Supplementary Information

Oligomeric state of β-coronavirus non-structural protein 10 stimulators studied by Small Angle X-ray Scattering

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Table S1. Summary of nsp10 constructs from β-CoVs used in this study.

Protein construct and name	Residue numbering	Original polypetide numbering	Calculated MW after cleavage [Da]	Calculated pI after cleavage
Short SARS-CoV-2 nsp10	10-133	Asn4264 - Gln4385	13,272	7.70
Long SARS-CoV-2 nsp10	1-133	Ala4255 - Gln4385	14,026	6.70
Full-length SARS-CoV-2 nsp10	1-139	Ala4255 - Gln4391	15,022	6.70
Full-length MERS nsp10	1-140	Ala4238 - Gln4378	15,122	7.70
Full-length SARS nsp10	1-139	Ala4231- Gln4369	15,075	6.70

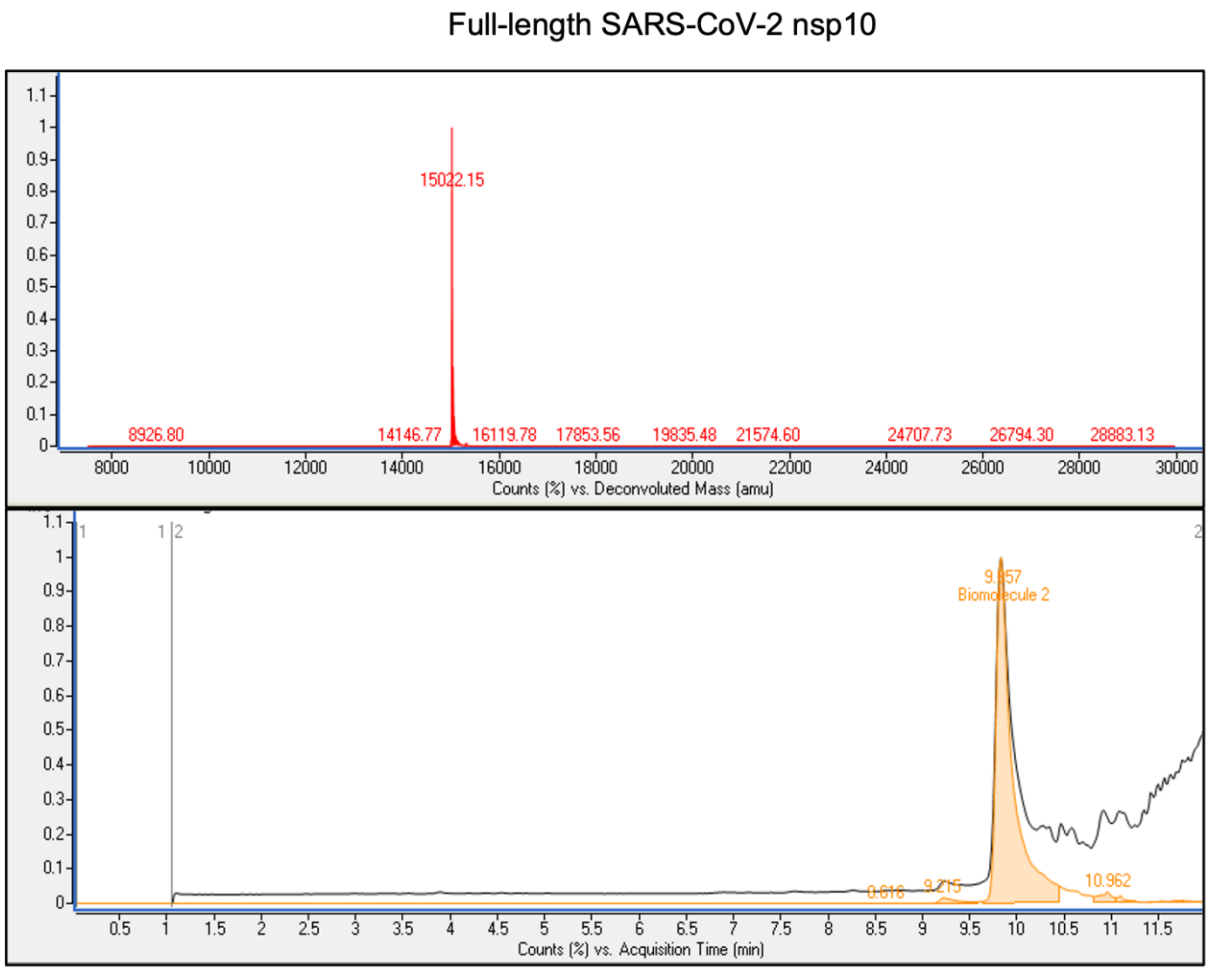
Table S2. SAXS data collection and scattering parameters for the β-CoV nsp10 proteins studied.

