# Figures

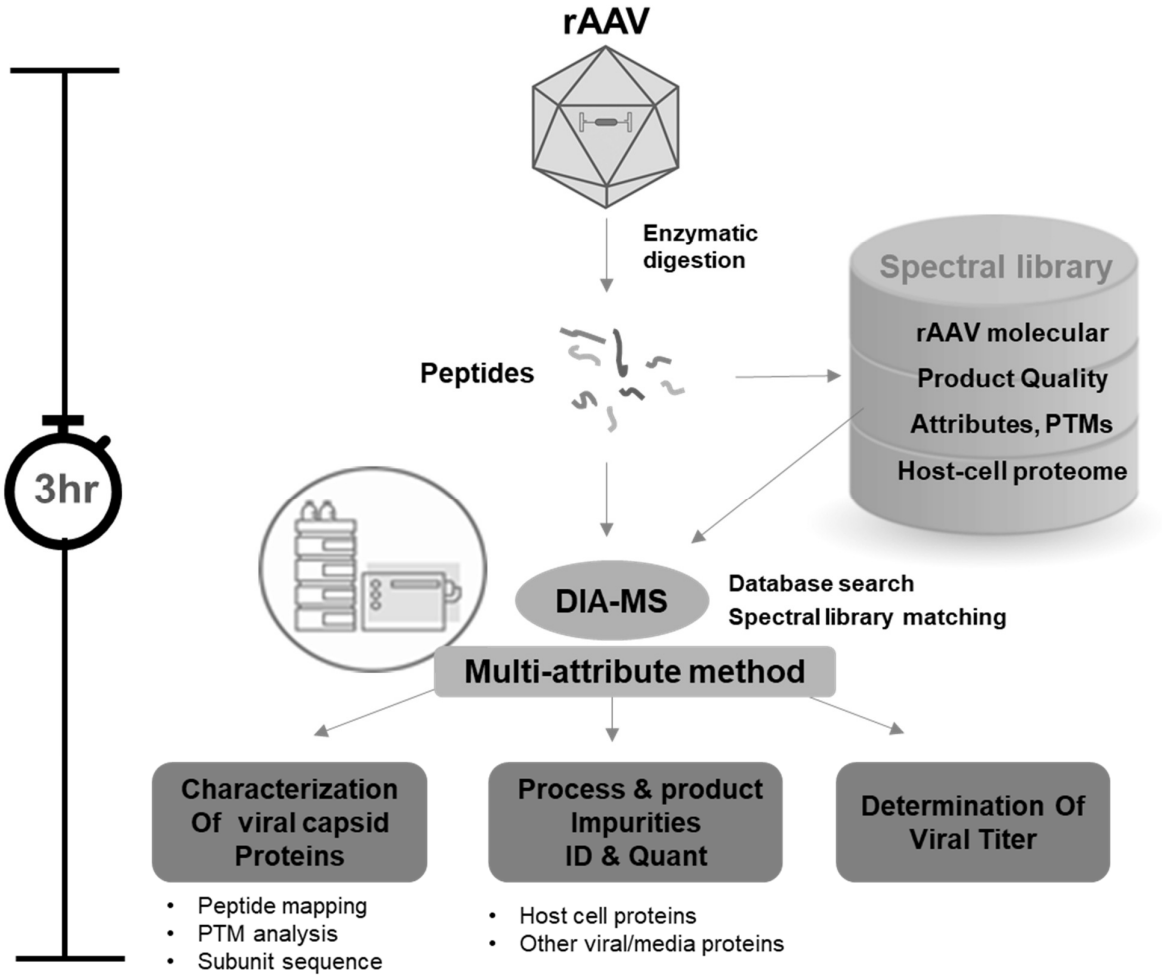



**Figure 1**

**> AAV2 Capsid Sequence- VP1 (Protein length 734 amino acids)**

```
  1 AADGYLPDWL EDTLSEGIRQ WWKLKPGPPP PKPAERHKDD SRGLVLPGYK YLGPFNGLDK GEPVNEADAA ALEHDKAYDR
 81 QLDSGDNPYL KYNHADAEFQ ERLKEDTSFG GNLGRAVFQA KKRVLEPLGL VEEPVKTAPG KKRPVEHSPV EPDSSSGTGK
161 AGQQPARKRL NFGQTGDADS VPDPQPLGQP PAAPSGLGTN TMATGSGAPM ADNNEGADGV GNSSGNWHCD STWMGDRVIT
241 TSTRTWALPT YNNHLYKQIS SQSGASNDNH YFGYSTPWGY FDFNRFHCHF SPRDWQRLIN NNWGFRPKRL NFKLFNIQVK
321 EVTQNDGTTT IANNLTSTVQ VFTDSEYQLP YVLGSAHQGC LPPFPADVFM VPQYGYLTLN NGSQAVGRSS FYCLEYFPSQ
401 MLRTGNNFTF SYTFEDVPFH SSYAHSQSLD RLMNPLIDQY LYYLSRTNTP SGTTTQSRLQ FSQAGASDIR DQSRNWLPGP
481 CYRQQRVSKT SADNNNSEYS WTGATKYHLN GRDSLVNPGP AMASHKDDEE KFFPQSGVLI FGKQGSEKTN VDIEKVMITD
561 EEEIRTTNPV ATEQYGSVST NLQRGNRQAA TADVNTQGVL PGMVWQDRDV YLQGPIWAKI PHTDGHFHPS PLMGGFGLKH
641 PPPQILIKNT PVPANPSTTF SAAKFASFIT QYSTGQVSVE IEWELQKENS KRWNPEIQYT SNYNKSVNVD FTVDTNGVYS
721 EPRPIGTRYL TRNL
```

