Supplementary Information

Inosine induces context-dependent recoding and translational stalling

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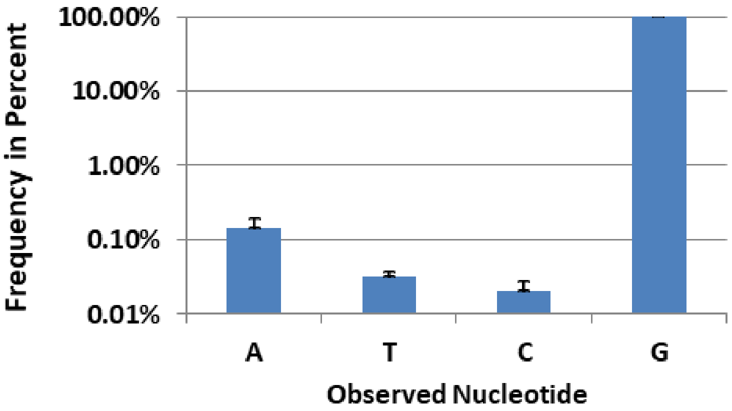


Supplementary Figure S1. The fidelity of *in vitro* transcription with inosine is very high. Using RT-PCR and amplicon-seq the rate of mis-incorporation of other nucleotides instead of inosine was tested. The frequency of all nucleotides detected at positions with inosine is given. n=11, error bars = s.e.m.

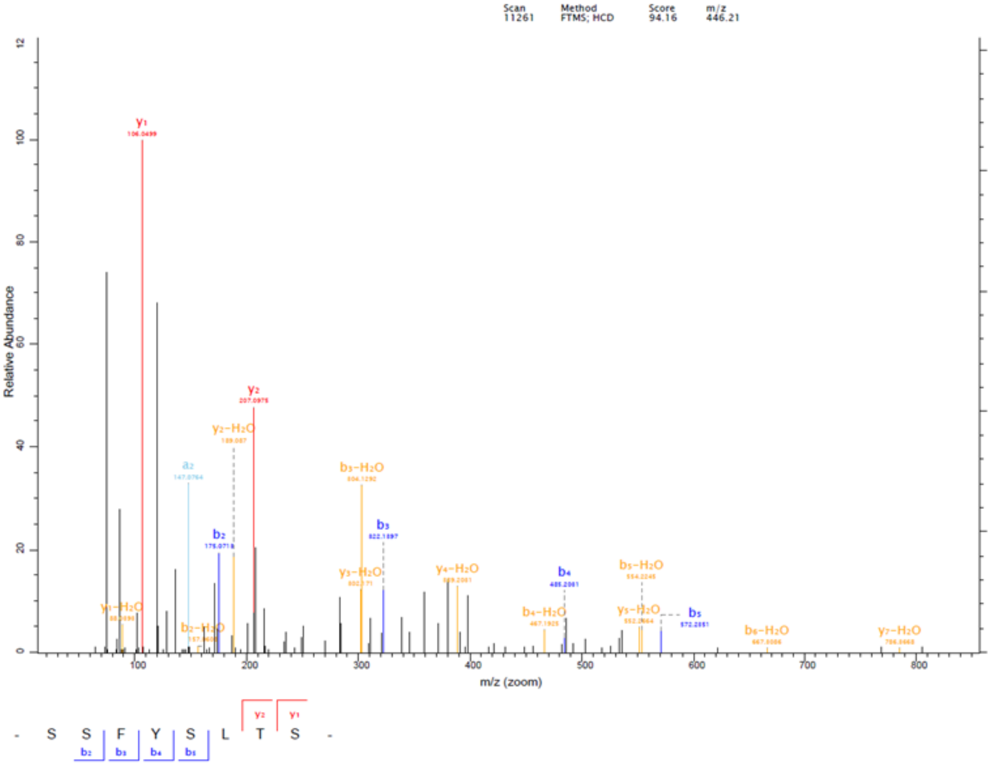
Supplementary Table S1. All measured peak intensities for the detected peptides after *in vitro* translation, affinity purification, and mass spectrometry of inosine-containing codons. Sample#: number of sample, reflects also the order of measurement. Codon: inosine-containing codon. Decoding/Interpretation: Codon (either A, U, G), truncation (truncated peptide), STOP (Stop-codon), carryover (in 3 samples a peptide from the previous run was detected. For these three samples we have analyzed the wash runs between samples and could detect the peptide-signal with decreasing intensities, strongly arguing for carryover). Peptide: peptides detected. Summed peak intensities (log2) using Skyline (the summed intensities of all fragments that could be assigned to the respective peptide, log2).

Sample#	Codon	Decoding/ Interpretation	Peptide	Summed peak intensities (log2) using Skyline	Remarks
1	AIC	AGC	SSFYSLTS**S**SNISK	32.34	
2	CIA	CGA	SSFYSLTS**R**SNISK	32.26	
3	CII	CGG	SSFYSLTS**R**SNISK	34.49	
	CII	truncation	SSFYSLTS*****	30.88	
4	UIA	STOP	SSFYSLTS*****	29.2	
5	IUA	GUA	SSFYSLTS**V**SNISK	33.62	
	IUA	truncation	SSFYSLTS*****	29.49	
6	IAA	GAA	SSFYSLTS**E**SNISK	33.16	
	IAA	AAA	SSFYSLTS**K**	27.33	
	IAA	truncation	SSFYSLTS*****	29.01	
7	ICC	GCC	SSFYSLTS**A**SNISK	33.24	
	ICC	ACC	SSFYSLTS**T**SNISK	25.39	
	ICC	truncation	SSFYSLTS*****	28.06	
8	IAU	GAU	SSFYSLTS**D**SNISK	34.23	
	IAU	truncation	SSFYSLTS*****	29.09	
9	ICU	GCU	SSFYSLTS**A**SNISK	33.78	
	ICU	ACU	SSFYSLTS**T**SNISK	24.54	
	ICU	truncation	SSFYSLTS*****	28.44	
10	AII	AGG	SSFYSLTS**R**SNISK	33.97	

	AII	truncation	SSFYSLTS*	29.5	
11	IUU	GUU	SSFYSLTSVSNISK	33.44	
	IUU	truncation	SSFYSLTS*	28.02	
12	IAC	GAC	SSFYSLTSDSNISK	32.93	
	IAC	AAC	SSFYSLTSNSNISK	31.19	
	IAC	truncation	SSFYSLTS*	27.08	
13	UAI	STOP	SSFYSLTS*	29.63	
	UAI	UAU	SSFYSLTSYSNISK	23.73	
14	UII	UGG	SSFYSLTSWSNISK	27.8	
	UII	truncation	SSFYSLTS*	28.23	
15	IUI	GUG	SSFYSLTSVSNISK	31.57	
	IUI	truncation	SSFYSLTS*	30.05	
16	AAI	AAG	SSFYSLTSK	35.87	
	AAI	truncation	SSFYSLTS*	27.83	
17	CIC	CGC	SSFYSLTSRSNISK	33.26	
	CIC	truncation	SSFYSLTS*	28.72	
	CIC	truncation	SSFYSLTSR	29.13	
	CIC	*carryover*	SSFYSLTSK	25.73	carryover from sample 16
18	AIU	AGU	SSFYSLTSSSNISK	32.57	
	AIU	truncation	SSFYSLTS*	27.65	
	AIU	*carryover*	SSFYSLTSK	23.73	carryover from sample 16
19	IUC	GUC	SSFYSLTSVSNISK	31.53	
	IUC	truncation	SSFYSLTS*	26.35	
20	ICA	GCA	SSFYSLTSASNISK	31.91	
	ICA	ACA	SSFYSLTSTSNISK	25.97	
	ICA	UCA	SSFYSLTSSSNISK	25.03	
	ICA	truncation	SSFYSLTS*	27.16	
21	IIU	GGU	SSFYSLTSGSNISK	31.19	
	IIU	truncation	SSFYSLTS*	26.71	
22	IAI	GAG	SSFYSLTSESNISK	29.56	
	IAI	AAG/AAA	SSFYSLTSK	26.03	
	IAI	truncation	SSFYSLTS*	32.08	

