
  - *P. malariae*
  - *P. knowlesi*
  - *P. vivax*

❑ *Plasmodium falciparum*

- Cause of Complicated/Severe Malaria
- Only species that develops Maurer's clefts (MC)
  - MC responsible for protein transport from red cell cytopalsm to erythrocyte membrane (RBCM)
- Virulence protein PfEMP1 is associated with knobs on the surface of RBC
- PfMC-2TM is localized to the MC

❑ Rationale

- The rationale behind this project is to determine if the full-length chemically synthesized gene, following bacterial transcription and subsequent translation, can yield a protein with immunogenic properties.

Objectives and Hypothesis

❑ Objectives

- Transform DH5α strain *E.coli* bacteria with full-length pET-28a(+) *PfMC-2TM* recombinant plasmid
- Induce expression of the PfMC-2TM protein in BL21 DE3 strain of *E. coli*
- Analyze bacterially synthesized protein by western blot analysis and determine potential immunogenicity

❑ Question

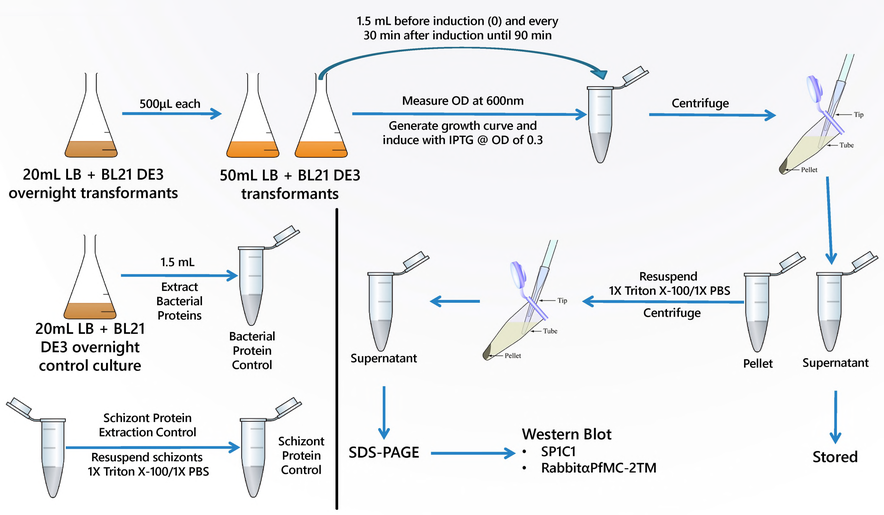
- Do *E. coli* transformed with chemically synthesized *PfMC-2TM* recombinant pET-28a(+) plasmid yield an immunogenic PfMC-2TM protein?

❑ Hypothesis

- *E. coli* bacteria transformed with chemically synthesized *PfMC-2TM* recombinant plasmid pET-28a(+) yield immunogenic PfMC-2TM protein

Methods

- ❑ Transformed recombinant *PfMC-2TM* into *E. coli* strains DH5α and BL21 DE3
- ❑ Selected 4 DH5α and 6 BL21 DE3 transformants
- ❑ Plated transformants on LB-Kanamycin plates and LB broth



