



EU FT-ICR MS

Sample preparation
FT-ICR MS sample analysis
Top down of proteins
Data interpretation

Petr Novák, Zdeněk Kukačka, Petr Man



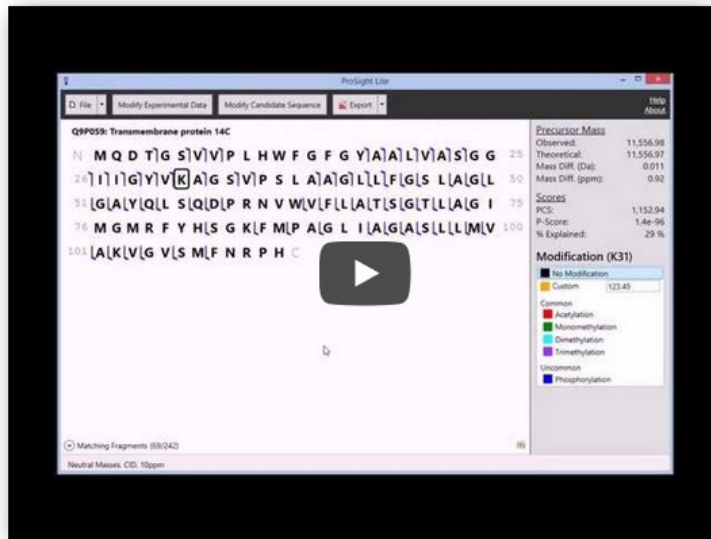
ProSight Lite – freely available

<http://prosightlite.northwestern.edu/>

- Groups of Neil L. Kelleher and Paul M. Thoms
- Windows operating systems



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ProSight Lite

ProSight Lite is a free Windows application for matching a single candidate protein sequence and its modifications against a set of mass spectrometric observations.

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ProSight Lite – Manual and software description

ProSight Lite: Graphical Software to Analyze Top-Down Mass Spectrometry Data

Ryan T. Fellers, Joseph B. Greer, Bryan P. Early, Xiang Yu, Richard D. LeDuc, Neil L. Kelleher*, and Paul M. Thomas*

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Abstract

Many top-down proteomics experiments focus on identifying and localizing post-translational modifications and other potential sources of “mass shift” on a known protein sequence. A simple application to match ion masses and facilitate the iterative hypothesis testing of PTM presence and location would assist with the data analysis in these experiments. ProSight Lite is a free software tool for matching a single candidate sequence against a set of mass spectrometric observations. Fixed or variable modifications, including both post-translational modifications and a select number of glycosylations, can be applied to the amino acid sequence. The application reports multiple scores and a matching fragment list. Fragmentation maps can be exported for publication in either PNG or SVG format. ProSight Lite can be freely downloaded from <http://proslightlite.northwestern.edu>, installs and updates from the web, and requires Windows 7 or higher.

Keywords

Top-down proteomics; proteomics software; MS Analysis; proteoform characterization

Top-down proteomics describes the study of intact proteins with mass spectrometry [1, 2]. Traditional bottom-up proteomics experiments are marked by the use of an enzyme, typically trypsin, to proteolyze intact proteins into more analytically manageable peptides (0.5-3 kDa)[3]. This proteolysis effects a loss of information between the ribosomally-expressed pro-protein, which in eukaryotes often contains RNA splice variants, and the post-translationally modified intact protein (termed a “proteoform”)[4]. If a modification has been identified on two separate peptides, a typical bottom-up proteomics experiment cannot know whether those modification existed singly on two separate proteoforms or in tandem on a single proteoform. While more analytically challenging, top-down proteomics provides that full information[5].

Bioinformatics Analysis of Top-Down Mass Spectrometry Data with ProSight Lite

Caroline J. DeHart, Ryan T. Fellers, Luca Fornelli, Neil L. Kelleher, and Paul M. Thomas

Abstract

Traditional bottom-up mass spectrometry-based proteomics relies on the use of an enzyme, often trypsin, to generate small peptides (typically < 25 amino acids long). In top-down proteomics, proteins remain intact and are directly measured within the mass spectrometer. This technique, while inherently simpler than bottom-up proteomics, generates data which must be processed and analyzed using software tools “purpose-built” for the job. In this chapter, we will show the analysis of intact protein spectra through deconvolution, deisotoping, and searching with ProSight Lite, a free, vendor-agnostic tool for the analysis of top-down mass spectrometry data. We will illustrate with two examples of intact protein fragmentation spectra and discuss the iterative use of the software to characterize proteoforms and discover the sites of post-translational modifications.