23	ICI	GCG	SSFYSLTS**A**SNISK	30.62	
	ICI	UCG	SSFYSLTS**S**SNISK	24.11	
	ICI	truncation	SSFYSLTS*	31.46	
24	UIC	UGC	SSFYSLTS**C**SNISK	31.84	
	UIC	truncation	SSFYSLTS*	27.63	
25	UIU	UGU	SSFYSLTS**C**SNISK	30.23	
	UIU	truncation	SSFYSLTS*	26.35	
26	CAI	CAG	SSFYSLTS**Q**SNISK	32.37	
	CAI	truncation	SSFYSLTS*	27.36	
27	IIC	GGC	SSFYSLTS**G**SNISK	32.22	
	IIC	truncation	SSFYSLTS*	26.36	
	IIC	*carryover*	SSFYSLTS**Q**SNISK	22.93	carryover from sample 26
28	III	GGG	SSFYSLTS**G**SNISK	33.08	
	III	truncation	SSFYSLTS*	32.25	
29	CIU	CGU	SSFYSLTS**R**SNISK	30.99	
	CIU	truncation	SSFYSLTS*	25.73	
30	AIA	AGA	SSFYSLTS**R**SNISK	29.39	
	AIA	truncation	SSFYSLTS*	25.73	
31	AUI	AUG	SSFYSLTS**M**SNISK	31.83	
	AUI	truncation	SSFYSLTS*	27	
32	IIA	GGA	SSFYSLTS**G**SNISK	32.17	
	IIA	AGA	SSFYSLTS**R**SNISK	26.51	
	IIA	truncation	SSFYSLTS*	28.41	
33	UUI	UUG	SSFYSLTS**L**SNISK	26.8	
	UUI	truncation	SSFYSLTS*	26.72	

