Supplemental Information

Supplemental Figures

Figure S1:

The detailed mechanisms involved in the chemical reactions. A: The guanidine group of arginine is blocked by malondialdehyde (MDA) in solution to form the 2-pyrimidinyl ring, with greatly reduced basicity and proton affinity. B: Ortho-phthalaldehyde (OPA) reacts with primary amine in the presence of sulfydryl. The primary product gradually decomposes first into an intermediate, then into a non-fluorescent stable product. C: The 1,1,3,3-Tetramethoxypropane (TMP) is converted into 1,1,3,3-tretra-isopropoxypropane (TiPP) as the source of MDA *in situ* to reduce the ester formation during the reaction with arginine.

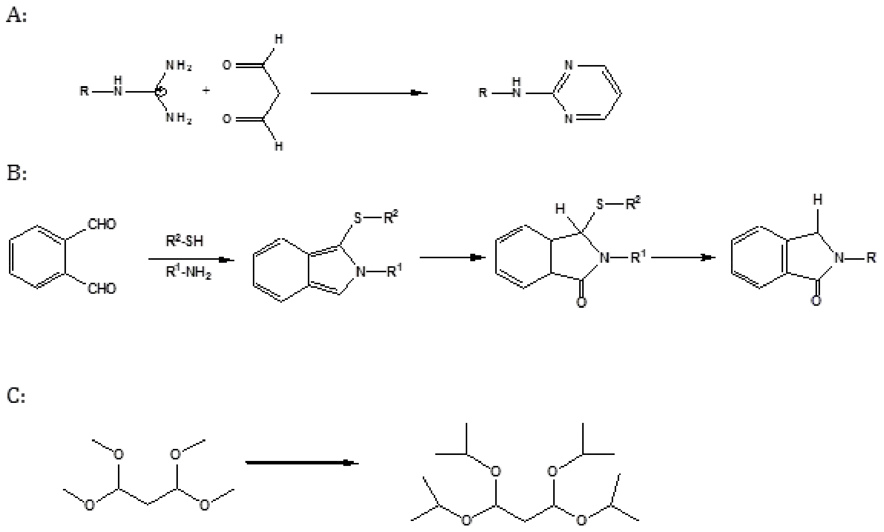
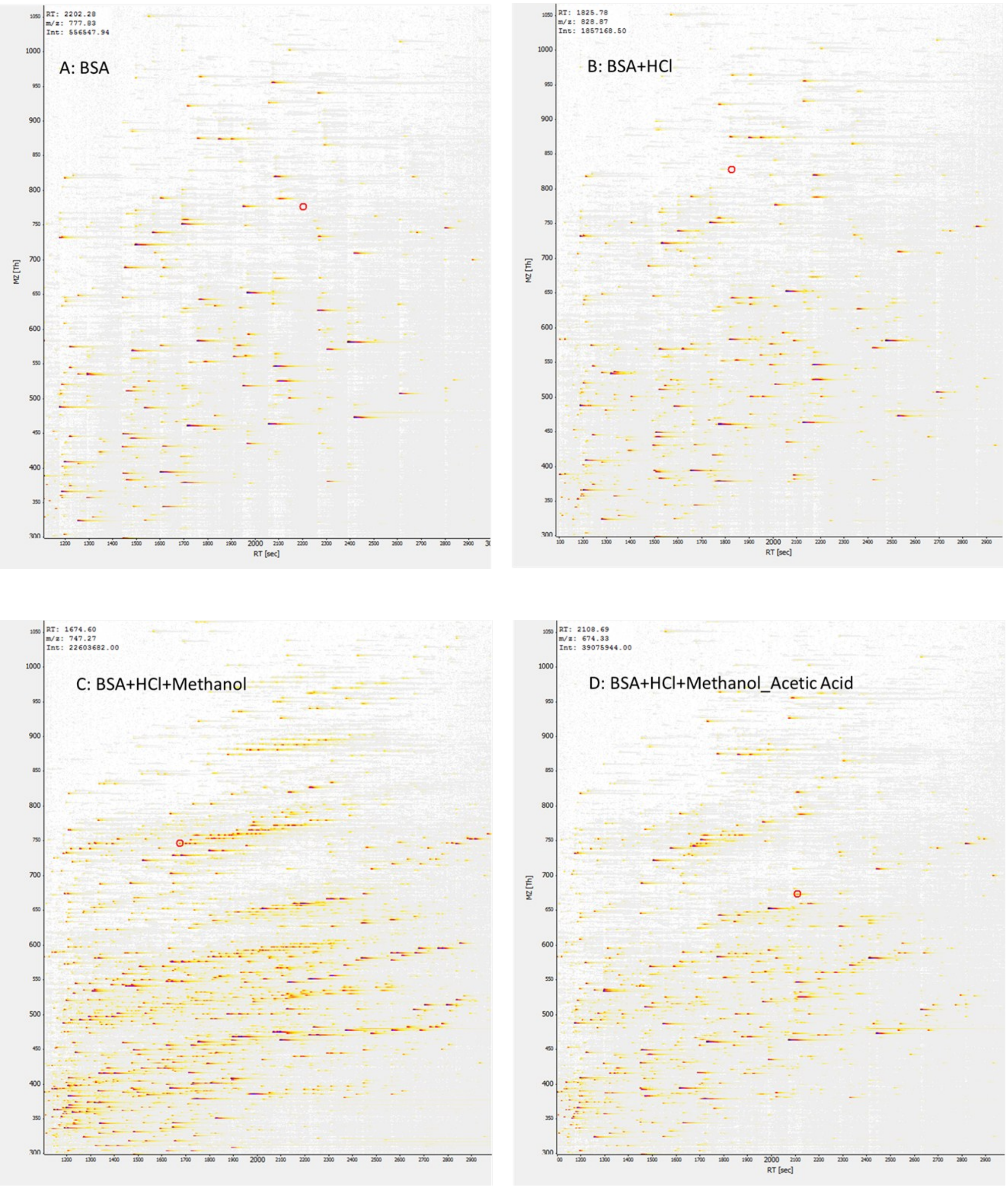


Figure S2:

Acetic acid can efficiently suppress protein esterification. Virtual 2D plot of BSA tryptic peptide analysis under different conditions, including BSA only as negative control, BSA+HCl, BSA+HCl+Methanol, BSA+HCl+Methanol+Acetic acid, BSA+HCl+Ethanol, BSA+HCl+Ethanol+Acetic acid. X axis is the retention time, y axis is the m/z. HCl incubation does not bring obvious degradation or modification judging from the 2D map. Acetic acid can efficiently reverse the esterification generated by adding methanol or ethanol.


A: BSA
RT: 2202.28
m/z: 777.83
Int: 556547.94
B: BSA+HCl
RT: 1825.78
m/z: 828.87
Int: 1857168.50
C: BSA+HCl+Methanol
RT: 1674.60
m/z: 747.27
Int: 22603682.00
D: BSA+HCl+Methanol_Acetic Acid
RT: 2108.69
m/z: 674.33
Int: 39075944.00
MZ [Th]
RT [sec]
300
350
400
450
500
550
600
650
700
750
800
850
900
950
1000
1050
1200
1300
1400
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2500
2600
2700
2800
2900

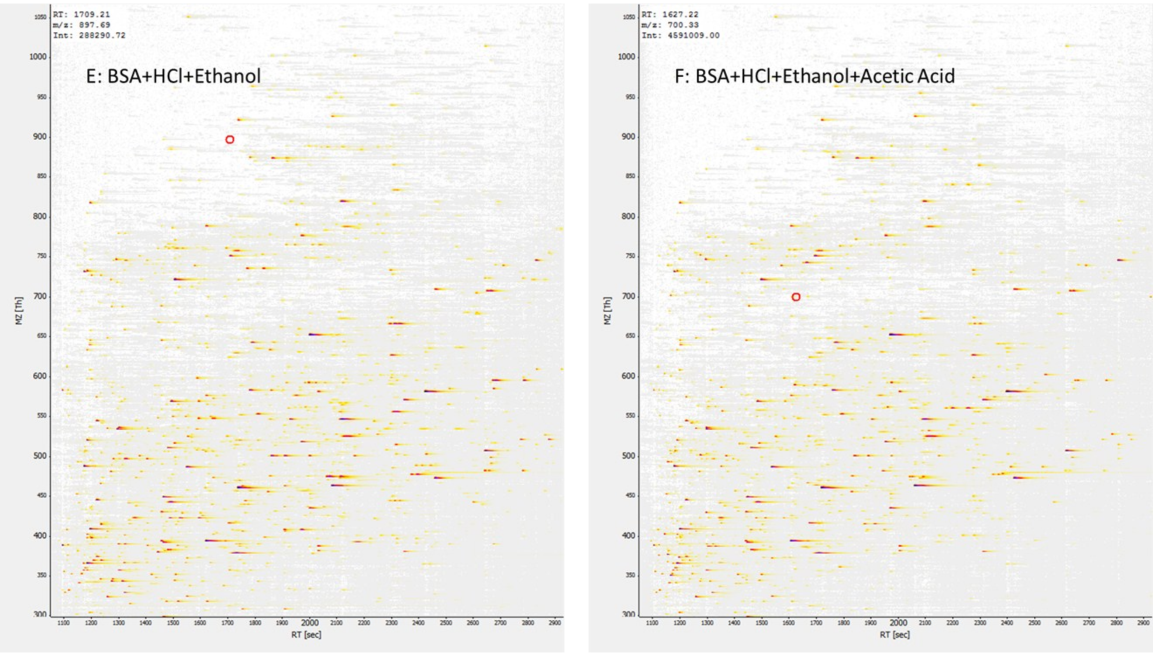
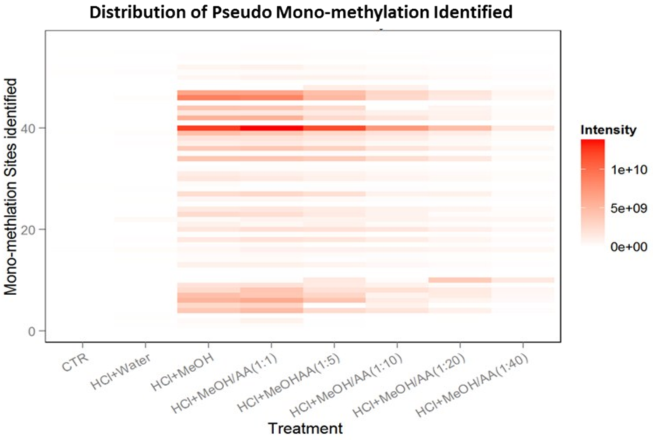


