Table S1: Overview of labs as assigned to protocols

Protein	System	Main protocol from group(s)	Protocol as „additional information“ from group(s)
nsp1	Bacterial	Carlomagno (fl), Schlundt (GD)	Schlundt (fl)
nsp2	Bacterial	Laurents (CtDR)	-
nsp3a	Bacterial	Blackledge (UBl+IDR), Schlundt (UBl)	-
nsp3b	Bacterial	Schwalbe (Macrodomain)	Alfano (Macrodomain)
nsp3c	Bacterial	Spyroulias (SUD-N, SUD-NM, SUD-M, SUD-MC, SUD-C)	-
nsp3d	Bacterial	Schwalbe (PL^{pro})	Schwalbe (PL^{pro})
nsp3e	Bacterial	Schlundt (NAB)	Schlundt (NAB)
nsp3Y	Bacterial	Hoch (CoV-Y)	-
nsp5	Bacterial	Schwalbe (fl)	Schwalbe (fl, **A**-**D**), Orts (fl, **E**), Varga (fl, **F**), Bax ((fl, **G**-**H**), Martin ((fl, **I**)
nsp7	Bacterial	Henzler-Wildman/Kirchdoerfer (fl)	-
nsp8	Bacterial	Henzler-Wildman/Kirchdoerfer (fl)	-
nsp9	Bacterial	Schlundt (fl)	Schlundt (fl, **A**), Alfano (fl, **B**)
nsp10	Bacterial	Schwalbe (fl)	Jaudzems (fl)
nsp13	Bacterial	Schwalbe (fl)	Schwalbe (fl)
nsp14	Bacterial	Jaudzems (fl, MTase)	Schwalbe (fl)
nsp15	Bacterial	Schwalbe (fl)	-
nsp16	Bacterial	Jaudzems (fl)	Jaudzems (fl)
ORF3a	Cell-free	Böckmann (fl)	-
Envelope (ORF4)	Cell-free	Böckmann/Meier (fl)	-
Membrane (ORF5)	Cell-free	Böckmann/Meier (fl)	Böckmann/Meier (fl)
ORF6	Cell-free	Böckmann (fl)	Böckmann (fl)
ORF7a	Bacterial	Muhle-Goll (ED)	-
ORF7b	Bacterial	Schwalbe (fl)	Schwalbe (fl, **A**-**E**)
	Cell-free	Böckmann (fl)	-

ORF8	Bacterial	Wiedemann/Ohlenschläger (fl-L84S) Alfano (w/o signal peptide (Δ))	Wiedemann/Ohlenschläger (fl)
	Cell-free	Böckmann (fl, Δ)	-
Nucleo-capsid (ORF9a)	Bacterial	Pierattelli/Felli (IDR1-NTD-IDR2), Almeida (NTD-SR, NTD), Schlundt (CTD)	-
ORF9b	Cell-free	Böckmann (fl)	Böckmann (fl, **A**-**B**)
ORF14	Cell-free	Böckmann/Meier (fl)	-
ORF10	Bacterial	Schwalbe (fl)	Schwalbe (fl, **A**-**D**)

Table S2: Abbreviations used throughout the SI

Abbreviation	**Full name**
aa	Amino acid
AC	Affinity chromatography
BEST	Band-selective Excitation Short-Transient
BisTris	2,2-Bis(hydroxymethyl)-2,2′,2″-nitrilotriethanol
bME	2-mercaptoethanol
BMRB	Biomagnetic Resonance Databank
Brij 58	Polyethylene glycol hexadecyl ether, Polyoxyethylene (20) cetyl ether
CFPS	Cell-free protein synthesis
CFS	Cell-free sample
CoV	Coronavirus
CTD	C-terminal domain
DDM	n-dodecyl β-D-maltoside
E. coli	*Escherichia coli* cells
ED	Ectodomain
fl	Full-length
GB1	Protein G B1 domain
GD	Globular domain
GST	Glutathione-S-transferase
His_6 (analog His_7)	Hexahistidine tag
HSQC	Heteronuclear single quantum coherence
IDR	Intrinsically disordered region
IEC	Ion exchange chromatography
IMAC	Immobilized metal ion affinity chromatography
Inv.	Inverse
IPTG	Isopropyl-β-d-thiogalactopyranoside
LB medium	Lysogeny broth medium
M9 medium	M9 minimal medium
MOPS	3-(N-morpholino)propanesulfonic acid, 4-morpholinepropanesulfonic acid
M^{pro}	Main protease

MTase	Methyltransferase
MWCO	Molecular weight cut-off
NAB	Nucleic acid-binding domain
NaPi/KPi	Sodium/potassium phosphate
NA	Not available
n.d.	Not defined/no information available
nsp	Non-structural protein
NTA	Nitrilotriacetic acid
NTD	N-terminal domain
o.n.	Overnight
OD_{600}	Optical density at 600 nm
ORF	Open reading frame
PDB	Protein Data Bank
PL^{pro}	Papain-like protease
rt	Room temperature
S, SARS	Severe acute respiratory syndrome
SD	Superdex
SEC	Size exclusion chromatography
SN	Soluble fraction, supernatant
SUD	SARS unique domain
SUMO	Small ubiquitin-like modifier
TCEP	Tris-(2-carboxyethyl)-phosphin
TEV	Tobacco etch virus
Triton X-100	4-(1,1,3,3-Tetramethylbutyl)-phenyl-polyethylenglykol
TROSY	Transverse relaxation-optimized spectroscopy
Trx	Thioredoxin
Ubl	ubiquitin-like domain
Ulp1	ubiquitin-like specific protease 1
WB	Western blot
WG(E)	Wheat germ (extract)
YT medium	Yeast extract-tryptone medium

SI1: nsp1

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp1
2	**Region/Name/Further Specification**
	nsp1 / Leader protein
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MESLVPGFNEKTHVQLSLPVLQVRDVLVRGFGDSVEEVLSEARQHLKDGTCGLVEVEKGVLPQ LEQPYVFIKRSDARTAPHGHVMVELVAELEGIQYGRSGETLGVLVPHVGEIPVAYRKVLLRKNG NKGAGGHSYGADLKSFDLGDELGTDPYEDFQENWNTKHSSGVTRELMRELNGG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
fl	aa 1-180 (fl nsp1)
GD	aa 13-127 of fl nsp1
5	**Ratio for construct design**
fl	fl sequence according to NCBI Reference Sequence YP_009725297.1
GD	In analogy to the available NMR structure (PDB 2GDT) of nsp1 SCoV 13-127
6	**Sequence homology (to SCoV)**
fl	Identity: 83%; similarity: 89%
GD	Identity: 85%; similarity: 90%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PBD 2GDT, 2HSX SCoV2: PBD 7K3N, 7K7P, 6ZN5, 7JQC, 7K5I
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 7014 SCoV2: BMRB 50620

Table 2: Protein Expression

1	**Expression vector**
fl	pETM11 (Gunter Stier, EMBL Heidelberg)
GD	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
fl	N-terminal His_6
GD	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**

fl	19.90 kDa / 12,950 M^{-1} cm^{-1} / 5.37
GD	12.93 kDa / 4,470 M^{-1} cm^{-1} / 6.22
5	**Comments on sequence of expressed construct**
fl	N-terminal „GA" two artificial residues due to TEV-cleavage and construct design
GD	N-terminal „GAMA" four artificial residues due to TEV-cleavage and construct design
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
fl	0.6 mM IPTG at OD_{600} 0.7
GD	1 mM IPTG
10	**Cultivation temperature and time**
fl	16°C for 18-20 h
GD	16°C for 18-20 h

Table 3a: Protein Purification (fl nsp1)

1	**Buffer List**
A	50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 100 mM Na_2SO_4, 5% (v/v) glycerol, 5 mM imidazole, 1 mM TCEP-HCl (cell disruption / immobilized metal affinity chromatography (IMAC) / TEV-cleavage).
B	50 mM Tris-HCl (pH 7.5), 500 mM NaCl, 100 mM Na_2SO_4, 1 mM EDTA, 1 mM TCEP-HCl (SEC).
C	50 mM NaPi (pH 6.5), 200 mM NaCl, 2 mM DTT, 2 mM EDTA (final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Roche), 100 µg of lysozyme (Carl Roth), and 50 µg of deoxyribonuclease (DNAse) (New England Biolabs)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA (Cytiva)), washed first with buffer **1A** and then with buffer **1A** containing additional 2 M LiCl, before eluting with 300 mM imidazole in buffer **1A**.
C	Desalting and TEV-cleavage (0.5 mg TEV protease per 1 L culture) o.n. in buffer **1A**.
D	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1B**.
E	NMR sample preparation in buffer **1C**.

Table 3b: Protein Purification (GD nsp1)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 4 mM DTT (cell disruption / IMAC/ dialysis after IMAC / TEV-cleavage).
B	25 mM NaPi (pH 7.0), 250 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) NaN_3 (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA), Elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in in buffer **1A**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1A**.
E	SEC on HiLoad SD 75 16/600 (GE Healthcare) in buffer **1B**.
F	NMR sample preparation in buffer **1B**.

Table 4: Final samples

1	**Yield**
fl	5 mg/L ^{13}C,^{15}N-M9 medium
GD	< 0.5 mg/L ^{15}N-M9 medium
2	**Stability**
fl	No significant precipitation or degradation observed after storage at 4°C for 3 weeks. Relatively stable during NMR measurements at 25°C for ~7 days, despite some proteolysis of disordered C-terminal tail.
GD	Stable during several weeks storage at 4°C.
3	**Comment on applicability**
fl	Suitable for NMR structure determination, fragment screening, interaction studies.
GD	purification needs optimization to obtain more soluble protein

Additional information

Constructs	**Conditions**	**Comments**
aa 1-180 (fl nsp1); His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal 2 artificial residues “GA“.	As above for GD nsp1.	Yields 2.4 mg/L ^{15}N,^{13}C-M9 medium. Obvious degradation during measurement. Storage at 4°C not advisable. Higher salt concentration seems to slightly improve stability.

SI2: nsp2

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp2
2	**Region/Name/Further Specification**
	C-terminal IDR (CtDR)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AYTRYVDNNFCGPDGYPLECIKDLLARAGKASCTLSEQLDFIDTKRGVYCCREHEHEIAWYTE RSEKSYELQTPFEIKLAKKFDTFNGECPNFVFPLNSIIKTIQPRVEKKKLDGFMGRIRSVYPVASP NECNQMCLSTLMKCDHCGETSWQTGDFVKATCEFCGTENLTKEGATTCGYLPQNAVVKIYCP ACHNSEVGPEHSLAEYHNESGLKTILRKGGRTIAFGGCVFSYVGCHNKCAYWVPRASANIGCN HTGVVGEGSEGLNDNLLEILQKEKVNINIVGDFKLNEEIAIILASFSASTSAFVETVKGLDYKAFK QIVESCGNFKVTKGKAKKGAWNIGEQKSILSPLYAFASEAARVVRSIFSRTLETAQNSVRVLQK AAITILDGISQYSLRLIDAMMFTSDLATNNLVVMAYITGGVVQLTSQWLTNIFGTVYEKLKPVL DWLEEKFKEGVEFLRDGWEIVKFISTCACEIVGGQIVTCAKEIKESVQTFFKLVNKFLALCADSII IGGAKLKALNLGETFVTHSKGLYRKCVKSREETGLLMPLKAPKEIIFLEGETLPTEVLTEEVVLK TGDLQPLEQPTSEAVEAPLVGTPVCINGLMLLEIKDTEKYCALAPNMMVTNNTFTLKGG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 557-601 of complete nsp2 (Ct-DR)
5	**Ratio for construct design**
	Based on disorder predictions (PrDOS (Ishida and Kinoshita, 2007))
6	**Sequence homology (to SCoV)**
	Identity: 55%; similarity: 68%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: 50687

Table 2: Protein Expression

1	**Expression vector**
	Home made plasmid derived from pET28b(+) (EMD Biosciences) containing the codifying sequence for thioredoxin A from *E. coli* and TEV protease cleavage site instead of thrombin.
2	**Purification-/Solubility-Tag**
	N-terminal His_6-Trx
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**

	4.92 kDa / - / 3.9
5	**Comments on sequence of expressed construct**
	N-terminal „G“, one artificial residue due to TEV-cleavage.
6	**Used expression strain**
	E. coli BL21 star (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.6
10	**Cultivation temperature and time**
	37ºC until induction. Following induction, incubation at 25ºC for 17 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole (cell lysis, IMAC1 and 2).
B	5 mM Tris-HCl (pH 8.0), 20 mM NaCl (dialysis after IMAC1/TEV cleavage).
C	5 mM histidine (pH 5.4), 5 mM NaCl (dialysis after IMAC2 and anionic IEC).
D	10 mM acetic acid (pH 4.3), 5 mM NaCl (dialysis after cationic IEC).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell lysis in **1A** (plus 5 µL Halt protease inhibitor (Thermo) and lysozyme 20 µg/mL).
B	IMAC1 (HisTrap crude 5 mL, Cytiva). Elution 10-500 mM imidazole in buffer **1A**.
C	Dialysis in buffer **1B** and TEV cleavage (4ºC, 17 h).
D	IMAC2 (after TEV cleavage) (HisTrap crude 5 mL, Cytiva). Elution 10-500 mM imidazole in buffer **1A** (protein expected in flow-through).
E	Dialysis in buffer **1C** (4ºC, 17 h).
F	Anionic IEC, elution 10-1,000 mM NaCl in buffer **1C**.
G	Dialysis in buffer **1C** (4ºC, 48 h).
H	Cationic IEC. Elution 10-1,000 mM NaCl in buffer **1D** (protein expected in flow-through).

Table 4: Final sample

1	**Yield**
	1.5 mg/L LB medium, 0.7-1.5 mg/L ^{13}C, ^{15}N-M9 medium
2	**Stability**
	No visible precipitation after two weeks at 4ºC.
3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.

SI3: nsp3a

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3a Ubiquitin-like domain (Ubl) + IDR
3	**Sequence of “fl” protein (aa 1-206 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	APTKVTFGDDTVIEVQGYKSVNITFELDERIDKVLNEKCSAYTVELGTEVNEFACVVADAVIKT LQPVSELLTPLGIDLDEWSMATYYLFDESGEFKLASHMYCSFYPPDEDEEEGDCEEEEFEPSTQY EYGTEDDYQGKPLEFGATSAALQPEEEQEEDWLDDDSQQTVGQQDGSEDNQTTTIQTIVEVQP QLEMELTPVVQTIE
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
Ubl+ IDR	aa 1-206 of complete nsp3
Ubl	aa 1-111 of complete nsp3
5	**Ratio for construct design**
Ubl+ IDR	Based on homologous structure from SCoV.
Ubl	Based on disorder prediction, folded domain and SCoV Ubl1.
6	**Sequence homology (to SCoV)**
Ubl+ IDR	Identity: 58%; Similarity: 75%
Ubl	Identity: 79%; Similarity: 89%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2GRI; 2IDY
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 7019 SCoV2: BMRB 50446

Table 2: Protein Expression

1	**Expression vector**
Ubl+ IDR	pET-TEV-Nco (GenScript)
Ubl	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
Ubl+ IDR	N-terminal His_6

Ubl	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
Ubl+ IDR	23.50 kDa / 24,410 M^{-1} cm^{-1} / 3.62
Ubl	12.72 kDa / 14,440 M^{-1} cm^{-1} / 4.08
5	**Comments on sequence of expressed construct**
Ubl+ IDR	N-terminal “GAM” three artificial residues due to TEV-cleavage and construct design.
Ubl	N-terminal “GAMG” four artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{600} 0.6-0.8
10	**Cultivation temperature and time**
Ubl+ IDR	37ºC for 5 h
Ubl	18°C for 18 h

Table 3a: Protein Purification (Ubl + IDR)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and complete EDTA-free tablet (cell disruption).
B	50 mM Tris-HCl (pH 8.0) and 150 mM NaCl (wash buffer).
C	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 500 mM imidazole (elution buffer).
D	50 mM Tris-HCl (pH 8.0), 150 mM NaCl and 5 mM bME (TEV cleavage).
E	50 mM NaPi (pH 6.5), 250 mM NaCl (final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Resuspension of cell pellet in 50 mL per liter of culture of **1A** at 4°C.
B	Cell disruption by sonication on ice.

