MECP2 Expression and Purification

Rationale:

As a result of a BioID experiment done to identify possible interaction partners of Huntingtin, MECP2 was flagged. This purification aims to provide a MECP2 sample for future experiments to confirm the possible interaction between these proteins.

Growth:

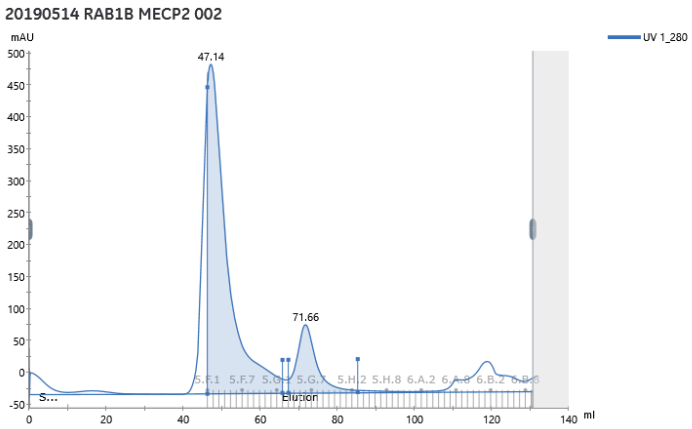
3 x 2 L LB media inoculated with *E. coli* BL21 codon plus RIL transformed with the plasmid detailed in the table, were grown at 37°C in LEX system. The cells were induced at OD_{600}~0.62 with 0.5mM IPTG. Cultures were grown overnight at 18°C.

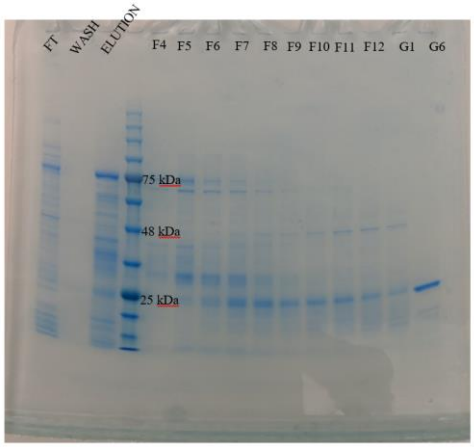
Construct ID	Vector	Cloned AA Sequence	Mol. Weight	PI
APC065:C05	pET28a-MHL	MHHHHHHSSGRENLYFQGMVAGMLGLREEKSEDQDLQGLKDKPL KFKKVKKDKKEEKEGKHEPVQPSAHHSAEPAEAGKAETSEGSGS APAVPEASASPKQRRSIIRDRGPMYDDPTLPEGWTRKLKQRKSG RSAGKYDVYLINPQGKAFRSKVELIAYFEKVGDTSLDPNDFDFT VTGRGSPSRREQKPPKKPKSPKAPGTGRGRGRPKGSGTTRPKAA TSEGVQVKRVLEKSPGKLLVKMPFQTSPGGKAEGGGATTSTQVM VIKRPGRKRKAEADPQAIPKKRGRKPGSVVAAAAAEAKKKAVKE SSIRSVQETVLPIKKRKTRETVSIEVKEVVKPLLVSTLGEKSGK GLKTCKSPGRKSKESSPKGRSSSASSPPKKEHHHHHHHSESPKA PVPLLPPLPPPPPEPESSEDPTSPPEPQDLSSSVCKEEKMPRGG SLESDGCPKEPAKTQPAVATAATAAEKYKHRGEGERKDIVSSSM PRPNREEPVDSRTPVTERVS	54.6 kDa (with tag)	9.9

Purification:

Collect cells from media culture using centrifugation at 4000 rpm for 10 mins at 4°C in JLA 8.1000. Resuspend cell pellet volume (8 mL) in a 1:10 dilution with lysis buffer (20 mM HEPES pH 7.4, 300 mM NaCl, 1 mM TCEP, 2.5% glycerol [v/v]). Add 5 µL of benzonase and 1 mL of protease inhibitors. Lyse cells using sonication and clarify lysate via centrifugation at 16000 rpm for 60 mins at 4°C in JLA16.250. Rock clarified lysate with equilibrated NiNTA (2.5 mL) for an hour at 4°C. Benchtop centrifuge the samples at 1000 rpm for 10 mins at 4°C to collect beads at the bottom of the container, collect supernatant (flow through - FT). Resin washed in open column (BioRad) with buffer + 20 mM Imidazole (wash - W). Elute beads with buffer + 300 mM Imidazole (elution - E). Concentrate elution to 5 mL with spin concentrator (MWCO 10,000). Load sample into AKTA using Superdex s75 16/60 column and run at 1 ml/min in buffer (20 mM HEPES pH 7.4, 120 mM NaCl, 0.5 mM TCEP). Fractions purity was assessed by SDS Page. The peak fractions were then collected and concentrated (MWCO 10,000).

Results:





Discussion:

Due to the impurity of the sample after Size-Exclusion chromatography, this purification was abandoned. In the elution, we can see that the NiNTA resin bound several proteins indiscriminately. This led to a considerable amount of impurities in our sample, inhibiting the further purification of this protein. Another purification will need to be attempted to obtain MECP2 for use in future experiments.