Experimental Report

17/02/2015

Proposal: **8-03-827** **Council:** 4/2014

Title: A Neutron Scattering Solution Study of Chromatin Regulatory Proteins in the Human Malaria Parasite

This proposal is resubmission of: 8-03-794

Researh Area: Biology

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Experimental Team: JORDAN Ashley

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Samples: PfAlba3 (H- and D- forms)
PfSir2a (H- and D- forms)

Instrument	Req. Days	All. Days	From	To
D11	2	1	27/11/2014	28/11/2014

Abstract:

This proposal concerns the malaria parasite Plasmodium falciparum, investigating a key biomolecular interaction that is believed to be central to the way the parasite evades the human immune system. To do this, it expresses many virulence genes in a highly controlled manner. Our work focuses on protein-protein and protein-DNA interactions that regulate the expression of these genes. The P. falciparum virulence genes are controlled epigenetically, by histone marks that activate or silence their expression. A key player in this is the 'sirtuin' histone deacetylase enzyme, PfSir2a. The enzyme is unlikely to bind DNA directly, but was recently shown to interact with a small DNA-binding protein called PfAlba3. We will use SANS methods, together with selective deuteration, to determine the solution structures of the sirtuin-alba complex. The work will improve our understanding of the basic biology of the human malaria parasite and the impact of this biology on virulence.

Experiment 8-03-827 Preliminary Report:

A Neutron Scattering Solution Study of Chromatin Regulatory Proteins in the Human Malaria Parasite

Background:

Human malaria is caused by infection with the parasitic protozoan *Plasmodium falciparum* that causes widespread morbidity and results in approximately 600,000 deaths each year, predominantly young children in the sub-Saharan African region. Illness occurs via the cyclical infection of red blood cells, whereby the parasite multiplies inside these cells and exports a protein called *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) that modifies the cell surface membrane. Infected cells become sequestered in small blood vessels via PfEMP1 and this is a key virulence trait for the parasite. It is of great interest to malaria researchers to understand the mechanisms behind PfEMP1 gene control and expression.

A large family of `*var*' genes encode different variants of PfEMP1. Switching between variants to evade host immune responses is controlled by altering the structure of the encoding chromatin regions. This is known as 'epigenetic switching'. Currently our understanding of how these biological mechanisms work suffers from a lack of molecular-level information. Targets for structural characterization are the sirtuin enzyme, PfSir2a, and its putative binding partner, PfAlba3.

Experiments:

To investigate the nature of the interaction between PfSir2a and PfAlba3 using small angle neutron scattering (SANS) in solution we designed two sets of experiments. Initially set out to confirm the conditions in which the hydrogenated and deuterated forms of the proteins are matched out in solvent via a contrast variation series with an increasing ratio of D_2O. Calculations for the scattering length density give values of 1.74×10^{-6} Å^{-2} and 1.85×10^{-6} Å^{-2} for PfSir2a and PfAlba3 respectively. When the match point for each protein has been experimentally determined, we will measure the following:

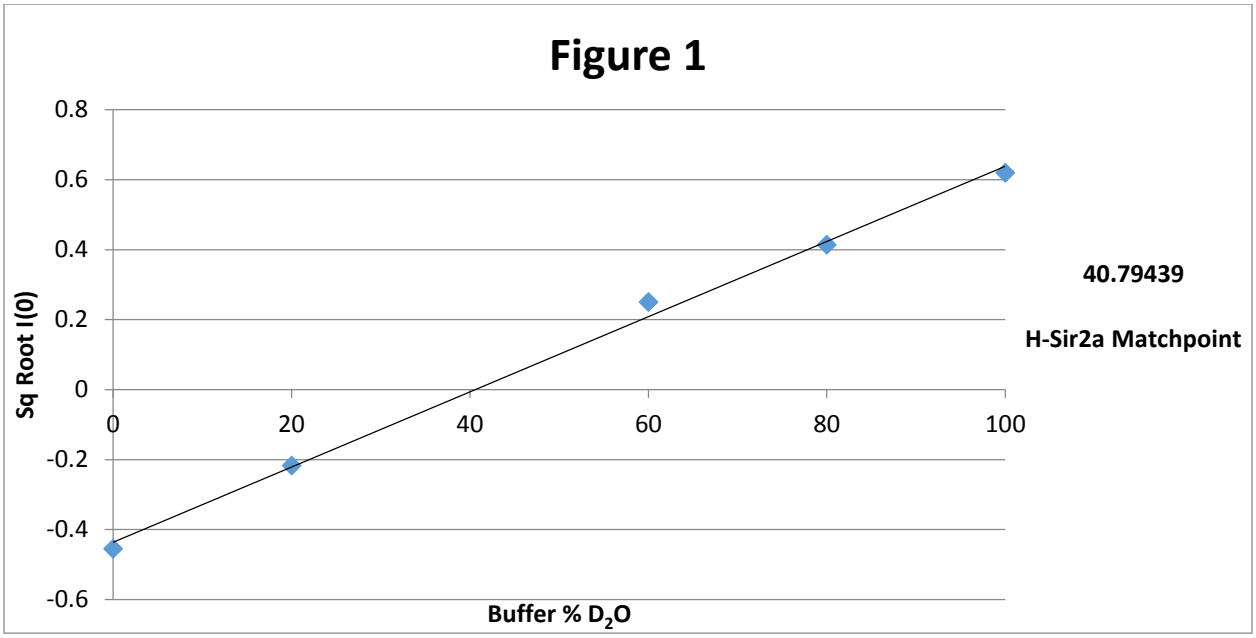
(i) <u>PfSir2a bound to d-PfAlba3 (43kDa) in match out solvent conditions.</u>

(ii) PfAlba3 bound to d-PfSir2a (43kDa) in match out solvent conditions.

Data will be measured in the Q-range 0.05-0.3Å^{-1} in order to carry out *ab initio* modeling for shape determination.

Results & Discussion:

The investigation so far has concentrated on the match point determination for PfSir2a and PfAlba3 in buffers containing different percentages of D_2O. This is necessary for producing conditions whereby one protein is 'matched-out' to enable visualization of the individual components whilst in complex. At a concentration of 3g/L we determined the match point of hydrogenated Sir2a to be at 40.79% D_2O (Figure 1). For deuterated Alba3 a concentration of 1g/L was used which resulted in low scattering intensity but we were able to confirm it was matched out in 100% D_2O conditions.



Future work will be required to investigate the PfSir2a-PfAlba3 protein complex using the experimentally determined match points mentioned here.