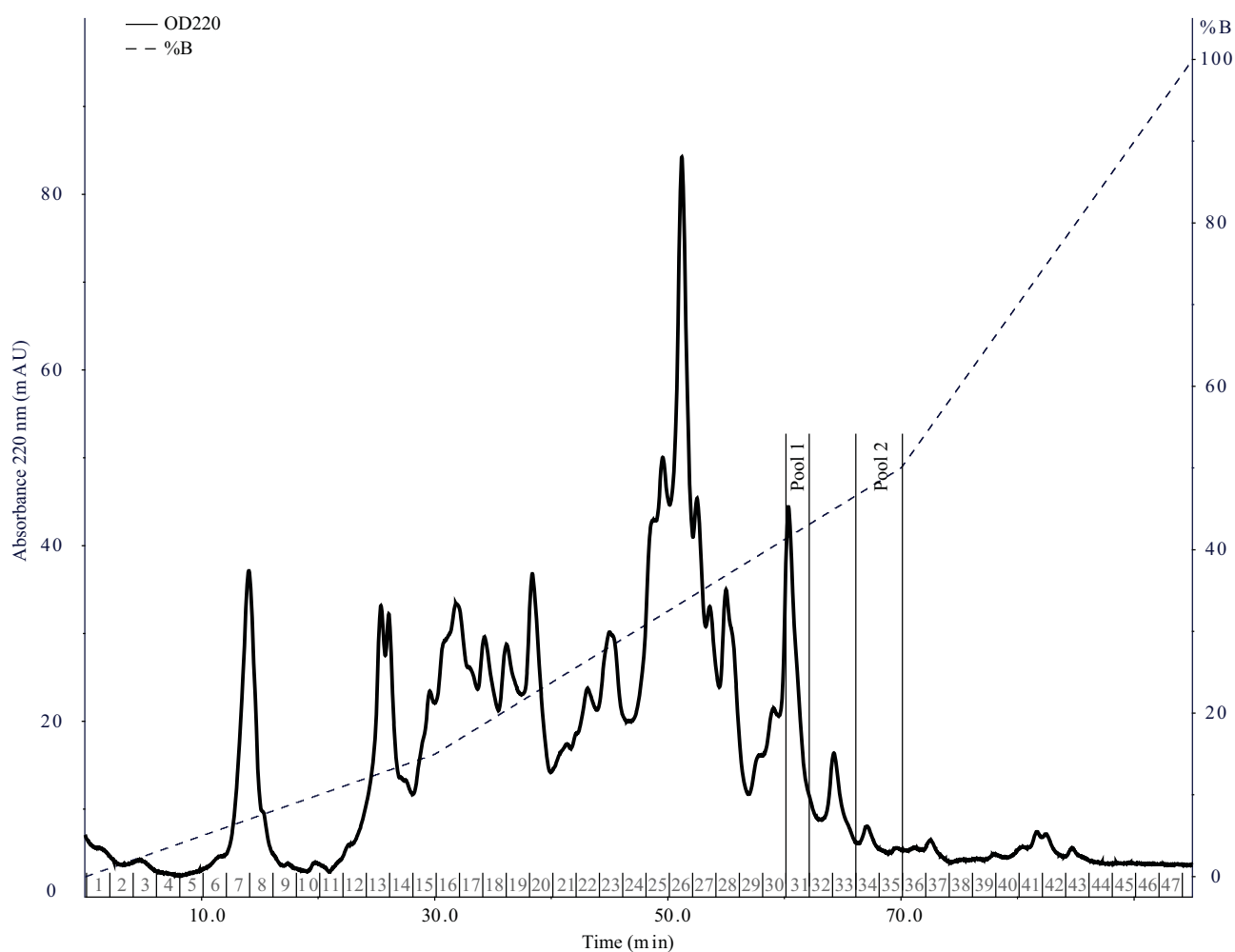


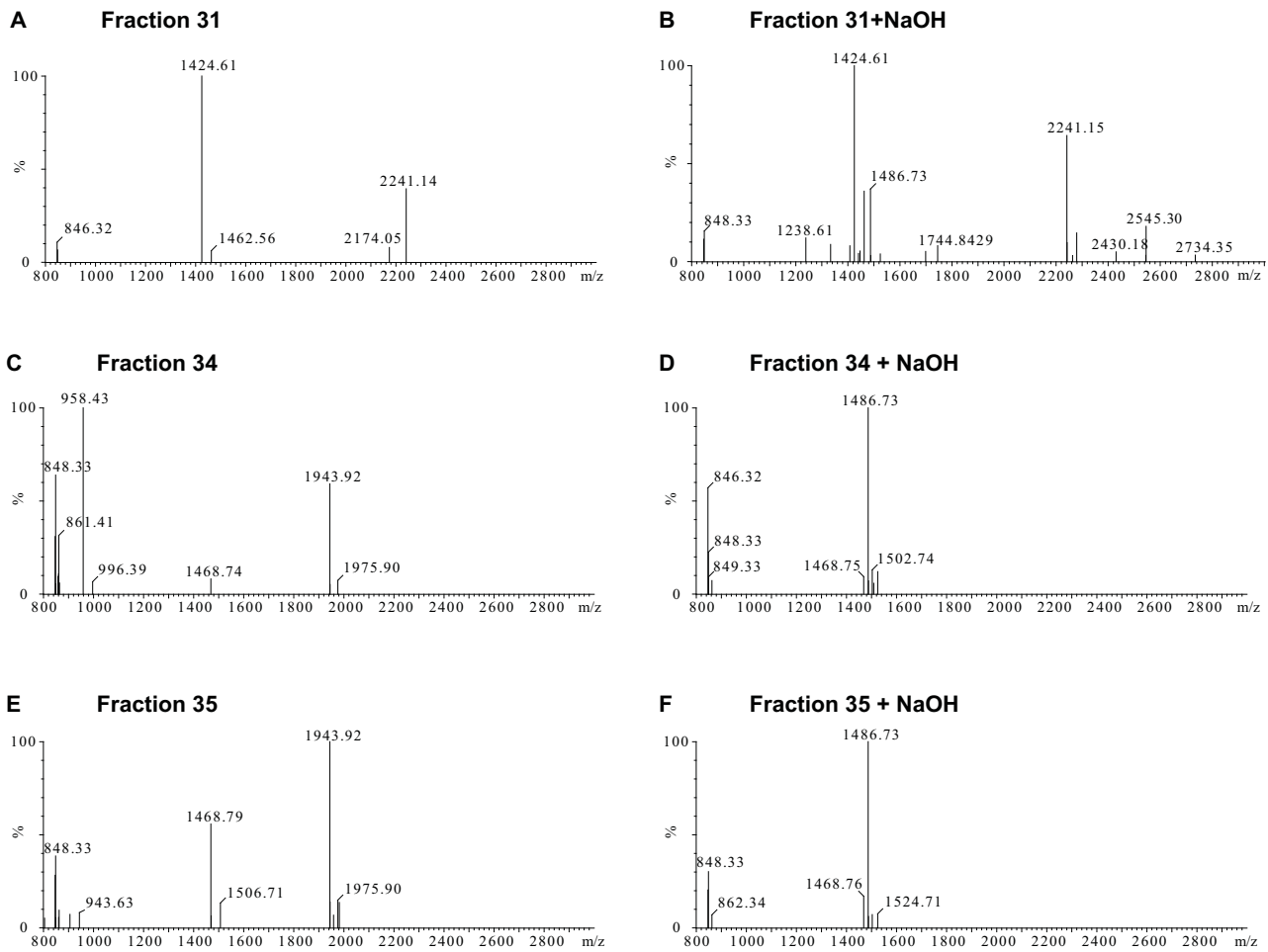
## TSG-6 TRANSFERS PROTEINS BETWEEN GLYCOSAMINOGLYCANS VIA A SER<sub>28</sub>-MEDIATED COVALENT CATALYTIC MECHANISM

Kristian W. Sanggaard, Carsten S. Sonne-Schmidt, Toke P. Krogager, Torsten Kristensen, Hans-Georg Wisniewski, Ida B. Thøgersen, and Jan J. Enghild.