(SEQ ID NO:1)

**Figure 2 (continued)**

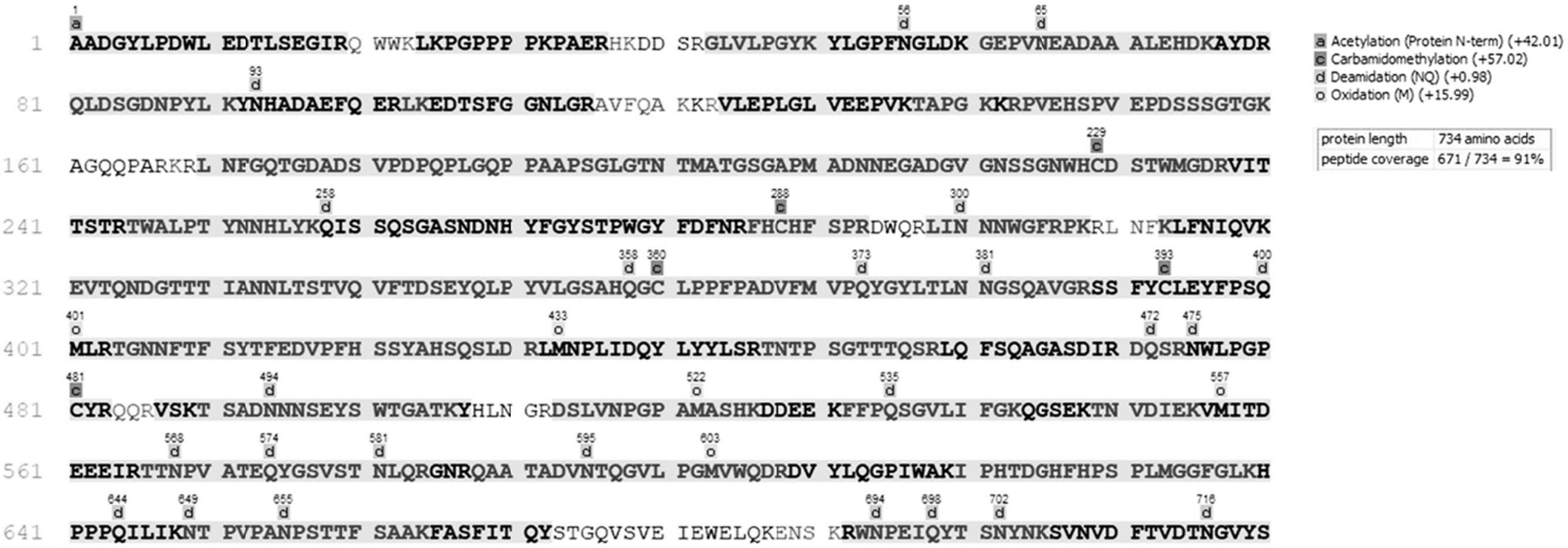
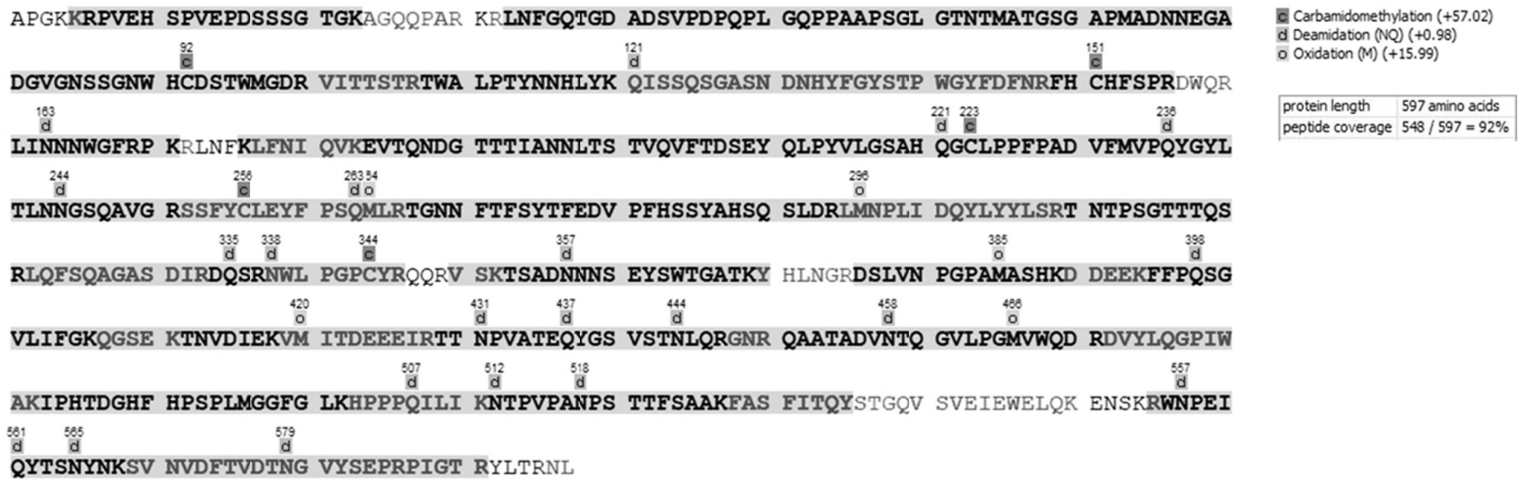