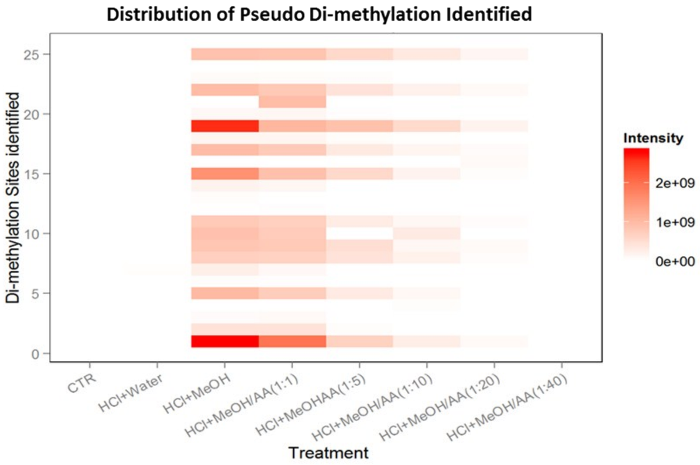
Figure S3:

Identification quantification results show excessive acetic acid can effectively suppress esterification. Different ratio between methanol/ethanol and acetic acid (1:1, 1:5, 1:10, 1:20, 1:40) were tested. A: Quantitative result from methyl esterification. B: Quantitative result from ethyl esterification. C: Result for the un-modified peptides.

A:



B:



C:

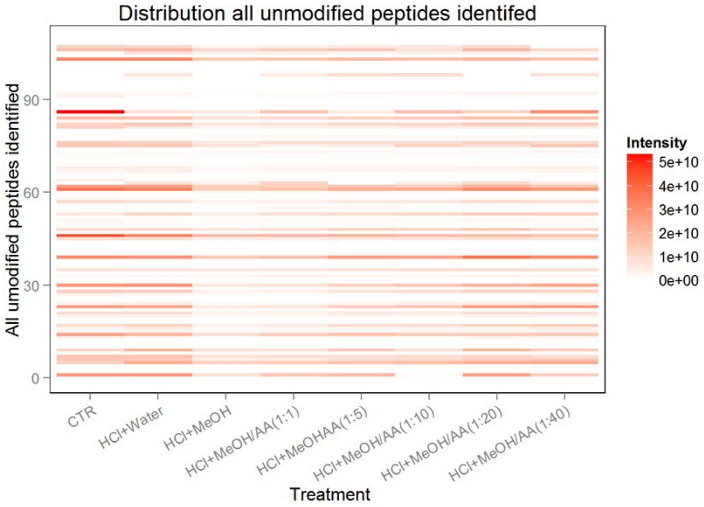


Figure S4:

The stability of the OPA reacted peptide product measured by 340nm UV absorbance and fluorescence at 455nm.

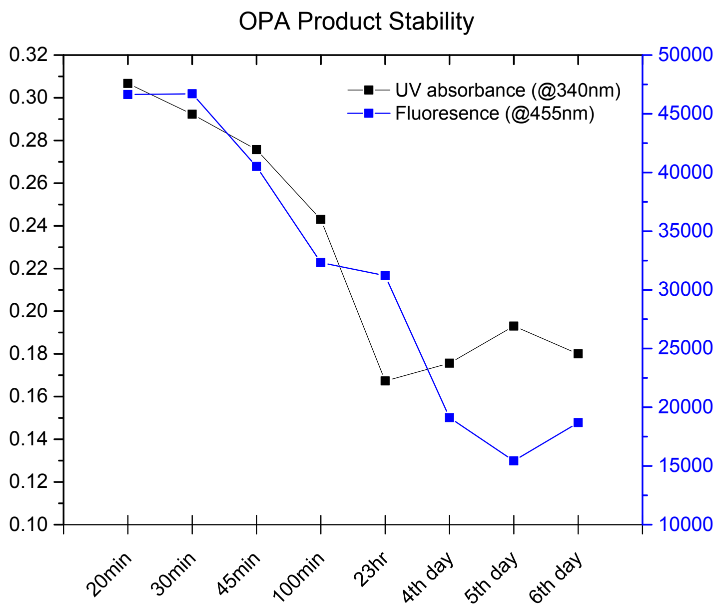


Figure S5:

Comparison between percentage of peptides with lysine (K) and arginine (R) as C-terminals from MS identification of regular proteomic profiling and OPA reacted sample. In-gel and In-solution dataset were calculated based on datasets from multiple proteomic experiments using a standard trypsin digestion protocol.

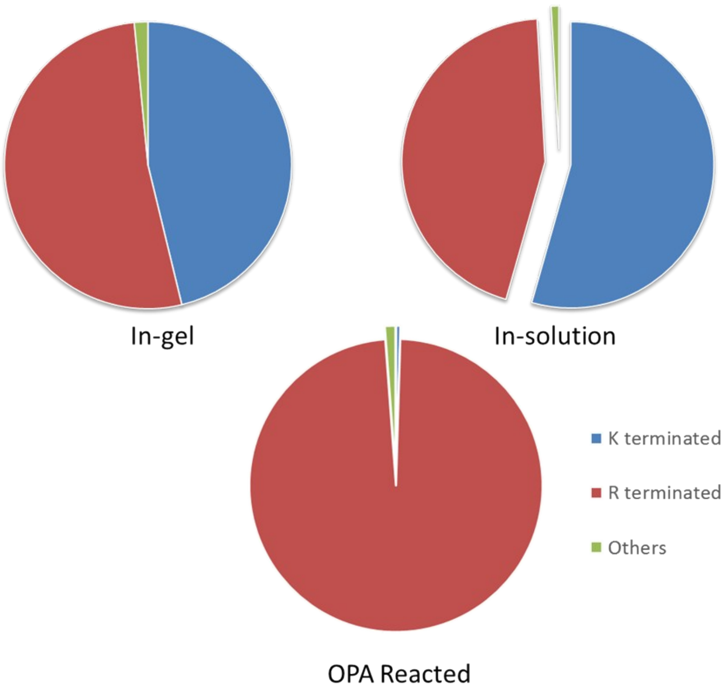
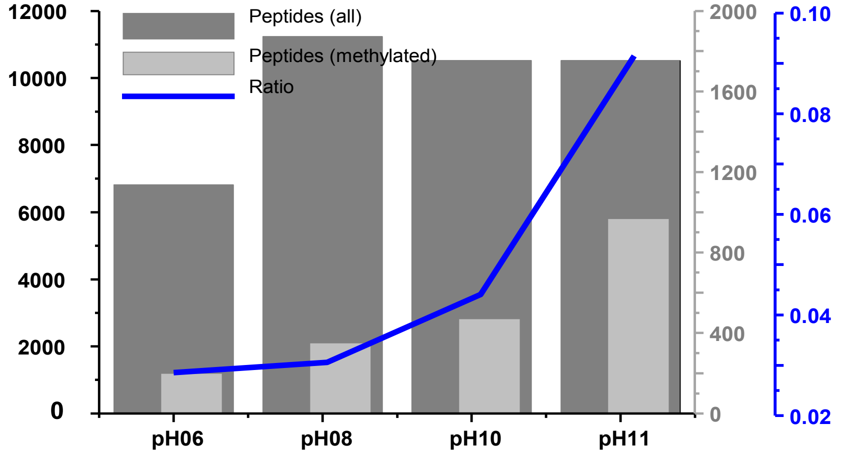


Figure S6:

Distribution of the peptide identification from MDA-OPA (A) and negative control experiment (B) for methylation enrichment. The methylated peptides account negligible portion (around 0.1%) of all the peptides identified, compared with the significant enrichment (up to 10%)by the MDA-OPA strategy.

A:



B:

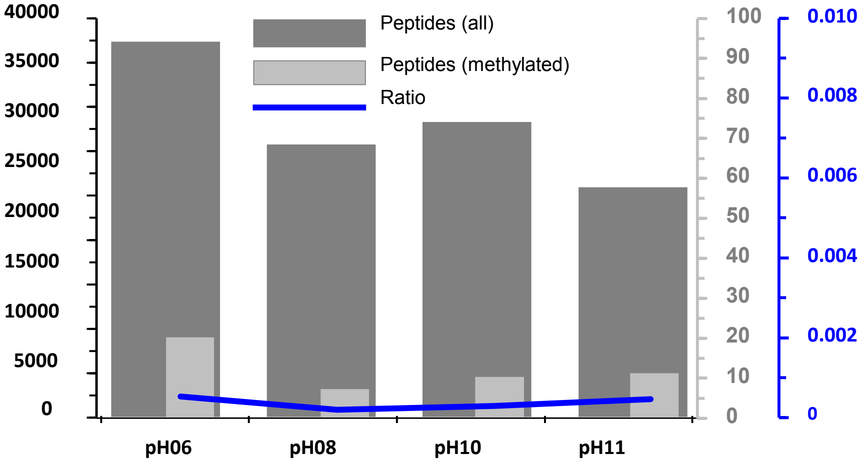


Figure S7:

Summary of identified methylation site profile for lysine, arginine, and histidine.