Sample	**SARS-CoV-2 nsp10**			**SARS nsp10**	**MERS nsp10**
	full-length	**long**	**short**	**full-length**	**full-length**
Data Collection parameters					
Instrument	B21 Beamline (Diamond Light Source, UK)				
Beam size at sample (mm^2)	1.0 × 0.25				
Wavelength (Å)	0.9464				
s-Range ($Å^{-1}$)	0.0045-0.3400				
Method	SEC-SAXS				
Sample to Detector D (mm)	3722.0				
Temperature (K)	288				
Structural parameters					
R_g (Å) (from Guinier)	17.10 (± 0.14)	16.35 (± 0.10)	15.20 (± 0.12)	17.19 (± 0.10)	17.27 (± 0.10)
R_g (Å) (from P(r))	17.31 (± 0.01)	16.64 (± 0.01)	15.46 (± 0.01)	17.40 (± 0.02)	17.32 (± 0.01)
D_{max} (Å)	59.0	57.0	51.4	59.4	53.3
Porod volume estimate ($Å^3$)*	26542	23162	20976	24726	24363
Molecular mass determination					
Molecular mass from Porod volume (Vp ∗ 0.6) (Da)	15925	13897	12585	14836	14618
Molecular mass from forward scattering (Da)	15395 (± 268)	13985 (± 291)	9324 (± 161)	20841 (± 63)	14328 (± 44)
Molecular mass from sequence (Da)	15151	14026	13272	14843	14891
Software					

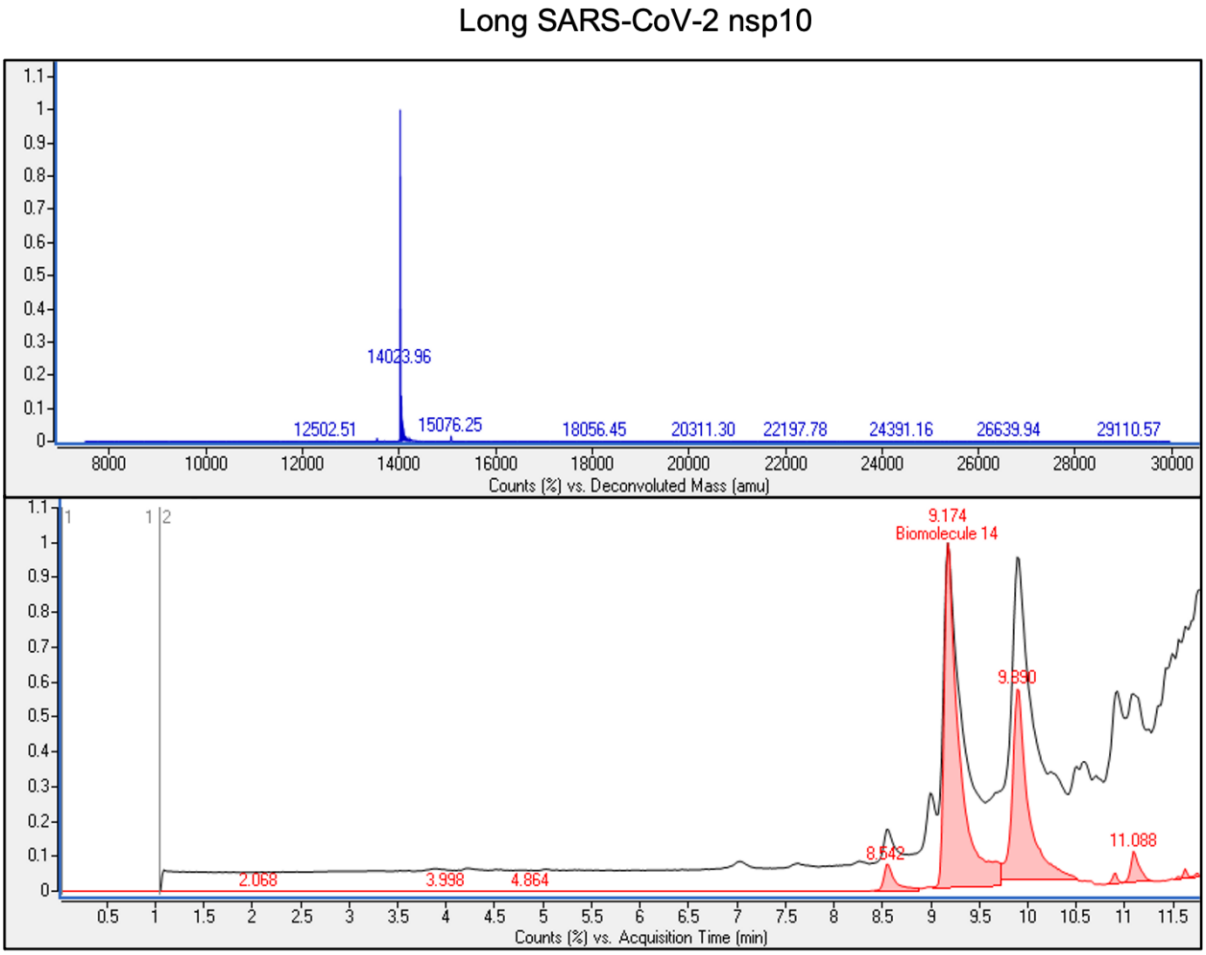
Data processing	ATSAS v3.2.1 (CHROMIX, PRIMUS, GNOM)
Modelling	DAMMIF, DAMMIN (*ab initio*), BUNCH (rigid body)
Atomic models scattering	CRYSOL

