

Differential static light scattering (DSLS) of full-length huntingtin samples of different polyQ lengths (Q19, Q23, Q42 and Q54) – 2018/05/09

Rationale

To investigate how increased polyQ length of the huntingtin protein affects its biophysical properties.

Experimental set up and data analysis

Full-length huntingtin protein samples purified from baculovirus expression system sf9 insect cell production were used at ~1 mg/mL (NB: use Expassy Protparam A₂₈₀ correction – 1 mg/mL reads at 0.78 mg/mL) in 20 mM HEPES pH7.4, 300 mM NaCl, 5 % (v/v) glycerol, 1 mM TCEP.

40 µL each protein solution was transferred to wells in black 384-well clear-bottom square-welled plate. For each Q-length, 5 repeat wells were set up with blank wells inbetween.

Each protein-containing well was topped with ~40 µL mineral oil.

The plate was spun at 1000 rpm for 1 min in bench-top centrifuge.

Images were taken every 0.5 °C from 25-85 °C in a StarGazer <https://www.epiphyte3.com/Stargazer-2> and then images were analysed using Intensity and data was subject to regression analysis via BioActive.

Results

Full intensity data and regression results can be seen in file: HTT_20180503.xls

In the table below is a summary of the regression data with mean and standard deviations (s.d.) calculated for the 5 replicates.

Start scatter intensity = A1 (arbitrary units)

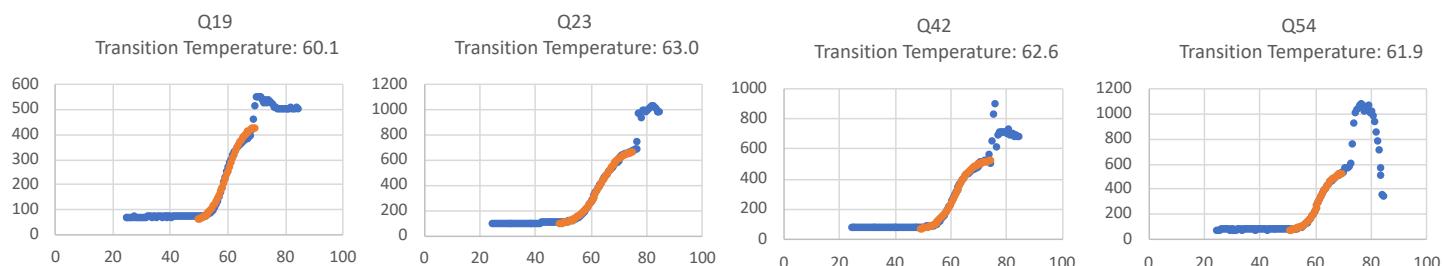
End scatter intensity = A2 (arbitrary units)

Slope = dx

Mid-point of regression = Tagg (temperature of aggregation in °C)

	Start scatter intensity mean	Start scatter intensity s.d.	End scatter intensity mean	End scatter intensity s.d.	Slope dx mean	Slope dx s.d.	Tagg (°C) mean	Tagg (°C) s.d.
Q19	68.6	18.1	669.4	347.7	2.3	0.3	60.1	0.2
Q23	116.0	30.9	993.3	229.9	3.3	0.2	63.0	0.4
Q42	50.1	6.8	739.8	150.7	3.1	0.2	62.6	0.5
Q54	60.1	8.0	722.5	118.7	2.4	0.1	61.9	0.3

Exemplary plots with regression fits can be seen on the next page with the average Tagg values in the titles.



Conclusions

There does not appear to be any correlation in increasing Q-length with start or end scatter intensity values, dx or Tagg. This probably reflects the large solenoidal structure of huntingtin which dominate the heat-dependent aggregation being measured in this assay. Effects of the polyQ length of the huntingtin protein on full-length in vitro protein aggregation are likely more subtle.