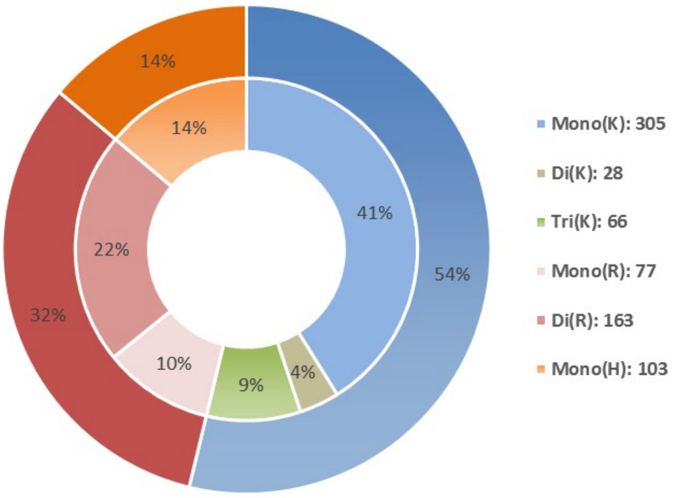


Figure S8:

The MS accuracy in ppm for peptide on Q-Exactive from the results reported in this manuscript.

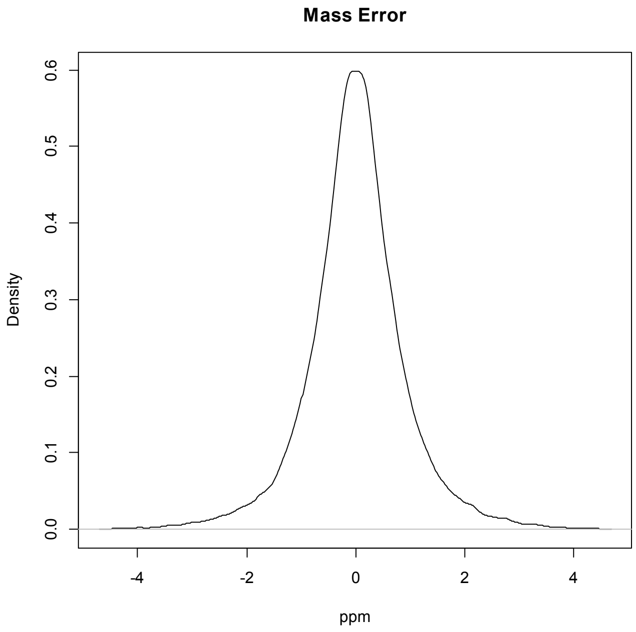


Figure S9:

Examples of surface accessibility of methylated amino acid. A: 14-3-3 protein theta (Uniprot: P27348). Red: K3 with trimethylation identified; Green: K49 with mono-methylation identified; B: Triosephosphate isomerase (Uniprot: P60174). Red: K106 with mono-methylation identified. Green: K212 with mono-methylation identified.

A:

B:

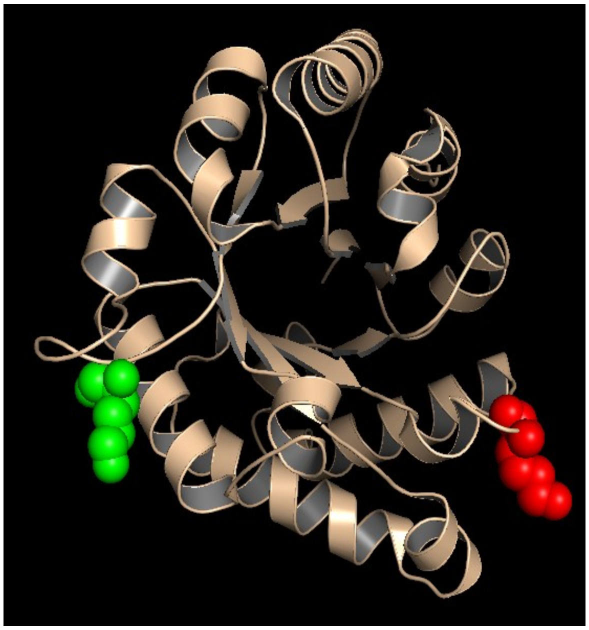


Figure S10:

Icelogo visualization of six flanking amino acid around methylation events. Sequences were aligned at methylated amino acid residue in the middle. Over presented (above) and under presented (under) amino acid are shown against human proteome with $p<0.05$.

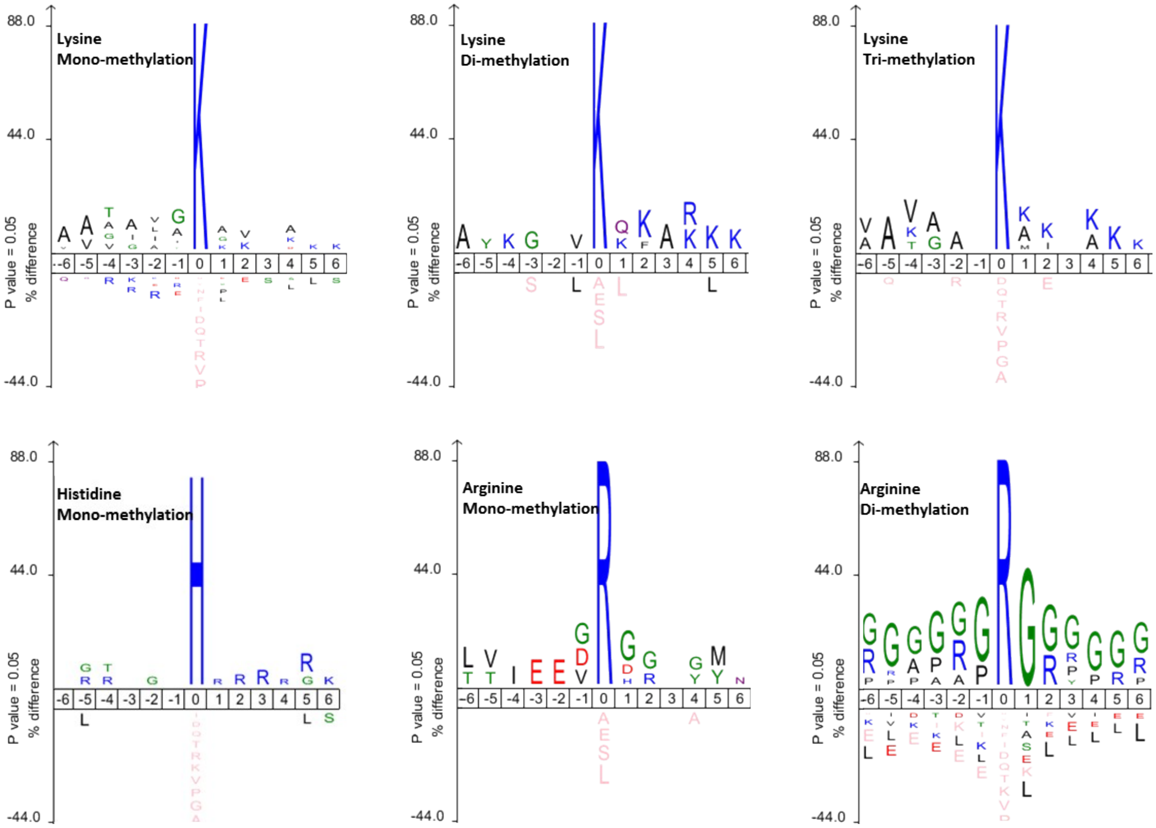
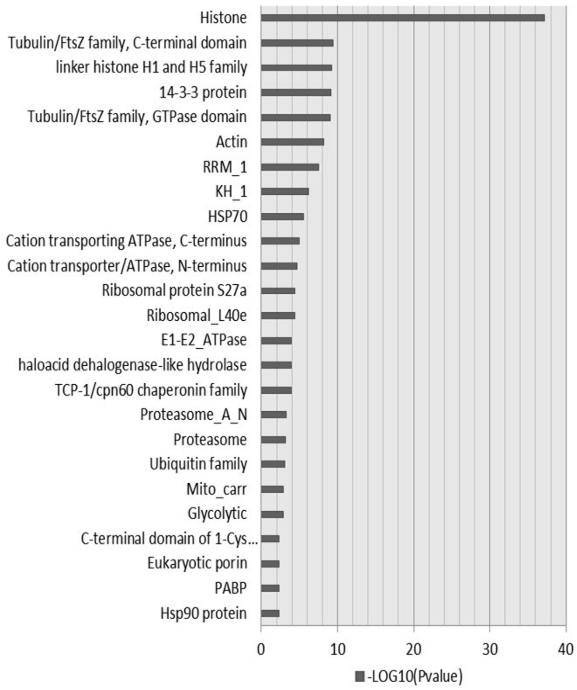


Figure S11:

Pfam protein domain analysis of identified methylated proteins.
A: Lysine methylation



