Improvement of Shotgun Proteomics in the Negative Mode by Carbamylation of Peptides and Ultraviolet Photodissociation Mass Spectrometry

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Supporting Figure 1. pI distribution of *in silico* generated tryptic peptides from *H. sapiens, E.coli,* and *S. cerevisiae* with up to two missed cleavages. Significant portions (52-58%) of these tryptic peptidomes are acidic.

Supporting Figure 2. Reaction scheme for carbamylation of a peptide bearing a lysine residue

Supporting Figure 3. Positive mode extracted ion chromatograms of carbamylated and unmodified $LVNELTEFAK^{2+}$, thus showing the extent of carbamylation of *H. salinarum* tryptic peptides.

Supporting Figure 4. Positive mode ESI spectrum of RPKPQQFFGLM (M_r 1347.72 Da) after carbamylation. The major species observed is doubly carbamylated (N-terminus and K), corresponding to a doubly carbamylated species (M_r 1433.72 Da).

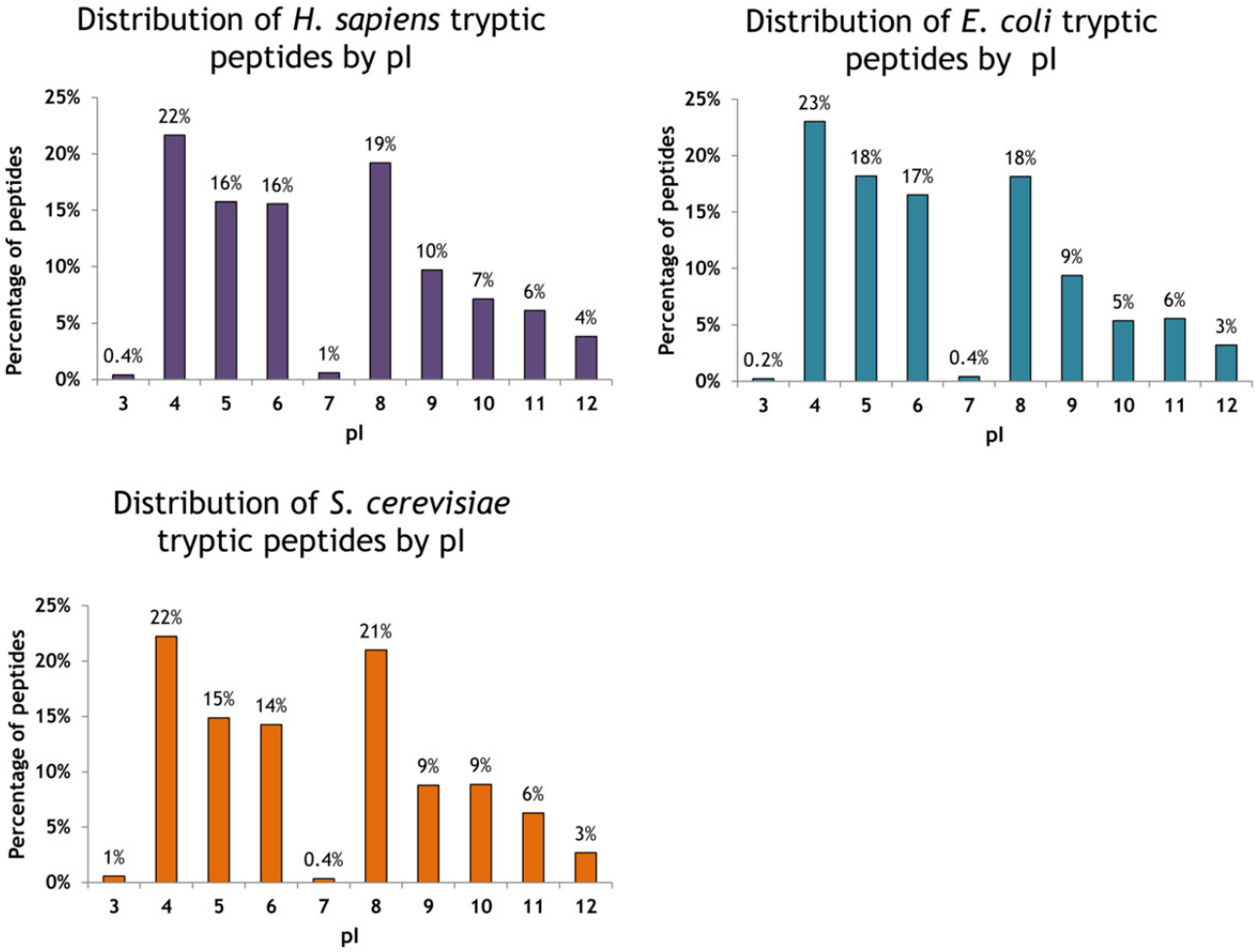
Supporting Figure 5. pI distribution of *in silico* generated tryptic peptides from *H. salinarum* with up to two missed cleavages.

