CVNSP3A-c011 small scale purification

PAGE24-00789

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Experiment Started:
Projects: **Purification**;**ASAP**
Related Pages: **PAGE23-00304**;**PAGE24-00534**
Referenced by:
Tags:

Expression and information

CVNSP3A-c011

Expression done by Ellie
CVNSP3A-e004
CVNSP3A-p004

Final pellet ~60g

MHHHHHHSSGVDLGTENLYFQ////SMVNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYLKHGGGVAGALNKATNNA
MQVESDDYIATNGPLKVGGSCVLSGHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPLLSAGIFGADPIHSLRVCVDT
VRTNVYLAVFDKNLYDKLVSSFL

Post-cleavage:
Molecular weight: 18157.79
Ext. coefficient 10430
Abs 0.1% (=1 g/l) 0.574, assuming all Cys residues are reduced

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Purification

Buffers

Lysis buffer - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP
Wash buffer 1 - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 20mM imidazole, 0.5mM TCEP
Elution Buffer (IMAC) – 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 500 mM imidazole, 50mM ArgGlu, 0.5mM TCEP
Gel Filtration Buffer (SEC) – 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP

Cell Lysis

1. Add lysis buffer to pellet until total volume is 400mL. Supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme, 1mM final MgSO4, and 1 PIC tablet.
2. Mix lysis mixture until homogenous.
3. Sonicated on ice at 40% amplitude for 10 minutes (2 seconds on 4 seconds off).
4. Clarified lysate by centrifugation at 18,000rpm, 4°C for 1 hour.

IMAC

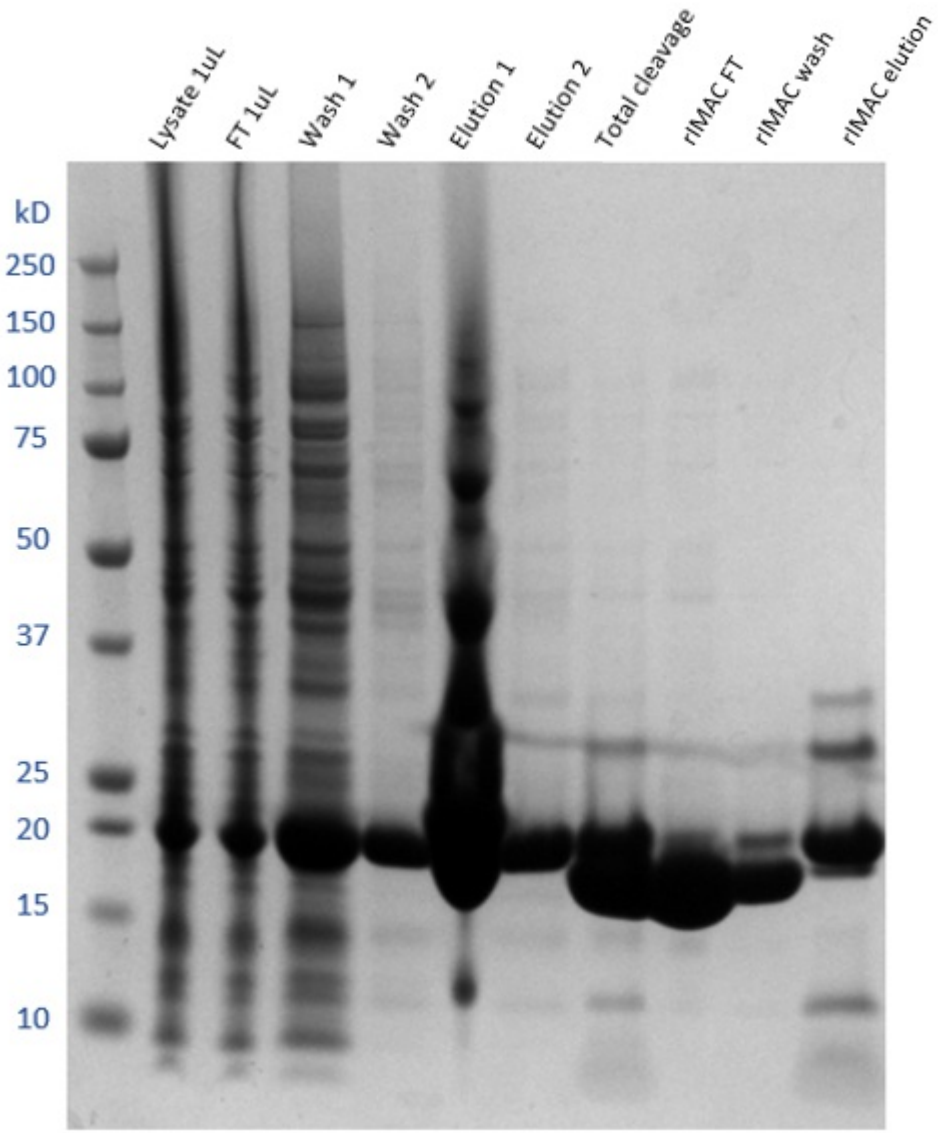
1. Wash and equlibrate 2mL bed volume of Ni sepharose resin on gravity flow column, first with distilled water, then with wash buffer. (use cut pipette tip)
2. Add equilibrated resin to clarified lysate. Incubate in cold room on rotation for 1hr.
3. Put stopper on column. Re-suspend resin with small volumes of the clarified supernatant, and pour into 500mL v-bottom centrifuge bottles.

