**Cryo-EM HTT Q23 (+/- DNA) sample generation (2017/06/05)**

**Previous work:**

HTT Q23 was shown to bind a 32 bp FAM tagged oligo in a concentration dependent manner <https://zenodo.org/record/801606>



C12 – dsDNA oligo tagged with 6-FAM

5’-FAM-TCTTCTGGTCCGGATGGTAGTTAAGTGTTGAG

5’-FAM-CTCAACACTTAACTACCATCCGGACCAGAAGA

Additionally, EM data suggested that the sample was too flexible to yield high resolution structural data <https://zenodo.org/record/569282>

Grafix treatment of the HTT sample was found in top ~1/4 of tube, meaning that the protein sample was not exposed to large amounts of glutaraldehyde. Additionally, the glutaraldehyde used in previous experiments was not EM grade.

**1st June 2017:**

To evaluate how stronger cross-linking might affect the sample the following procedure was followed:

6 x 30 mL gradients of 5-15 % (w/v) sucrose, 0-0.2 % (v/v) glutaraldehyde (EM grade, Sigma - G5882) in 20 mM Hepes pH 7.4, 300 mM NaCl were poured.

~300 µg of HTT1-3144 Q23 (TOC009:C01) (860 pmol) was applied each to tubes 1, 2 and 3.

~300 µg of HTT1-3144 Q23 (TOC009:C01) (860 pmol) incubated with 40 µg of C12 oligo (~1700 pmol – 2:1 DNA:protein ratio) was applied each to tubes 4, 5 and 6.

Tubes spun in SW32-Ti for 18 hours, 4 ºC, 28,000 rpm (~ 100,000xg average compared to 75,000xg used previously at 25,000 rpm).

**2nd June 2017:**

Each tube fractionated into ~15 x 2 mL fractions. 30 µL fraction + 10 µL 4 x loading dye run on 4-20 % Tris-Glycine SDS-PAGE at 200 V, 2 hours.



Fractions 6-9 pooled from tubes 1-3 and 4-6, then buffer exchanged into 50 mM Tris pH 7, 150 mM NaCl, concentrated and flash frozen in liquid nitrogen. ~ 30 µL @ 2 mg/mL each. Samples shipped to Susan on dry ice.

EM examination of this sample will be needed to assess whether the cross-linking conditions have affected the protein sample. However, as sample found in half-way point of tube, can expect greater cross-linking.

Samples:

A – 4 µL 8 µM C12 oligo (FAM labelled) + 1 µL 6 x loading dye supplemented 1:10000 cybrsafe

B – 10 µL 2 mg/mL concentrated HTT Q23-C12 grafix treated complex + 2 µL 6 x loading dye supplemented 1:10000 cybrsafe

C – 10 µL 2 mg/mL concentrated HTT Q23 grafix treated + 2 µL 6 x loading dye supplemented 1:10000 cybrsafe

Samples run on 1.5 % (w/v) agarose-TAE gel at 135 V for 20 minutes in 0.5 % TAE buffer.

Visualisation of the gel shows a clear band for the C12 oligo in lane A. Lane B shows a gel shifted DNA species, not resolved to a single band. No shift can be seen for the control sample grafix’d without DNA.



This suggests that the HTT Q23 sample is bound to the C12 oligo in some fashion. EM examination of this sample will be needed to assess whether DNA is bound in a physiologically relevant and homogenous manner.