Activation mode of the eukaryotic m^{2}G10 tRNA methyltransferase Trm11 by its partner protein Trm112

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Running title: Insights into Trm11-Trm112 tRNA methyltransferase

Materials and methods

Circular dichroism

CD spectra were recorded using a Jobin-Yvon Mark VI circular dichrograph at a scan speed of 0.2 nm/s using a Quartz cuvette with 0.1 cm path length. Blanks were run before each spectrum and subtracted from the raw data. Three spectra were averaged to increase the signal-to-noise ratio. The final protein concentration was 10 μM in 10 mM Tris-HCl buffer (pH 8) containing 150 mM NaCl and the assays were carried out at 20°C. The results are presented as normalized Δε values on the basis of the amino acid mean residue mass of 110 Da for protein. Taking into account a sensitivity of $\delta(\Delta A) = 10^{-6}$ for the apparatus, the biomolecule concentration and the optical path-length of the cuvette, measurements were obtained at a precision of $\delta(\Delta\varepsilon) = +/- 0.01\ M^{-1}.cm^{-1}$ per residue.

Size-exclusion chromatography – Multi-angle laser light scattering analysis (SEC-MALLS)

A sample of 100 μL of ScTrm11-Trm112 complex (1 mg/mL) was injected at a flow rate of 0.75 mL/min on a Superdex^{TM} 200 Increase 10/300 GL column (GE-Healthcare) equilibrated in buffer A (20 mM Tris-HCl pH 7.5, 200 mM NaCl, 5 mM β-mercaptoethanol). Elution was followed by a UV-Visible spectrophotometer, a RID-20A refractive index detector (Shimadzu), a MiniDawn TREOS detector (Wyatt Technology). The data were collected and processed with the program AS-TRA 6.1 (Wyatt Technology). M_w was directly calculated from the absolute light scattering measurements using a dn/dc value of 0.183.

Figure legends

Figure S1: Sequence alignment between *Tko*Trm11 and *S. cerevisiae* Trm11.

Strictly conserved residues are in white on a black background. Partially conserved amino acids are boxed. Secondary structure elements assigned from the *Tko*Trm11 crystal structure are indicated above the alignment. Black stars indicate *Tko*Trm11 residues mutated by Hirata *et al.* (1).

Figure S2: Biophysical characterization of *S. cerevisiae* Trm11 protein and Trm11-Trm112 complex.

A. CD spectrum of yeast Trm11 purified in the absence of Trm112.
B. The Trm11-Trm112 complex is heterodimeric in solution. Left: SEC-MALLS analysis of yeast Trm11-Trm112 complex. The refractive index for the eluted sample is shown in blue, revealing the presence of a single peak. Right: Zoom on the main peak (refractive index in blue on the left y axis) and distribution of the molecular mass calculated from light scattering along this peak (red curve, right y axis).

Figure S3: Binding of SAM to *S. cerevisiae* Trm11-Trm112 complexes.

Upper panels: ITC data obtained by injecting SAM into corresponding Trm11-Trm112 complexes at 20°C. Lower panels show the fit to the binding curve using a binding site model.

Figure S4: Characterization of Trm11-Trm112 complex formation and of the effect of Trm112 on SAM binding to Trm11 by ITC.

A. Control experiment showing that the injection of Trm112 into buffer at 10°C does not generate a signal comparable to that observed for the injection of Trm112 into Trm11 (Fig.3A).
B. The Trm11 D286R mutant does not interact with wild-type Trm112. This experiment was performed at 10°C.
C. The Trm112 R53E mutant does not interact with wild-type Trm11. This experiment was performed at 10°C.
D. Titration of Trm11 (40 μM; left) or Trm112 (39 μM; right) by SAM (400 μM) at 10°C.

Figure S5: Schematic representation of Trm11 (top) and Trm112 (bottom) peptides monitored in our HDX-MX experiments.

Figure S6: HDX kinetics of all Trm11 peptides.

Red circles and blue boxes correspond to data extracted from Trm11 alone and Trm11-Trm112 complex, respectively. Error bars represent standard deviations from the mean of three replicates.

Figure S7: HXD kinetics of all Trm112 peptides.

Red circles and blue boxes correspond to data extracted from Trm112 alone and Trm11-Trm112 complex, respectively. Error bars represent standard deviations from the mean of three replicates.

Table S1: Primers used in this study.

Name	Purpose	Sequences
oMG267	Cloning of $tRNA^{Ile}$ and transcript A (F)	TAATACGACTCACTATAGGGGGTCTCTTGGCCC
oMG335	Production of Transcript A (R)	TGGTCTCTAGCGGGATCGAACC
oMG78	Production of Transcript B (F)	TAATACGACTCACTATAGGGGGTCTCTTGGCCCAG
oMG285	Production of Transcript B (R)	TCTCTAGCGGGATCGAACC
oMG318	Mutation $tRNA^{Ile}$-G10C/C25G (F)	CTTGCCCCAGTTGGTTAAGGGACCGTGCTAA-TAACG
oMG319	Mutation $tRNA^{Ile}$-G10C/C25G (R)	CACGGTCCCTTAACCAACTGGGGCAAGA-GACCCCC
oMG301	Mutation D238A Trm11 (F)	GGTTCGGCTATAGATGGCAGAATGATTCG
oMG302	Mutation D238A Trm11 (R)	GCCATCTATAGCCGAACCAATCACTAAAGAACC
oMG303	Mutation D286R Trm11 (F)	CTTGTCATTCGTACTATTTTGTGTGATCCTCC
oMG304	Mutation D286R Trm11 (R)	CAAAATAGTACGAATGACAAGATTGTTTC
oMG305	Mutation D291A Trm11 (F)	GTGTGCTCCTCCATATGGTATC
oMG306	Mutation D291A Trm11 (R)	CCATATGGAGGAGCACACAAAATAGTATC
y-trm11-eag1-poly	Cloning of ScTrm11 in pKHS (F)	TTTTC GGCCGATTAATTTAAGAAGGAGATATATAT ATG AAGAAATATCTGCTG
y-trm11-not1-poly	Cloning of ScTrm11 in pKHS (R)	TTTTTGC GGC CGC GTTAAAATTATTGAAGTACCG

Table S2: Plasmids used in this study

Name	Vector	Product	Marker	Tag	Reference
pMG526	pKHS	Trm11	Kanamycin	His6 (Cter)	This work
pVH451	pET11a	Trm112	Kanamycin	His6 (Cter)	(2)
pMG513	pACYC-Duet1	Trm112	Chloramphenicol	No	Kind gift from Dr V. Heurgué-Hamard
pMG729	pET11a	Trm112 N123R	Ampicillin	No	(3)
pMG730	pET11a	Trm112 A106E	Ampicillin	No	(3)
pMG731	pET11a	Trm112 I118E	Ampicillin	No	(3)
pMG732	pET11a	Trm112 Y120D	Ampicillin	No	(3)
pMG735	pET11a	Trm112 E107K	Ampicillin	No	(3)
pMG745	pKHS	Trm11 D238A	Kanamycin	His6 (Cter)	This work
pMG476	pKHS	Trm11 D286R	Kanamycin	His6 (Cter)	This work
pMG747	pKHS	Trm11 D291A	Kanamycin	His6 (Cter)	This work
pILE	pUC18	$tRNA^{Ile}$	Ampicillin		Kind gift from Dr B. Collinet
pMG755	pUC18	$tRNA^{Ile}$ G10C/C25G	Ampicillin		This work

Bibliography

1. Hirata, A., Nishiyama, S., Tamura, T., Yamauchi, A. and Hori, H. (2016) Structural and functional analyses of the archaeal tRNA m2G/m22G10 methyltransferase aTrm11 provide mechanistic insights into site specificity of a tRNA methyltransferase that contains common RNA-binding modules. *Nucleic Acids Res*, **44**, 6377-6390.
2. Heurgue-Hamard, V., Graille, M., Scrima, N., Ulryck, N., Champ, S., van Tilbeurgh, H. and Buckingham, R.H. (2006) The zinc finger protein Ynr046w is plurifunctional and a component of the eRF1 methyltransferase in yeast. *J Biol Chem*, **281**, 36140-36148.
3. Liger, D., Mora, L., Lazar, N., Figaro, S., Henri, J., Scrima, N., Buckingham, R.H., van Tilbeurgh, H., Heurgue-Hamard, V. and Graille, M. (2011) Mechanism of activation of methyltransferases involved in translation by the Trm112 'hub' protein. *Nucleic Acids Res*, **39**, 6249-6259.