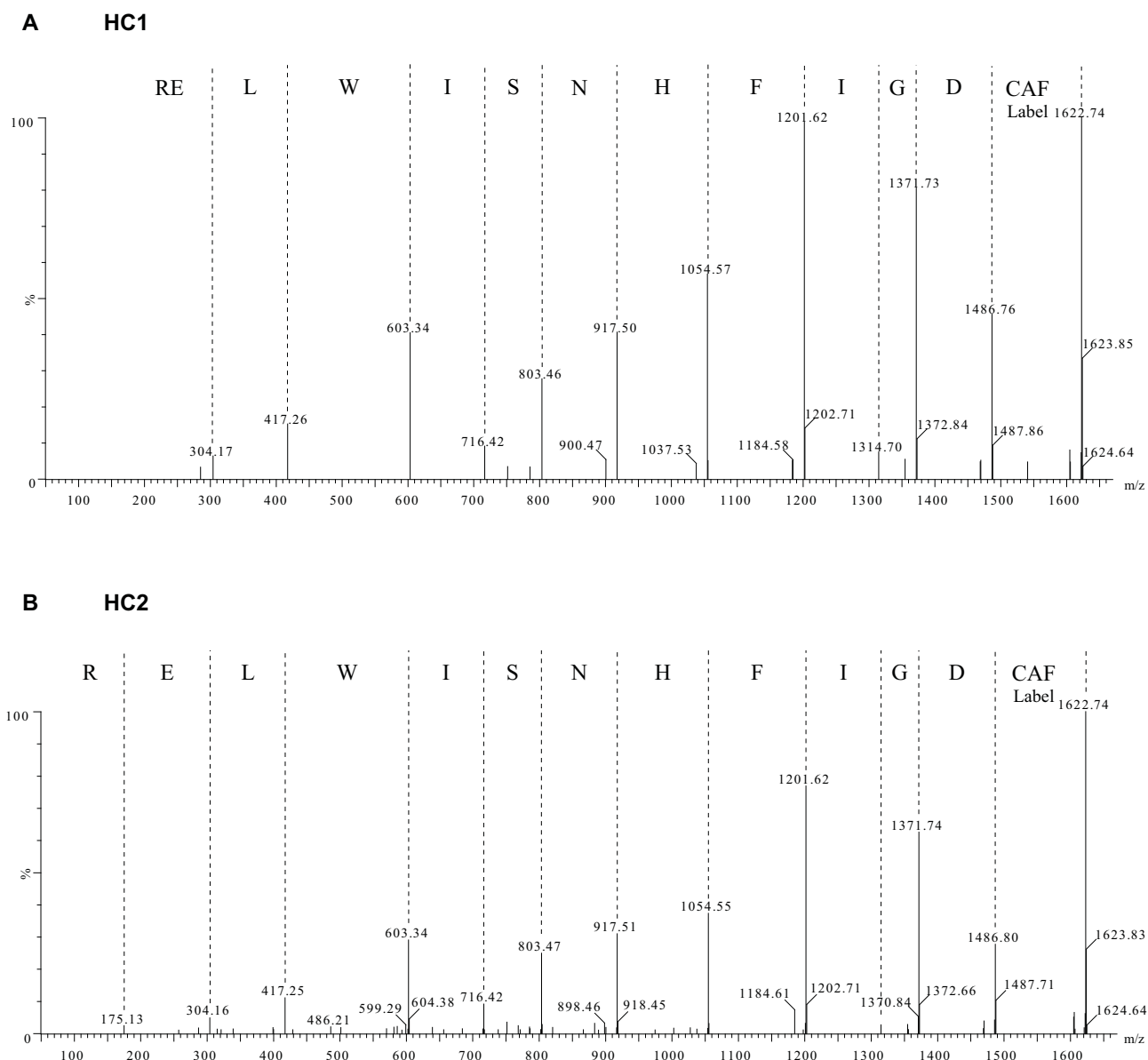
### SUPPLEMENTAL DATA



**Fig. S1.** Purification of HC•TSG-6 cross-links using strong cation exchange-HPLC. Purified I $\alpha$ I and TSG-6 were incubated to generate HC•TSG-6 complexes. Subsequently the proteins were denatured and digested with trypsin, and the resulting peptides were applied to a strong cation exchange column. The column was developed by a gradient of increasing KCl concentration and the eluting peptides monitored at 220 nm. The figure shows the strong cation exchange-HPLC trace. The fraction numbers are indicated above the x-axis. Pool 1 and pool 2 were used for subsequently purification of HC1•TSG-6 and HC2•TSG-6, respectively.



**Fig. S2.** MS analyses of strong cation exchange-HPLC fractions. Fractions 22-44 (+/- NaOH treatment) from the strong cation exchange-HPLC were analyzed by MALDI-TOF MS. The fractions sensitive to NaOH treatment are shown. (A) Fraction 31, (B) fraction 31 after NaOH treatment, (C) fraction 34, (D) fraction 34 after NaOH treatment, (E) fraction 35, and (F) fraction 35 after NaOH treatment. The observed  $MH^+$ 's correspond to the presence of  $HC1 \bullet TSG-6$  cross-link in fraction 31 ( $m/z$  2174.05) and the presence of  $HC2 \bullet TSG-6$  cross-link in fraction 34-35 ( $m/z$  1943.92).



**Fig. S3.** Tandem MS analyses of the tryptic TSG-6 peptide that participates in the cross-links. The tryptic TSG-6 peptide released after NaOH treatment of the cross-links was purified using RP-HPLC, subsequently the peptide was modified using the CAF derivatization protocol, and finally analyzed by tandem MS. (A) MS/MS analysis of the TSG-6 peptide (Fig. 2B, peak  $\beta$ ) released from the