4. Incubate resin and supernatant on rotator for ~30min. Pour mixture back on gravity flow column and rinse remaining resin from bottle with ~30mL wash buffer. Load all onto column and collect FT.
5. Wash resin 2x with 50mL wash buffer each time. Collect both washes as wash 1 and 2.
6. Elute with 7.5mL elution buffer, 10mL incubation. Collect both elutions as elution 1 and 2.

Desalting and Cleavage
Imidazole was removed from eluate using HiPrep Desalting column into gel filtration buffer.
His-2Strep-TEV was added in 1:20 molar ratio and incubated in cold room overnight.

rIMAC
Imidazole was added to final concentration of 10mM to thecleavage mix. Cleavage mix passed over washed IMAC resin 3 times. FT collected.
Washed with 10mL wash buffer.
Elution with 10mL elution buffer to see what stuck to the resin.

IMAC and cleavage results



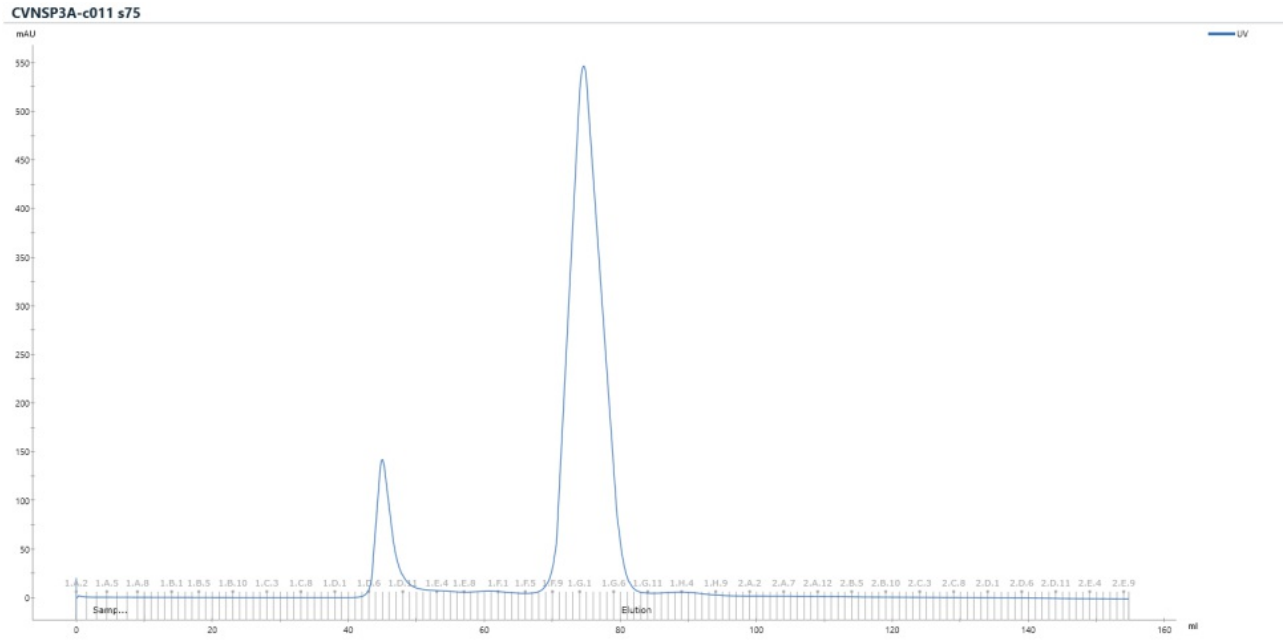
Gel filtration

FT and wash was concentrated in Vivaspin 15 10kDa MWCO concentrators to ~1mL
ACCIDENTALLY SPILLED HALF