Supplementary Dataset S1. Representative spectra for all detected peptides. Spectra (taken from MaxQuant) are shown. If the same peptide occurred in multiple samples, one spectra was randomly chosen.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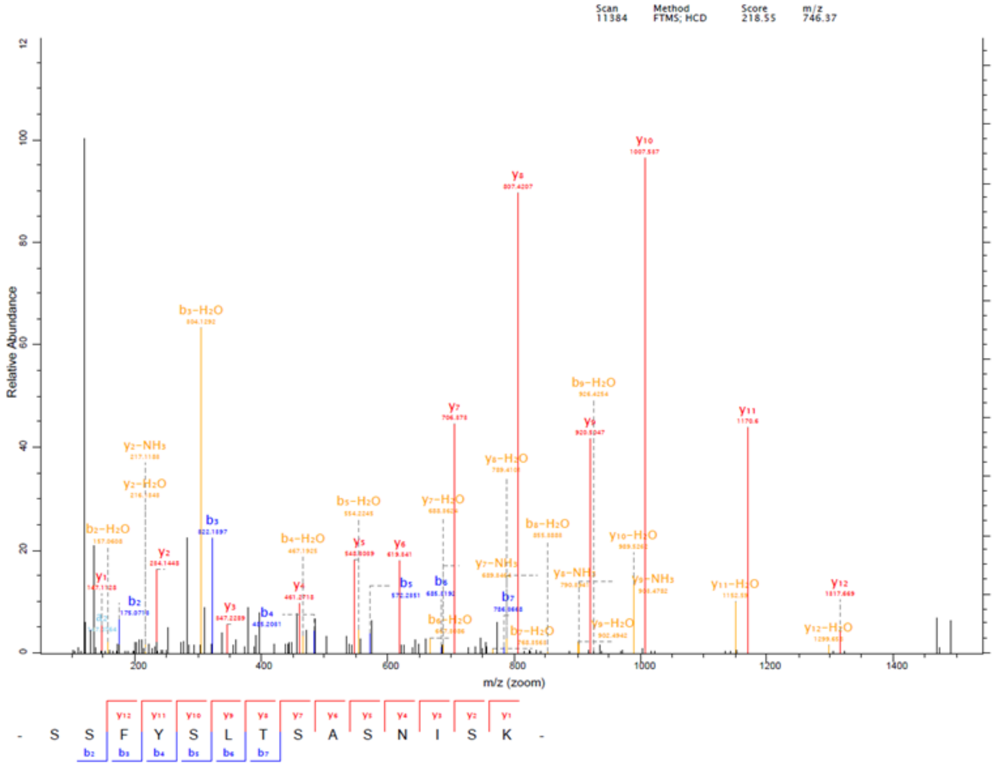
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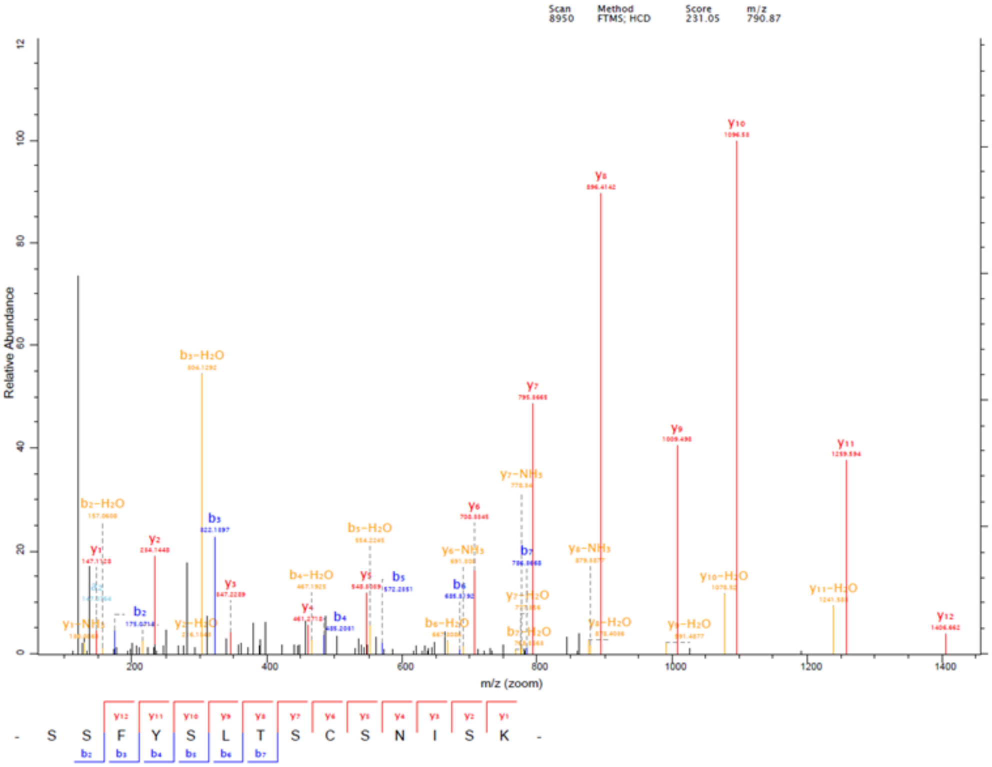
