Large-scale full-length HTT Q23 purification from HEK293T – 2018/04/30

Rationale:

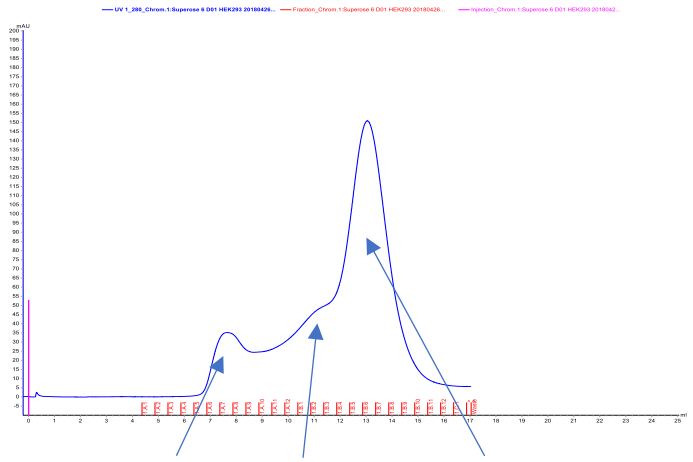
Previously, small scale expression of constructs TOC009-D01, D02, D04 and D06 (Q23, Q54, Q78 and Q145 respectively) was completed https://zenodo.org/record/1239023 and showed good expression for the 2 shorter polyQ-length huntingtin constructs. Next this protocol was scaled for large scale purification of huntingtin Q19 and Q42 and was successful https://zenodo.org/record/1239789. Now the protocol is scaled for Q23.

Protocol:

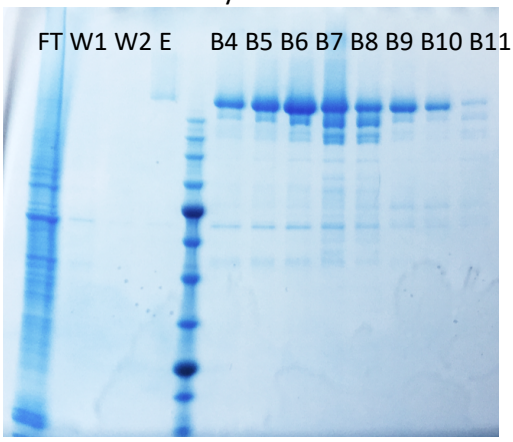
~2000 mL HEK293T production for TOC009-D01 (HTT^{1-3144} pBACMAM C-terminal FLAG – Q23) – completed with baculoviral transduction protocol by Alma Seitova https://zenodo.org/record/1194724. Cells were harvested by centrifugation at 1500 rpm, 30 mins at 4 ˚C in a benchtop centrifuge and were stored at -80 ˚C prior to purification.

Cell pastes were thawed and resuspended in ~400 mL 50 mM Tris pH 8, 500 mM NaCl supplemented with 1 x protease inhibitors supplemented with benzonase. NB: freeze-thaw cycle for cells is sufficient for lysis. The lysate was clarified by centrifugation and then bound to 5 mL anti-FLAG resin (Sigma M2) at 4 ˚C with rocking for 2 hours. Resin was washed with 800 mL 50 mM Tris pH 8, 500 mM NaCl. HTT protein was eluted with ~15 mL resuspension buffer supplemented with 200 µg/mL 3xFLAG peptide. The sample was concentrated to 1 mL and run on Superose6 column. Samples were run on SDS-PAGE 4-20 % tris-glycine gel of the purification process.

Superose6 elution profile



4-20 % Tris-Glycine SDS-PAGE



Comments

Peak shape from elution is the typical huntingtin distribution. Yields very good and samples clean.

➔ 5.0 mg/mL – 18 x 20 µL aliquots. Some degradation of sample visible on SDS-PAGE.