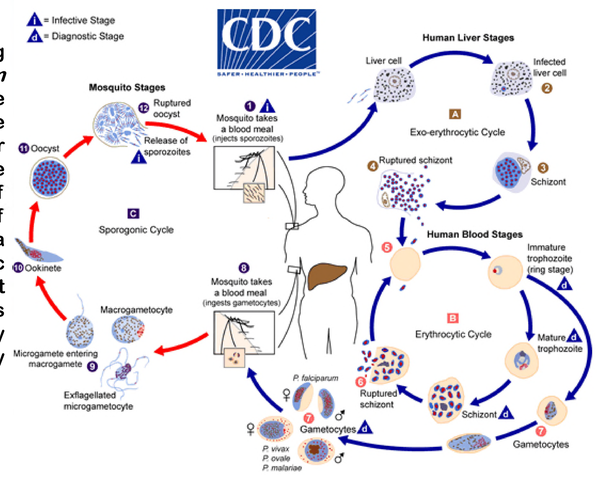


Figure 1. General Plasmodium Life Cycle. Obtained from www.cdc.gov

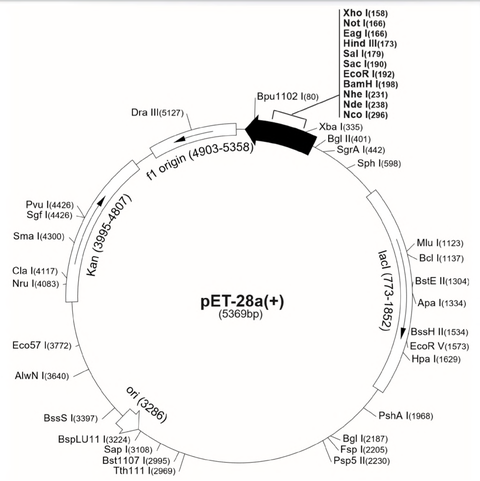


Figure 2. pET-28a(+) plasmid map showing available restriction sites and Kanamycin resistance gene.

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Gene sequence for cloning project

MC-2TM + P

NdeI

XhoI

Backbone of pET28

TATACCATGGGCAGCAGCCATCATCATCATCATCACAGCAGCGGCCTGGTGCCGCGCGGCAGCCATATGTTTCATTATA
TTTATAAAATATATATTTTTACCATAATACTATGTGCATCTAATCTATTTAATAACAAT
GTAGTAGAAATTGGAACATACAAATTATCATACCATAATGGAGGGATACAATTCAGAAT
GTTAGCACAAAAAAACACAAATAAAAAACCATTCGGAAATACCTTAACGAATATATTGT
TTAAGGACAAAAAAAAAAAAAAAATCTTGATCCTCAAATTTCATCTCTAGTCAGTTTAGTA
GATAATATGAATATAACTCAAGAAAAAAAAGATAAAATCAAAAATCTCTCATTAAAATA
TATAAATAGTAGAGATGTAAAAGAAAAAAATGAATCAATTAATGAACTTCAAAAATATA
GTAATAACGAAGAATGTAAAGAATATATGGATAGTTATTTAATGCATCTTCGTATGCAA
AATGATATAAAATGTTTAAAAAGAAAAAATTTGTGGAATAATATTTGGATTGTTTCAAC
GACCTTATTATTAATCATTATAATGATAGTATGTTTATTTTCGGTTGATATTACATCAT
CTTCTGCACTTGGCCCTGCATTTATTCTTTTAATTTTTATTATCCATATAGTTGCTCGT
TATTTTCCTGACATGAAAATAGGTTTTTAAAAAATTAAAAACAAAATTGAACACATTTTT
TCAAAACAAAAAGCAAATAACAAAATAACTCGAGCACCACCACCACCACCACTGAGATCCGGCTGCTAACAA
AGCCC
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Figure 3. Sequence of chemically synthesized *PfMC-2TM* gene containing added restrictions sites and pET-28a(+) plasmid backbone.

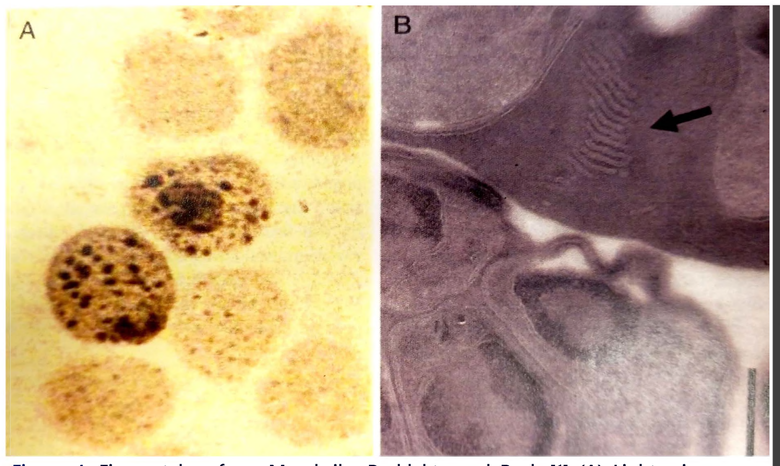


Figure 4. Figure taken from Mundwiler-Pachlakto and Beck [1] (A) Light microscope image from Georg Maurer's publication of Maurer's clefts. (B) Electron microscopy of Maurer's clefts (scale bar 500nm).