*Porod Volume was calculated through Dammif.

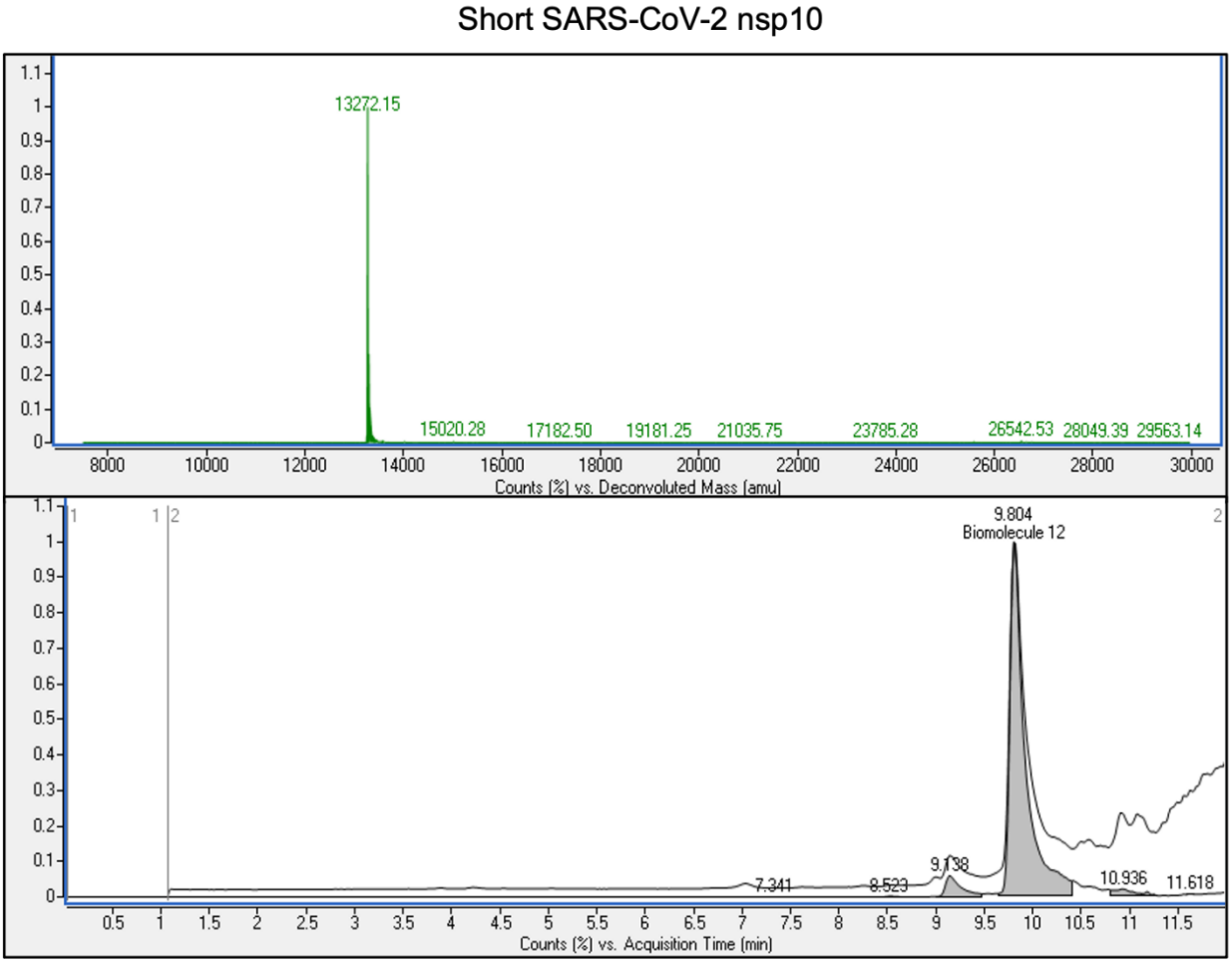
A


Full-length SARS-CoV-2 nsp10
15022.15
8926.80
14146.77
16119.78
17853.56
19835.48
21574.60
24707.73
26794.30
28883.13
Counts (%) vs. Deconvoluted Mass (amu)
Biomolecule 2
10.962
Counts (%) vs. Acquisition Time (min)