HC1•TSG-6 complex. (B) MS/MS analysis of the TSG-6 peptide (Fig. 3B, peak  $\lambda$ ) released from the HC2•TSG-6 complex. The two spectra ((A) and (B)) are very similar and the TSG-6 sequence D<sub>22</sub>GIFHNSIWLER<sub>33</sub> was easily derived from both spectra. In contrast to the MS/MS analysis of the cross-link (Fig. 4) the Ser in the NaOH-released TSG-6 peptide did not lose water upon fragmentation. It supports that Ser<sub>28</sub> is involved in the cross-link.

TABLE S1

*Analysis of fragment ions from MS/MS of the CAF modified HC1•TSG-6 cross-link*

The purified HC1•TSG-6 cross-link (Fig. 2A, peak  $\alpha$ ) was modified using the CAF derivatization protocol and subjected to MS/MS analysis (Fig. 4A-B). The resulting fragment ions were subsequently examined. The CAF protocol favors the formation of Y-ions and only “weak” B-ions were detected. The detected fragment ion series correlate with an ester bond between the internal Ser residue in the TSG-6 peptide and the terminal Asp residue of HC1.

**Y-ions:****Sequence: DGIFHNSIWLER, where Ser is dehydrated.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	175,13	-	-	R
Y2"	304,16	304,18	0,02	ER
Y3"	417,25	417,26	0,01	LER
Y4"	603,33	603,34	0,01	WLER
Y5"	716,41	716,42	0,01	IWLER
Y6"	785,42	785,45	0,03	S(-H <sub>2</sub> O)IWLER
Y7"	899,46	899,49	0,03	NS(-H <sub>2</sub> O)IWLER
Y8"	1036,52	1036,56	0,04	HNS(-H <sub>2</sub> O)IWLER
Y9"	1183,59	1183,62	0,03	FHNS(-H <sub>2</sub> O)IWLER
Y10"	1296,68	-	-	IFHNS(-H <sub>2</sub> O)IWLER
Y11"	1353,70	1353,73	0,03	GIFHNS(-H <sub>2</sub> O)IWLER
Y12"	1468,72	1468,76	0,04	DGIFHNS(-H <sub>2</sub> O)IWLER
Intact	1604,72	1604,73	0,01	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLER

**Sequence: DGIFHNSIWLER, with intact Ser.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y2"	304,16	304,18	0,02	ER
Y3"	417,25	417,26	0,01	LER
Y4"	603,33	603,34	0,01	WLER
Y5"	716,41	716,42	0,01	IWLER
Y6"	803,44	-	-	SIWLER
Y7"	917,48	-	-	NSIWLER
Y8"	1054,54	1054,56	0,02	HNSIWLER
Y9"	1201,61	1201,62	0,01	FHNSIWLER
Y10"	1314,70	-	-	IFHNSIWLER
Y11"	1371,72	1371,76	0,04	GIFHNSIWLER
Y12"	1486,74	1486,79	0,05	DGIFHNSIWLER
Intact	1622,74	1622,73	-0,01	"CAF"DGIFHNSIWLER

**Sequence: VTGVDTD (cross-linked to DGIFHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1737,77*	1737,80	0,03	D
Y2"	1838,82	1838,81	-0,01	TD
Y3"	1953,85	1953,86	0,01	DTD
Y4"	2052,91	2052,91	0,00	VDTD
Y5"	2109,94	2109,93	-0,01	GVDTD
Y6"	2210,98	2210,98	0,00	TGVDTD
Y7"	2310,05	2310,07	0,02	VTGVDTD
Intact	2446,05	2446,05	0,00	"CAF"VTGVDTD

\* = TSG-6 peptide (1485,74) + CAF (136) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**Sequence: VTGVDTD (cross-linked to GIFHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1486,74*	1486,79	0,05	D
Y2"	1587,79	1587,85	0,06	TD
Y3"	1702,82	1702,84	0,02	DTD
Y4"	1801,88	1801,95	0,07	VDTD
Y5"	1858,91	1858,95	0,04	GVDTD
Y6"	1959,95	1960,00	0,05	TGVDTD
Y7"	2059,02	-	0,00	VTGVDTD
Intact	2195,02	2195,01	-0,01	"CAF"VTGVDTD

\* = TSG-6 peptide (1370,71) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**Sequence: VTGVDTD (cross-linked to FHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1316,63*	1316,68	0,05	D
Y2"	1417,68	1417,73	0,05	TD
Y3"	1532,70	1532,73	0,02	DTD
y4"	1631,77	-	-	VDTD
Y5"	1688,79	1688,86	0,07	GVDTD
Y6"	1789,83	1789,89	0,06	TGVDTD
Y7"	1888,91	-	-	VTGVDTD
Intact	2024,91	2024,93	0,02	"CAF"VTGVDTD

\* = TSG-6 peptide (1200,60) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup>(1,01)

**Sequence: VTGVDTD (cross-linked to HNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1169,57*	1169,61	0,04	D
Y2"	1270,62	1270,65	0,03	TD
Y3"	1385,64	1385,68	0,04	DTD
Y4"	1484,71	-	-	VDTD
Y5"	1541,73	1541,76	0,03	GVDTD
Y6"	1642,78	1642,85	0,07	TGVDTD
Y7"	1741,85	-	-	VTGVDTD
Intact	1877,85	1877,86	0,01	"CAF"VTGVDTD

\* = TSG-6 peptide (1053,54) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup>(1,01)

**Sequence: VTGVDTD (cross-linked to NSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1032,51*	1032,53	0,02	D
Y2"	1133,56	1133,59	0,03	TD
Y3"	1248,59	1248,61	0,02	DTD
Y4"	1347,66	-	-	VDTD
Y5"	1404,68	1404,70	0,02	GVDTD
Y6"	1505,73	1505,74	0,01	TGVDTD
Y7"	1604,80	-	-	VTGVDTD
Intact	1740,80	1740,83	0,03	"CAF"VTGVDTD