Figure S1

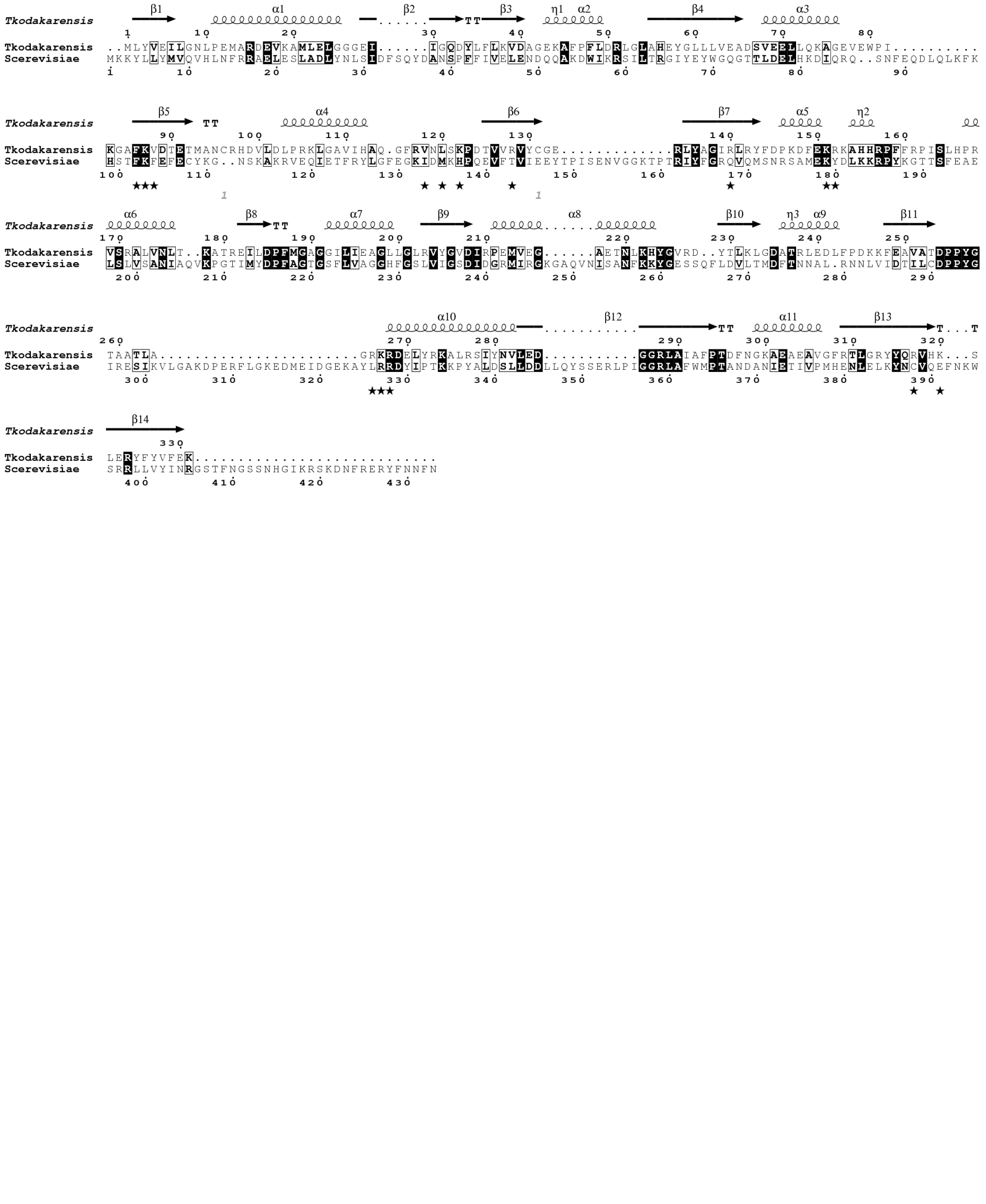
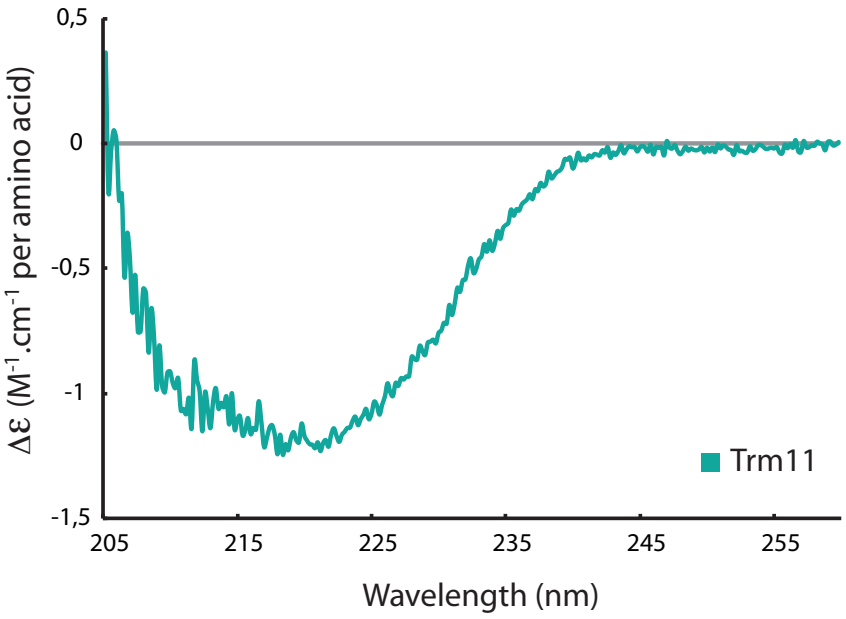
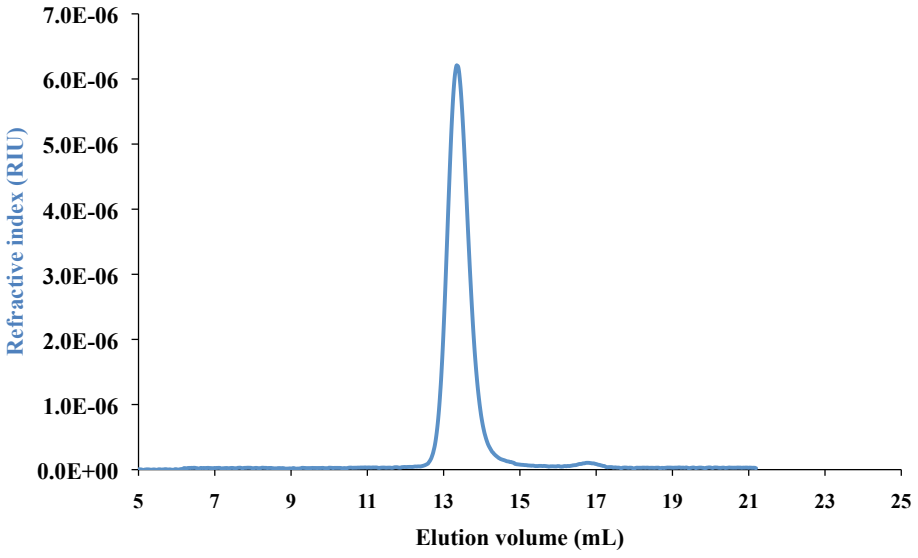

Tkodakarensis
Scerevisiae
β1 α1 β2 β3 η1 α2 β4 α3
β5 α4 β6 β7 α5 η2
α6 β8 α7 β9 α8 β10 η3 α9 β11
α10 β12 α11 β13
β14

Figure S2

A


0,5
0
-0,5
-1
-1,5
Δε (M-1.cm-1 per amino acid)
205
215
225
235
245
255
Wavelength (nm)
Trm11

B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6.0E-06
5.0E-06
4.0E-06
3.0E-06
2.0E-06
1.0E-06
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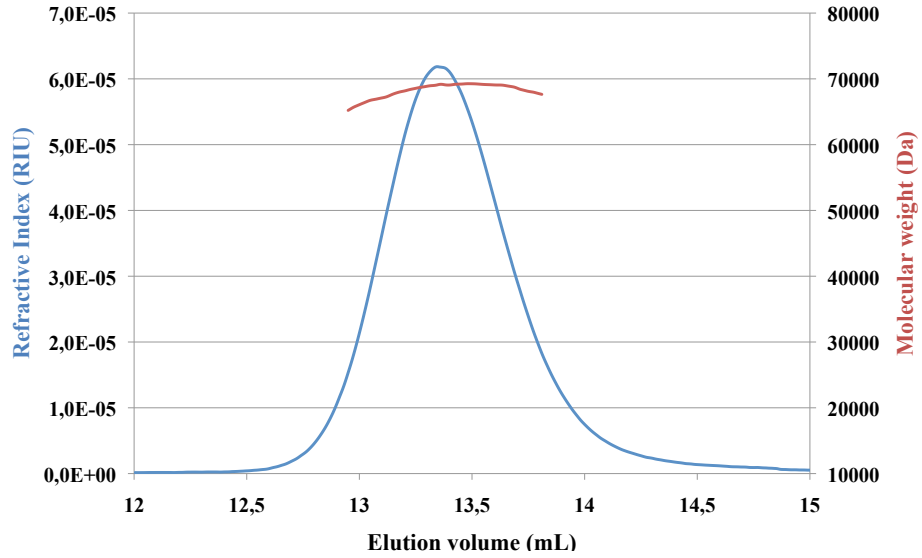
Refractive index (RIU)
5
7
9
11
13
15
17
19
21
23
25
Elution volume (mL)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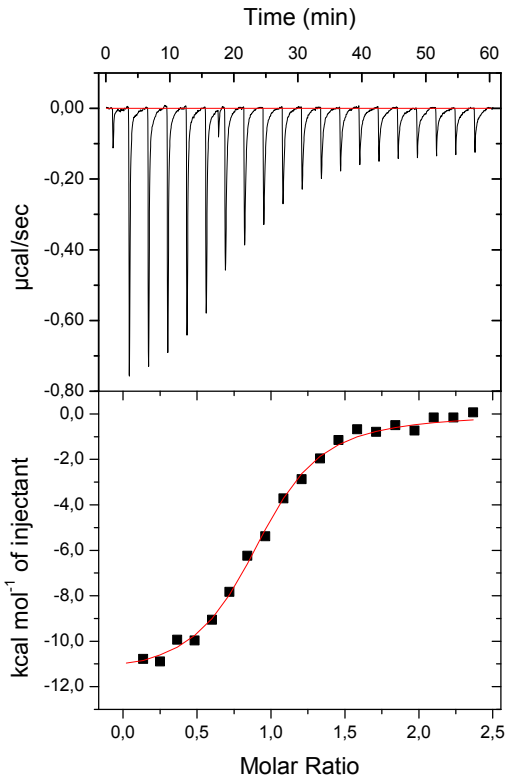
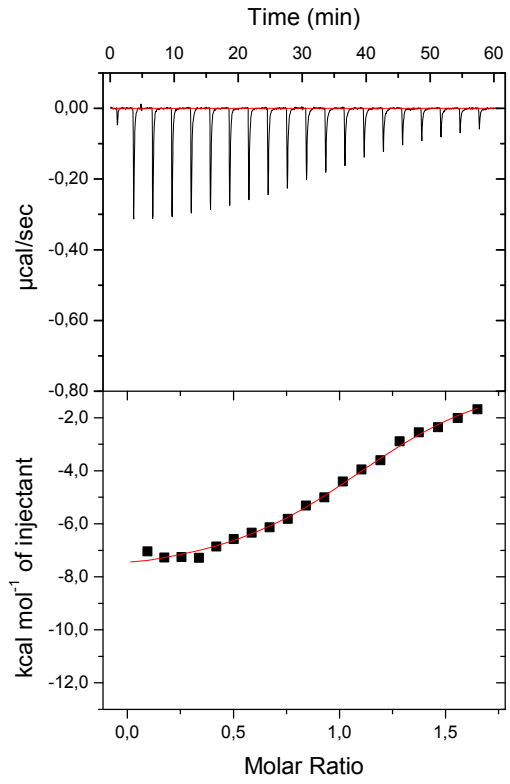
Refractive Index (RIU)
80000
70000
60000
50000
40000
30000
20000
10000
Molecular weight (Da)
12
12,5
13
13,5
14
14,5
15
Elution volume (mL)