Key words Top-down, Mass spectrometry, Proteomics, ProSight Lite, Intact protein, Bioinformatics

1 Introduction

Complementing the speed and sensitivity of bottom-up proteomics [1, 2], top-down proteomics [3] offers a comprehensive view of proteoforms present in the sample (for a more detailed description of a proteoform, *see Note 1*) [4]. It is important to distinguish between top-down mass spectrometry and top-down proteomics. In top-down mass spectrometry, one or a few proteoforms are isolated and studied. This technique is typified by studies of histones [5–7] and immunoglobulins [8–10], among others, including the routine analysis of small (< 40 kDa) proteins. In top-down proteomics, a whole, unknown proteome is typically isolated, fractionated, and analyzed by mass spectrometry [11–13]. The tools used for the study of top-down mass spectrometry and top-down proteomics are related, but fundamentally different. Top-down proteomics requires searching tandem MS data against a database of known proteins and proteoforms [14, 15], while top-down mass

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ProSight Lite – User interface

ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

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ProSight Lite v1.4
Simple, Targeted, Top-Down Proteomics.


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To match mass fragment data against a known protein sequence:

- ! [Add Experimental Data](#)
- ! [Add Candidate Sequence](#)

ProSight Lite – Sequence Uniprot/fasta

Modify Sequence

UniProt Accession:

Sequence

Fixed Modifications

Cysteine

- No Modification
- S-carboxamidoethyl-L-cysteine
- S-pyridylethyl-L-cysteine
- S-carboxamidomethyl-L-cysteine
- cysteine mercaptoethanol
- half cystine

Methionine

- No Modification
- L-methionine sulfoxide
- L-methionine sulfone

Modify Sequence

UniProt Accession:

Sequence

```
GLSDGEWQQVLNVWGKVEADIAGHGQEVLRIRLFTGHPETLEKFDKFKHLK  
TEAEMKASEDLKKHGTVVLTALGGILKKKGHHEALKPLAQSHATKKKIPIK  
YLEFISDAIIHVLHSHKHPGDFGADAQGAMTKALELFRNDIAAKYKELGFQG
```

Fixed Modifications

Cysteine

- No Modification
- S-carboxamidoethyl-L-cysteine
- S-pyridylethyl-L-cysteine
- S-carboxamidomethyl-L-cysteine
- cysteine mercaptoethanol
- half cystine

Methionine

- No Modification
- L-methionine sulfoxide
- L-methionine sulfone

ProSight Lite – Experimental data

Experimental Data

Precursor

Precursor Mass Type
 Monoisotopic Average

Fragments (one per line)
Line Count:

✖

Fragmentation Methods
 CID
 HCD
 SID
 ECD
 ETD
 EThcD
 IRMPD
 BY and CZ*
 UVPD 4
 UVPD 6
 UVPD 9

Fragmentation Tolerance
10 ppm

Save Cancel

Experimental Data

Precursor

Precursor Mass Type
 Monoisotopic Average

Fragments (one per line)
Line Count: 480

310.6183
321.2544
336.1558
349.1478
351.9706
390.1988
408.1881
447.2201
460.8899
465.5631
490.2582
505.2537
524.9506
532.2493
538.5565
576.2628

Fragmentation Methods
 CID
 HCD
 SID
 ECD
 ETD
 EThcD
 IRMPD
 BY and CZ*
 UVPD 4
 UVPD 6
 UVPD 9

Fragmentation Tolerance
2 ppm

Save Cancel

First Example

Myoglobin – modification by NHS-propionate (mass shift 56.026)

