

Experimental report

06/09/2022

Proposal: 8-03-1050

Council: 4/2021

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

Research area: Biology

This proposal is a continuation of 8-03-1020

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Samples: Huntingtin Exon-1

Instrument	Requested days	Allocated days	From	To
D22	2	2	15/09/2021	17/09/2021

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.

Experiment 8-03-1050 Report

14. – 16. September 2021 performed at D22, ILL.

Introduction and objectives

Huntington's Disease (HD) is a genetically inheritable disease caused by mutations in the gene encoding the protein Huntingtin (Htt). The manifestation of disease symptoms caused by an abnormal increase in the number of CAG trinucleotides in the N-terminal exon-1 (HttEx1) that causes an elongation of the poly-glutamine tract (Poly-Q) of the protein. Individuals with more than 35 consecutive glutamines develop the disease. The age of onset and severity are correlated with the Poly-Q length [1,2].

In order to further investigate the overall structure of HttEx1, we combine small angle x-ray scattering (SAXS) and small angle neutron scattering (SANS) data using ensemble fitting with atomistic models in order to elucidate differences between the non-pathogenic (Htt16-16 glutamines in the Poly-Q) and the pathogenic (Htt36-36 glutamines in the Poly-Q) forms of the protein. Note that, in addition of the Poly-Q, HttEx1 also contains a Proline Rich Region (PRR) (figure 1).

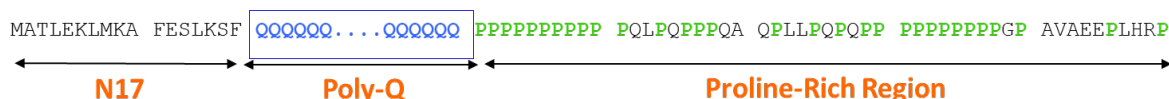


Figure 1: Sequence of the Huntingtin Exon-1 indicating the three regions of the protein. The N17 N-terminal, The poly-Q tract and the proline rich region.

Our protein constructs are C-terminally fused to super folder green fluorescent protein (sfGFP) and a His-tag to improve sample stability and purification. Cell-free expression is used for protein preparation, enabling an excellent control of the amino acid composition (hydrogen/deuterium) of the protein and perform contrast variation SANS experiments [3]. We have developed a strategy to combine SAXS and SANS data measured for HttEx1 with different deuteration patterns using the ensemble optimization method (EOM) [4]. The objective of this beamtime was to obtain additional measurements of segmentally labelled samples of both Htt-Q16 and Htt-Q36 to incorporate in our data analysis strategy.

Measurements

Previous experiments (8-03-1020 & 9-13-984) have shown that the SEC-SANS setup is preferred to prevent aggregation during measurements.

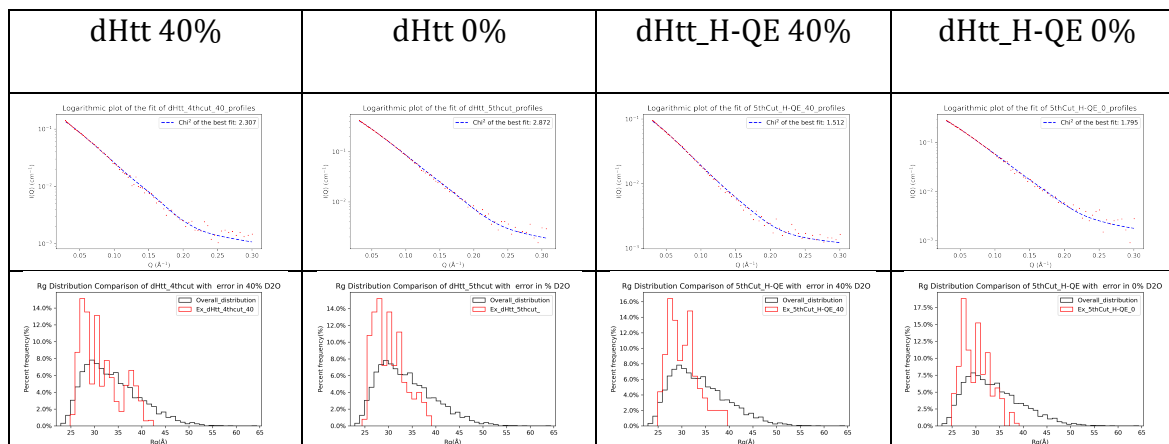
At the 8-03-1050 experiment six samples were measured (Table 1). By removing a few initial points, the measured R_g s were very similar to those of the simulated SANS profiles. Each of the experimental profiles was evaluated by fitting a sub-ensemble of 50 conformations selected from a large pool of atomistic models.

