

**Project:**

Biophysical investigation of purified HTT protein samples

**Experiment:**

Mass spectrometry analysis of contaminating band in HTT samples

**Date completed:**

2019/10/31

**Rationale:**

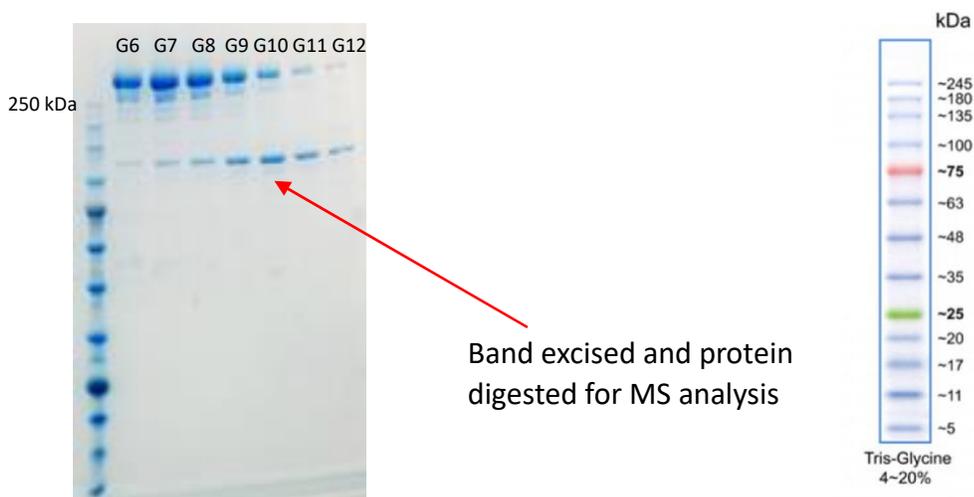
To determine the identity of ~100 kDa band seen on SDS-PAGE in HTT preps – see

<https://zenodo.org/record/3555378>

**Experimental approach:**

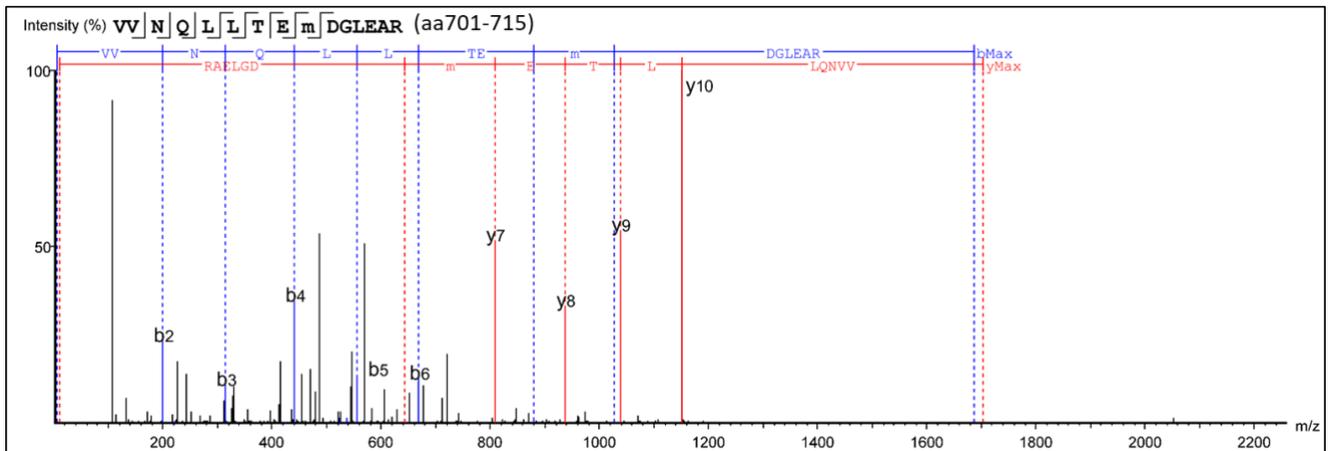
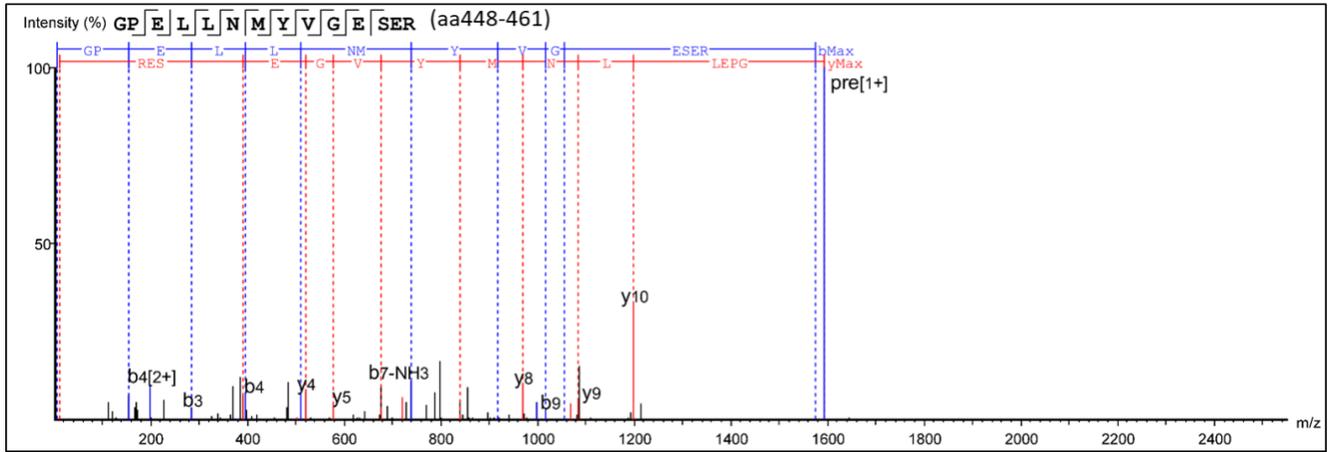
A contaminating band in HTT purifications was identified in previous experiments e.g.