C	Clarification of lysate by centrifugation at 16,000 g for 30 min at 4°C.
D	Loading of lysate on Ni^{2+}-loaded IMAC resin (ThermoFisher scientific) pre-equilibrated with **1B** at 22°C.
E	Wash IMAC resin with 50 bed volumes of **1B**.
F	Elute protein from IMAC resin with 5 bed volumes of **1C**.
G	TEV cleavage with 1 mg TEV per 50 mg protein by dialysis against **1D** for 18 h at 4°C.
H	Removal of uncleaved protein and tag by elution through Ni^{2+}-loaded IMAC resin pre-equilibrated with **1B** at 22°C.
I	Wash with 5 bed volumes of **1B**.
J	SEC with HiLoad SD 75 pg column (GE Healthcare) pre-equilibrated with **1E** at 4°C.

Table 3b: Protein Purification (Ubl)

1	**Buffer List**
A	50 mM NaPi (pH 6.5), 300 mM NaCl, 10 mM imidazole, 2 mM TCEP-HCl (Cell disruption / IMAC)
B	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM DTT, 0.02% NaN3 (dialysis after IMAC / TEV-cleavage)
C	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl, 0.02% NaN_3, pH7 (SEC / final NMR buffer)
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA), Elution with 150-500 mM imidazole in buffer **1A**
C	Dialysis o.n. in in buffer **1B**
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1B**
E	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1C**
F	NMR sample preparation in buffer **1C**

Table 4: Final sample

1	**Yield**
Ubl+ IDR	0.7 mg/L ^{15}N-M9 medium
Ubl	2-3 mg/L ^{15}N-M9 medium
1b	**A260/280 ratio**
Ubl+ IDR	0.57
Ubl	0.6
2	**Stability**
Ubl+	2 weeks at 25°C.

IDR	
Ubl	Very stable over weeks.
3	**Comment on applicability**
Ubl+ IDR	Stable for NMR assignments and screening
Ubl	Stable for NMR assignments and screening (spectra overlay with folded part of nsp3a Ubl + IDR above.)

SI3: nsp3b

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3b / Macrodomain
3	**Sequence of “fl” protein (aa 207-376 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	VNSFSGYLKLTDNVYIKNADIVEEAKKVKPTVVVNAANVYLKHGGGVAGALNKATNNAMQV ESDDYIATNGPLKVGGSCVLSGHNLAKHCLHVVGPNVNKGEDIQLLKSAYENFNQHEVLLAPL LSAGIFGADPIHSLRVCVDTVRTNVYLAVFDKNLYDKLVSSFLEMK
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 207-376 of complete nsp3
5	**Ratio for construct design**
	Based on homologous structure from SCoV (PDB 6VXS).
6	**Sequence homology (to SCoV)**
	Identity: 74%; similarity: 84%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6W6Y, 6YWM, 6YWL, 6YWK, 6WEY, 7KG3, 6W02, 6WOJ, 6WEN, 6WCF, 6VXS, 7JME
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV2: BMRB 50387 (apo), 50388 (holo)

Table 2: Protein Expression

1	**Expression vector**
	pET28a(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	18.65 kDa / 10,430 M^{-1} cm^{-1} / 7.20
5	**Comments on sequence of expressed construct**
	N-terminal “GHM” three artificial residues due to TEV-cleavage and construct design.

6	**Used expression strain**
	E. coli T7 Express
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME (cell disruption / IMAC).
B	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM bME (dialysis after IMAC / TEV-cleavage).
C	25 mM BisTris (pH 6.5), 150 mM NaCl, 3 mM TCEP-HCl (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A.**
C	TEV-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1B.**
D	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with 500 mM imidazole in buffer **1A**.
E	SEC (HiLoad 26/600 SD 200 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1C** (elution volume 245-290 mL).
F	NMR sample preparation in buffer **1C.**

Table 4: Final sample

1	**Yield**
	94 mg/L ^{15}N-M9 medium, 9 mg/L ^{13}C,^{15}N-M9 medium
2	**Stability**
	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 2 weeks.
3	**Comment on applicability**

	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

Constructs	Conditions	Comments
aa 206-374 of complete nsp3; His_6-GST (mod pET9d), TEV-cleavage site, N-terminal “GAM“ three artificial residues. Based on boundaries from crystal structure (PDB 6W6Y).	**IMAC buffer:** 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 5% (v/v) glycerol, 50 mM imidazole, 1 mM DTT. **Cleavage buffer:** 50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 1 mM DTT. **SEC/final buffer:** 20 mM NaPi (pH 7.4), 150 mM NaCl, 3 mM TCEP-HCl.	Yields 30 mg/L LB medium. No significant precipitation or degradation observed after storage at 4°C for 10 days. Suitable for NMR studies, fragment-based screening, interaction studies.

SI3: nsp3c

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
SUD-N	nsp3c / SARS Unique Domain (SUD) -N
SUD-NM	nsp3c / SUD-NM
SUD-M	nsp3c / SUD-M
SUD-MC	nsp3c / SUD-MC
SUD-C	nsp3c / SUD-C
3	**Sequence of "fl" protein (aa 409-743 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	QDDKKIKACVEEVTTTLEETKFLTENLLLYIDINGNLHPDSATLVSDIDITFLKKDAPYIVGDVV QEGVLTAVVIPTKKAGGTTEMLAKALRKVPTDNYITTYPGQGLNGYTVEEAKTVLKKCKSAFY ILPSIISNEKQEILGTVSWNLREMLAHAEETRKLMPVCVETKAIVSTIQRKYKGIKIQEGVVDYG ARFYFYTSKTTVASLINTLNDLNETLVTMPLGYVTHGLNLEEAARYMRSLKVPATVSVSSPDA VTAYNGYLTSSSKTPEEHFIETISLAGSYKDWSYSGQSTQLGIEFLKRGDKSVYYTSNPTTFHLD GEVITFDNLKTLLS
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
SUD-N	aa 409-548 of complete nsp3
SUD-NM	aa 409-675 of complete nsp3
SUD-M	aa 551-675 of complete nsp3
SUD-MC	aa 551-743 of complete nsp3
SUD-C	aa 680-743 of complete nsp3
5	**Ratio for construct design**
SUD-N	Based on X-ray structure of homologue nsp3c from SCoV (PDB 2W2G).
SUD-NM	Based on X-ray structure of homologue nsp3c from SCoV (PDB 2W2G).
SUD-M	Based on X-ray structure of homologue nsp3c from SCoV (PDB 2W2G).
SUD-MC	Based on NMR structure of homologue nsp3c from SCoV (PDB 2KQV, 2KQW).
SUD-C	Based on NMR structure of homologue nsp3c from SCoV (PDB 2KAF).
6	**Sequence homology (to SCoV)**

SUD-N	Identity: 69%, similarity: 81.6%
SUD-NM	Identity: 74%, similarity: 85.4%
SUD-M	Identity: 82%, similarity: 89.6%
SUD-MC	Identity: 79%, similarity: 88.7%
SUD-C	Identity: 73%, similarity: 87.7%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
SUD-N	SCoV2: BMRB 50448
SUD-NM	Ongoing
SUD-M	SCoV2: BMRB 50516 SUD-M
SUD-MC	Ongoing
SUD-C	SCoV2: BMRB 50517 SUD-C

Table 2: Protein Expression

1	**Expression vector**
SUD-N	pGEX4T1 (Addgene)
SUD-NM	pGEX4T1 (Addgene)
SUD-M	pET28a(+) (Addgene)
SUD-MC	pET28a(+) (Addgene)
SUD-C	pGEX4T1 (Addgene)
2	**Purification-/Solubility-Tag**
SUD-N	N-terminal GST
SUD-NM	N-terminal GST
SUD-M	N-terminal His_6
SUD-MC	N-terminal His_6
SUD-C	N-terminal GST

3	**Cleavage Site**
	Thrombin
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
SUD-N	15.54 kDa / 8,940 M^{-1} cm^{-1} / 5.04
SUD-NM	29.60 kDa / 26,360 M^{-1} cm^{-1} / 6.03
SUD-M	14.27 kDa / 17,420 M^{-1} cm^{-1} / 8.71
SUD-MC	21.94 kDa / 28,880 M^{-1} cm^{-1} / 6.58
SUD-C	7.42 kDa / 11,460 M^{-1} cm^{-1} / 4.82
5	**Comments on sequence of expressed construct**
SUD-N	N-terminal „GS" two artificial residues due to thrombin-cleavage
SUD-NM	N-terminal „GS" two artificial residues due to thrombin-cleavage
SUD-M	N-terminal „GSHM" four artificial residues due to thrombin-cleavage and cloning
SUD-MC	N-terminal „GSHM" four artificial residues due to thrombin-cleavage and cloning
SUD-C	N-terminal „GS" two artificial residues due to thrombin-cleavage
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{600} 0.6-0.8
10	**Cultivation temperature and time**
	18˚C for 18-20 h

Table 3a: Protein Purification (SUD-N and SUD-NM)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl (cell disruption / affinity chromatography (AC)).
B	50 mM NaPi (pH 7.2), 50 mM NaCl, 2 mM EDTA, 2 mM DTT (SEC / NMR buffer).

C	50 mM Tris-HCl (pH 8.0), 10 mM reduced glutathione (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 25 µL protease inhibitor cocktail (Sigma Aldrich P8849) and 2 mM DTT) by sonication, after sonication incubation with 25 µL DNase (1 mg/mL) for 10 min on ice.
B	AC - GSTrap (GE Healthcare) (wash buffer **1A**).
C	Cleavage on column (100 µL thrombin (10 mg/mL) per 0.5 L culture) at 4˚C for 16 h.
D	Elution of SUD-N, SUD-NM after cleavage with buffer **1A**, elution of GST with buffer **1C** and buffer exchange with Amicon Ultra 15 mL centrifugal filter membrane (10,000 MWCO) (Merck Millipore) to buffer **1B**.
E	SEC - SD Increase 75 10/300 GL (GE Healthcare) in buffer **1B**.
F	NMR sample preparation in buffer **1B**.

Table 3b: Protein Purification (SUD-M and SUD-MC)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 500 mM NaCl (Cell disruption / IMAC).
B **SUD-M**	50 mM NaPi (pH 7.2), 50 mM NaCl, 2 mM EDTA, 2 mM DTT (SEC / NMR buffer).
B **SUD-MC**	50 mM NaPi (pH 7.6), 50 mM NaCl, 2 mM EDTA, 2 mM DTT (SEC / NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 10 mM imidazole and 25 µL protease inhibitor cocktail (Sigma Aldrich P8849) and 2 mM DTT) by sonication, before and after sonication incubation with 50 µL DNase (1 mg/mL) for 15 min on ice.
B	IMAC - HisTrap (Ni^{2+}) (GE Healthcare), a step gradient elution of imidazole in buffer **1A** (10, 20, 40, 100, 200, 400 mM). **SUD-M** eluted mostly in 100 mM imidazole in buffer **1A** and a small amount in fraction 200 mM imidazole in buffer **1A**. **SUD-MC** eluted mostly in 100 mM imidazole in buffer **1A** and a small amount in 40 mM imidazole in buffer **1A**.
C	Buffer exchange with Amicon Ultra 15 mL centrifugal filter membrane (10,000 MWCO) (Merck Millipore) in buffer **1B SUD-M** and **SUD-MC** respectively.
D	Cleavage in solution (100 µL thrombin (10 mg/mL) per 0.5 L culture) for **SUD-M**: 1 h at 4˚C and then 1 h at rt; **SUD-MC**: 16 h at 4˚C.
E	SEC - Superdex Increase 75 10/300 GL (GE Healthcare) in buffer **1C-SUD-M**, **1C-SUD-MC**.
F	NMR sample preparation in buffer **1C-SUD-M, 1C-SUD-MC**.

Table 3c: Protein Purification (SUD-C)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10% (v/v) glycerol (cell disruption / AC).
B	50 mM Tris-HCl (pH 8.0), 300 mM NaCl (AC).

C	50 mM Tris-HCl (pH 8.0), 10 mM reduced glutathione (elution buffer).
D	50 mM NaPi (pH 7.2), 50 mM NaCl, 2 mM EDTA, 2 mM DTT (SEC / NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 25 µL protease inhibitor cocktail (Sigma Aldrich P8849) and 2 mM DTT) by sonication, after sonication incubation with 25 µL DNase (1 mg/mL) for 10 min on ice.
B	AC with GSTrap (GE Healthcare) (wash buffer **1A** and then wash with buffer **1B**).
C	Elution with buffer **1C**, buffer exchange with Amicon Ultra 15 mL centrifugal filter membrane (10,000 MWCO) (Merck Millipore) to buffer **1D**.
D	Cleavage in solution (350 µL thrombin (10 mg/mL) per 0.5 L culture) at 37˚C for 5 h.
E	SEC on SD Increase 75 10/300 GL (GE Healthcare) in buffer **1D**.
F	NMR sample preparation in buffer **1D**.

Table 4: Final sample

1	**Yield**
SUD-N	13.92 mg/L ^{15}N or $^{13}C,^{15}N$-M9 medium
SUD-NM	17.25 mg/L ^{15}N or $^{13}C,^{15}N$-M9 medium
SUD-M	8.47 mg/L ^{15}N or $^{13}C,^{15}N$-M9 medium
SUD-MC	12.06 mg/L ^{15}N or $^{13}C,^{15}N$-M9 medium
SUD-C	4.70 mg/L ^{15}N or $^{13}C,^{15}N$-M9 medium
1b	**A260/280 ratio**
SUD-N	0.55
SUD-NM	0.50
SUD-M	0.81
SUD-MC	0.62
SUD-C	0.71
2	**Stability**
SUD-N	Stable throughout NMR spectra acquisition (10 days, 298 K). No significant precipitation or degradation observed after thawing from -80˚C. Very stable construct.
SUD-NM	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after defrosting from -80˚C.
SUD-M	Not very stable throughout spectra acquisition, 10 days 298 K. Significant precipitation observed after thawing from storage at -80˚C. Forms dimers without reducing agent observable even by SDS-page.

SUD-MC	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after thawing from -80˚C.
SUD-C	Stable throughout measurement (10 days, 298 K). No significant precipitation or degradation observed after thawing from -80˚C. Stable construct.
3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.

SI3: nsp3d

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3d / papain-like protease / PL^{pro}
3	**Sequence of "fl" protein (aa 743-1060 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	SLREVRTIKVFTTVDNINLHTQVVDMSMTYGQQFGPTYLDGADVTKIKPHNSHEGKTFYVLPN DDTLRVEAFEYYHTTDPSFLGRYMSALNHTKKWKYPQVNGLTSIKWADNNCYLATALLTLQQ IELKFNPPALQDAYYRARAGEAANFCALILAYCNKTVGELGDVRETMSYLFQHANLDSCKRVL NVVCKTCGQQQTTLKGVEAVMYMGTLSYEQFKKGVQIPCTCGKQATKYLVQQESPFVMMSA PPAQYELKHGTFTCASEYTGNYQCGHYKHITSKETLYCIDGALLTKSSEYKGPITDVFYKENSY TTTIK
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 743-1060 of complete nsp3
5	**Ratio for construct design**
	Based on homologous structure from SCoV (PDB 4M0W)
6	**Sequence homology (to SCoV)**
	Identity: 83%; similarity: 91%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 4M0W, 2FE8 SCoV2: PDB 6W9C
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pE-SUMO (LifeSensors)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-SUMO
3	**Cleavage Site**
	Ulp1
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	35.99 kDa / 45,270 M^{-1} cm^{-1} / 8.17
5	**Comments on sequence of expressed construct**

	No artificial residues due to Ulp1-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7 (addition of 50 µM $ZnCl_2$)
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 50 µM $ZnCl_2$, 10 mM bME (cell disruption / IMAC).
B	10 mM HEPES (pH 7.4), 100 mM NaCl, 50 µM $ZnCl_2$, 10 mM bME (dialysis after IMAC / TEV-cleavage).
C	10 mM HEPES (pH 7.4), 100 mM NaCl, 50 µM $ZnCl_2$, 5 mM DTT (SEC).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (addition of 50 µM $ZnCl_2$) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A.**
C	Ulp1-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1B.**
D	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with 500 mM imidazole in buffer **1A**.
E	SEC (HiLoad 26/600 SD 75 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1C** (elution volume 180-220 mL).

