Expression and purification of SARS-Cov-2 Nsp13

Construct info

Construct CVNSP13A-c000

Vector pNIC-ZB

Sequence

MHHHHHHSSGVDNKFNKERRRARREIRHLPNLNREQRRAFIRSLRDDPSQSANLLAEAKKLNDAQPKGTENLYFQSM
AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKPPIS
FPLCANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLKLFAAETLKATEETFKLSYGIATVREVLSD
RELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQIGEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPT
LVPQEHYVRITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDALCE
KALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNARLRAKHYVYIGD
PAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCPAEIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVIT
HDVSSAINRPQIGVVREFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNRF
NVAITRAKVGILCIMSDRDLYDKLQFTSLEIPRRNVATLQ

Sequence (no-tag)

SMAVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVTDVTQLYLGGMSYYCKSHKP
PISFPLCANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTCTERLKLFAAETLKATEETFKLSYGIATVREVL
SDRELHLSWEVGKPRPPLNRNYVFTGYRVTKNSKVQIGEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSA
PTLVPQEHYVRITGLYPTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDAL
CEKALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNYDLSVVNARLRAKHYVYI
GDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCPAEIVDTVSALVYDNKLKAHKDKSAQCFKMFYKG
VITHDVSSAINRPQIGVVREFLTRNPAWRKAVFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNV
NRFNVAITRAKVGILCIMSDRDLYDKLQFTSLEIPRRNVATLQ

Expression conditions

Constructs were transformed in to E.coli Rosetta cells, and cultures were grown in TB media at 37 degrees until they reached an O.D. of 2-3 at which point the temperature was reduced to 18 degrees and expression was induced with the addition 0.1 mM IPTG. Cells were harvested after overnight induction.

Purification protocol

Purified from 4 Litres of culture (45g cell mass).

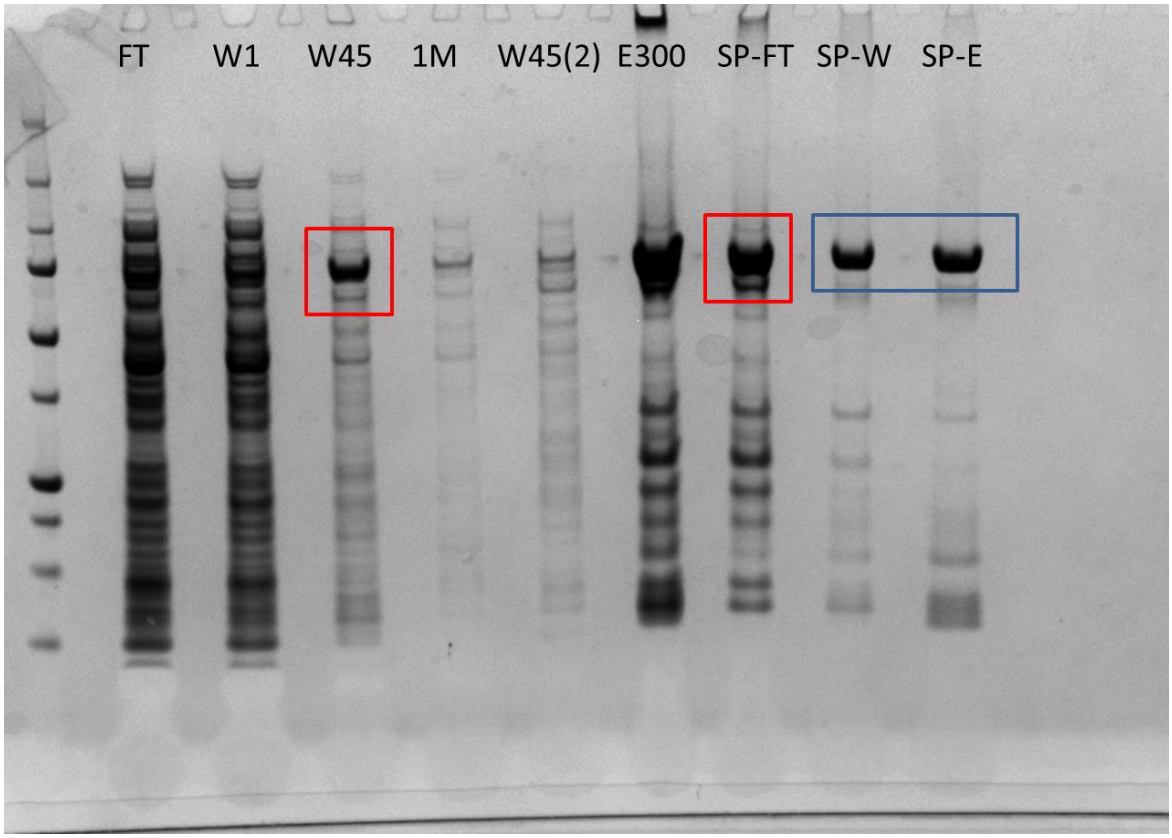
Resuspended in 200 ml lysis buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Gol, 10 mM Imidazole, 0.5 mM TCEP) with protease inhibitors (500 Merck set III). Cells were disrupted by sonication for 15 mins 10sec on 5sec off, spin in JA25.5 24500 RPM for 30 mins.