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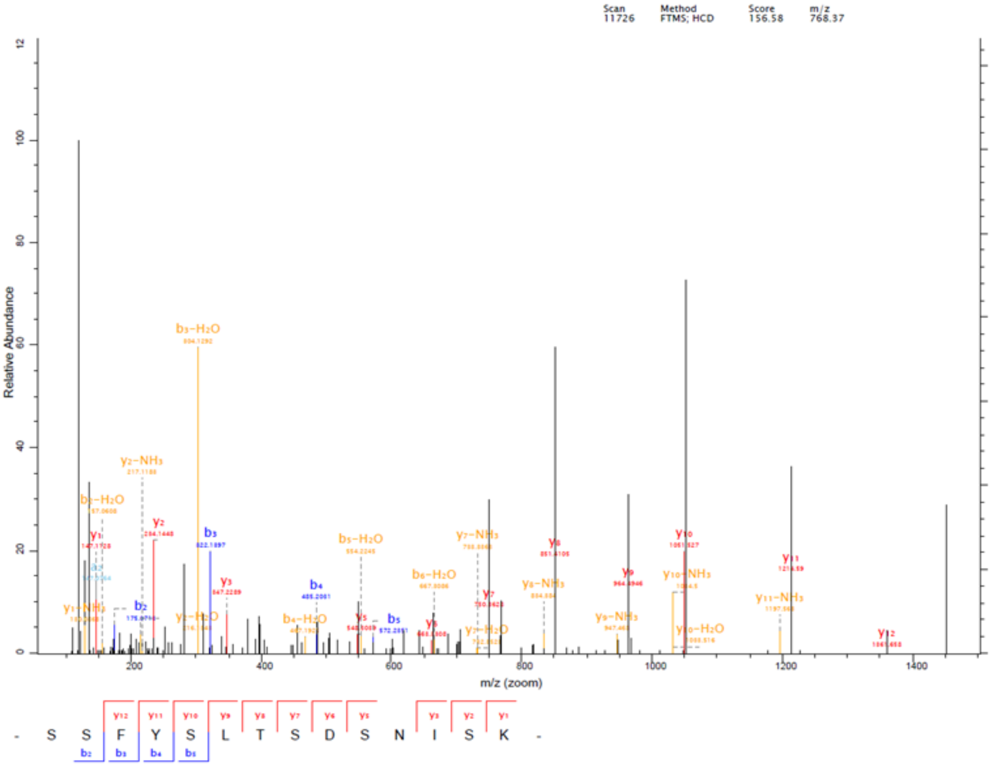
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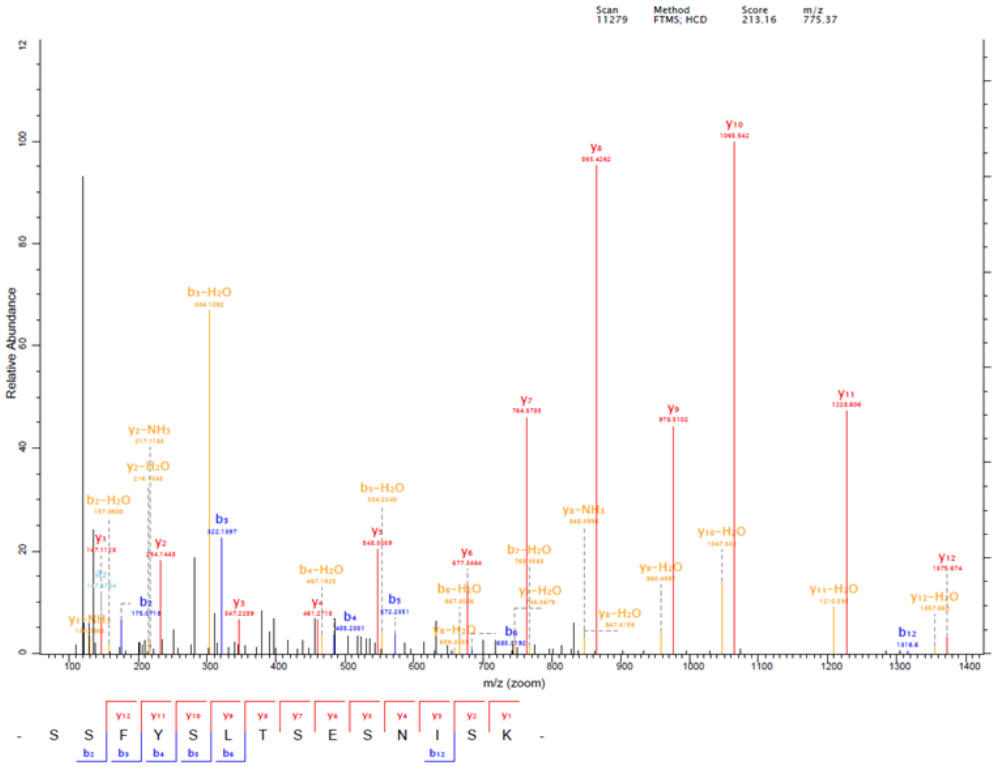
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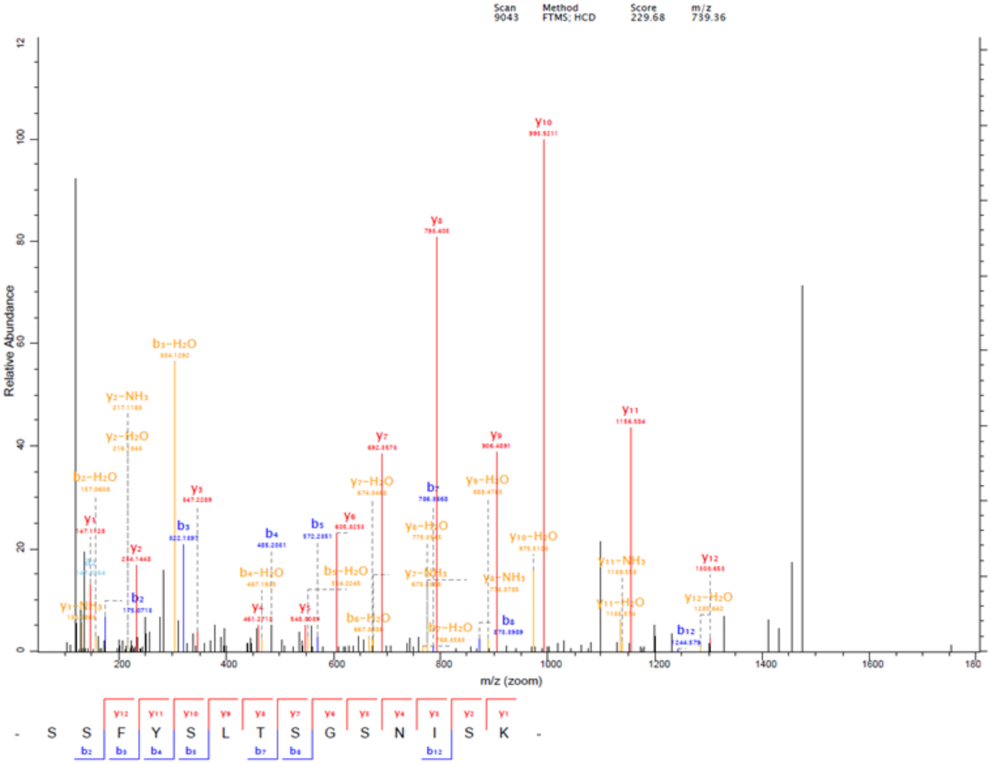
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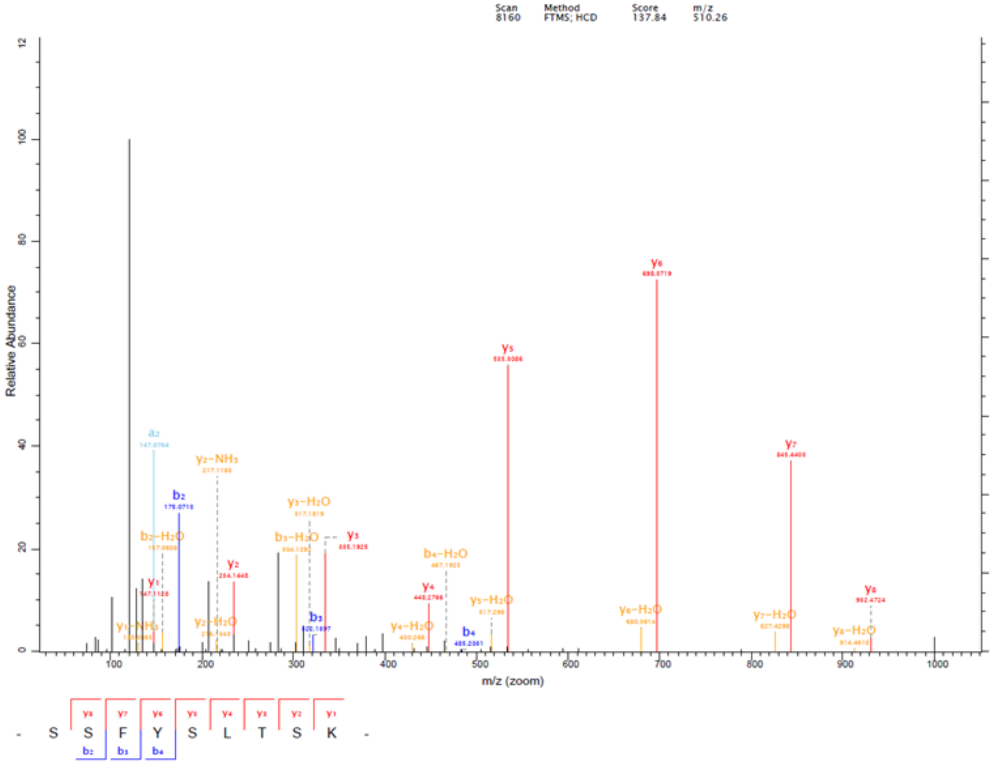
