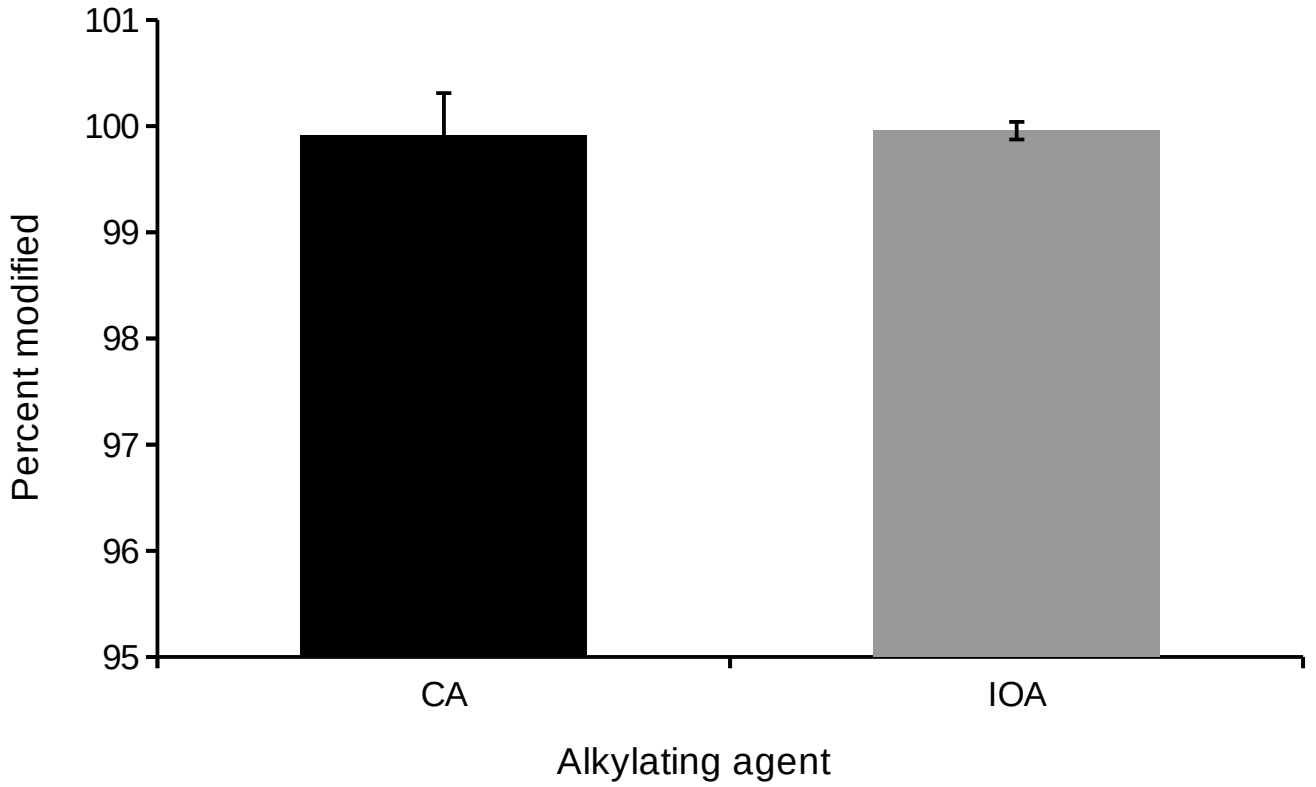
Supporting Information

The impact of commonly used alkylating agents on artefactual peptide modification

Peter G. Hains[1] and Phillip J. Robinson[1]

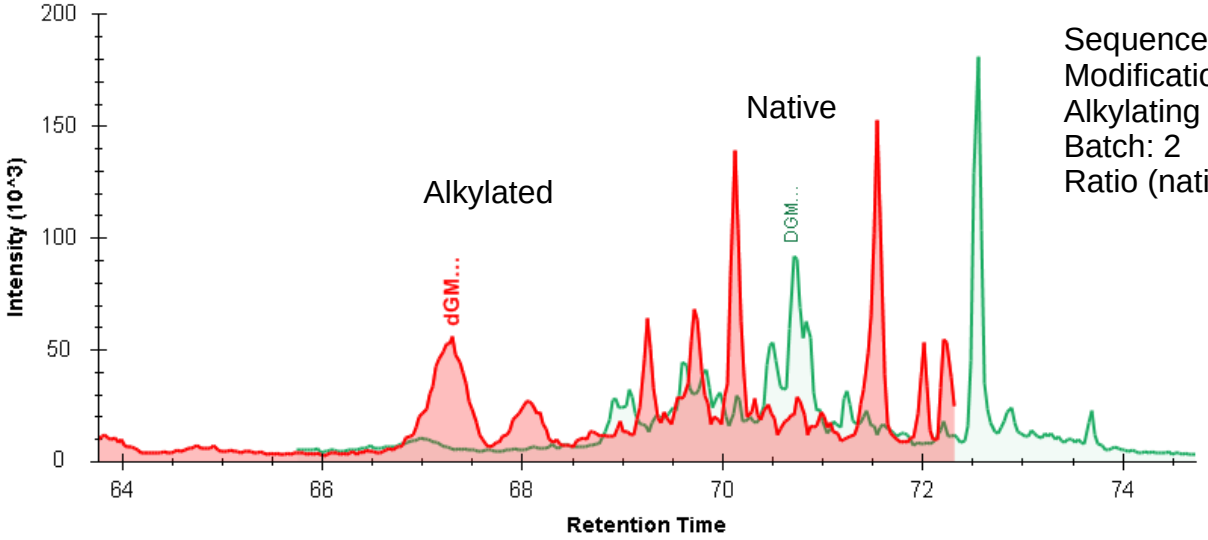
1- ProCan and the Cell Signalling Units, Children's Medical Research Institute, The University of Sydney, 214 Hawkesbury Rd, Westmead, NSW Australia, 2145.

Figure/Table	Description
Table S1	Tabulated peak area data for randomly selected representative peptide modifications.
Table S2	ProteomeXchange file names.
Figure S1	Percent alkylation following reaction with 2-chloroacetamide (CA) or iodoacetamide (IOA)
Figure S2A-I	Skyline MS1 extracted peak areas for alkylation side reaction peptides alkylated with either CA or IOA.
Figure S3	Level of deamidation (N,Q) and pyroGlu(Q,E) following reaction with CA or IOA.
Figure S4	Level of carbamylation (N-terminal or K) following reaction with CA or IOA.
Figure S5	Level of N-terminal carbamylation for samples processed under pressure (PCT), or at 1 atm (bench).
Figure S6	Level of pyro-Glu(Q) for samples processed under pressure (PCT), or at 1 atm (bench).
Figure S7A-J	Skyline MS1 extracted peak areas of oxidised (MSO, Wox, DiWox) and native peptides alkylated with either CA or IOA.
Figure S8	Level of MSO for samples processed using CA sourced from three different suppliers.
Figure S9	Level of oxidised W or dioxidised W for samples processed using CA sourced from three different suppliers.
Figure S10	Digestion statistics for treatment with three different suppliers of CA.
Figure S11	Level of MSO for data sourced from 2 published studies using CA.
Figure S12	Level of oxidised W or dioxidised W for data sourced from 2 published studies using CA.

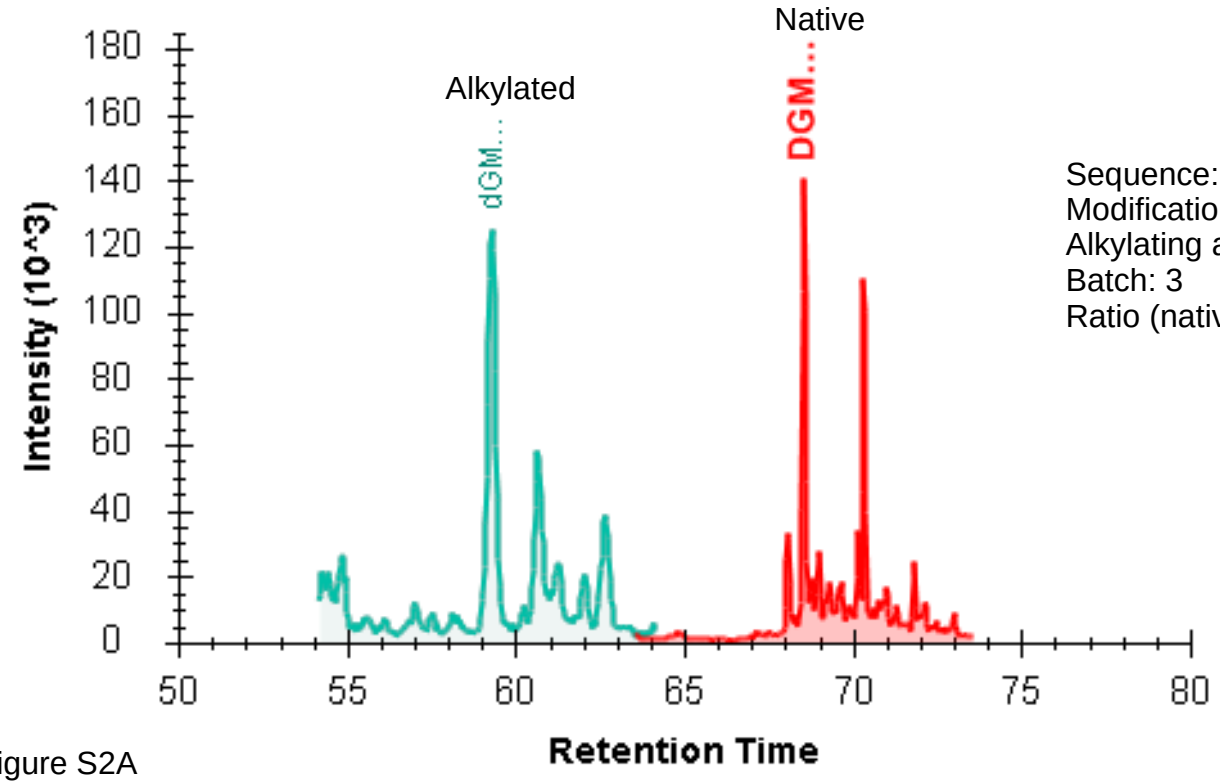


Percent alkylation following reaction with 2-chloroacetamide (CA) or iodoacetamide (IOA). Data is mean ±SD for 3 independent experiments. Note the scale used on the Y-axis.

Figure S1



Sequence: **D**GMYANLGIGIPVLASNYISPK
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 0.6



Sequence: **D**GMYANLGIGIPVLASNYISPK
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 3
Ratio (native/modified): 0.4

Figure S2A

Sequence: **N**QVAMNPTNTIFDAK
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 3.6

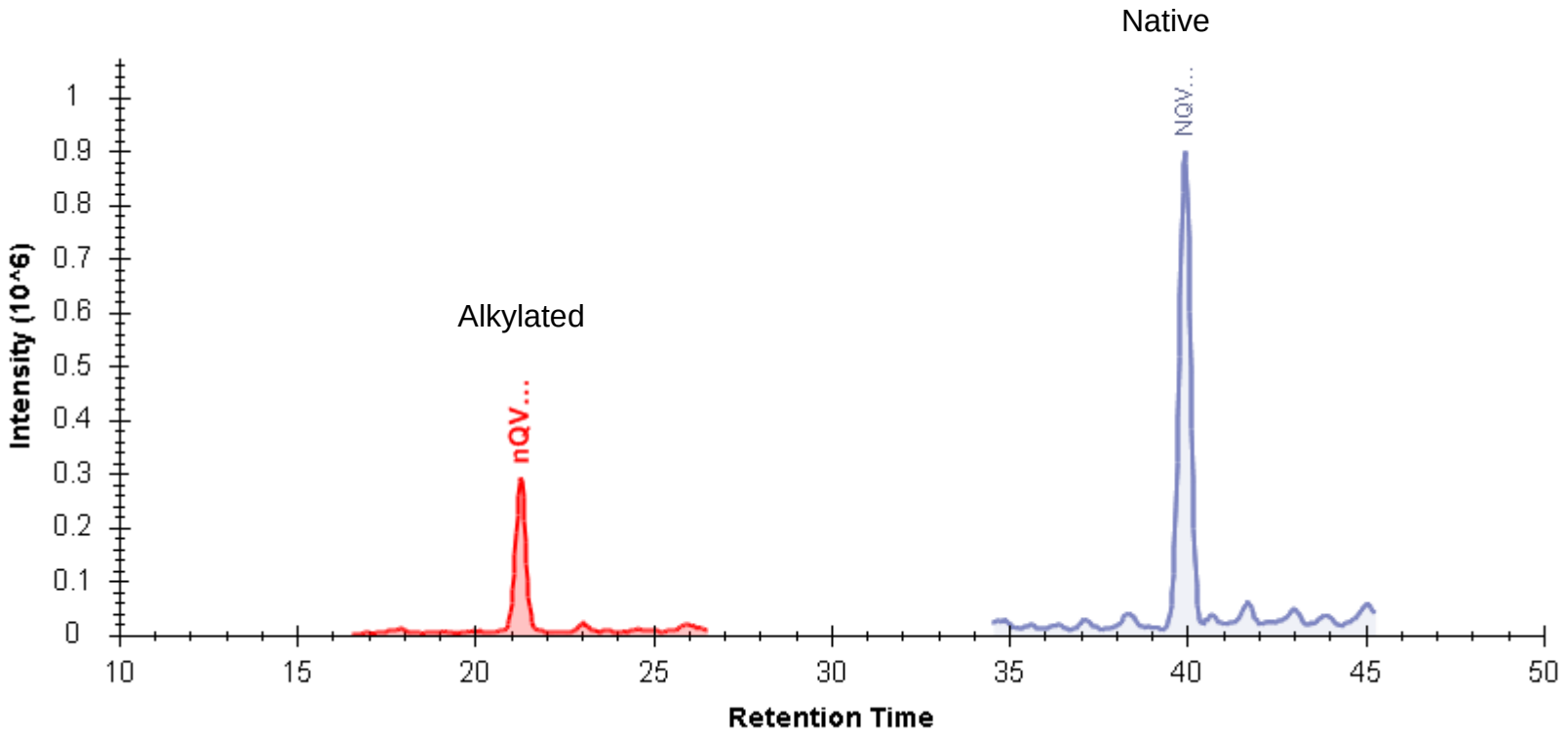


Figure S2B

Sequence: IINEPTAAAIAYGLDK
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 99.5