B: Arginine methylation

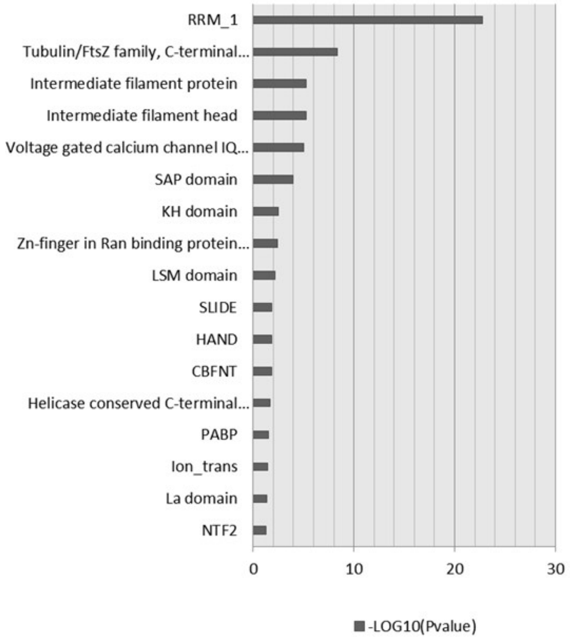
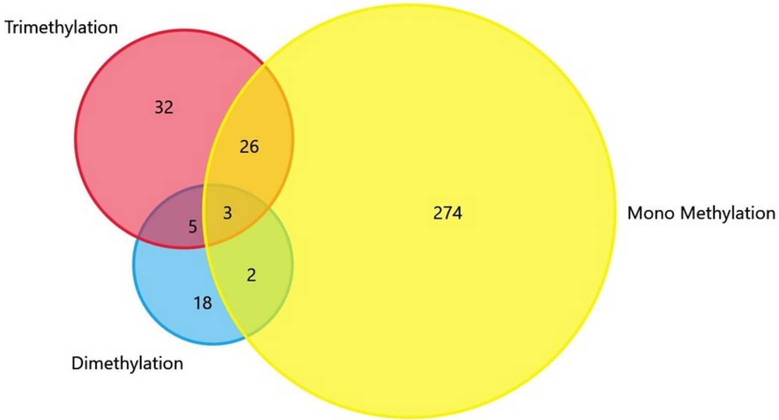


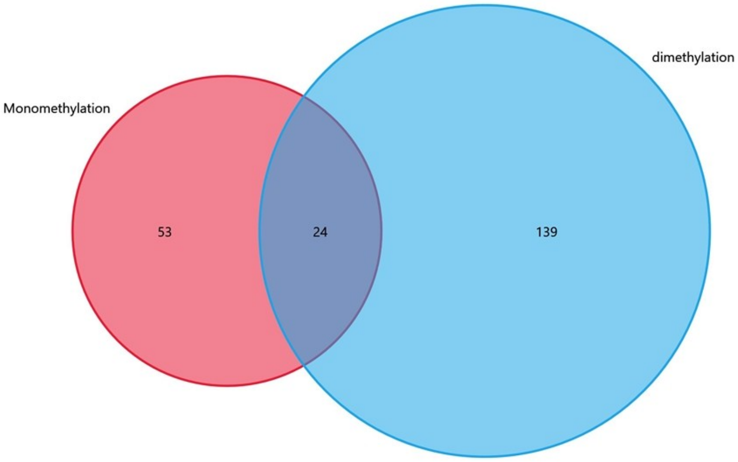
Figure S12:

Methylation events overlap on the same amino acid.

A: Lysine methylation:



B: Arginine methylation



Experimental Methods

Chemicals

All standard proteins(beta casein, myoglobulin, bovine albumin, alpha casein and lysozyme), 1,1,3,3-tetramethoxypropane, Amberlite IR-120 resin, urea, dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate (ABC), formic acid (FA), isopropanol, citric acid, β-2-mercatoethanol were obtained from Sigma Aldrich (St. Louis, MO). Water and Acetonitrile (ACN) for HPLC were obtained from JT Baker, Phillipsburg NJ, USA). Trypsin was purchased from Worthington Biochemical Corp (USA). Bio-Rad protein assay kit II (500-0002) and DC protein assay kit II (500-0112) were from Bio-Rad. All of the chemicals were of analytical purity grade except ACN and FA, which were of HPLC grade. All the water used in the experiment was prepared using a Milli-Q system (Millipore, Bedford, MA).

Sample preparation

Briefly, HeLa and HEK293 cells were grown to 80% confluence in 15cm dishes and harvested in RIPA buffer (25 mM Tris-HCl (pH 7.6), 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.5% SDS) after two washes with PBS. Cells were sonicated for 1 min with 20 seconds pulse using 30% power on Sonic Dismembrator 500 w/ Branson 1020 Sonicator (Fisher Scientific) to increase protein recovery. Proteins were precipitated to remove detergent by adding 5X volume of cold acetone overnight followed by two washes with cold acetone as well. The protein pellet was then reconstituted in 8 M urea in 50 mM ammonium bicarbonate (ABC) and quantified by the DC protein assay kit (BioRad). Reduction and alkylation were done by adding dithiothreitol (DTT) to a final concentration of 10 mM at 56°C for 30min followed by 20 mM iodoacetamide (IAA) at room temperature. The solution was then diluted 5 times by 50mM ABC. Digestion was performed by adding trypsin at a protein-enzyme ratio of 50:1, 37 °C overnight, with continuous head-to-end rotating. Digested peptides were then desalted on Sep-Pak C18 SPE column (Waters), aliquoted and dried down by SpeedVac (ThermoFisher Scientific, San Jose, CA).

To confirm the stable product from the OPA reactions, we used a tryptic digest of five standard proteins (beta casein, myoglobulin, bovine albumin, alpha casein and lysozyme). The digestion was done with the same protocol as described above for cell lysate digestion. Bovine serum albumin (BSA) tryptic digest was prepared with the same protocol for the MDA derivatization and optimization.

Bovine serum albumin methylation

BSA for the methylation spike-in was prepared according to the protocols described previously [1]. Briefly, 100 µg BSA protein was dissolved in 2M urea in 50 mM ABC, then reduced by 10 mM DTT at 56°C for 30 min, then alkylated by 20 mM iodoacetamide for 45min in dark. Then the sample was labeled by addition of 20 mM formaldehyde and 10 mM $NaCNBH_3$ (Sigma Aldritch) for 16 hrs. The labeling reaction was quenched by 100 mM ABC. BSA protein was then precipitated and reconstituted in 1 M urea and aliquoted into 1 µg for spike-in experiment, or digested overnight by trypsin. For BSA methylation spike-in experiment, HeLa lysate was digested along with methylated BSA.

Reaction Reagent preparation

The synthesis of 1,1,3,3-tetraisopropoxypropane (TiPP) was adapted from Foettinger, A. *et al*[2]. Briefly, Amberlite IR-120 resin (5.5 g, strong cation exchanger, SCX, HC-form, Sigma) was thoroughly washed with isopropanol to remove the yellow color residue, and 4.95 ml (30 mmol) 1,1,3,3-tetramethoxypropane (Sigma), dissolved in 182 ml (2.4 mol) isopropanol, was added to the resin. The mixture was shaken for 2 hours. The solvent was decanted; the isopropanol and released methanol were then removed by rotary evaporation at 60 °C and the residue was re-dissolved in 182 mL isopropanol and again added to the SCX resin. This whole procedure (shaking, evaporating, re-dissolving) was repeated four times, and approximately 3.5mL of pale yellow TiPP solution was obtained. The solution was aliquoted in amber tubes, stored in -80°C before usage. For the OPA reaction, 1 mL of 10X stock solution of OPA reaction buffer was prepared by adding 5mg OPA (MW:134.13) and 5μL β-2-mercatoethanol (BME, with density of 1.11 g/mL and MW of 78.13, molar ratio of OPA and BME is about 2:1) into 100 μL pure ethanol, then diluted in 900 μL 50 mM carbonate buffer (pH10.5). This stock solution was always freshly made in amber tube.

MDA derivatization and optimization

Twenty picomolar BSA trypsin digestion was used for each MDA derivatization optimization. Ten microgram Hela digest was used for real sample application. Peptide was incubated with TiPP and HCl at room temperature in dark for 1 hr, then diluted 20X before RP-SPE purification. For the esterification check experiment, 5μl methanol or ethanol, 200μl HCl, along with 5, 10, 50, 100, 200μl acetic acid were tested.

OPA product confirmation

One milligram of HeLa protein tryptic digest was reacted with the 1X OPA reaction buffer. The product was transferred onto 96-well plate with three replicates, along with 1X OPA reaction buffer and tryptic peptides of the same final concentration in bicarbonate buffer, which was used make the OPA reaction buffer. The UV absorbance at 340nm and the fluorescence at 455nm were recorded over time to monitor the products up to 6 days. Tryptic digest from five standard proteins (as described above) was subjected to the OPA reaction, SCX purification and desalting. To confirm which OPA reaction products can be detected by mass spectrometry, two variable modifications for the intermediate product (C(10)H(8)OS with MW of 176.0295845689, named as OPA-large) and the expected stable product (C(8)H(4)O with MW of 116.0262147505, named as OPA-small) were added as extra variable modification for database searching. The OPA reaction efficiency was tested on 10 μg HeLa digest, using the same protocol described above. The product was desalted and analyzed on LTQ mass spectrometer.

Tandem chemical reaction and SCX fractionation on large scale methylation profiling

The core chemical modification of the workflow consists of two derivatization reactions on arginine and lysine residues sequentially. The malondialdehyde (MDA) reaction was adapted from Foettinger, A[2]. To exhaust any residual alcohol and provide excessive carboxyl group 70µl acetic acid was mixed with 30 µL TiPP and 200 µL 12 M HCl for 10min in room temperature. Five hundred microgram of Hela tryptic digest was mixed with the pre-mixed reaction buffer, vortexed and kept in dark for 1 hr to introduce the MDA modification on un-methylated arginine residues. The solution was then diluted 20 times, and loaded onto activated RP SPE column (50mg SepPak™, Waters). The column was eluted by 80% ACN with 0.1% formic acid (FA). The eluent was then loaded onto SCX SPE column (50mg HyperSep™ SCX, Thermo) to remove polymer side products. The SCX column was eluted by 2 mL 50 mM Na_2CO_3 and 2 mM 50 mM NaOH to recovery the bound peptides. Then 450 µL of 10X OPA reaction buffer was added to the 4 mL SCX elutiion for OPA reaction. The reaction was kept in dark and at room temperature for another 2 hours. After OPA reaction, 5% of FA was used to lower the pH to 3. The solution was then loaded onto an activated SCX SPE column and washed by 80% ACN. Britton & Robinson buffers at different pH (20 mM CH_3COOH, 20 mM H_3PO_4, 20 mMH_3BO_3, adjusted to pH 6, 8, 10, 11, 12 by NaOH) were used to elute the peptides off the column sequentially. The eluent was desalted and dried down for MS analysis. The OPA derivatization peptides was also desalted and loaded to online SCX fractionation coupled to MS analysis to minimize sample loss. The online SCX fractionation was achieved by step elution of up to 1M ammonium formate.