**Figure 2 (continued)**

**> AAV2 Capsid Sequence- VP2 (Protein length 597 amino acids)**

```
  1 APGKKRPVEH SPVEPDSSSG TGKAGQQPAR KRLNFGQTGD ADSVPDPQPL GQPPAAPSGL GTNTMATGSG APMADNNEGA
 81 DGVGNSSGNW HCDSTWMGDR VITTSTRTWA LPTYNNHLYK QISSQSGASN DNHYFGYSTP WGYFDFNRFH CHFSPRDWQR
161 LINNNWGFRP KRLNFKLFNI QVKEVTQNDG TTTIANNLTS TVQVFTDSEY QLPYVLGSAH QGCLPPFPAD VFMVPQYGYL
241 TLNNGSQAVG RSSFYCLEYF PSQMLRTGNN FTFSYTFEDV PFHSSYAHSQ SLDRLMNPLI DQYLYYLSRT NTPSGTTTQS
321 RLQFSQAGAS DIRDQSRNWL PGPCYRQQRV SKTSADNNNS EYSWTGATKY HLNGRDSLVN PGPAMASHKD DEEKFFPQSG
401 VLIFGKQGSE KTNVDIEKVM ITDEEEIRTT NPVATEQYGS VSTNLQRGNR QAATADVNTQ GVLPGMVWQD RDVYLQGPIW
481 AKIPHTDGHF HPSPLMGGFG LKHPPPQILI KNTPVPANPS TTFSAAKFAS FITQYSTGQV SVEIEWELQK ENSKRWNPEI
561 QYTSNYNKSV NVDFTVDTNG VYSEPRPIGT RYLTRNL
```

(SEQ ID NO:2)



**> AAV2 Capsid Sequence- VP3 (Protein length 534 amino acids)**

```
  1 ATGSGAPMAD NNEGADGVGN SSGNWHCDST WMGDRVITTS TRTWALPTYN NHLYKQISSQ SGASNDNHYF GYSTPWGYFD
 81 FNRFHCHFSP RDWQRLINNN WGFRPKRLNF KLFNIQVKEV TQNDGTTTIA NNLTSTVQVF TDSEYQLPYV LGSAHQGCLP
161 PFPADVFMVP QYGYLTLNNG SQAVGRSSFY CLEYFPSQML RTGNNFTFSY TFEDVPFHSS YAHSQSLDRL MNPLIDQYLY
241 YLSRTNTPSG TTTQSRLQFS QAGASDIRDQ SRNWLPGPCY RQQRVSKTSA DNNNSEYSWT GATKYHLNGR DSLVNPGPAM
321 ASHKDDEEKF FPQSGVLIFG KQGSEKTNVD IEKVMITDEE EIRTTNPVAT EQYGSVSTNL QRGNRQAATA DVNTQGVLPG
401 MVWQDRDVYL QGPIWAKIPH TDGHFHPSPL MGGFGLKHPP PQILIKNTPV PANPSTTFSA AKFASFITQY STGQVSVEIE
481 WELQKENSKR WNPEIQYTSN YNKSVNVDFT VDTNGVYSEP RPIGTRYLTR NL
```

(SEQ ID NO:3)

