Production and Purification of GAPDH

Jacob McAuley

June 31, 2019

Objective:

Huntington's Disease (HD) is a hereditary neurodegenerative disease. The cause of this disease is a CAG repeat extension in the HTT Gene. This extension is then translated into an elongation of axon 1 which is primarily composed of a disordered PolyQ repeat. Although the cause is known, the mechanism by which this extension affects the function of Huntingtin (HTT), the protein produced by the HTT Gene, has yet to be understood. A part of this difficulty is our lack of understanding of the role of normal HTT in our cells. To further our understanding of the role of HTT, we at the SGC have set out to explore the HTT interactome, which might give us an idea of what specific interactions are modulated in HD. Our first step on this journey was to conduct a literature review alongside a BioID experiment to come up with a list of putative huntingtin interaction partners. One of the hits on the list was GAPDH. This means that we must obtain a pure sample of GAPDH for use in future experiments to further validate the claim that these proteins interact.

Methods & Results:

Plasmid Harvest:

Plasmid obtained from Addgene (Plasmid #83910) was inoculated in overnight culture of 4 mL by Yanjun. This culture consisted of DH5α cells housing the pET30-2-GAPDH plasmid. To harvest the plasmid, the QIAprep Spin Miniprep Kit was used, with methods described by the manufacturer. This resulted in a final yield of 50 µL of 30 ng/µL plasmid.

Expression/ Growth:

4 L of LB was inoculated with E. coli BL 21 codon plus RIL transformed with the plasmid described above. Chloramphenicol and Ampicillin were also added to working concentrations of 20 µg/mL and 50 µg/mL respectively. This culture was then grown at 37°C in the LEX system until an OD_{600} measurement read .948, at which point the plasmid was induced by .5 mM ITPG and grown for another ~16 hours at 18°C.

Harvest:

The cell culture was spun down at 6000 rpm for 6 minutes in the Beckman JLA 8.1000. Cells were collected to a final wet cell pellet volume of approximately 40 mL, this was spun further in a benchtop centrifuge at 4500 rpm for 15min to remove all residual LB. These cells were then flash frozen and stored at -80°C.

Lysis:

The cell pellet was thawed and resuspended into 400 mL of lysis buffer [20 mM HEPES pH 7.4, 300 mM NaCl, 1 mM TCEP, 2.5% Glycerol]. Subsequently 5 µL Benzonase and 4 mL of 1 mM Protease Inhibitors were added to the resuspended cells. This was followed with sonication. A cadence of 5 seconds on 7 seconds off for a total of 10 minutes of sonication at power level 8.5 was used to lyse the

cells. This was followed by the clarification of the lysate via centrifugation (Beckman JLA 16.250) at 15000 rpm for 1 hour.

Nickel Purification:

Clarified lysate was Rocked with 5 mL of NiNTA resin for 45 minutes, this slurry was then spun down on a benchtop centrifuge at 1000 rpm for 5 min. The supernatant was decanted off the resin and collected (Ni Flow Though). Resin was then resuspended and washed in an open column (BioRad) with 250 mL wash buffer (lysis buffer + 15 mM Imidazole) (Wash). Beads eluted with elution buffer (lysis buffer + 300 mM Imidazole) (Nickel Elution).

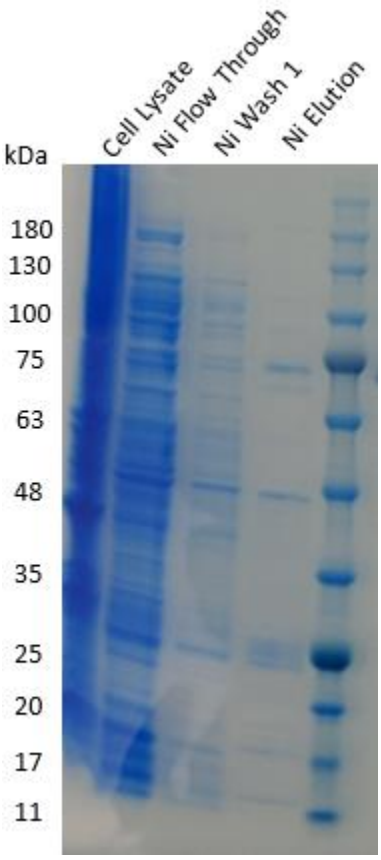

Figure 1. SDS Page, Tris-Glycine 4-20% Gel. Analysis of Steps of Nickel Purification

Size Exclusion Chromatography:

Nickel Elution was concentrated to 5 mL with a spin concentrator (MWCO 30 000). This sample was then loaded into the AKTA using the HiLoad Superdex 75pg 16/60 column and run at 1 mL/min in lysis buffer. Peak fractions were analyzed by SDS Page, those that were found to be roughly the proper molecular weight (37 kDa) and sufficiently pure were collected, concentrated and flash frozen in liquid N_2. A portion of the sample was used for MS analysis.