Figure S3: Titration of various Trm11-trm112 complexes by SAM

Trm112-Trm11 WT



Trm112-Trm11 D291A



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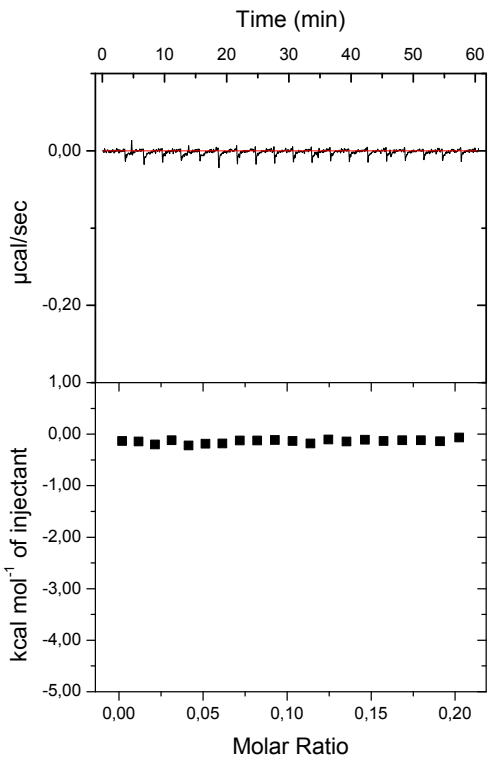
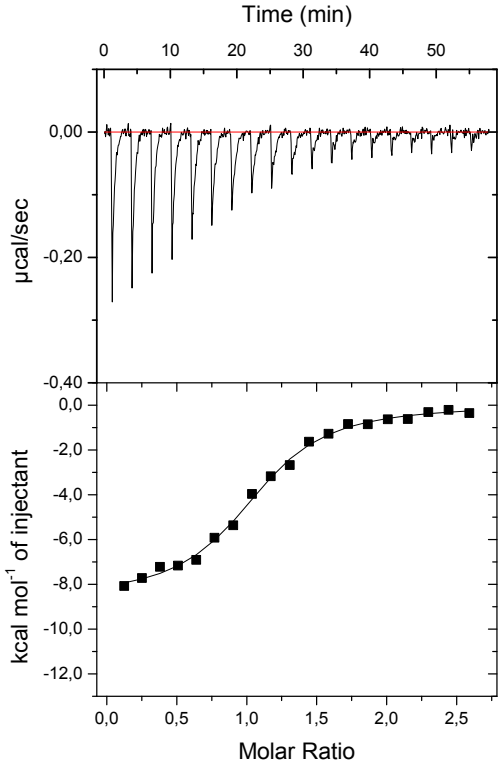
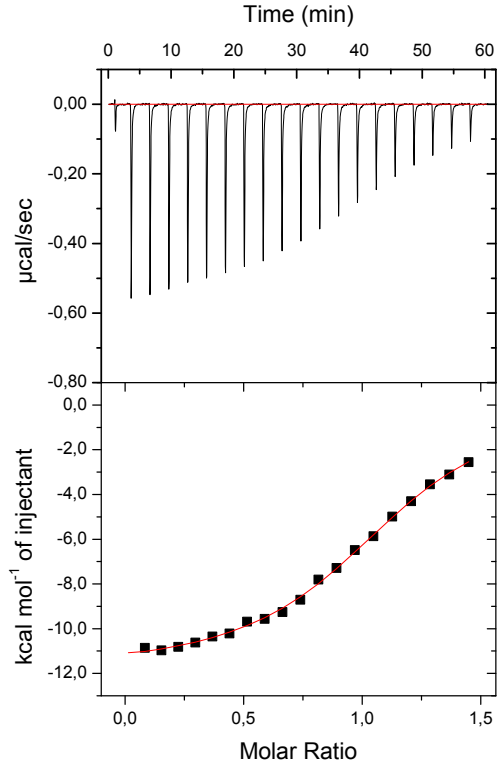


Trm112 A106E-Trm11



Trm112 E107K-Trm11



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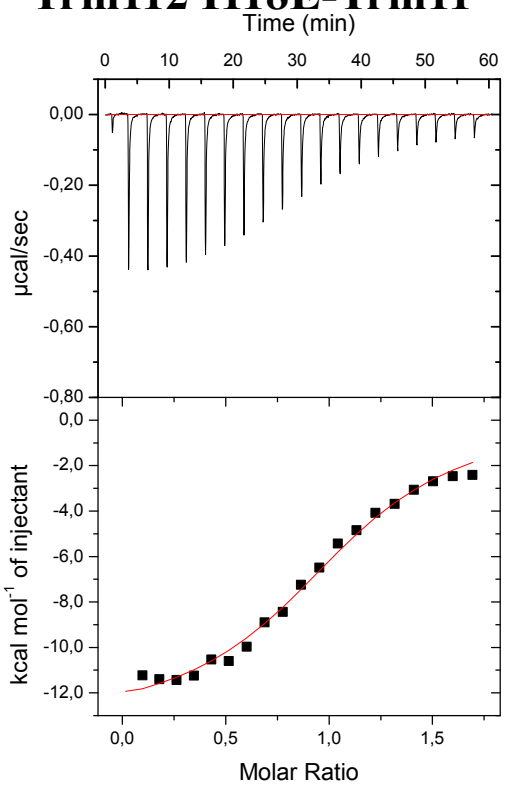

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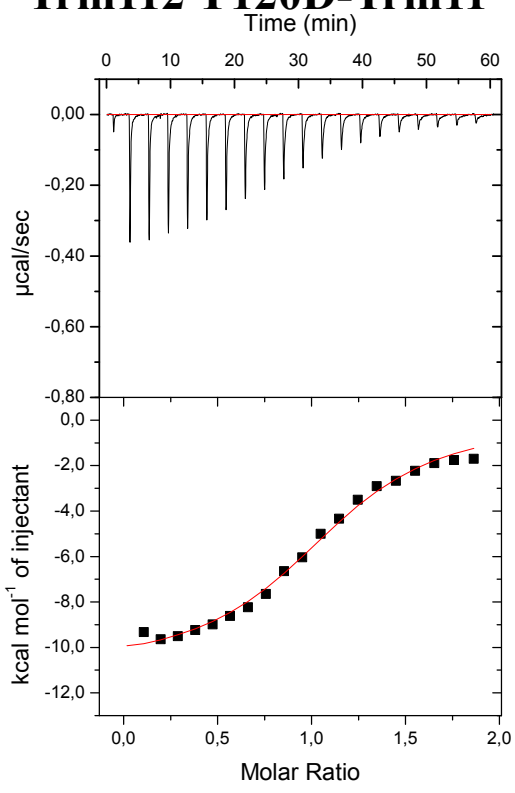
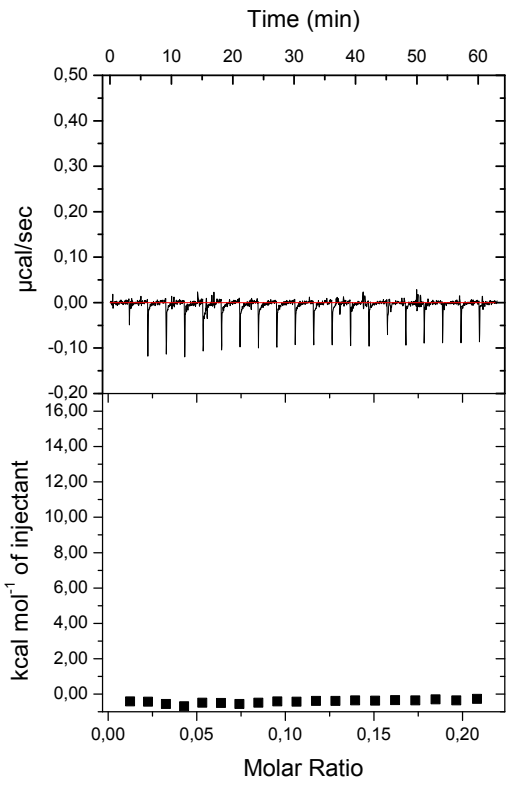
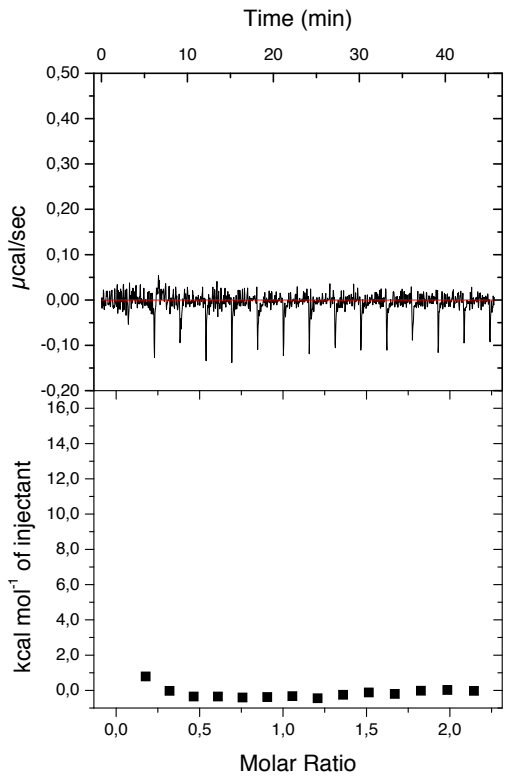