Supporting Figure 6. Distribution of ion types resulting from UVPD of carbamylated and unmodified tryptic peptide anions from *H. salinarum*

Supporting Figure 7. Mass distribution of peptides identified uniquely in the carbamylated peptide data set (light bars) and peptides identified uniquely in the unmodified peptide data set (dark bars) for UVPD of a tryptic digest of *H. salinarum*.

Supporting Figure 8. Number of peptides identified uniquely in the unmodified data set sorted by elution time for a tryptic digest of *H. salinarum*.

Supporting Table 1. List of carbamylated BSA peptides and the chromatographic peak areas of the carbamylated peptide and its corresponding unmodified peptide. For the case of multiple (double and triple) carbamylations, each sequential modification is shown. CARB indicates the peptide modification at the N-terminus, and CARB(K) indicates the modification at the lysine side-chain.



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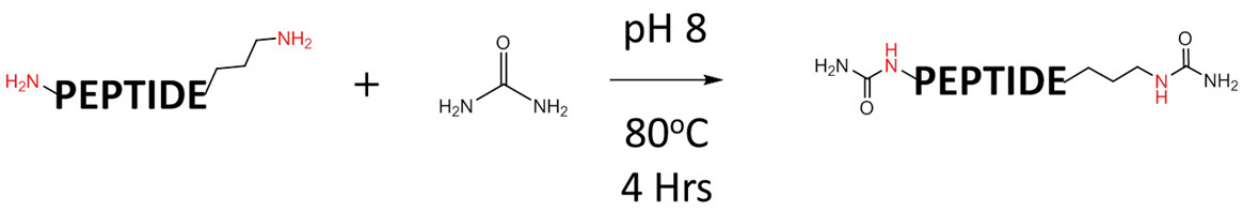

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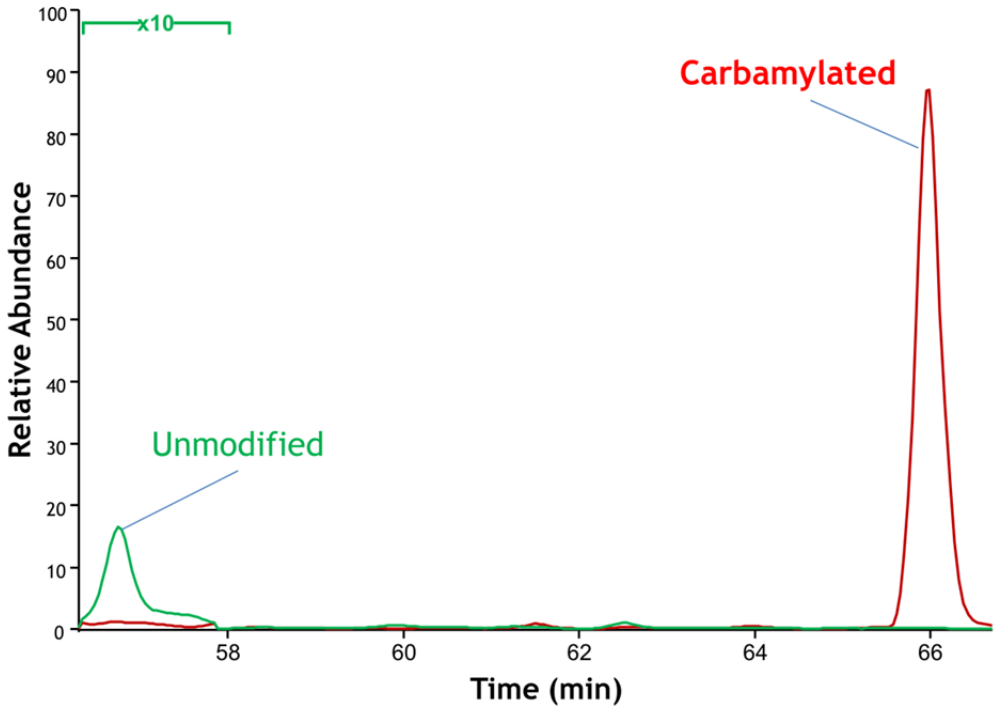

