

SRF-Bio- ASAP 68 and 71 3C protease expression and purification

PAGE23-00979

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Experiment Started:

Projects: **Expression;Purification;ASAP**

Related Pages: **PAGE22-01976;PAGE23-00563**

Referenced by: **PAGE23-01016;PAGE23-01458**

Tags:

Scale-up and expression

Expression done with Jess in TB autoinduction media (granuel with trace metal used)

Glycerol stock straight into 100mL 2xLB +Kan

Grew until OD~4, temperature reduced to 18oC, 200rpm ON for autoinduction
6L expression, pellet harvested in 2L aliquots into falcon tubes.

D68EV3CPROA-p003

A71EV3CPROA-p003

Info

Mike ran into issues concentrating 71 in HEPEs buffer (pH7.5), but bicine buffer seems to work better.

ASAP-1, well G01:

D68EV3CPROA-c001

MGPGFDFAQAIMKKNTVIARTEKGEFTMLGVYDRVAVIPTHASVGEIIYINDVETRVLDACALRDLTDTNLEITIVKLDNRNQKFR
DIRHFLPRCEDDYNDVAVLSVHTSKFPNMYIPVGQVTNYGFLNLSGGTPTHRILMYNFPTRAGQCQGGVTTTGVKIGIHVGGNGA
QGFAAMLLHSYFTDTQKHHHHHHH

21283.3 Da

10430 mM-1cm-1

PI=7.21

ASAP-1, well F12:

A71EV3CPROA-c001

MGPSLDFALSLLRRNIRQVQTDQGHFTMLGVRDRLAVLPRHSQPDKTIWVEHKLINILDAVELVDEQGVNLELTLVTLDTNEKF
RDITKFIPENISAASDATLVINTEHMPMSMFVPVGDVVQYGFLNLSGKPTHRMMPNFPKAGQCQGGVTSVGVKIGIHIGGNG
RQGFCAGLKRSYFASEQLEHHHHHHH

21331.5 Da

9970 mM⁻¹ cm⁻¹

PI=7.22

Cell lysis and IMAC

71 and 68 done in parallel, so same procedure x2

Buffers:

Lysis buffer - 50 mM HEPES pH 7.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP

Wash buffer - 10 mM Bicine pH 8.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP, 30mM imidazole

Elution Buffer - 10 mM Bicine pH 8.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP, 300mM imidazole

Gel Filtration Buffer (SEC) - 10 mM Bicine pH 8.5, 500 mM NaCl, 5% glycerol, 0.5mM TCEP

1. 1 falcon tube (2L) of pellet was thawed and incubated 30min in cold room with lysis buffer supplement with 1:4000 dilution of benzonase, 0.5mg/mL lysozyme, and 1mM MgSO₄. Final volume ~200mL.
1. Sonicated on ice at 35% amplitude for a total of 15-minute sonication time (2 seconds on 4 seconds off) with thick probe.
2. Clarified lysate by centrifugation at 18,000rpm, 4°C for 1 hour.

IMAC

1. Wash and equilibrate 15mL bed volume of Ni Sepharose resin on gravity flow column, first with distilled water, then with wash buffer. (use cut pipette tip)
2. Put stopper on column. Re-suspend resin with small volumes of the clarified supernatant, and pour into 500mL v-bottom centrifuge bottles.
3. Incubate resin and supernatant on rotator for ~30min. Pour mixture back on gravity flow column and rinse remaining resin from bottle with ~10mL wash buffer. Load all onto column and collect FT.
4. Wash resin 2x with 100mL wash buffer. Collect both washes as wash 1 and 2.
5. Elute with 30mL elution buffer, 10min incubation. 3 elutions carried out