\* = TSG-6 peptide (916,48) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**Sequence: VTGVDTD (cross-linked to SIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	918,46*	918,49	0,03	D
Y2"	1019,52	1019,53	0,01	TD
Y3"	1134,55	1134,54	-0,01	DTD
Y4"	1233,62	-	-	VDTD
Y5"	1290,64	1290,67	0,03	GVDTD
Y6"	1391,69	1391,74	0,05	TGVDTD
Y7"	1490,76	-	-	VTGVDTD
Intact	1626,76	1626,77	0,01	"CAF"VTGVDTD

\* = TSG-6 peptide (802,43) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**B-ions:**

**Sequence: DGIFHNSIWLER, where Ser is dehydrated.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
B1"	252,04	-	-	"CAF"D
B2"	309,06	-	-	"CAF"DG
B3"	422,14	-	-	"CAF"DGI
B4"	569,21	-	-	"CAF"DGIF
B5"	706,27	-	-	"CAF"DGIFH
B6"	820,31	830,30	-0,01	"CAF"DGIFHN
B7"	889,32	889,34	0,02	"CAF"DGIFHNS(-H <sub>2</sub> O)
B8"	1002,41	1002,41	0,00	"CAF"DGIFHNS(-H <sub>2</sub> O)I
B9"	1188,49	-	-	"CAF"DGIFHNS(-H <sub>2</sub> O)IW
B10"	1301,57	-	-	"CAF"DGIFHNS(-H <sub>2</sub> O)IWL
B11"	1430,61	-	-	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLE
Intact	1604,72	1604,73	0,01	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLER

TABLE S2

*Analysis of fragment ions from MS/MS of CAF modified HC2•TSG-6 cross-link*

The purified HC2•TSG-6 cross-link (Fig. 3A, peak  $\gamma$ ) was modified using the CAF derivatization protocol and subjected to MS/MS analysis (Fig. 4C-D). The resulting fragment ions were subsequently examined. The CAF protocol favors Y-ions and only a single “weak” B-ion series was detected. The detected fragment ion series correlate with an ester bond between the internal Ser residue in the TSG-6 peptide and the terminal Asp residue of HC2.

**Y-ions:****Sequence: DGIFHNSIWLER, where Ser is dehydrated.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	175,13	175,14	0,01	R
Y2"	304,16	304,17	0,01	ER
Y3"	417,25	417,24	-0,01	LER
Y4"	603,33	603,32	-0,01	WLER
Y5"	716,41	716,40	-0,01	IWLER
Y6"	785,42	785,42	0,00	S(-H <sub>2</sub> O)IWLER
Y7"	899,46	899,47	0,01	NS(-H <sub>2</sub> O)IWLER
Y8"	1036,52	1036,51	-0,01	HNS(-H <sub>2</sub> O)IWLER
Y9"	1183,59	1183,58	-0,01	FHNS(-H <sub>2</sub> O)IWLER
Y10"	1296,68	-	-	IFHNS(-H <sub>2</sub> O)IWLER
Y11"	1353,70	1353,70	0,00	GIFHNS(-H <sub>2</sub> O)IWLER
Y12"	1468,72	1468,70	-0,02	DGIFHNS(-H <sub>2</sub> O)IWLER
Intact	1604,72	1604,69	-0,03	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLER

**Sequence: DGIFHNSIWLER, with intact Ser.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y2"	304,16	304,17	0,01	ER
Y3"	417,25	417,24	-0,01	LER
Y4"	603,33	603,32	-0,01	WLER
Y5"	716,41	716,40	-0,01	IWLER
Y6"	803,44	803,43	-0,01	SIWLER
Y7"	917,48	-	-	NSIWLER
Y8"	1054,54	1054,53	-0,01	HNSIWLER
Y9"	1201,61	1201,59	-0,02	FHNSIWLER
Y10"	1314,70	-	-	IFHNSIWLER
Y11"	1371,72	1371,70	-0,02	GIFHNSIWLER
Y12"	1486,74	1486,73	-0,01	DGIFHNSIWLER
Intact	1622,74	1622,70	-0,04	"CAF"DGIFHNSIWLER