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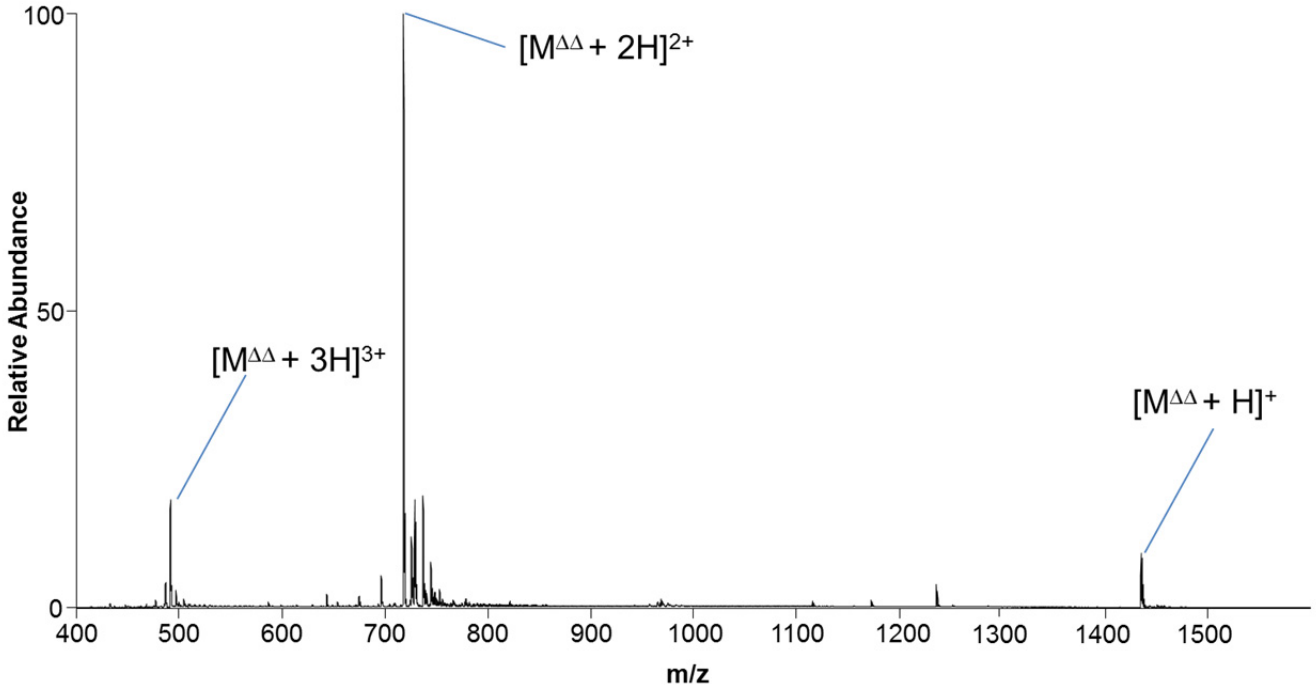