Figure S4

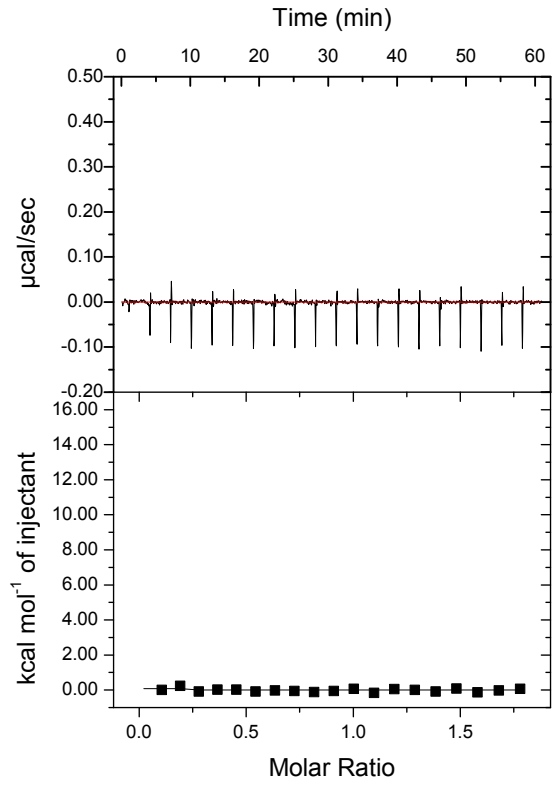
A **Trm112 vs Buffer**



B **Trm112 vs Trm11 D286R**

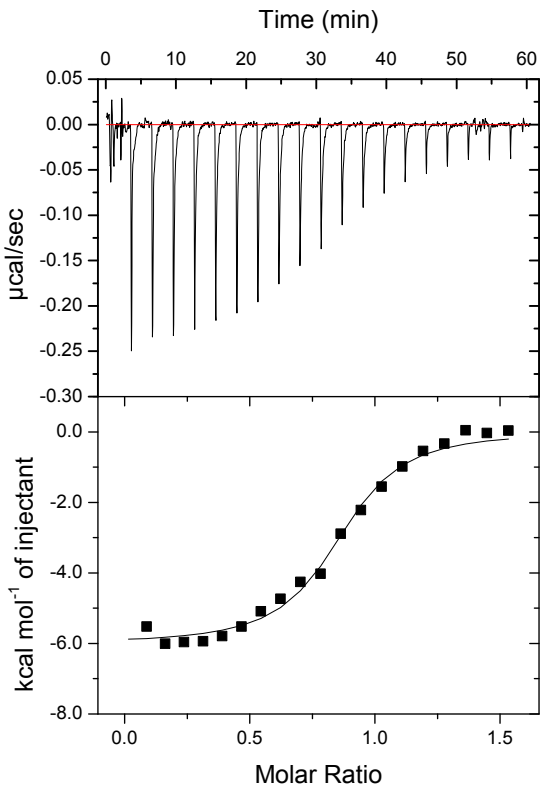


C **Trm112 R53E vs Trm11**

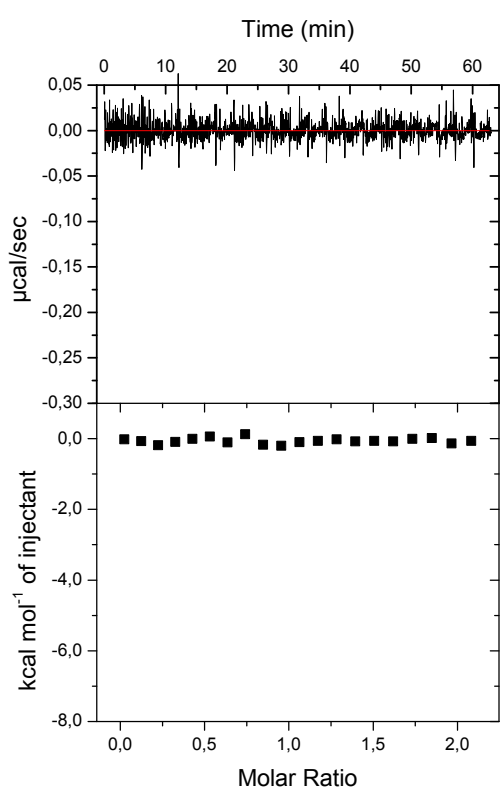


D

Trm11-Trm112 vs SAM



Trm11 vs SAM



Trm112 vs SAM

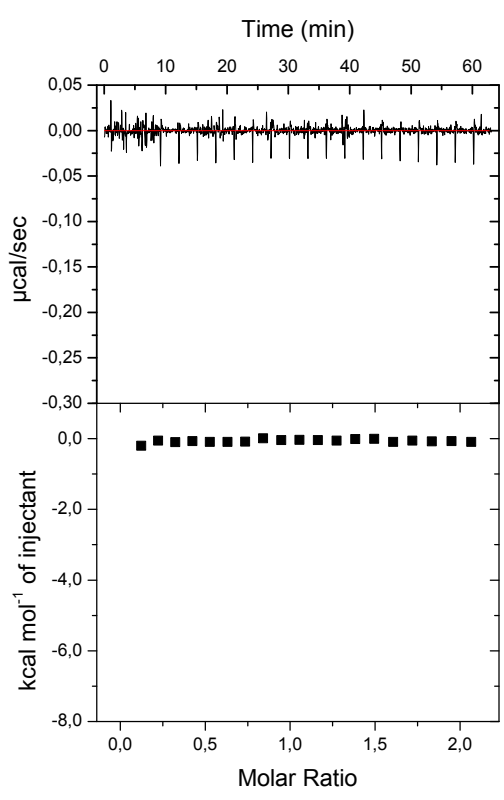
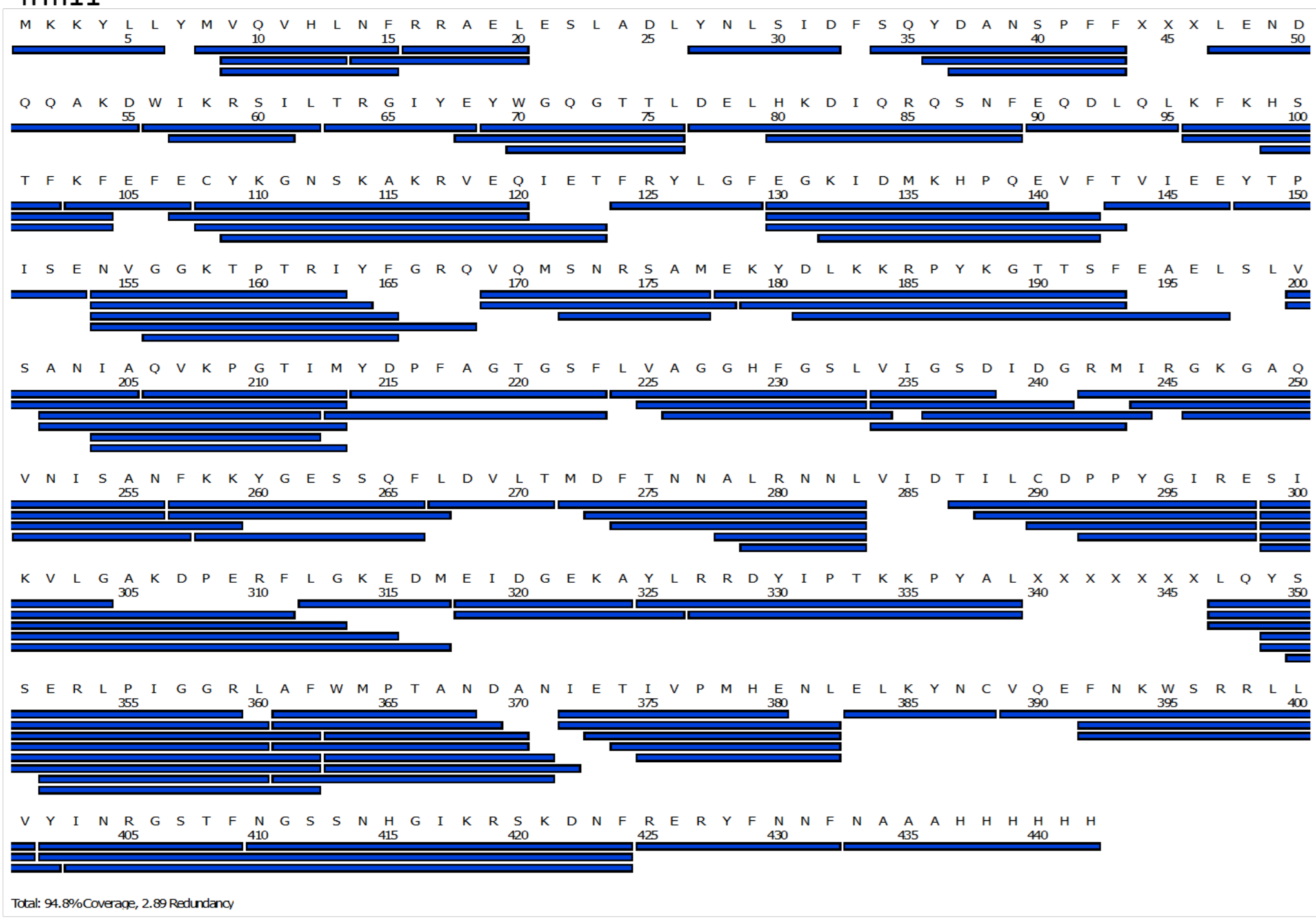


Figure S5


Trm11
Total: 94.8%Coverage, 2.89 Redundancy

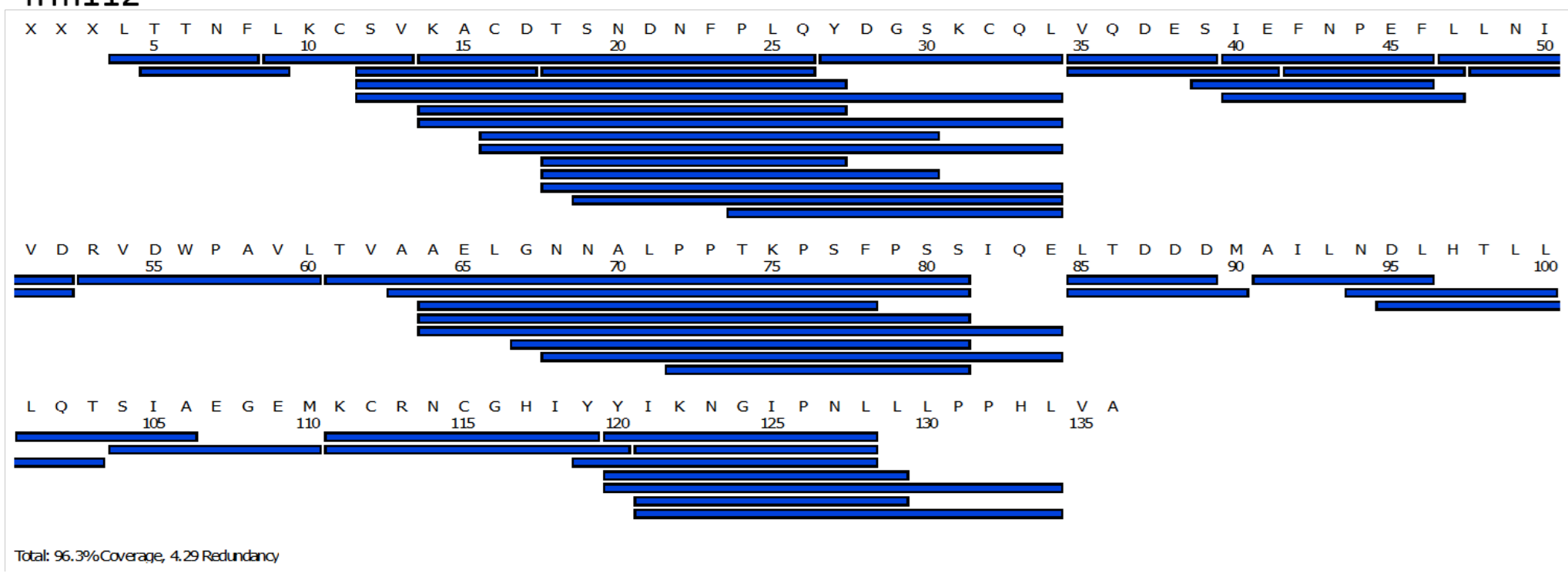

Trm112
Total: 96.3%Coverage, 4.29 Redundancy

Figure S6 (to be continued)