<https://zenodo.org/record/3555378>



Both the excised band and solution protein sample from this purification were processed for bottom up proteomics using the procedure described in Harding et al. [doi: 10.1074/jbc.RA118.007204].

Two peptides corresponding to NVL (Nuclear Valosin-containing protein-like) were identified (<https://www.uniprot.org/uniprot/O15381>) with high confidence (and mass accuracy < 10 ppm). However, the confidence in identifying this protein is low due to the poor sequence coverage. An overview of the protein family may be found here<sup>1,2</sup>.



```

1  MKPRPAGFVD NKLKQRFVIQY LTSNKCQKYV DIGVLASDLQ RVYSIDYGRR KRNAFRIQVE KVFSIISSEK ELKNLTELED
81  EHLAKRARQG EEDNEYTESY SDDSSMEDY PDPQSANHMN SLLSLYRKG NPDSVSNTPE MEQRETTSSST PRISSKTGS I
161 PLKTPAKDSE GGWFIDKTPS VKKDSFFLDL SCEKSNPKKP ITEIQDSKDS SLLSDMKRK GKLKNKGSKR KKEDLQEV DG
241 EIEAVLQKKA KARGLEFQIS NVKFEDVGGN DMTLKEVCKM LIHMRHPEVY HHLGVVPPRG VLLHGPPGCG KTL LAHAIAIG
321 ELDLPILKVA APEIVSGVSG ESEQKLRELF EQAVSNAPCI IFIDEIDAIT PKREVASKDM ERRIVAQLLT CMDDLNNVAA
401 TARVLVIGAT NRPDSLDPAL RRAGRFDREI CLGIPDEASR ERILQTLCRK LRLPQAFDFC HLAHLTPGFV GADLMALCRE
481 AAMCAVNRVL MKLQEQQKKN PEMEDLPSKG VQEERLGTEP TSETQDELQR LLGLLRDQDP LSEEQMQGLC IELNDFIVAL
561 SSVQPSAKRE GFVTPVNV TW ADIGALEDIR BELTMAILAP VRNPQFKAL GLVTPAGVLL AGPPGCGKTL LAKAVANESG
641 LNFISVKGPE LLNMYVGESE RAVRQVFQRA KNSAPCVIFF DEVDALCPRR SDRETGASVR VVNQLLT709EMD715GLEARQQVFI
721 MAATNRPDII DPAILRPGRL DKTLFVGLPP PADRLAILKT ITKNGTKPPL DADVNLEAIA GDLRCD709CYTG ADLSALVREA
801 SICALRQEMA RQKSGNEKGE LKVSHKHFEF AFKKVRS709SSIS KKDQIMYERL QESLSR

```

1 <http://www.jbc.org/content/early/2019/04/21/jbc.RA119.007585.full.pdf>  
2 <https://jcs.biologists.org/content/joces/early/2014/08/18/jcs.093831.full.pdf>