Huntingtin Exon 1 Q16 and Q46 pET32a thioredoxin tagged expression and purification 2018/04/02

Rationale:

To express and purify huntingtin exon 1 protein samples to investigate DNA binding properties of these portions of the huntingtin protein.

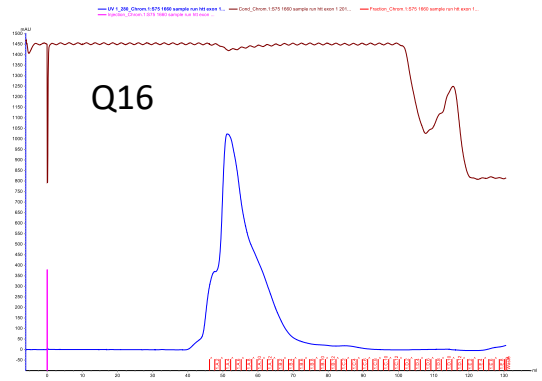
Constructs:

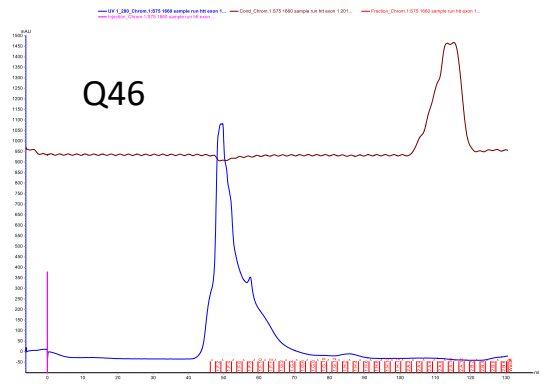
Huntingtin exon 1 Q16 https://www.addgene.org/11487/ and huntingtin exon 1 Q46 https://www.addgene.org/11515/ from Bennet et al (2002) PNAS, Pubmed ID: 12193654.

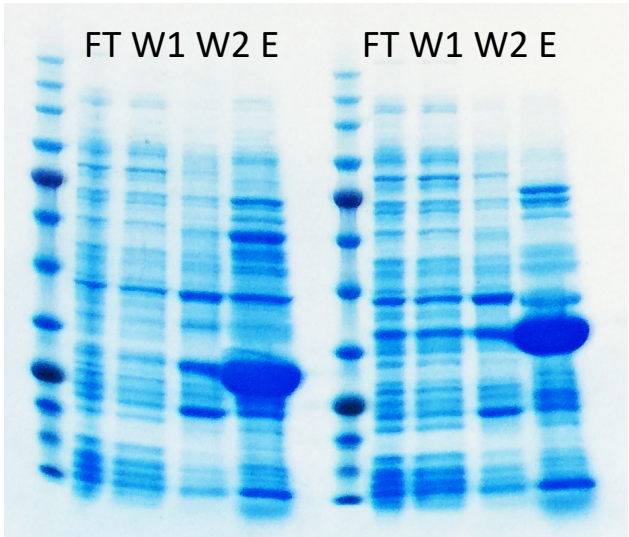
Protocol:

12 L each LB culture grown of each exon 1 construct in LEX system at 37 ˚C until OD_{600} ~ 0.8 then induced with 1 mM IPTG and grown at 25 ˚C. Cells were harvested by centrifugation and then the pastes stored at -80 ˚C prior to purification.

Cell pastes were resuspended in 500 mL 50 mM Tris pH8, 300 mM NaCl, 5% glycerol, 1mM TCEP, supplemented with benzonase and 1 µM PMSF and 1 µM benzamidine. The lysate was clarified by centrifugation at 15000 rpm in JLA16.250 for 60 mins, supplemented with 5 mM imidazole and then bound to 5 mL Ni-NTA resin per lysate at 4 ˚C with rocking for 1 hour. Resin was washed with 500 mL lysis buffer supplemented with 25 mM imidazole. Exon 1 proteins were eluted with ~20 mL lysis buffer supplemented with 300 mM imidazole. The sample was concentrated to 5 mL and run on S75 16/60 in 50 mM Hepes pH 7.4, 500 mM NaCl.







Next steps:

Another 12 L cells grown to repurify and follow the buffer conditions as per the protocol detailed in PNAS manuscript.