Experimental report

18/02/2021

Proposal: 8-03-994 **Council:** 10/2019

Title: Small-Angle Neutron Scattering studies of bacterial colonization factors

Research area: Biology

This proposal is a new proposal

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Samples: chitin
Deuterated GbpA and homologous proteins
GbpA and homologous proteins

Instrument	Requested days	Allocated days	From	To
D33	2	0		
D22	2	0		
D11	2	2	18/08/2020	20/08/2020

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, which we plan to study with Small-Angle Neutron and X-ray Scattering.

This is a continuation of proposal 8-03-988, taking place in September (after proposal submission). After completion of the experiments with GbpA and chitin, we plan to extend the work to GbpA homologues from other bacteria and viruses.

The chitin match point was already established at an earlier beamtime (21/09/2019-23/09/2019), where we also measured the scattering of GbpA in the chitin-bound state at the chitin match point. However, the form factor of chitin-bound GbpA could not be deduced from these data, due to considerable aggregation of GbpA on chitin, yielding a significant structure factor in the data.

For the current experiment, we decided to measure mixtures of deuterated and non-deuterated GbpA with chitin at the chitin match point, with the aim to determine the structure of an isolated GbpA molecule in the complex. Since the match point of non-deuterated GbpA (≈44 % D_2O) is very close to the match point of chitin (≈47 % D_2O), we hoped to essentially match out both chitin and non-deuterated GbpA at the same time, and increase the distance between the strongly scattering deuterated GbpA, lowering the structure factor. We confirmed that non-deuterated GbpA in complex with chitin was well matched out, but the mixtures of deuterated and non-deuterated GbpA on chitin did not eliminate the structure factor we saw in the experiments with only deuterated GbpA on chitin. It just lowered the overall signal.

We also tried to increase the ratio of chitin to GbpA in one measurement, to decrease the level of crowding on chitin. This also did not eliminate the structure factor, however, the scattering at moderate q-values was significantly different in this case, which could be interesting for future modeling.