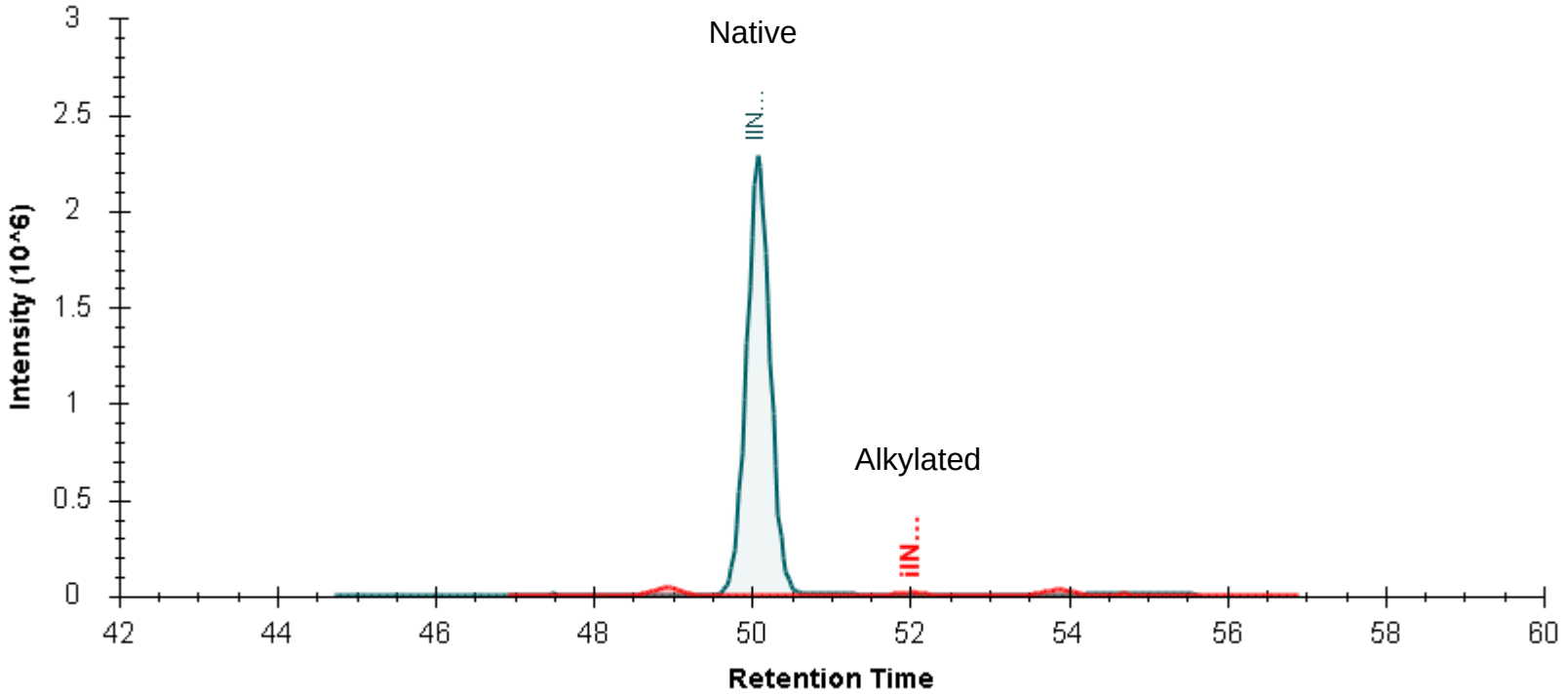


Figure S2C

Sequence: **P**MILGYWNVR
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 31.7

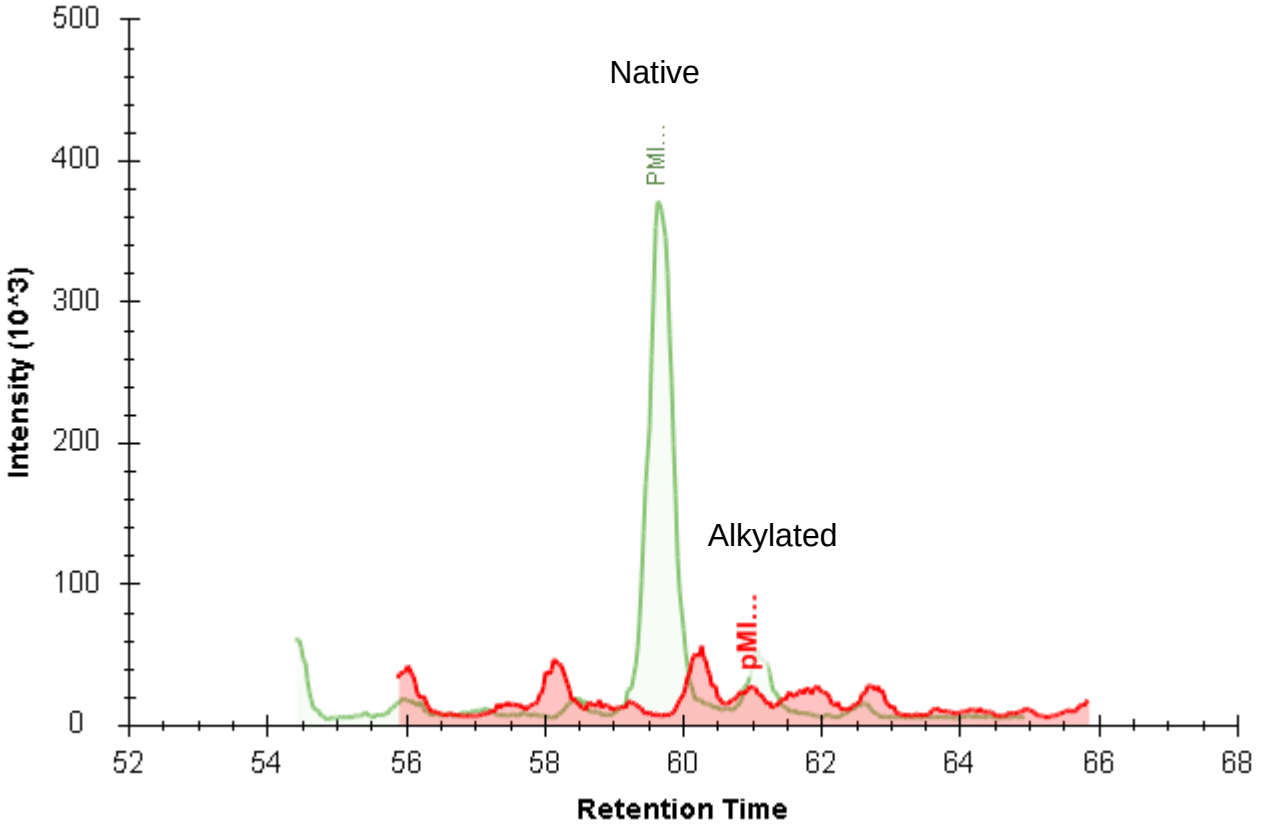


Figure S2D

Sequence: **A**MGIMNSFVNDIFER
Modification: N-terminal alkylation
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 2.0

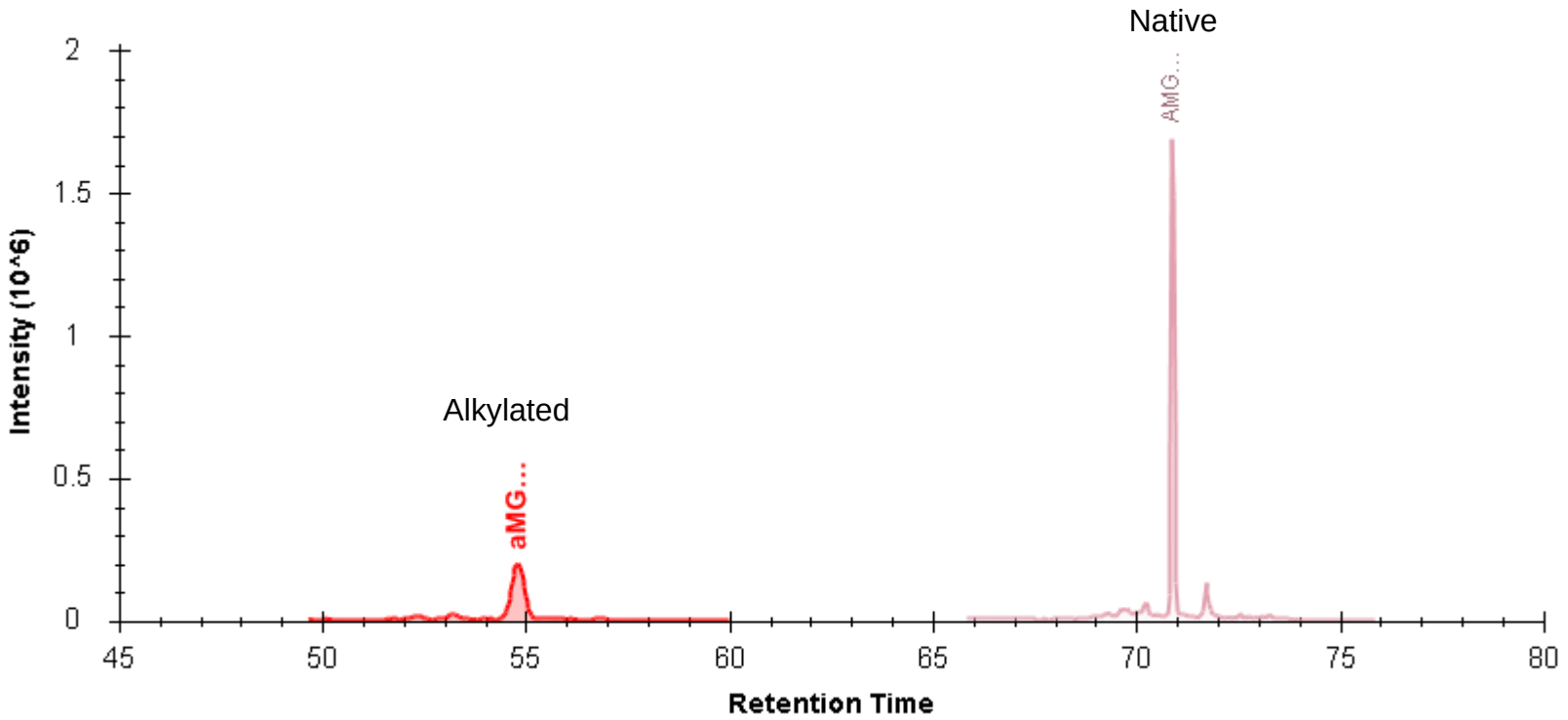


Figure S2E

Sequence: LGAGYPMGPF**E**LLDYVGLDTTK
Modification: Alkylated E
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 1.1

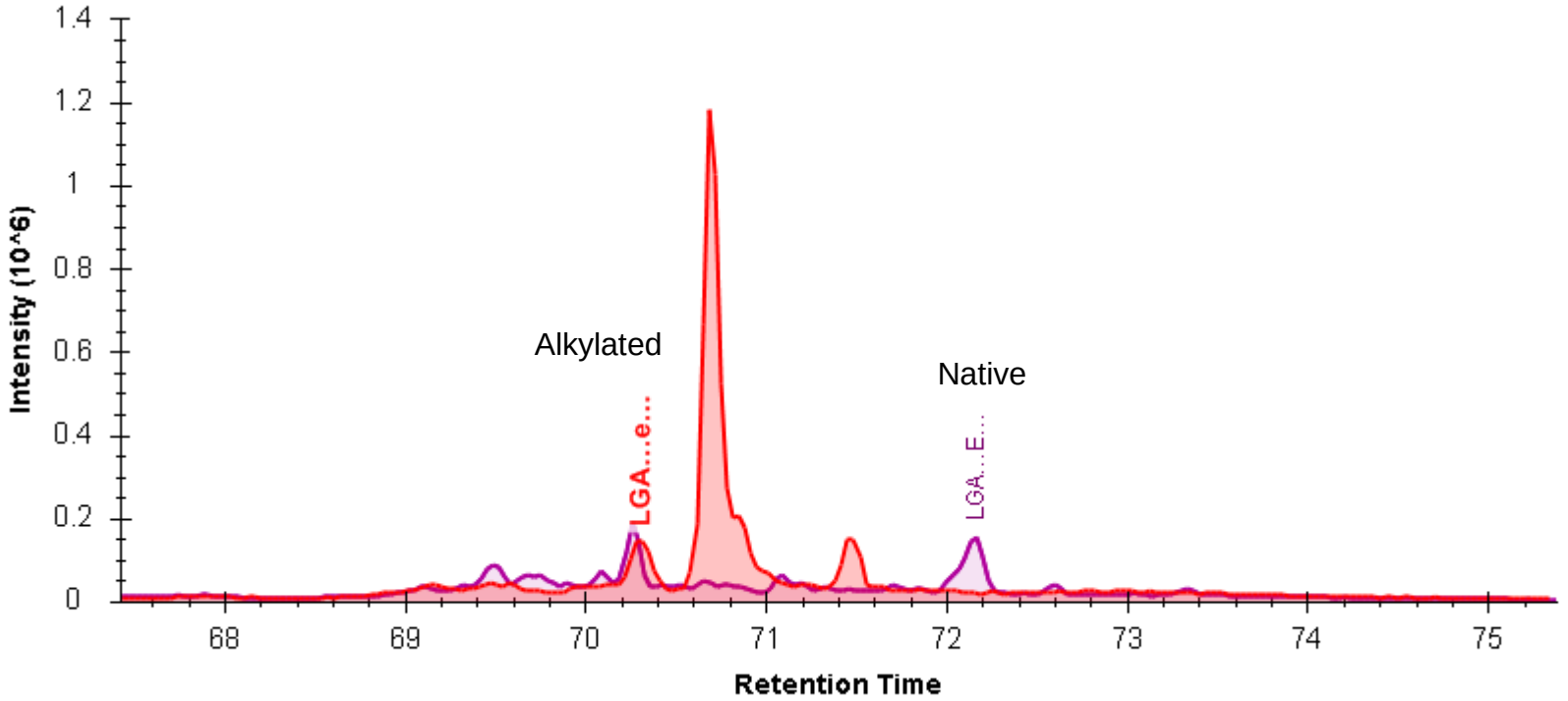


Figure S2F

Sequence: LEL**E**MTPQGTLAER
Modification: Alkylated E
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 2.1

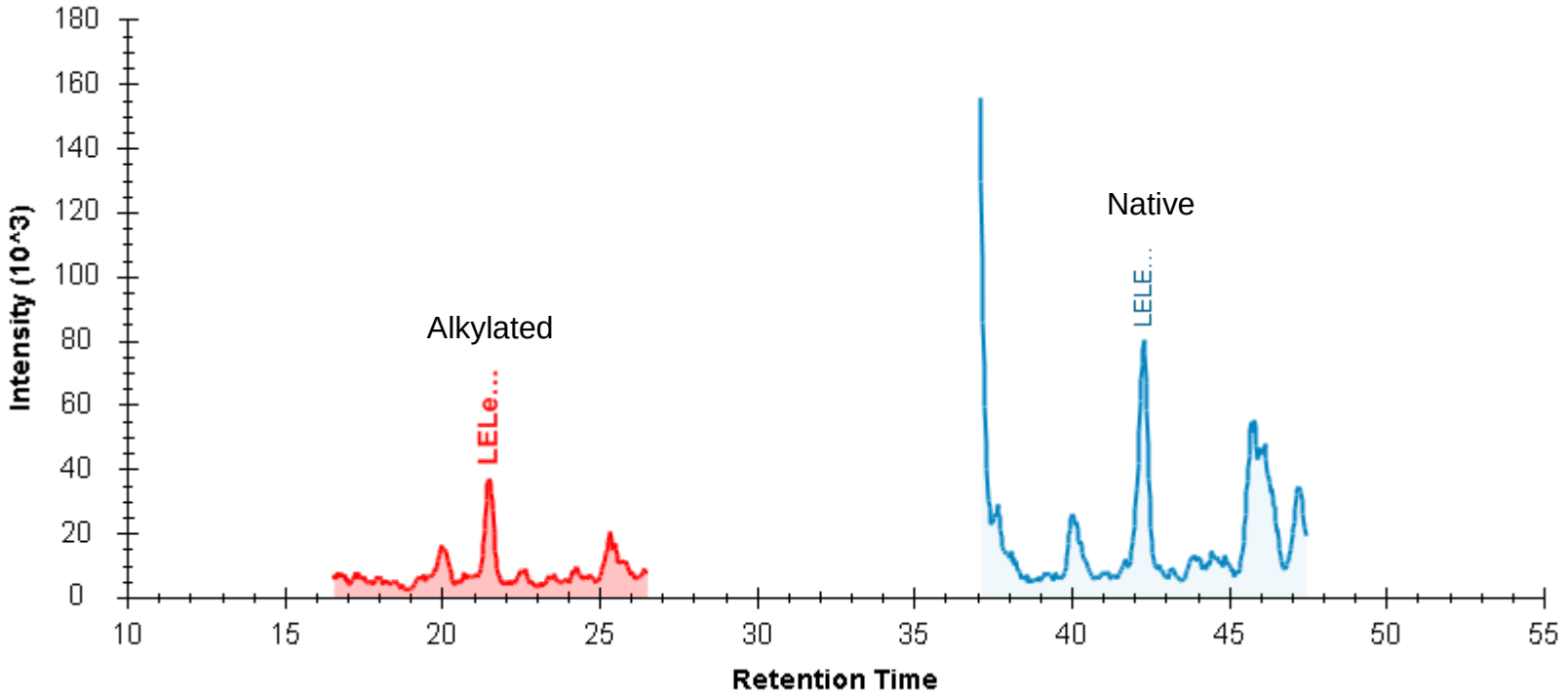


Figure S2G

Sequence: NLHNITGVLM**T**DSDFVSAVK
Modification: Alkylated T
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 2.3