Added 5ml of Ni resin (IAMC sepharose) for batch binding 40 mins rotating in cold room.

Wash with 40 ml lysis buffer, 25 ml wash buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Gol, 45 mM Imidazole, 0.5 mM TCEP) and transfer to column. Wash with 10 ml Hi-salt buffer (50 mM HEPES pH 7.5, 1 M NaCl, 5% Gol, 0.5 mM TCEP) and again with another 10 ml of wash buffer. Proteins were eluted with addition of 15ml of elution buffer (50 mM HEPES pH 7.5, 500 mM NaCl, 5% Gol, 300 mM Imidazole, 0.5 mM TCEP).

Elution fraction was immediately applied to a 5ml HItrap SP column using a syringe, collecting flow through. Column was washed with 10ml elution buffer and proteins were eluted with 15ml Hi-salt buffer.

Gel

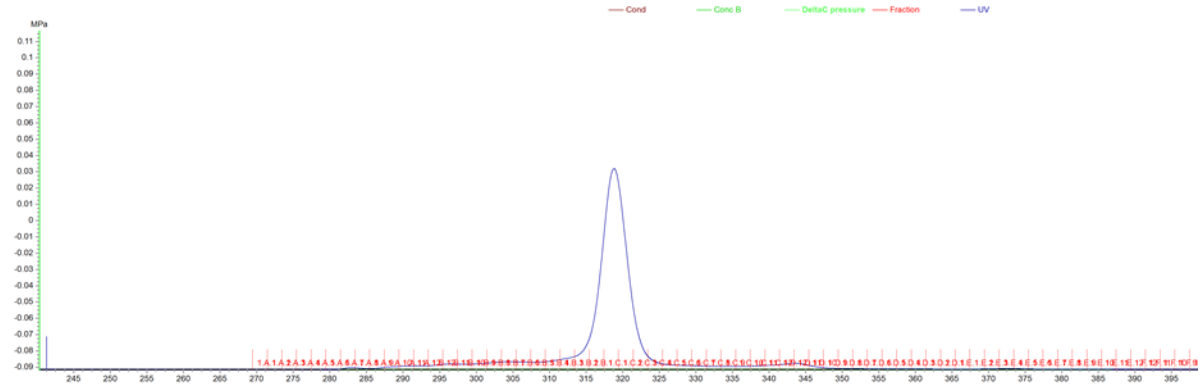


Nsp13 protein was present in several fractions and these were pooled separately with SP column wash and elution fractions (PoolA) and Nickel wash and SP-flow through fractions (PoolB) treated separately from this point onward.

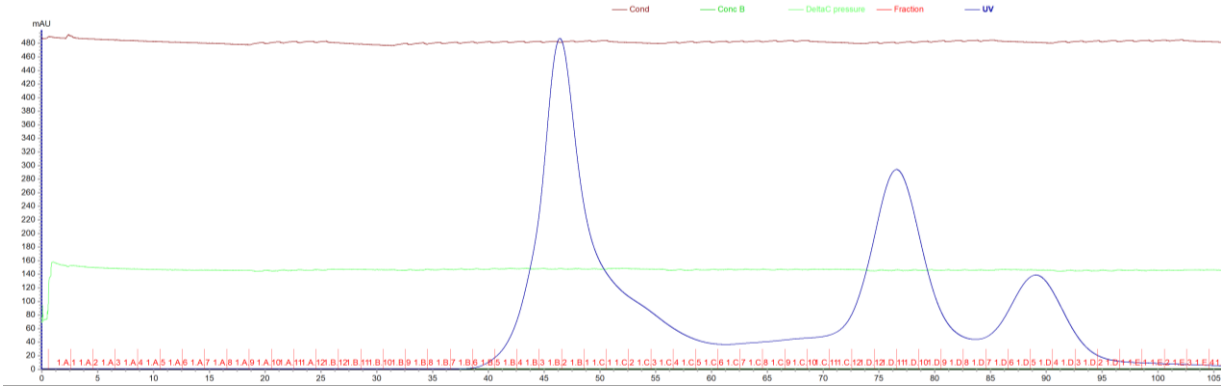
N.B. Both poolA and PoolB were found to crystallize with the majority of the crystals coming from poolB which had greater yield although slightly more dirty.