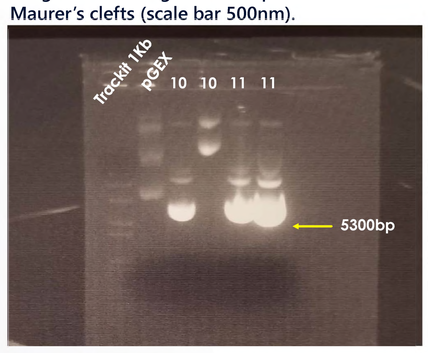
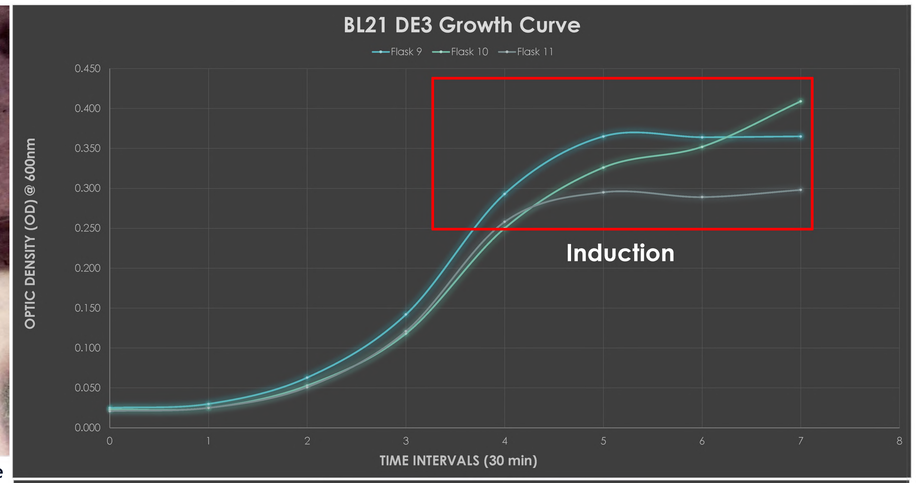


Figure 5. Recombinant PfMC-2TM pET-28a(+) plasmid isolated from DH5α strain E. coli analyzed on 1% agarose gel electrophoresis. DNA ladder used was TrackIt™ 1Kb Plus DNA Ladder (Invitrogen). Plasmid isolation was performed using the Qiagen Plasmid Mini Kit. The recombinant plasmid was constructed and verified by GenScript (Piscataway, NJ) using restriction enzyme analysis and DNA sequencing to validate insert location in the pET-28a plasmid (see plasmid map).



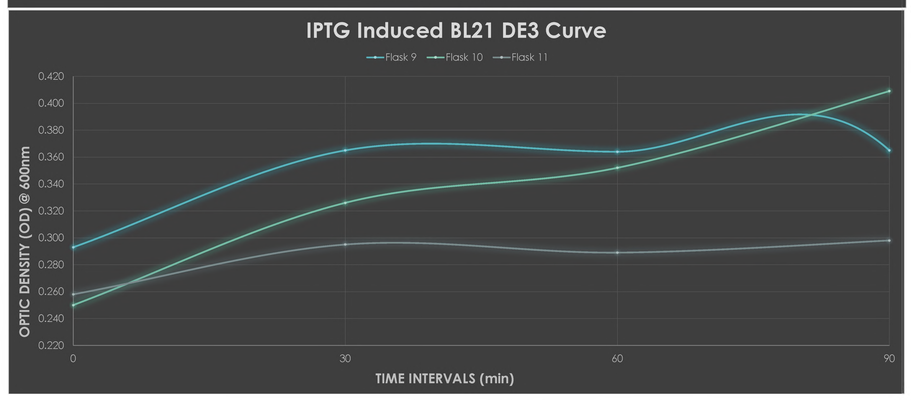


Figure 6. BL21 DE3 growth curves and 1mM isopropylthiol-β-D-galactoside (IPTG) induction. Optical density (OD) readings of bacteria broth cultures were done in triplicate and repeated twice by UV-Vis Spectroscopy in Fixed View settings at 600nm. Readings were taken every 30 minutes for a total of 2 hours. Shaker/Incubator was set at a range of 36-38ºC and shaking intensity 4.