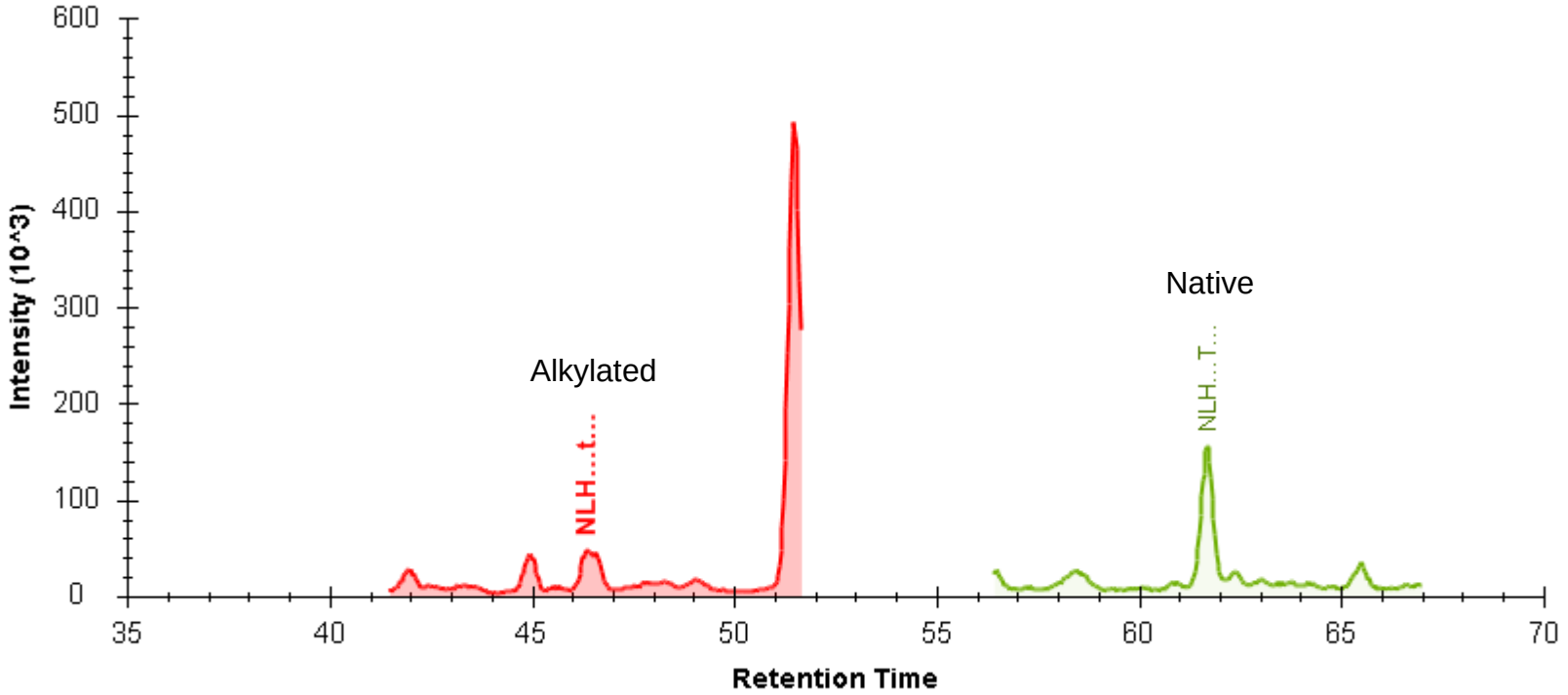


Figure S2H

Sequence: IQLM**Y**IWVDGTGEGLR
Modification: Alkylated Y
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 0.6

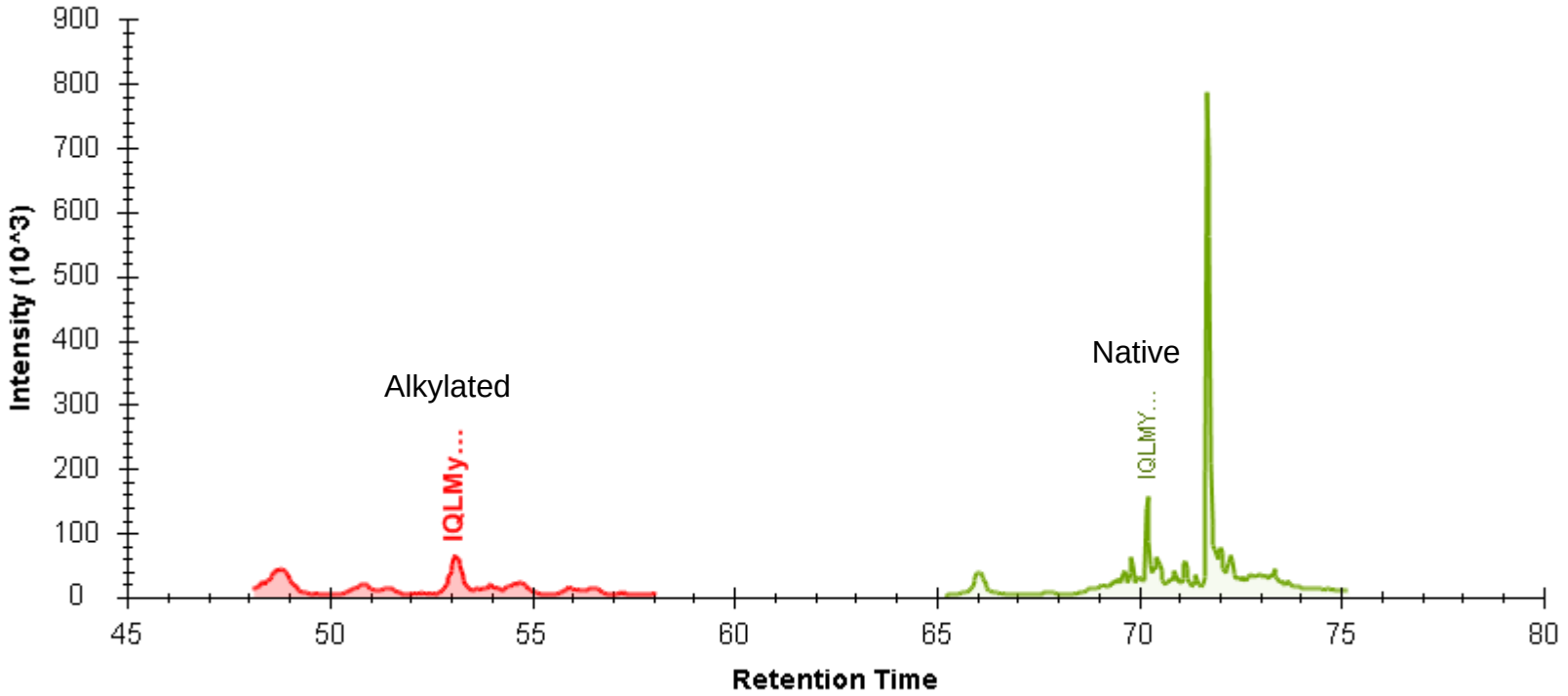
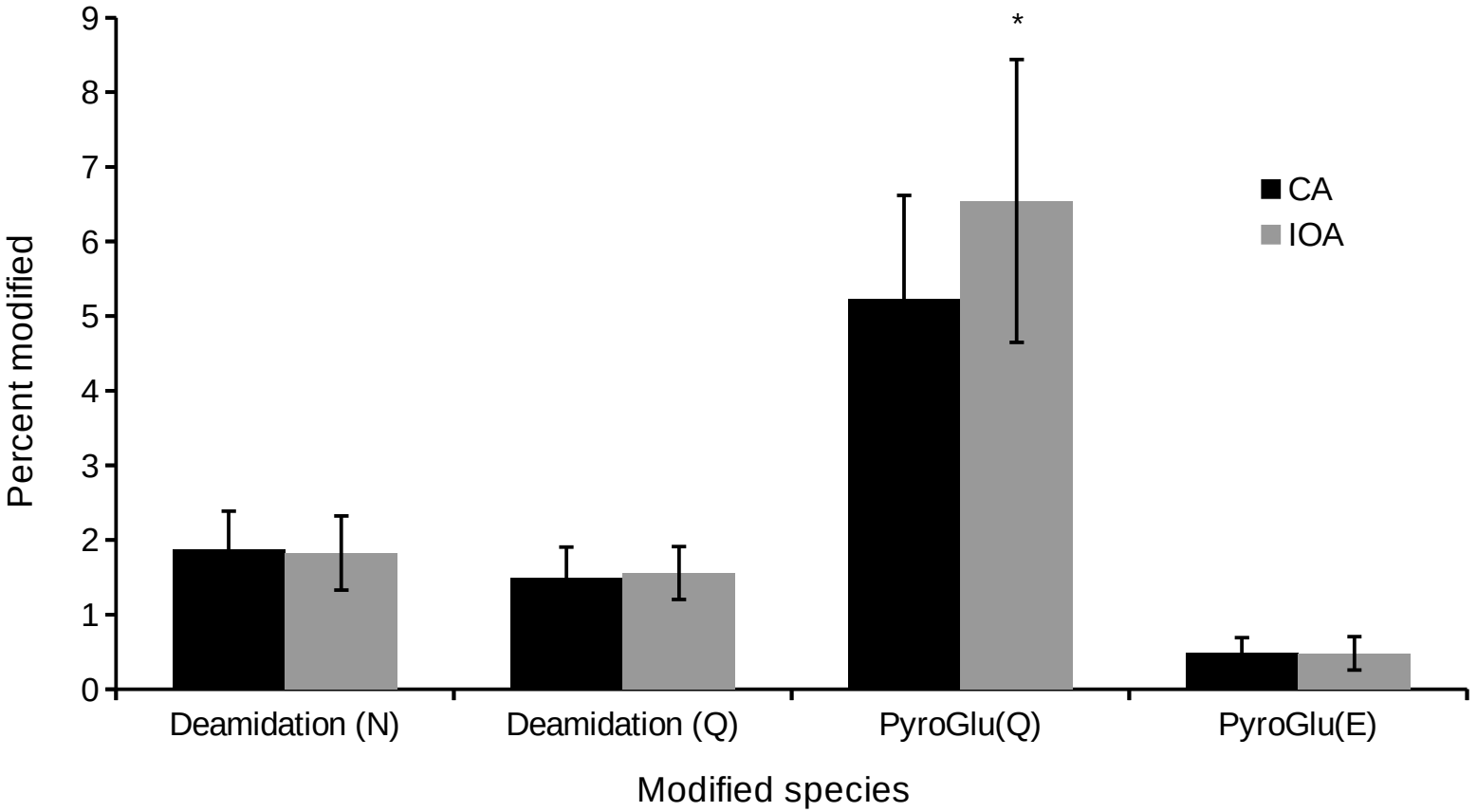
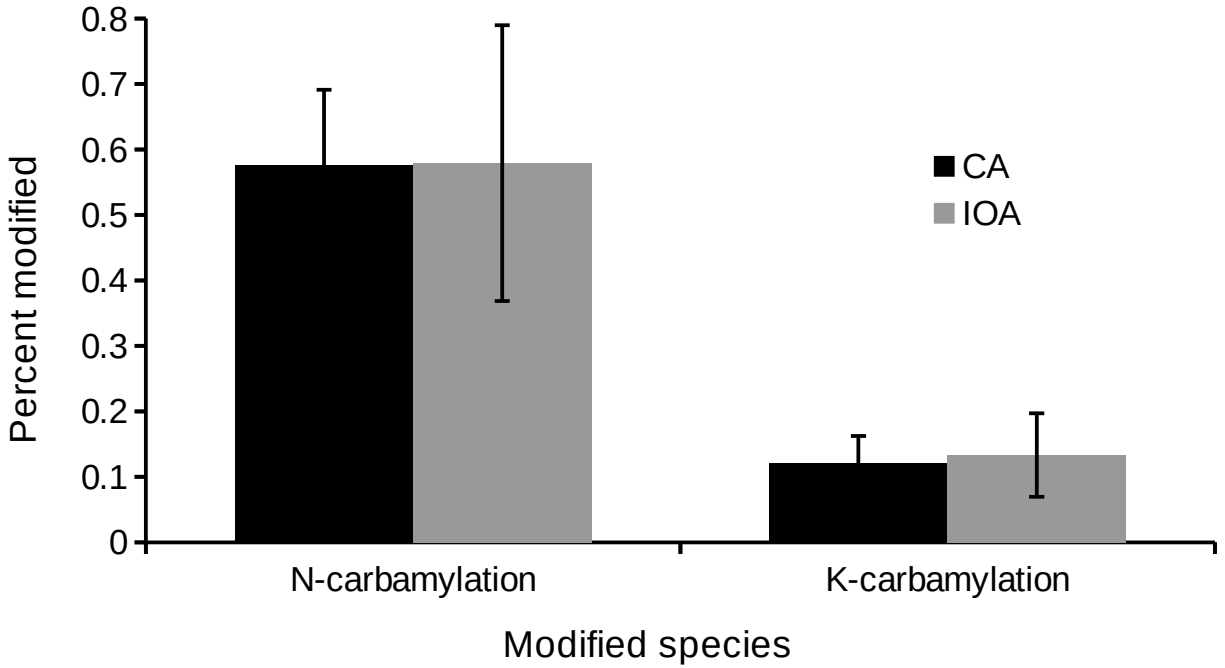