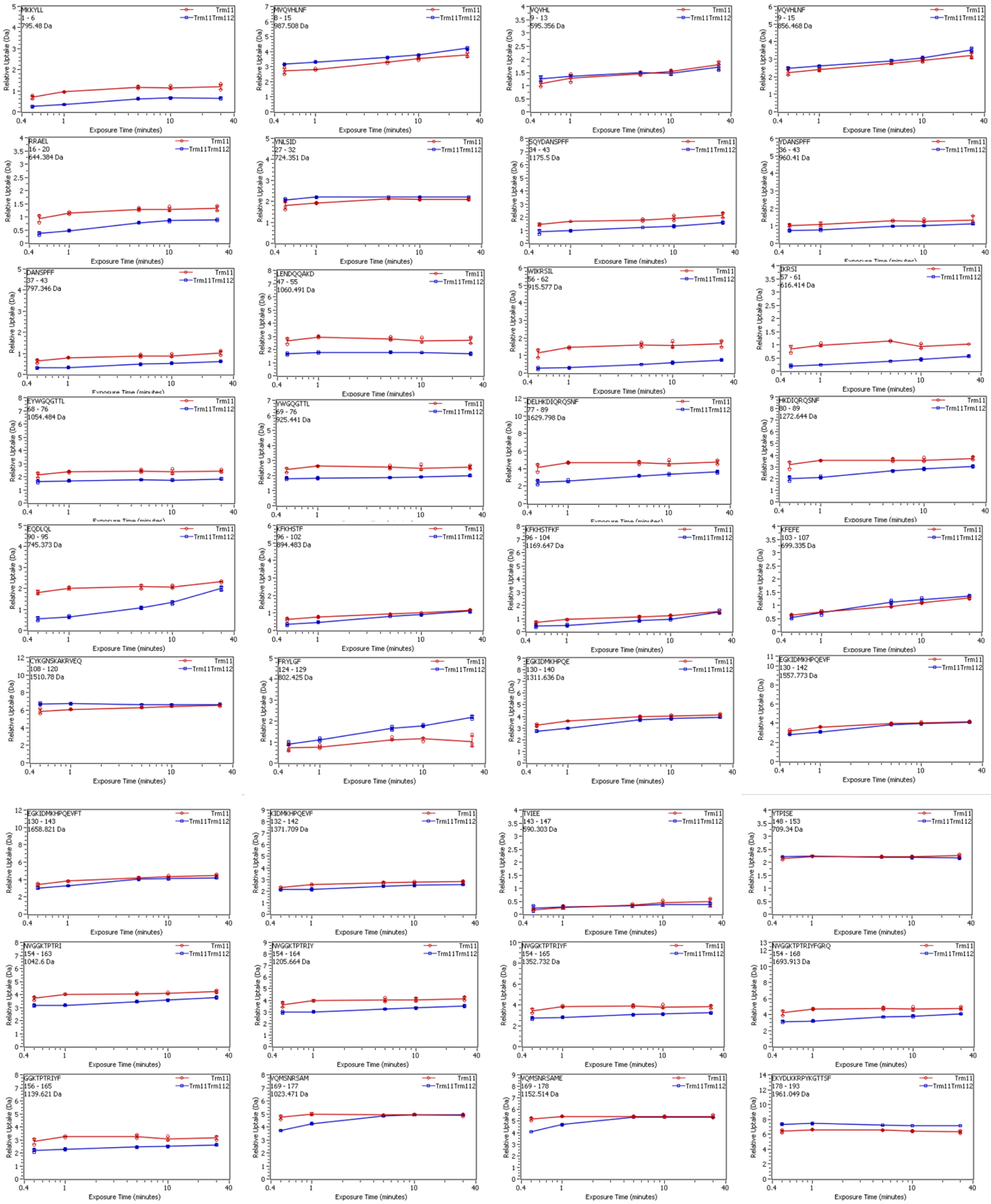
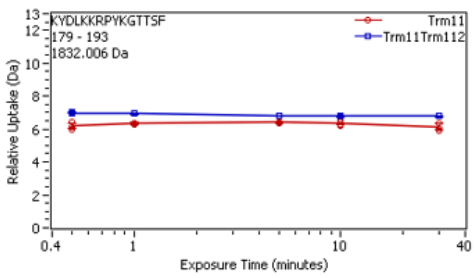
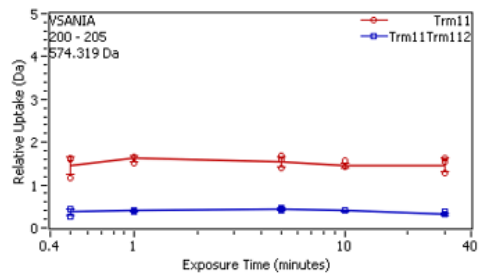
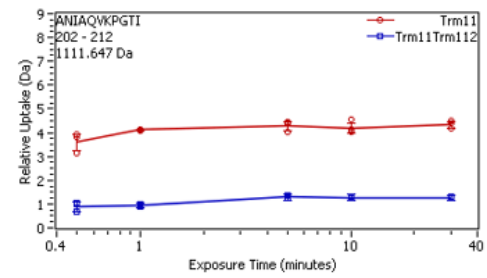
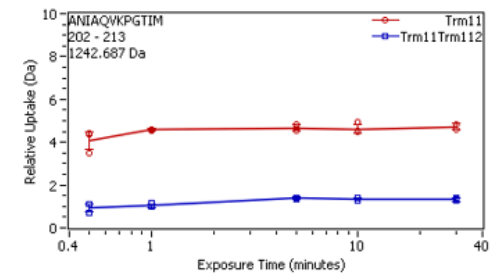


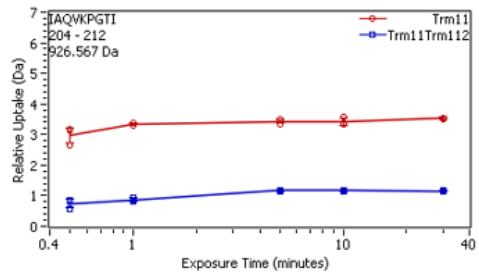
Figure S6 (continued)