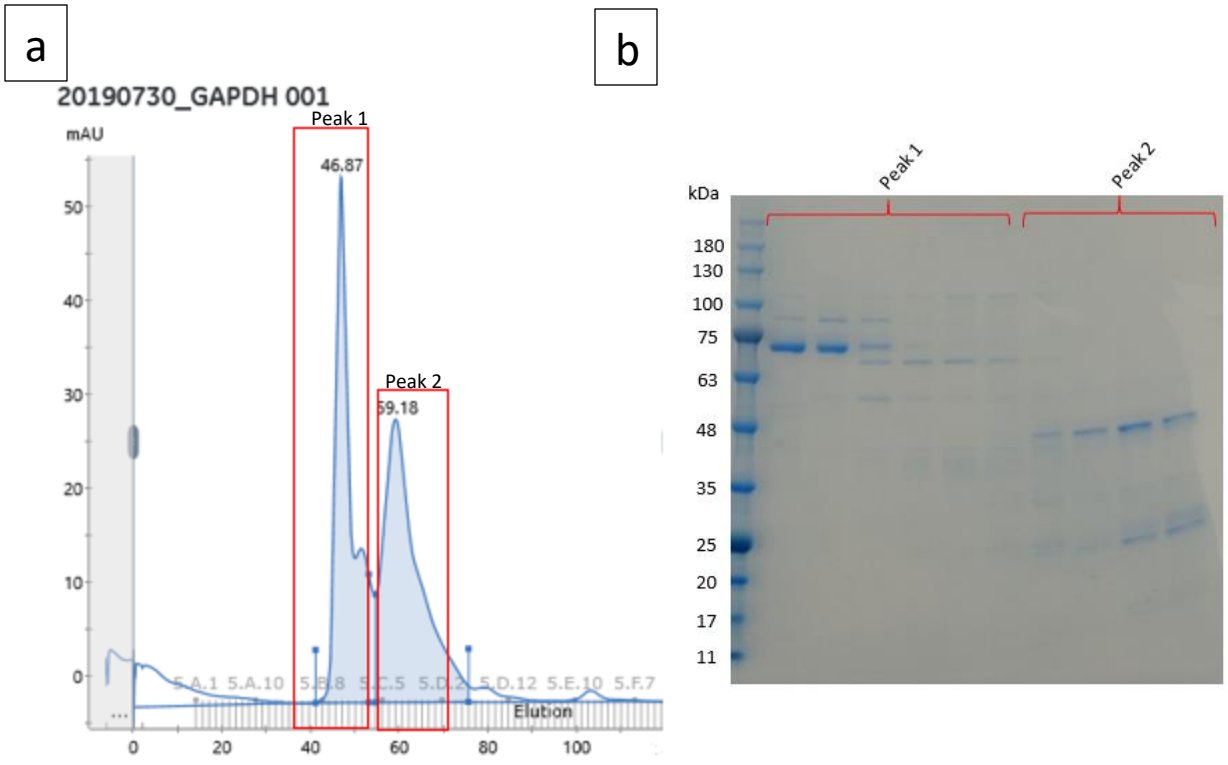

Figure 2.a. UV280 absorption from size exclusion chromatography, peak fractions were analyzed on an SDS Page (figure 2.b.)

Figure 2.b. SDS Page analysis of fractions, Tris-Glycine 4-20% Gel.

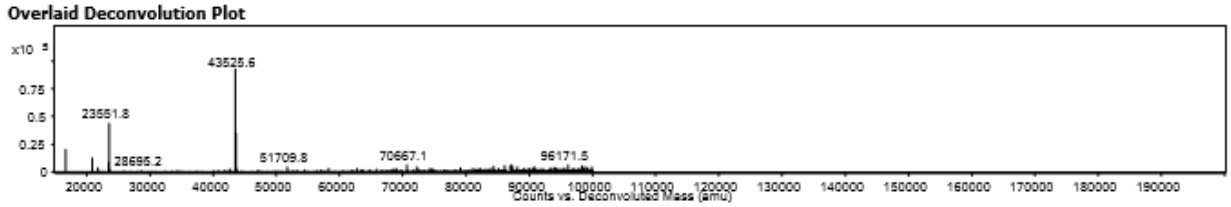

Figure 3. Deconvoluted MS Data

Discussion:

Confirmation of identity via Mass Spec showed the improper molecular weight by 6 kDa. This is beyond the margin of error of the machine and means that it is likely that the sample I have collected is not GAPDH but in fact some contaminant that has a similar molecular weight. Going forward, an attempted purification should take more measures to ensure stringent binding and purification of GAPDH.