Figure S2I



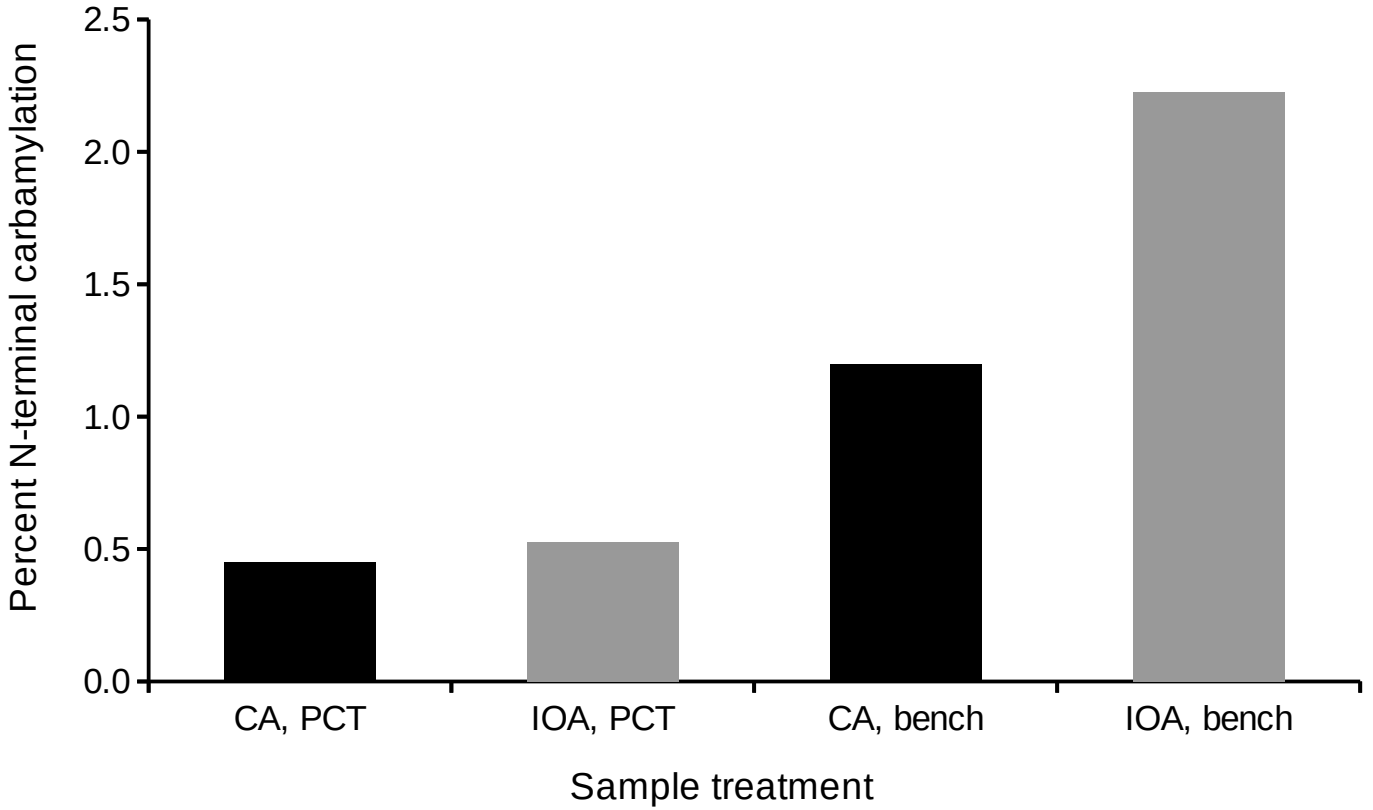
Level of deamidation (N,Q) and pyroGlu(Q,E) following reaction with CA or IOA. Data is mean ±SD for 3 independent experiments. An asterisk (*) indicates a significant difference between CA and IOA treated samples. Significance is at least $p < 0.05$. It is likely the result is only significant given the large number of individual samples used in the study, as the standard deviation clearly overlaps between the two datasets.

Figure S3



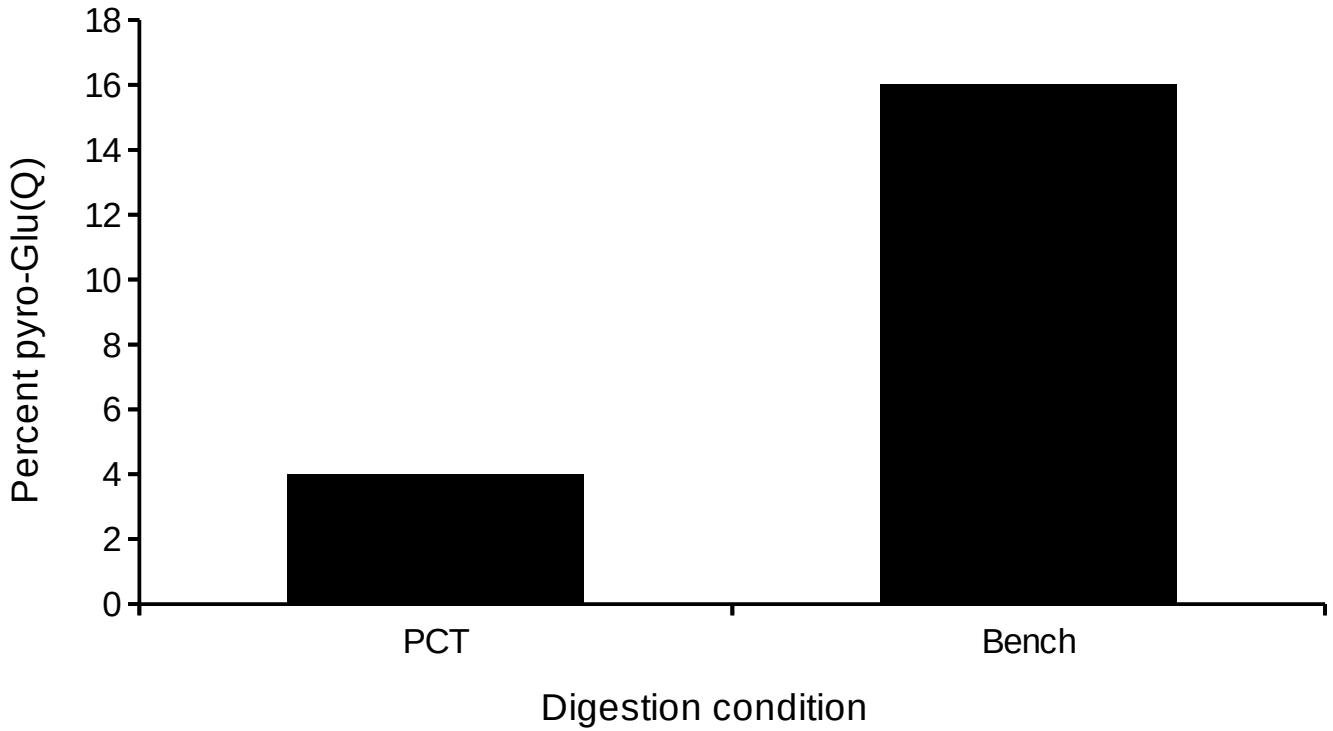
Level of carbamylation (N-terminal or K) following reaction with CA or IOA. Data is mean ±SD for 3 independent experiments.

Figure S4



Level of N-terminal carbamylation for samples processed under pressure (PCT), or at 1 atm (bench). Samples are further divided into CA or IOA alkyated. Data is presented as the mean of 4 samples performed in 1 experiment.
Whilst the IOA treated bench samples do show higher levels of carbamylation compared to the CA treated samples, a greater number of experiments is required to test this hypothesis. Note there was no significant difference for the CA vs IOA treated samples (N-terminal carbamylation), with 3 separate sets of experiments and 8 samples in each group (Fig S4).

Figure S5



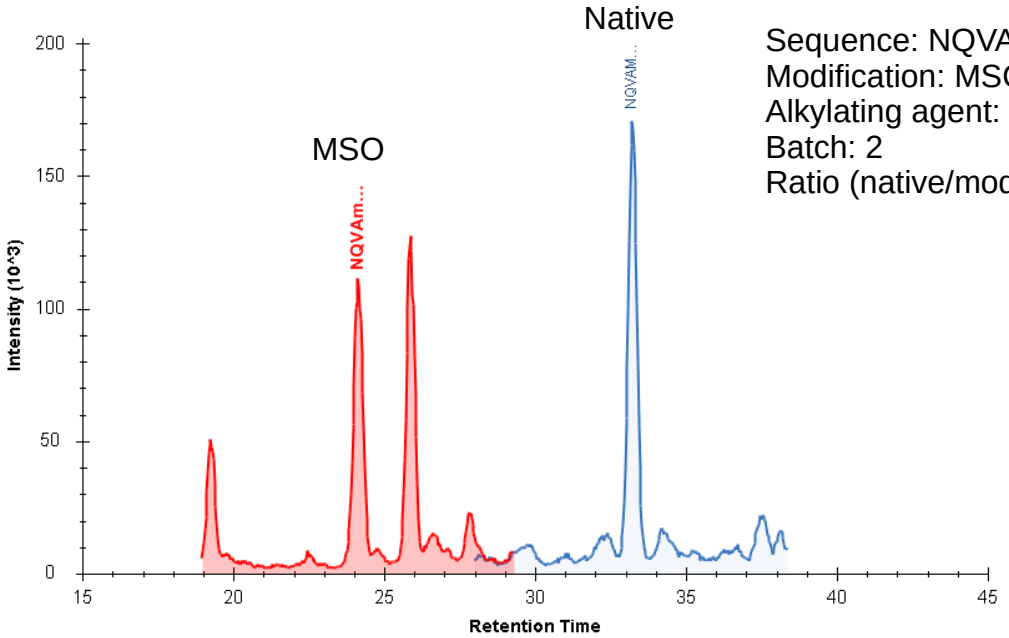
Level of pyro-Glu(Q) for samples processed under pressure (PCT), or at 1 atm (bench). Data is presented as the mean of 8 samples performed in 1 experiment. Note the CA and IOA alkylated samples were pooled after no significant difference (*t*-test) was found between the groups.

Figure S6

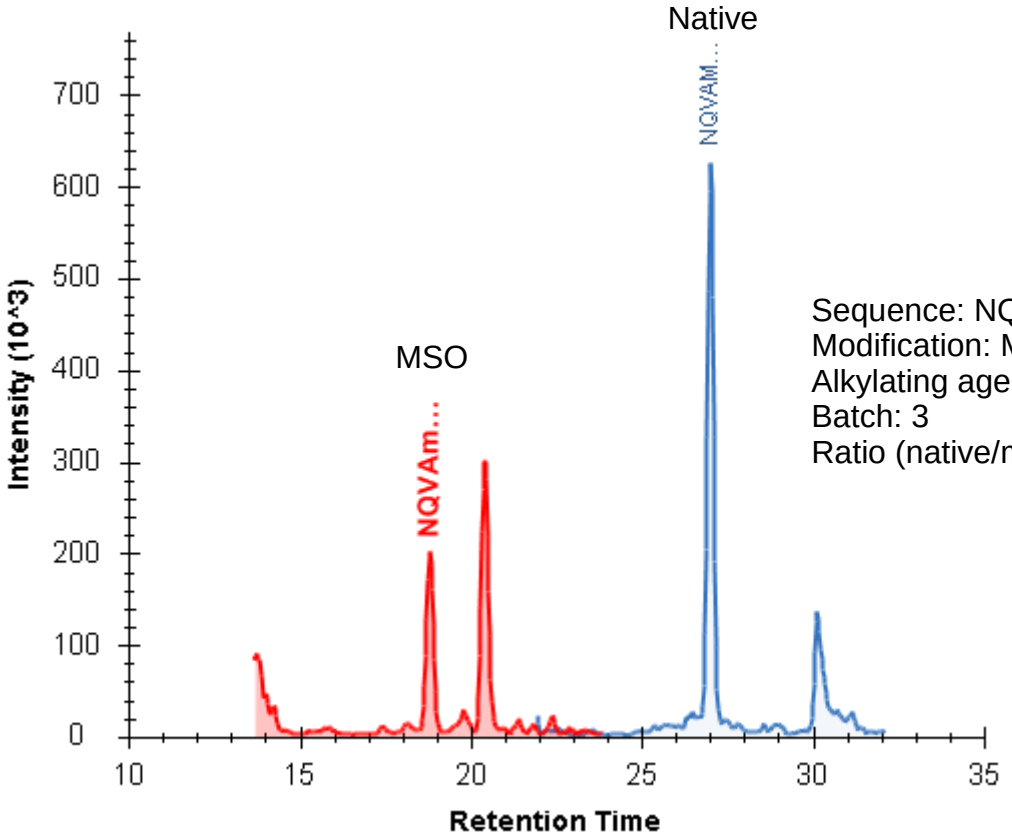
Note that two identical LC-MS/MS systems were used to collect the data. Whilst identical in terms of hardware and methods, we still see variations in peptide retention time between the systems.

Each system ran both CA and IOA treated samples, they were not used exclusively.

These variations are systematic and reproducible.



Sequence: NQVA**M**NPTNTVFDAK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 1.5



Sequence: NQVA**M**NPTNTVFDAK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 2.7

