

## JEDI Program

### Compound Screening for SARS-CoV-2 Proteins Using MST/Dianthus

### Reconstitution and purification of the RNA-dependent RNA Polymerase complex

JDI01\_3

April 30, 2021



## Aim:

Screening for binders of the following SARS-CoV-2 proteins using MST/Dianthus

- RNA-dependent RNA polymerase complex (nsp12-nsp7-nsp8)
- Spike (S)
- Nucleoapsid (N)

## Status:

- ACE2, Spike and Nucleocapsid proteins purchased from external vendor
- tool compounds (Remdesivir metabolite GS-443902 trisodium, Ribavirin, Favipiravir) available
- RNA-dependent RNA polymerase complex has been purified, optimization ongoing

# Purification summary - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

- The complex of nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1) and an RNA was reconstituted from its individual subunits according to Hillen et al., Nature, 2020 ([doi.org/10.1038/s41586-020-2368](https://doi.org/10.1038/s41586-020-2368))
- Tagged protein complex was purified by SEC
  - Excess of individual subunits was removed and fractions of intact complex were pooled and concentrated
- Quality control after one freeze-thaw cycle shows pure protein complex (>95% purity) that elutes as multiple peaks from analytical SEC
  - Individual peaks correspond to individual subunits → complex appears to be almost completely dissociated
  - Analytical SEC of quality control is performed at room temperature → complex is temperature-sensitive
- Intact mass analysis confirms the protein integrity of DVT1 and DZT1; no signal could be detected for DZU1
- Next step: Reconstitution under different buffer conditions to stabilize complex

# Protein data sheet - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

Protein: nsp12:nsp8:nsp7 (Uniprot ID: P0DTD1)  
Construct ID: DVT1:DZT1:DZU1  
Lot-ID: PC14847

Protein Purification	Concentration (A260, mg/mL) *	Aliquot size (µL)	A260/A280	Modification / Ligand	Tag
	2.4	15	1.47	none	C-terminal on DVT1: Tb-His <sub>6</sub> -Strep
	Expression system	Purification steps	Storage buffer		
	SF21	Complex reconstitution, SEC	20 mM HEPES / NaOH, 100 mM NaCl, 1 mM MgCl2, 1 mM TCEP, pH 7.4		
Quality control *	Analytical SEC	Purity based on SDS-PAGE	Intact mass (Da)		
	heterogeneous	>95%	109361.2 / 22153.8		
Protein parameters	Theoretical MW (Da)	Theoretical extinction coefficient (= 1 g/L)	Construct boundaries	Mutations	Theoretical pI
	DVT1: 109449.3; DZT1: 22153.3; DZU1: 9512.1	1.85	DVT1: 4393-5324; DZT1: 3943-4140; DZU1: 3860-3942	none	6.7
	Amino acid sequence				
	DVT1: MGSADAQSFLNRVCGVSAARLTPCGTGTSTDVVYRAFDIYNDKVAGFAKFLKTNCCRFQEKKDEDDNLIDSYFVVKRHTFSNYQHEETIYNLLKDCPAVAKHDFKFRIDGDMVPHISRQRLTKYTMADLVYALRHFEDEGNCDTLKEILVTYNCCDDDYFNKKDWYDFVENPDILRVYANLGERVRQALLKTVQFCDAMRNAGIVGVLTLDNQDLNGNWDYDFGDFIQTPGSGVPVVDSSYSLMPILTALTAESHVDTDLTKPYIKWDLKDYDFTEERLKLFDRIYFKYWDQTYHPNCVNCLEDDRCILHCANFNVLSTVFPPTSFGPLVRKIFVDGVPFVSTGYHFRELGVVHNQDVNLHSSRLSFKELLVYAADPAMHAASGNLLLDKRTTCFSAALTNNVAFQTVKPGNFNKFDFYDAVSKGFFKEGSSVELKHFFFAQDGNAAISDYDYRYNLPMTCDIRQLLFVVEVVDKYFDCYDGGCINANQVIVNNLDKSAGFPFNKWGKARLYYDSMSYEDQDALFAYTKRNVIPITITQMNLYAISAKNRARTVAGVSICSTMTRNQFHQKLLKSIAATRGATVVIKTSKFGGWHNMLKTVYSDVENPHLMGWDPKCDRAMPNMLRIMASVLARKHTTCCSLSHRFYRLANCAQVLSEMVCMCGGSLYVKPGGTSSGDATTAYANSVFNICQAVTANVNALLSTDGNGKIADKYVRNLQHRLEYCLYRNRDVTDFVNEFYAYLRKHFSMMILSDDAVVCFNSTYASQGLVASIKNFKSVLYYQNNVFMSEAKCWTETDLTKGPHEFCSQH TMLVKQGDDYVYLPYDPSPRILGAGCFVDDIVKTDGTLMIERFVSLAIDAYPLTKHPNQEYADVHLYLQYIRKLHDELTDGMLDMYSVMLTNDNTSRYWEPEFYEAMYPHTVLQLVPRGSGHHHHHHHAWSHQPQFEK DZT1: SNAAIASEFSSLPYAAFATAQEAYEQAVANGDSEVVLKKLKKSLNVAKSEFDRDAAMQRKLEKMADQAMTQMYKQARSEDKRAKVTSAMQTMFTMLRKLNDALNNIINNARDGCVPLNIIPLTTAAKLMVIPDYNTYKNTCDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVTALRANSVKKLQ DZU1: SNASKMSDVKCTSVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEAFEKMSVLLSVLLSMQGAVDINKLCEEMLDNRATLQ RNA: 5'-UUUUCAUGCUCGCGUAGUUUUUCUACGCG-3'				