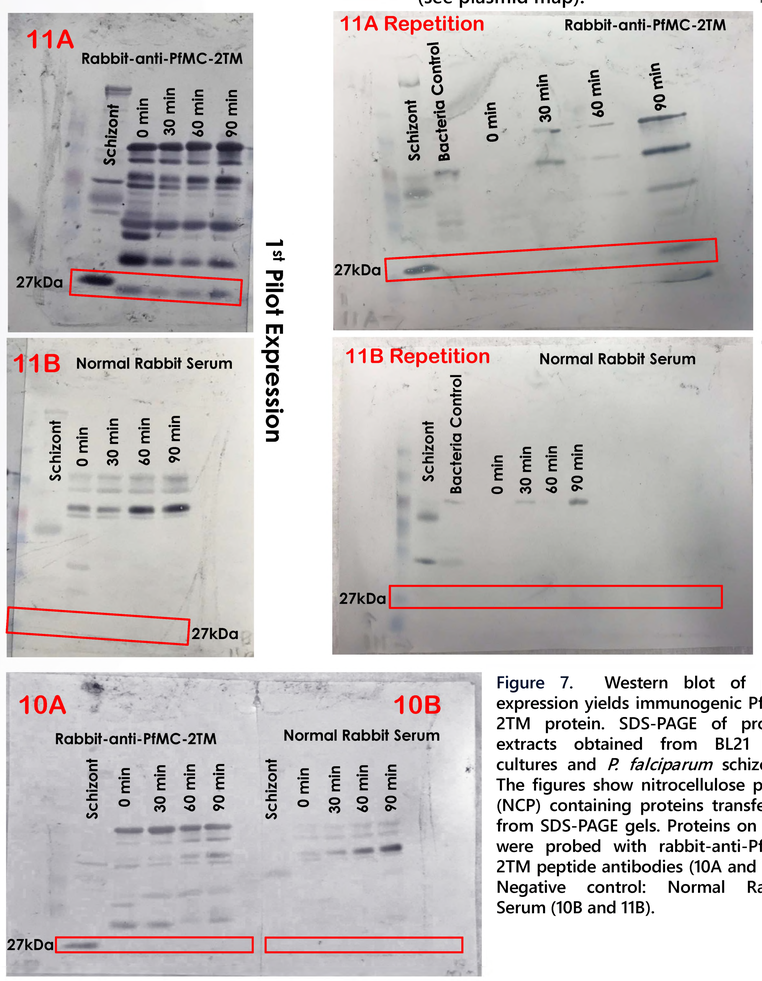


Figure 7. Western blot of pilot expression yields immunogenic PfMC-2TM protein. SDS-PAGE of protein extracts obtained from BL21 DE3 cultures and *P. falciparum* schizonts. The figures show nitrocellulose paper (NCP) containing proteins transferred from SDS-PAGE gels. Proteins on NCP were probed with rabbit-anti-PfMC-2TM peptide antibodies (10A and 11A). Negative control: Normal Rabbit Serum (10B and 11B).

Results and Conclusions

- ✓ pET28a plasmid was isolated from DH5α *E. coli* cultures
- ✓ PfMC-2TM was successfully expressed in BL21-DE3 *E. coli* cultures
- ✓ Full-length recombinant PfMC-2TM reacted strongly with rabbit-anti-PfMC-2TM antibodies

Future Directions

- ✓ Repeat pilot expression to obtain more protein
- ✓ Express protein for purification
- ✓ Purified protein will be used for antibody production
- ✓ Antibodies will be used for domain and epitope characterization

References

1. Mundwiler-Pachlatko, E., Beck, HP. Maurer's clefts, the enigma of *Plasmodium falciparum*. *PNAS* (2013) 110:19987-19994.
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3. Sam-Yellowe, T. *et. al.* A *Plasmodium falciparum* protein located at Maurer's clefts underneath knobs and protein localization in association with Rhop-3 and SERA in the intracellular network of infected erythrocytes. *Parasitol Res* (2001) 87:173-185.
4. Sam-Yellowe, T. A *Plasmodium* Gene Family Encoding Maurer's Cleft Membrane Proteins: Structural Properties and Expression Profiling. *Genome Res* (2004) 14:1052–1059.

Acknowledgements

❖ We want to thank Cleveland State University's 2018 Undergraduate Summer Research Award Program for providing the support and funding for the realization of this research project.