**Sequence: VEND (cross-linked to DGIFHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1737,77*	1737,72	-0,05	D
Y2"	1851,81	1851,78	-0,03	ND
Y3"	1980,86	1980,80	-0,06	END
Y4"	2079,92	2079,88	-0,04	VEND
Intact	2215,92	2215,86	-0,06	"CAF"VEND

\* = TSG-6 peptide (1485,74) + CAF (136) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**Sequence: VEND (cross-linked to GIFHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1486,74*	1486,73	-0,01	D
Y2"	1600,78	1600,75	-0,03	ND
Y3"	1729,83	1729,82	-0,01	END
Y4"	1828,89	1828,89	0,00	VEND
Intact	1964,89	1964,87	-0,02	"CAF"VEND

\* = TSG-6 peptide (1370,71) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)

**Sequence: VEND (cross-linked to FHNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1316,63*	1316,62	-0,01	D
Y2"	1430,67	1430,65	-0,02	ND
Y3"	1559,72	1559,71	-0,01	END
y4"	1658,78	1658,76	-0,02	VEND
Intact	1794,78	1794,75	-0,03	"CAF"VEND

\* = TSG-6 peptide (1200,60) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup>(1,01)

**Sequence: VEND (cross-linked to HNSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1169,57*	1169,56	-0,02	D
Y2"	1283,61	1283,60	-0,01	ND
Y3"	1412,66	1412,63	-0,03	END
Y4"	1511,72	1511,71	-0,01	VEND
Intact	1647,72	1647,68	-0,04	"CAF"VEND

\* = TSG-6 peptide (1053,54) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup>(1,01)

**Sequence: VEND (cross-linked to NSIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	1032,51*	1032,49	-0,02	D
Y2"	1146,55	1146,54	-0,01	ND
Y3"	1275,60	1275,58	-0,02	END
Y4"	1374,66	1374,63	-0,03	VEND
Intact	1510,66	1510,63	-0,03	"CAF"VEND

\* = TSG-6 peptide (916,48) + Asp (133,04) - H<sub>2</sub>O (18,02) + H<sup>+</sup> (1,01)



**Sequence: VEND (cross-linked to SIWLER)**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
Y1"	918,46*	918,45	-0,01	D
Y2"	1032,50	1032,49	-0,01	ND
Y3"	1161,55	1161,55	0,00	END
Y4"	1260,61	1260,61	0,00	VEND
Intact	1396,61	-	-	"CAF"VEND

\* = TSG-6 peptide (802,43) + Asp (133,04) - H2O (18,02) + H+ (1,01)

**B-ions:**

**Sequence: DGIFHNSIWLER, where Ser is dehydrated.**

Ion	MH+		$\Delta$ MH+	Peptide
	Theoretical	Observed		
B1"	252,04	-	-	"CAF"D
B2"	309,06	-	-	"CAF"DG
B3"	422,14	-	-	"CAF"DGI
B4"	569,21	-	-	"CAF"DGIF
B5"	706,27	-	-	"CAF"DGIFH
B6"	820,31	820,28	-0,03	"CAF"DGIFHN
B7"	889,32	889,31	-0,01	"CAF"DGIFHNS(-H <sub>2</sub> O)
B8"	1002,41	1002,39	-0,02	"CAF"DGIFHNS(-H <sub>2</sub> O)I
B9"	1188,49	-	-	"CAF"DGIFHNS(-H <sub>2</sub> O)IW
B10"	1301,57	-	-	"CAF"DGIFHNS(-H <sub>2</sub> O)IWL
B11"	1430,61	1430,65	0,04	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLE
Intact	1604,72	1604,69	-0,03	"CAF"DGIFHNS(-H <sub>2</sub> O)IWLER

TABLE S3

*NH<sub>2</sub>-terminal protein sequencing of the purified HC•TSG-6 cross-links and unreacted TSG-6*The cross-linked HC1 and TSG-6 peptides (peak  $\alpha$ ):