71

E1: 26.1 mg/mL

E2: 19.6 mg/mL

E3: 3.60 mg/mL

68

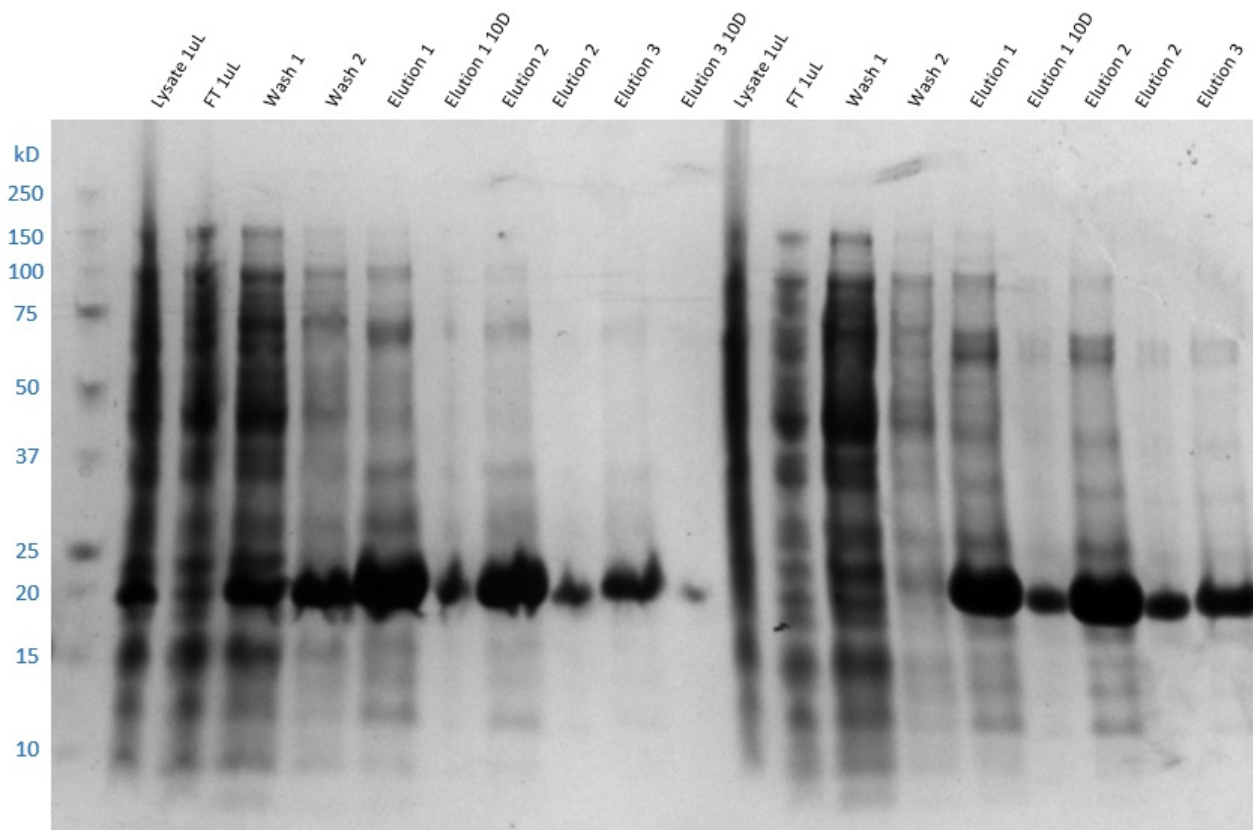
E1: 5.94 mg/mL

E2: 4.77 mg/mL

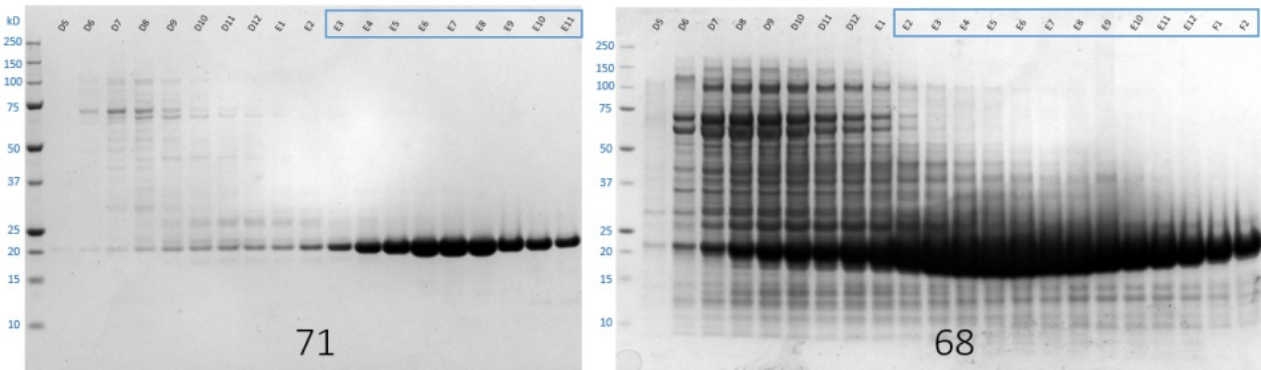
E3: 1.21 mg/mL

