Experimental report

06/09/2022

Proposal: 8-03-997 **Council:** 10/2019

Title: Contrast Variation SANS Experiments to Differentiate Shape Profile ofGlyco- and Protein-Part of HIV-1 gp141 Envelope Trimer -/+ CD4 or mAb

Research area: Biology

This proposal is a new proposal

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Experimental team: Amin SAGAR

Local contacts: Lionel PORCAR

Samples: Glycoprotein GP120
Human protein CD4
GP120 binding antibody

Instrument	Requested days	Allocated days	From	To
D22	6	1	24/08/2020	25/08/2020

Abstract:

Being a heavily glycosylated protein in its native state, HIV-1 gp120/41 envelope protein is still poorly understood by biophysical methods. Since it is key to both viral entry and replication, and a candidate for practical vaccine against HIV-1, clear understanding of how it is folded and binds to its receptor CD4 or antibodies is highly sought. By acquiring SANS data at different deuteration contrast points, we aim to resolve which portions of trimeric assembly are sugar and protein and how they are correlated in shape. Also, data will be obtained to refine what shape changes occur in solution when the protein core opens-up to bind receptor or antibody. These are first of their kind experiments, and any success would be a big achievement in structural biology of HIV entry mechanism or vaccine design understanding.

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 correleated with the Poly-Q length [1,2].
In order to further investigate the ovearall 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 sontains a Proline Riich Region (PRR) (figure 1).

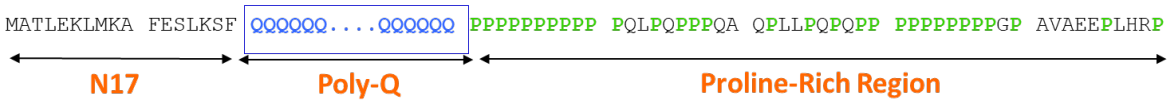


Figure 1: Sequence of the Huntingtin Exon-1 indicating the three regions of the protein. The N17 N-terminal, The poly-Q tract and the proline rich region.

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

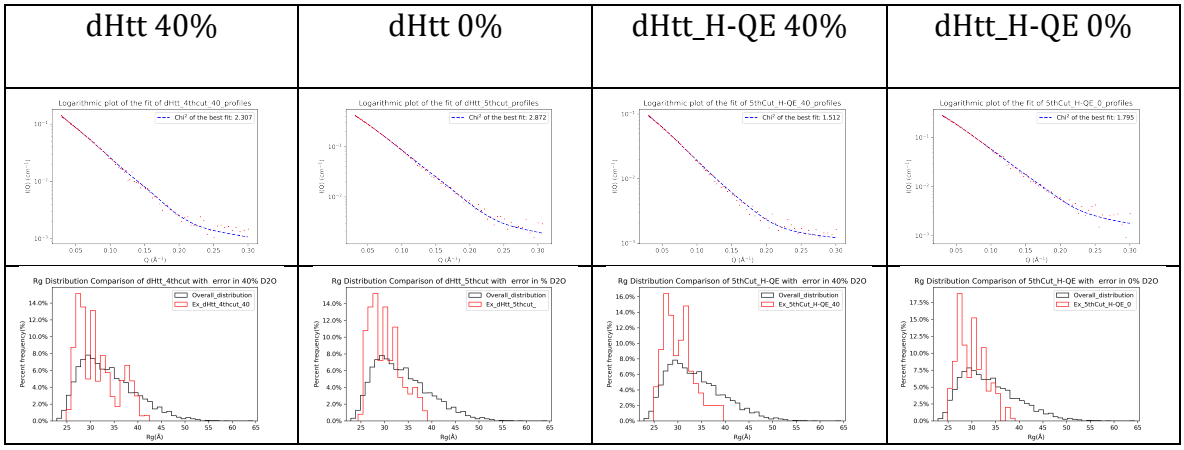
Previous experiments (8-03-1020 & 9-13-984) have shown that the SEC-SANS setup is preferred to prevent aggregation during measurements.
At the 8-03-1050 experiment six samples were measured (Table 1). By removing a few initial points, the measured R_gs were very similar to thse of the simulated SANS profiles. The 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_2O	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

he 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) chi²	SAXS chi²	hHtt chi²	D-QE chi²	H-QE 40% chi²	H-QE 0% chi²	dHtt 40% chi²	dHtt 0% chi²
	Singular fit	0.99	3.18	2.07	2.20	2.75	2.31	2.84
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.

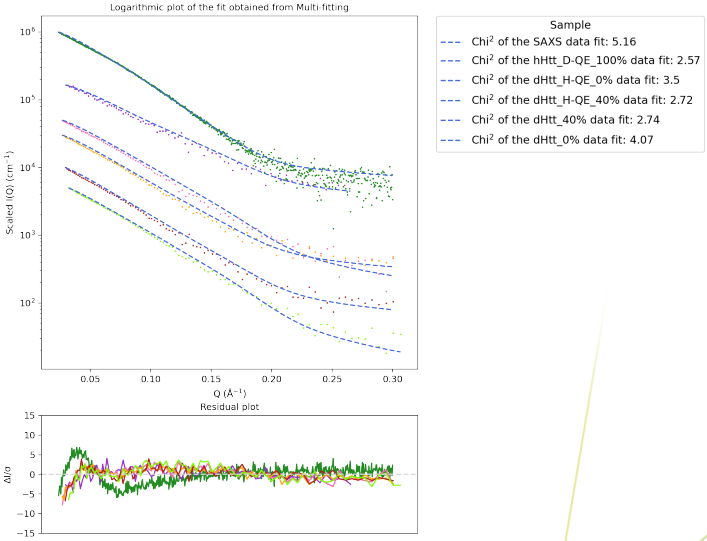


Figure 2: Plot showing the 8th combination from Table 3. The initial points of the SAXS (green) data deviates from the ensemble profiles according to the residual plot.

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.