Figure S7A

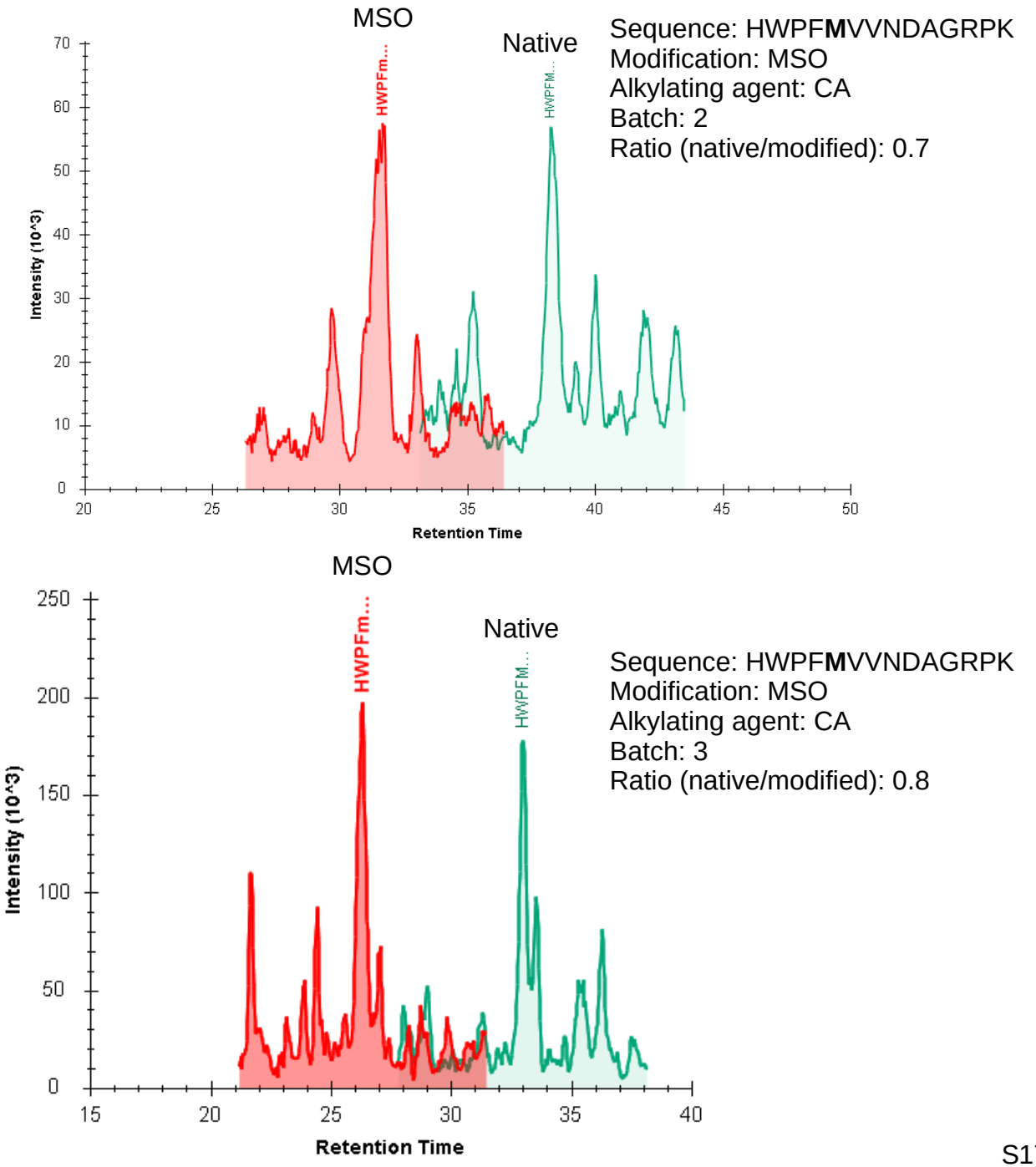
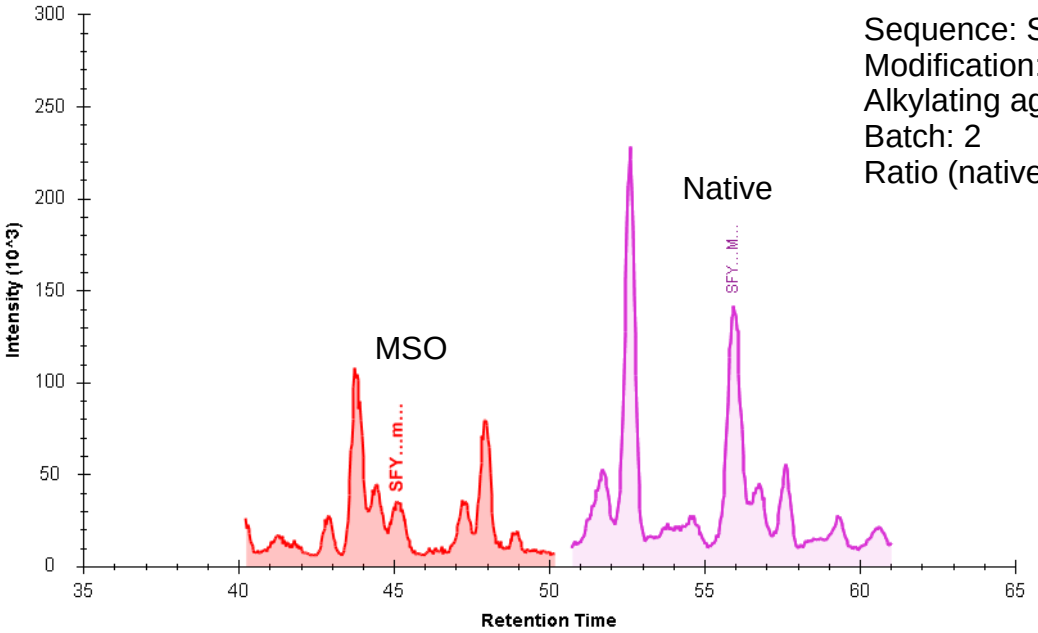
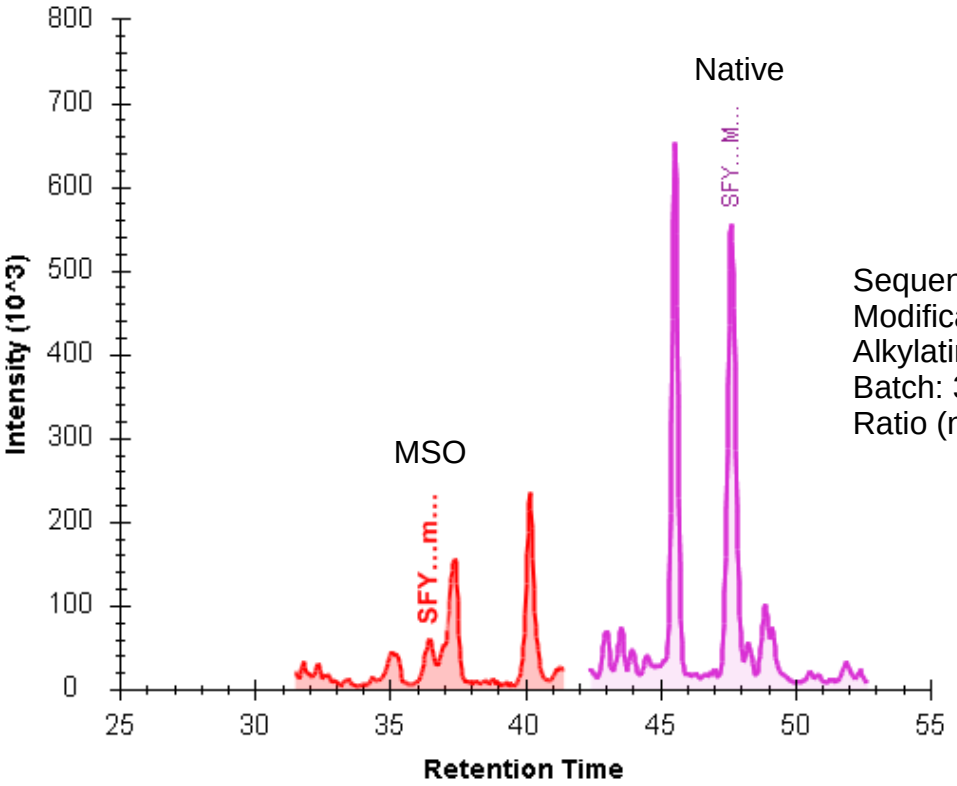

MSO
Native
Sequence: HWPFMVVNDAGRPK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 0.7
HWPFm...
HWPFM...
Intensity (10^3)
Retention Time
MSO
Native
Sequence: HWPFMVVNDAGRPK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 0.8
HWPFm...
HWPFM...
Intensity (10^3)
Retention Time

Figure S7B

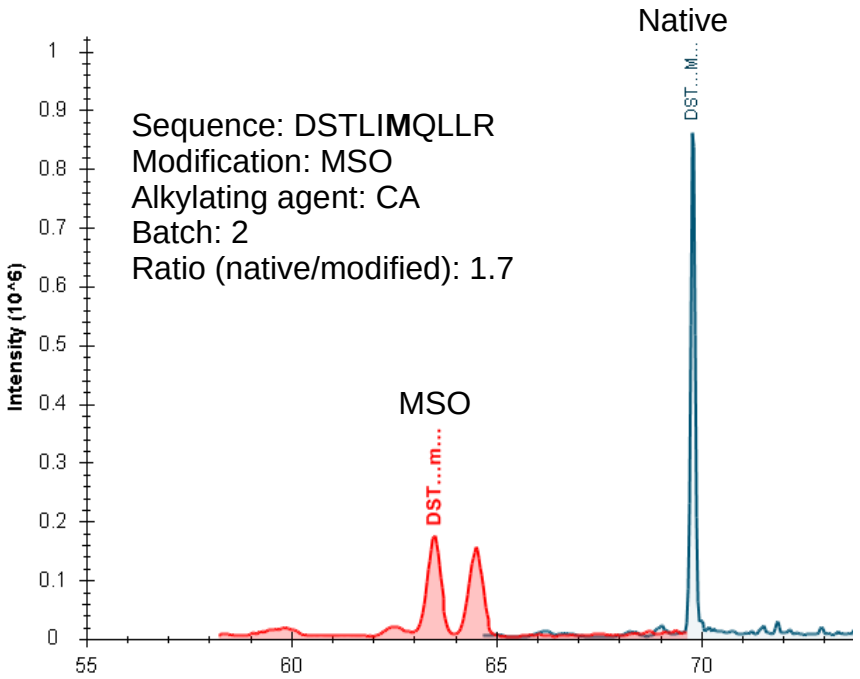


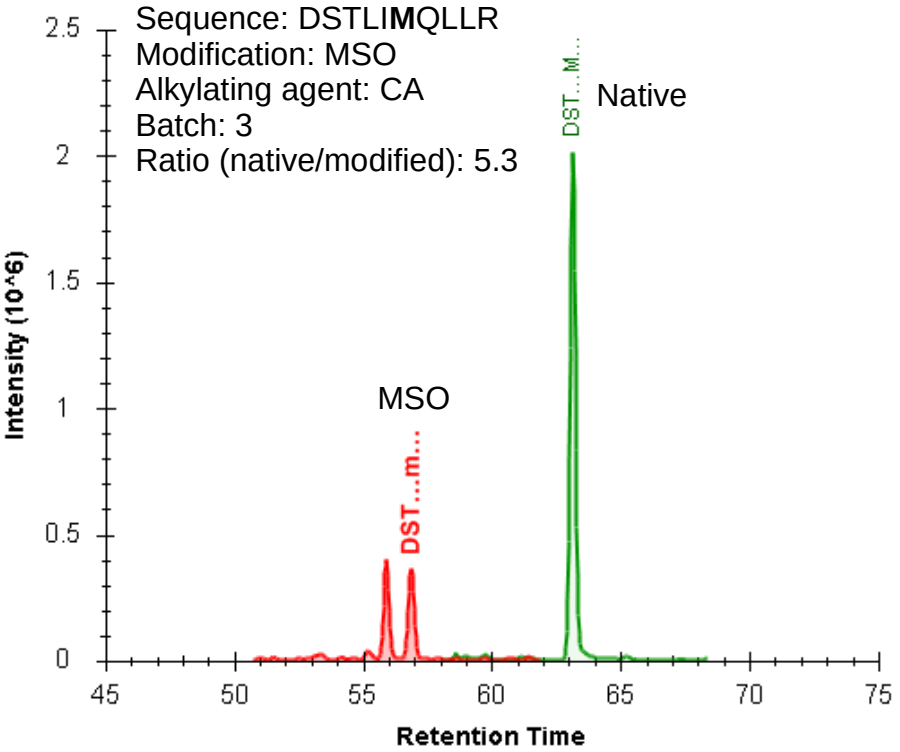
Sequence: SFYPEEVSS**M**VLTK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 4.6



Sequence: SFYPEEVSS**M**VLTK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 5.8

Figure S7C


Native
Sequence: DSTLIMQLLR
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 1.7
MSO
DST...m...
DST...M...
Intensity (10^6)


Sequence: DSTLIMQLLR
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 5.3
Native
MSO
DST...m...
DST...M...
Intensity (10^6)
Retention Time

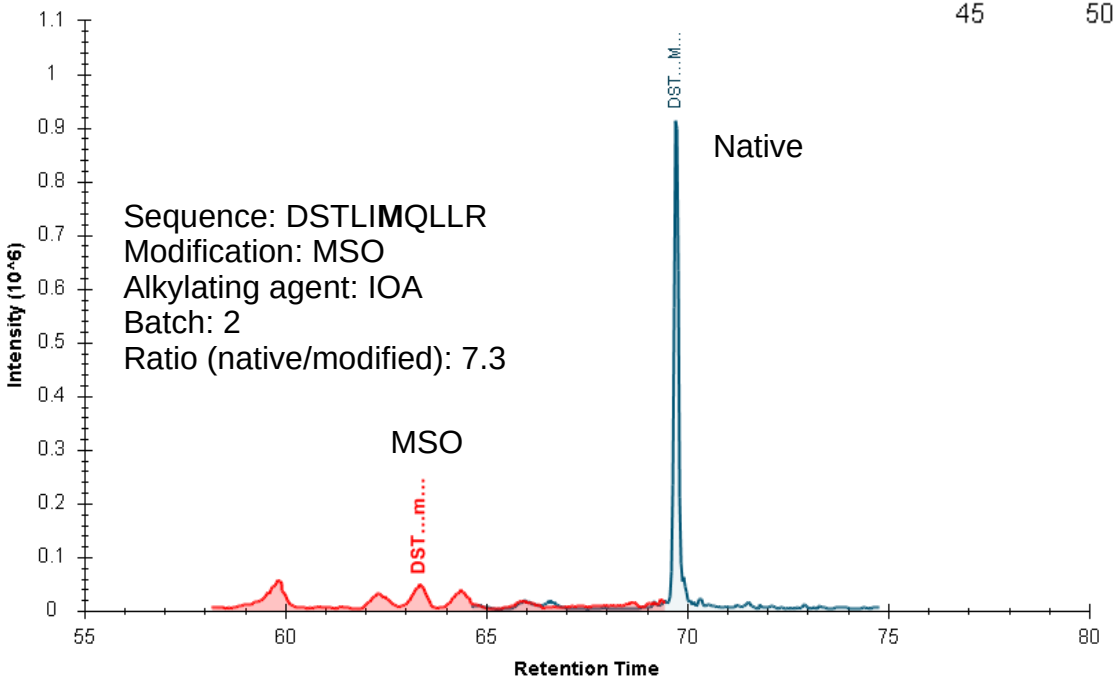
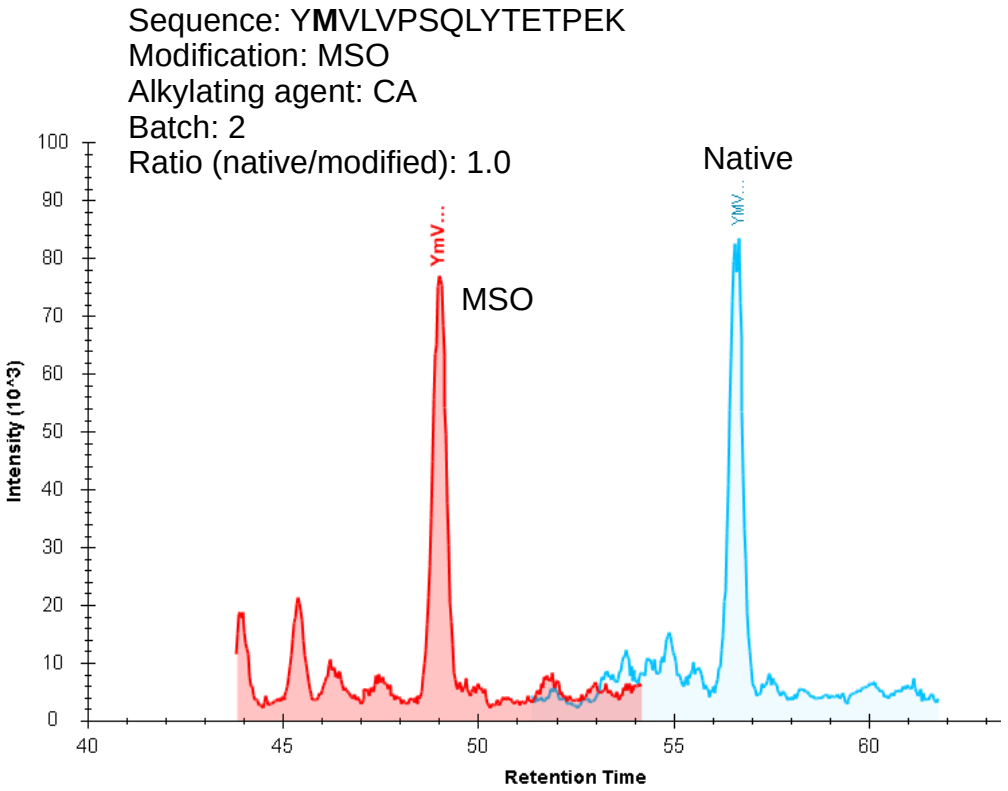

Native
Sequence: DSTLIMQLLR
Modification: MSO
Alkylating agent: IOA
Batch: 2
Ratio (native/modified): 7.3
MSO
DST...m...
DST...M...
Intensity (10^6)
Retention Time

Figure S7D


Sequence: YMVLVPSQLYTETPEK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 1.0
Native
MSO
YmV...
YMV...
Intensity (10^3)
Retention Time