MS analysis

MS platforms used in the current manuscript includes one with Agilent 1100 capillary-HPLC system (Agilent Technologies, Santa Clara, CA, USA) coupled to LTQ-Orbitrap (ThermoFisher Scientific, San Jose, CA), the other with Eksigent Nano-2D plus nano LC (ABSciex) coupled to Q-Exactive (ThermoFisher Scientific, San Jose, CA). All mass spectectrometers were equipped with a nano-electrospray interface operated in positive ion mode. The mobile phases consisted of 0.1% (v/v) FA in water as buffer A and 0.1% (v/v) FA in acetonitrile as buffer B. Peptide separation was performed on a 75μm × 150 mm analytical column packed in-house with reverse phase Magic C18AQ resins (1.9 µm; 100-Å pore size; Dr. Maisch GmbH, Ammerbuch, Germany). Dried peptide samples were reconstituted in 20 µL 0.5% FA and 4 µL was loaded using 98% buffer A at a flow rate of 300 nL/min for 20min. Then, a gradient from 10% to 50% buffer was performed in 60min, 120min or 180min at a flow rate of ~300nL/min obtained from splitting a 20µL/min through a restrictor or directly from the nano-HPLC. The MS method consisted of one full MS scan from 350 to 1,700 *m/z* followed by 5 data-dependent MS/MS CID scan of the most intense ions in linear ion trap, or 10 HCD MS2 scans on Q-Exactive, with dynamic exclusion repeat count of 2, and a repeat duration of 90s. The resolution of full MS was set 60,000 defined at m/z 400 on LTQ-Orbitrap or 7,500 defined at m/z 200 on Q-Exactive. To improve the mass accuracy, all the measurements in Orbitrap mass analyzer were performed with internal recalibration ("Lock Mass") at 445.1205.[3] The charge state rejection function was enabled, with single and "unassigned" charge states rejected.

Database search for protein identification

The database search was done either by MaxQuant 1.3.0.5 [4] for quantitative anlysis and large scale sample profiling, or Mascot 2.3 [5] for quick result readout. The raw files were searched against UniProt protein fasta database (2014, July version), including commonly observed contaminants defined by MaxQuant. For OPA modification efficiency test, we used yeast database plus 5 standard proteins (beta casein, myoglobulin, bovine albumin, alpha casein and lysozyme, named as YAL01SD to YAL05SD in the database). Most of the parameters used for both search engines are the same: cysteine carbamidomethylation (+57.021463) was selected as fixed modification; variable modifications were set as follows: methionine oxidation, protein N-terminal acetylation, MDA modification on arginine residue, OPA modification on peptide N-terminal and lysine, mono- and dimethylation on both lysine and arginine residue, trimethylation on lysine residue. Methionine oxidation, protein N-terminal acetylation and MDA modification on arginine residue were set for the MDA reaction efficiency test, while methionine oxidation, protein N-terminal acetylation and OPA on modification on peptide N-terminal and lysine were set for OPA reaction efficiency test. Mono methylation on histidine was also included for histidine methylation. Enzyme specificity was always set to trypsin for all searches. Up to two missing cleavages of trypsin were allowed. Precursor ion mass tolerances were 7 ppm, and fragment ion mass tolerance was 0.5 Da for CID MS/MS spectra, 20 ppm for HCD spectra for MaxQuant search, while 20 mmu for Mascot search. The false discovery rate (FDR) for modified peptide, peptide and protein was all set at 1% and a minimum length of 7 amino acids was used for peptides filtration.

Bioinformatics analysis

Pfam protein domain analysis is done by DAVID[6]. Consensus sequence analysis was performed with the iceLogo web tool using standard settings[7]. Protein methylation modeling was displayed by PyMOL. NetSurfP[8] was used to calculate the surface accessibility of all the amino acid for identified proteins, defined as buried or exposed. The enrichment of the methylated amino acid was assessed by the hypergeometric p-values by "phyper" in R (https://www.r-project.org/). Public dataset of methylation was downloaded from Uniprot and PhosphositsPlus[9]. UniProt methylation list was extracted from downloaded gff format human database. The unique methylation site was assigned back to all possible proteins, the compared with the format reorganized PhosphositePlus database (only site with definite sites localization information were used) and UniProt database.

To test how much the increased number of variable modifications affected the FDR for methylation identification, protein extract from HEK 293 was processed using our workflow with the exceptions that no MDA-OPA reactions were performed. The MS results were searched with the same parameters which assumed that the chemical modifications are present. Any MSMS spectra matched to a peptide with MDA or OPA modification would therefore represent false positives as the peptides are not chemically modified. In this case, the FDR of MDA-OPA from this background search can be used to estimate the random FDR brought by multiple-variable-modification search for MDA-OPA reaction, as well as other modifications.

Other Supplemental information

Supplemental table captions

Please see supplemental file "Supplemental_tables.xlsx", for all supplemental tables in different spread sheets:

Table S1: MDA efficiency exemplified by unique peptide identification.Peptides with arginine residuals and MDA modification are marked by "+". Result from MaxQuant search.

Table S2: OPA stable product confirmation. Result from MaxQuant search with different forms of OPA modification, OPA-large, OPA-small as detailed in method section.

Table S3: BSA di-methylation events identified after reductive demethylation reaction on protein level.

Table S4: BSA di-methylation events identified from reductive demethylation reacted
BSA spiked in Hela total lysate background.

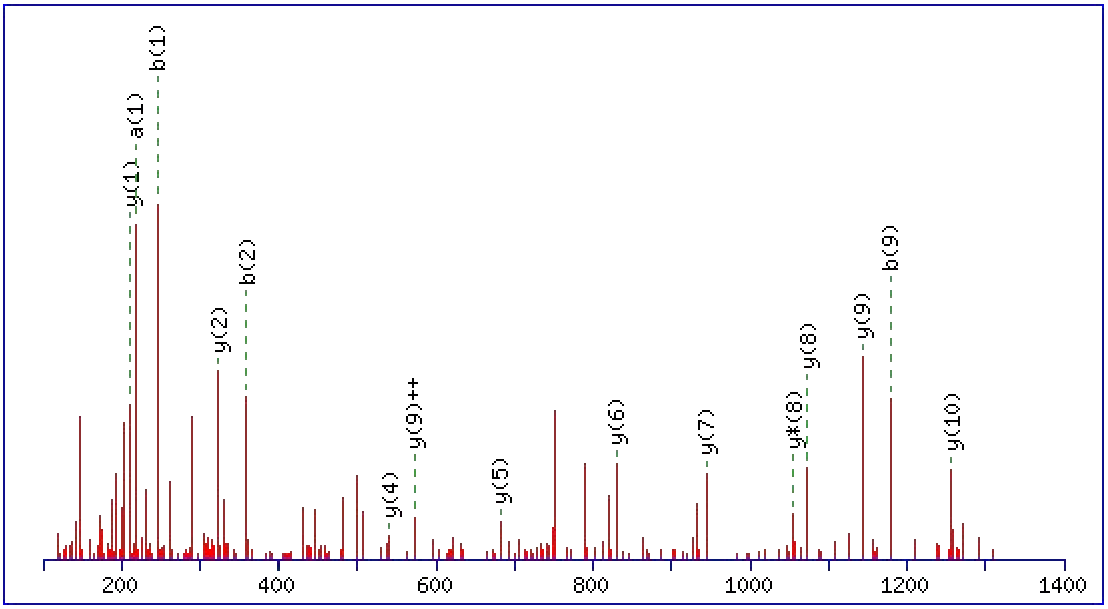
Table S5: High confidence methylation events identified.

Information of synthesized peptide identification

ID	Peptide	Comment	Validated?	Identified Sequence
1	EIAQDFK(mono-meth)TDLR	MONO-methyl on K	yes	R.$EIAQDFK&TDLR#.F
2	EIAQDFK(tri-meth)TDLR	tri-methyl on K	yes	R.$EIAQDFK^TDLR#.F
3	QYK(Di-meth)GIIDCVVR	DI-methyl on K	No	K.$QYK@GIIDCVVR#.I
4	QYK(tri-meth)GIIDCVVR	tri-methyl on K	yes	K.$QYK^GIIDCVVR#.I
5	MDSTEPPYSQK(Di-meth)R	DI-methyl on K	yes	K.$MDSTEPPYSQK@R#.Y

Peptide 1: R.$EIAQDFK&TDLR#.F

Spectrum from Large scale ID:



Monoisotopic mass of neutral peptide Mr(calc): 1500.7249

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K7 : Methyl-Mono (K)