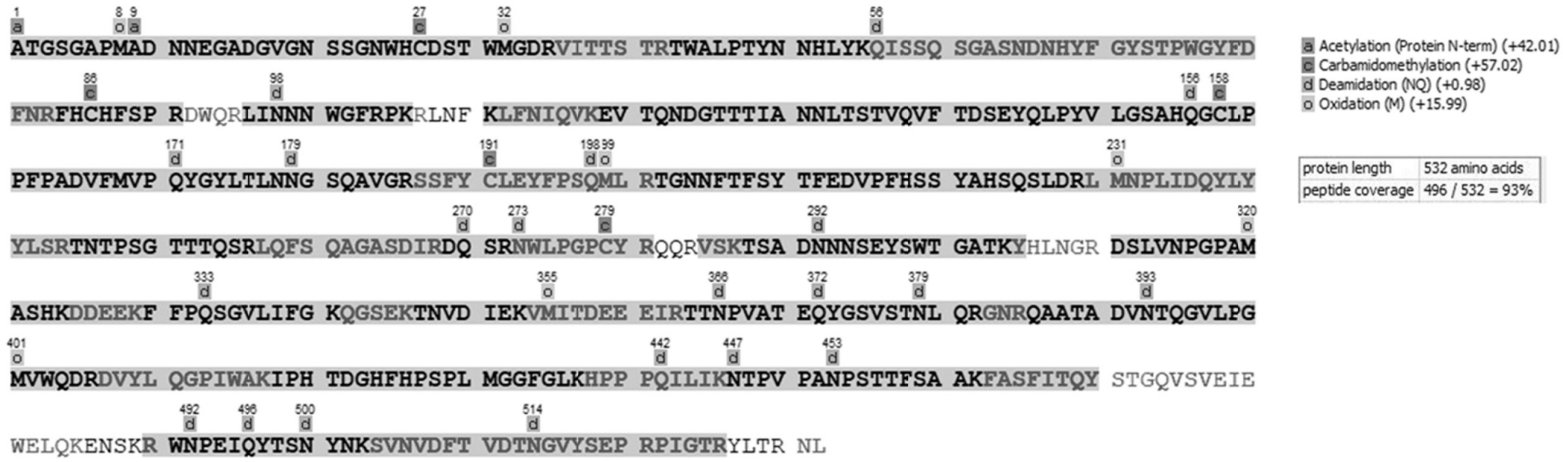



Figure 2 (end)

