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	On going
SUD-M	SCoV2: BMRB 50516 SUD-M
SUD-MC	On going
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

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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 / AC).
B	50 mM NaPi (pH 7.2), 50 mM NaCl, 2 mM EDTA, 2 mM DTT (SEC / NMR buffer).

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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 **1B-SUD-M**, **1B-SUD-MC**.
F	NMR sample preparation in buffer **1B-SUD-M, 1B-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.

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