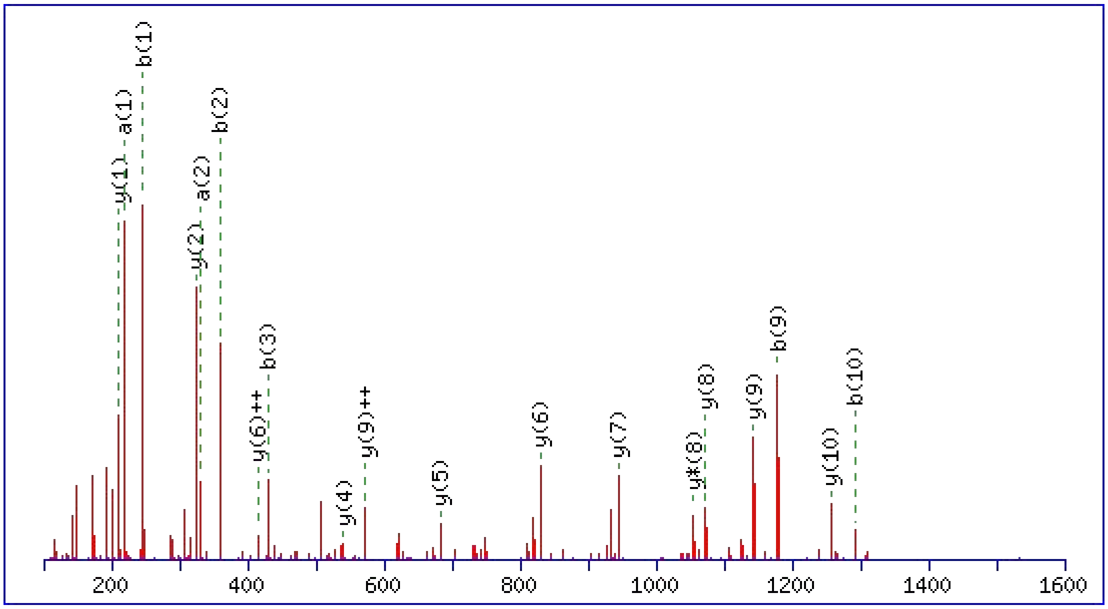
R11 : MDA (R)

Ions Score: 66 Expect: 0.00036

Matches : 15/108 fragment ions using 24 most intense peaks

#	b	b^{++}	b*	Seq.	y	y^{++}	y*	#
1	**246.0761**	123.5417		E				11
2	**359.1601**	180.0837		I	***1256.6634***	628.8353	1239.6368	10
3	430.1973	215.6023		A	***1143.5793***	572.2933	1126.5528	9
4	558.2558	279.6316	541.2293	Q	***1072.5422***	536.7747	1055.5156	8
5	673.2828	337.1450	656.2562	D	***944.4836***	472.7454	927.4571	7
6	820.3512	410.6792	803.3246	F	***829.4567***	415.2320	812.4301	6
7	962.4618	481.7345	945.4353	K	***682.3883***	341.6978	665.3617	5
8	1063.5095	532.2584	1046.4829	T	***540.2776***	270.6425	523.2511	4
9	**1178.5364**	589.7719	1161.5099	D	439.2300	220.1186	422.2034	3
10	1291.6205	646.3139	1274.5939	L	***324.2030***	162.6051	307.1765	2
11				R	***211.1190***	106.0631	194.0924	1

Spectrum from Validation:



Monoisotopic mass of neutral peptide Mr(calc): 1500.7249

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

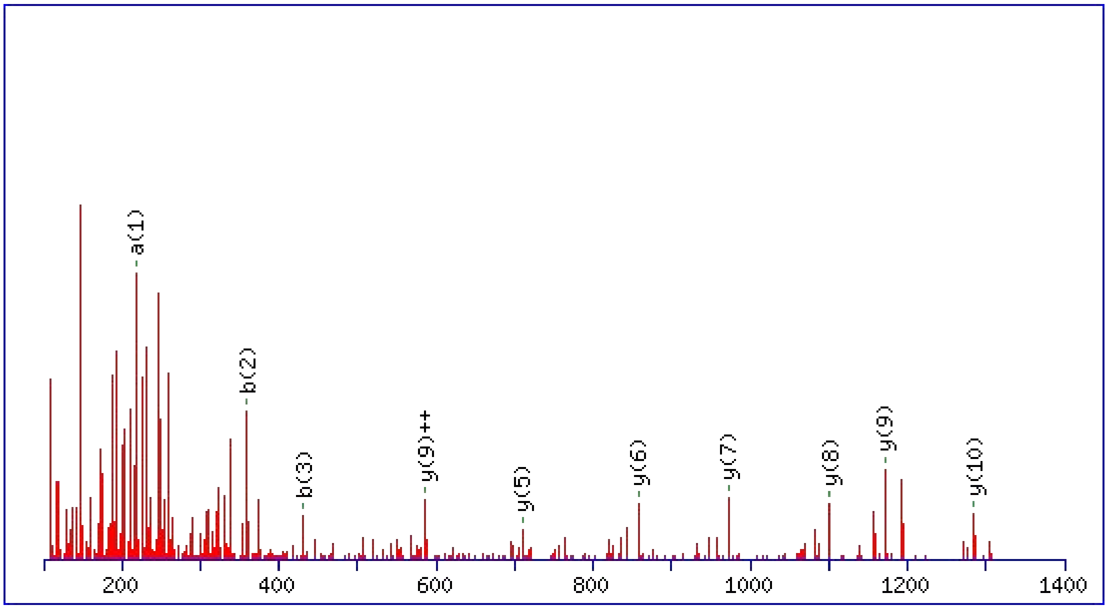
Variable modifications:

N-term : OPA (N-term)
K7 **:** Methyl-Mono (KR)
R11 **:** MDA (R)
Ions Score: 55 **Expect:** 9.2e-005
Matches : 19/108 fragment ions using 37 most intense peaks

#	**b**	**b^{++}**	**b***	**Seq.**	**y**	**y^{++}**	**y***	**#**
1	**246.0761**	123.5417		**E**				**11**
2	**359.1601**	180.0837		**I**	***1256.6634***	628.8353	1239.6368	**10**
3	**430.1973**	215.6023		**A**	***1143.5793***	572.2933	1126.5528	**9**
4	558.2558	279.6316	541.2293	**Q**	***1072.5422***	536.7747	1055.5156	**8**
5	673.2828	337.1450	656.2562	**D**	***944.4836***	472.7454	927.4571	**7**
6	820.3512	410.6792	803.3246	**F**	***829.4567***	415.2320	812.4301	**6**
7	962.4618	481.7345	945.4353	**K**	***682.3883***	341.6978	665.3617	**5**
8	1063.5095	532.2584	1046.4829	**T**	***540.2776***	270.6425	523.2511	**4**
9	**1178.5364**	589.7719	1161.5099	**D**	439.2300	220.1186	422.2034	**3**
10	**1291.6205**	646.3139	1274.5939	**L**	***324.2030***	162.6051	307.1765	**2**
11				**R**	***211.1190***	106.0631	194.0924	**1**

Peptide 2: R.$EIAQDFK^TDLR#.F

Spectrum from Large scale ID:



Monoisotopic mass of neutral peptide Mr(calc): 1528.7562

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K7 : Methyl-Tri (K)

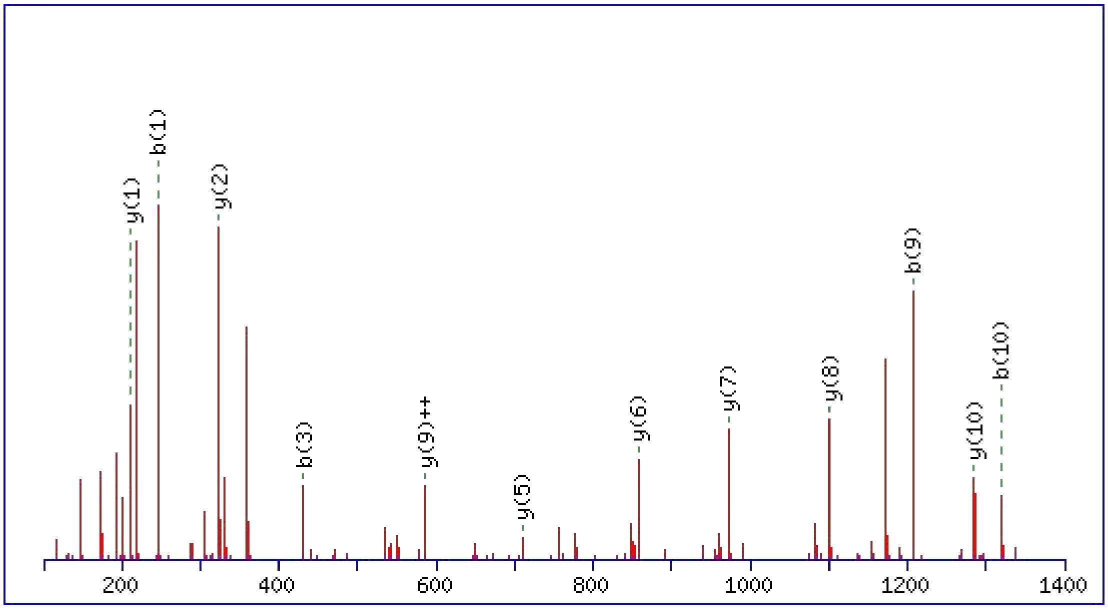
R11 : MDA (R)

Ions Score: 44 Expect: 0.066

Matches : 10/108 fragment ions using 12 most intense peaks

#	b	b++	b*	Seq.	y	y++	y*	#
1	246.0761	123.5417		E				11
2	**359.1601**	180.0837		I	***1284.6947***	642.8510	1267.6681	10
3	**430.1973**	215.6023		A	***1171.6106***	586.3089	1154.5841	9
4	558.2558	279.6316	541.2293	Q	***1100.5735***	550.7904	1083.5469	8
5	673.2828	337.1450	656.2562	D	***972.5149***	486.7611	955.4884	7
6	820.3512	410.6792	803.3246	F	***857.4880***	429.2476	840.4614	6
7	990.4931	495.7502	973.4666	K	***710.4196***	355.7134	693.3930	5
8	1091.5408	546.2740	1074.5142	T	540.2776	270.6425	523.2511	4
9	1206.5677	603.7875	1189.5412	D	439.2300	220.1186	422.2034	3
10	1319.6518	660.3295	1302.6252	L	324.2030	162.6051	307.1765	2
11				R	211.1190	106.0631	194.0924	1

Spectrum from Validation:



Monoisotopic mass of neutral peptide Mr(calc): 1528.7562

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K7 **:** Methyl-Tri (K)

R11 **:** MDA (R)

