INSTITUT MAX VON LAUE - PAUL LANGEVIN

NEUTRONS FOR SCIENCE ®

Structural biology: A powerful tool to gain insight into the biology of the malaria parasite, *Plasmodium falciparum*

Theresa L. Coetzer[a], Manuel Blanc[b], Daniel R Meyersfeld[a], Juliette Devos[b], Kubendran Naidoo[a], Malene Ringkjøbing Jensen[c], Sonja B Lauterbach[a], Martin Blackledge[c], Michael Haertlein[b], V. Trevor Forsyth[b,d], Edward P. Mitchell[e]

[a] WITS Medical School , NHLS, Johannesburg, Republic of South Africa.
[b] Life Sciences Group, Institut Laue-Langevin, 71 Avenue des Martyrs, 38000 Grenoble, France.
[c] IBS, 71 Avenue des Martyrs, 38000 Grenoble, France.
[d] Faculty of Natural Sciences, Keele University, Staffordshire, ST5 5BG, United Kingdom.
[e] ESRF, 71 Avenue des Martyrs, 38000 Grenoble, France.

INTRODUCTION

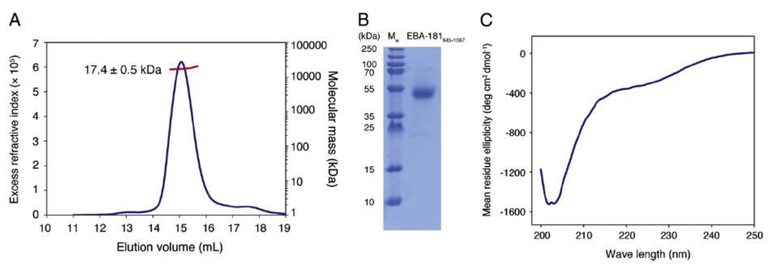
- ***Plasmodium falciparum*** **is responsible for the vast majority of malaria cases and deaths, especially in sub-Saharan Africa.**
- **Progress towards malaria elimination is hampered by the lack of an effective vaccine, the rapid development of drug-resistant parasites and insecticide-resistant *Anopheles* mosquito vectors, and insufficient knowledge of the biology of the parasite, in particular of the proteome and gene regulation.**
- **The array of sophisticated structural biology techniques available at ESRF and ILL provides a powerful tool to study critically important *P. falciparum* proteins and their interactions within the parasite.**

AIM

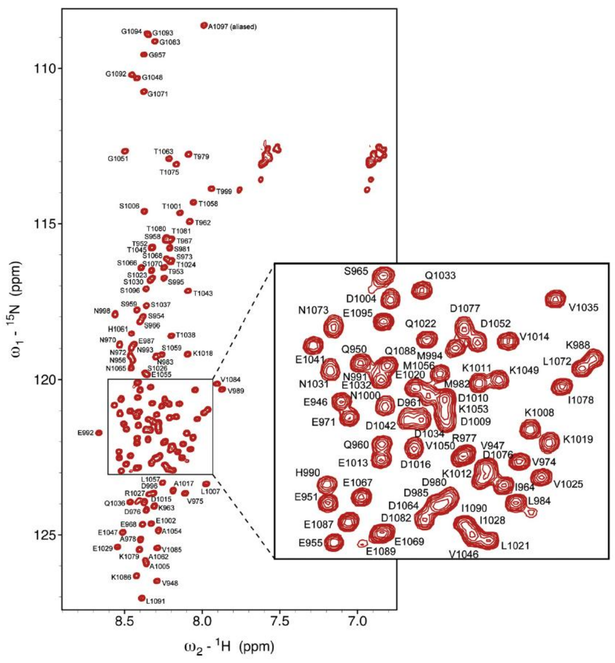
To characterise two potential therapeutic target proteins:

- **Erythrocyte Binding Antigen, EBA-181, which is essential for the invasion of erythrocytes**
- **PfMyb2, a DNA-binding protein and putative transcription factor.**

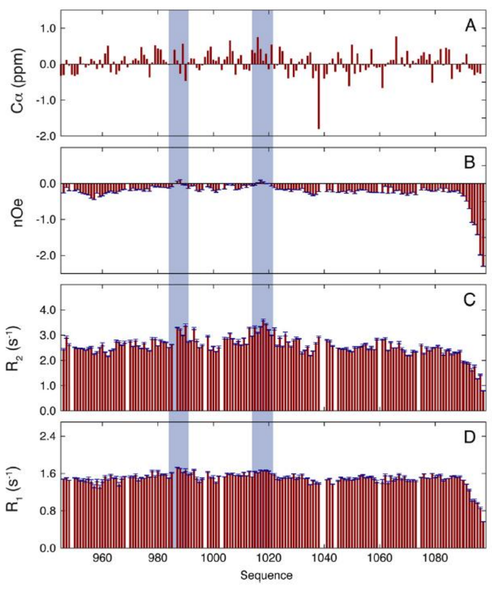
CHARACTERISATION OF EBA-181



Studies on EBA-181$_{945–1097}$ in solution
(A) Determination of the molecular mass **(B)** SDS-PAGE of purified protein **(C)** Far-UV CD spectrum