For further purification both proteins were incubated overnight with TEV protease (1:40 mass ratio) and loaded onto ge filtration using a superdex 200 16/60 column equilibrated in 50 mM Hepes, 500 mM NaCl, 0.5 mM TCEP.

Pool A s200 trace



Pool B s200 trace (some aggregates)



Protein was concentrated to 20 mg /ml and diluted in half with water for initial crystallization trials at 10 mg/ml using a combination of screens and nucleotide combinations. Total yield was around 6 mg.

Crystallization

Initial diffracting crystals were found in the Morpheus screen from Molecular dimensions for the apo form protein at 10 mg/ml.

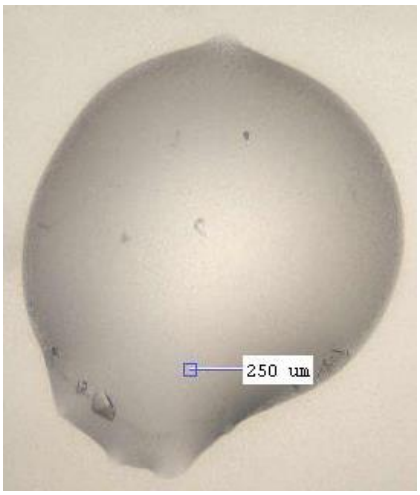
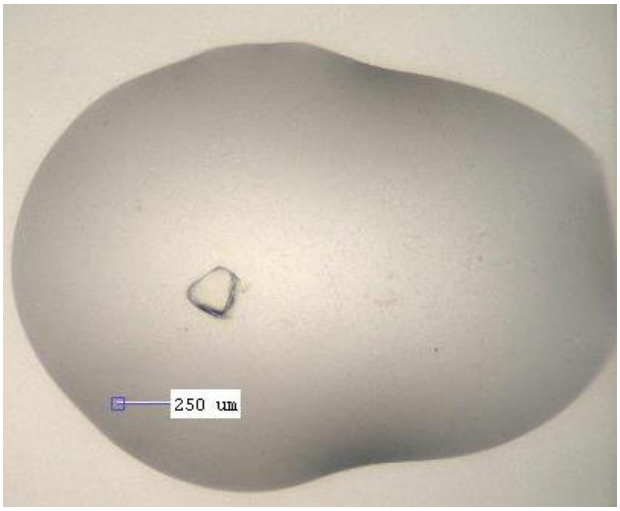


Image of initial crystals after 7 days

Initial crystals grew at 20 degrees from well C6 of the Morpheus screen. Condition is, 20 % Ethylene Glycol, 10 % PEG 8K, 0.05 M HEPES, 0.05 M MOPS, 0.03 M Sodium Nitrate, 0,03 M Sodium Phosphate, 0.03 M Ammonium Sulphate.

For crystal optimization seeding was performed, around 5-10 crystals were crushed with glass probe and transferred to 25 ul of well solution. Seed bead was added ant the mixture was sonicated for around 30-60 seconds with pulsing. Final seed dilution should be optimized empirically. Best way is to prepare and freeze serial dilutions of seeds in well solution and flash freeze and test a range of seed dilutions to have optimal result. For me around 1 in 400 dilution worked best.

Final plates were set up with protein at 5 mg/ml (diluted 4 fold in water from 20 mg/ml stock) with a slightly reduced precipitant concentration (16 % Ethylene Glycol, 8 % PEG 8K, 0.05 M HEPES, 0.05 M MOPS, 0.03 M Sodium Nitrate, 0,03 M Sodium Phosphate, 0.03 M Ammonium Sulphate), using 300 nl drops (1:1 ratio) with 20 nl seeds (added last).



Optimized crystallization after 10 days