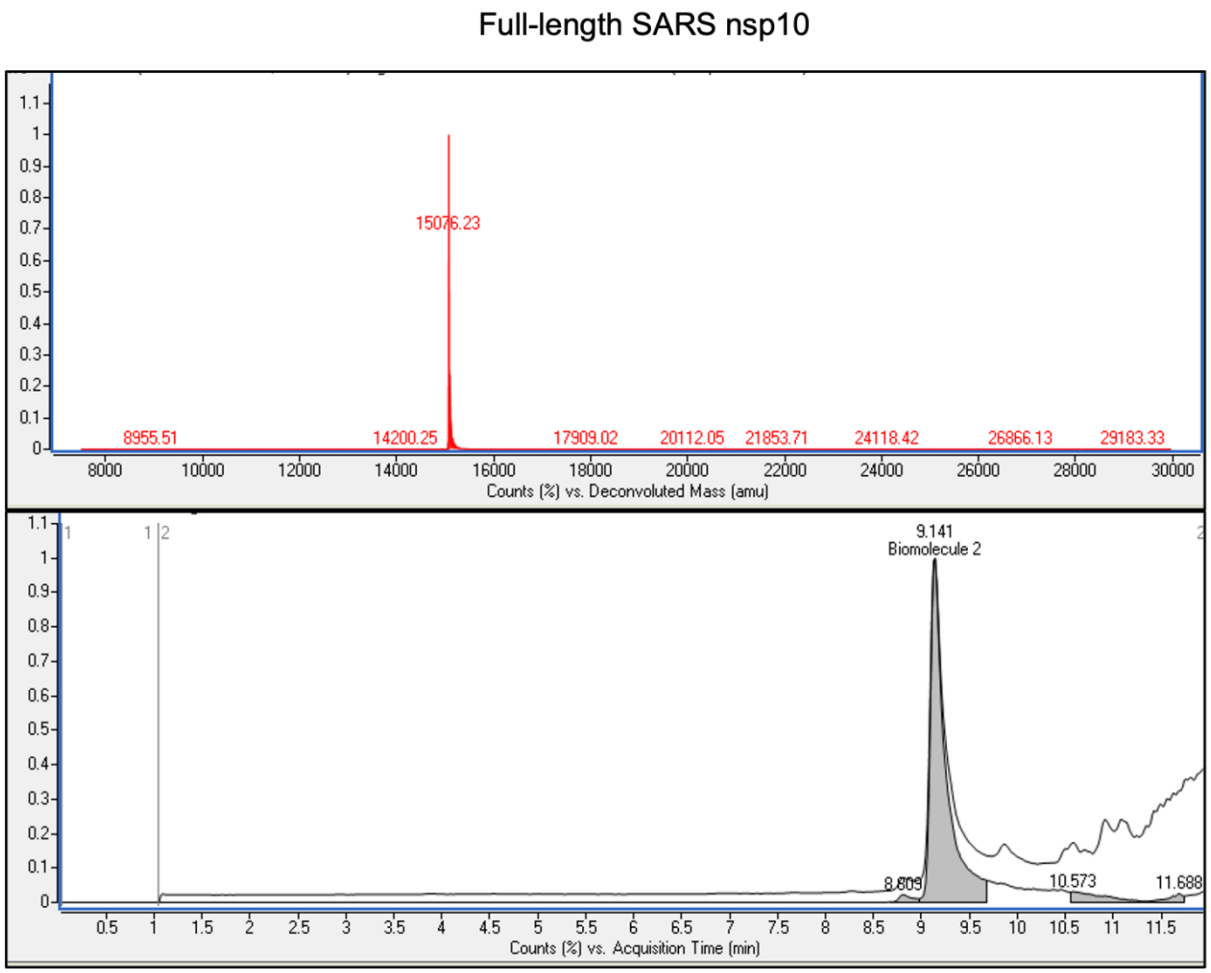
B


Long SARS-CoV-2 nsp10
14023.96
12502.51
15076.25
18056.45
20311.30
22197.78
24391.16
26639.94
29110.57
Counts (%) vs. Deconvoluted Mass (amu)
9.174
Biomolecule 14
9.890
11.088
8.542
2.068
3.998
4.864
Counts (%) vs. Acquisition Time (min)