ECD – fragmentation of singly modified protein

ProSight Lite – Experimental data from mgf file

```
Myo_ECD_NHSP.mgf - Notepad
File Edit Format View Help
### C:\Users\Administrator\Desktop\Myo_ECD_NHSP.mgf
### D:\Biocev\Data\MultiCASI_ECD_NHSP_apo_MY0.d\analysis.baf
### MultiCASI_ECD_NHSP_apo_MY0.d
### Instrument: solarix XR
### apo_MY0 NHSP
### MultiCASI_ECD
### MultiCASI_ECD_NHSP_+19/+18/+17/+16/+15
### Quality 0.50, S/N 0, BB C 4.9384 N 1.3577 O 1.4773 S 0.0417 H 7.7583, BB2 , RelIntens 0, MinIntens 0, MaxCharge 15, SNAP2 1
### Retain Residuals no, Create neutrally no,
### FullScan Parameters: AdductIon (+)+H (-)-H, LowMass 250, HighMass 4000, MaxCharge charge decon 4, MinPeaks 3
### Perf. isotope decon yes, Max Charge Istopic decon 10, MW agreement 0.00, Abund. cutoff 10.00, Envelope cutoff 75.00
### MaxRes Parameters: AdductIon (+)+H (-)-H, LowMass 250, HighMass 4000
### Perf. isotope decon yes, Max Charge Istopic decon 10, Abund. cutoff 2.00
### MSn Parameters: AdductIon (+)+H (-)-H, LowMass 50, HighMass 2000
### Perf. isotope decon yes, Max Charge Istopic decon 8, Abund. cutoff 2.00
### Global Charge Limit: no 3, Prefer FullScan Result: yes, Export what: 1 (0=non deconv/1=deconv only/2=mixed), NoMSMSignals: 2000, Intensity threshold: 2000,
NoMSMSignals (deconv): 2000, NoMSMSignals (non-deconv): 2000, NormalizedData: no, SingleCharged: yes
### +MS(CASI 895.58580)

####FS: #m/z: 895.58580 #charge 19
####MaxRes:#m/z: 0.000000 #charge-128
####MS:
####MSMS:
BEGIN IONS
TITLE=+MS2(qCID 895.58580)
RTINSECONDS=0
SCANS=MS: MSMS:
PEPMASS=895.58580 148506160

310.6183 1032778 1+
321.2544 625904 1+
336.1558 12251523 1+
349.1478 767920 1+
351.9706 1008276 1+
390.1988 30597142 1+
408.1881 4508194 1+
447.2201 137446480 1+
460.8899 812333 1+
465.5631 890910 1+
490.2582 930141 1+
505.2537 8062103 1+
524.9506 859948 1+
532.2493 4148949 1+
```


ProSight Lite – Results window

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N **G** L S **[D]** **[G]** **[E]** **[W]** **[Q]** **[Q]** V L N V W **[G]** **[K]** V E A D I **[A]** **[G]** H G 25

26 **[Q]** **[E]** V L I **[R]** L F T **[G]** **[H]** P **[E]** **[T]** L **[E]** **[K]** **[F]** **[D]** **[K]** **[F]** **[K]** H L K 50

51 **[T]** **[E]** A E **[M]** **[K]** A S **[E]** **[D]** L **[K]** **[K]** H **[G]** T V V L T A L **[G]** G I 75

76 L **[K]** **[K]** K G H H E A E L K P L A Q **[S]** **[H]** A T **[K]** **[H]** **[K]** I P 100

101 I K Y **[L]** **[E]** **[F]** I S **[D]** A I I H V **[L]** **[H]** **[S]** **[K]** H P **[G]** **[D]** **[F]** **[G]** A 125

126 **[D]** A **[Q]** **[G]** A **[M]** T **[K]** A L **[E]** **[L]** **[F]** **[R]** **[N]** **[D]** I A **[A]** **[K]** **[Y]** K E L G 150

151 F **[Q]** G C

Matching Fragments (Count: 123)

MH+, ECD, 2ppm

Precursor Mass
Type: Monoisotopic
Observed: n/a
Theoretical: 16,940.96

Scores
PCS: 2,390.93
P-Score: 4.1e-190
% Fragments Explain... 25%
% Residue Cleavages: 68%