all elutions have very high A260, DNA contamination, but still very high protein from gel

IMAC result



SEC result



SEC test

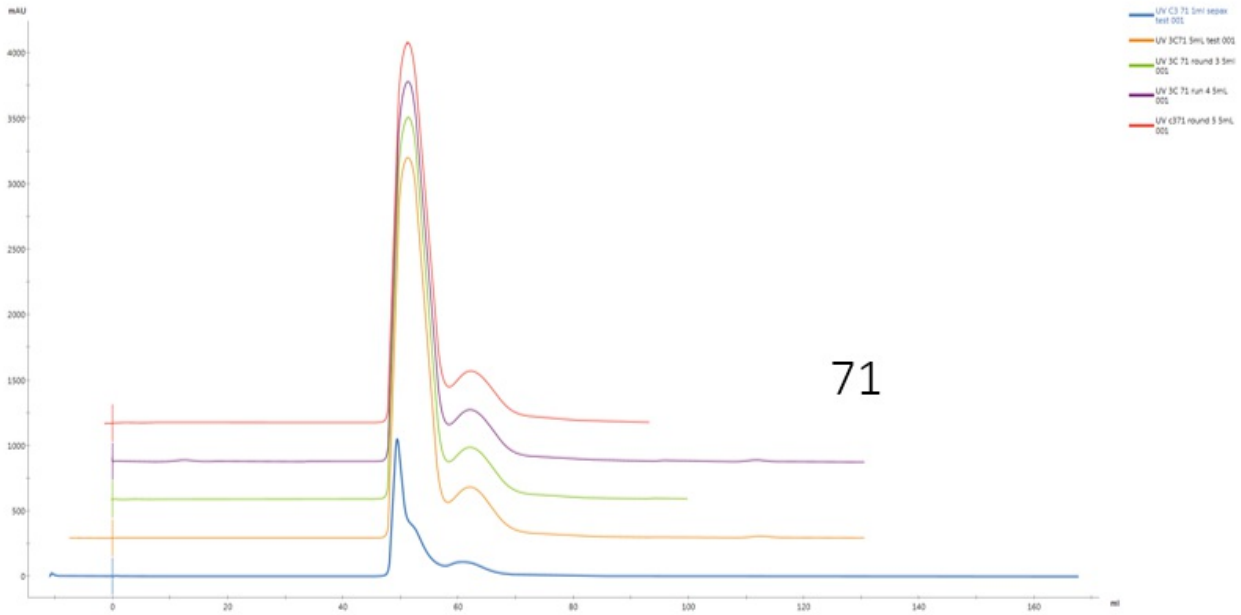