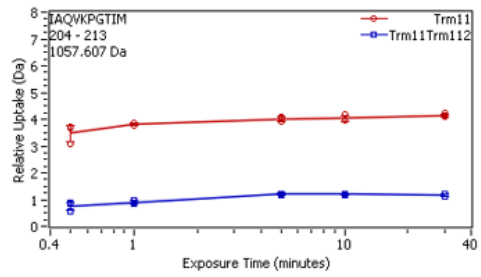


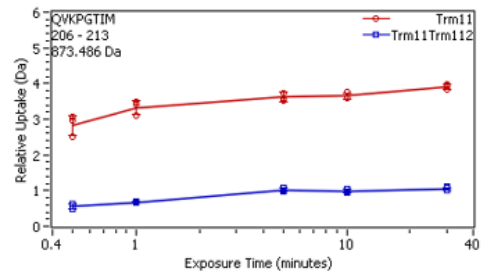


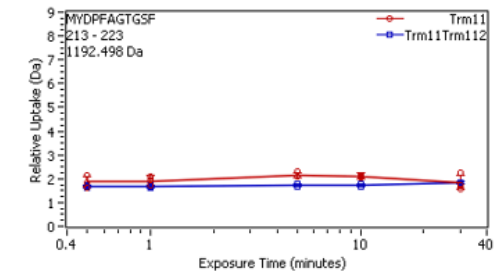


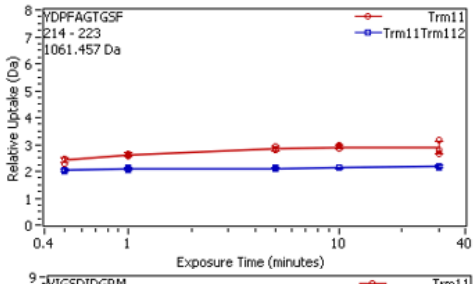


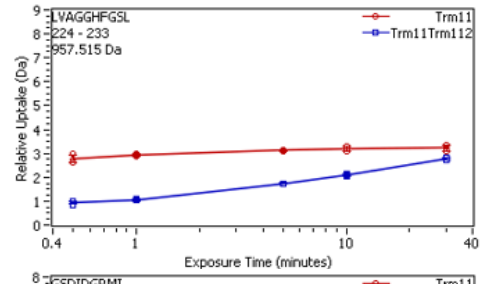


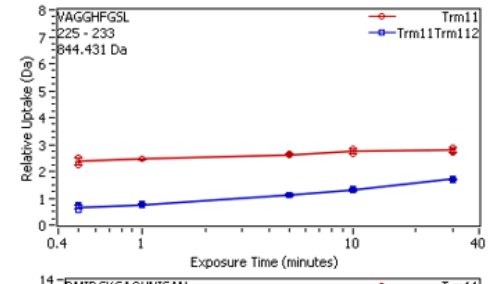


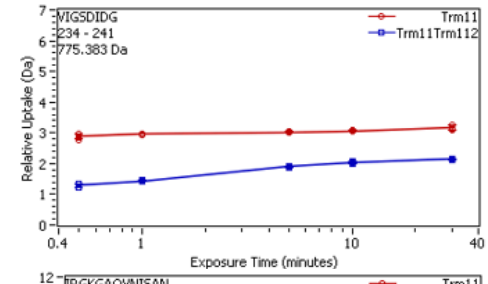


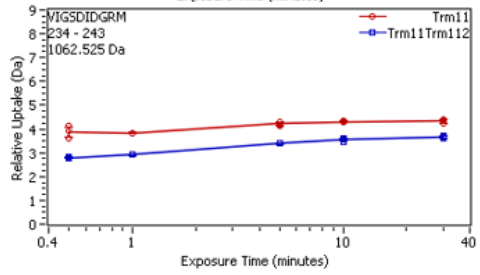


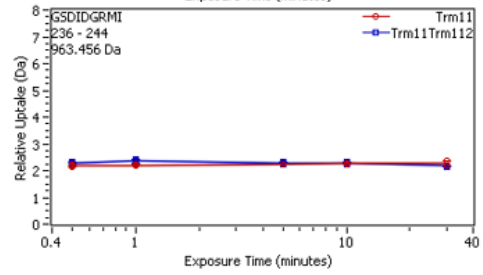


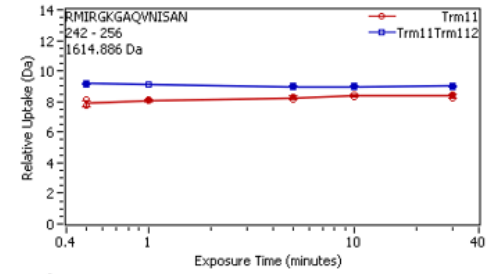


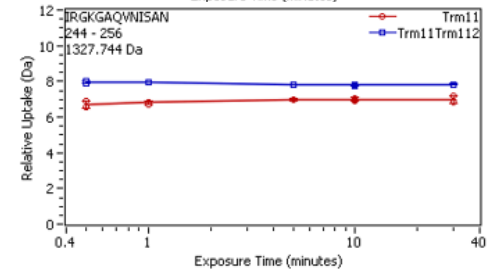


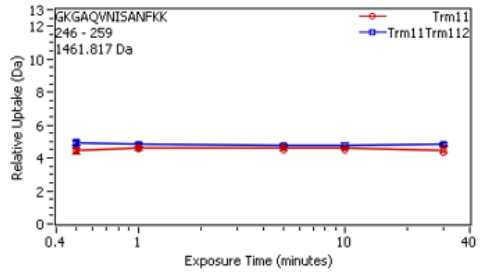


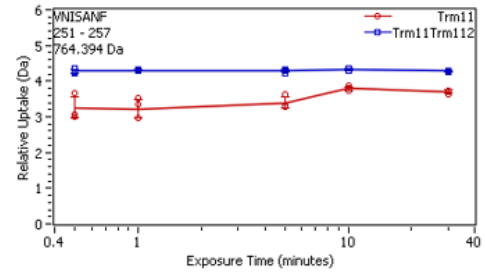


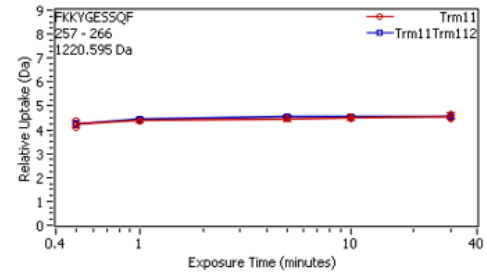


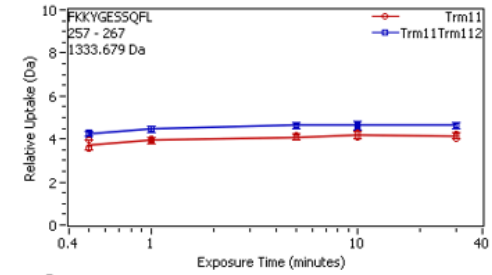


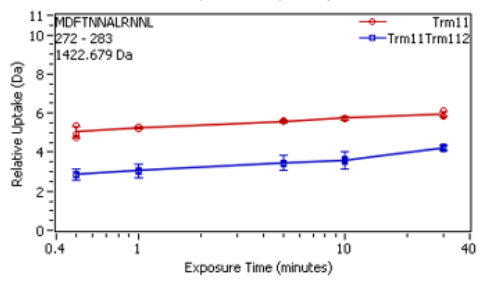


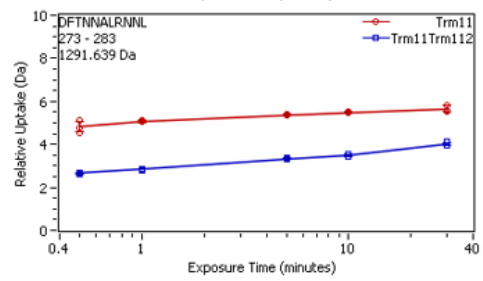


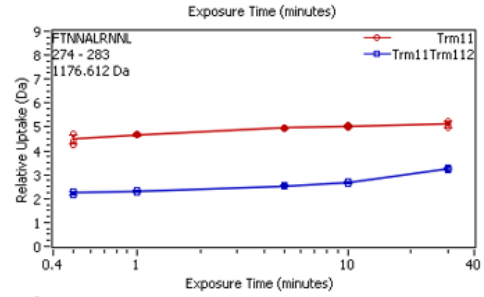


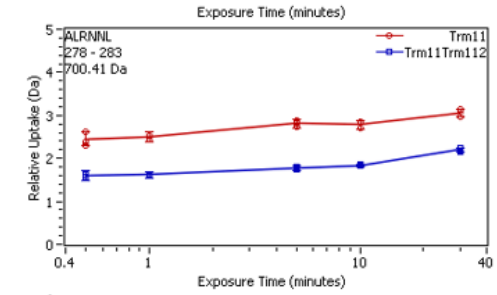


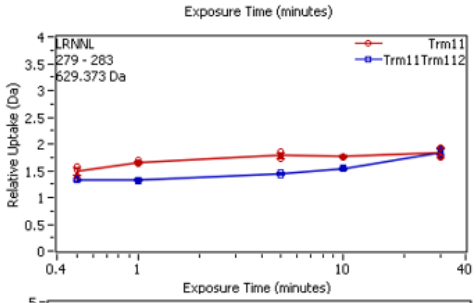


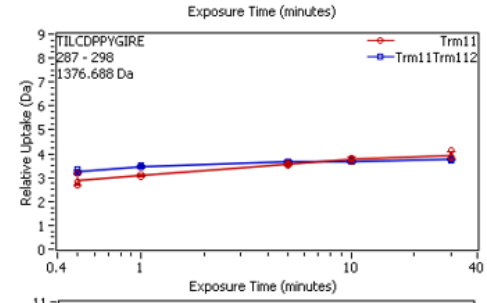


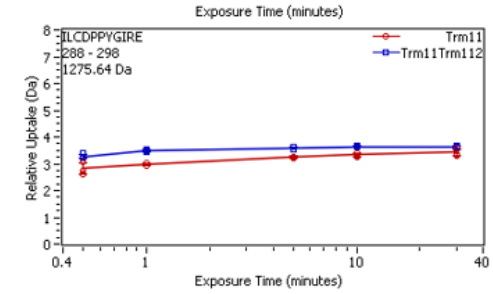


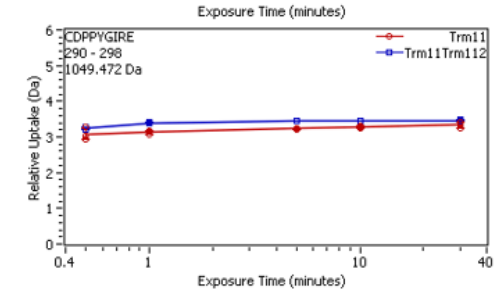


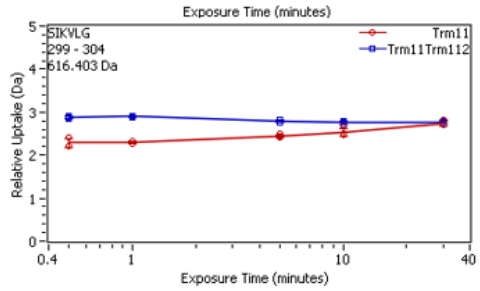


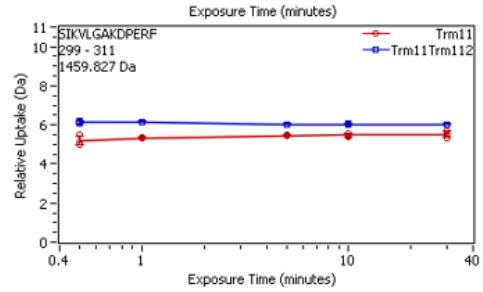


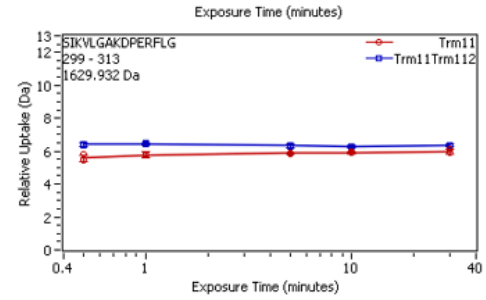


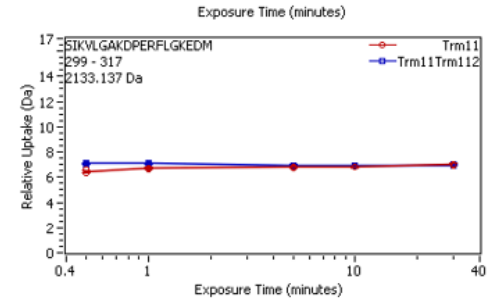


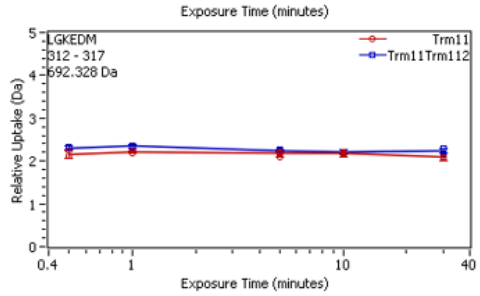


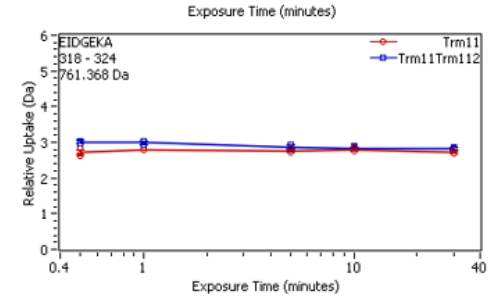


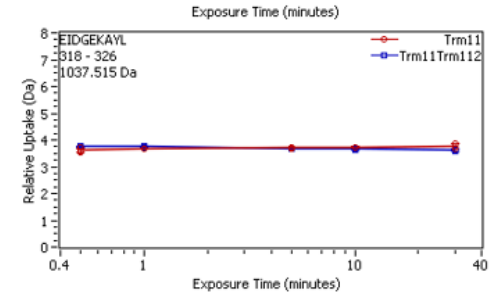












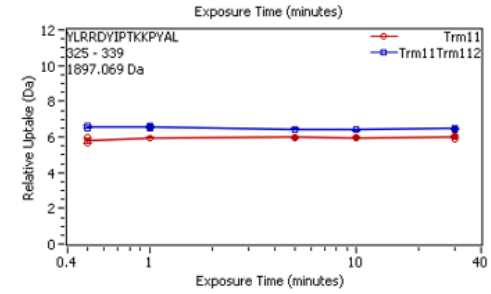


Figure S6

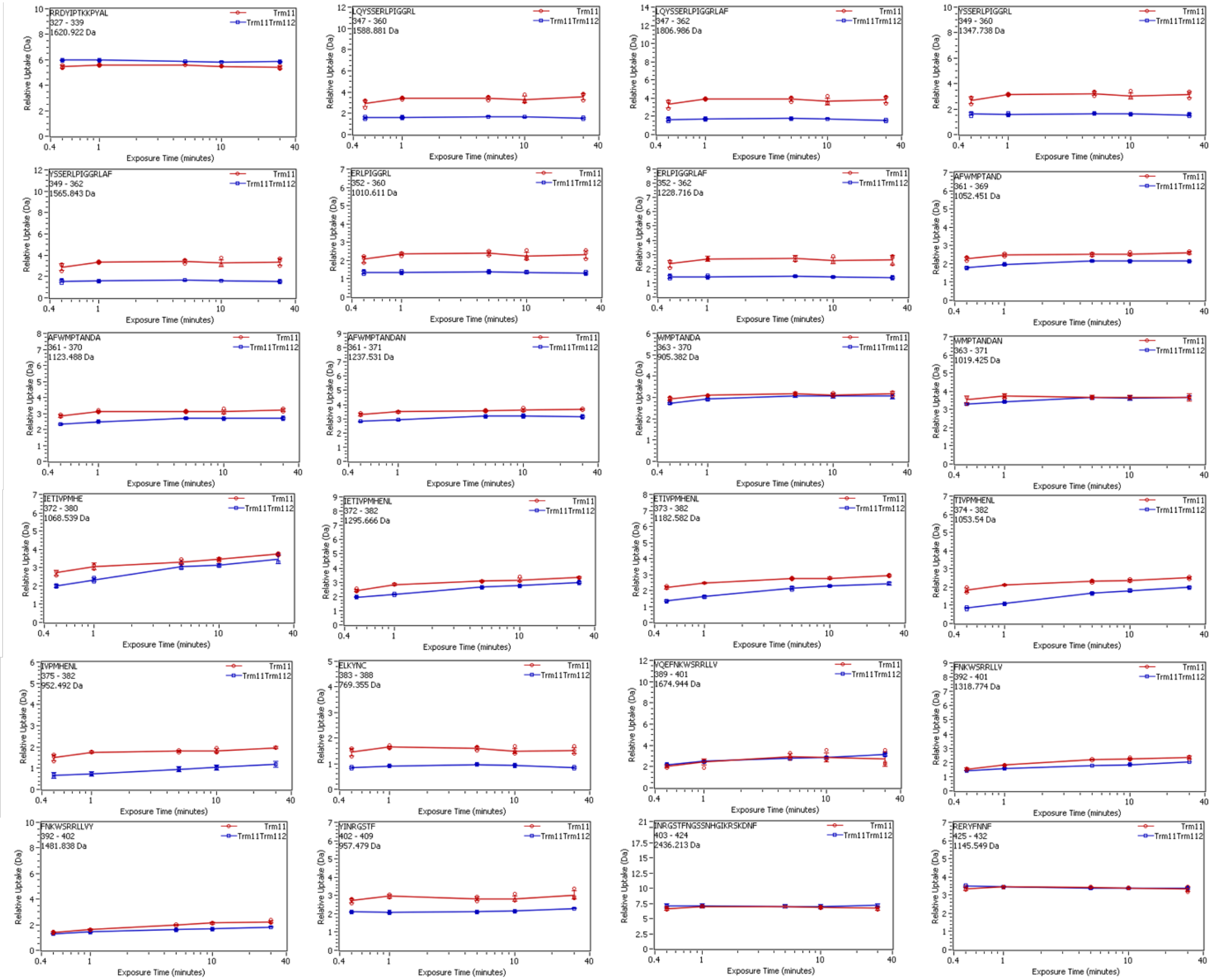
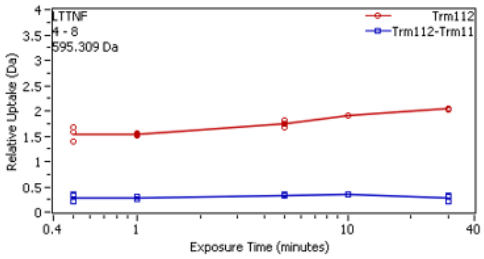
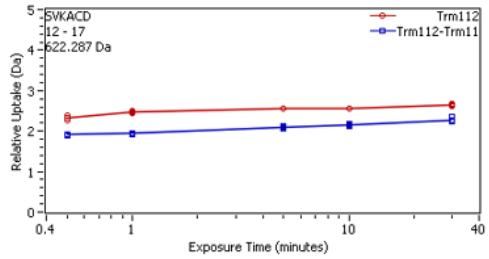
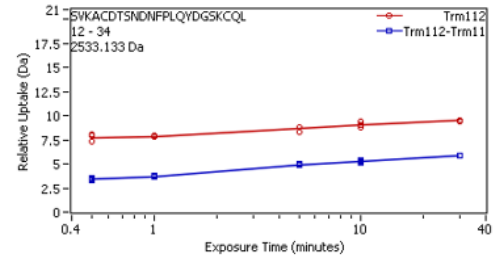
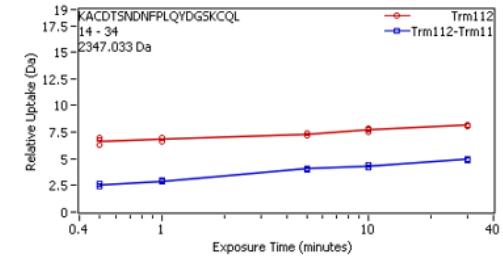

RRDYIPTKKPYAL
327 - 339
1620.922 Da
LQYSSERLPIGGRL
347 - 360
1588.881 Da
LQYSSERLPIGGRLAF
347 - 362
1806.986 Da
YSSERLPIGGRL
349 - 360
1347.738 Da
YSSERLPIGGRLAF
349 - 362
1565.843 Da
ERLPIGGRL
352 - 360
1010.611 Da
ERLPIGGRLAF
352 - 362
1228.716 Da
AFWMPTAND