Modification (G1)
No Modification
Custom
Uncommon
Monomethylation

ProSight Lite – List of matching fragments

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help About

N **G** L S [D] [G] [E] [W] [Q] [Q] V [L] [N] [V] [W] [G] [K] V [E] A D [I] [A] [G] [H] G 25
 26 [Q] [E] [V] L [I] [R] [L] F [T] [G] [H] P [E] [T] [L] [E] [K] [F] [D] [K] [F] [K] [H] L K 50
 51 [T] [E] [A] E [M] [K] [A] S [E] [D] [L] [K] [K] [H] [G] [T] [V] [V] L [T] [A] L [G] [G] I 75
 76 L [K] [K] [K] G H H E [A] E [L] K P L A Q [S] [H] [A] T [K] [H] [K] [I] P 100
 101 I K Y [L] [E] [F] [I] S [D] [A] I I H V [L] [H] [S] [K] [H] P G [D] [F] [G] [A] 125
 126 [D] [A] [Q] [G] [A] [M] [T] [K] A L [E] [L] [F] [R] [N] [D] [I] [A] [A] [K] [Y] [K] E [L] G 150
 151 F Q [G] C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 16,940.96

Scores
 PCS: 2,390.93
 P-Score: 4.1e-190
 % Fragments Explain... 25%
 % Residue Cleavages: 68%

Modification (G1)
 No Modification
 Custom
 Uncommon
 Monomethylation

Matching Fragments (Count: 123)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
C4	C	4	389.19	389.19	0.001	1.88
C5	C	5	446.21	446.21	0.001	1.29
C6	C	6	575.25	575.26	0.001	1.19
C7	C	7	761.33	761.34	0.001	1.15
C8	C	8	889.39	889.39	0.001	0.89
C10	C	10	1,116.52	1,116.52	0.001	0.54

MH+, ECD, 2ppm

ProSight Lite – Modification of N-terminus

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N **G** L S **D** G E W **Q** Q V L N V W G K V E A D I A G H G 25
 26 Q E V L I R L F T **G** H P E **T** L E K F D K F K H L K 50
 51 T **E** **A** E M K A S E **D** L K **K** H **G** T V V L T A L G G I 75
 76 L K K **K** G H H E A E **L** K P L A **Q** **S** **H** A T **K** **H** **K** I P 100
 101 I K Y **L** **E** **F** I S **D** A I I H V **L** **H** **S** **K** **H** P G **D** **F** **G** A 125
 126 **D** A **Q** **G** A **M** T **K** A L **E** **L** **F** **R** **N** **D** I A **A** **K** Y **K** E **L** G 150
 151 **F** **Q** **G** C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 16,996.99

Scores
 PCS: 1,169.56
 P-Score: 7.8e-98
 % Fragments Explaine... 15%
 % Residue Cleavages: 38%

Modification (G1)
 No Modification
 Custom 56,026
 Uncommon
 Monomethylation

Matching Fragments (Count: 72)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
C90	C	90	10,013.36	10,013.36	-0.001	-0.11
C92	C	92	10,228.45	10,228.45	0.006	0.62
C93	C	93	10,365.51	10,365.51	0.003	0.26
C97	C	97	10,802.74	10,802.75	0.002	0.16
C98	C	98	10,930.84	10,930.84	0.003	0.27
C109	C	109	12,249.56	12,249.56	0.005	0.37

MH+, ECD, 2ppm

ProSight Lite – Modification of K16

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help About

N G L S [D] [G] [E] [W] [Q] [Q] V [L] [N] [V] [W] [G] [K] V E A D I A G H G 25
 26 Q E V L I R L F T [G] [H] [P] [E] [T] L E K F D K F K H L K 50
 51 T [E] [A] E M K A S E [D] L K [K] H [G] T V V L T A L G G I 75
 76 L K K [K] G H H E A E [L] K P L A [Q] [S] [H] [A] T [K] [H] [K] I P 100
 101 I K Y [L] [E] [F] [I] S [D] A I I H V [L] [H] [S] [K] [H] P G [D] [F] [G] [A] 125
 126 [D] [A] [Q] [G] [A] [M] [T] [K] A L [E] [L] [F] [R] [N] [D] [I] [A] [A] [K] [Y] [K] E [L] G 150
 151 F [Q] [G] C

Matching Fragments (Count: 81)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
C4	C	4	389.19	389.19	0.001	1.88
C5	C	5	446.21	446.21	0.001	1.29
C6	C	6	575.25	575.26	0.001	1.19
C7	C	7	761.33	761.34	0.001	1.15
C8	C	8	889.39	889.39	0.001	0.89
C10	C	10	1,116.52	1,116.52	0.001	0.54