Table 4: Final sample

1	**Yield**
	12 mg/L ^{15}N-M9 medium
2	**Stability**
	Tendency to aggregate.
3	**Comment on applicability**

	Suitable for fragment screening, interaction studies.

Additional information

Constructs	**Conditions**	**Comments**
aa 743-1060 of complete nsp3; His_6 (pET28a(+) (GenScript)), TEV-cleavage site, N-terminal “GHM“ three artificial residues.	Native (as above)	Weak expression, less protein.

SI3: nsp3e

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3e / NAB globular domain
3	**Sequence of "fl" protein (aa 1080-1203 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	YFTEQPIDLVPNQPYPNASFDNFKFVCDNIKFADDLNQLTGYKKPASRELKVTFFPDLNGDVVA IDYKHYTPSFKKGAKLLHKPIVWHVNNATNKATYKPNTWCIRCLWSTKPVET
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1088-1203 of complete nsp3
5	**Ratio for construct design**
	Based on boundaries from NMR structure of homologue nsp3e from SARS-CoV (2K87).
6	**Sequence homology (to SCoV)**
	Identity: 82%; similarity: 89%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2K87
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 15723; SCoV2: BMRB 50334

Table 2: Protein Expression

1	**Expression vector**
	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	13.75 kDa / 25,565 M^{-1} cm^{-1} / 8.9
5	**Comments on sequence of expressed construct**
	N-terminal „GAMG" four artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**

	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or $^{13}C,^{15}N$-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	20-22°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM NaPi (pH 6.5), 300mM NaCl, 10 mM imidazole, 2 mM TCEP-HCl (cell disruption / IMAC).
B	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM DTT, 0.02% (w/v) NaN_3 (dialysis after IMAC / TEV-cleavage).
C	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) NaN_3 (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA) (Carl Roth, Germany), elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in buffer **1B**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1B**.
E	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1C**.
F	NMR sample preparation in buffer **1C**.

Table 4: Final sample

1	**Yield**
	3.5 mg/L ^{13}C, ^{15}N-M9 medium
2	**A260/280 ratio**
	0.74
3	**Stability**
	Stable throughout measurement (7 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 5 weeks.
4	**Comment on applicability**

	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

Constructs	Conditions	Comments
NAB (aa 1088-1203) of complete nsp3; His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal “GAMG“ four artificial residues.	As above.	Works as well, but slightly less expression and yield.

SI3: nsp3Y

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp3
2	**Region/Name/Further Specification**
	nsp3-Y / Cov-Y
3	**Sequence of “fl” protein (aa 1638-1945 of complete nsp3, according to NCBI Reference Sequence NC_045512.2)**
	DTFCAGSTFISDEVARDLSLQFKRPINPTDQSSYIVDSVTVKNGSIHLYFDKAGQKTYERHSLSHF VNLDNLRANNTKGSLPINVIVFDGKSKCEESSAKSASVYYSQLMCQPILLLDQALVSDVGDSAE VAVKMFDAYVNTFSSTFNVPMEKLKTLVATAEAELAKNVSLDNVLSTFISAARQGFVDSDVET KDVVECLKLSHQSDIEVTGDSCNNYMLTYNKVENMTPRDLGACIDCSARHINAQVAKSHNIAL IWNVKDFMSLSEQLRKQIRSAAKKNNLPFKLTCATTRQVVNVVTTKIALKGG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1638-1945 of complete nsp3
5	**Ratio for construct design (detailed and comprehensible)**
	We took the C-terminal part of nsp3 after predicted transmembrane region and Y1 domain that consists of two sequential zinc finger motifs.
6	**Sequence homology (to SCoV)**
	Identity: 89%; similarity: 96%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pET28b(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	34 kDa / 17,420 M^{-1} cm^{-1} / 6.66
5	**Comments on sequence of expressed construct**

	N-terminal „G" one artificial residue due to TEV-cleavage.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labeling)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	18°C for 15-16 h

Table 3: Protein Purification

1	**Buffer List**
A	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 0.1 mM PMSF, 5 mM bME, 0.1 mg/mL lysozyme, cOmplete EDTA-free inhibitor (Cell disruption).
B	20 mM Tris-HCl (pH 8.0), 300 mM NaCl, 20 mM imidazole (IMAC).
C	50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 2 mM DTT (TEV-cleavage).
D	50 mM HEPES (pH 6.9), 200 mM LiBr, 5 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA) (Thermo Scientific), wash with buffer **1B** and elution with 250 mM imidazole in buffer **1B**.
C	TEV-cleavage (5% (w/w) TEV protease per approximate amount of the protein) in buffer **1C** o.n. at rt.
D	Inv. IMAC (gravity flow Ni^{2+}-NTA) in buffer **1C**.
E	SEC on 10/300 GL SD 200 (GE Healthcare) in buffer **1D**.

Table 4: Final sample

1	**Yield**
	12 mg/L ^{13}C,^{15}N-M9 medium
2	**Stability**
	Stable at 25°C at protein concentration below 0.4 mM for 3 to 5 days or at 30°C o.n.. The protein gradually degrades at rt. After one week, we observe an additional band on SDS gel at ~27 kDa.
3	**Comment on applicability**

	The protein is suitable for NMR assignment and protein interaction studies at low temperature (20-25°C) and reasonably low concentration (< 0.2 mM).

SI4: nsp5

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp5
2	**Region/Name/Further Specification**
	3C-like protease ($3CL^{pro}$) / main protease (M^{pro})
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	SGFRKMAFPSGKVEGCMVQVTCGTTTLNGLWLDDVVYCPRHVICTSEDMLNPNYEDLLIRKSN HNFLVQAGNVQLRVIGHSMQNCVLKLKVDTANPKTPKYKFVRIQPGQTFSVLACYNGSPSGVY QCAMRPNFTIKGSFLNGSCGSVGFNIDYDCVSFCYMHHMELPTGVHAGTDLEGNFYGPFVDRQ TAQAAGTDTTITVNVLAWLYAAVINGDRWFLNRFTTTLNDFNLVAMKYNYEPLTQDHVDILG PLSAQTGIAVLDMCASLKELLQNGMNGRTILGSALLEDEFTPFDVVRQCSGVTFQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-306 (fl nsp5)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 96%; similarity: 99.7%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 1P9U, 6LU7 SCoV2: PDB 6Y2E, 5R7Y, 6Y84, 7K3T
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 17251

Table 2: Protein Expression

1	**Expression vector**
	pE-SUMO (LifeSensors)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-SUMO
3	**Cleavage Site**
	Ulp1
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	33.80 kDa / 32,890 M^{-1} cm^{-1} / 5.95
5	**Comments on sequence of expressed construct**
	No artificial residues due to TEV-cleavage and construct design.

6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM NaPi (pH 7.5), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME (cell disruption / IMAC).
B	50 mM NaPi (pH 7.0), 300 mM NaCl, 10 mM bME, 5% (v/v) glycerol (dialysis after IMAC / Ulp1-cleavage).
C	25 mM NaPi (pH 7.5), 150 mM NaCl, 2 mM TCEP-HCl (SEC buffer).
D	10 mM NaPi (pH 7.0), 0.5 mM TCEP-HCl (final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A**.
C	Ulp1-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1B**.
D	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with 500 mM imidazole in buffer **1A**.
E	SEC (HiLoad 26/600 SD 75 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1C** (elution volume 170-210 mL).
F	NMR sample preparation in buffer **1D**.

Table 4: Final sample

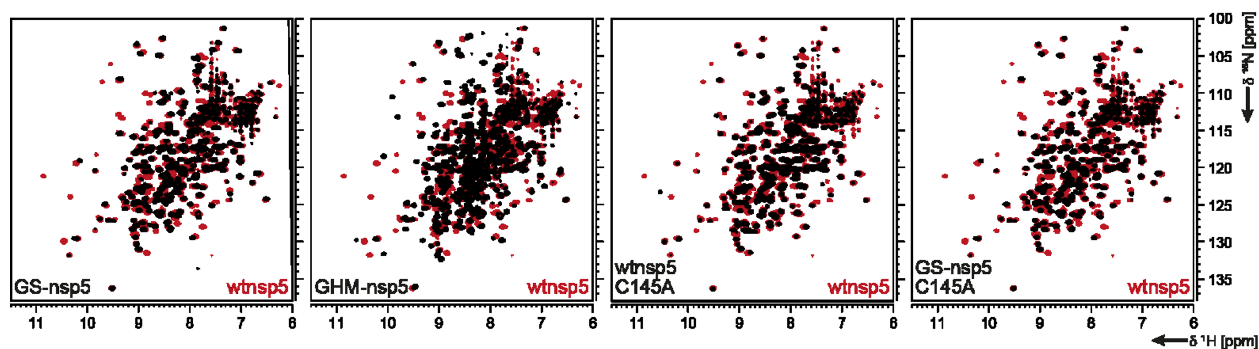
1	**Yield**
	55 mg/L ^{15}N-M9 medium
2	**Stability**
	No significant precipitation or degradation observed after storage at -80°C for a month.

3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

	Constructs	**Conditions**	**Comments**
A	aa 1-306 (fl nsp5) C145A mutation; His_6-SUMO (pE-SUMO (LifeSensors)), Ulp1-cleavage site, no N-terminal artificial residues.	Native (as above)	Comparable to fl nsp5 expression and purification, similar yield (80 mg/L ^{15}N-M9 medium).
B	aa 1-306 (fl nsp5); His_6-SUMO (pE-SUMO (LifeSensors)), Ulp1-cleavage site, N-terminal “GS“ two artificial residues.	Native (as above)	Comparable to fl nsp5 expression and purification, similar yield (55 mg/L ^{15}N-M9 medium, 36 mg/L ^{13}C,^{15}N-M9 medium, 20 mg/L ^{2}H,^{13}C,^{15}N E. coli-OD2 CDN medium (Silantes)).
C	aa 1-306 (fl nsp5) C145A mutation; His_6-SUMO (pE-SUMO (LifeSensors)), Ulp1-cleavage site, N-terminal “GS“ two artificial residues.	Native (as above)	Comparable to fl nsp5 expression and purification, similar yield (55 mg/L ^{15}N-M9 medium).
D	aa 1-306 (fl nsp5); His_6 (pet28a(+) (GenScript)), TEV-cleavage site; N-terminal “GHM“ three artificial residues.	Native (as above) **IMAC buffer (1A)**: 25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME	Comparable to fl nsp5 purification, however, less expression/yield (35 mg/L ^{15}N-M9 medium, 10 mg/L ^{13}C,^{15}N-M9 medium).
E	aa 1-306 (fl nsp5); GST and His_6-tag (pET-28a(+) (GenScript)), TEV and auto cleavage site for M^{pro}, N-terminal „GS“ and C-terminal “GPHHHHHH“ ten artificial residues.	**IMAC buffer:** 50 mM Tris-HCl (pH 8.0), 200 mM NaCl, 20 mM imidazole. **SEC-buffer:** 50 mM NaPi (pH 7.6), 50 mM NaCl, 0.02% (w/v) NaN_3. **NMR-buffer:** 50 mM NaPi (pH 7.6), 50 mM NaCl, 0.02% (w/v) NaN_3, 5 mM bME.	Yields 20 mg/L ^{15}N-M9 medium. The protein is stable up to 350 µM in NMR buffer at 25°C for at least 7 days. At 50 µM and at 4°C, the protein is stable for ~15 days. The protein is not suitable for freeze/thaw and results in precipitation.
F	aa 1-306 (fl nsp5); C-terminal His_6-tag (pET21b(+) (GenScript)), human rhinovirus 3-C protease cleavage site, N-terminal “M” additional aa, however our mass spectrum results suggest that M1 was removed by *E. coli* methionine aminopeptidase.	**IMAC buffer:** 20 mM Tris-HCl (pH 7.33), 150 mM NaCl, 20 mM imidazole. **Storage buffer:** 20 mM Tris-HCl (pH 7.33), 150 mM NaCl.	Yields 5 mg/L ^{15}N-M9 medium. Stable for 2-3 weeks at 4°C at low micromolar concentration.
G	aa 1-306 (fl nsp5) C145A mutation; His_6-GB1 (pET24a(+) (GenScript)), TEV-cleavage site, no artificial residues.	**IMAC buffer:** 20 mM Tris-HCl (pH 7.5), 150 mM NaCl, 20 mM imidazole, 0.5 mM TCEP-HCl. **SEC/NMR buffer:** 10 mM NaPi (pH 7.0), 0.5 mM TCEP-HCl.	Yields ≥ 70 mg/L ^{15}N, ^{2}H,^{15}N-M9, and ^{2}H,^{13}C,^{15}N-M9 medium. 1-2 mM sample stable for several weeks at 25°C. Negligible precipitation on freeze-thaw. Samples stable for ≥ 3 months at 80°C. Sample precipitation in buffer: 10 mM NaPi (pH 7.0), 0.4 M GdnHCl.

H	aa 1-306 (fl nsp5); His6-GB1 (pET24a(+) (GenScript)), TEV-cleavage site, no artificial residues.	As above (**G**).	Negligible expression when induced in ^{15}N-M9 medium at 25˚C, 30˚C, and 37˚C, with 0.5-1 mM IPTG.
I	aa 1-306 (fl nsp5); His6-GST (pGEX-6p-1 (Genewiz)), autolytic and HRV 3C cleavage site, no artificial residues.	**IMAC buffer:** 25 mM Tris-HCl (pH 7.8), 150 mM NaCl, 5 mM imidazole, 1 mM bME. **Cleavage buffer:** 25 mM Tris-HCl (pH 7.8), 150 mM NaCl, 1 mM DTT. **SEC buffer:** 25 mM Tris-HCl (pH 7.8), 150 mM NaCl, 1 mM DTT, 1 mM EDTA.	40-60 mg/mL autoinduction Media ZYM-5052. Stored at 1 mg/mL at -20°C with 30% v/v) glycerol in SEC buffer. Also stored at 25 mg/mL at -80˚C in SEC buffer. Flash frozen. Neither show loss of activity compared to non-frozen samples.



Overlays of [^{15}N, ^{1}H]-BEST TROSY spectra of wtnsp5 (red) with the other constructs (black). From left to right: N-terminally GS added nsp5 (GS-nsp5), GHM added (GHM-nsp5), the active site mutants C145A with native N-terminus (wtnsp5 C145A), and GS added mutant (GS-nsp5 C145A).

SI5: nsp7

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp7
2	**Region/Name/Further Specification**
	nsp7
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	SKMSDVKCTSVVLLSVLQQLRVESSSKLWAQCVQLHNDILLAKDTTEAFEKMVSLLSVLLSMQ GAVDINKLCEEMLDNRATLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-83 (fl nsp7)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 98.8%; similarity: 100%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2KYS, 1YSY, 6NUS, 6NUR, 2AHM, SCoV2: PDB 7BV2, 7BV1, 6YYT, 7BTF, 6WQD, 6WTC, 6WIQ, 6M71, 6YHU, 6XEZ, 6M5I, 7CTT, 7C2K, 7BW4, 7BZF, 7JLT, 7AAP, 6XIP, 6XQB
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: PDB 1YSY, BMRB 6513, PDB 2KYS, BMRB 16981 SCoV2: BMRB 50337

Table 2: Protein Expression

1	**Expression vector**
	pET46
2	**Purification-/Solubility-Tag**
	N-terminal His_6, enterokinase
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	9.24 kDa / 5500 cm^{-1} M^{-1} / 5.2
5	**Comments on sequence of expressed construct**
	N-terminal “G” an artificial residue due to TEV-cleavage.