Ions Score: 64 **Expect:** 0.00015

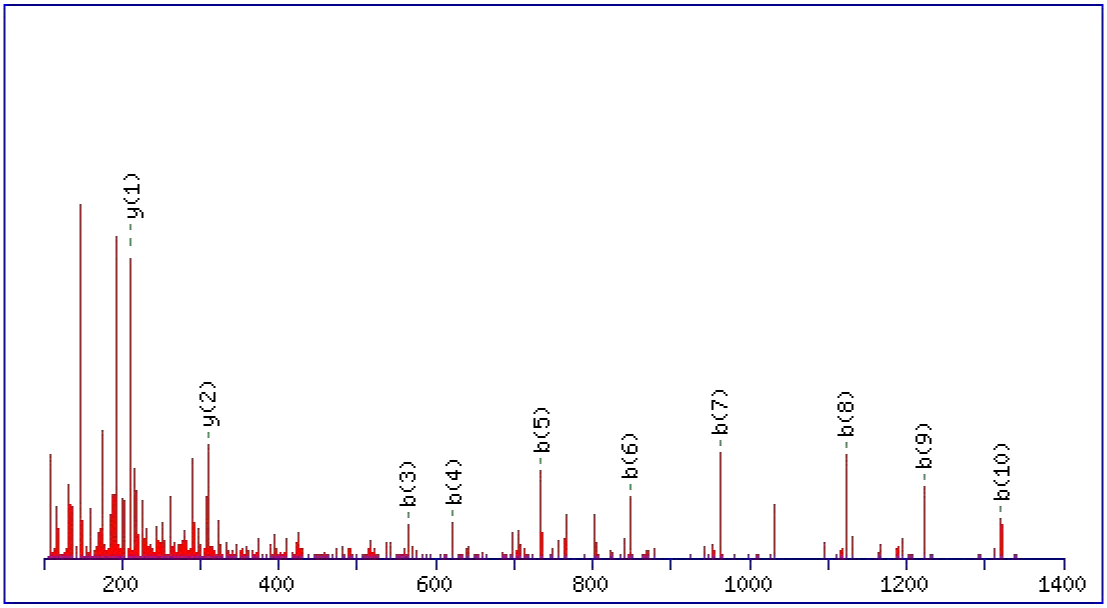
Matches : 12/108 fragment ions using 13 most intense peaks

#	**b**	**b^{++}**	**b***	**Seq.**	**y**	**y^{++}**	**y***	**#**
1	**246.0761**	123.5417		**E**				**11**
2	359.1601	180.0837		**I**	***1284.6947***	642.8510	1267.6681	**10**
3	**430.1973**	215.6023		**A**	1171.6106	586.3089	1154.5841	**9**
4	558.2558	279.6316	541.2293	**Q**	***1100.5735***	550.7904	1083.5469	**8**
5	673.2828	337.1450	656.2562	**D**	***972.5149***	486.7611	955.4884	**7**
6	820.3512	410.6792	803.3246	**F**	***857.4880***	429.2476	840.4614	**6**
7	990.4931	495.7502	973.4666	**K**	***710.4196***	355.7134	693.3930	**5**
8	1091.5408	546.2740	1074.5142	**T**	540.2776	270.6425	523.2511	**4**
9	**1206.5677**	603.7875	1189.5412	**D**	439.2300	220.1186	422.2034	**3**
10	**1319.6518**	660.3295	1302.6252	**L**	***324.2030***	162.6051	307.1765	**2**

11				R	***211.1190***	106.0631	194.0924	1

Peptide 3: K.$QYK@GIIDCVVR#.I

Spectrum from Large scale ID:



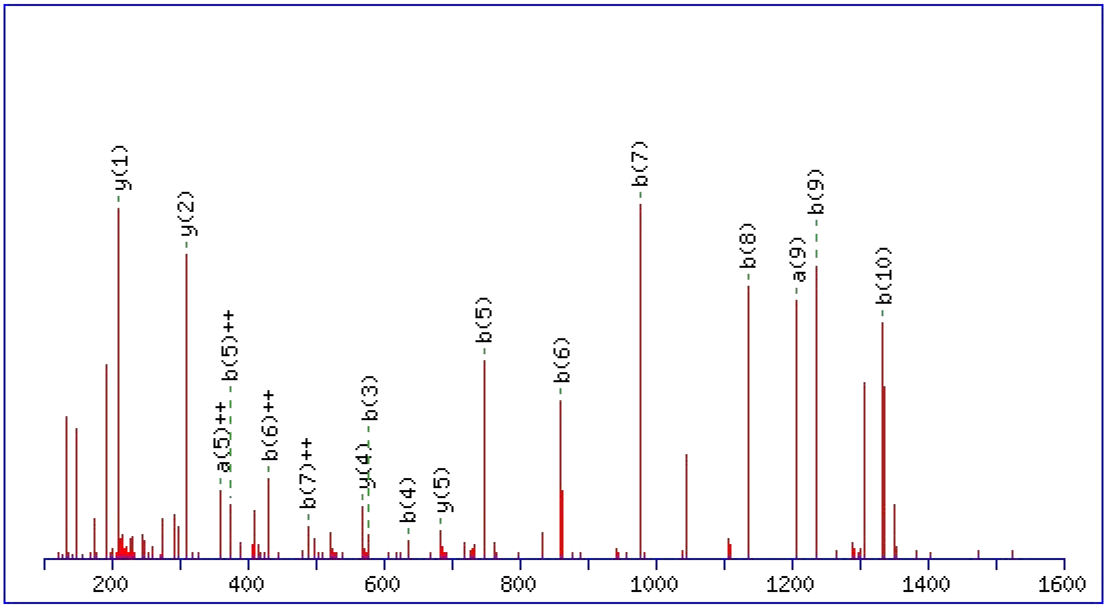
Monoisotopic mass of neutral peptide Mr(calc): 1529.7701
Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)
Variable modifications:
N-term : OPA (N-term)
K3 : Methyl-Di (K)
R11 : MDA (R)
Ions Score: 66 Expect: 0.00054
Matches : 10/120 fragment ions using 13 most intense peaks

#	b	b^{++}	b*	Seq.	y	y^{++}	y*	#
1	245.0921	123.0497	228.0655	Q				11
2	408.1554	204.5813	391.1288	Y	1286.6926	643.8499	1269.6660	10
3	***564.2817***	282.6445	547.2551	K	1123.6292	562.3183	1106.6027	9
4	***621.3031***	311.1552	604.2766	G	967.5030	484.2551	950.4764	8
5	***734.3872***	367.6972	717.3606	I	910.4815	455.7444	893.4550	7

6	***847.4713***	424.2393	830.4447	I	797.3974	399.2024	780.3709	6
7	***962.4982***	481.7527	945.4716	D	684.3134	342.6603	667.2868	5
8	***1122.5288***	561.7681	1105.5023	C	569.2864	285.1469	552.2599	4
9	***1221.5973***	611.3023	1204.5707	V	409.2558	205.1315	392.2292	3
10	***1320.6657***	660.8365	1303.6391	V	**310.1874**	155.5973	293.1608	2
11				R	**211.1190**	106.0631	194.0924	1

Peptide 4: K.$QYK^GIIDCVVR#.I

Spectrum from Large scale ID:



Monoisotopic mass of neutral peptide Mr(calc): 1543.7857

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K3 : Methyl-Tri (K)

R11 : MDA (R)

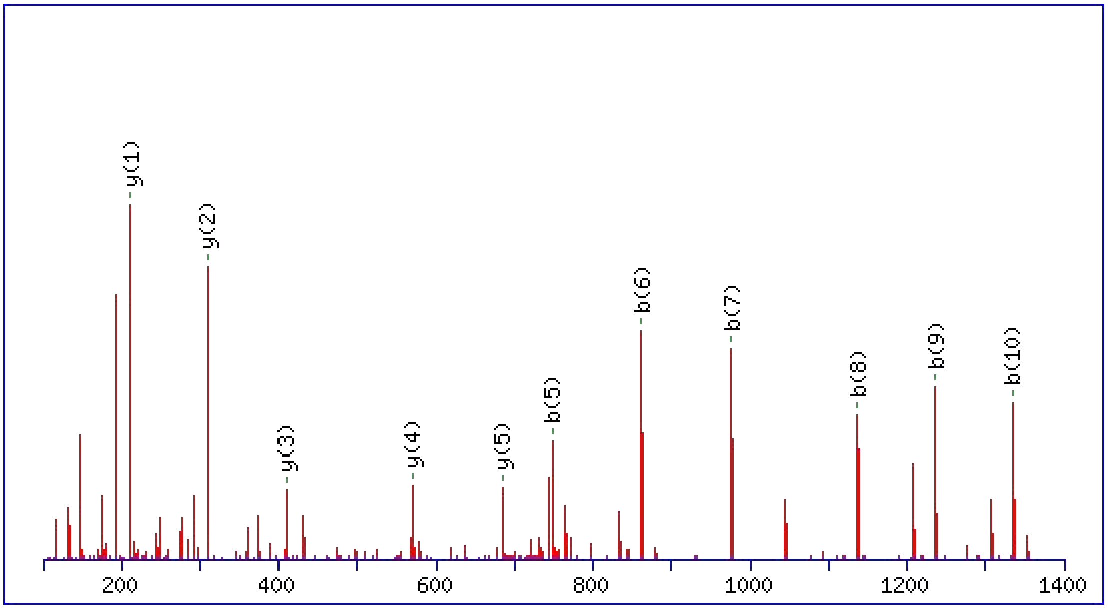
Ions Score: 53 Expect: 0.012

Matches : 17/120 fragment ions using 28 most intense peaks

#	b	b++	b*	Seq.	y	y++	y*	#
1	245.0921	123.0497	228.0655	Q				11

2	408.1554	204.5813	391.1288	Y	1300.7082	650.8577	1283.6817	10
3	***578.2973***	289.6523	561.2708	K	1137.6449	569.3261	1120.6183	9
4	***635.3188***	318.1630	618.2922	G	967.5030	484.2551	950.4764	8
5	***748.4028***	**374.7051**	731.3763	I	910.4815	455.7444	893.4550	7
6	***861.4869***	**431.2471**	844.4604	I	797.3974	399.2024	780.3709	6
7	***976.5138***	**488.7606**	959.4873	D	**684.3134**	342.6603	667.2868	5
8	***1136.5445***	568.7759	1119.5179	C	**569.2864**	285.1469	552.2599	4
9	***1235.6129***	618.3101	1218.5864	V	409.2558	205.1315	392.2292	3
10	***1334.6813***	667.8443	1317.6548	V	**310.1874**	155.5973	293.1608	2
11				R	**211.1190**	106.0631	194.0924	1