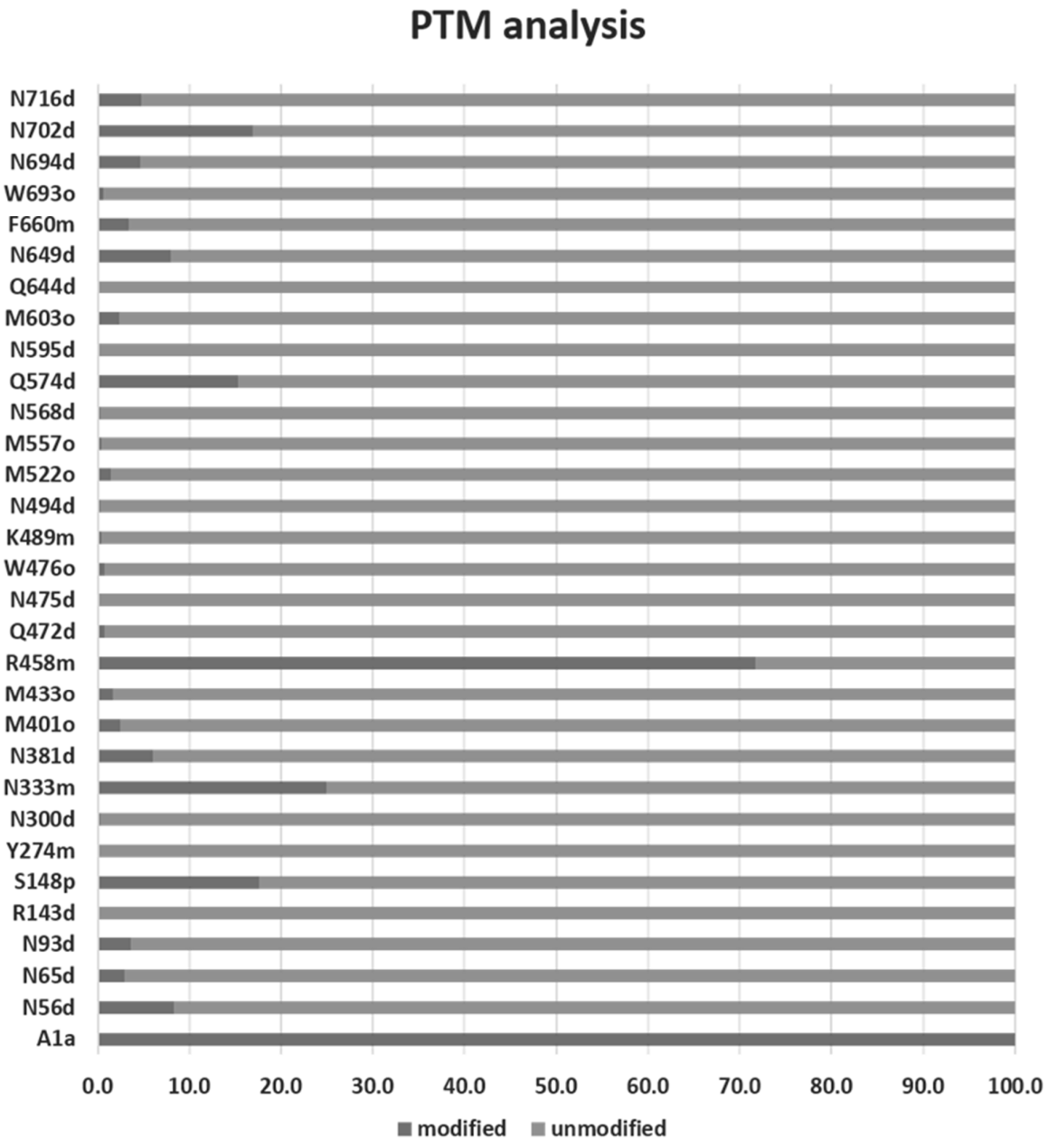

PTM analysis
N716d
N702d
N694d
W693o
F660m
N649d
Q644d
M603o
N595d
Q574d
N568d
M557o
M522o
N494d
K489m
W476o
N475d
Q472d
R458m
M433o
M401o
N381d
N333m
N300d
Y274m
S148p
R143d
N93d
N65d
N56d
A1a
0.0
10.0
20.0
30.0
40.0
50.0
60.0
70.0
80.0
90.0
100.0
modified
unmodified

**Figure 3**

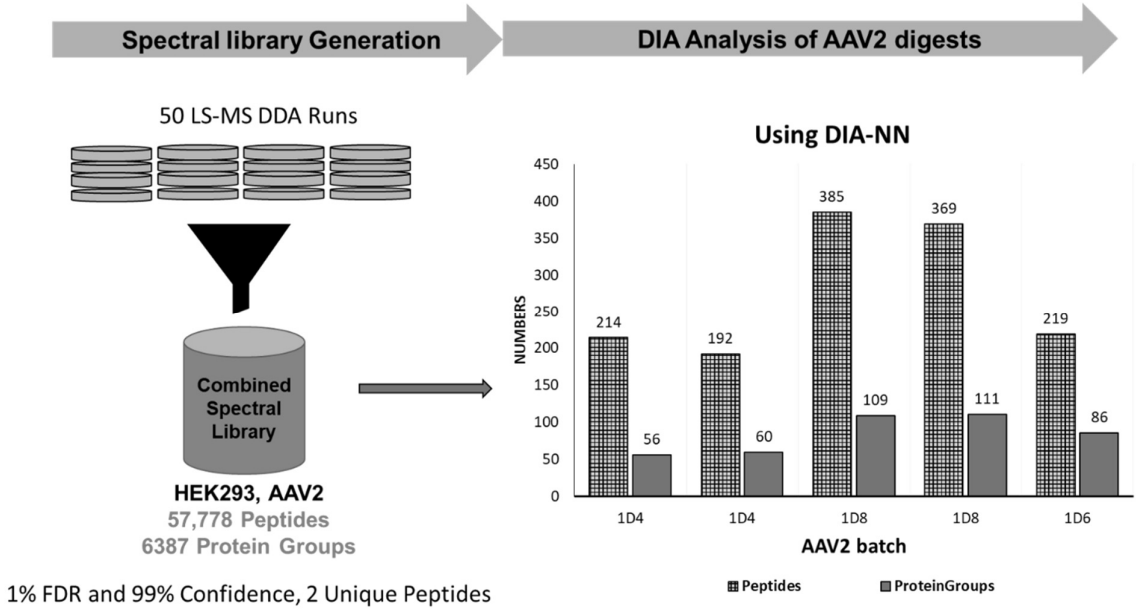

Spectral library Generation
DIA Analysis of AAV2 digests
50 LS-MS DDA Runs
Combined Spectral Library
HEK293, AAV2
57,778 Peptides
6387 Protein Groups
1% FDR and 99% Confidence, 2 Unique Peptides
Using DIA-NN
NUMBERS
450
400
350
300
250
200
150
100
50
0
214
56
192
60
385
109
369
111
219
86
1D4
1D4
1D8
1D8
1D6
AAV2 batch
Peptides
ProteinGroups