NMR studies of EBA-181$_{945–1097}$
The limited signal dispersion is characteristic of an intrinsically disordered protein



Structure and dynamics of EBA-181$_{945–1097}$
(A) Secondary Cα chemical shifts.
(B) {1H}–15N heteronuclear nOes.
(C) 15N R2 and **(D)** 15N R1 spin relaxation rates.
Blue shading indicates regions of the protein for which a rigidification is observed

Conclusion

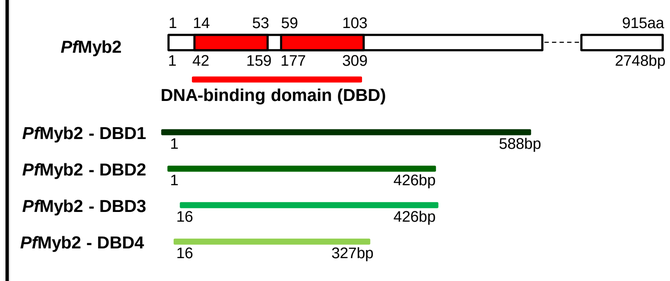
- **The RIII–V region of EBA-181 (EBA-181$_{945–1097}$) is an intrinsically disordered structure**
- **It is essentially a statistical coil with several turn motifs**
- **It does not possess transiently populated secondary structures commonly seen in intrinsically disordered proteins that fold via specific, pre-formed molecular recognition elements**
- **The binding region with its macromolecular receptor was identified**

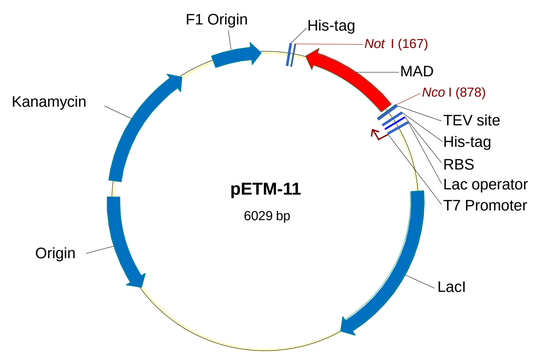
CHARACTERISATION OF PfMYB2

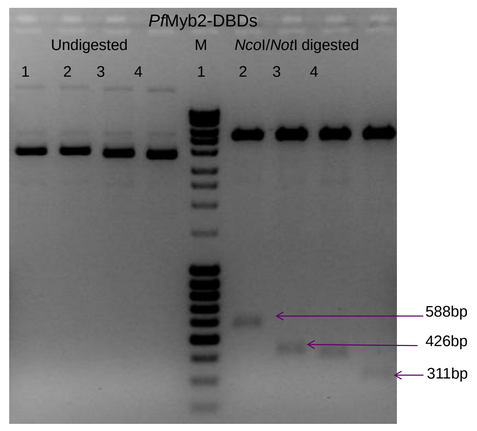
- **PfMyb2 has two DNA Binding Domains (DBD) at the N terminal**
- **It localises to the nucleus as demonstrated by indirect immunofluorescence**
- **Electrophoretic mobility shift assays (EMSAs) using recombinant PfMybDBD revealed binding to a consensus DNA sequence**
- **Gene knockout studies showed the gene is essential for parasite survival**

Cloning of PfMyb2 DBDs

- **Culture 3D7 *P. falciparum* parasites**
- **Extract DNA and amplify PfMyb2 DBDs using high fidelity PCR**
- **Ligate fragments into the *Nco*I and *Not*I sites of the pETM-11 vector**
- **Transform competent DH5α *E. coli***
- **Identifiy plasmid constructs by restriction digest analysis**
- **Verify accuracy of constructs by DNA sequencing**







Conclusion

- **Four PfMyb DBDs have been cloned into pETM-11**
- **This construct will be used to express deuterated PfMyb2 proteins**
- **The structure of the PfMyb2-DNA complex will be analysed**

FUTURE PROSPECTS

- **Envisaged future studies will focus on the role of structural biology in a malaria drug development pipeline.**
- **The interaction of lead anti-malarial compounds with their target proteins will provide valuable information to guide optimisation of the lead compounds**

REFERENCE
Manuel Blanc, Theresa L. Coetzer, Martin Blackledge, Michael Haertlein, Edward P. Mitchell , V. Trevor Forsyth, Malene Ringkjøbing Jensen. **Biochimica et Biophysica Acta** 1844 (2014) 2306–2314
Intrinsic disorder within the erythrocyte binding-like proteins from *Plasmodium falciparum*

National Research Foundation