361 - 369
1052.451 Da
AFWMPTANDA
361 - 370
1123.488 Da
AFWMPTANDAN
361 - 371
1237.531 Da
WMPTANDA
363 - 370
905.382 Da
WMPTANDAN
363 - 371
1019.425 Da
IETIVPMHE
372 - 380
1068.539 Da
IETIVPMHENL
372 - 382
1295.666 Da
ETIVPMHENL
373 - 382
1182.582 Da
TIVPMHENL
374 - 382
1053.54 Da
IVPMHENL
375 - 382
952.492 Da
ELKYNC
383 - 388
769.355 Da
VQEFNKWSRRLLV
389 - 401
1674.944 Da
FNKWSRRLLV
392 - 401
1318.774 Da
FNKWSRRLLVY
392 - 402
1481.838 Da
YINRGSTF
402 - 409
957.479 Da
INRGSTFNGSSNHGIKRSKDNF
403 - 424
2436.213 Da
RERYFNNF
425 - 432
1145.549 Da
Trm11
Trm11Trm112
Relative Uptake (Da)
Exposure Time (minutes)

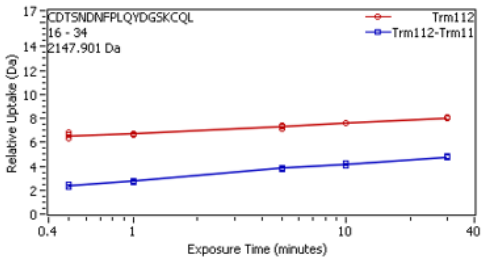
Figure S7

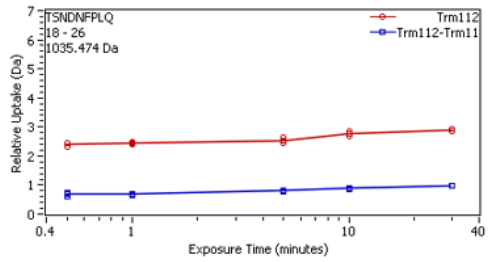

LTTNF
4 - 8
595.309 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

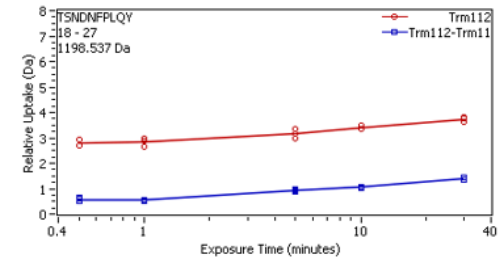

SVKACD
12 - 17
622.287 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

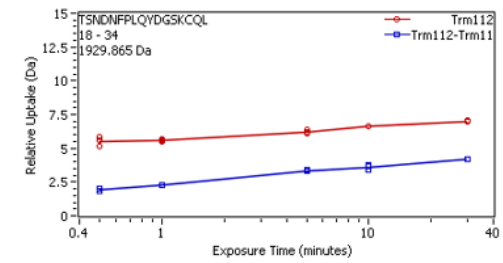

SVKACDTSNDNFPLQYDGSKCQL
12 - 34
2533.133 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

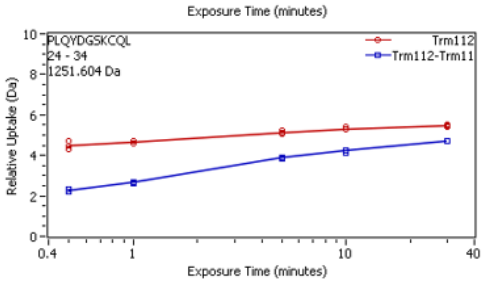

KACDTSNDNFPLQYDGSKCQL
14 - 34
2347.033 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

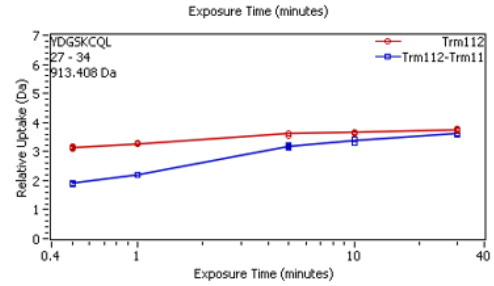

CDTSNDNFPLQYDGSKCQL
16 - 34
2147.901 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

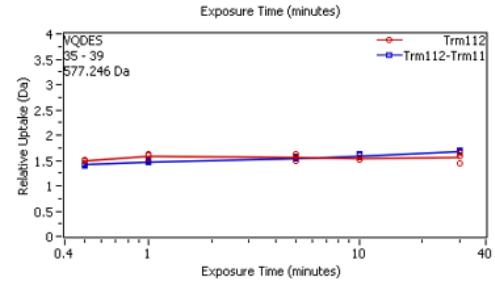

TSNDNFPLQ
18 - 26
1035.474 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

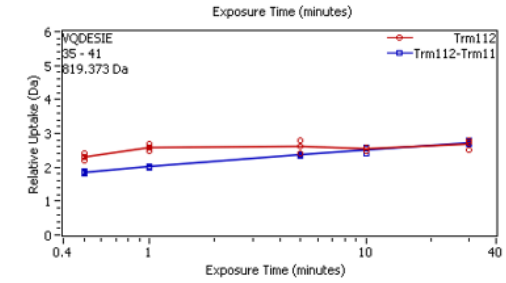

TSNDNFPLQY
18 - 27
1198.537 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

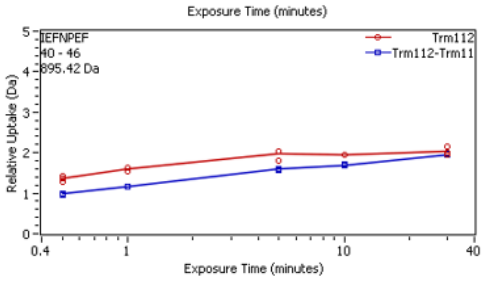

TSNDNFPLQYDGSKCQL
18 - 34
1929.865 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

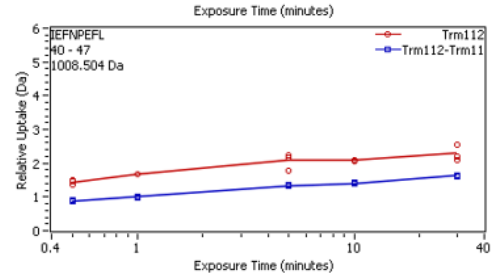

PLQYDGSKCQL
24 - 34
1251.604 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