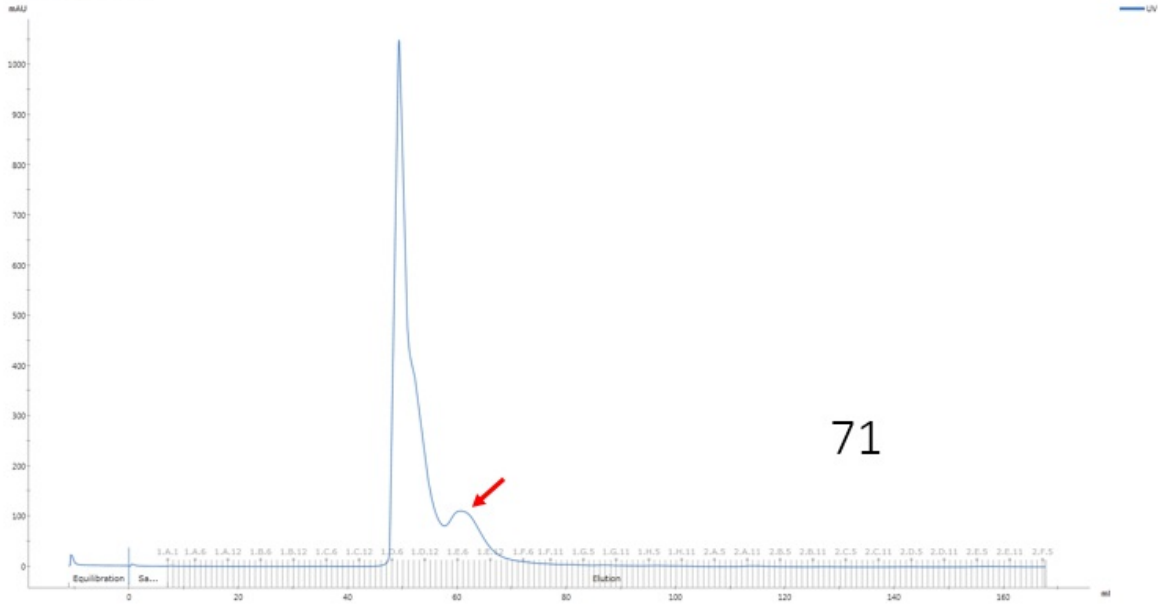
All elutions pooled and concentrated in Amicon 10kDa concentrators. Relatively slow, especially 71. Probably due to high DNA concentration.