6	**Used expression strain**
	E. coli Rosetta2 pLysS
7	**Cultivation medium**
	M9 (uniformly ^{15}N, ^{13}C-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.8
10	**Cultivation temperature and time**
	16˚C for 14-16 h

Table 3: Protein Purification

1	**Buffer List**
A	10 mM HEPES (pH 7.4), 300 mM NaCl, 30 mM imidazole, 2 mM DTT.
B	10 mM HEPES (pH 7.4), 300 mM NaCl, 300 mM imidazole, 2 mM DTT.
C	10 mM MOPS (pH 7.0), 150 mM NaCl, 2 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell lysis in buffer **1A** by microfluidizer operating at 20,000 psi. Lysates were cleared by centrifugation at 25,000 g for 30 min and then filtered through a 0.45 µm filter. Ni-NTA Agarose beads (Qiagen) were added to cleared lysates and incubated for 30 min. Beads were collected by centrifugation and then loaded onto a gravity column. Beads were washed twice with 10 column volumes of buffer **1A**. Protein was eluted with 5 column volumes of buffer **1B.**
B	Eluted protein was cleaved with 1% (w/w) TEV protease o.n. at rt while dialyzing the protein into 1 L buffer **1C**. Uncleaved protein was removed by inv. Ni-NTA binding.
C	Protein was concentrated using a 10 kDa MWCO (Amicon) concentrator and purified on an SD 200 Increase 10/300 (GE Life Sciences) size exclusion column, AKTApure (GE Life Sciences) using buffer **1C**.

Table 4: Final sample

1	**Yield**
	17 mg/L ^{13}C,^{15}N-M9 medium
1b	**A260/280 ratio**
	0.5
2	**Stability**
	NMR sample stable at 4°C for a month, at 35°C for several days before degradation occurs.
3	**Comment on applicability**

	Suitable for NMR-based screening applications.

SI6: nsp8

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp8
2	**Region/Name/Further Specification**
	nsp8
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AIASEFSSLPSYAAFATAQEAYEQAVANGDSEVVLKKLKKSLNVAKSEFDRDAAMQRKLEKM ADQAMTQMYKQARSEDKRAKVTSAMQTMLFTMLRKLDNDALNNIINNARDGCVPLNIIPLTT AAKLMVVIPDYNTYKNTCDGTTFTYASALWEIQQVVDADSKIVQLSEISMDNSPNLAWPLIVT ALRANSAVKLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-198 (fl nsp8)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 97%; similarity: 98%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 6NUS, 6NUR, 2AHM, SCoV2: PDB 7C2K, 7BV2, 7BV1, 7CTT, 6M5I, 7BW4, 6XEZ, 7BZF, 6XQB, 6M71, 6YYT, 7BTF, 7JLT, 7AAP, 6WIQ, 6XIP, 6WQD, 6WTC, 6YHU
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pET46
2	**Purification-/Solubility-Tag**
	N-terminal His_6, enterokinase
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	21.94 kDa / 19,940 $cm^{-1}M^{-1}$ / 6.5
5	**Comments on sequence of expressed construct**
	N-terminal “G” an artificial residue due to TEV-cleavage.

6	**Used expression strain**
	E. coli Rosetta2 pLysS
7	**Cultivation medium**
	M9 (uniformly ^{15}N-, ^{13}C-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.8
10	**Cultivation temperature and time**
	16˚C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	10 mM HEPES (pH 7.4), 300 mM NaCl, 30 mM imidazole, 2 mM DTT.
B	10 mM HEPES (pH 7.4), 300 mM NaCl, 300 mM imidazole, 2 mM DTT.
C	10 mM MOPS (pH 7.0), 300 mM NaCl, 2 mM DTT.
D	10 mM MOPS (pH 7.0), 150 mM NaCl, 2 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell lysis in buffer **1A** by microfluidizer operating at 20,000 psi. Lysates were cleared by centrifugation at 25,000 g for 30 min and then filtered through a 0.45 µm filter. Ni-NTA Agarose beads (Qiagen) were added to cleared lysates and incubated for 30 min. Beads were collected by centrifugation and then loaded onto a gravity column. Beads were washed twice with 10 column volumes of buffer **1A**. Protein was eluted with 5 column volumes of buffer **1B.**
B	Eluted protein was cleaved with 1% (w/w) TEV protease o.n. at rt while dialyzing the protein into 1 L buffer **1C**. Uncleaved protein was removed by inverse Ni-NTA binding.
C	Protein was concentrated using a 10 kDa MWCO (Amicon) concentrator and purified on an SD 200 Increase 10/300 (GE Life Sciences) size exclusion column, AKTApure (GE Life Sciences) using buffer **1D**.

Table 4: Final sample

1	**Yield**
	17 mg/L ^{13}C,^{15}N-M9 medium
1b	**A260/280 ratio**
	0.5
2	**Stability**
	Concentration dependent aggregation of nsp8 observed in the range of 0.1-1.1 mM by NMR.

3	**Comment on applicability**
	Suitable for NMR-based screening approach.

SI7: nsp9

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp9
2	**Region/Name/Further Specification**
	nsp9
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	NNELSPVALRQMSCAAGTTQTACTDDNALAYYNTTKGGRFVLALLSDLQDLKWARFPKSDGT GTIYTELEPPCRFVTDTPKGPKVKYLYFIKGLNNLNRGMVLGSLAATVRLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-113 (fl nsp9)
5	**Ratio for construct design (detailed and comprehensible)**
	In analogy to the available crystal structure (PDB 1QZ8) of nsp9 SCoV, fl sequence.
6	**Sequence homology (to SCoV)**
	Identity: 97%; similarity: 97%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 3EE7 (G104E), 1UW7, 1QZ8 SCoV2: PDB 6WXD, 6W4B, 6W9Q
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 6501 SCoV2: BMRB 50621, 50622

Table 2: Protein Expression

1	**Expression vector**
	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GST
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	12,7 kDa / 13,075 M^{-1} cm^{-1} / 9.1
5	**Comments on sequence of expressed construct**
	N-terminal „GAMG" four artificial residues due to TEV-cleavage and construct design
6	**Used expression strain**

	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or $^{13}C,^{15}N$-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	20-22°C for 18-20 h

Table 3: Protein Purification

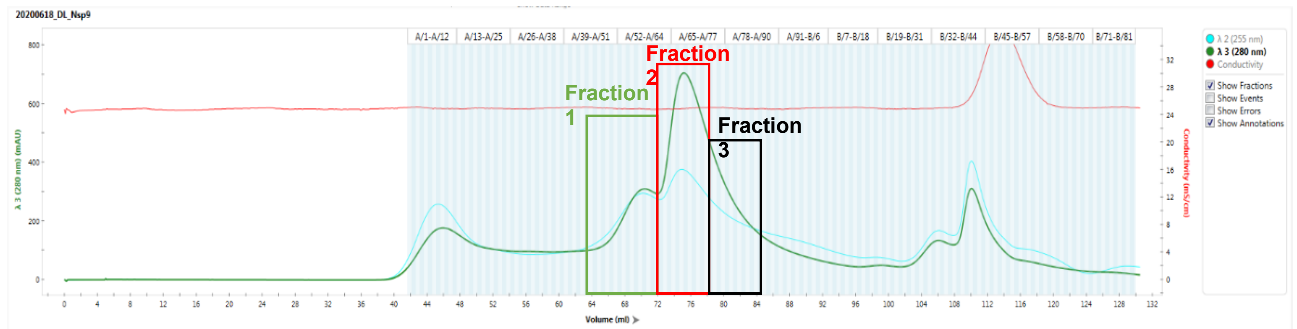
1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 4 mM DTT (cell disruption / IMAC/ dialysis after IMAC / TEV-cleavage).
B	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) NaN_3 (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA (Carl Roth)), Elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in in buffer **1A**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1A**.
E	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1B**. See relevant peak in attached SEC profile.
F	NMR sample preparation in buffer **1B**.

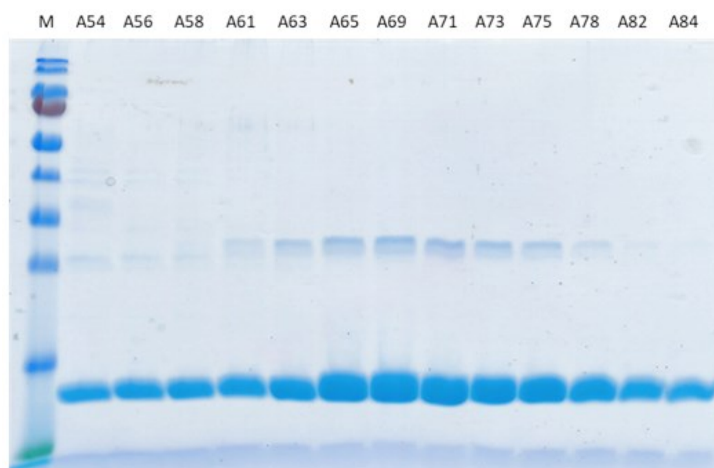
Table 4: Final sample

1	**Yield**
	4.5 mg/L ^{13}C, ^{15}N-M9 medium
1b	**A260/280 ratio**
	0.7
2	**Stability**
	Stable dimer. Storage at 4°C possible.
3	**Comment on applicability**
	Conditions for NMR structure determination may need to be optimized (concerning line width due to dimeric state). Backbone assignment and screening successful.

Additional information

	Constructs	Conditions	Comments
A	aa 1-113 (fl nsp9); His_7 (pET-TEV-Nco (GenScript)), TEV-cleavage site, N-terminal "GAMG" four artificial residues.	As above.	Expression and purification as for GST-tagged fl nsp9, but lower expression and yield.
B		**IMAC buffer:** 25 mM NaPi (pH 7.4), 300 mM NaCl, 20 mM imidazole, 1 mM DTT. **Cleavage buffer:** 25 mM NaPi (pH 7.4), 150 mM NaCl, 1 mM DTT. **SEC/NMR buffer A:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 1 mM DTT, 150 mM NaCl, 2 mM TCEP-HCl. **SEC/NMR buffer B:** 25 mM NaAc (pH 5.0), 150 mM NaCl, 2 mM TCEP-HCl.	3 mg/L ^{13}C,^{15}N-M9 medium. Sample in Buffer **A** looked degraded (from the ^{15}N HSQC) after 5 days of ^{13}C 3D NMR experiments at 298 K. Less degradation was observed for sample in Buffer **B** after same period. Suitable for NMR studies, fragment-based screening, interaction studies.





SEC profile of TEV-cleaved His_6-GST-fl_nsp9 (HiLoad 16/600 SD 75, GE Healthcare) **and SDS gel of corresponding fractions.** (Ladder: PageRuler™ prestained, Thermo Fischer)
Main peak (fraction 2 - corresponding to SEC fractions A 61 to A73) was subsequently used for NMR.

SI8: nsp10

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1a and ORF1ab; nsp10
2	**Region/Name/Further Specification**
	nsp10
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AGNATEVPANSTVLSFCAFAVDAAKAYKDYLASGGQPITNCVKMLCTHTGTGQAITVTPEAN MDQESFGGASCCLYCRCHIDHPNPKGFCDLKGKYVQIPTTCANDPVGFTLKNTVCTVCGMWK GYGCSCDQLREPMLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-139 (fl nsp10)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 97%; similarity: 99%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 5C8S, 5NFY, 2FYG, 2XYQ, 2XYV, 2XYV SCoV2: PDB 6W4H, 6W61, 7JYY, 7C2I, 7BQ7, 2G9T
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV2: BMRB 50392

Table 2: Protein Expression

1	**Expression vector**
	pET21b(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	16.24 kDa / 12,950 M^{-1} cm^{-1} / 6.72
5	**Comments on sequence of expressed construct**
	N-terminal “MGSDKIHHHHHH” twelve artificial residues due to construct design
6	**Used expression strain**

	E. coli T7 Express
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.5 mM IPTG at OD_{600} 0.6-0.7 (addition of 50 µM $ZnCl_2$)
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME (cell disruption / IMAC)
B	50 mM NaPi (pH 7.5), 50 mM NaCl, 5 mM DTT (SEC / final NMR buffer)
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck) and addition of 50 µM $ZnCl_2$) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A.**
C	SEC (HiLoad 26/600 SD 75 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1B** (elution volume 175-225 mL).
D	NMR sample preparation in buffer **1B.**

Table 4: Final sample

1	**Yield**
	25 mg/L ^{15}N-M9 medium, 15 mg/L ^{13}C,^{15}N-M9 medium
2	**Stability**
	Stable throughout measurement (6 days, 298 K). No significant precipitation or degradation observed after storage at -80°C for 2 months.
3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.

Additional information

Constructs	**Conditions**	**Comments**
aa 1-139 (fl nsp10); His_6 (pMCSG53 (BEI Resources, cat.	**IMAC-buffer:** 50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME,	Yields 30-40 mg/L 2xTY medium. Can be flash-frozen in liquid

NR-52425)), TEV cleavage site, N-terminal "SNM" three artificial residues.	2 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 5-10% (v/v) glycerol, 50 mM imidazole. **SEC-buffer:** 20 mM HEPES (pH 8.5), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 20 mM imidazole.	nitrogen and stored at 20°C, used for nsp14 and nsp16 stabilization at 1:1 molar ratios.

SI9: nsp13

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp13
2	**Region/Name/Further Specification**
	NTPase / helicase domain / RNA 5'-triphosphatase
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AVGACVLCNSQTSLRCGACIRRPFLCCKCCYDHVISTSHKLVLSVNPYVCNAPGCDVTDVTQL YLGGMSYYCKSHKPPISFPLCANGQVFGLYKNTCVGSDNVTDFNAIATCDWTNAGDYILANTC TERLKLFAAETLKATEETFKLSYGIATVREVLSDRELHLSWEVGKPRPPLNRNYVFTGYRVTKN SKVQIGEYTFEKGDYGDAVVYRGTTTYKLNVGDYFVLTSHTVMPLSAPTLVPQEHYVRITGLY PTLNISDEFSSNVANYQKVGMQKYSTLQGPPGTGKSHFAIGLALYYPSARIVYTACSHAAVDAL CEKALKYLPIDKCSRIIPARARVECFDKFKVNSTLEQYVFCTVNALPETTADIVVFDEISMATNY DLSVVNARLRAKHYVYIGDPAQLPAPRTLLTKGTLEPEYFNSVCRLMKTIGPDMFLGTCRRCPA EIVDTVSALVYDNKLKAHKDKSAQCFKMFYKGVITHDVSSAINRPQIGVVREFLTRNPAWRKA VFISPYNSQNAVASKILGLPTQTVDSSQGSEYDYVIFTQTTETAHSCNVNRFNVAITRAKVGILCI MSDRDLYDKLQFTSLEIPRRNVATLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	1-601 aa (fl nsp13)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 99.8%; similarity: 100%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6ZSL, 6JYT, 6XEZ
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pE-SUMO (LifeSensors)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-SUMO
3	**Cleavage Site**
	Ulp1
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**

	66.85 kDa / 67,160 M^{-1} cm^{-1} / 8.66
5	**Comments on sequence of expressed construct**
	No artificial residues due to Ulp1-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7 (addition of 50 µM $ZnCl_2$)
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris (pH 8.0), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME (cell disruption / IMAC).
B	20 mM BisTris (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl (SEC/ final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck) and addition of 50 µM $ZnCl_2$) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A**.
C	SEC (HiLoad 26/600 SD 200 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1B** (elution volume 210-240 mL).
D	NMR sample preparation in buffer **1B.**

Table 4: Final sample

1	**Yield**
	0.5 mg/L ^{15}N-M9 medium
2	**Stability**
	Aggregation at > 20 µM under these conditions.
3	**Comment on applicability**
	Not suitable for NMR experiments.

Additional information

Constructs	**Conditions**	**Comments**
aa 1-601 (fl nsp13); His_6 (pET28a(+) (GenScript)), TEV-cleavage site, N-terminal "GHM" three artificial residues.	Native (as above)	Weak expression, instable protein.