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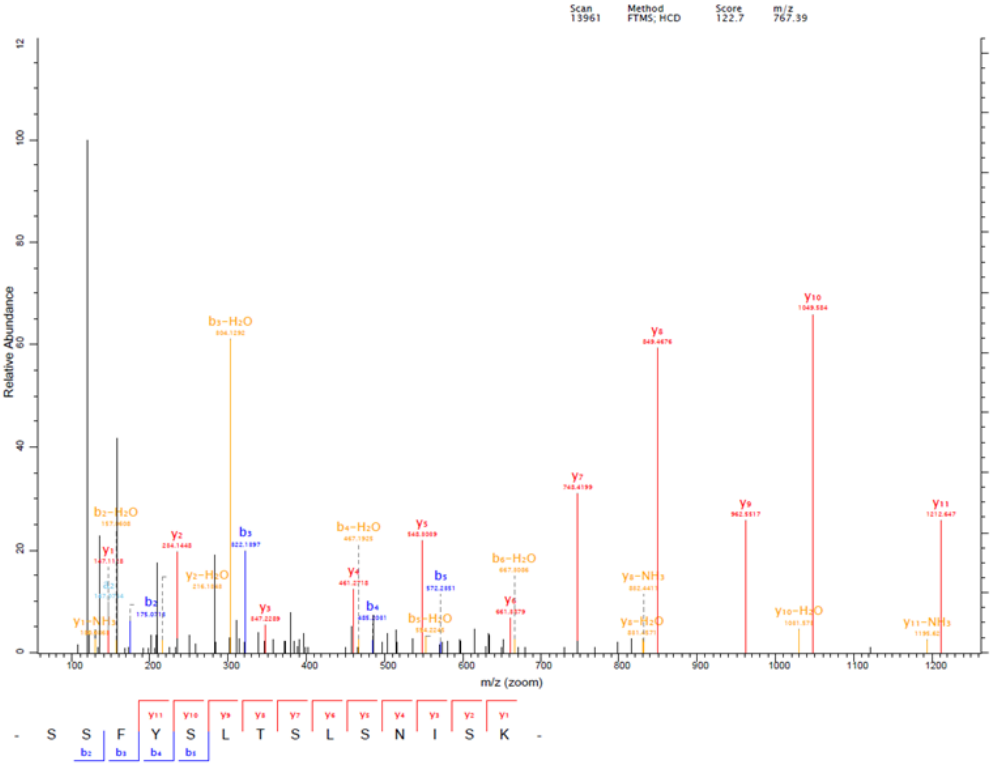
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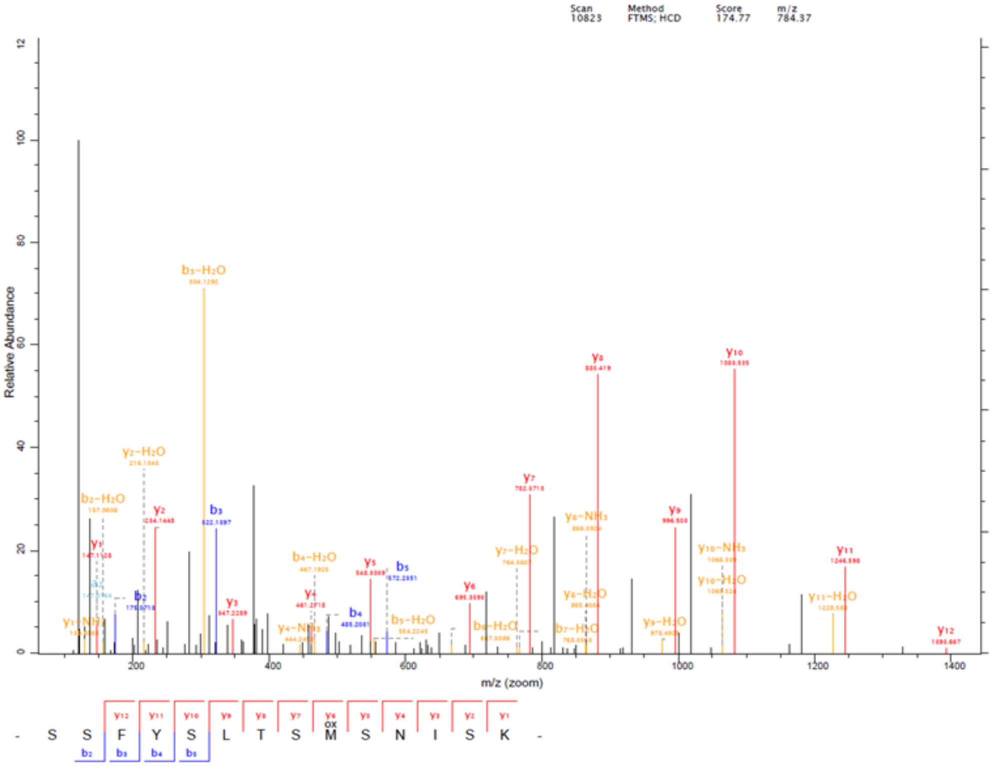
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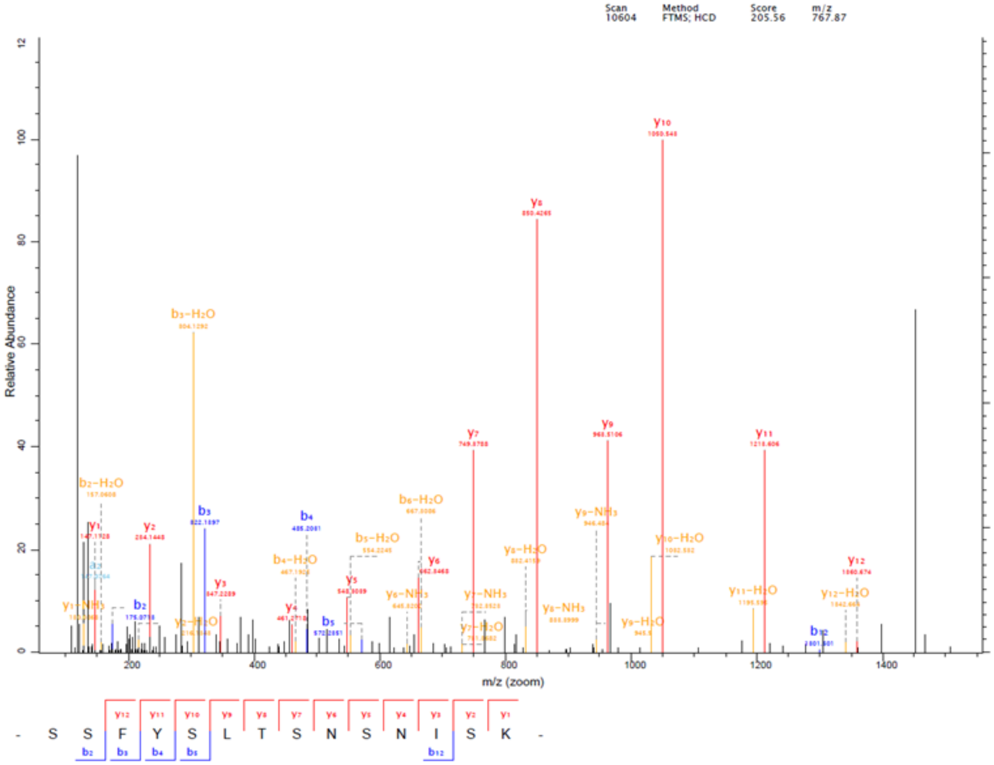