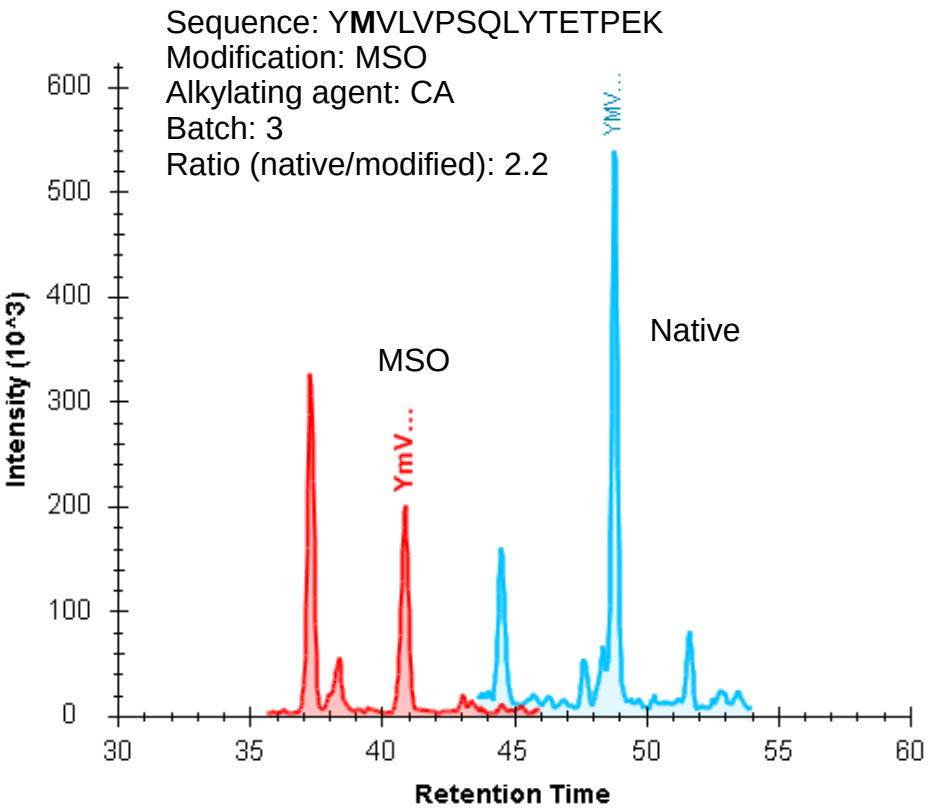
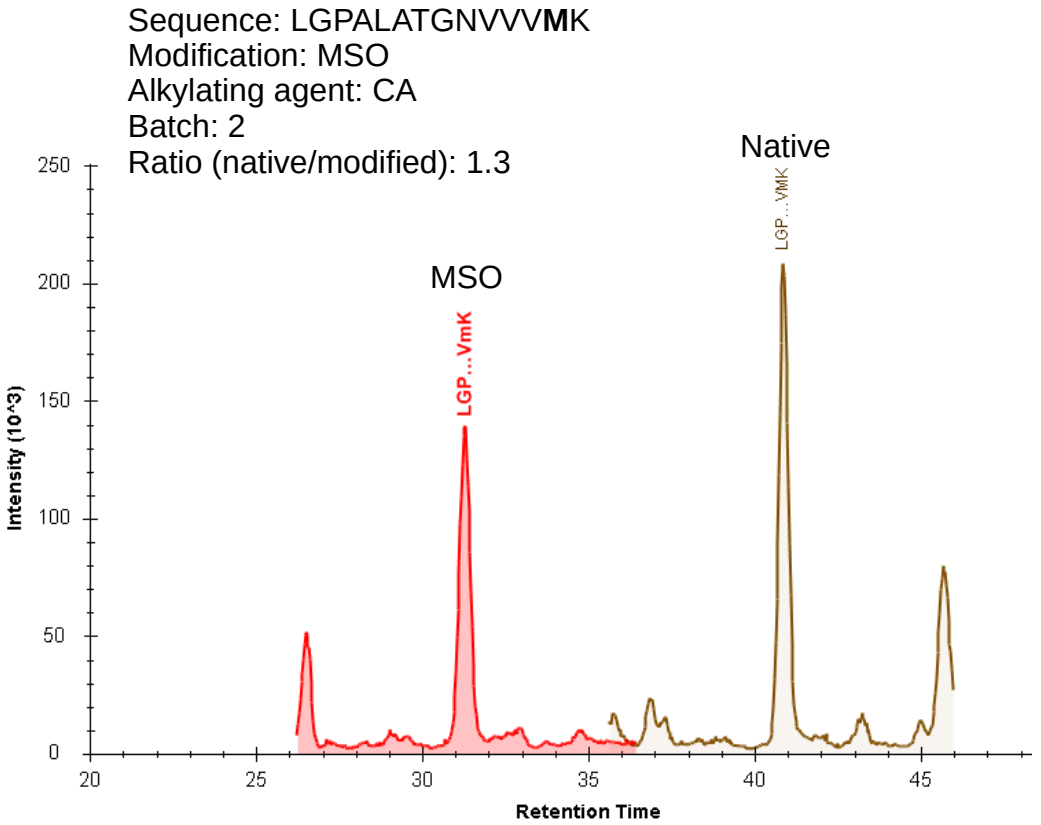

Sequence: YMVLVPSQLYTETPEK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 2.2
Native
MSO
YmV...
YMV...
Intensity (10^3)
Retention Time

Figure S7E


Sequence: LGPALATGNVVV**M**K
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 1.3
Native
MSO
LGP...VmK
LGP...VMK
Intensity (10^3)
Retention Time

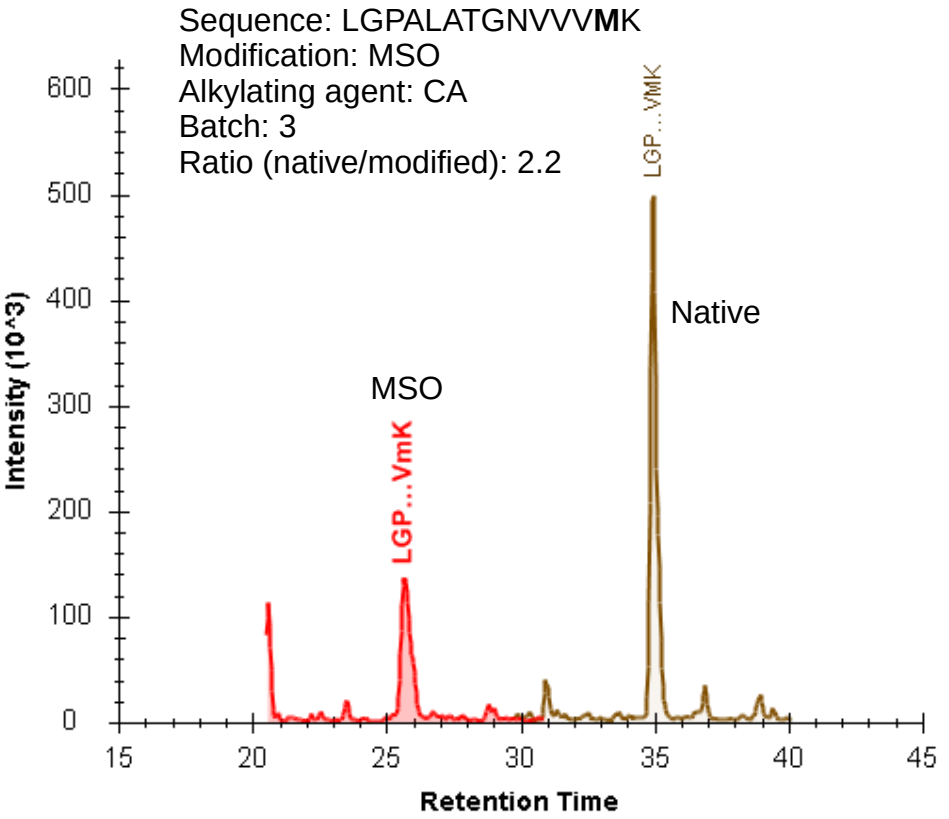
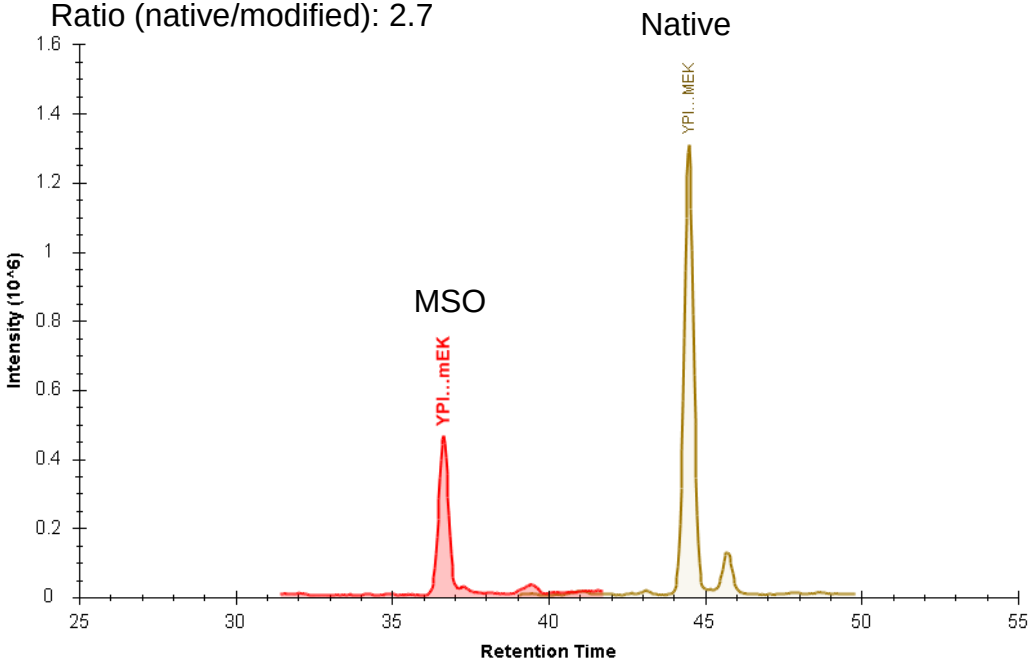

Sequence: LGPALATGNVVV**M**K
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 2.2
LGP...VMK
Native
MSO
LGP...VmK
Intensity (10^3)
Retention Time

Figure S7F

Sequence: YPIEHGIITNWDD**M**EK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 2.7



Sequence: YPIEHGIITNWDD**M**EK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 3.2

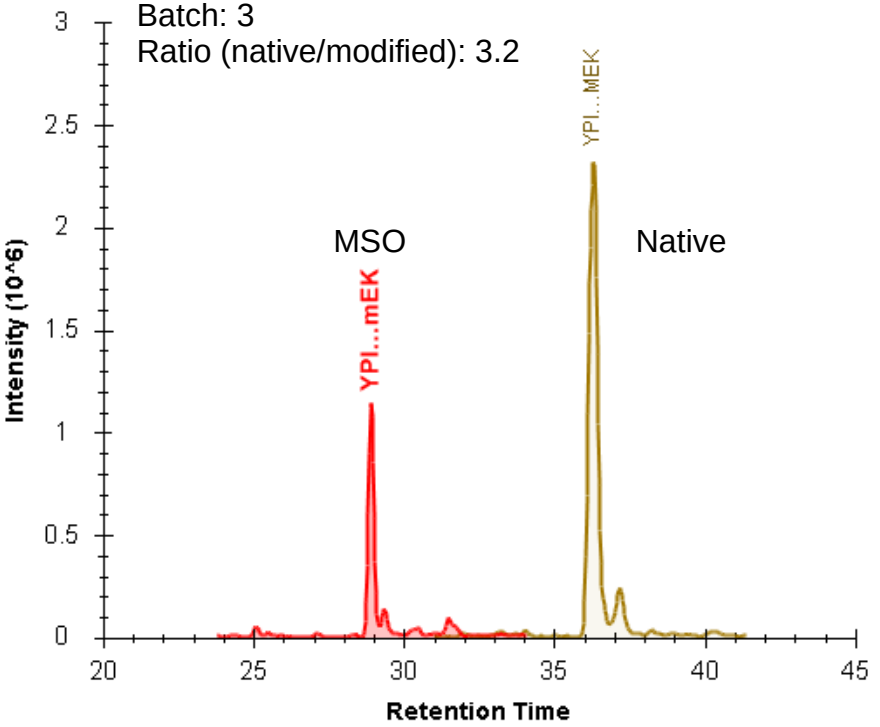
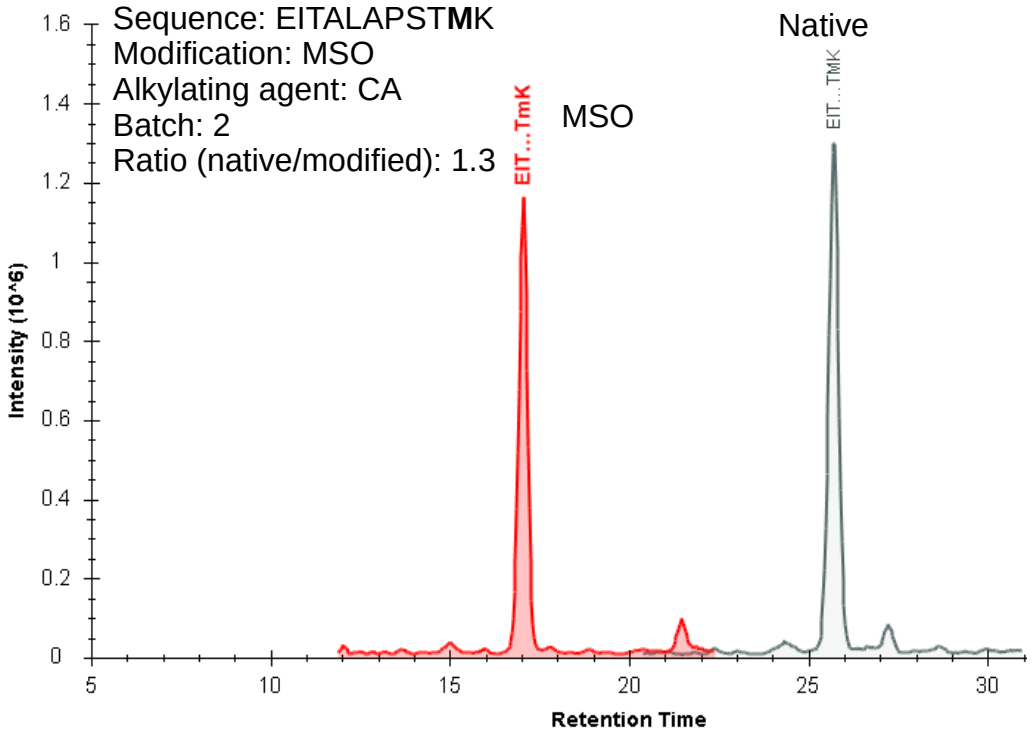
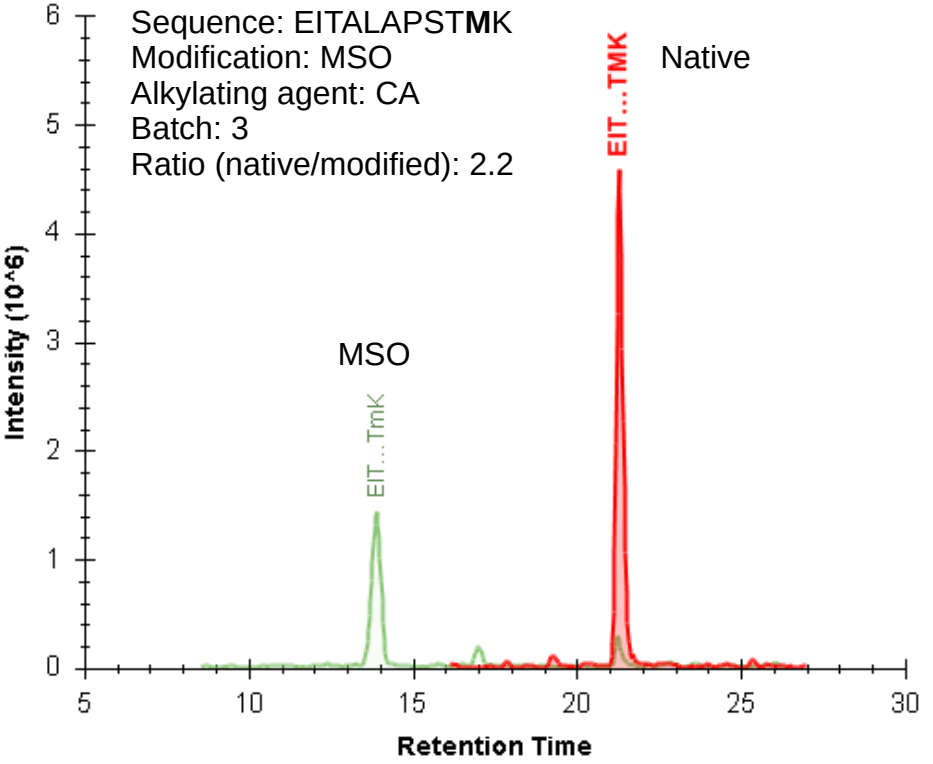