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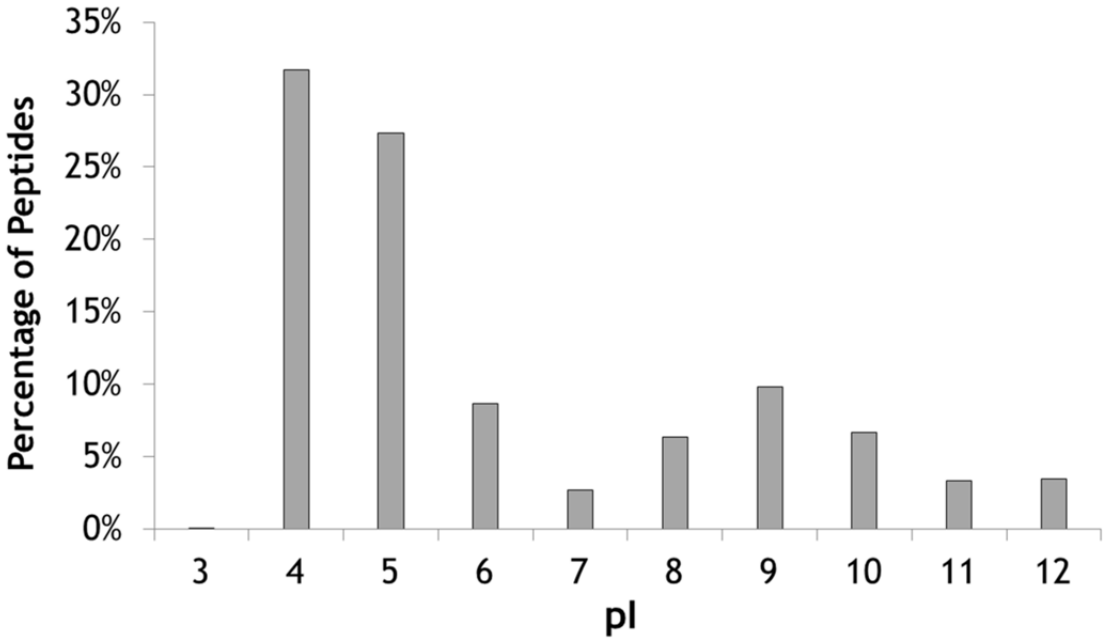

