# Experimental report

13/09/2024

**Proposal:** 8-04-945 **Council:** 10/2022

**Title:** The influence of ligand binding onthe dynamics of bovine carbonic anhydrase

**Research area:** Biology

**This proposal is a new proposal**


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**Samples:** BCA I C20H10N2O4
ligand C6H6FNO2S
ligand C6H2F5NO2S

| Instrument | Requested days | Allocated days | From | To |
|---|---|---|---|---|
| IN16B | 4 | 3 | 14/04/2023 | 16/04/2023 |
| | | | 01/06/2023 | 02/06/2023 |


## Abstract:

Changes of protein conformational entropy ¿S_conf and hydration layers are relevant for ligand-binding. The protein-ligand binding is governed by equilibrium thermodynamics ¿G=¿H-T¿S, it occurs if ¿G is minimised. Previous work showed entropy-entropy compensation occurs between the protein streptavidin and its hydration layer. Another interesting system is bovine carbonic anhydrase II (BCAII). This protein has many compatible ligands. Two of these are 4-fluorobenzenesulfonamide (4-FBS) and pentafluorobenzenesulfonamide (PFBS). Analysing BCAII with 4-FBS and PFBS creates the opportunity to analyse different ligand binding affinities and their ¿S_conf. For this we aim to perform quasielastic neutron scattering experiments on BCAII in the ligand free and bound state for each ligand at different temperatures. We expect to see a negative ¿S_conf, as the ligand reduces the proteins flexibility