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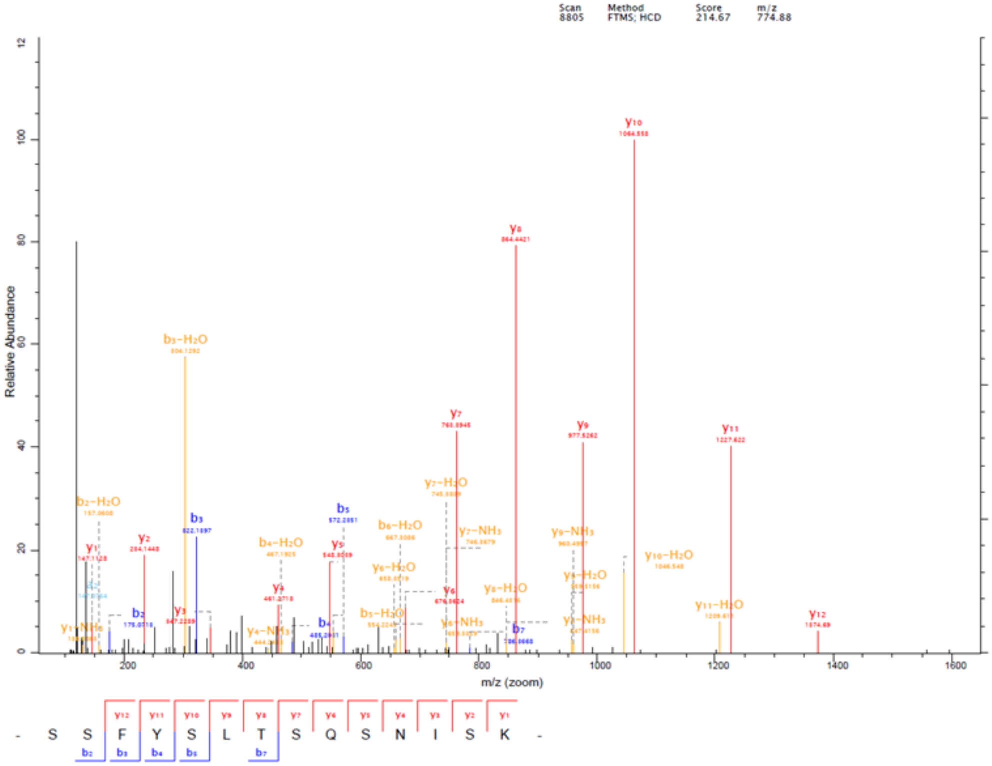
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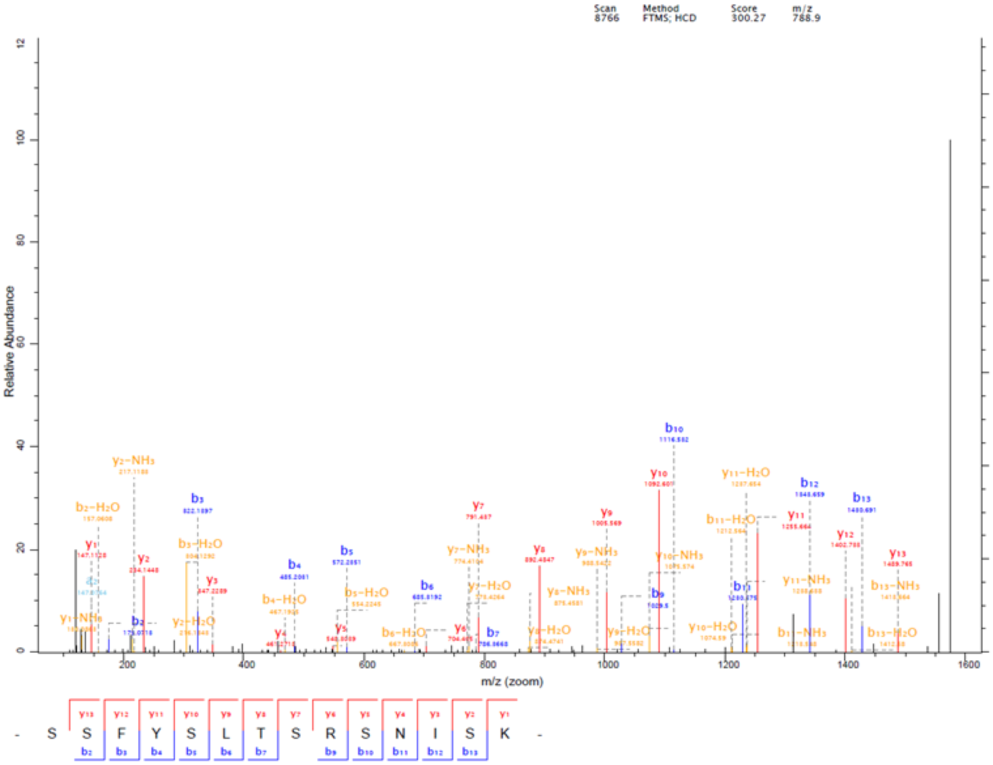
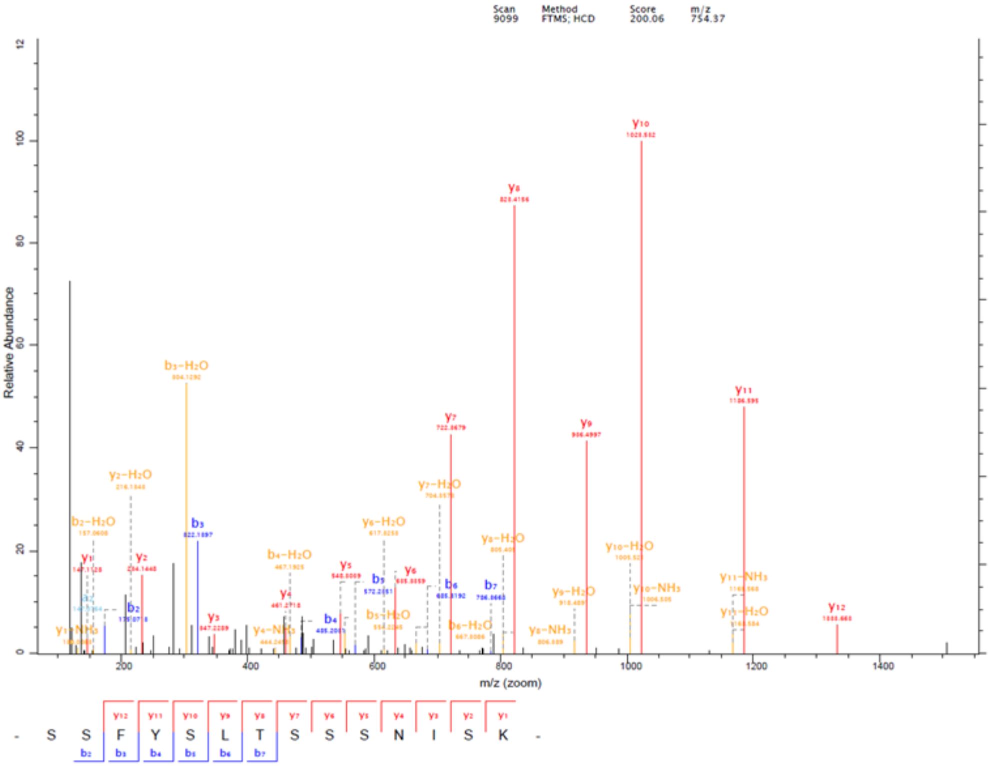
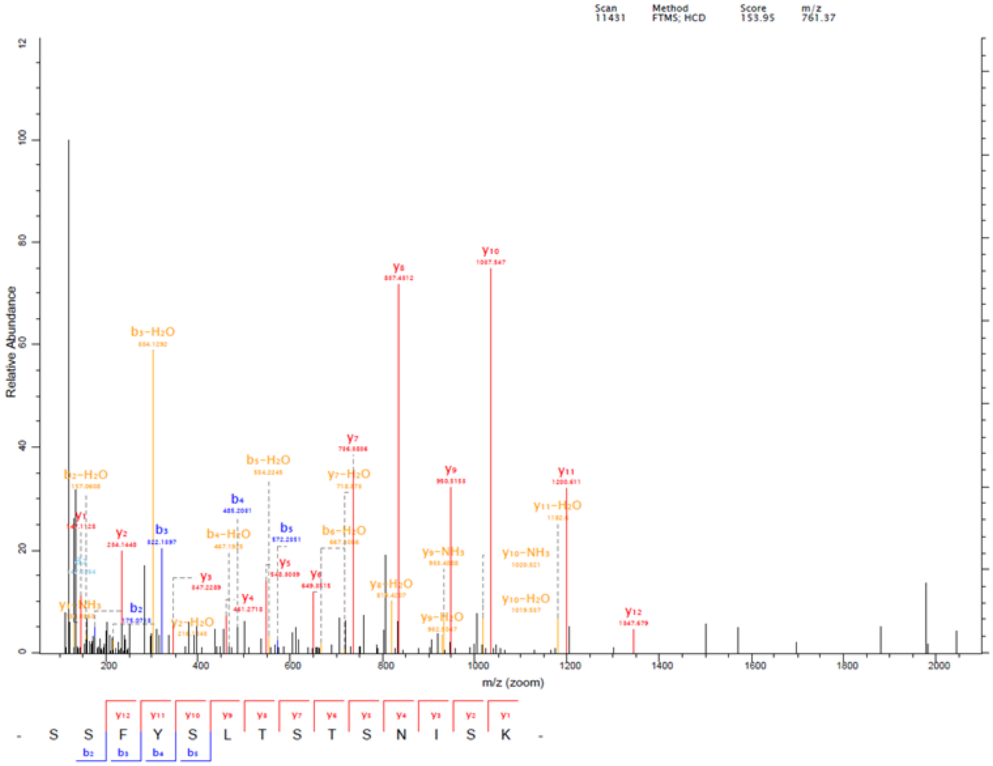
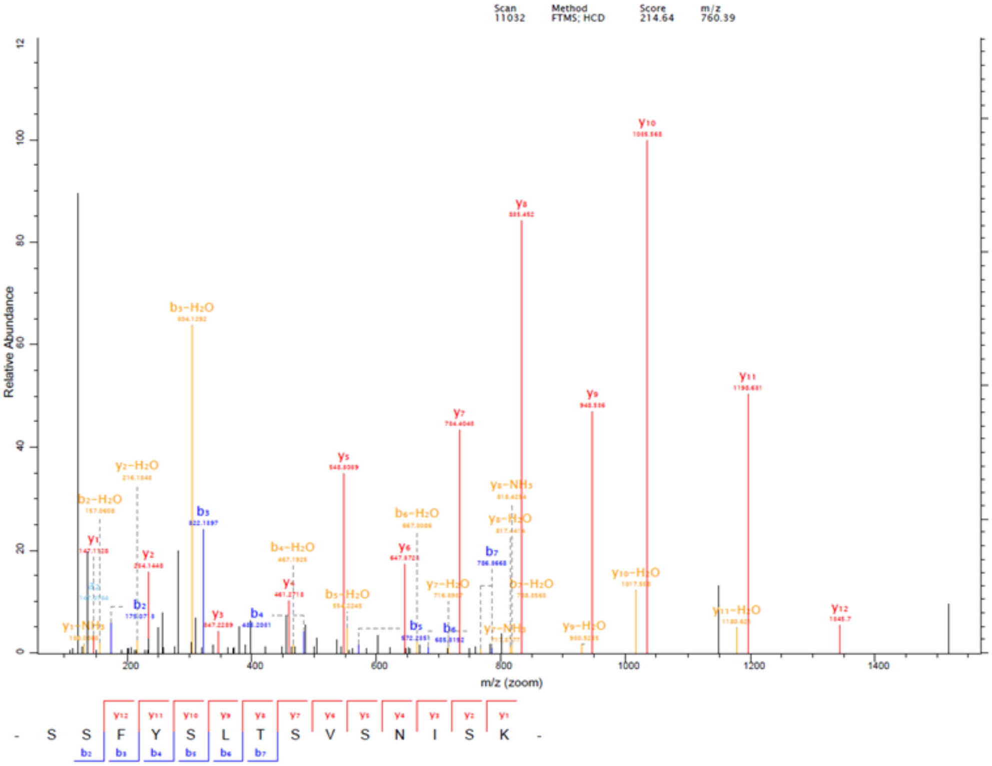
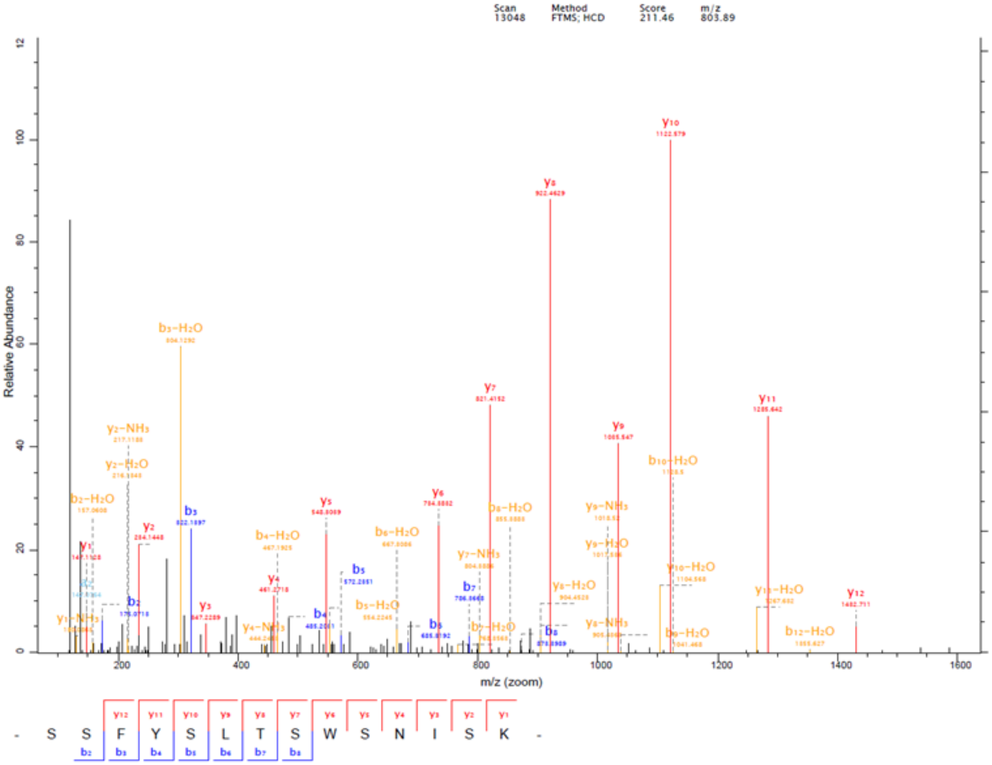
1600
y10
1064.555
y8
864.4421
b3-H2O
304.1292
y7
763.3945
y9
977.5262
y11
1227.622
y12
1374.69
b2-H2O
157.0606
b3
b5
y7-H2O
b4-H2O
b6-H2O
y7-NH3
y9-NH3
y2
254.1446
y1
147.1128
y5
y6-H2O
y10-H2O
y4
461.2718
y6
676.3624
y8-H2O
y9-H2O
b2
175.0718
y3
b5-H2O
y11-H2O
y1-NH3
y4-NH3
b4
y6-NH3
b7
y8-NH3
- S S F Y S L T S Q S N I S K -
y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
b2 b3 b4 b5 b7