Position	22	23	24	25	26	27	<b>28</b>	29	30	31	32	33
TSG-6, residue:	D	G	I	F	H	N	<b>S</b>	I	W	L	E	R
Amount (pmol):	25.1	14.6	7.0	9.0	3.8	5.9	-	1.6	-	1.3	0.9	-
% of initial yield	100	58.2	27.9	35.9	15.1	23.5	-	6.4	-	5.2	3.6	-

Position	632	633	634	635	636	637	638
HC1, residue:	V	T	G	V	D	T	D
Amount (pmol):	25.5	12.0	18.8	8.5	11.7	3.2	-
% of initial yield	100	47.1	73.7	33.3	45.9	12.5	-

The cross-linked HC2 and TSG-6 peptides (peak  $\gamma$ ):

Position	22	23	24	25	26	27	<b>28</b>	29	30	31	32	33
TSG-6, residue:	D	G	I	F	H	N	<b>S</b>	I	W	L	E	R
Amount (pmol):	15.9	17.1	9.5	9.4	3.5	6.2	-	1.7	-	1.2	1.1	-
% of initial yield	100	108	59.7	59.1	22.0	39.0	-	10.7	-	7.5	6.9	-

Position	645	646	647	648
HC2, residue:	V	E	N	D
Amount (pmol):	34.5	23.6	16.1	-
% of initial yield	100	68.4	46.7	-

## TSG-6 (unreacted):

Position	18	19	20	21	22	23	24	25	26	27	<b>28</b>	29
Residue:	W	G	F	K	D	G	I	F	H	N	<b>S</b>	I
Amount (pmol):	-	36.0	31.8	7.7	26.5	23.1	15.7	17.3	4.2	7.8	<b>7.2</b>	8.1
% of initial yield	-	100	88.3	21.4	73.6	64.2	43.6	48.1	11.7	21.7	<b>20.0</b>	22.5

Position	30	31	32	33	34	35	36
Residue:	W	L	E	R	A	A	G
Amount (pmol):	-	7.1	5.0	-	9.6	10.8	9.2
% of initial yield	-	19.7	13.9	-	26.7	30.0	25.6

TABLE S4

*LC-MS/MS analyses of TSG-6 expressed in mammalian cells.*

Concentrated medium from mammalian cells transfected with respectively wild type TSG-6 cDNA, S28A mutant TSG-6 cDNA, and extracellular superoxide dismutase cDNA (control) were resolved by SDS-PAGE. Bands of interest were excised, digested with trypsin and analyzed by LC-MS/MS. Several proteins were positively identified by the Mascot search engine in each of the three analyzed media samples. TSG-6 was identified in medium from both the transfection with wild type cDNA and from the transfection with mutant cDNA, but not in the medium from the control transfection. The identification of TSG-6 is shown below. The data demonstrates that both wild type and mutant TSG-6 have been expressed. *Mr (Expt)*, the experimental derived relative molecular mass. *Mr (Calc)*, calculated relative molecular mass.

Expression of wild type TSG-6:

Protein score: 260

MS/MS spectrum number: 45

Matched MS/MS spectrum: 6

Sequence coverage: 23 %

Peptides	Mr (Expt)	Mr (Calc)	$\Delta$ Mr	Peptide score
LTYAEAK	794.33	794.42	-0.09	25
TGIIDYGIR	1006.42	1006.54	-0.12	55
ECGGVFTDPK	1122.37	1122.50	-0.13	38
WDAYCYNPHAK	1437.42	1437.61	-0.19	24
DGIFHNSIWLER	1485.56	1485.74	-0.18	48
FLSDASVTAGGFQIK	1539.60	1539.79	-0.20	70

Expression of mutant S28A TSG-6:

Protein score: 103

MS/MS spectrum number: 38

Matched MS/MS spectrum: 3

Sequence coverage: 10 %

Peptides	Mr (Expt)	Mr (Calc)	$\Delta$ Mr	Peptide score
TGIIDYGIR	1006.44	1006.54	-0.10	54
YVAMDPVSK	1008.39	1008.49	-0.10	17
ECGGVFTDPK	1122.39	1122.50	-0.12	32