**Figure 4**

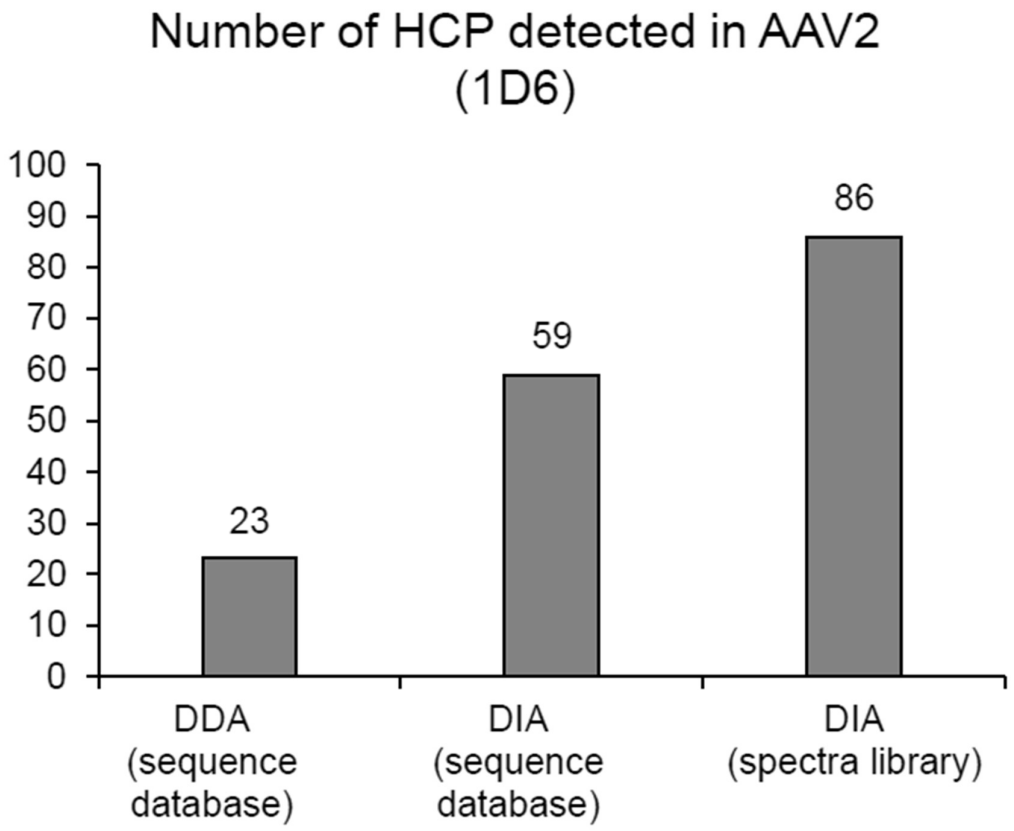
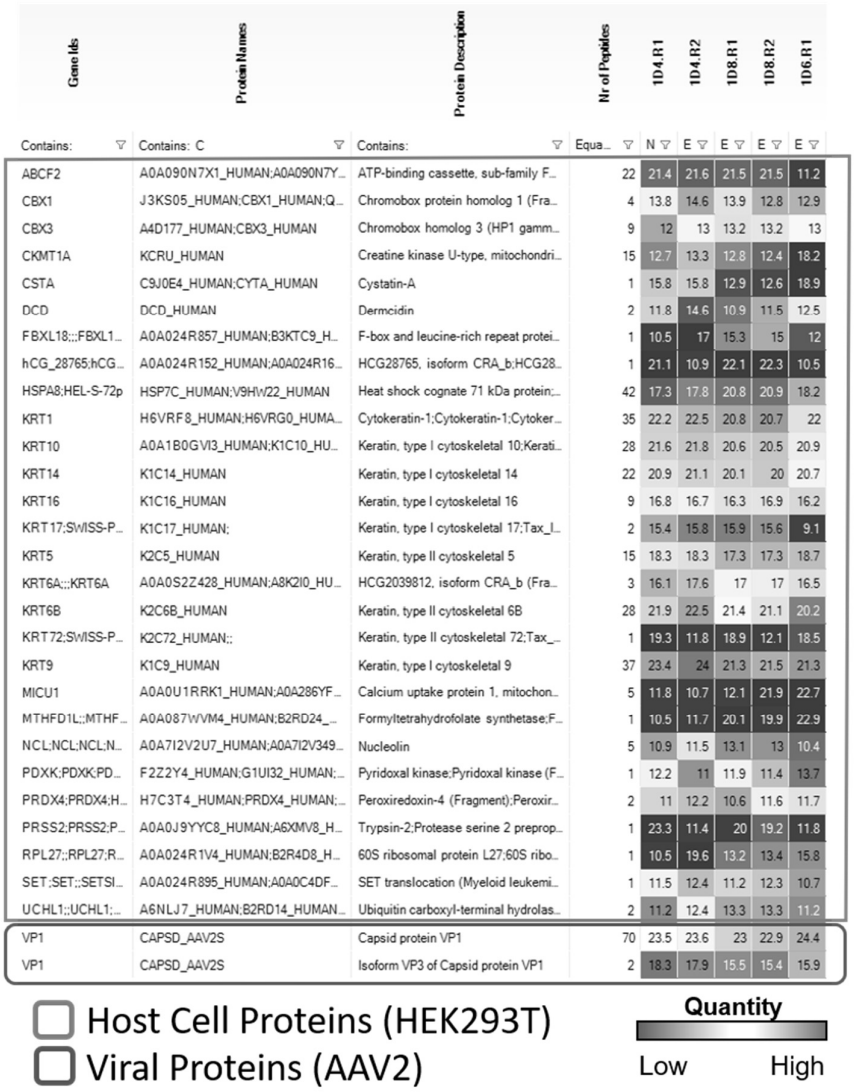

Number of HCP detected in AAV2 (1D6)
100
90
80
70
60
50
40
30
20
10
0
23
59
86
DDA (sequence database)
DIA (sequence database)
DIA (spectra library)