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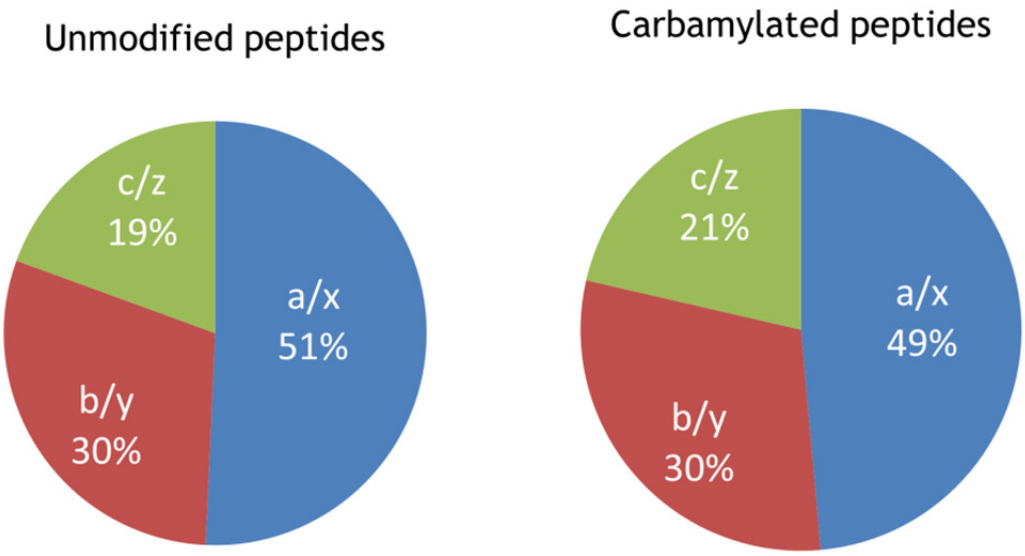
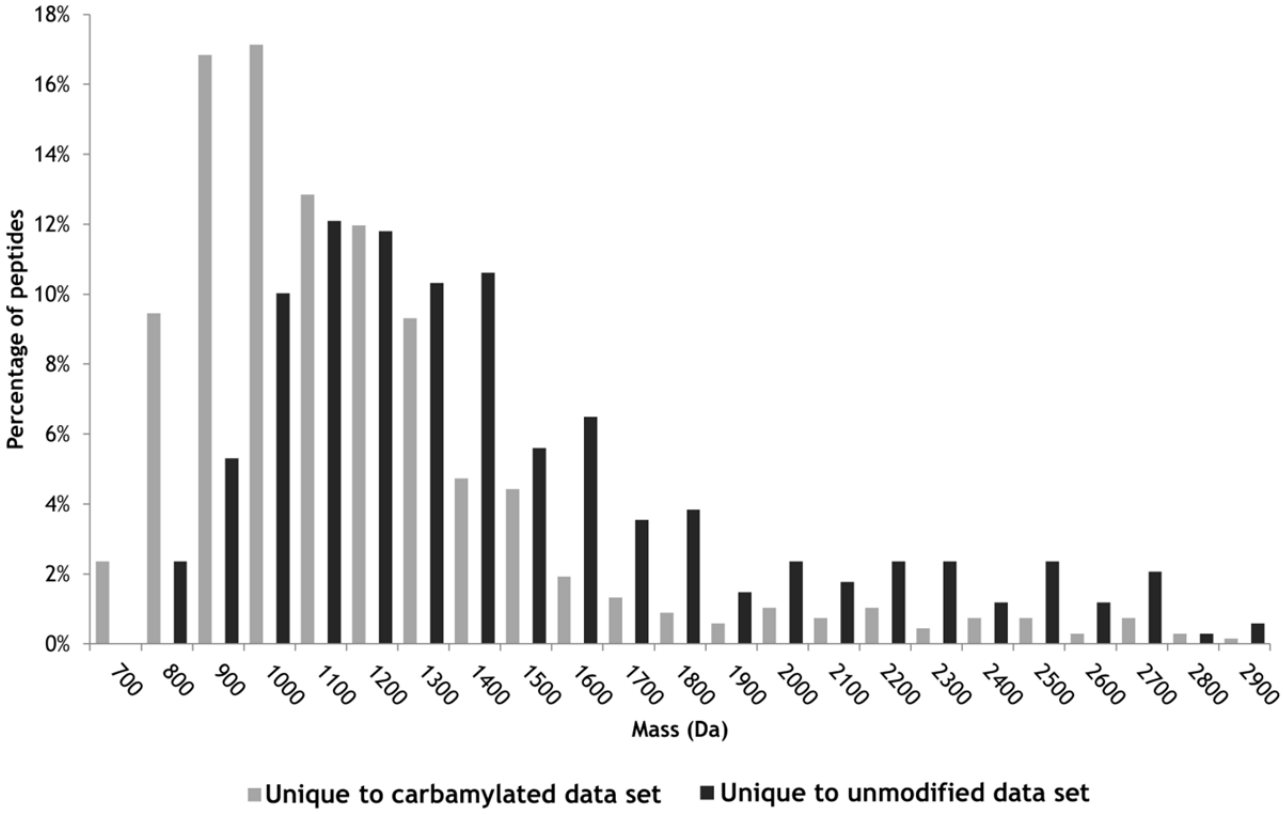