Had around ~21 mL of 71 and 10mL of 68

Decided to use sepax SEC SRT-100 rather than superdex cuz it would take too long for multiple runs. Did test run of 1mL 71 and 5mL 68 on SEPAX first to make sure the protein doesn't stick.

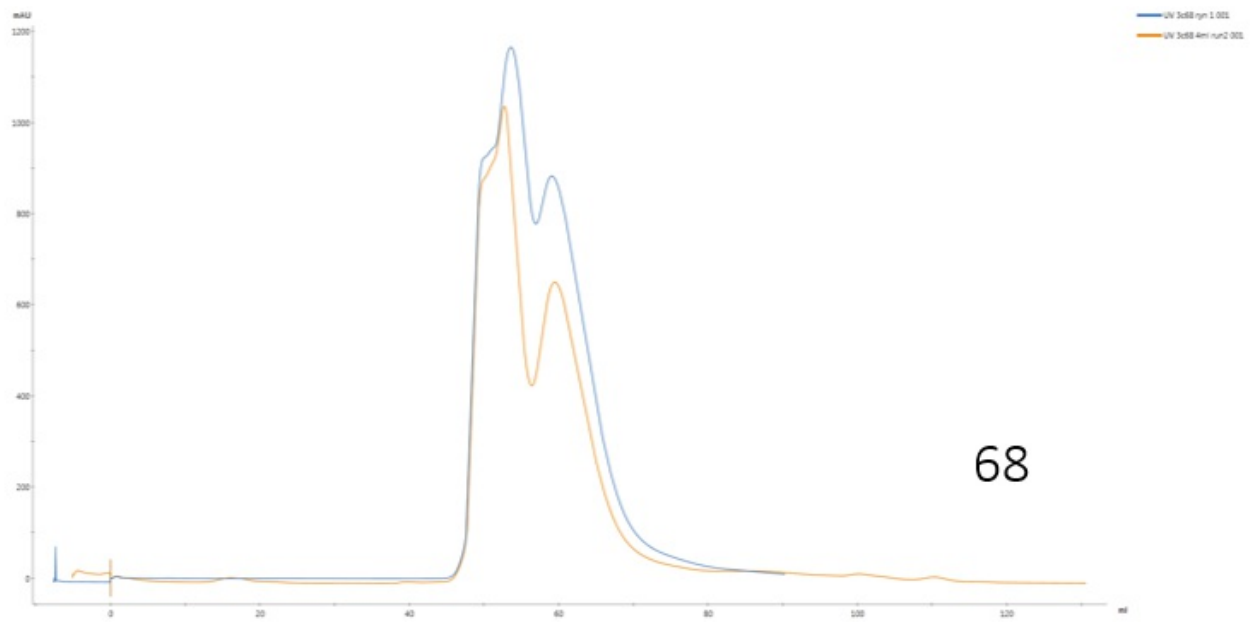
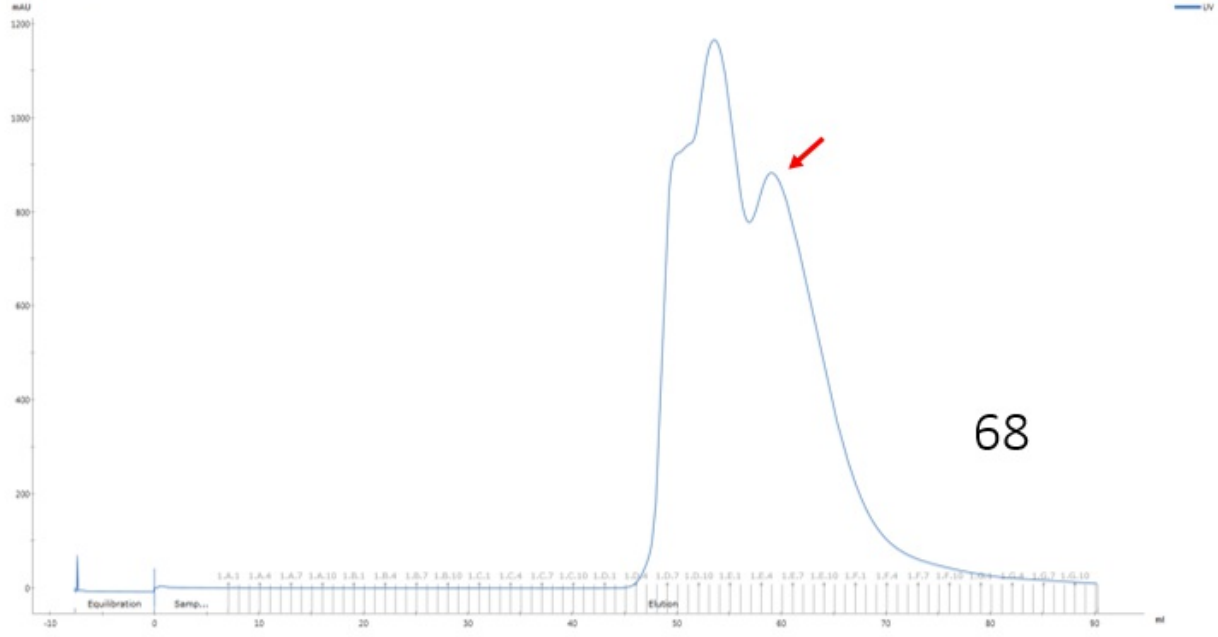
71 chromatogram