SI10: nsp14

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp14
2	**Region/Name/Further Specification**
	nsp14 / 3'-to-5' exonuclease / guanine N7-methyltransferae (MTase)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	AENVTGLFKDCSKVITGLHPTQAPTHLSVDTKFKTEGLCVDIPGIPKDMTYRRLISMMGFKMNY QVNGYPNMFITREEAIRHVRAWIGFDVEGCHATREAVGTNLPLQLGFSTGVNLVAVPTGYVDT PNNTDFSRVSAKPPPG
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
fl	aa 1-527 (fl nsp14)
MTase	aa 288-527 (MTase domain)
5	**Ratio for construct design**
fl	fl protein
MTase	In analogy to SCoV structure (PDB 5C8U)
6	**Sequence homology (to SCoV)**
fl	Identity: 95%; similarity: 99%
MTase	Identity: 95%, similarity: 97%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 5C8U, 5C8S, 5C8T, 5NFY
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
fl	pRSF-Duet1 (Novagen)
MTase	pET28a (Novagen)
2	**Purification-/Solubility-Tag**
fl	N-terminal His_6
MTase	N-terminal His_6
3	**Cleavage Site**
fl	TEV

MTase	Thrombin
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
fl	60.01 kDa / 91,660 M^{-1} cm^{-1} / 7.79
MTase	27.82 kDa / 48,970 M^{-1} cm^{-1} / 7.19
5	**Comments on sequence of expressed construct**
fl	N-terminal "GSM" three artificial residues due to construct design.
MTase	N-terminal "GSHM" four artificial residues due to construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	2xTY for protein production, LB for transformation and maintenance
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{540} 0.5-0.6
10	**Cultivation temperature and time**
	20°C for 18-20 h

Table 3: Protein Purification (fl nsp14 and nsp14 MTase)

1	**Buffer List**
A	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol, 50 mM imidazole (cell disruption).
B	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 50 mM imidazole (IMAC).
C	50 mM Tris-HCl (pH 9.0), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 1 M imidazole (IMAC).
D	20 mM HEPES (pH 8.5), 0.5 M NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 20 mM imidazole (SEC).
E	20 mM potassium phosphate (pH 8.0), 0.25 M KCl (Screening).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication in pulse mode (0.5 s on /0.5 s off) for 10 min.
B	IMAC (gravity flow or batch Ni^{2+}-NTA) (GE Healthcare), washing with buffer **1B**, elution with **1C**.
C-**fl**	[Optional] Overnight incubation with TEV protease at 4°C. The ratio was 1 mg of TEV protease per 20-40 mg of nsp14 protein.

C-**MTase**	[Optional] Overnight incubation with thrombin protease at 4°C. The ratio was 1-2 U of thrombin protease per 3-4 mg of MTase nsp14 protein.
D	SEC on SD 200 16/600 column (GE Healthcare) in buffer **1D** (elution volume 75-95 mL).
E-**fl**	[Optional] Separation of TEV protease and uncleaved nsp14 material with IMAC, collection of flow through in buffer **1D**.
E-**MTase**	[Optional] Separation of thrombin protease and uncleaved MTase nsp14 material with IMAC, collection of flow through in buffer **1D**.
F	For fragment screening the buffer is exchanged to **1E**.
G	[Optional] If higher concentrations or increased stability of fl nsp14 is desired, nsp10 should be added at 1:1 molar ratio.

Table 4: Final sample

1	**Yield**
fl	6 mg/L 2xTY medium
MTase	~ 10 mg/L 2xTY medium
1b	**A260/280 ratio**
fl	0.6
MTase	0.6
2	**Stability**
fl	The fl nsp14 construct tends to be unstable at concentrations above 3 mg/mL without reducing agent (TCEP-HCl or bME). Unstable at 4°C longer than one week. Freezing is not advisable; storage in 50% (v/v) glycerol at -20°C is preferable.
MTase	The MTase construct is even more unstable, and requires the presence of reducing agent (TCEP-HCl or bME) and NaCl at least in 400 mM concentration.
3	**Comment on applicability**
	Suitable for fragment screening and enzymatic activity assays.

Additional information

Constructs	**Conditions**	**Comments**
Fl nsp14; His_6 (pETDuet (GenScript)), no cleavage site, N-terminal “MGSSHHHHHHSQDP” 14 artificial residues.	**IMAC-buffer:** 25 mM Tris/HCl (pH 8.5), 300 mM NaCl, 5 mM imidazole, 10 mM bME, 5% (v/v) glycerol. **SEC-buffer:** 25 mM Tris/HCl (pH 8.5), 300 mM NaCl, 5 mM DTT, 5% (v/v) glycerol	Yields 14 mg/L ^{15}N-M9 medium. Tendency to aggregate.

SI11: nsp15

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp15
2	**Region/Name/Further Specification**
	nsp15 / NendoU / Endonucleasee
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	SLENVAFNVVNKGHFDGQQGEVPVSIINNTVYTKVDGVDVELFENKTTLPVNVAFELWAKRNI KPVPEVKILNNLGVDIAANTVIWDYKRDAPAHISTIGVCSMTDIAKKPTETICAPLTVFFDGRVD GQVDLFRNARNGVLITEGSVKGLQPSVGPKQASLNGVTLIGEAVKTQFNYYKKVDGVVQQLPE TYFTQSRNLQEFKPRSQMEIDFLELAMDEFIERYKLEGYAFEHIVYGDFSHSQLGGLHLLIGLAK RFKESPFELEDFIPMDSTVKNYFITDAQTGSSKCVCSVIDLLLDDFVEIIKSQDLSVVSKVVKVTI DYTEISFMLWCKDGHVETFYPKLQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-346 (fl nsp15)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 89%; similarity: 98%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 2H85 SCoV2: PDB 6W01
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pET28a(+) (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	39.14 kDa / 32,890 M^{-1} cm^{-1} / 5.12
5	**Comments on sequence of expressed construct**

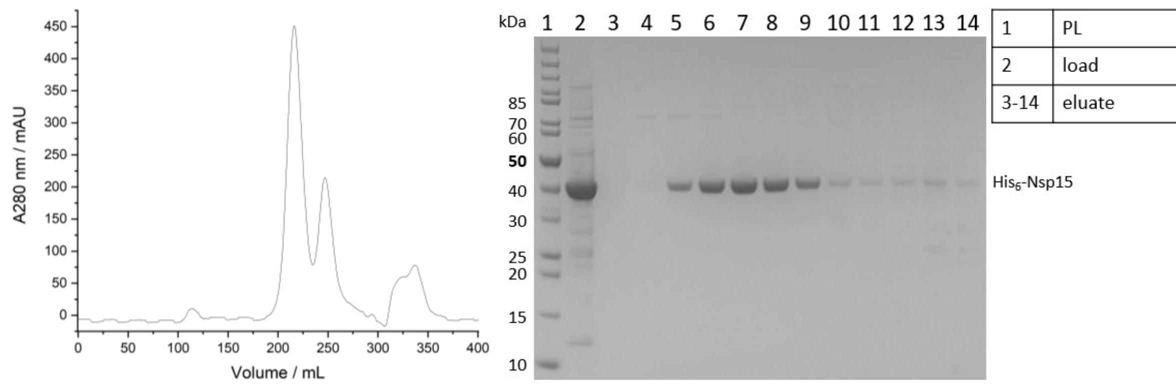
	N-terminal “GHM” three artificial residues due to TEV-cleavage and construct design
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 5% (v/v) glycerol, 10 mM bME (cell disruption / IMAC).
B	25 mM NaPi (pH 7.5), 300 mM NaCl, 2 mM TCEP-HCl (SEC/ final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)) by microfluidization.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1A**.
C	SEC (HiLoad 26/600 SD 200 pg (GE Healthcare), ÄKTApurifier (GE Healthcare)) in buffer **1B** (elution volume 200-260 mL).
D	NMR sample preparation in buffer **1B**.

Table 4: Final sample

1	**Yield**
	5 mg/L ^{15}N-M9 medium
2	**Stability**
	Tendency to aggregate at rt.
3	**Comment on applicability**
	Suitable for fragment screening and interaction studies.



Analytical SEC of nsp15. Protein was eluted from 200-260 mL (left panel) with corresponding SDS-PAGE of SEC with fractions analyzed from 190-260 mL (right panel).

SI12: nsp16

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF1ab; nsp16
2	**Region/Name/Further Specification**
	nsp16 / 2'-O-ribose methyltransferase (2'-O-MTase)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	SSQAWQPGVAMPNLYKMQRMLLEKCDLQNYGDSATLPKGIMMNVAKYTQLCQYLNTLTLAV PYNMRVIHFGAGSDKGVAPGTAVLRQWLPTGTLLVDSDLNDFVSDADSTLIGDCATVHTANK WDLIISDMYDPKTKNVTKENDSKEGFFTYICGFIQQKLALGGSVAIKITEHSWNADLYKLMGHF AWWTAFVTNVNASSSEAFLIGCNYLGKPREQIDGYVMHANYIFWRNTNPIQLSSYSLFDMSKFP LKLRGTAVMSLKEGQINDMILSLLSKGRLIIRENNRVVISSDVLVNN
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-298 (fl nsp16)
5	**Ratio for construct design**
	Based on fl annotation boundaries of YP_009725311.1 protein entry in NC_045512.2.
6	**Sequence homology (to SCoV)**
	Identity: 93%; similarity: 99%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 3R24, 2XYR, 2XYQ SCoV2: PDB 7JYY, 6W4H, 6YZ1, 7BQ7, 7C2I, 6W61
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pRSF-Duet1 (Novagen)
2	**Purification-/Solubility-Tag**
	N-terminal His_6
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	33.67 kDa / 55,790 M^{-1} cm^{-1}/ 7.76
5	**Comments on sequence of expressed construct**
	N-terminal „GSMA" - four artificial residues due to TEV-cleavage and construct design.

6	**Used expression strain**
	E. coli BL21(DE3)
7	**Cultivation medium**
	2xTY
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	1 mM IPTG at OD_{540} 0.5-0.6
10	**Cultivation temperature and time**
	20°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	50 mM Tris-HCl (pH 9.0), 500 mM NaCl, 10 mM bME, 2 mM $MgCl_2$, 0.1% (v/v) Triton X-100, 10% (v/v) glycerol, 50 mM imidazole (cell disruption).
B	50 mM Tris-HCl (pH 9.0), 500 mM NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 50 mM imidazole (IMAC).
C	50 mM Tris-HCl (pH 9.0), 500 mM NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 1 M imidazole (IMAC).
D	20 mM HEPES (pH 8.5), 500 mM NaCl, 10 mM bME, 2 mM $MgCl_2$, 5% (v/v) glycerol, 20 mM imidazole (SEC).
E	20 mM KPi (pH 8.0), 200 mM KCl, 1 mM $MgCl_2$, 2 mM DTT (Screening).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication in pulse mode (0.5 s on /0.5 s off) for 10 min.
B	IMAC (gravity flow or batch Ni^{2+}-NTA) (GE Healthcare), washing with buffer **1B**, elution with **1C**.
C	[Optional] Overnight incubation with TEV protease at 4°C. The ratio was 1 mg of TEV protease per 20-40 mg of nsp16 protein.
D	SEC on SD 200 16/600 column (GE Healthcare) in buffer **1D** (elution volume 90-100 mL).
E	[Optional] Separation of TEV protease and uncleaved nsp16 material with IMAC, collection of flow through in buffer **1D**.
F	nsp10 is added at 1:1 molar ratio – necessary for stability and activity.
G	For fragment screening the buffer is exchanged to **1E**.

Table 4: Final sample

1	**Yield**
	~ 10-15 mg/L 2xTY medium.

1b	**A260/280 ratio**
	0.55
2	**Stability**
	Extremely unstable in non-reducing conditions, presence of reducing agents is essential. Presence of 5% (v/v) glycerol is also desirable for increased stability. Can be flash-frozen in liquid nitrogen and stored at -20°C.
3	**Comment on applicability**
	Suitable for fragment screening.

Additional information

Constructs	**Conditions**	**Comments**
Fl nsp16; His_6 (pMCSG53 (BEI Resources, cat. NR-52427)), TEV-cleavage site, N-terminal „SNM" three artificial residues.	As above	~ 5 mg/L 2xTY medium). Purity and stability is comparable to the “GSMA” construct above.

SI13: ORF3a

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF3a
2	**Region/Name/Further Specification**
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MDLFMRIFTIGTVTLKQGEIKDATPSDFVRATATIPIQASLPFGWLIVGVALLAVFQSASKIITLK KRWQLALSKGVHFVCNLLLLFVTVYSHLLLVAAGLEAPFLYLYALVYFLQSINFVRIIMRLWLC WKCRSKNPLLYDANYFLCWHTNCYDYCIPYNSVTSSIVITSGDGTTSPISEHDYQIGGYTEKWE SGVKDCVVLHSYFTSDYYQLYSTQLSTDTGVEHVTFFIYNKIVDEPEEHVQIHTIDGSSGVVNPV MEPIYDEPTTTTSVPL
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-275 (fl ORF3a)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 72.4%; similarity: 90.2%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6XDC
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	32.32 kDa / 64,205 M^{-1} cm^{-1} / 5.67
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.

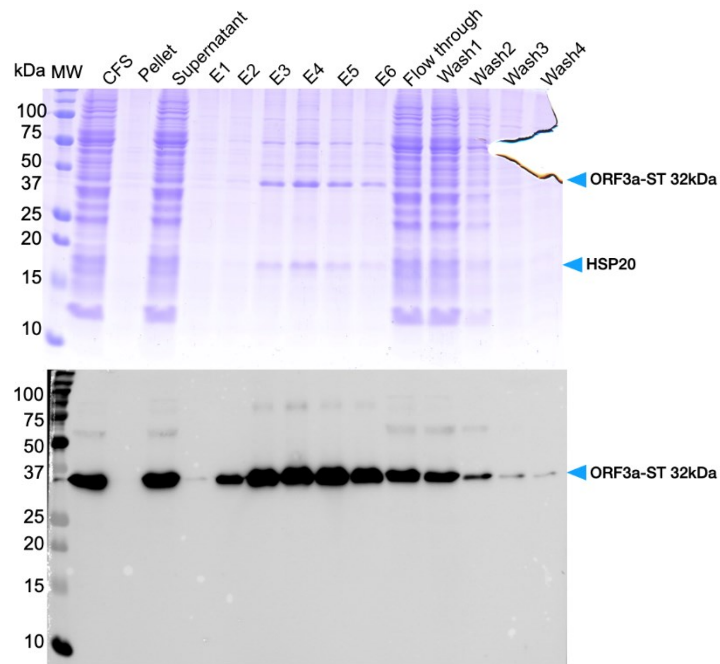
6	**Feeding buffer**
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.6 mg/mL WGE
1b	**A260/280 ratio**
	1.08
2	**Stability**
	Stable at 4°C for at least 2 weeks.
3	**Comment on applicability**
	ORF3a-ST is eluted with small heat shock protein (SHSP, 18 kDa) from wheat.



WG-CFPS in the presence of detergent, and Strep-tag purification of ORF3a. SDS-PAGE (upper panel) and WB (lower panel).

SI14: ORF4 (Envelope (E) protein)

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF 4; Envelope (E) protein
2	**Region/Name/Further Specification**
	E protein
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MYSFVSEETGTLIVNSVLLFLAFVVFLLVTLAILTALRLCAYCCNIVNVSLVKPSFYVYSRVKNL NSSRVPDLLV
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-75 (fl ORF4)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 94.7%; similarity: 97.4%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 5X29
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 36049

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	9.56 kDa / 11,460 M^{-1} cm^{-1} / 8.55
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.
6	**Feeding buffer**

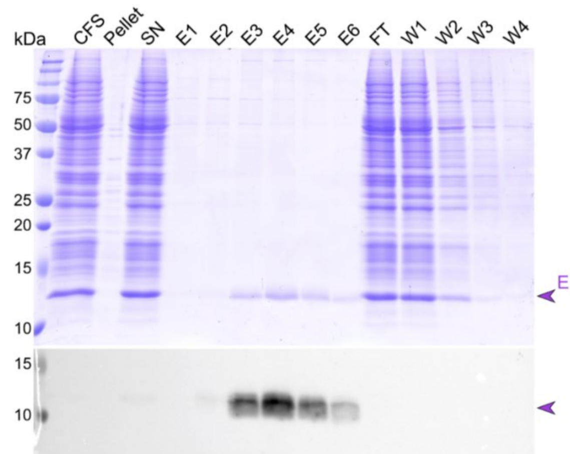
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.45 mg/mL WGE
1b	**A260/280 ratio**
	1.52
2	**Stability**
	Stable at least a few days at rt.
3	**Comment on applicability**
	E protein cannot be sedimented and is thus not directly available for solid-state NMR. Lipid reconstitution will be needed.