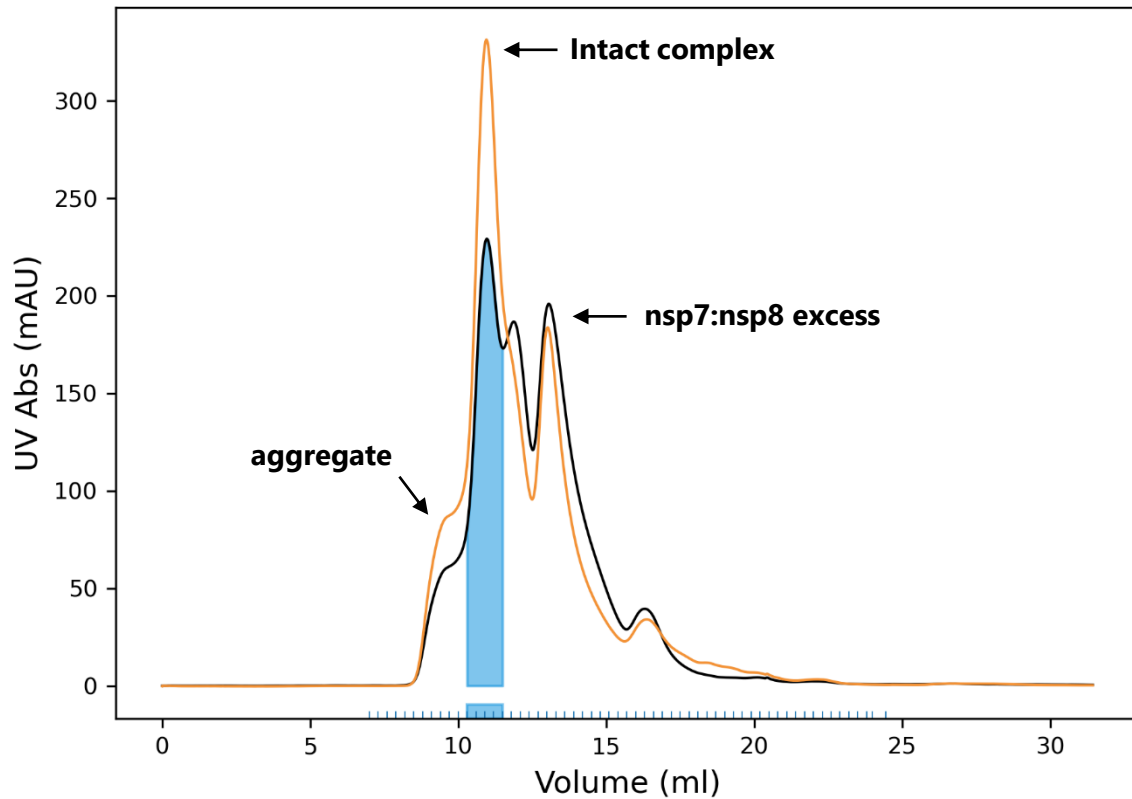
Handling information: Store at – 80 °C, use immediately after thawing, do not re-freeze.