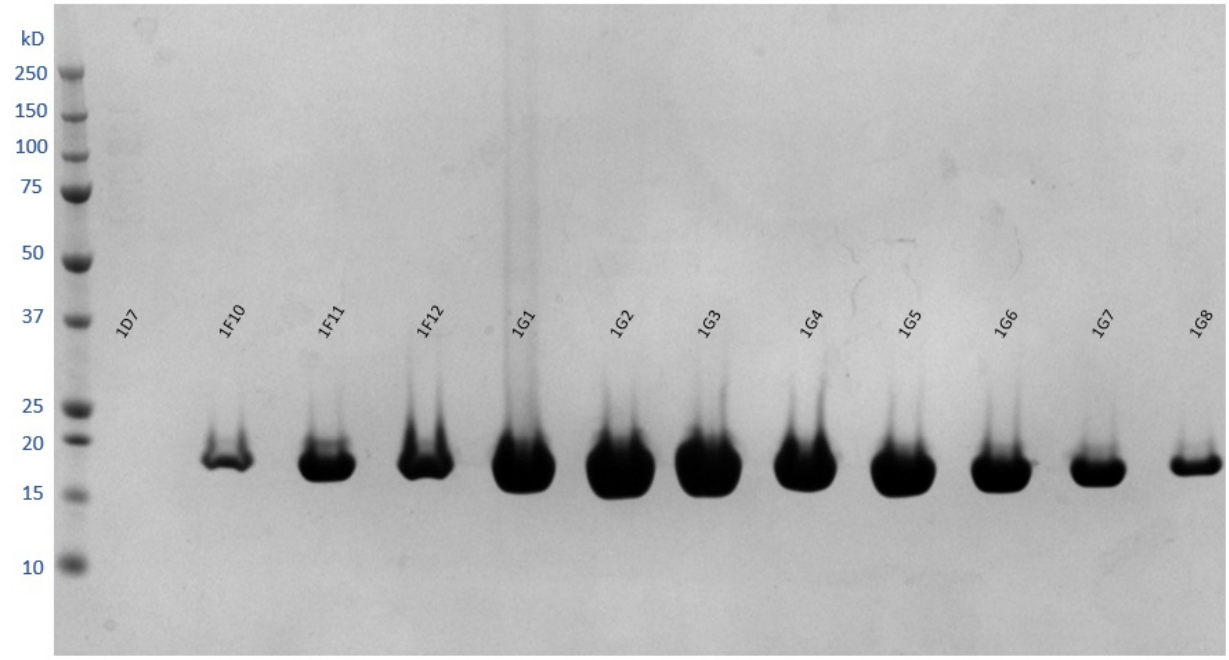
Solid aggregates pelleted by centrifugation at 27,000xg, 10 mins, 4oC

Sample injected onto superdex 75 16/600 on AKTA pure, 1mL/min

s75 chromatogram



SEC results



Final sample

Fraction F9-G8 were pooled and concentrated in Vivaspin 15 10kDa MWCO concentrators.

Final concentratrion: 58.17 mg/mL, 8x50uL
Total yield: 46.5mg

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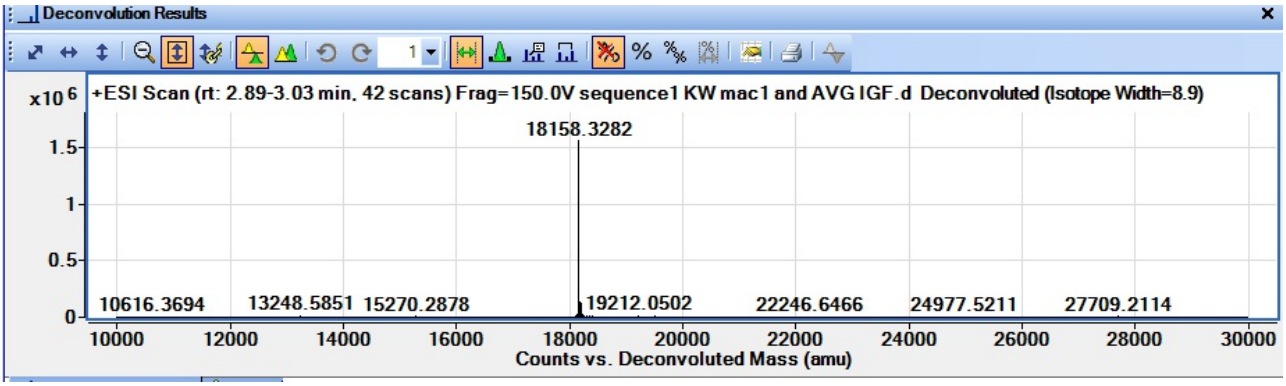
MS


Deconvolution Results
+ESI Scan (rt: 2.89-3.03 min, 42 scans) Frag=150.0V sequence1 KW mac1 and AVG IGF.d Deconvoluted (Isotope Width=8.9)
x10 6
1.5
1
0.5
0
18158.3282
10616.3694
13248.5851
15270.2878
19212.0502
22246.6466
24977.5211
27709.2114
10000
12000
14000
16000
18000
20000
22000
24000
26000
28000
30000
Counts vs. Deconvoluted Mass (amu)