Experimental report

15/02/2021

Proposal: 8-03-1020 **Council:** 4/2020

Title: Structure and Dynamics of Huntingtin. A Segmental Labelling Approach

Research area: Biology

This proposal is a new proposal

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Samples: Huntingtin-H16

Instrument	Requested days	Allocated days	From	To
D22	2	1	03/02/2021	04/02/2021

Abstract:

Huntington's disease (HD) is a neurodegenerative disorder caused when the number of consecutive glutamines in the poly-glutamine (poly-Q) tract of the huntingtin exon1 (httex1) exceeds 35. In addition of the poly-Q, httex1 contains two poly-Proline (poly-P) tracts. Due to its flexibility and the low complexity of its sequence, the structural characterization of httex1 is challenging, and the structural bases of its pathological threshold remain poorly understood. In this project we will overcome these problems by applying contrast variation SANS experiments to multiple segmentally labelled versions of the protein. We will exploit the control of the amino acid composition offered by the Cell-Free protein synthesis in order to selectively deuterate httex1 repeats. By deuterating the repeats individually we will obtain information about their structural properties. When simultaneously deuterating both repeats we will probe the relative localization of the homopolymeric regions. The ensemble of SANS profiles will be combined with SAXS and NMR data in order to derive ensemble models of httex1 reporting on the overall shape of the protein and insights into the structural bases of HD.

Final Report Experiment 8-03-1020 (03/02/2021)

Structure and Dynamics of Huntingtin. A Segmental Labelling Approach

Background and Objectives:

Huntington's disease (HD) is one of nine hereditary neurodegenerative disorders caused by an expansion of CAG triplet repeats beyond a pathological threshold. For HD, this expansion is located in the first exon of the *huntingtin* gene and results in an abnormally long polyglutamine (poly-Q) tract within the N-terminus of the huntingtin protein (Httex1), which causes cytotoxicity [1]. The aim of the project is unveiling the structural mechanisms leading to this cytotoxicity. For that we are going to measure SANS data on Httex1 in which the long Poly-Q and Poly-P tracts will be segmentally deuterated. We expect that different deuteration patterns will provide complementary information about these regions, which are extremely challenging to study with other methods. This corresponds to the thesis project for an ILL PhD student (Xamuel Loft Lund) who is co-supervised by Anne Martel (ILL), Frank Gabel (IBS) and the main proposer (CBS).

Experiments:

The first experiments of the project were measured on the 3rd of February 2021 (8-03-1020) at the D22 beamline of the ILL. Sample deuteration patterns, concentrations, buffer composition, measuring modes and exposure times of the experiments performed are displayed in Table 1. The resulting profiles for the different samples are displayed in Figure 1.

Table 1. Description of the experiments performed

Sample	%D_2O	Concentration (mg/mL)	Measuring Mode	Exp. Time (min)	*Exp.* R_g (Å)	Theor. R_g (Å)
H-HttEx1	100	6.00	SEC-SANS	85	35.1	32.7
H-HttEx1	20	6.00	SEC-SANS	120	41.2	34.0
H-HttEx1, D-Pro	100	2.25	SEC-SANS	360	26.1	29.4
H-HttEx1, D-Pro D-Gln	100	0.82	Batch	30	30.0	20.3
H-HttEx1, D-Pro D-Gln	20	0.82	Batch	120	48.5	37.3

Due to the short time between the measurement and the submission of this report, only preliminary analyses of the raw data have been done. From these analyses we can extract several conclusions:

- Our Cell-Free protein production strategy is able to provide segmentally labelled samples for SANS.

- Aggregation is observed in our measurements in batch mode. Note that HttEx1 is an aggregation-prone protein. Therefore, SEC-SANS should be privileged, even if the signal to noise is lower. In that sense, **H-HttEx1, D-Pro D-Gln** will have to be measured again with after reaching higher concentrations.

- Some of the samples provided good data that will be useful for subsequent structural analyses. However, other did not provide high quality data and will have to be repeated in subsequent visits to the ILL (H-HttEx1 in 20% D_2O).

- As expected, the R_g values change depending on the deuteration pattern and the buffer composition. Importantly, the resulting values for good samples are in all cases similar to these obtained by SAXS and these derived from simulated curves for the same experimental conditions.

- We have compared the experimental curves with these computed from atomistic ensembles based on NMR data in the different experimental conditions (deuteration pattern and buffer composition) [2]. Note that it is just a comparison and not a fit. From this comparison we can conclude that the structural model based on the NMR data is in broad agreement with the experimental curves. However, some difference are observed, suggesting that the SANS data contain information about the structural features that are not captured by the NMR data.

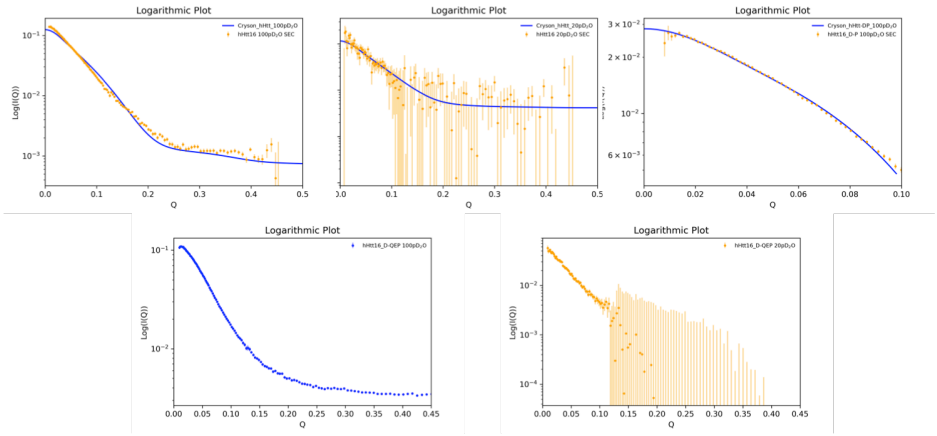

Figure 1: SANS profiles measured for (top, from left to right) H-HttEx1 (100% D_2O), H-HttEx1 (20% D_2O) and H-HttEx1, D-Pro (100% D_2O) and (bottom, from left to right) H-HttEx1, D-Pro D-Gln (100% D_2O) and H-HttEx1, D-Pro D-Gln (200% D_2O). Solid lines in the top panels correspond to comparison to the theoretical curves of HttEx1 in the same experimental conditions.

Future experiments:

- Higher concentrations must be achieved for samples with deuterated glutamines to improve the signal to noise in the SEC-SAXS experiments. An optimization of the Cell-Free protein sysnthesis conditions is envisaged [3]. With the expected increase in concentration, some of the samples will be remeasured.

- Other deuteration patterns will be measured in order to improve the structural description of Httex1. Concretely the fully deuterated sample at low D_2O percentage will be interesting.

- The simultaneous analysis of multiple curves measured along several visits using Ensemble Optimization Method (EOM) should provide a better definition of the structural determinants of Httex1 [4,5].

References

[1] Walker, F. O. *Lancet* **369,** 218 (2007).
[2] Urbanek, A. *et al. Structure.* **28**, 733-746 (2020).
[3] Morató, A. et al. *Biomolecules*, 10, 1458 (2020).
[4] Bernadó, P. *et al. J. Am. Chem. Soc.* **129**, 5656-5664 (2017).
[5] Yabukarski, F. *et al. J. Mol. Biol*. **428**, 2671-2694 (2016).