Released by: KS

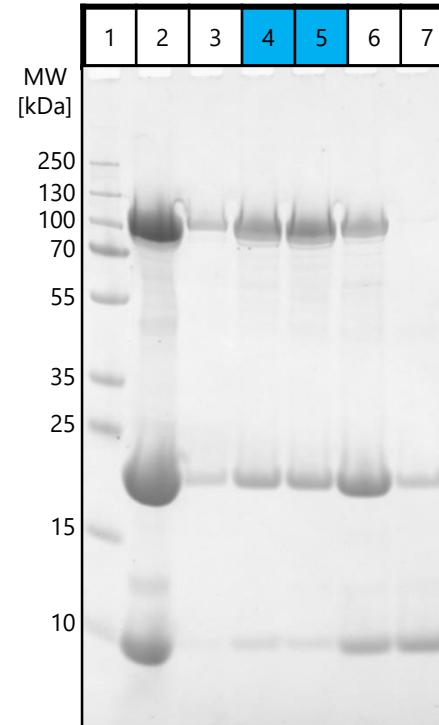
\* Measured after one freeze-thaw cycle

# Preparative SEC - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

Chromatogram / CHR1 SEC



SDS-PAGE / Coomassie stained

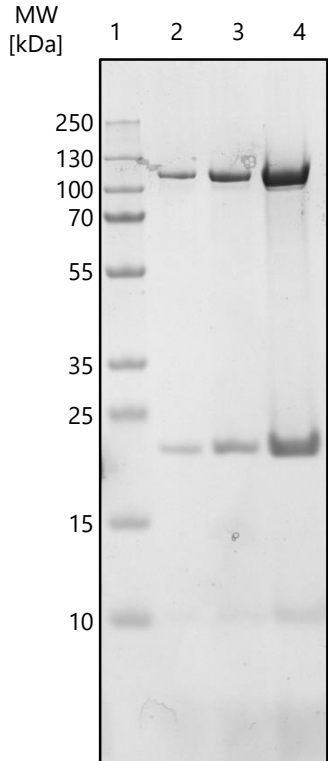


Lane	Sample
1	Marker: PageRuler Plus
2	Input
3	Aggregate
4	Complex #1
5	Complex #2
6	Shoulder
7	nsp7:nsp8 excess

- Pooled fractions are highlighted in blue

# Quality control - nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

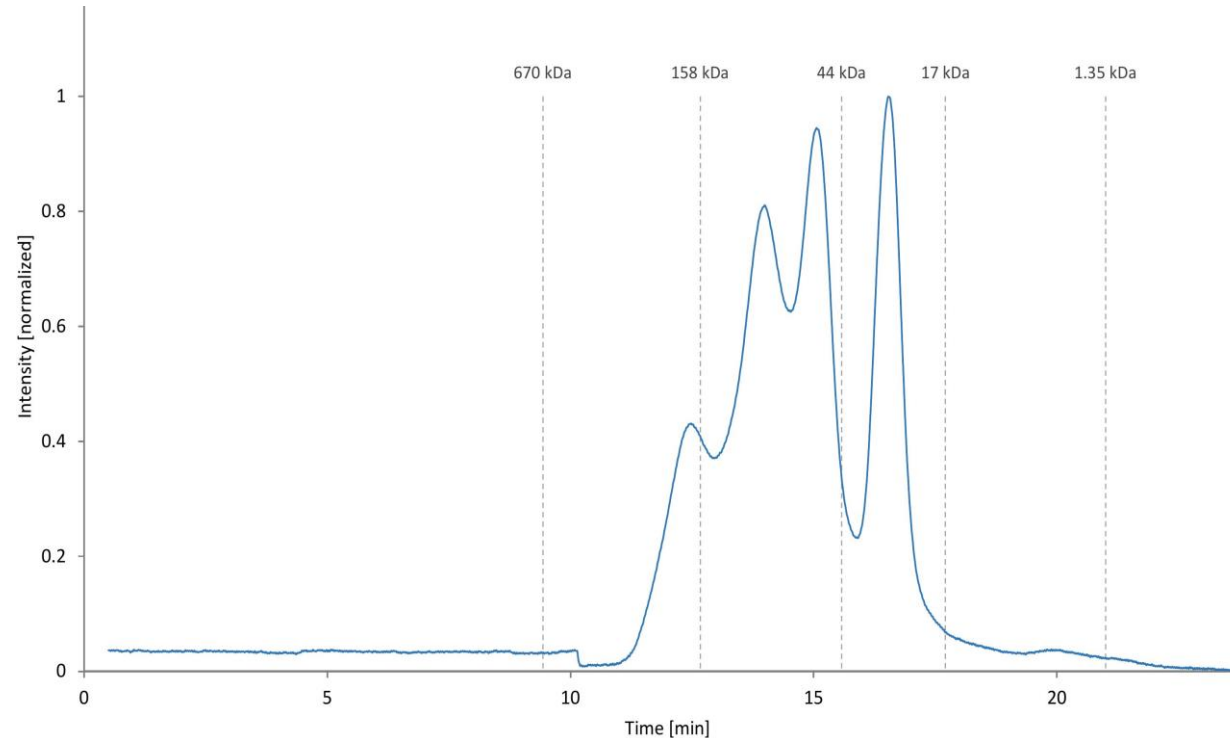
## SDS-PAGE



- 10% Tris-Glycine gel
- Reducing condition
- Coomassie stained
- Sample: nsp12:nsp8:nsp7 - DVT1:DZT1:DZU1, PC14847
- Theoretical MW [kDa]  
Complex: 150.3  
DVT1: 109.5  
DZT1: 22.2  
DZU1: 9.5