**Figure 5**

## List of HCPs and AAV proteins detected



| Gene Ids | Protein Names | Protein Description | Nr of Peptides | 1D4.R1 | 1D4.R2 | 1D8.R1 | 1D8.R2 | 1D6.R1 |
|---|---|---|---|---|---|---|---|---|
| Contains: | Contains: C | Contains: | Equa... | N | E | E | E | E |
| ABCF2 | A0A090N7X1_HUMAN;A0A090N7Y... | ATP-binding cassette, sub-family F... | 22 | 21.4 | 21.6 | 21.5 | 21.5 | 11.2 |
| CBX1 | J3KS05_HUMAN;CBX1_HUMAN;Q... | Chromobox protein homolog 1 (Fra... | 4 | 13.8 | 14.6 | 13.9 | 12.8 | 12.9 |
| CBX3 | A4D177_HUMAN;CBX3_HUMAN | Chromobox homolog 3 (HP1 gamm... | 9 | 12 | 13 | 13.2 | 13.2 | 13 |
| CKMT1A | KCRU_HUMAN | Creatine kinase U-type, mitochondri... | 15 | 12.7 | 13.3 | 12.8 | 12.4 | 18.2 |
| CSTA | C9J0E4_HUMAN;CYTA_HUMAN | Cystatin-A | 1 | 15.8 | 15.8 | 12.9 | 12.6 | 18.9 |
| DCD | DCD_HUMAN | Dermcidin | 2 | 11.8 | 14.6 | 10.9 | 11.5 | 12.5 |
| FBXL18;;;FBXL1... | A0A024R857_HUMAN;B3KTC9_H... | F-box and leucine-rich repeat protei... | 1 | 10.5 | 17 | 15.3 | 15 | 12 |
| hCG_28765;hCG... | A0A024R152_HUMAN;A0A024R16... | HCG28765, isoform CRA_b;HCG28... | 1 | 21.1 | 10.9 | 22.1 | 22.3 | 10.5 |
| HSPA8;HEL-S-72p | HSP7C_HUMAN;V9HW22_HUMAN | Heat shock cognate 71 kDa protein;... | 42 | 17.3 | 17.8 | 20.8 | 20.9 | 18.2 |
| KRT1 | H6VRF8_HUMAN;H6VRG0_HUMA... | Cytokeratin-1;Cytokeratin-1;Cytoker... | 35 | 22.2 | 22.5 | 20.8 | 20.7 | 22 |
| KRT10 | A0A1B0GVI3_HUMAN;K1C10_HU... | Keratin, type I cytoskeletal 10;Kerati... | 28 | 21.6 | 21.8 | 20.6 | 20.5 | 20.9 |
| KRT14 | K1C14_HUMAN | Keratin, type I cytoskeletal 14 | 22 | 20.9 | 21.1 | 20.1 | 20 | 20.7 |
| KRT16 | K1C16_HUMAN | Keratin, type I cytoskeletal 16 | 9 | 16.8 | 16.7 | 16.3 | 16.9 | 16.2 |
| KRT17;SWISS-P... | K1C17_HUMAN; | Keratin, type I cytoskeletal 17;Tax_I... | 2 | 15.4 | 15.8 | 15.9 | 15.6 | 9.1 |
| KRT5 | K2C5_HUMAN | Keratin, type II cytoskeletal 5 | 15 | 18.3 | 18.3 | 17.3 | 17.3 | 18.7 |
| KRT6A;;;KRT6A | A0A0S2Z428_HUMAN;A8K2I0_HU... | HCG2039812, isoform CRA_b (Fra... | 3 | 16.1 | 17.6 | 17 | 17 | 16.5 |
| KRT6B | K2C6B_HUMAN | Keratin, type II cytoskeletal 6B | 28 | 21.9 | 22.5 | 21.4 | 21.1 | 20.2 |
| KRT72;SWISS-P... | K2C72_HUMAN;; | Keratin, type II cytoskeletal 72;Tax_... | 1 | 19.3 | 11.8 | 18.9 | 12.1 | 18.5 |
| KRT9 | K1C9_HUMAN | Keratin, type I cytoskeletal 9 | 37 | 23.4 | 24 | 21.3 | 21.5 | 21.3 |
| MICU1 | A0A0U1RRK1_HUMAN;A0A286YF... | Calcium uptake protein 1, mitochon... | 5 | 11.8 | 10.7 | 12.1 | 21.9 | 22.7 |
| MTHFD1L;;MTHF... | A0A087WVM4_HUMAN;B2RD24_... | Formyltetrahydrofolate synthetase;F... | 1 | 10.5 | 11.7 | 20.1 | 19.9 | 22.9 |
| NCL;NCL;NCL;N... | A0A7I2V2U7_HUMAN;A0A7I2V349... | Nucleolin | 5 | 10.9 | 11.5 | 13.1 | 13 | 10.4 |
| PDXK;PDXK;PD... | F2Z2Y4_HUMAN;G1UI32_HUMAN;... | Pyridoxal kinase;Pyridoxal kinase (F... | 1 | 12.2 | 11 | 11.9 | 11.4 | 13.7 |
| PRDX4;PRDX4;H... | H7C3T4_HUMAN;PRDX4_HUMAN;... | Peroxiredoxin-4 (Fragment);Peroxir... | 2 | 11 | 12.2 | 10.6 | 11.6 | 11.7 |
| PRSS2;PRSS2;P... | A0A0J9YYC8_HUMAN;A6XMV8_H... | Trypsin-2;Protease serine 2 preprop... | 1 | 23.3 | 11.4 | 20 | 19.2 | 11.8 |
| RPL27;;RPL27;R... | A0A024R1V4_HUMAN;B2R4D8_H... | 60S ribosomal protein L27;60S ribo... | 1 | 10.5 | 19.6 | 13.2 | 13.4 | 15.8 |
| SET;SET;;SETSI... | A0A024R895_HUMAN;A0A0C4DF... | SET translocation (Myeloid leukemi... | 1 | 11.5 | 12.4 | 11.2 | 12.3 | 10.7 |
| UCHL1;;UCHL1;... | A6NLJ7_HUMAN;B2RD14_HUMAN... | Ubiquitin carboxyl-terminal hydrolas... | 2 | 11.2 | 12.4 | 13.3 | 13.3 | 11.2 |
| VP1 | CAPSD_AAV2S | Capsid protein VP1 | 70 | 23.5 | 23.6 | 23 | 22.9 | 24.4 |
| VP1 | CAPSD_AAV2S | Isoform VP3 of Capsid protein VP1 | 2 | 18.3 | 17.9 | 15.5 | 15.4 | 15.9 |