Figure S7G


Sequence: EITALAPSTMK
Modification: MSO
Alkylating agent: CA
Batch: 2
Ratio (native/modified): 1.3
Native
MSO
EIT...TMK
EIT...TmK
Intensity (10^6)
Retention Time


Sequence: EITALAPSTMK
Modification: MSO
Alkylating agent: CA
Batch: 3
Ratio (native/modified): 2.2
Native
MSO
EIT...TMK
EIT...TmK
Intensity (10^6)
Retention Time

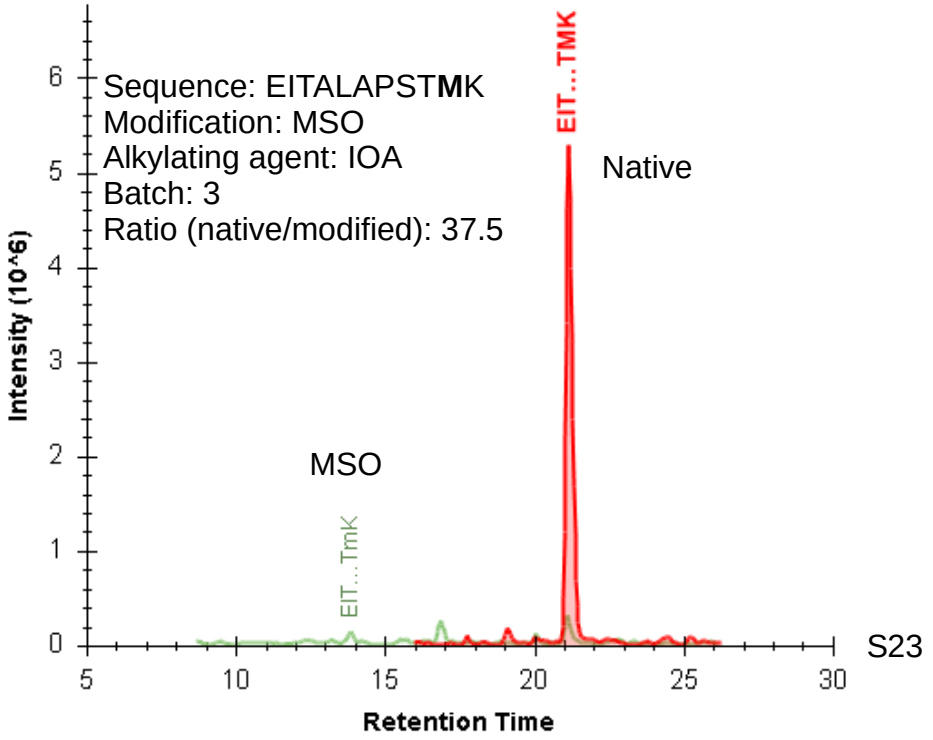
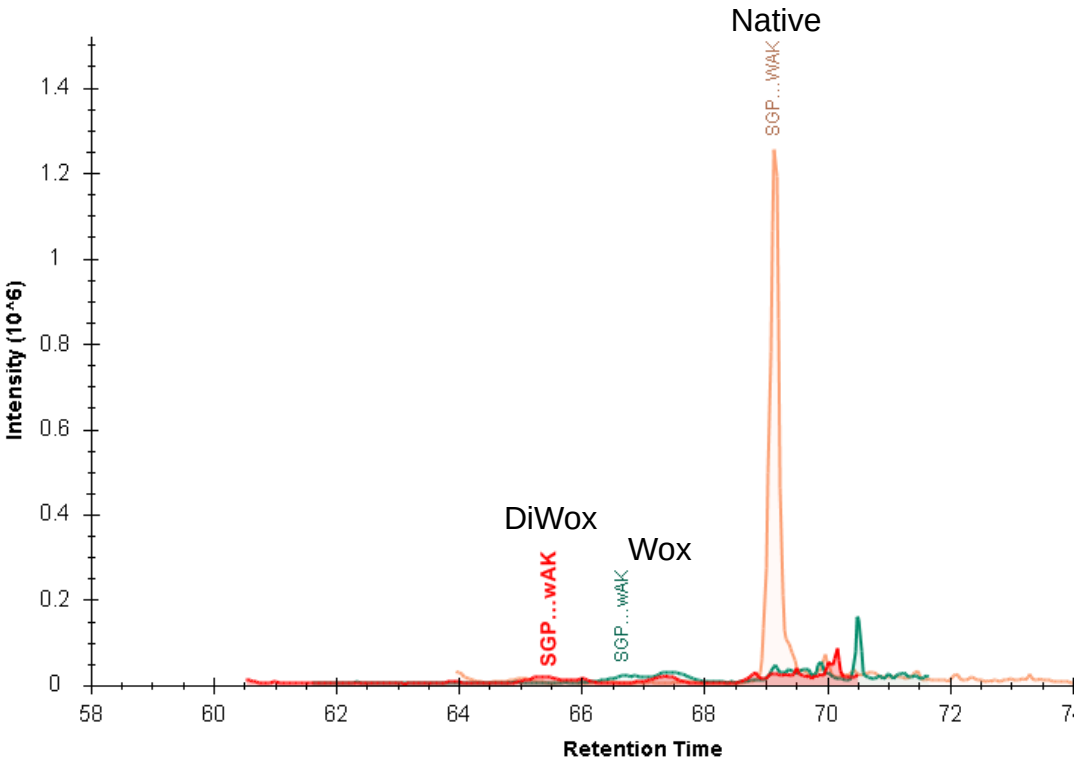

Sequence: EITALAPSTMK
Modification: MSO
Alkylating agent: IOA
Batch: 3
Ratio (native/modified): 37.5
Native
MSO
EIT...TMK
EIT...TmK
Intensity (10^6)
Retention Time

Figure S7H

Sequence: SGPFGQIFRPDNFVFGQSGAGNN**W**AK
Modification: Wox, DiWox
Alkylating agent: CA
Batch: 2
Ratio (native/modified) Wox: 18.1
Ratio (native/modified) DiWox: 22.4



Sequence: SGPFGQIFRPDNFVFGQSGAGNN**W**AK
Modification: Wox
Alkylating agent: CA
Batch: 3
Ratio (native/modified) Wox: 33.1

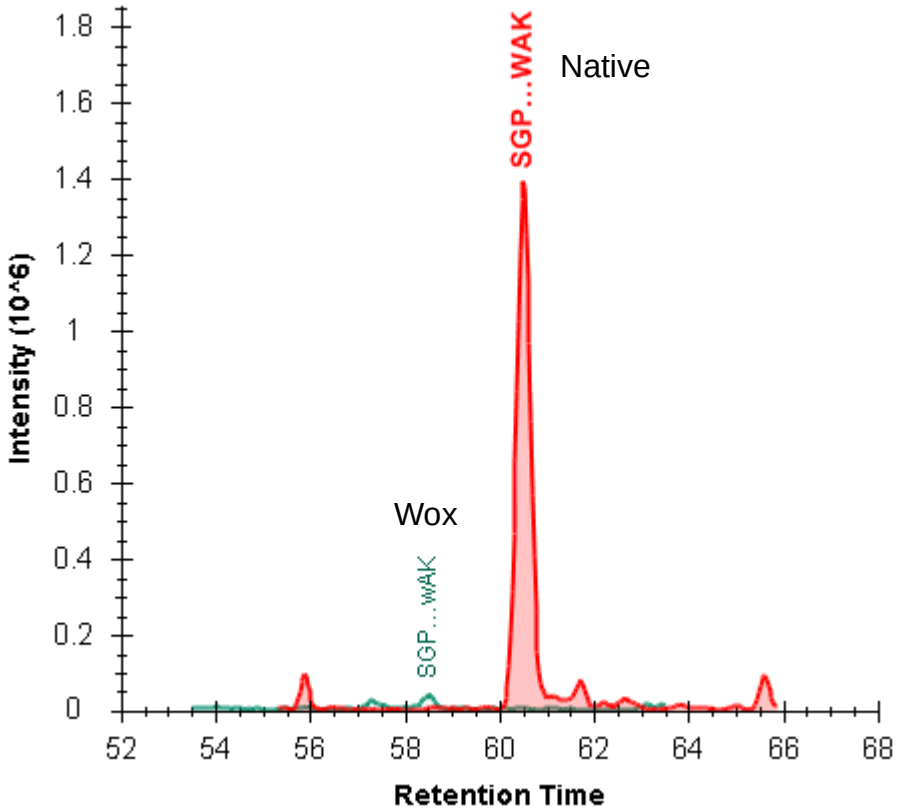
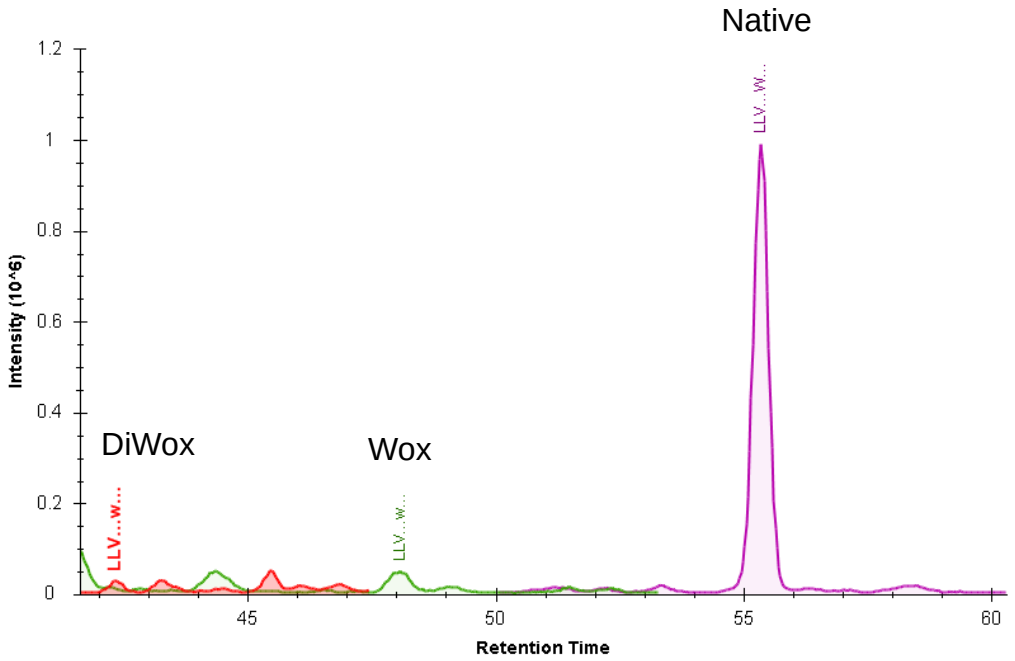


Figure S7I

Sequence: LLVVYP**W**TQR
Modification: Wox, DiWox
Alkylating agent: CA
Batch: 2
Ratio (native/modified) Wox: 16.2
Ratio (native/modified) DiWox: 45.3



Sequence: LLVVYP**W**TQR
Modification: Wox, DiWox
Alkylating agent: CA
Batch: 3
Ratio (native/modified) Wox: 13.9
Ratio (native/modified) DiWox: 34.5

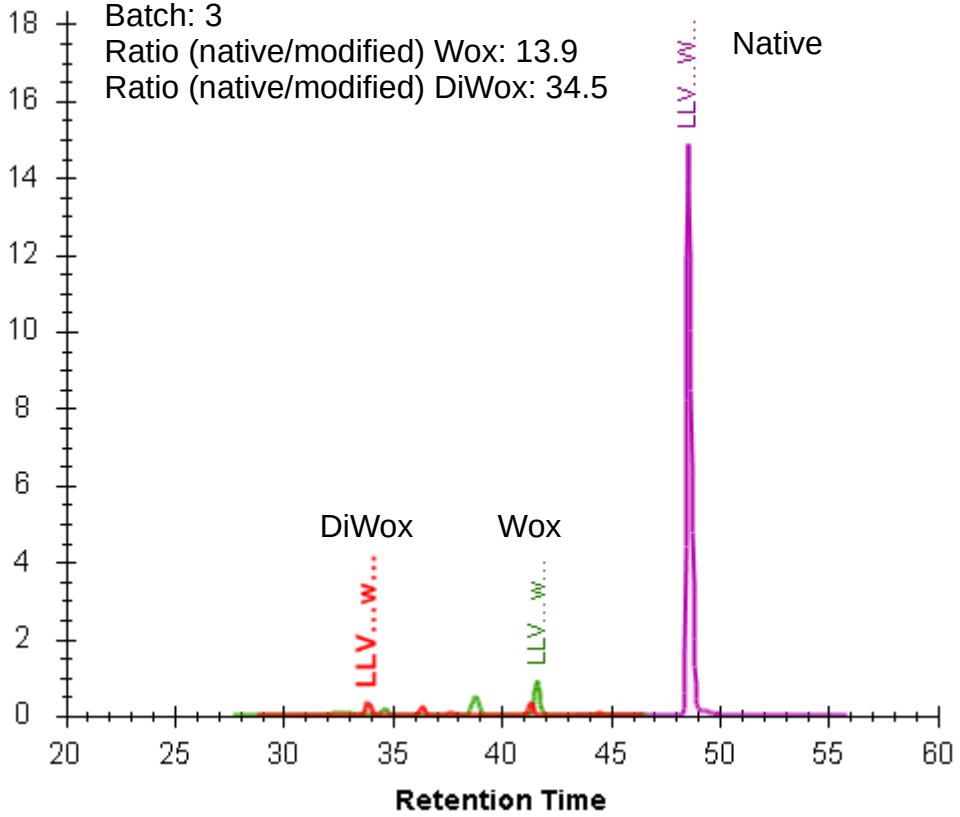
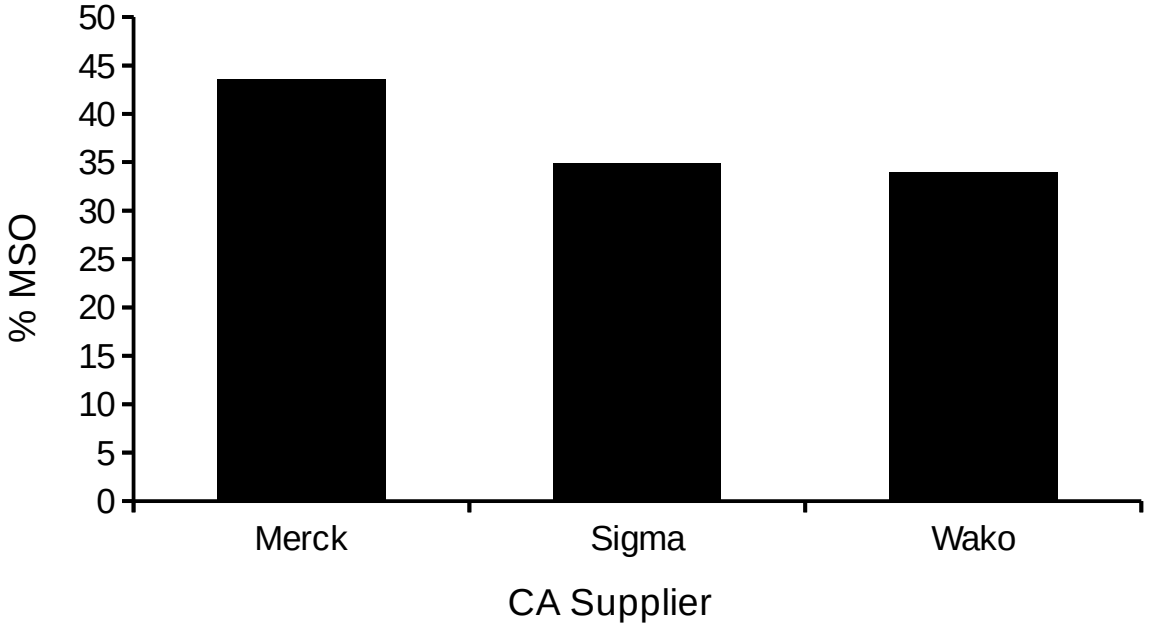
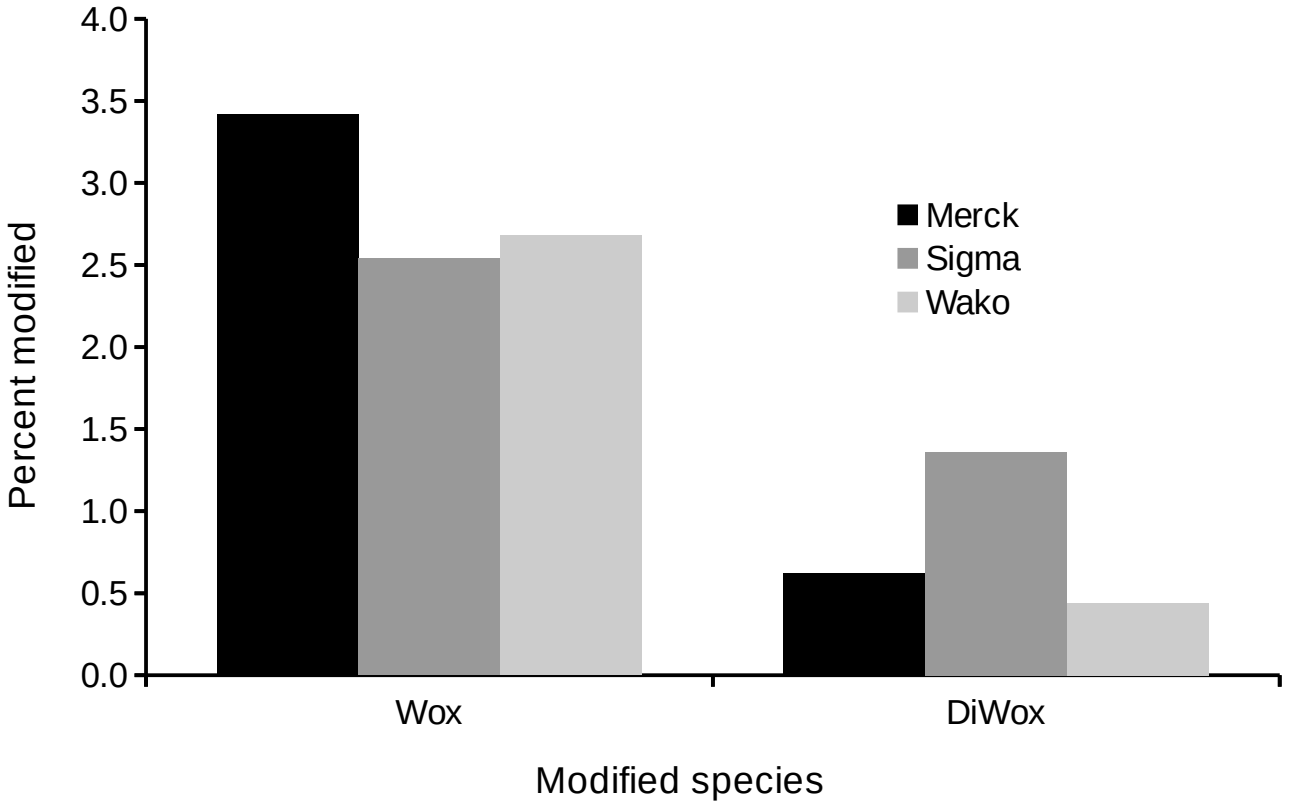