C


Short SARS-CoV-2 nsp10
13272.15
15020.28
17182.50
19181.25
21035.75
23785.28
26542.53
28049.39
29563.14
Counts (%) vs. Deconvoluted Mass (amu)
9.804
Biomolecule 12
9.138
7.341
8.523
10.936
11.618
Counts (%) vs. Acquisition Time (min)

D


Full-length SARS nsp10
15076.23
8955.51
14200.25
17909.02
20112.05
21853.71
24118.42
26866.13
29183.33
Counts (%) vs. Deconvoluted Mass (amu)
9.141
Biomolecule 2
8.809
10.573
11.688
Counts (%) vs. Acquisition Time (min)

E

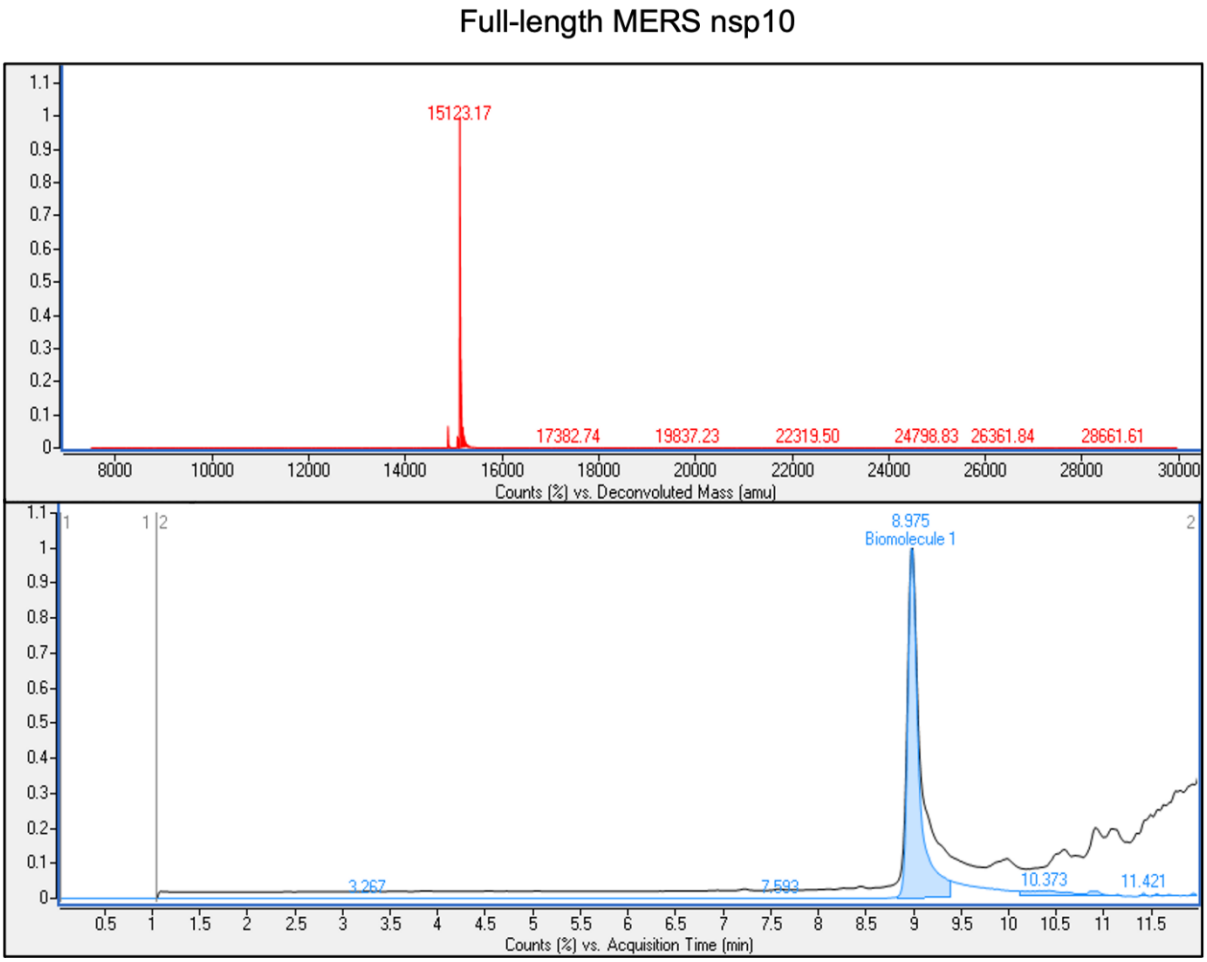


Figure S1. Denatured High-resolution mass spectra (HRMS) of **A)** full-length SARS-CoV-2 nsp10, **B)** long SARS-CoV-2 nsp10, **C)** short SARS-CoV-2 nsp10, **D)** full-length SARS nsp10 and **E)** full-length MERS nsp10. Deconvoluted