WG-CFPS in the presence of detergent, and Strep-tag purification of E (ORF4). SDS-PAGE (upper panel) and WB (lower panel).

SI15: ORF5 (M protein)

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF5; Membrane glycoprotein (M)
2	**Region/Name/Further Specification**
	M protein
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MADSNGTITVEELKKLLEQWNLVIGFLFLTWICLLQFAYANRNRFLYIIKLIFLWLLWPVTLACF VLAAVYRINWITGGIAIAMACLVGLMWLSYFIASFRLFARTRSMWSFNPETNILLNVPLHGTILT RPLLESELVIGAVILRGHLRIAGHHLGRCDIKDLPKEITVATSRTLSYYKLGASQRVAGDSGFAA YSRYRIGNYKLNTDHSSSSDNIALLVQ
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-222 (fl ORF5)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 90.5%; similarity: 98.2%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	26.35 kDa / 57,660 M^{-1} cm^{-1} / 9.48
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.

6	**Feeding buffer**
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT, 6 mM (average concentration) amino acid mix, and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, 6 mM (average concentration), and amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

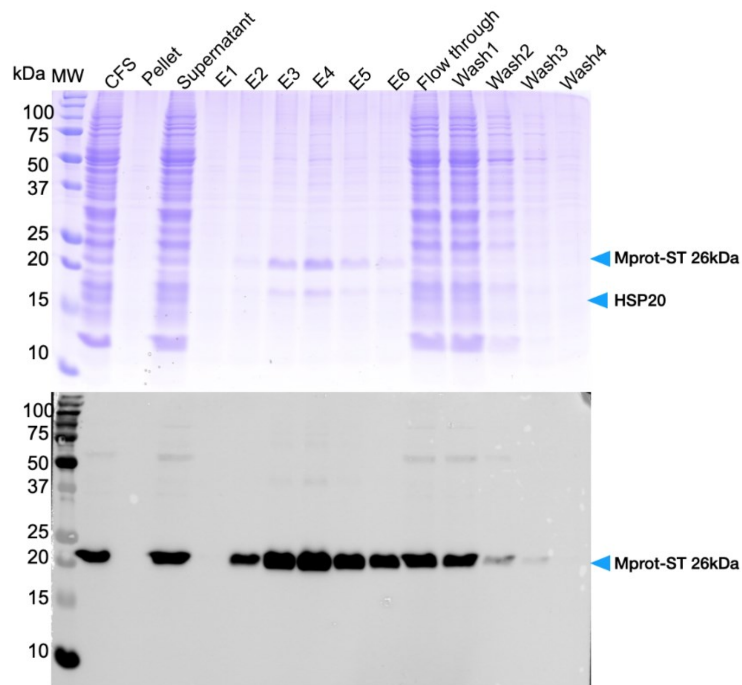
1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.33 mg/mL WGE
1b	**A260/280 ratio**
	1.16
2	**Stability**
	Stable at 4°C for at least 2 weeks.
3	**Comment on applicability**
	Mprotein-ST (and ST-Mprot) is eluted with small heat shock protein (SHSP 18 kDa) from wheat.

Additional information

Constructs	Conditions	Comments
Fl ORF5; Strep tag II (pEU-E01-MCS (Cell-Free Sciences)); no cleavage site; N-terminal "WSHPQFEK" eight artificial residues.	As above, but: - Purification: 1 mM DTT was added in purification buffers 1A and 1B. - Tab. 3.2B: 0.25% (w/v) DDM is added and incubated on the wheel for 1 h. - Tab. 3.2C: 40,000 g for 40 min. - Tab. 3.2E: added Strep beads for batch purification (200 µL 50% (w/v) suspension per well) and incubated on the wheel for 1.5 h.	Works as well with similar yield (0.39 mg/mL) and purity.



WG-CFPS in the presence of detergent, and Strep-tag purification of M (ORF5). SDS-PAGE (upper panel) and WB (lower panel).

SI16: ORF6

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF6
2	**Region/Name/Further Specification**
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MFHLVDFQVTIAEILLIIMRTFKVSIWNLDYIINLIIKNLSKSLTENKYSQLDEEQPMEID
4	**Protein boundaries - amino acid numbering (according to NCBI Reference Sequence NC_045512.2):**
	aa 1-61 (fl ORF6)
5	**Ratio for construct design (detailed and comprehensible)**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 68.9%; similarity: 93.4%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	8470.85 kDa / 13,980 M^{-1} cm^{-1} / 4.89
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.
6	**Feeding buffer**

	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

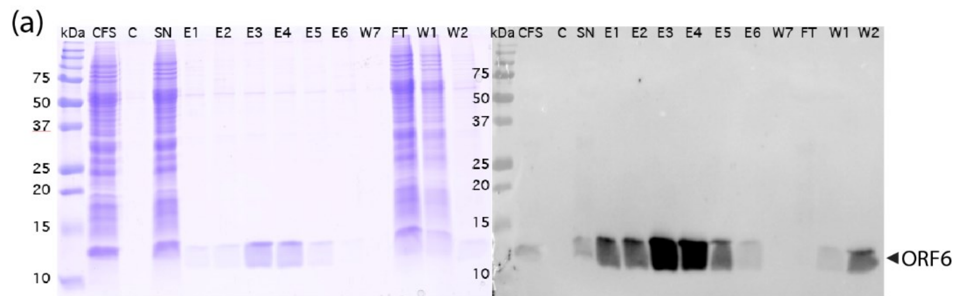
1	**Buffer List**
A	20 mM NaPi (pH 6.5), 50 mM NaCl (wash buffer).
B	20 mM NaPi (pH 6.5), 50 mM NaCl, 2.5 mM desthiobiotin (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

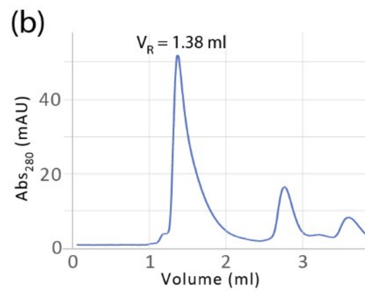
Table 4: Final sample

1	**Yield**
	0.27 mg/mL of WGE and total production of 875 µg for NMR samples
1b	**A260/280 ratio**
	1.36
2	**Stability**
	stable
3	**Comment on applicability**
	Positioning the Strep tag at the N-terminus abolished synthesis.

Additional information

Constructs	Conditions	Comments
Fl ORF6; Strep tag II (pEU-E01-MCS (Cell-Free Sciences)), no cleavage site, N-terminal "WSHPQFEK " eight artificial residues.		No expression observed.





(a) WG-CFPS and Strep-tag purification of ORF6. SDS-PAGE (left panel) and WB (right panel). **(b) SEC profile of ORF6.**

SI17: ORF7a

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF7a
2	**Region/Name/Further Specification**
	Ectodomain (ED)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MKIILFLALITLATCELYHYQECVRGTTVLLKEPCSSGTYEGNSPFHPLADNKFALTCFSTQFAFA CPDGVKHVYQLRARSVSPKLFIRQEEVQELYSPIFLIVAAIVFITLCFTLKRKTE
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 16-81 (ectodomain of ORF7a)
5	**Ratio for construct design (detailed and comprehensible)**
	Only the ectodomain without signaling peptide. Transmembrane helix is also not included in the construct.
6	**Sequence homology (to SCoV)**
	Identity: 85.3%; similarity: 95.9%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV: PDB 1XAK, 1YO4
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 6824

Table 2: Protein Expression

1	**Expression vector**
	pET24d-GB1 (Novagen, modified by G. Stier (Bogomolovas et al., 2009))
2	**Purification-/Solubility-Tag**
	N-terminal His_6-GB1
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	7.49 kDa / 6,210 M^{-1} cm^{-1} / 6.99
5	**Comments on sequence of expressed construct**
	N-terminal „G" one artificial residue due to TEV-cleavage and construct design.
6	**Used expression strain**

	E.coli (DE3) BL21
7	**Cultivation medium**
	M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	25°C for 18-20 h

Table 3: Protein Purification

1	**Buffer List**
A	20 mM Tris-HCl (pH 8.0), 6 M GdnHCl, 500 mM NaCl, 5 mM imidazole, 2 mM bME (Cell disruption / solubilization of pellet).
B	20 mM Tris (pH 8.0), 6 M GdnHCl, 500 mM NaCl, 10 mM imidazole, 2 mM bME (IMAC1).
C	50 mM NaPi (pH 8.0), 300 mM NaCl, 10 mM imidazole, 2 mM bME (IMAC2).
D	1 mM acetate-D4 (pH 5.0) (final NMR-buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption and solubilization of pellet in buffer **1A**.
B	IMAC, gravity flow Ni^{2+}-NTA (Qiagen), elution with 200 mM imidazole in buffer **1B**.
C	Dialysis against buffer **1C**.
D	TEV-cleavage (1 mg TEV protease per 10 mL protein solution) o.n. in buffer **1C**.
E	Inv. IMAC, elution with 200 mM imidazole in buffer **1C**.
F	Dialysis of flow-through of inv. IMAC against **1D** and concentrate (NMR-sample).

Table 4: Final sample

1	**Yield**
	0.4 mg/L ^{15}N-M9 medium
1b	**A260/280 ratio**
	0.7
2	**Stability**
	Stable throughout measurement (1 day, 298/315 K). No precipitation or degradation observed after four days at rt.

3	**Comment on applicability**
	Suitable for NMR structure determination, fragment screening, interaction studies.

SI18: ORF7b

Tabel 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF7b
2	**Region/Name/Further Specification**
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MIELSLIDFY LCFLAFLLFL VLIMLIIFWF SLELQDHNET CHA
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-43 (fl ORF7b)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: 85.4%; similarity: 97.2%
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Bacterial

Table 2: Protein Expression

1	**Expression vector**
	pThiored (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-Trx
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	5.37 kDa / 6,990 M^{-1} cm^{-1} / 4.17
5	**Comments on sequence of expressed construct**
	N-terminal “GA(M)G” three artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**

	E. coli BL21 (DE3)
7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification with detergent

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME (cell disruption).
B	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME, 1.5% (w/v) DDM (Solubilization of pellet).
C	25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 10 mM imidazole, 10 mM bME, 0.02% (w/v) DDM (IMAC).
D	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl, 0.02% (w/v) DDM (SEC/final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)) by microfluidization.
B	Solubilization of pellet after lysis **1B** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)).
C	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1C.**
D	TEV-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1C**
E	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with 500 mM imidazole in buffer **1C**.
F	Rebuffer flow-through of inv. IMAC in buffer **1D** (NMR sample).
G	Analytical SEC (SD 75 Increase 10/300 GL (GE Healthcare), ÄKTA start (GE Healthcare)) in buffer **1D**.

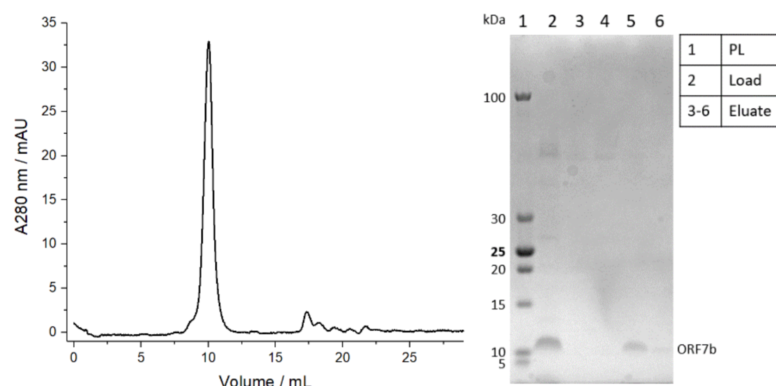
Table 4: Final sample

1	**Yield**
	0.6 mg/L ^{15}N-M9 medium
2	**Stability**
	Stable throughout measurement (2 days, 283/298 K). No significant precipitation or degradation observed after storage at 4°C for 3 months.

3	**Comment on applicability**
	Due to necessity of solubilizing agent and tendency to oligomerize structure determination, fragment screening, and interaction studies are hindered.

Additional information

	Constructs	**Conditions**	**Comments**
A	As above	Native **IMAC buffer:** 25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl	Nearly no protein was extracted in soluble fraction.
B		Denaturing **Solubilizing buffer:** 25 mM Tris-HCl (pH 8.0), 6 M GdnHCl, 300 mM NaCl, 5 mM imidazole. **IMAC wash buffer:** 25 mM Tris-HCl (pH 8.0), 8 M urea, 300 mM NaCl, 5 mM imidazole. **Renaturing buffer:** 25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME. **IMAC elution buffer:** 25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 500 mM imidazole, 10 mM bME.	After refolding and cleavage degradation of protein.
C	Fl ORF7b; His_6-SUMO (pE-SUMO (GenScript)), Ulp1-cleavage site, no artificial residues.	Native **IMAC buffer:** as above **SEC buffer:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl.	Protein is soluble with fusion, runs in exclusion volume of SD 200 columns, degrades after cleavage. NMR shows SUMO is mostly unfolded.
D		Detergent **IMAC buffer:** 50 mM NaPi (pH 7.0), 200 mM NaCl, 0.1% (v/v) Triton X-100, 5 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 6.0), 50 mM NaCl, 0.01% (v/v) Triton X-100, 2 mM TCEP-HCl.	Copurification of impurities, runs in exclusion volume of SD 200 columns. NMR shows severely broadened and poorly dispersed resonances hinting to oligomerization.
E		Semi-denaturing **IMAC buffer:** 50 mM Tris-HCl **(pH 8.0)**, 2 M urea, 300 mM NaCl, 10 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 6.5), 50 mM NaCl, 2 M urea, 5 mM DTT.	Degrades after cleavage.



Analytical SEC of ORF7b. Protein was in exclusion volume (9-11 mL, left panel) with corresponding SDS-PAGE of SEC with fractions analyzed from 7-11 mL elution volume (right panel).

Cell-free

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	5.37 kDa / 6,990 M^{-1} cm^{-1} / 4.17
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.
6	**Feeding buffer**
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.1% (w/v) MNG-3.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix, 0.1% (w/v) MNG-3.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

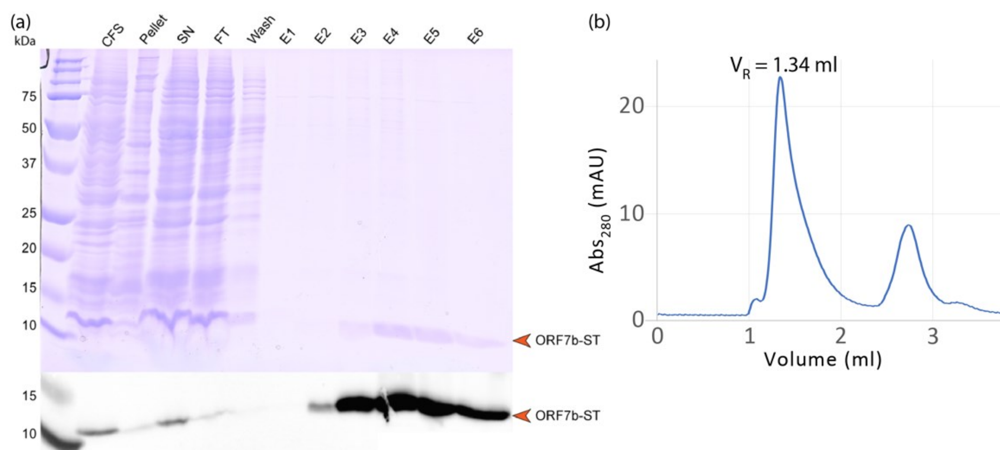
Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).