MH+, ECD, 2ppm

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 16,996.99

Scores
 PCS: 1,415.87
 P-Score: 1.5e-116
 % Fragments Explain... 17%
 % Residue Cleavages: 44%

Modification (K16)

- No Modification
- Custom 56,026
- Common
 - Acetylation
 - Monomethylation
 - Dimethylation
 - Trimethylation
- Uncommon
 - Phosphorylation

ProSight Lite – Modification of K87

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N G L S [D] [G] [E] [W] [Q] [Q] V [L] [N] [V] [W] [G] [K] V [E] A [D] [I] [A] [G] [H] G 25
 26 [Q] [E] [V] L [I] [R] [L] F [T] [G] [H] P [E] [T] [L] [E] [K] [F] [D] [K] [F] [K] [H] L K 50
 51 [T] [E] [A] E [M] [K] [A] S [E] [D] [L] [K] [K] [H] [G] [T] [V] [V] L [T] [A] L [G] [G] I 75
 76 L K [K] [K] G [H] H E [A] E L [K] P L A [Q] [S] [H] [A] T [K] [H] [K] [I] P 100
 101 I K Y [L] [E] [F] [I] S [D] [A] I I H V [L] [H] [S] [K] [H] P G [D] [F] [G] [A] 125
 126 [D] [A] [Q] [G] [A] [M] [T] [K] A L [E] [L] [F] [R] [N] [D] [I] [A] [A] [K] [Y] [K] E [L] G 150
 151 F [Q] [G] C

Matching Fragments (Count: 140)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
C4	C	4	389.19	389.19	0.001	1.88
C5	C	5	446.21	446.21	0.001	1.29
C6	C	6	575.25	575.26	0.001	1.19
C7	C	7	761.33	761.34	0.001	1.15
C8	C	8	889.39	889.39	0.001	0.89
C10	C	10	1,116.52	1,116.52	0.001	0.54

MH+, ECD, 2ppm

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 16,996.99

Scores
 PCS: 2,922.26
 P-Score: 5e-230
 % Fragments Explaine... 29%
 % Residue Cleavages: 71%

Modification (K87)


- No Modification
- Custom 56,026
- Common
 - Acetylation
 - Monomethylation
 - Dimethylation
 - Trimethylation
- Uncommon
 - Phosphorylation

Second Example

Ubiquitin – modification by NHS-propionate (mass shift 56.026)

CID – fragmentation of singly modified protein

ProSight Lite – Experimental data

 Experimental Data

Precursor

Fragments (one per line)
Line Count: 466

215.139074
229.118376
243.079890
243.134016
260.106428
287.171512
298.176274
310.139876
316.132704
326.171164
340.150443
342.202466
343.234063
344.181762
358.161046
360.228340

Precursor Mass Type
 Monoisotopic Average

Mass Mode
 M (neutral) MH+

Fragmentation Methods
 CID
 HCD
 SID
 ECD
 ETD
 EThcD
 IRMPD
 BY and CZ*
 UVPD 4
 UVPD 6
 UVPD 9

Fragmentation Tolerance
 ppm

Save Cancel

ProSight Lite – Result window

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

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About

N **M** Q I I F V K T L T G K T I T L E V E P S D T I E N 25
26 V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50
51 E D G R T L S D Y N I Q K E S T L L H L V L R L R G 75
76 G C

Precursor Mass
Type: Monoisotopic
Observed: n/a
Theoretical: 8,559.62

Scores
PCS: 1,099.54
P-Score: 1.7e-92
% Fragments Explaine... 12%
% Residue Cleavages: 57%

Modification (M1)
No Modification
Custom
Common
Oxidation
Uncommon
Monomethylation

Matching Fragments (Count: 55)

MH+, CID, 2ppm

ProSight Lite – Matching fragments

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N **M** Q I F V K T L T G K T I T L E V E P S D T I E N 25
 26 V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50
 51 E D G R T L S D Y N I Q K E S T L L H L V L R L R G 75
 76 G C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 8,559.62

