**Project:**

Biophysical investigation of purified HTT protein samples

**Experiment:**

A time-course analysis using Differential Static Light Scattering (DSLS) of purified HTT1-3144 Q23

**Date completed:­**

2019/01/28

**Rationale:**

Time and resources in the HD field have been primarily focussed on understanding HTT aggregation looking as caspase cleavage products spanning aa. 1-586 or exon 1 spanning aa. 1-90. However, we know that HTT protein purified in its apo form is able to self-associate into larger oligomeric species and that monomer, dimer and larger species are found following FLAG-affinity chromatography as determined by size-exclusion chromatography (SEC) and SEC-multi-angle light scattering (SEC-MALS). This experiment aimed to begin to investigate how HTT self-associates and aggregates over time in a range of different conditions.

**Previous work:**

Two conditions have been previously tested by other groups which show HTT does not aggregate until 14 days or 24 days respectively.

|  |  |  |
| --- | --- | --- |
|  | Condition #1 | Condition #2 |
| Temperature | 4°C. | 37°C. |
| Time | Up to 14 days | Up to 24-30 days |
| Concentration | 0.56 μM | 2.28 μM |
| Buffer | 50 mM Tris, 500 mM NaCl, 5 mM DTT, 5% glycerol, 0.5% CHAPS, pH 8 | 50 mM Tris, 220 mM NaCl, 0.2 mM TCEP, 1% glycerol, 0.1% CHAPS, pH 8 |

NB: PolyQ length, solution volume and method of assessing protein aggregation not yet disclosed.

Previously assessed aggregation of full-length HTT as a function of temperature using DSLS <https://zenodo.org/record/1248163>. A Tagg ~ 55-60˚C was calculated for all Q-lengths tested and did not change with HAP40-bound HTT samples. These experiments were run in 20 mM HEPES pH 7.5, 300 mM NaCl, 5 % (v/v) glycerol, 1 mM TCEP with protein at ~1 mg/mL.

**Experimental approach:**

Plate set up and analysis:

* 20 mM HEPES pH 7.5, 5 % (v/v) glycerol, 1 mM TCEP with HTT1-3144 Q23 protein at ~1 mg/mL (2.5 μM) with either 250 mM NaCl, 500 mM NaCl, 250 mM KCl, 500 mM KCl. Also run conditions #1 and #2.
* Experiment run with 4 replicates for each buffer condition.
* Using Epiphyte Stargazer instrument, collect an image every ~3 hours and maintain plate at 25˚C







##### DSLS plots of light scattering (measured in arbitrary units) over a 14 day time-course of different conditions, each run with 4 repeats – Y-axis scaled to best represent each plot

****

****

****

##### DSLS plots of light scattering (measured in arbitrary units) over a 14 day time-course of different conditions, each run with 4 repeats – Y-axis scaled 0-1000 AU for each plot

**Conclusions:**

* In all conditions, sample stable for minimum of 2-3 days
* Well-to-well variation across replicates very high – reflect well variations e.g. dust/addition of mineral oil
* CHAPS in buffer gives a replicate consistent and linear increase in scatter over time
* Conditions with KCL and no CHAPS show the greatest protein stability with all conditions (excluding row 4 run 4 which shows anomalous high light scattering from day 0, indicative of well contamination with dust etc, common issue with this methodology) showing no protein aggregation for at least 5 days.

**Next steps:**

* Repeat with additional filter steps to ensure reduced dust/particulate matter in samples and plate
* Protease inhibitors to remove HTT proteolysis related protein aggregation