B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.27 mg/mL of WGE and total production of 880 µg for NMR samples
1b	**A260/280 ratio**
	1.36
2	**Stability**
	Stable in detergent over several days.
3	**Comment on applicability**
	Needs reconstitution into membranes for further structural analysis.



(a) WG-CFPS in presence of detergent and Strep-tag purification of ORF7b. SDS-PAGE (upper panel) and WB (lower panel). **(b) SEC profile of ORF7b.**

SI19: ORF8

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF8
2	**Region/Name/Further Specification**
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MKFLVFLGIITTVAAFHQECSLQSCTQHQPYVVDDPCPIHFYSKWYIRVGARKSAPLIELCVDEA GSKSPIQYIDIGNYTVSCSPFTINCQEPKLGSLVVRCSFYEDFLEYHDVRVVLDFI
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
ORF8	aa 1-121 (fl ORF8 = ORF8)
ORF8 m	aa 1-121 (fl ORF8) with L84S mutation (~ isolate 2019-nCoV_HKU-SZ-002a_2020).
ΔORF 8	aa 16-121 (without signal peptide = ΔORF8)
5	**Ratio for construct design (detailed and comprehensible)**
ORF8	fl protein
ΔORF 8	Protein after the hypothetical cleavage of the N-terminal Signal Peptide
6	**Sequence homology (to SCoV)**
ORF8	Identity: 31.7%; similarity: 70.7%
ΔORF 8	Identity: 40.5%; similarity: 66.7%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: 7JTL, 7JX6
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Bacterial

Table 2: Protein Expression

1	**Expression vector**
ORF8 m	pPK1154 (GenScript)
ΔORF 8	pET22b (+) (Merck/Novagen)
2	**Purification-/Solubility-Tag**

ORF8m	N-terminal His_6-SUMO
ΔORF8	N-terminal His_6-GST
3	**Cleavage Site**
ORF8m	Ulp1
ΔORF8	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
ORF8m	13.80 kDa / 15,930 M^{-1} cm^{-1} / 5.42
ΔORF8	12.54 kDa / 15,930 M^{-1} cm^{-1} / 5.15
5	**Comments on sequence of expressed construct**
ORF8m	No artificial residues due to Ulp1-cleavage and construct design.
ΔORF8	N-terminal “GAMG” three artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**
ORF8m	*E. coli* BL21 (DE3)
ΔORF8	*E. coli* BL21 (DE3) pLysS
7	**Cultivation medium**
ORF8m	LB / M9 (uniformly ^{15}N-labelled)
ΔORF8	LB
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
ORF8m	0.5 mM IPTG at OD_{600} 0.6
ΔORF8	0.5 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
ORF8m	16-20°C for 16-18 h
ΔORF8	18°C for 16-18 h

Table 3a: Protein Purification (ORF8)

1	**Buffer List**
A	10 mM NaPi (pH 8.0), 300 mM NaCl, 10 mM imidazole, 0.5 mM DTT (Cell disruption).
B	10 mM NaPi (pH 8.0), 300 mM NaCl, 10 mM imidazole, 0.5 mM DTT (Solubilization of pellet).
C	10 mM NaPi (pH 8.0), 300 mM NaCl, 0.5 mM DTT (IMAC).
D	50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM DTT, 0.2% (w/v) NP40.
E	50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM DTT.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)) by French-press.
B	Solubilization of pellet after lysis **1B** (plus one tablet of EDTA free protease inhibibitor cocktail (Merck)).
C	IMAC (Nickel-NTA-Agarose, QIAGEN) by hand, elution with 250 mM imidazole in buffer **1C**.
D	Ulp1-cleavage (Protein/Ulp1 ratio 10:1) o.n. at 21°C in buffer **1D**.
E	Rebuffer in buffer **1E**.

Table 3b: Protein Purification (ΔORF8)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 5% (v/v) glycerol, 50 mM imidazole (cell disruption/IMAC).
B	50 mM Tris-HCl (pH 8.0), 150 mM NaCl (TEV-cleavage).
C	20 mM NaPi (pH 7.4), 150 mM NaCl, 1 mM EDTA (SEC final buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** (supplemented with 0.5 mg/mL lysozyme, 10 µg/mL DNaseI, 5 mM $MgCl_2$, cOmplete™ EDTA-free protease inhibitors) by incubation for 30 min at RT followed by sonication at 43% amplitude for 2 minutes (1 s on, 1 s off). Extraction of the periplasmatic fraction: added 0.1% (v/v) Triton to the total sample after sonication, and incubated 15 min at 4°C. Centrifugation at 24.700 g for 40 min at 4°C. Recovering of the soluble fraction and filtration using 0.45 µm syringe filters.
B	IMAC (HisTrap FF Crude (GE Healthcare), ÄKTA Pure 25 M1 (GE Healthcare)), binding with buffer **1A** supplemented with 50 mM imidazole, elution with imidazole gradient up to 500 mM in buffer **1A**.
C	TEV-cleavage (Protein/TEV ratio 1:10) at 4°C, o.n. in buffer **1B**.
D	Inv. IMAC (HisTrap FF Crude (GE Healthcare), ÄKTA Pure 25 M1 (GE Healthcare)), binding with buffer **1A** supplemented with 50 mM imidazole, elution with imidazole gradient up to 500 mM in buffer **1A**.
E	SEC on Increase 10/300 S75 (GE Healthcare) at 4°C in buffer **1C**.

Table 4: Final sample

1	**Yield**
ORF8m	<0.5 mg/L LB mg/mL ^{15}N-M9 medium

ΔORF 8	0.5 mg/L LB medium
2	**Stability**
ORF8 m	Not determined.
ΔORF 8	No significant precipitation or degradation observed after storage at 4°C for 1 week.
3	**Comment on applicability**
ORF8 m	Weak expression into solube fraction, 30%/70% soluble/inclusion bodies. After purification extremely low yield for NMR studies.
ΔORF 8	Very low yield. It would be very expensive to prepare a labelled sample for NMR studies.

Additional information (bacterial expression)

Constructs	**Conditions**	**Comments**
ORF8 with L84S mutation; His_6 (pPK1151 (Genscript)), TEV-cleavage site, N-terminal "GS" two artificial residues.	As above for ORF8m, only LB medium.	No expression.

Cell-free

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
ORF8	C-terminal Strep tag II (WSHPQFEK)
ΔORF 8	N-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
ORF8	15.00 kDa / 21,805 M^{-1} cm^{-1} / 5.64
ΔORF 8	13.53 Da / 21,805 M^{-1} cm^{-1} / 5.39
5	**Comments on sequence of expressed construct**
ORF8	C-terminal "SAWSHPQFEK" ten artificial residues due to construct design.
ΔORF 8	N-terminal "M" and C-terminal "SAWSHPQFEK" eleven artificial residues due to construct design.

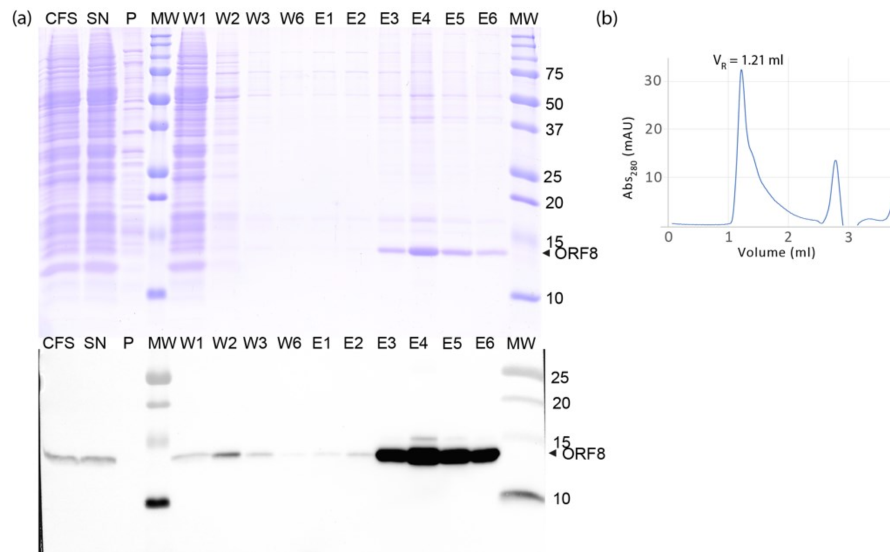
6	**Feeding buffer**
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 μg/mL creatine kinase, and 6 mM (average concentration) amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification (ORF8a and ORF8b)

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.62 mg/mL WGE after purification. Total of 683 µg for the NMR samples
1b	**A260/280 ratio**
	0.7
2	**Stability**
	Stable at 4ºC for weeks.
3	**Comment on applicability**
	Protein very sensitive to dilution-concentration steps. Purity is sufficient for NMR as other cell-free proteins are not labelled.



(a) WG-CFPS in presence of detergent and Strep-tag purification of ORF8. SDS-PAGE (upper panel) and WB (lower panel). **(b) SEC profile of ORF8.**

SI20: ORF9a (Nucleocapsid (N) protein)

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF9a; Nucleocapsid (N) phosphoprotein
2	**Region/Name/Further Specification**
	N-terminal disordered region (aa 1-43, IDR1) / N-terminal RNA binding domain (aa 44-180, NTD) / serine-arginine (SR) rich motif (aa 181-212, SR) / central disordered linker (aa 181-248, IDR2) / C-terminal dimerization domain (247-364) / C-terminal disordered region (aa 365-419, IDR3)
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MSDNGPQNQRNAPRITFGGPSDSTGSNQNGERSGARSKQRRPQGLPNNTASWFTALTQHGKED LKFPRGQGVPINTNSSPDDQIGYYRRATRRIRGGDGKMKDLSPRWYFYYLGTGPEAGLPYGAN KDGIIWVATEGALNTPKDHIGTRNPANNAAIVLQLPQGTTLPKGFYAEGSRGGSQASSRSSSRSR NSSRNSTPGSSRGTSPARMAGNGGDAALALLLLDRLNQLESKMSGKGQQQQGQTVTKKSAAE ASKKPRQKRTATKAYNVTQAFGRRGPEQTQGNFGDQELIRQGTDYKHWPQIAQFAPSASAFFG MSRIGMEVTPSGTWLTYTGAIKLDDKDPNFKDQVILLNKHIDAYKTFPPTEPKKDKKKKADET QALPQRQKKQQTVTLLPAADLDDFSKQLQQSMSSADSTQA
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
IDR1-NTD-IDR2	aa 1-248 (of fl ORF9a)
NTD-SR	aa 44-212 (of fl ORF9a)
NTD	aa 44-180 (of fl ORF9a)
CTD	aa 247-364 (of fl ORF9a)
5	**Ratio for construct design (detailed and comprehensible)**
IDR1-NTD-IDR2	Based on boundaries from SCoV homolog.
NTD-SR	In analogy to the available NMR (PDB 6YI3) and crystal (6M3M) structures of N-NTD SCoV2.
NTD	In analogy to the available NMR (PDB 6YI3) and crystal (6M3M) structures of N-NTD SCoV2.
CTD	In analogy to the available NMR structure (PDB 2JW8) of N-CTD from SCoV.
6	**Sequence homology (to SCoV)**
IDR1-NTD-IDR2	Identity: 90%; similarity: 94%
NTD-SR	Identity: 92%; similarity: 96%
NTD	Identity: 93%; similarity: 97%
CTD	Identity: 96%; similarity: 98%
7	**Published structures (SCoV2 or homologue variants)**

	SCoV: PDB 2JW8, 2CJR SCoV2: PDB 6YI3, 6M3M, 6VYO, 6WKP, 6WZO, 6WJI, 6YUN, 6ZCO, 7CE0, 7C22
8	**(Published) assignment (SCoV2 or homologue variants)**
	SCoV: BMRB 15511 (CoV) SCoV2: PDB 6YI3, BMRB 34511 (NTD), BMRB 50518 (CTD), BRMB 50619 (IDR1), BMRB 50618 (IDR2), BMRB 50557 (IDR1), BMRB 50558 (IDR2).

Table 2: Protein Expression

1	**Expression vector**
IDR1-NTD-IDR2	pET29a(+) (Twistbioscience)
NTD-SR	pET-28a(+) (GenScript)
NTD	pET-28a(+) (GenScript)
CTD	pKM263 (GenScript)
2	**Purification-/Solubility-Tag**
IDR1-NTD-IDR2	-
NTD-SR	N-terminal His_6
NTD	N-terminal His_6
CTD	N-terminal His_6-GST
3	**Cleavage Site**
IDR1-NTD-IDR2	-
NTD-SR	TEV
NTD	TEV
CTD	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
IDR1-NTD-IDR2	26.52 kDa / 26,930 M^{-1} cm^{-1} / 10.57
NTD-SR	18.10 kDa / 26,930 M^{-1} cm^{-1} / 10.35
NTD	14.85 kDa / 26,930 M^{-1} cm^{-1} / 9.60
CTD	13.56 kDa / 16,960 M^{-1} cm^{-1} / 9.77

5a	**Comments on sequence of expressed construct**
IDR1-NTD-IDR2	No artificial residues due to construct design.
NTD-SR	No artificial residues due to TEV-cleavage and construct design.
NTD	No artificial residues due to TEV-cleavage and construct design.
CTD	N-terminal „GAMG" four artificial residues due to TEV-cleavage and construct design.
6	**Used expression strain**
	E. coli BL21 (DE3)
7	**Cultivation medium**
IDR1-NTD-IDR2	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
NTD-SR	LB / M9 (uniformly ^{15}N-labelled)
NTD	LB / M9 (uniformly ^{15}N-labelled)
CTD	LB / M9 (uniformly ^{15}N or ^{13}C,^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
IDR1-NTD-IDR2	0.2 mM IPTG at OD_{600} 0.8
NTD-SR	0.2 mM IPTG at OD_{600} 0.7
NTD	0.2 mM IPTG at OD_{600} 0.7
CTD	1 mM IPTG at OD_{600} 0.7
10	**Cultivation temperature and time**
IDR1-NTD-IDR2	Cells are grown at 37°C in 1 L LB until OD_{600} 0.8, then transferred in 250 mL labelled minimal medium (4x). After 1 h of metabolite clearance, the culture is induced at 18°C for 16-18 h. For unlabelled protein, culture is induced at OD_{600} 0.9.
NTD-SR	16-18°C for 16-18 h
NTD	16-18°C for 16-18 h
CTD	20-22°C for 18-20 h

Table 3a: Protein Purification (IDR1-NTD-IDR2)

1	**Buffer List**
A	25 mM Tris-HCl (pH 8.0), 1 M NaCl, 5% (v/v) glycerol, RNAse, DNAse, proteases inhibitor cocktail (SIGMA*FAST*™ tablet, 500 µL of 100x stock) (lysis buffer).
B	25 mM Tris-HCl (pH 7.2) (dialysis after lysis and binding buffer).
C	25 mM Tris-HCl (pH 7.2), 1 M NaCl (elution buffer).
D	25 mM Tris-HCl (pH 7.2), 450 mM NaCl, 0.02% (w/v) NaN_3 (NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell lysis in Buffer **1A** by sonication (30 min with pulse 1 s on, 10 s off). It is crucial to add a cocktail of proteases inhibitors in lysis buffer; this step is crucial to preserve construct integrity.
B	Dialysis O/N at 4°C in Buffer **1B** for buffer exchange.
C	Ion Exchange chromatography with HiTrap SP FF 5 mL column (GE Healthcare), gradient elution with buffer **1C**. The protein eluted at 45-50% gradient.

Table 3b: Protein Purification (NTD and NTD-SR)

1	**Buffer List**
A	50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20 mM imidazole, 10% (v/v) glycerol, 0.01 mg/mL DNase, 5 mM $MgCl_2$ and protease inhibitor cocktail (Sigma) (cell disruption).
B	50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 20 mM imidazole, 10% (v/v) glycerol (IMAC).
C	50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 500 mM imidazole, 10% (v/v) glycerol (IMAC).
D	50 mM Tris-HCl (pH 8.0), 500 mM NaCl, 1 mM DTT (dialysis after IMAC / TEV-cleavage).
E	20 mM Na_2HPO_4 (pH 6.5), 50 mM NaCl, 500 µM PMSF, 3 mM NaN_3, 3 mM EDTA (final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Cell disruption in buffer **1A** by sonication.
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1B** and **1C**.
C	TEV-cleavage (1:10 (v/v) TEV:protein solution) during dialysis o.n. in buffer **1D**.
D	Inv. IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), elution with imidazole gradient up to 500 mM in buffer **1B** and **1C**.
E	NMR sample preparation in buffer **1E**.