C3 71 1ml sepax test 001

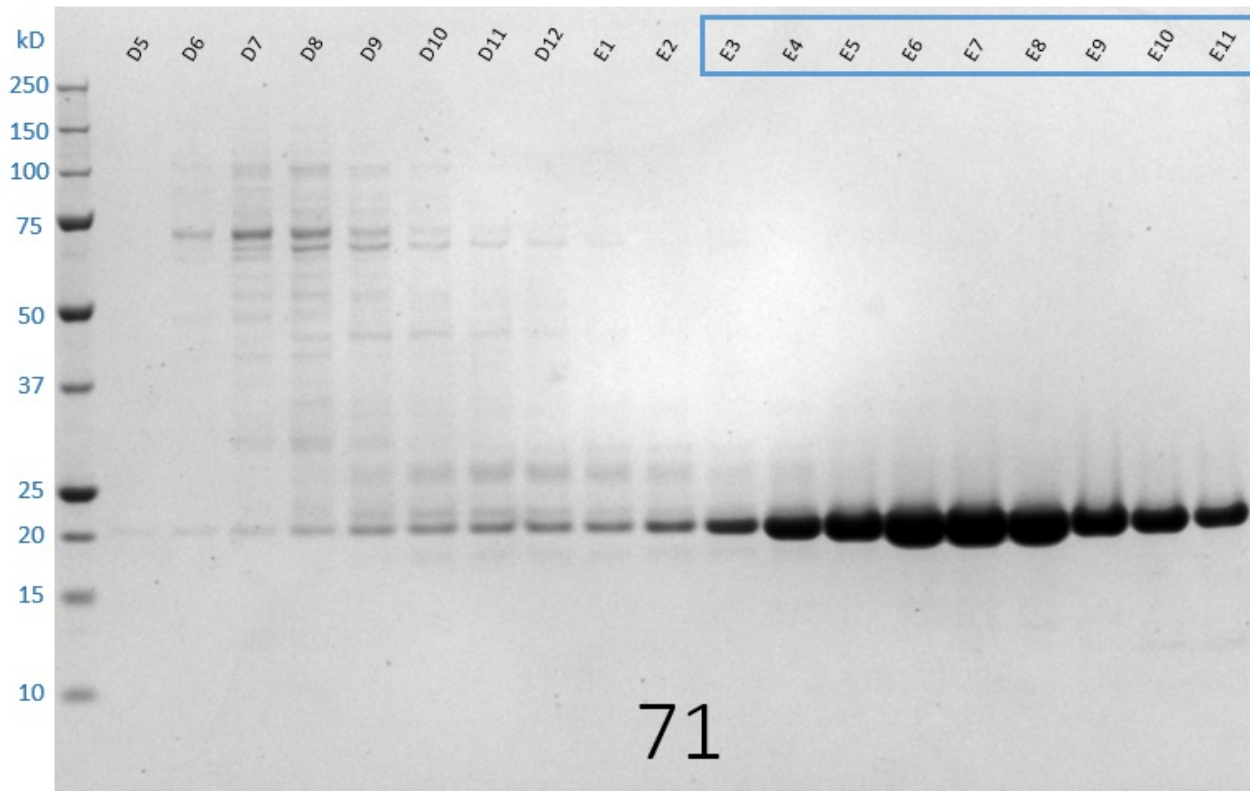


68 chromatogram

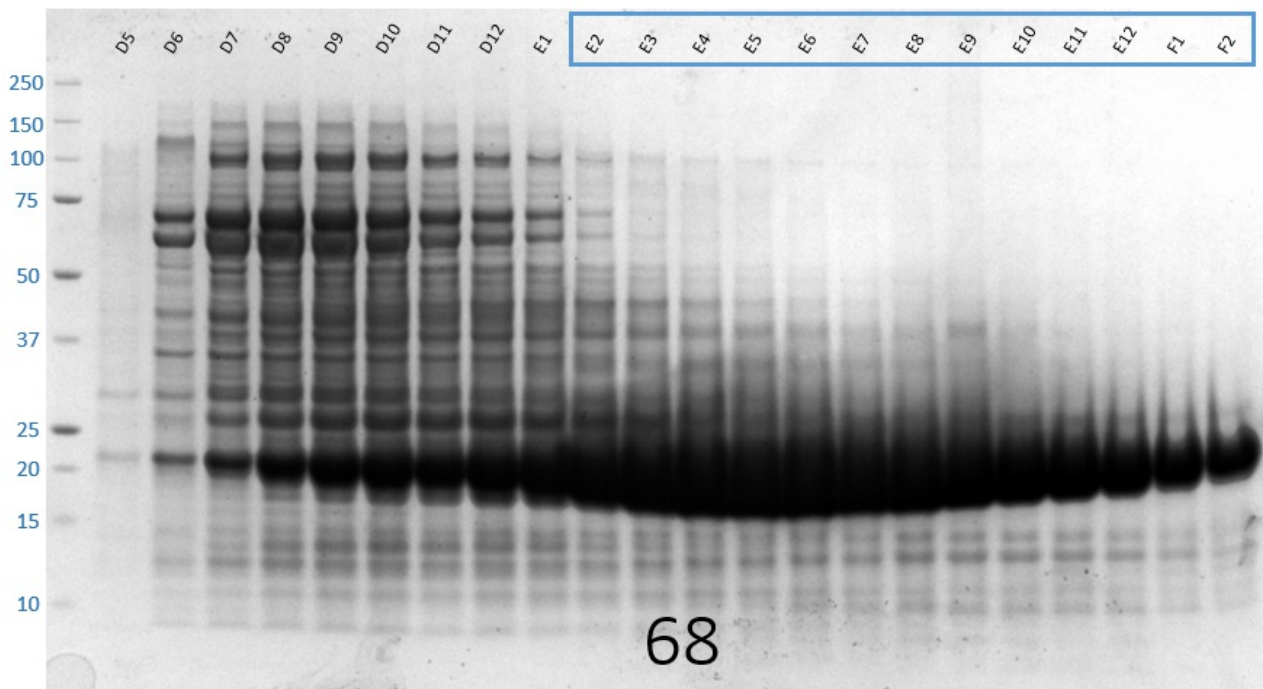
3c68 rjn 1 001



71 SEC fraction



68 SEC fractions



Note

The high peak around 50mL in all runs is probably from bleed-through of A280 absorbance from high DNA concentration.

SDS-PAGE analysis of these fractions (as seen in 71 SEC fractions) show very little protein.

Fraction pooling

71

Run 1: E3-G1

Run 2: E4-G1