Lane	Quantity
1	Marker
2	1 µg
3	2 µg
4	5 µg

## aSEC chromatogram



- Column: S200 increase 10/300
- Temperature: RT
- Running buffer corresponds to storage buffer
- Retention time of globular standard proteins is indicated by dotted lines

- SDS-PAGE shows pure sample (>95% purity); no indication of degradation
- Analytical SEC after one freeze-thaw cycle shows multiple peaks corresponding to individual subunits  
→ Complex dissociates at room temperature

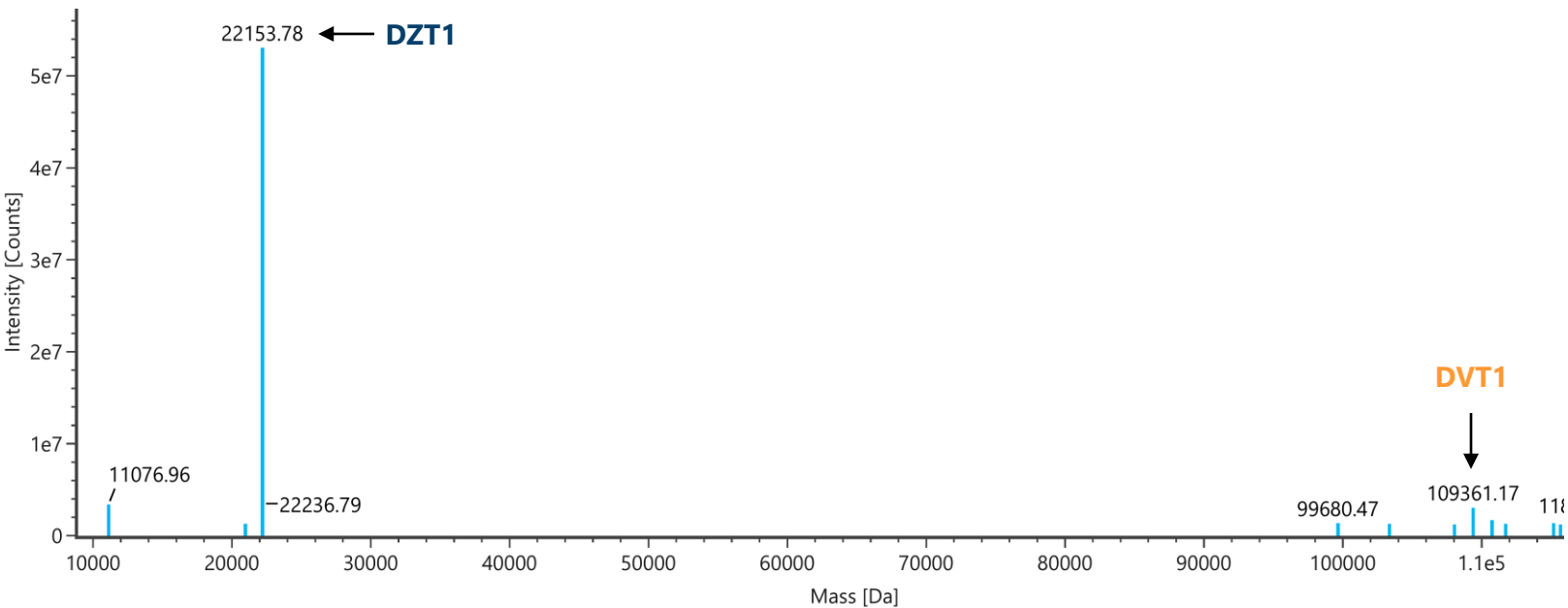
# LC-MS Intact Mass Analysis – nsp12:nsp8:nsp7 (DVT1:DZT1:DZU1)

- Sample: nsp12 (SARS-CoV-2) - DVT1 – PC14847

## Mass spectrum intact mass analysis

Item name: DVT1.33 PC14847

Item name: DVT1.33 PC14847



Theoretical weight DVT1 [Da]	109449.3
Observed weight (main species) [Da]	109361.2
Mass difference [Da]	-88.1

Theoretical weight DZT1 [Da]	22153.3
Observed weight (main species) [Da]	22153.8
Mass difference [Da]	+0.5

Theoretical weight DZU1 [Da]	9512.1
Observed weight (main species) [Da]	n.a.
Mass difference [Da]	

- Waters BioAccord LC-MS / ESI-TOF system
- Denaturing LC conditions
- Deconvolution range: theoretical MW +/- 10 kDa

- Intact mass analysis confirms the overall integrity of DVT1 and DZT1
  - Observed mass difference of DVT1 indicates N-terminal methionine excision (-131 Da) and acetylation (+42 Da) of this subunit
- No signal could be detected for DZU1



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