Experimental report

03/04/2020

Proposal: 8-03-988 **Council:** 4/2019

Title: Neutron crystallography, small-angle neutron scattering and neutron reflectometry on a bacterial adhesin

Research area: Biology

This proposal is a new proposal

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Samples: N-acetyl glycosamin-binding protein A
N-acetyl glycosamin-binding protein A (perdeuterated)
N-acetyl glycosamin-binding protein A domain 1 (perdeuterated)
Chitin coated silica plates
Beta-chitin
N-acetyl glycosamin-binding protein A domain 1

Instrument	Requested days	Allocated days	From	To
D11	2	2	21/09/2019	23/09/2019
FIGARO	3	0		
D17	3	0		
D33	2	0		
D22	2	0		
LADI	15	0		

Abstract:

Cholera is an ancient and deadly diarrheal disease that is caused by the pathogen Vibrio cholerae. The bacterium can easily survive in the ocean, where it binds to plankton and crustaceans. Upon ingestion of contaminated water or food, the bacteria colonize the human small intestine, where they secrete their major virulence factor, the cholera toxin. Currently, many of these processes are poorly understood. Insight into the underlying molecular mechanisms may help us to develop new medications, which are urgently needed given the increase in antibiotic resistance. This proposal targets the bacterial adhesin N-acetyl glucosamine binding protein A (GbpA) and its interaction with chitin. Methods used will be Small-Angle Neutron and X-ray Scattering, Neutron reflectometry and Neutron crystallography.

ILL report

In order to find the contrast match point for chitin, we measured chitin at 0 % D_2O, 20 % D_2O, 42 % D_2O, 66 % D_2O, 80 % D_2O and 100 % D_2O. At 42 % D_2O, the chitin was already quite well matched out, and from the data analysis we found the contrast match point to be at 47 % D_2O. Indeed, when measuring chitin nanofibers at 47 % D_2O, chitin was matched out except at very low q. Since hydrogenated proteins have a contrast match point around 44 % D_2O, this suggest the need for deuterated protein for interaction studies between GbpA and chitin.

In order to get a good reference measurement of chitin alone before the interaction studies with the protein, we also measured chitin at 100 % D_2O.

We also measured GbpA alone, using hydrogenated GbpA in D_2O, and deuterated GbpA in H_2O, D_2O and 47 % D_2O. These data confirmed that the deuterated GbpA strongly scatters at the chitin match point. These data will be used for an article we are writing on GbpA deuteration.

Chitin and deuterated GbpA were then measured together at 47 % D_2O at different molar ratios. The data showed approximately how GbpA bound to the fibers. However, the forward scattering could not be determined, hence limiting the amount of information that could be derived from the scattering.

We then diluted the chitin-GbpA sample in D_2O to the expected overall match points for the complex at low q. The results were similar to 47 % D_2O, suggesting that the complex does not have an overall match point.

Follow-up experiments should include varying salt concentrations, pH and temperature. Future experiments will also include GbpA homologues as well as the interaction of GbpA with other biologically relevant ligands.