QTOF-MS spectrums of each sample are showing in the upper panels. The signal-counts of the main peaks were normalised to 1. The calculated mass values were labelled on the top of main peaks. Chromatograms of each sample using reversed-phase chromatography are in the lower panels. The signal-counts of the main peaks were normalised to 1. The acquisition time was labelled on the top of main peaks.

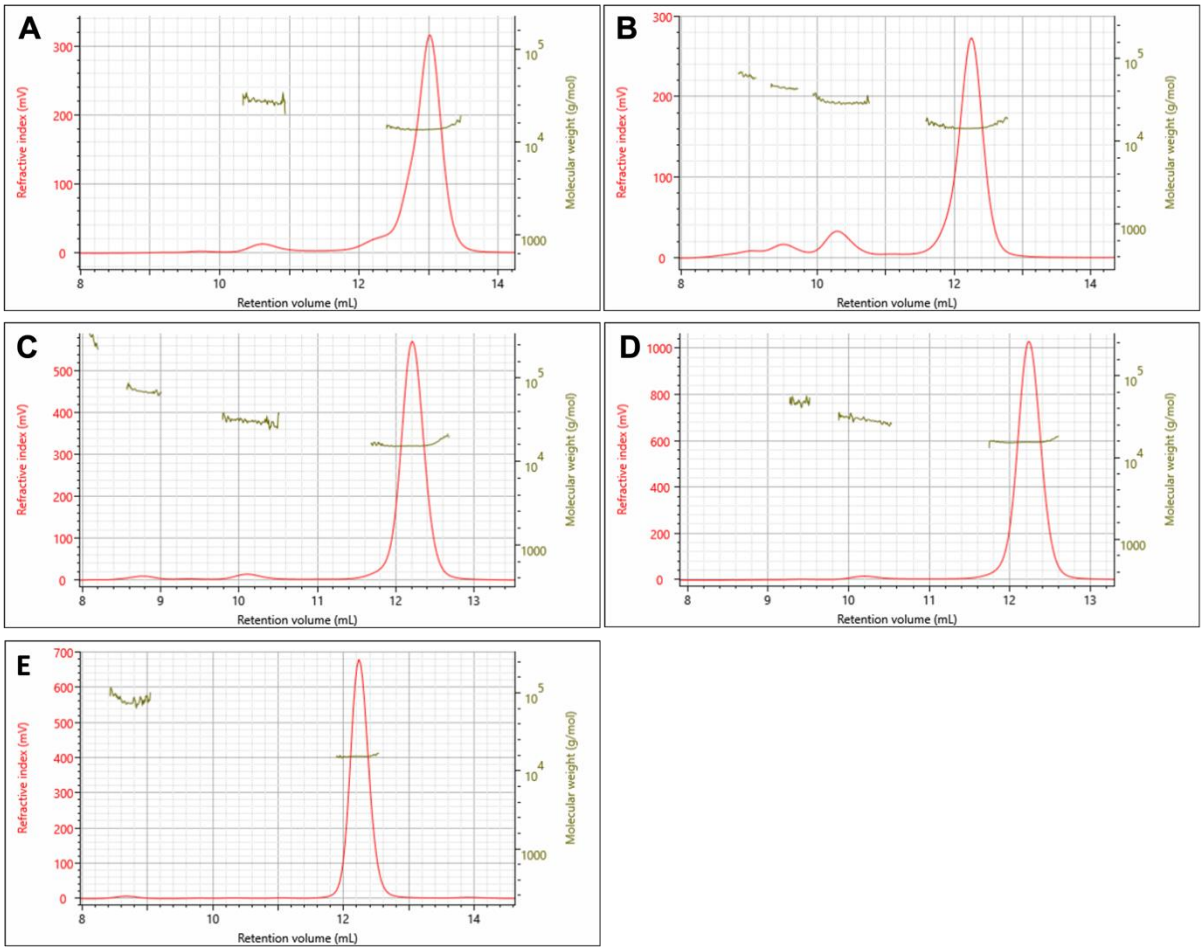


Figure S2. Representative OmniSEC traces. **A.** Short SARS-CoV-2 nsp10. **B.** Long SARS-CoV-2 nsp10. **C.** Full-length SARS-CoV-2 nsp10. **D.** Full-length SARS nsp10. **E.** Full-length MERS nsp10. The refractive indexes are shown in red; the determined molecular weight of the peaks are in green.