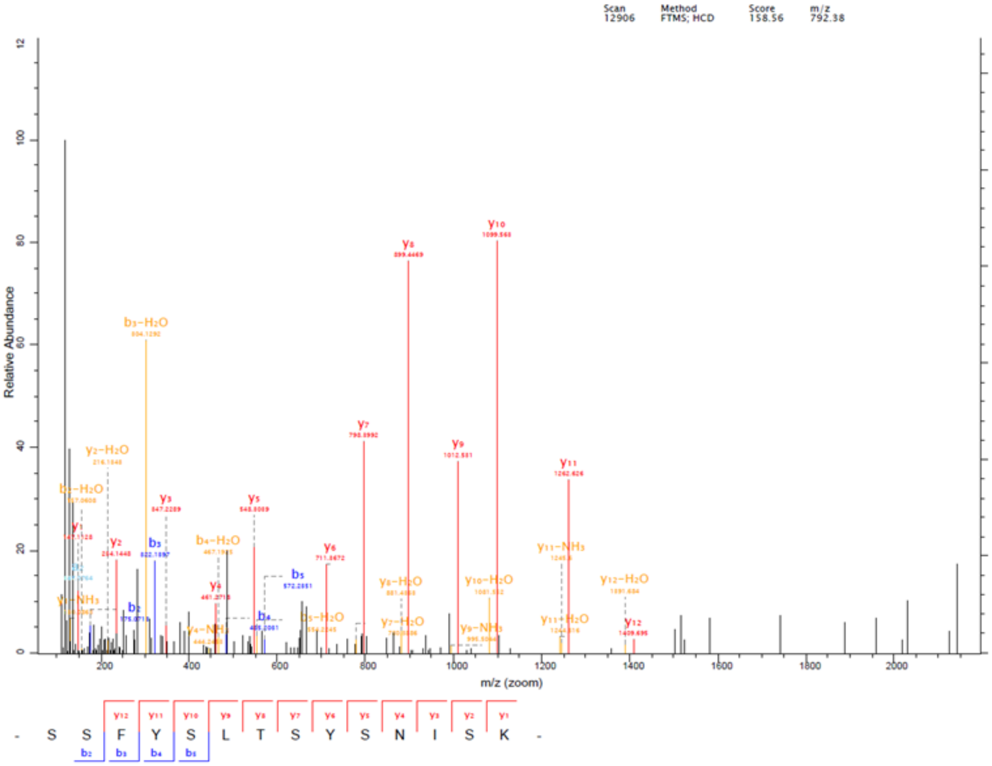

Scan 8766
Method FTMS; HCD
Score 300.27
m/z 788.9
Relative Abundance
0
20
40
60
80
100
12
m/z (zoom)
200
400
600
800
1000
1200
1400
1600
y1 147.1128
y1-NH3
b2-H2O 157.0608
y2-NH3 217.1180
y2 234.1448
b2 175.0718
b3 322.1897
b3-H2O
y2-H2O
y3 347.2289
b4 485.2081
b4-H2O 467.1935
y4
b5 572.2851
b5-H2O 554.2245
y5
b6 685.8192
b6-H2O 667.0085
y6
b7 786.3665
y7 791.457
y7-NH3 774.4104
y7-H2O 773.4264
y8 892.4847
y8-NH3 875.4581
y8-H2O 874.4741
y9 1005.569
y9-NH3 988.5422
y9-H2O 987.5582
b9 1029.5
y10 1092.607
y10-NH3 1075.574
y10-H2O 1074.59
b10 1116.582
b11-H2O 1212.564
y11-H2O 1237.654
y11 1255.664
y11-NH3 1238.638
b11
b11-NH3
b12 1343.659
y12 1402.738
b13 1430.691
b13-NH3 1413.664
b13-H2O 1412.68
y13 1489.765
- S S F Y S L T S R S N I S K -
y13 y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
b2 b3 b4 b5 b6 b7 b9 b10 b11 b12 b13


Scan
9099
Method
FTMS; HCD
Score
200.06
m/z
754.37
Relative Abundance
0
20
40
60
80
100
12
m/z (zoom)
200
400
600
800
1000
1200
1400
y₁
y₂
y₃
y₄
y₅
y₆
y₇
y₈
y₉
y₁₀
y₁₁
y₁₂
b₂
b₃
b₄
b₅
b₆
b₇
a₂
b₂-H₂O
b₃-H₂O
b₄-H₂O
b₅-H₂O
b₆-H₂O
y₁-NH₃
y₂-H₂O
y₄-NH₃
y₆-H₂O
y₇-H₂O
y₈-H₂O
y₈-NH₃
y₉-H₂O
y₁₀-H₂O
y₁₀-NH₃
y₁₁-NH₃
y₁₁-H₂O
- S S F Y S L T S S S N I S K -


Scan
11431
Method
FTMS; HCD
Score
153.95
m/z
761.37
Relative Abundance
0
20
40
60
80
100
12
m/z (zoom)
200
400
600
800
1000
1200
1400
1600
1800
2000
b2-H2O
b3-H2O
304.1292
y1
y1-NH3
y2
b2
b3
322.1397
y2-H2O
y3
b4
485.2081
b4-H2O
y4
b5-H2O
b5
572.2351
y5
y6
b6-H2O
y7
y7-H2O
y8
y8-H2O
y9
y9-NH3
y9-H2O
y10
y10-NH3
y10-H2O
y11
1200.611
y11-H2O
y12
1347.679
y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
- S S F Y S L T S T S N I S K -
b2 b3 b4 b5


Scan
11032
Method
FTMS; HCD
Score
214.64
m/z
760.39
Relative Abundance
0
20
40
60
80
100
12
m/z (zoom)
200
400
600
800
1000
1200
1400
y1-NH3
y1
147.1128
b2-H2O
157.0608
y2-H2O
216.1040
y2
234.1448
b2
175.0718
b3-H2O
304.1292
b3
322.1397
y3
347.2289
b4
485.2061
b4-H2O
467.1925
y4
461.2718
y5
548.3089
b5-H2O
554.2245
b5
572.2351
y6
647.3723
b6-H2O
667.3086
b6
685.3192
y7-H2O
716.3907
y7
734.4048
y7-NH3
b7
786.3668
b7-H2O
768.3568
y8-NH3
818.4284
y8-H2O
817.4419
y8
835.452
y9-H2O
930.5235
y9
948.536
y10-H2O
1017.568
y10
1035.568
y11-H2O
1180.621
y11
1198.631
y12
1345.7
y12 y11 y10 y9 y8 y7 y6 y5 y4 y3 y2 y1
- S S F Y S L T S V S N I S K -
b2 b3 b4 b5 b6 b7


Scan
13048
Method
FTMS; HCD
Score
211.46
m/z
803.89
Relative Abundance
0
20
40
60
80
100
120
m/z (zoom)
200
400
600
800
1000
1200
1400
1600
y₁ 147.1128
y₁-NH₃
b₂-H₂O 157.0608
b₂ 175.0718
y₂-NH₃ 217.1188
y₂-H₂O 216.1348
y₂ 234.1448
b₃-H₂O 304.1292
b₃ 322.1397
y₃ 347.2289
y₄-NH₃
y₄ 461.2718
b₄-H₂O 467.1925
b₄ 485.2031
y₅ 548.3089
b₅-H₂O 554.2245
b₅ 572.2351
b₆-H₂O 667.3086
b₆ 685.3192
y₆ 734.3882
b₇
y₇-NH₃
y₇ 821.4152
b₇-H₂O
b₈-H₂O
b₈
y₈-H₂O 904.4523
y₈ 922.4629
y₈-NH₃
y₉-NH₃
y₉-H₂O
y₉ 1035.547
b₉-H₂O 1041.468
y₁₀-H₂O 1104.568
b₁₀-H₂O 1128.5
y₁₀ 1122.579
y₁₁-H₂O 1267.632
y₁₁ 1285.642
b₁₂-H₂O
y₁₂ 1432.711
- S S F Y S L T S W S N I S K -
y₁₂ y₁₁ y₁₀ y₉ y₈ y₇ y₆ y₅ y₄ y₃ y₂ y₁
b₂ b₃ b₄ b₅ b₆ b₇ b₈


Scan
12906
Method
FTMS; HCD
Score
158.56
m/z
792.38
Relative Abundance
0
20
40
60
80
100
12
m/z (zoom)
200
400
600
800
1000
1200
1400
1600
1800
2000
y10
1099.568
y8
899.4469
b3-H2O
304.1292
y7
798.0992
y9
1012.581
y11
1262.626
y2-H2O
216.1048
b2-H2O
157.0608
y3
347.2289
y5
548.3069
y1
147.1128
y2
234.1448
b3
322.1397
b4-H2O
467.1915
y6
711.3672
b5
572.2051
y4
461.2718
b2
175.0713
b4
485.2061
b5-H2O
554.2345
y4-NH3
444.2453
y1-NH3
130.0862
y8-H2O
881.4358
y7-H2O
780.3886
y10-H2O
1081.552
y9-NH3
995.5064
y11-NH3
1245.6
y11-H2O
1244.616
y12-H2O
1391.684
y12
1409.695
y12
y11
y10
y9
y8
y7
y6
y5
y4
y3
y2
y1
-
S
S
F
Y
S
L
T
S
Y
S
N
I
S
K
-
b2
b3
b4
b5