Figure S7J



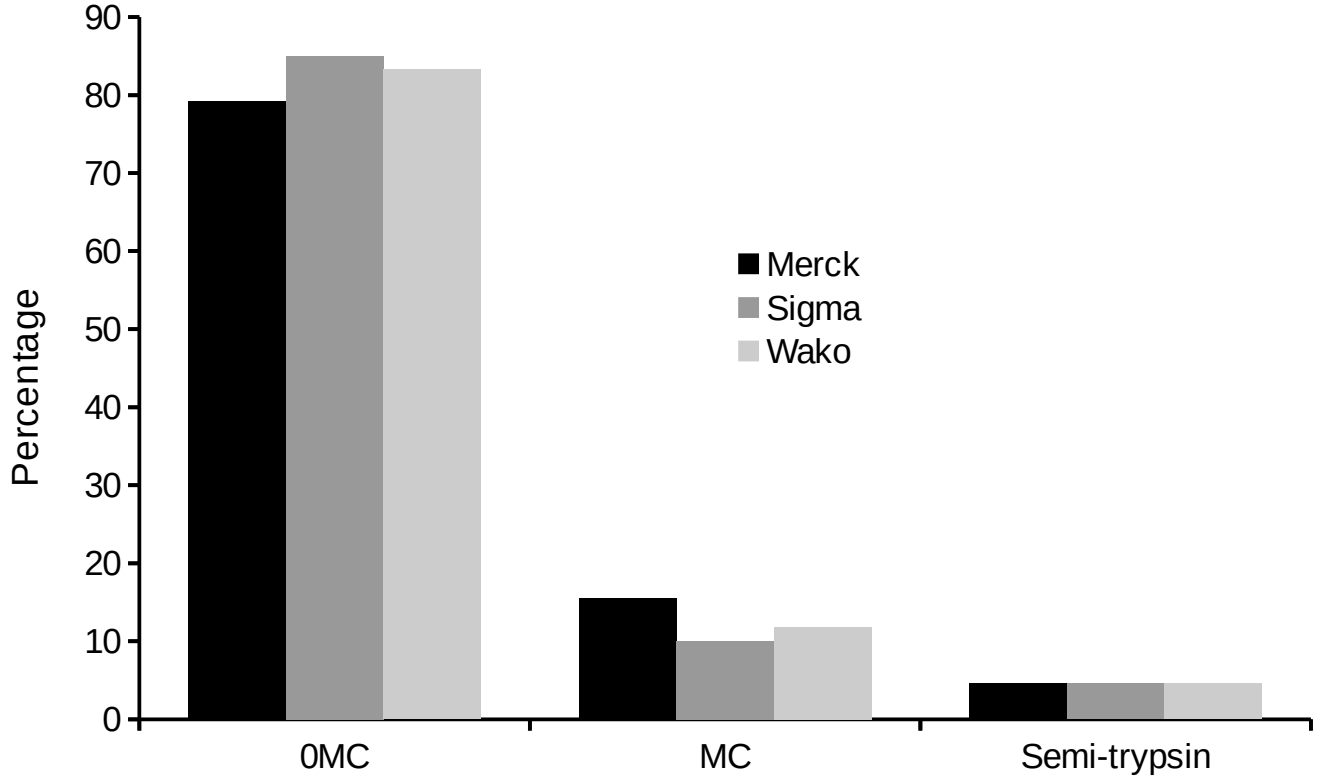
Level of MSO for samples processed using CA sourced from three different suppliers. Data is presented as the mean of 5 samples performed in 1 experiment.

Figure S8



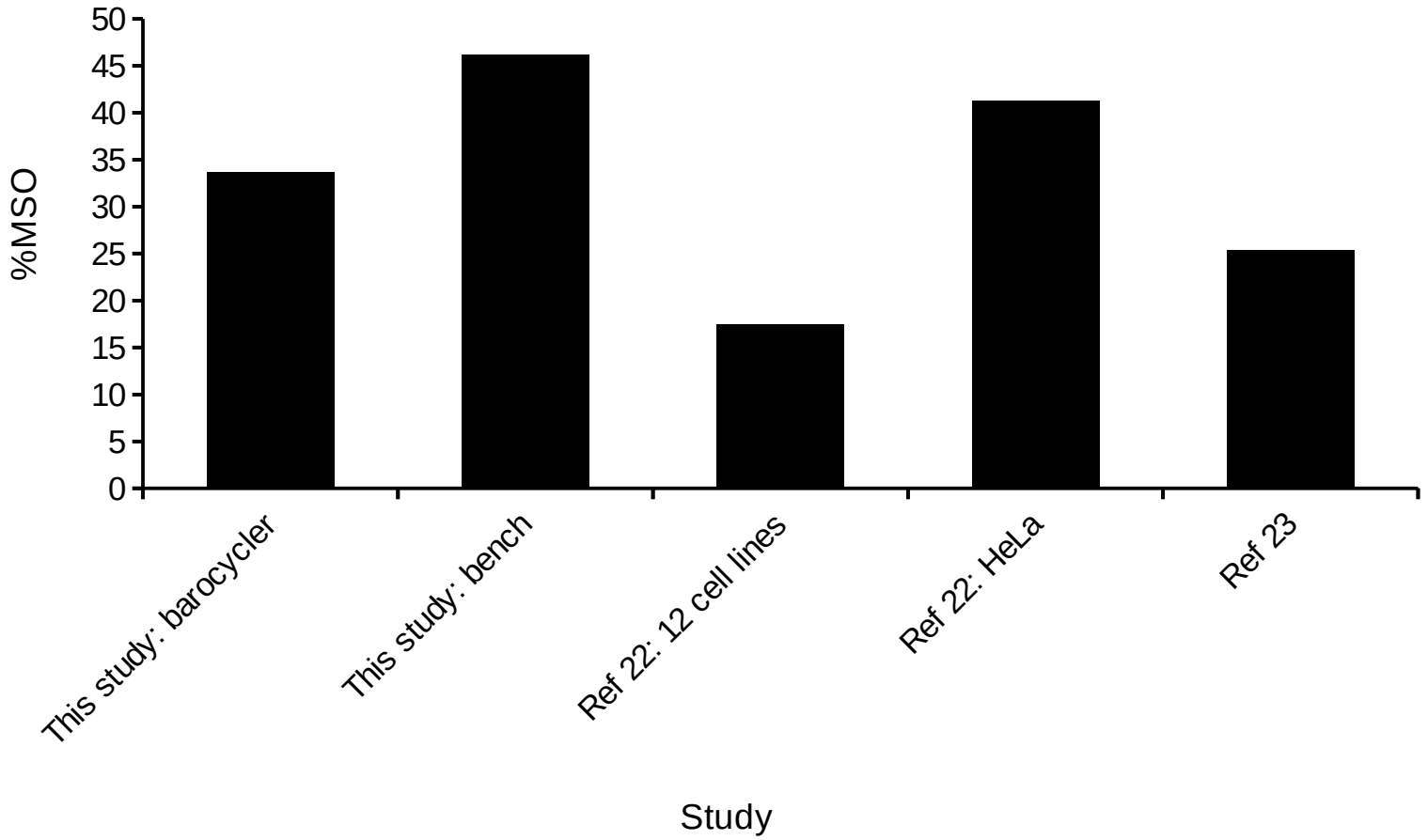
Level of oxidised W (Wox) or dioxidised W (DiWox) samples processed using CA sourced from three different suppliers. Data is presented as the mean of 5 samples performed in 1 experiment.

Figure S9



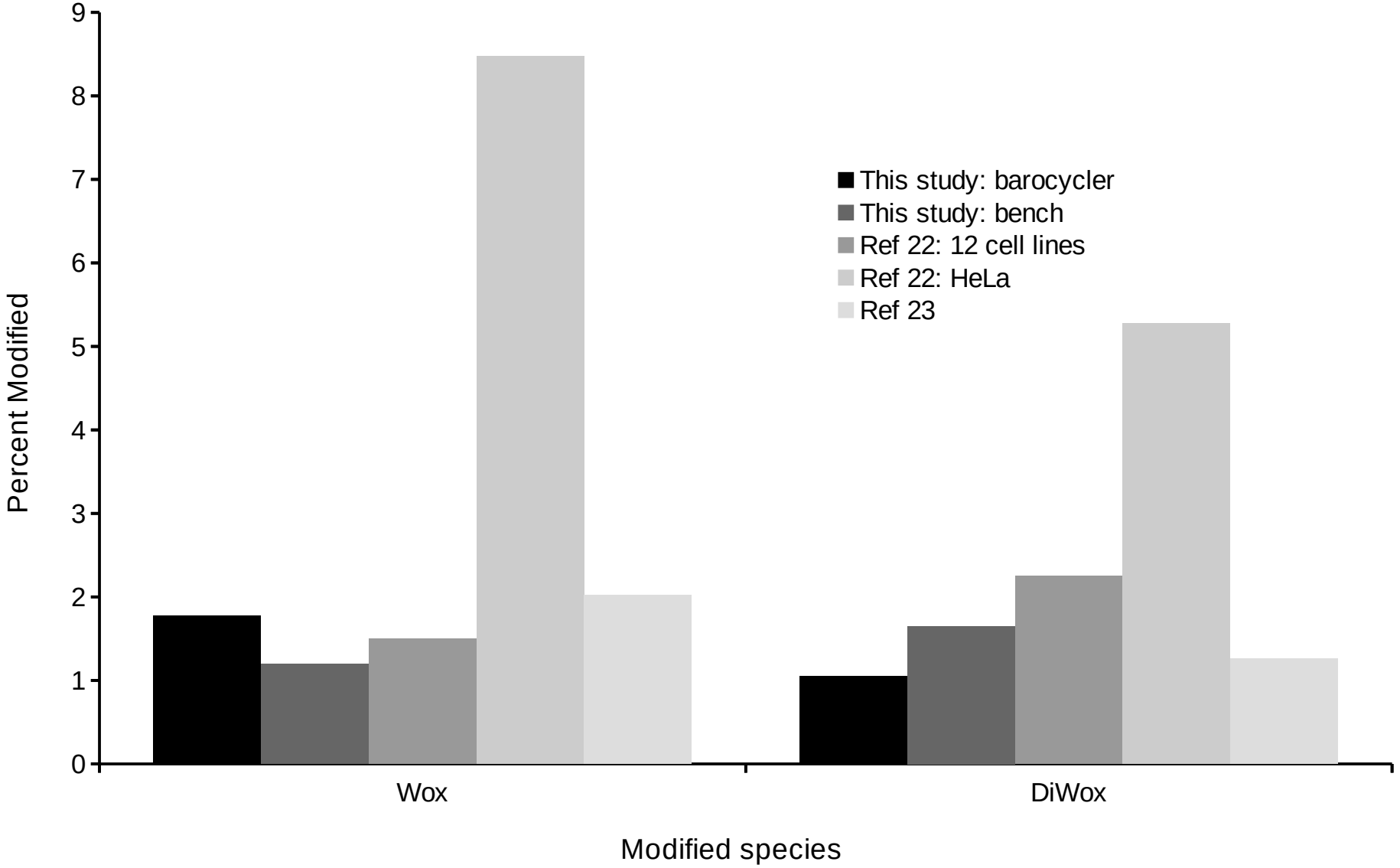
Digestion statistics for treatment with three different suppliers of CA where; 0MC, no missed cleavages; MC, missed cleaved peptides; semi-trypsin, semi-tryptic peptides. Data is presented as the mean of 5 samples performed in 1 experiment.

Figure S10



Level of MSO for data sourced from 2 published studies using CA. In all cases data is presented as the mean of 4 samples.

Figure S11



Level of oxidised W or dioxidised W for data sourced from 2 published studies using CA. In all cases data is presented as the mean of 4 samples.

Figure S12