Table 1: List of samples measured at the 8-03-1050 experiment. Initial h or d indicates protonation or deuteration.

Sample	%D ₂ O	Concentration (mg/mL)	Measuring Mode	Exp. Time (min)	Exp. R_g (Å)
dHtt(16Q)	40	4.8	SEC-SANS	68	31.8
dHtt(16Q)	0	4.8	SEC-SANS	125	30.2
dHtt_H-QE(16Q)	40	4.5	SEC-SANS	70	30.8

dHtt_H-QE(16Q)	0	4.5	SEC-SANS	67	30.3
hHtt(36Q)	100	2.3	SEC-SANS	81	32.6
hHtt_D-QE(36Q)	100	1.7	SEC-SANS	480	27.0

Table 2: Experimental profiles measured for Htt16 at the 8-03-1050.



When using the experimental data to optimize an ensemble of atomistic structures the range of R_g values selected (red distribution) is narrower than the initial pool of structures (black distribution).

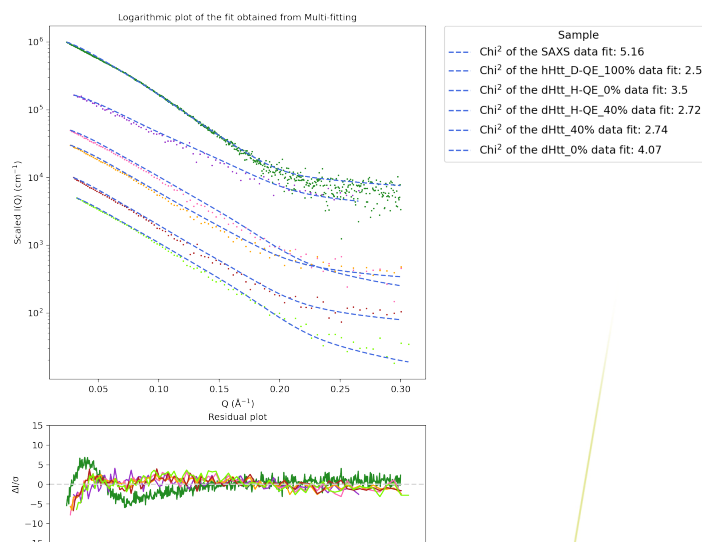
Simultaneous fitting of datasets

The average χ^2 -value varies depending on which profiles are included in the multiple fitting. By plotting the 8th combination comprising SAXS data and 5 SANS profiles (Fig. 3) the fit looks correct, but some disagreement is observed at low angles.

Sample	Cumulative (Average) χ^2	SAXS χ^2	hHtt χ^2	D-QE χ^2	H-QE 40% χ^2	H-QE 0% χ^2	dHtt 40% χ^2	dHtt 0% χ^2
Singular fit		0.99	3.18	2.07				
1	4.07 (2.04)	1.33		2.75				
2	13.42 (6.71)	6.18	7.24					
3	8.02 (2.67)	1.94		2.58	3.49			
4	12.57 (3.14)	3.10		2.44	2.92	4.11		
5	15.78 (3.16)	3.67		2.47	2.80	3.85	2.99	
6	19.96 (4.99)	6.20	7.74		3.05		2.96	
7	19.49 (4.87)	6.50	7.40	2.52	3.06			
8	20.75 (3.46)	5.16		2.57	2.72	3.50	2.74	4.07
9	7.64 (2.55)			2.40	2.35	2.89		
10	20.62 (3.44)		5.37	2.58	2.64	3.20	3.21	3.62
11	13.82 (2.76)			2.69	2.40	2.99	2.60	3.15

Table 3: Simultaneous fitting of multiple SANS profiles. Average χ^2 per number of curves used in fitting changes depending on chosen profiles.

Figure 2: Plot showing the 8th combination from Table 3. The initial points of the SAXS (green) data deviates from the ensemble profiles according to the residual plot.



References

- [1] Zuccato et al. *Physiol Rev.* 2010;90(3):905–81.
- [2] Saudou et al. *Neuron* 2016;89(5):910–26.
- [3] Urbanek et al. *Angew Chem Int Ed Engl.* 2018, 57(14):3598–601.
- [4] Bernado et al. *J Am Chem Soc.* 2007 May 1;129(17):5656–64.