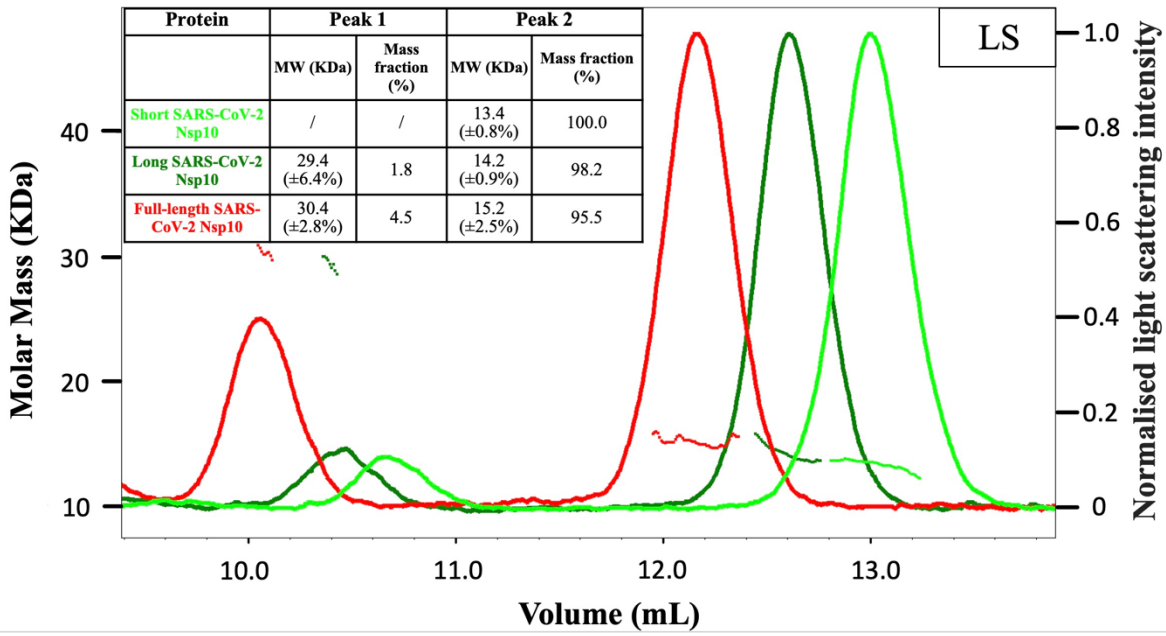


Figure S3. SEC-MALS analysis of three SARS-CoV-2 nsp10 constructs. Normalised light scattering (LS) peaks of short, long, and full-length SARS-CoV-2 nsp10s are coloured in light green, dark green, and red, respectively. The inset report on the molecular weight and mass fractions measured experimentally using SEC-MALS, where the lower peaks are categorised as peak 2 and the main peaks are named peak 2. The molecular weights for the second peak of short SARS-CoV-2 nsp10 was not statistically significant and therefore not included in the table.