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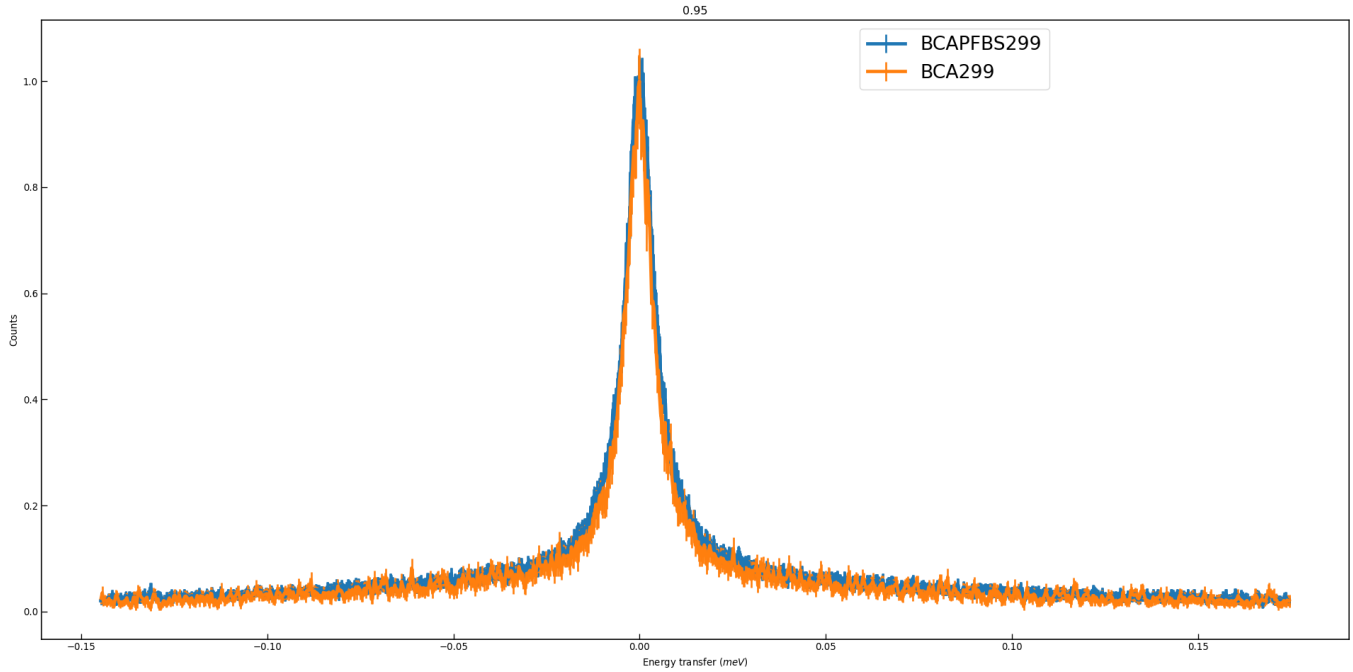
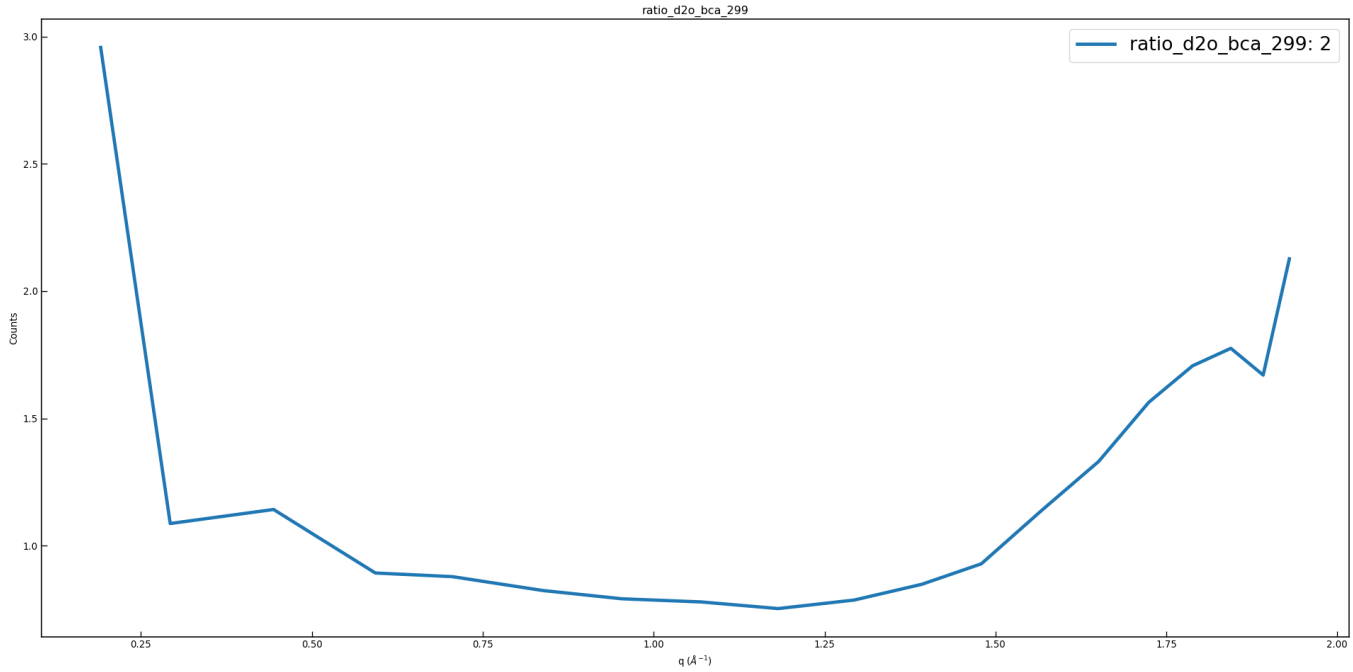
The experiment was performed on IN16B in BATS mode. Initially it was planned as a 2 day experiment. However, due to chopper problems during the experiment it was completed in two separate experiments. The experiments went well and apart from the chopper issues we encountered no technical issues. BCA was measured in buffer at 299 K and 309 K, it was also measured in a buffer with PFBS to ensure complex formation under the same conditions. The ligand free and ligand containing buffers were measured at each temperature as well.

During the experiment it was confirmed, that the presence of PFBS in the buffer alters the buffers dynamics, therefore the buffer and the buffer with the ligand were measured to allow for accurate data analysis.

The normalised spectra for free BCA and BCA liganded with PFBS indicate a change in dynamics between the spectra, with the data analysis still ongoing at this point.




For the analysis the ratio of the integrated intensity of the buffer and the proteins signal were compared in order to analyse the q-range where the coherent contribution is negligible.




This resulted in an accessible q-range of 0.59 $Å^{-1}$ – 1.29 $Å^{-1}$. The data will be fitter with 2 Lorentzians, from this the effective diffusion coefficient, internal dynamics, EISF and MSD will be determined and the change in conformational entropy calculated.

To back up the chosen model a Bayesian likelihood analysis was performed, this backs up the model chosen for the relevant q-ranges.