Scores
 PCS: 1,099.54
 P-Score: 1.7e-92
 % Fragments Explaine... 12%
 % Residue Cleavages: 57%

Modification (M1)
 No Modification
 Custom
 Common
 Oxidation
 Uncommon
 Monomethylation

Matching Fragments (Count: 55)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
B2	B	2	259.10	259.10	0.000	0.31
B3	B	3	372.18	372.18	0.000	0.42
B4	B	4	519.25	519.25	0.000	0.48
B5	B	5	618.32	618.32	0.000	0.38
B6	B	6	746.41	746.42	0.000	0.47
B7	B	7	847.46	847.46	0.000	-0.08

MH+, CID, 2ppm

ProSight Lite – Modification of N-terminus

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N **M** Q I I F V K T L T G K T I T L E V E P S D T I E N 25
 26 V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50
 51 E D G R T L S D Y N I Q K E S T L L H L V L R L R G 75
 76 G C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 8,615.64

Scores
 PCS: 1,072.29
 P-Score: 2e-90
 % Fragments Explaine... 12%
 % Residue Cleavages: 56%

Modification (M1)
 No Modification
 Custom 56,026
 Common
 Oxidation
 Uncommon
 Monomethylation

Matching Fragments (Count: 55)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
B2	B	2	315.13	315.13	0.000	1.13
B3	B	3	428.21	428.21	0.000	0.79
B4	B	4	575.28	575.28	0.000	0.67
B5	B	5	674.35	674.35	0.000	0.66
B6	B	6	802.44	802.44	0.000	0.38
B6	B	6	802.44	802.44	0.000	0.57

MH+, CID, 2ppm

ProSight Lite – Modification of K6

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N M Q I F V **K** T L T G K T I T L E V E P S D T I E N 25
 26 V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50
 51 E D G R T L S D Y N I Q K E S T L L H L V L R L R G 75
 76 G C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 8,615.64

Scores
 PCS: 1,154.38
 P-Score: 1.1e-96
 % Fragments Explaine... 13%
 % Residue Cleavages: 57%

Modification (K6)

No Modification
 Custom 56,026

Common
 Acetylation
 Monomethylation
 Dimethylation
 Trimethylation

Uncommon
 Phosphorylation

Matching Fragments (Count: 58)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
B2	B	2	259.10	259.10	0.000	0.31
B3	B	3	372.18	372.18	0.000	0.42
B4	B	4	519.25	519.25	0.000	0.48
B5	B	5	618.32	618.32	0.000	0.38
B6	B	6	802.44	802.44	0.000	0.38
B6	B	6	802.44	802.44	0.000	0.57

MH+, CID, 2ppm

ProSight Lite – Modification of K63

*Untitled.di - ProSight Lite

Modify Experimental Data Modify Candidate Sequence Export

Help
About

N M Q I F V K T L T G K T I T L E V E P S D T I E N 25
 26 V K A K I Q D K E G I P P D Q Q R L I F A G K Q L 50
 51 E D G R T L S D Y N I Q K E S T L L H L V L R L R G 75
 76 G C

Precursor Mass
 Type: Monoisotopic
 Observed: n/a
 Theoretical: 8,615.64

Scores
 PCS: 1,045.17
 P-Score: 2.3e-88
 % Fragments Explaine... 11%
 % Residue Cleavages: 53%

Modification (K63)

No Modification
 Custom 56,026

Common
 Acetylation
 Monomethylation
 Dimethylation
 Trimethylation

Uncommon
 Phosphorylation

Matching Fragments (Count: 53)

Name	Ion Type	Ion Number	Theoretical Mass	Observed Mass	Mass Difference (Da)	Mass Difference (ppm)
B2	B	2	259.10	259.10	0.000	0.31
B3	B	3	372.18	372.18	0.000	0.42
B4	B	4	519.25	519.25	0.000	0.48
B5	B	5	618.32	618.32	0.000	0.38
B6	B	6	746.41	746.42	0.000	0.47
B7	B	7	847.46	847.46	0.000	-0.08

MH+, CID, 2ppm



EU FT-ICR MS

Sample preparation
FT-ICR MS sample analysis
Top down of proteins
Data interpretation

Petr Novák, Zdeněk Kukačka, Petr Man