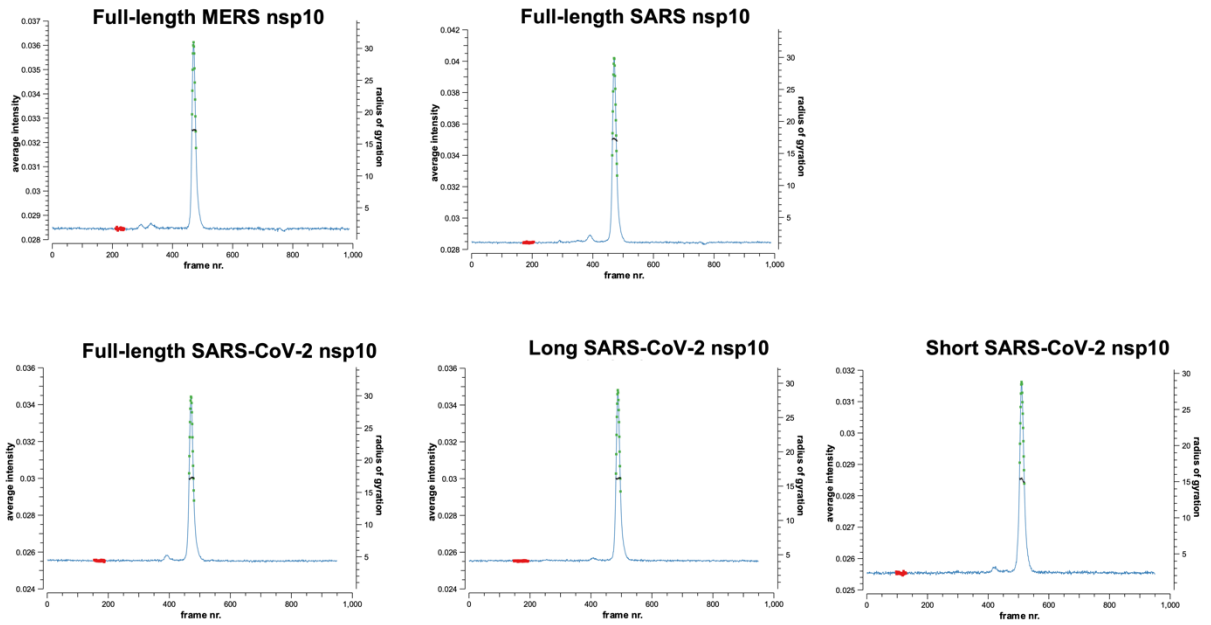


Figure S4. Scattering profiles for the nsp10 samples, measured by SEC/SAXS. The frames selected for buffer and peak calculations are shown as red dots. Peak frames were adjusted to generate a consistent R_g value (shown as black dots).

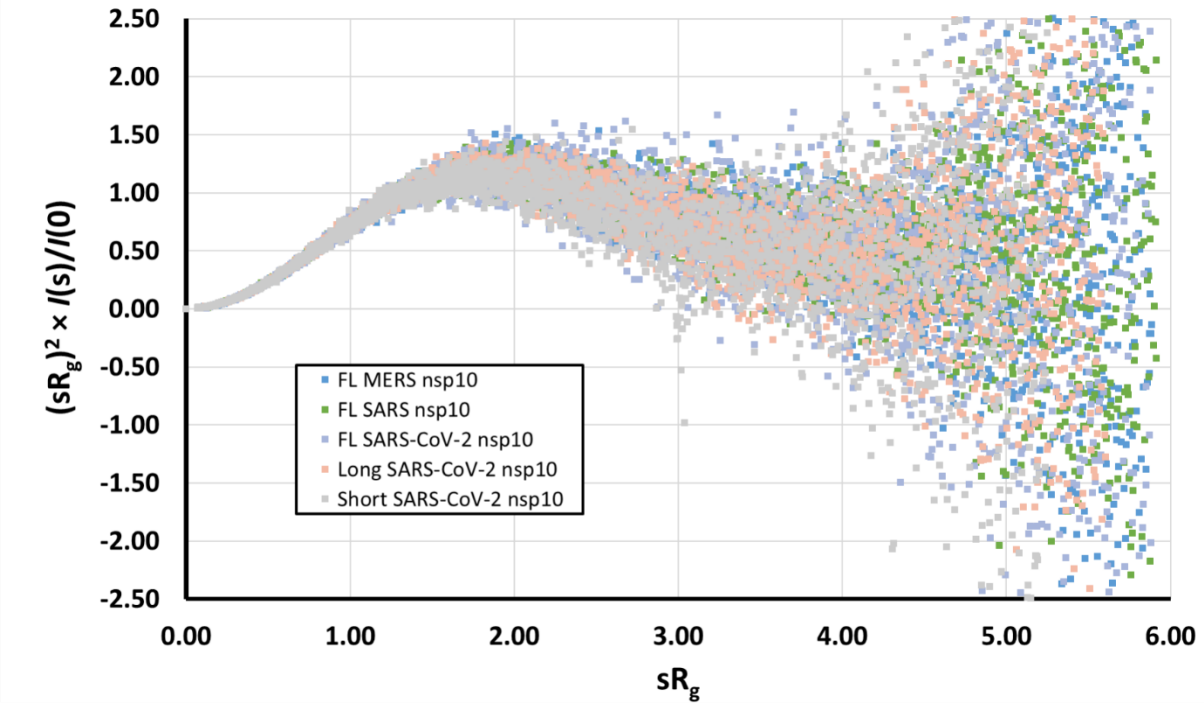


Figure S5. Dimensionless Kratky plots for the nsp10s measured by SAXS as shown in Fig 2.