Supporting Figure 6. Distribution of ion types resulting from UVPD of unmodified and carbamylated tryptic peptide anions from *H. salinarum*



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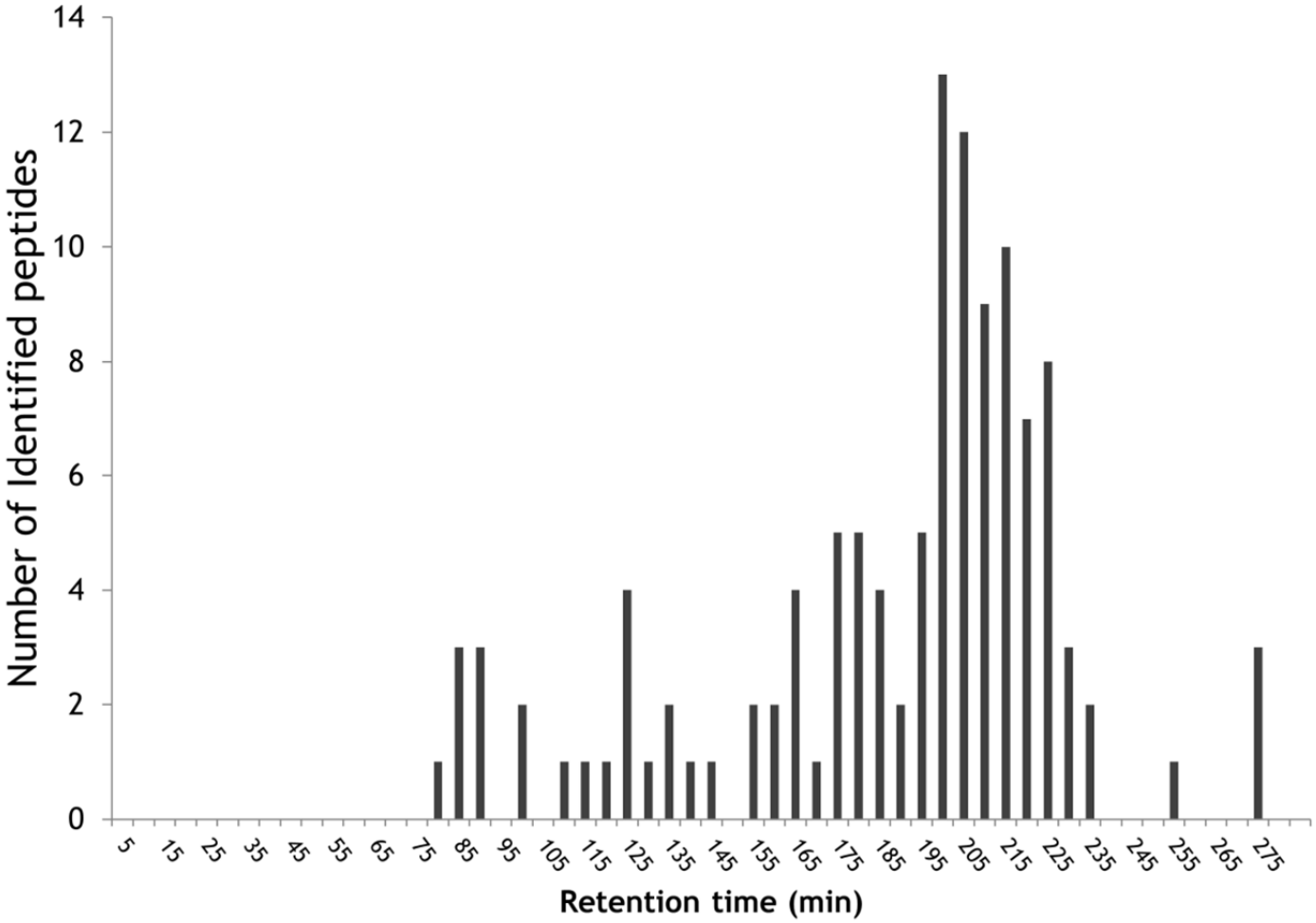

Supporting Figure 8. Number of peptides identified uniquely in the unmodified peptide data set sorted by elution time for a tryptic digest of *H. salinarum*.

Extent of Carbamylation of tryptic BSA peptides measured by (+) nano LC/MS/MS chromatographic peak areas										
Singly Carbamylated										
Peptide+modification	**Charge**	**m/z**	**Peak Area**	**Unmodified**	**Charge**	**m/z**	**Peak Area**	**Retention Time (min)**		
								shift from carbamylation	**%Unmod**	**%Mod**
LGEYGFQNALIVR + CARB	2+	761.90	654327139	LGEYGFQNALIVR	2+	740.40	5126023	*+16.25*	<1%	99%
DAFLGSFLYEYSR + CARB	2+	805.88	904112797	DAFLGSFLYEYSR	2+	784.37	4882691	*+24.01*	<1%	99%
LVVSTQTALA+ CARB	1+	1045.59	92343433	LVVSTQTALA	1+	1002.58	216255	*+17.20*	<1%	99%
LVVSTQTALA+ CARB	2+	523.29	1304867769	LVVSTQTALA	2+	501.79	4033497	*+17.09*	<1%	99%
Doubly Carbamylated										
LVNELTEFAK	2+	582.31	Not detected					Not observed		0
LVNELTEFAK + CARB	2+	603.82	4496449					65.93		1%
LVNELTEFAK + CARB + CARB(K)	2+	625.32	333178921					72.30		99%
Triply Carbamylated										
LKPDPNTLCDEFK	2+	760.37	1284970					42.83		<1%
LKPDPNTLCDEFK + CARB	2+	810.41	247578					48.05		<1%
LKPDPNTLCDEFK + CARB+ CARB(K)	2+	831.90	10378373					49.52		5%
LKPDPNTLCDEFK + CARB+CARB(K) +CARB(K)	2+	853.39	179823475					54.3		94%

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