Run 3: E5-G1

Run 4: E5-G1

Run 5: E5-G1

68

Run 1: E3-G1

Run 2: E2-F12

All fractions pooled and concentrated in Amicon 10kDA MWCO concentrators.

Desired concentration: 1mM for both. That would be around 21.3 mg/mL for both.

Final:

3C71: 30.0mg/mL, 39x100uL + 1x~80uL aliquots

3C68: 37.1mg/mL, 34x100uL + 1x~10uL aliquots

Update

UPDATE:

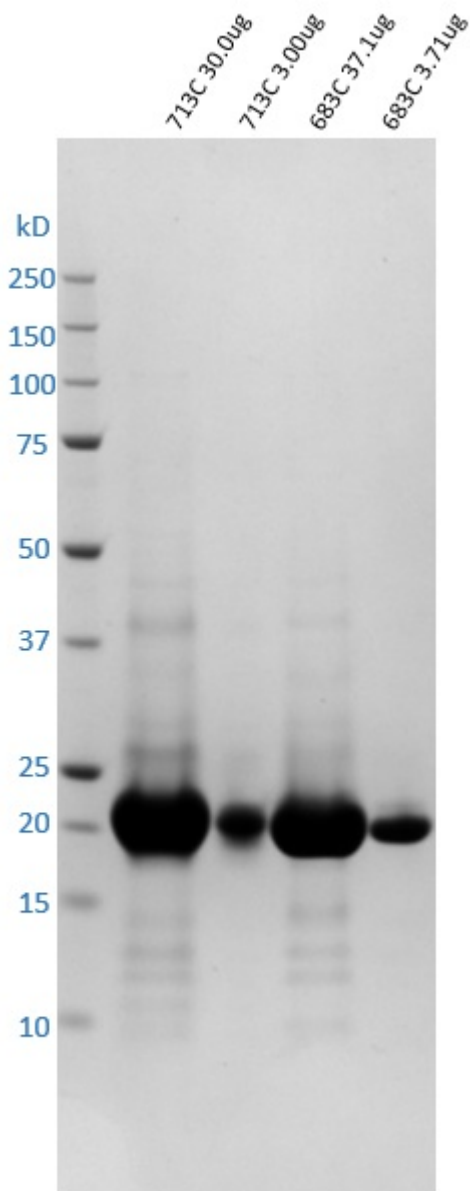
8 aliquots of each sent to Ryan Lithgo from Diamond on 16 Jun 2023