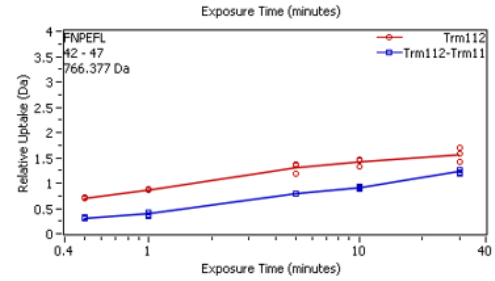

YDGSKCQL
27 - 34
913.408 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

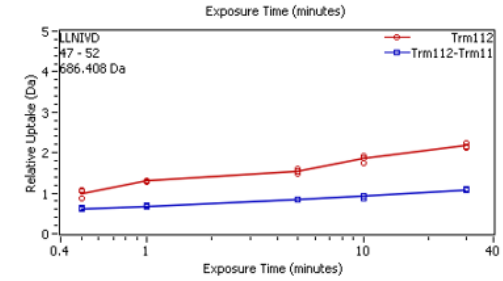

MQDES
35 - 39
577.246 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

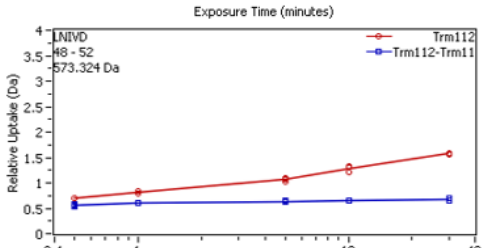

MQDESIE
35 - 41
819.373 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

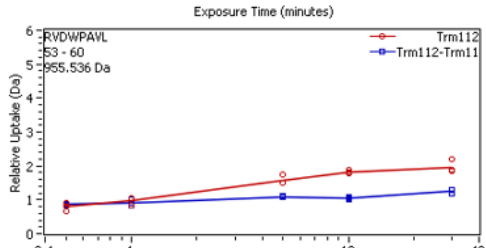

IEFNPEF
40 - 46
895.42 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

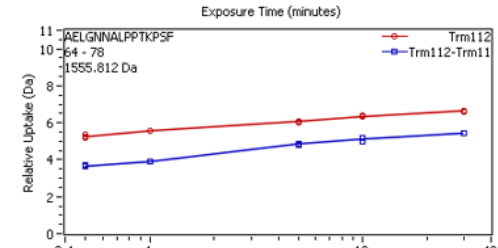

IEFNPEFL
40 - 47
1008.504 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

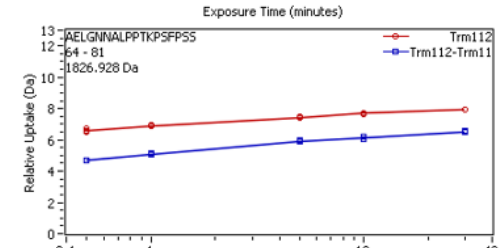

FNPEFL
42 - 47
766.377 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

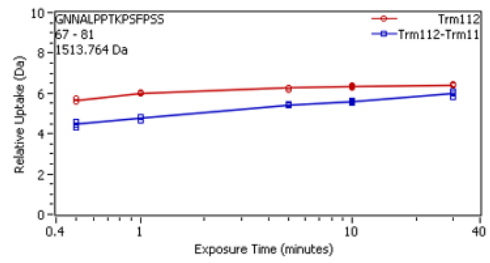

LNIVD
47 - 52
686.408 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

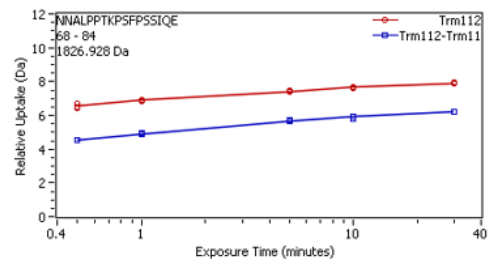

LNIVD
48 - 52
573.324 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

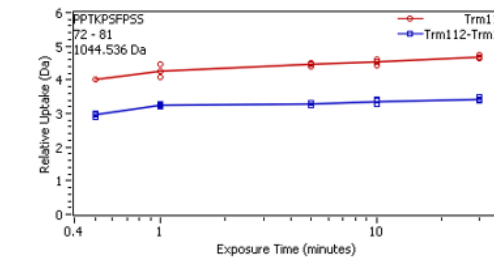

RVDWPAVL
53 - 60
955.536 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

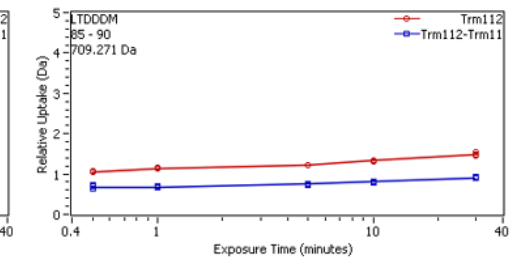

AELGNNALPPTKPSF
64 - 78
1555.812 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

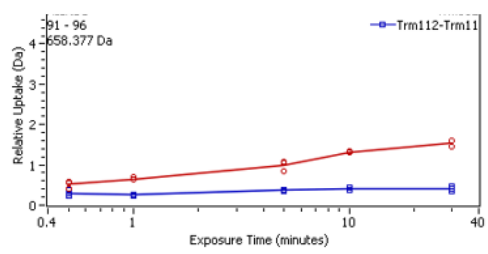

AELGNNALPPTKPSFPSS
64 - 81
1826.928 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

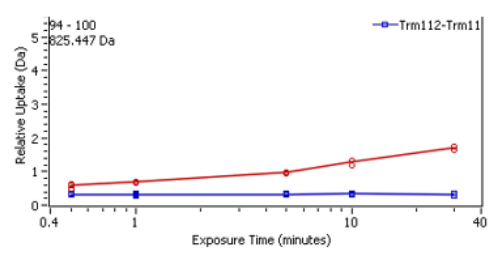

GNNALPPTKPSFPSS
67 - 81
1513.764 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

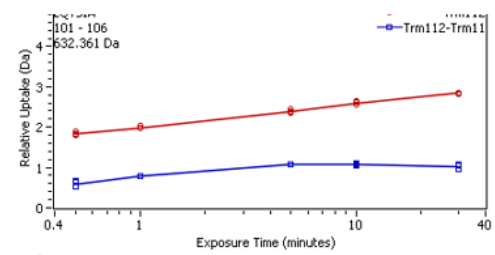

NNALPPTKPSFPSSIQE
68 - 84
1826.928 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

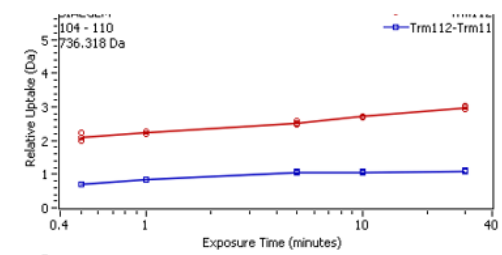

PPTKPSFPSS
72 - 81
1044.536 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

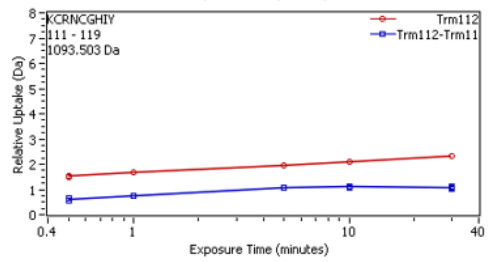

LTDDDM
85 - 90
709.271 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

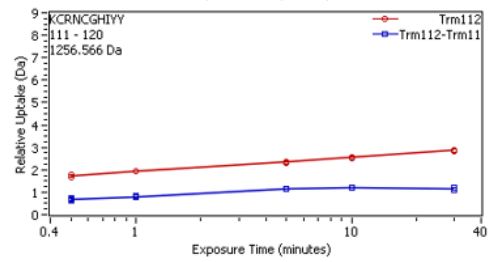

91 - 96
658.377 Da
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

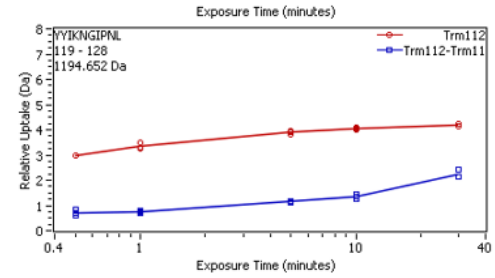

94 - 100
825.447 Da
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

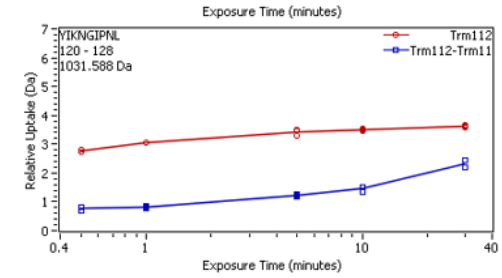

101 - 106
632.361 Da
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

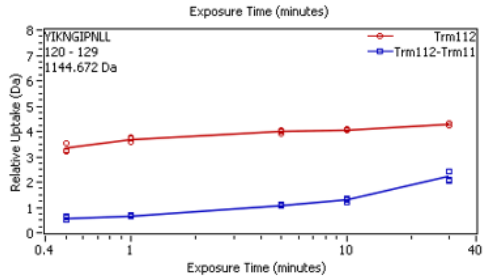

104 - 110
736.318 Da
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

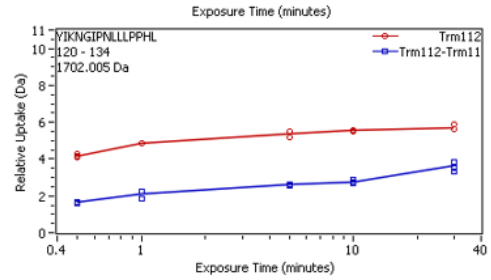

KCRNCGHIY
111 - 119
1093.503 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

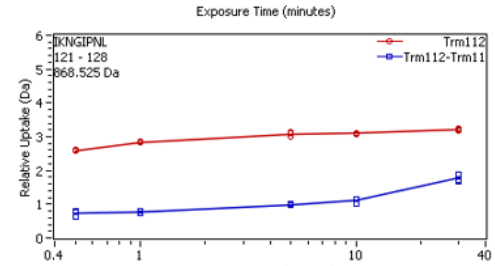

KCRNCGHIYY
111 - 120
1256.566 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

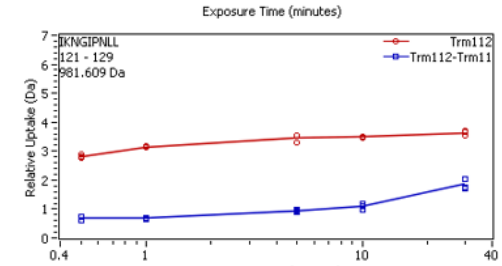

YYIKNGIPNL
119 - 128
1194.652 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)

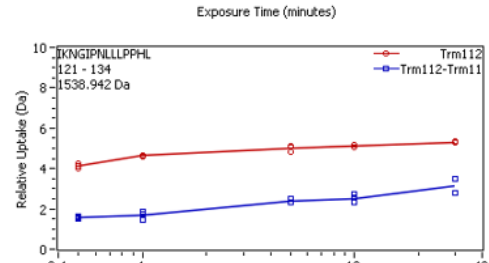

YIKNGIPNL
120 - 128
1031.588 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)


YIKNGIPNLL
120 - 129
1144.672 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)


YIKNGIPNLLLPPHL
120 - 134
1702.005 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)


IKNGIPNL
121 - 128
868.525 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)


IKNGIPNLL
121 - 129
981.609 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)


IKNGIPNLLLPPHL
121 - 134
1538.942 Da
Trm112
Trm112-Trm11
Relative Uptake (Da)
Exposure Time (minutes)