Spectrum from Validation:



Monoisotopic mass of neutral peptide Mr(calc): 1543.7857

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K3 : Methyl-Tri (K)

R11 : MDA (R)

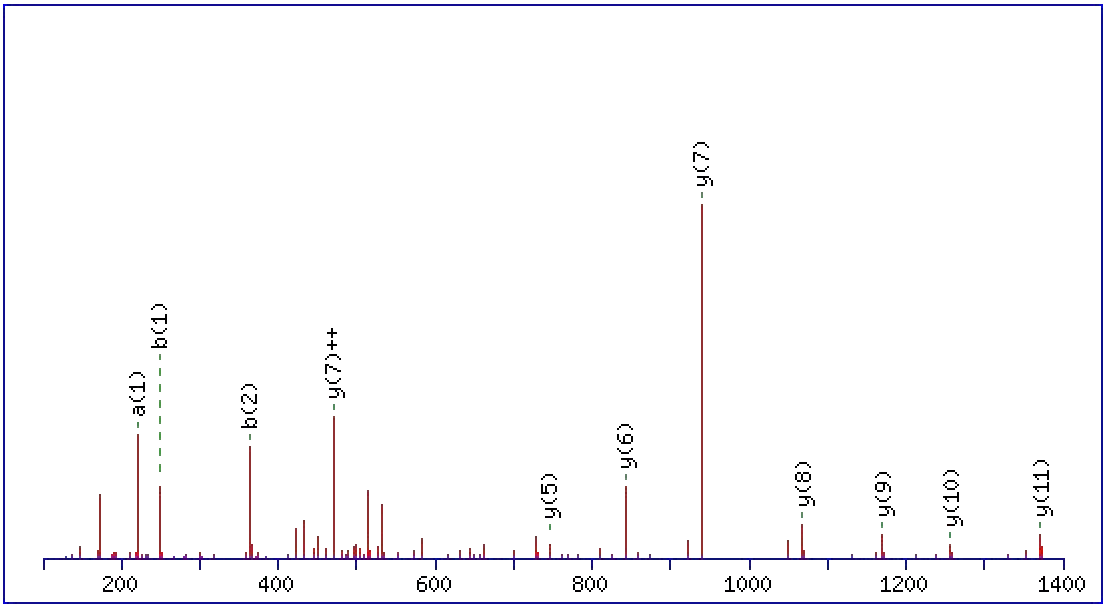
Ions Score: 48 **Expect:** 0.0021

Matches : 11/120 fragment ions using 13 most intense peaks

#	**b**	**b^{++}**	**b***	**Seq.**	**y**	**y^{++}**	**y***	**#**
1	245.0921	123.0497	228.0655	**Q**				**11**
2	408.1554	204.5813	391.1288	**Y**	1300.7082	650.8577	1283.6817	**10**
3	578.2973	289.6523	561.2708	**K**	1137.6449	569.3261	1120.6183	**9**
4	635.3188	318.1630	618.2922	**G**	967.5030	484.2551	950.4764	**8**
5	***748.4028***	374.7051	731.3763	**I**	910.4815	455.7444	893.4550	**7**
6	***861.4869***	431.2471	844.4604	**I**	797.3974	399.2024	780.3709	**6**
7	***976.5138***	488.7606	959.4873	**D**	**684.3134**	342.6603	667.2868	**5**
8	***1136.5445***	568.7759	1119.5179	**C**	**569.2864**	285.1469	552.2599	**4**
9	***1235.6129***	618.3101	1218.5864	**V**	**409.2558**	205.1315	392.2292	**3**
10	***1334.6813***	667.8443	1317.6548	**V**	**310.1874**	155.5973	293.1608	**2**
11				**R**	**211.1190**	106.0631	194.0924	**1**

Peptide 5: K.$MDSTEPPYSQK@R#.Y

Spectrum from Large scale ID:



Monoisotopic mass of neutral peptide Mr(calc): 1617.7133

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K11 **: Methyl-Di (K)**

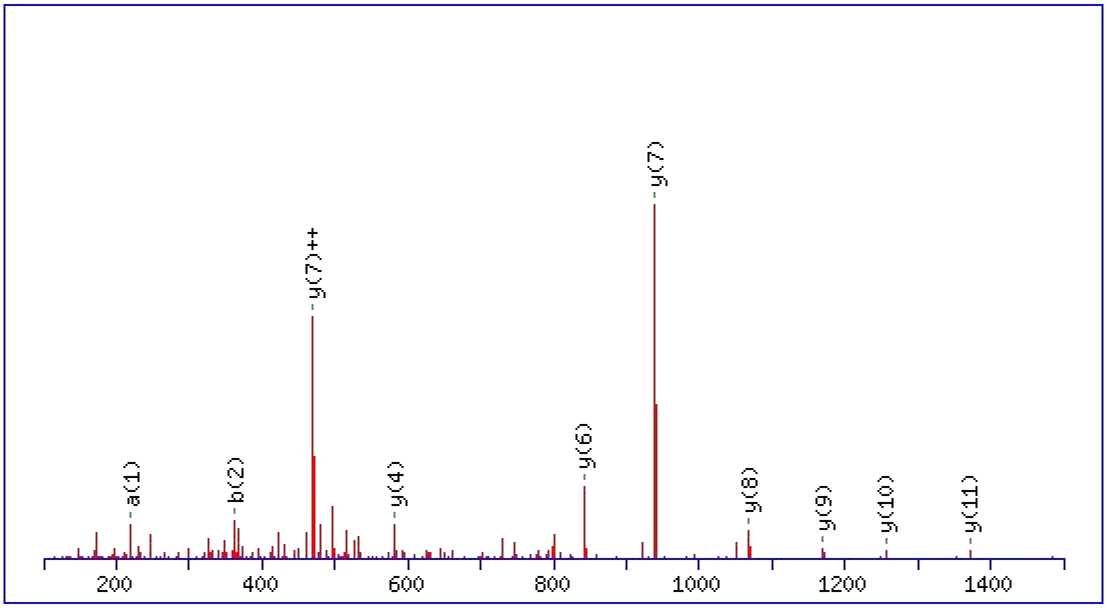
R12 **: MDA (R)**

Ions Score: 60 Expect: 0.00043

Matches : 11/96 fragment ions using 13 most intense peaks

#	b	b^{++}	b*	Seq.	y	y^{++}	y*	#
1	**248.0740**	124.5406		**M**				**12**
2	**363.1009**	182.0541		**D**	***1371.6539***	686.3306	1354.6274	**11**
3	450.1329	225.5701		**S**	***1256.6270***	628.8171	1239.6004	**10**
4	551.1806	276.0940		**T**	***1169.5950***	585.3011	1152.5684	**9**
5	680.2232	340.6152		**E**	***1068.5473***	534.7773	1051.5207	**8**
6	777.2760	389.1416		**P**	***939.5047***	470.2560	922.4781	**7**
7	874.3287	437.6680		**P**	***842.4519***	421.7296	825.4254	**6**
8	1037.3921	519.1997		**Y**	***745.3992***	373.2032	728.3726	**5**
9	1124.4241	562.7157		**S**	582.3358	291.6715	565.3093	**4**
10	1252.4827	626.7450	1235.4561	**Q**	495.3038	248.1555	478.2772	**3**
11	1408.6089	704.8081	1391.5824	**K**	367.2452	184.1262	350.2187	**2**
12				**R**	211.1190	106.0631	194.0924	**1**

Spectrum from Validation:



Monoisotopic mass of neutral peptide Mr(calc): 1617.7133

Fixed modifications: Carbamidomethyl (C) (apply to specified residues or termini only)

Variable modifications:

N-term : OPA (N-term)

K11 : Methyl-Di (KR)

R12 : MDA (R)

Ions Score: 52 **Expect:** 1.3e-005

Matches : 10/96 fragment ions using 14 most intense peaks

#	**b**	**b^{++}**	**b***	**Seq.**	**y**	**y^{++}**	**y***	**#**
1	248.0740	124.5406		**M**				**12**
2	**363.1009**	182.0541		**D**	***1371.6539***	686.3306	1354.6274	**11**
3	450.1329	225.5701		**S**	***1256.6270***	628.8171	1239.6004	**10**
4	551.1806	276.0940		**T**	***1169.5950***	585.3011	1152.5684	**9**
5	680.2232	340.6152		**E**	***1068.5473***	534.7773	1051.5207	**8**
6	777.2760	389.1416		**P**	***939.5047***	470.2560	922.4781	**7**
7	874.3287	437.6680		**P**	***842.4519***	421.7296	825.4254	**6**
8	1037.3921	519.1997		**Y**	745.3992	373.2032	728.3726	**5**
9	1124.4241	562.7157		**S**	***582.3358***	291.6715	565.3093	**4**
10	1252.4827	626.7450	1235.4561	**Q**	495.3038	248.1555	478.2772	**3**
11	1408.6089	704.8081	1391.5824	**K**	367.2452	184.1262	350.2187	**2**
12				**R**	211.1190	106.0631	194.0924	**1**

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