5 more of each (68 in HEPES) to Ryan on 1 Aug 2023

Gave some of both to Charline but forgot how much

Send 4 aliquot 71 to Weissman on 25 Aug 2023

Final



68 buffer exchange

Diamond said Bicine for 68 doesnt work. So buffer exchange into Mike's buffer:
10mM HEPES, 500mM NaCl, 5% glycerol, 0.5mM TCEP.

Thawed all remaining samples (30x100uL) and pooled 2.5mL. Passed through equilibrated PB10 and eluted as manual instruct.

Remaining 500uL kept as they were just in case buffer exchange causes it to crash.
All looks good. Straight off the PD10 is ~19mg/mL.

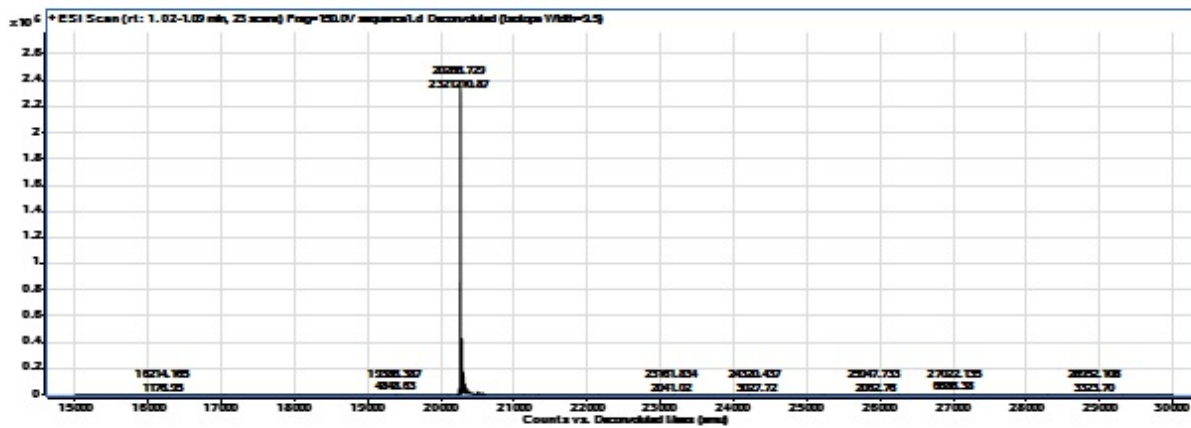
Concentrated into Amicon 10kDa MWCO concentrators.

Final concentration: 35.6 mg/mL, 19x100uL + ~70uL

Sent Ryan 5 aliquot of each (71 in bicine, 68 in HEPES)

A71EV3CPROA-p003

Mass is 20266.7 and it should be 21331.45



Self-cleavage after -80 storage PAWS result

A71EV3C

[1-192] mass = 21331.4

mass to match = 20266.0 ± 2.0

M[1-184]Q = 20266.3

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1  M G P S L D F A L S L L R R N I R Q V Q T D Q G H F T M L G 30
31  V R D R L A V L P R H S Q P G K T I W V E H K L I N I L D A 60
61  V E L V D E Q G V N L E L T L V T L D T N E K F R D I T K F 90
91  I P E N I S A A S D A T L V I N T E H M P S M F V P V G D V 120
121 V Q Y G F L N L S G K P T H R T M M Y N F P T K A G Q C G G 150
151 V V T S V G K V I G I H I G G N G R Q G F C A G L K R S Y F 180
181 A S E Q L E H H H H H H H 192

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