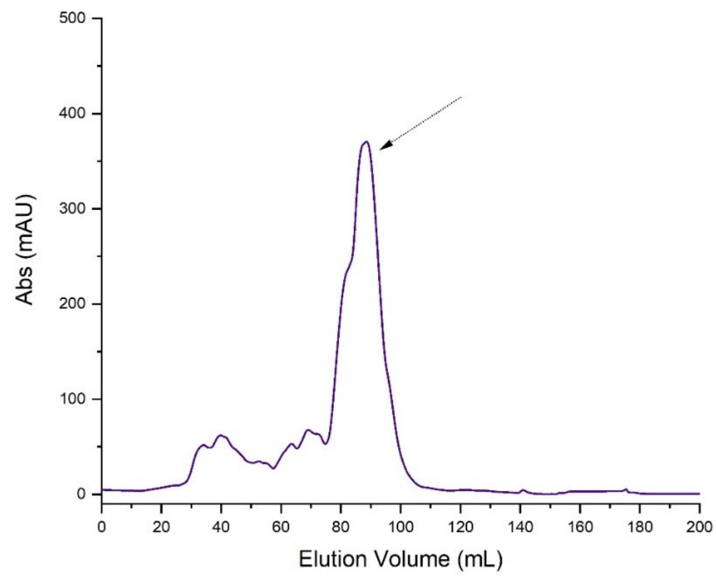
Table 3c: Protein Purification (CTD)

1	**Buffer List**
A	50 mM NaPi (pH 7.4), 150 mM NaCl, 10 mM imidazole (cell disruption / IMAC/ dialysis after IMAC / TEV-cleavage).
B	25 mM NaPi (pH 6.0), 50 mM NaCl, 0.5 mM EDTA, 0.02% (w/v) NaN_3 (SEC / final NMR buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**

A	Cell disruption in buffer **1A** (plus 100 µL protease inhibitor (Serva)) by sonication.
B	IMAC (gravity flow Ni^{2+}-NTA), Elution with 150-500 mM imidazole in buffer **1A**.
C	Dialysis o.n. in in buffer **1A**.
D	TEV-cleavage (0.5 mg TEV protease per 1 L culture) in buffer **1A**.
E	SEC on HiLoad 16/600 SD 75 (GE Healthcare) in buffer **1B**.
F	NMR sample preparation in buffer **1B**.

Table 4: Final sample

1	**Yield**
IDR1-NTD-IDR2	12 mg/L ^{13}C,^{15}N M9 medium
NTD-SR	3 mg/L ^{15}N M9 medium
NTD	3 mg/L ^{15}N M9 medium
CTD	2 mg/L ^{13}C,^{15}N-M9 medium
1b	**A260/280 ratio**
IDR1-NTD-IDR2	0.63
NTD-SR	0.7
NTD	0.7
CTD	0.55
2	**Stability**
IDR1-NTD-IDR2	Protein is stable for at least one1 week at working conditions (298 K).
NTD-SR	Stable throughout measurement (15 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 5 weeks.
NTD	Stable throughout measurement (15 days, 298 K). No significant precipitation or degradation observed after storage at 4°C for 5 weeks.
CTD	Stable throughout measurement (7 days, 303 K). No significant precipitation or degradation observed after storage at 4°C for 8 weeks. Tolerates temperature up to 315 K.
3	**Comment on applicability**
	All suitable for NMR structure determination, fragment screening, interaction studies.



Chromatogram of IEC of aa 1-248 construct. Protein is eluted at 45% gradient of Buffer 1B, fractions from 85-100 mL were collected.

SI21: ORF9b

Table 1: General Information

1	**Protein Name**
	ORF9b
2	**Region/Name/Further Specification**
3	**Sequence of fl protein**
	MDPKISEMHP ALRLVDPQIQ LAVTRMENAV GRDQNNVGPK VYPIILRLGS PLSLNMARKT LNSLEDKAFQ LTPIAVQMTK LATTEELPDE FVVVTVK
4	**Protein boundaries of expressed construct**
	aa 1-97 (fl ORF9b)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity : 72.4%; similarity: 95.0%
7	**Published structures (SCoV2 or homologue variants)**
	SCoV2: PDB 6Z4U
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	C-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	11.99 kDa / 6,990 M^{-1} cm^{-1} / 6.73
5	**Comments on sequence of expressed construct**
	C-terminal “SAWSHPQFEK” ten artificial residues due to construct design.
6	**Feeding buffer**

	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA.
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.64 mg/mL WGE and total production of 1338 µg for NMR samples.
1b	**A260/280 ratio**
	0.76
2	**Stability**
	Stable at 4ºC for a week.
3	**Comment on applicability**
	Protein studied at pH 6, 7.5 and pH 8. Methionine gets oxidized without DTT in the buffer.

Additional information

	Constructs	Conditions	Comments
A	Fl ORF9b; Strep tag II (pEU-E01-MCS (Cell-Free Sciences)); no cleavage site; C-terminal “WSHPQFEK” eight artificial residues.	As above with 0.1% (w/v) DDM	NMR shows severely broadened resonances due to oligomerization or protein micelles.
B		As above without DTT	Methionines get oxidated.

SI22: ORF14

Table 1: General Information

1	**Protein Name**
	ORF14
2	**Region/Name/Further Specification**
3	**Sequence of fl protein**
	MLQSCYNFLKEQHCQKASTQKGAEAAVKPLLVPHHVVATVQEIQLQAAVGELLLLEWLAMA VMLLLLCCCLTD
4	**Protein boundaries of expressed construct**
	aa 1-73 (fl ORF14)
5	**Ratio for construct design**
	fl protein
6	**Sequence homology (to SCoV)**
	Identity: NA; similarity: NA
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Cell-free Protein Synthesis

1	**Expression vector**
	pEU-E01-MCS (Cell-Free Sciences)
2	**Purification-/Solubility-Tag**
	N-terminal Strep tag II (WSHPQFEK)
3	**Cleavage Site**
	-
4	**Molecular weight / Extinction coefficient / pI - of protein**
	9.26 kDa / 12,490 M^{-1} cm^{-1} / 6.01
5	**Comments on sequence of expressed construct**
	N-terminal “WSHPQFEKGGG” eleven artificial residues due to construct design.
6	**Feeding buffer**

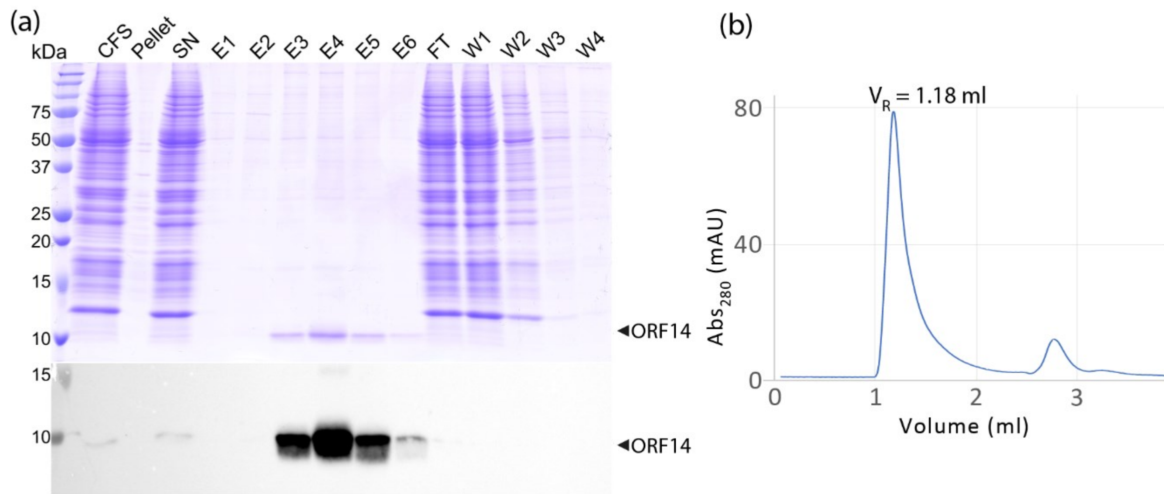
	30 mM HEPES-KOH (pH 7.6), 100 mM potassium acetate, 2.7 mM magnesium acetate, 16 mM creatine phosphate, 0.4 mM spermidine, 1.2 mM ATP, 0.25 mM GTP, 4 mM DTT and 6 mM (average concentration) amino acid mix and 0.05% (w/v) Brij-58.
7	**Translation mix**
	50% (v/v) mRNA, 50% (v/v) home-made WGE, 40 µg/mL creatine kinase, and 6 mM (average concentration) amino acid mix 0.05% (w/v) Brij-58.
8	**Protein synthesis temperature and time**
	22°C for 16 h without agitation (bilayer method).

Table 3: Protein Purification

1	**Buffer List**
A	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 0.1% (w/v) DDM (wash buffer).
B	100 mM Tris-HCl (pH 8.0), 150 mM NaCl, 1 mM EDTA, 2.5 mM desthiobiotin, and 0.1% (w/v) DDM (elution buffer).
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Harvest total CFS.
B	Incubate with benzonase for 30 min on a wheel, at rt.
C	Centrifuge for 30 min at 20,000 g, 4°C.
D	Harvest the soluble fraction (SN).
E	Equilibrate the Strep-Tactin column (IBA Lifesciences) with 2 CV of **1A** (all steps performed on the bench by gravity).
F	Load SN onto the column.
G	Wash the column with 5 CV of **1A**.
H	Elute the protein of interest with **1B**.

Table 4: Final sample

1	**Yield**
	0.43 mg/mL WGE
1b	**A260/280 ratio**
	1.06
2	**Stability**
	protein has proved unstable during lipid insertion using cyclodextrin for detergent removal
3	**Comment on applicability**
	Solution NMR shows severely broadened resonances hinting to oligomerization or too big protein micelles. Lipid reconstitution is ongoing.



(a) WG-CFPS in presence of detergent and Strep-tag purification of ORF14. SDS-PAGE (upper panel) and WB (lower panel). **(b) SEC profile of ORF14.**

SI23: ORF10

Table 1: General Information

1	**Protein Name (according to NCBI Reference Sequence NC_045512.2)**
	ORF10
2	**Region/Name/Further Specification**
3	**Sequence of fl protein (according to NCBI Reference Sequence NC_045512.2)**
	MGYINVFAFPFTIYSLLLCRMNSRNYIAQVDVVNFNLT
4	**Protein boundaries of expressed construct (according to NCBI Reference Sequence NC_045512.2)**
	aa 1-38 (fl ORF10)
5	**Ratio for construct design**
	Hypothetical fl protein.
6	**Sequence homology (to SCoV)**
	Identity: 29%; similarity: 52% with ORF9b
7	**Published structures (SCoV2 or homologue variants)**
	-
8	**(Published) assignment (SCoV2 or homologue variants)**
	-

Table 2: Protein Expression

1	**Expression vector**
	pThiored (GenScript)
2	**Purification-/Solubility-Tag**
	N-terminal His_6-Trx
3	**Cleavage Site**
	TEV
4	**Molecular weight / Extinction coefficient / pI - of cleaved protein**
	4.45 kDa / 4,470 M^{-1} cm^{-1} / 7.93
5	**Comments on sequence of expressed construct**
	N-terminal “GA” two artificial residues due to TEV-cleavage and construct design
6	**Used expression strain**
	E. coli BL21 (DE3)

7	**Cultivation medium**
	LB / M9 (uniformly ^{15}N-labelled)
8	**Induction system**
	IPTG inducible T7 promoter
9	**Induction of protein expression**
	0.2 mM IPTG at OD_{600} 0.6-0.7
10	**Cultivation temperature and time**
	18-20°C for 16-18 h

Table 3: Protein Purification

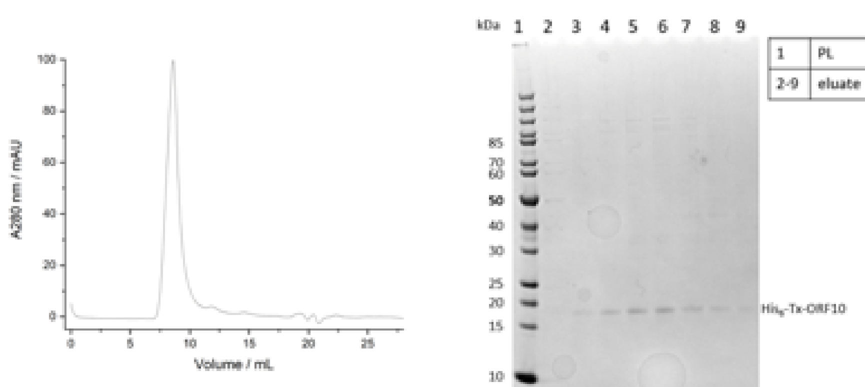
1	**Buffer List**
A	25 mM Tris (pH 8.0), 6 M GdnHCl, 300 mM NaCl, 5 mM imidazole (Solubilization)
B	25 mM Tris (pH 8.0), 8 M urea, 300 mM NaCl, 5 mM imidazole (IMAC - wash)
C	25 mM Tris (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME (IMAC - elution)
D	25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl.
2	**Purification steps (with corresponding buffer(s) and incubation times)**
A	Solubilization of cell pellet and inclusion bodies in **1A** (plus one tablet of EDTA free protease inhibitor cocktail (Merck)).
B	IMAC (HisTrap HP (GE Healthcare), ÄKTA start (GE Healthcare)), washed with buffer **1B**, refolded on column in buffer **1C**, elution with imidazole gradient up to 500 mM in buffer **1C**.
C	Analytic TEV-cleavage (1 mg TEV protease per 50 mL protein solution) o.n. in buffer **1C**.
D	Analytical SEC (SD 75 Increase 10/300 GL (GE Healthcare), ÄKTA start (GE Healthcare)) in buffer **1D**.

Table 4: Final sample

1	**Yield**
	2 mg/L (^{15}N-M9) His_6-SUMO-fused
2	**Stability**
	Degrades after cleavage
3	**Comment on applicability**
	Tendency to oligomerize (exclusion volume of SD 75 column).

Additional information

	Constructs	Conditions	Comments
A	As above	Native **IMAC buffer:** 25 mM Tris-HCl (pH 8.0), 300 mM NaCl, 5 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl.	Nearly no protein was extracted in soluble fraction (in inclusion bodies)
B	Fl ORF10; His_6-SUMO (pE-SUMO (GenScript)), Ulp1-cleavage site, no artificial residues.	Native **IMAC buffer:** as above **SEC buffer:** 25 mM NaPi (pH 7.0), 150 mM NaCl, 2 mM TCEP-HCl.	Protein is mostly soluble with fusion, partial degradation (copurification of His_6-SUMO), runs in exclusion volume of SD 200 columns, degrades after cleavage. NMR shows SUMO is mostly unfolded.
C		Detergent **IMAC buffer:** 50 mM NaPi (pH 7.0), 200 mM NaCl, 0.1% (v/v) Triton X-100, 5 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 6.0), 50 mM NaCl, 0.01% (v/v) Triton X-100, 2 mM TCEP-HCl.	Copurification of impurities, runs in exclusion volume of SD 75 columns hinting to oligomerization. Degrades after cleavage.
D		Semi-denaturing **IMAC buffer:** 50 mM Tris-HCl **(pH 8.0)**, 2 M urea, 300 mM NaCl, 10 mM imidazole, 10 mM bME. **SEC buffer:** 25 mM NaPi (pH 6.5), 50 mM NaCl, 2 M urea, 5 mM DTT.	Degrades after cleavage.



Analytical SEC of His_6-Trx-ORF10. Protein was in exclusion volume (8.5-12 mL, left panel) with corresponding SDS-PAGE of SEC with fractions analyzed from 8-12 mL elution volume (right panel).