**Figure 6 (continued)**

## **Post-translational modifications** in Capsid Protein VP1

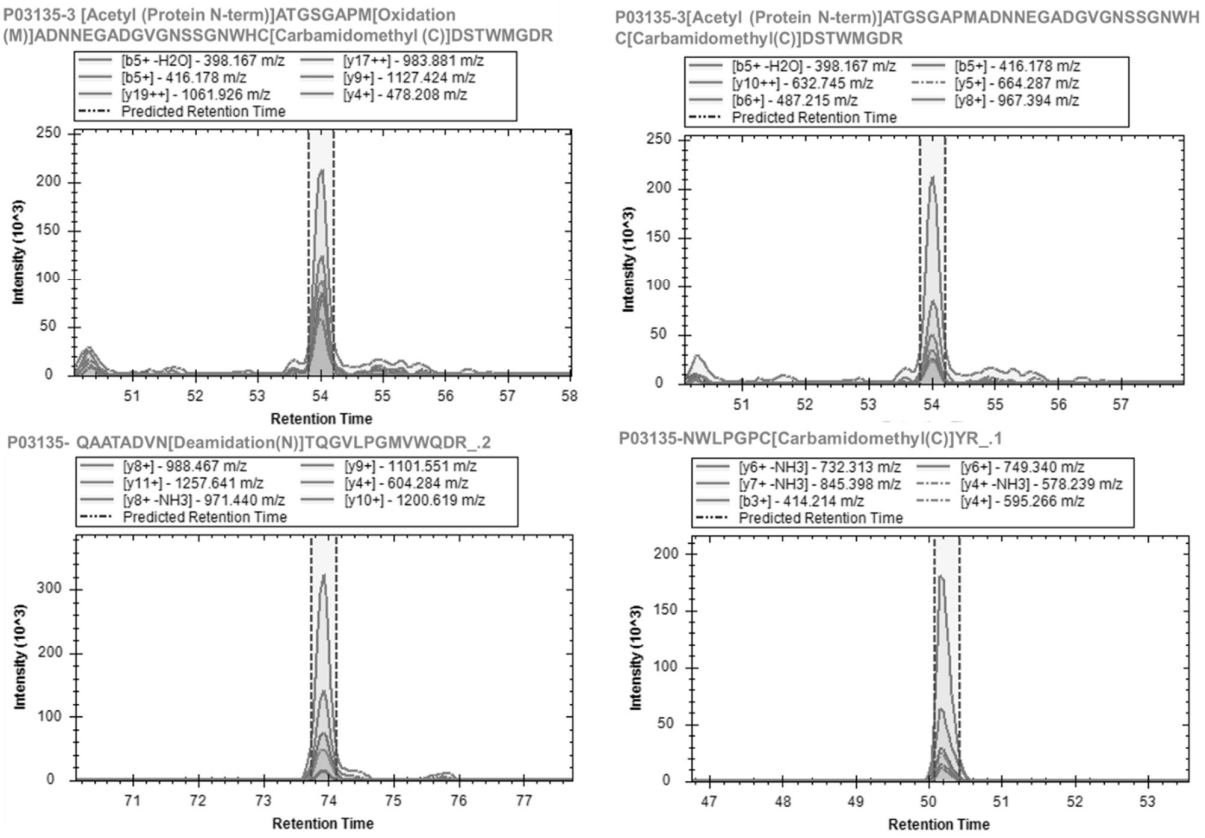



**Figure 6 (end)**

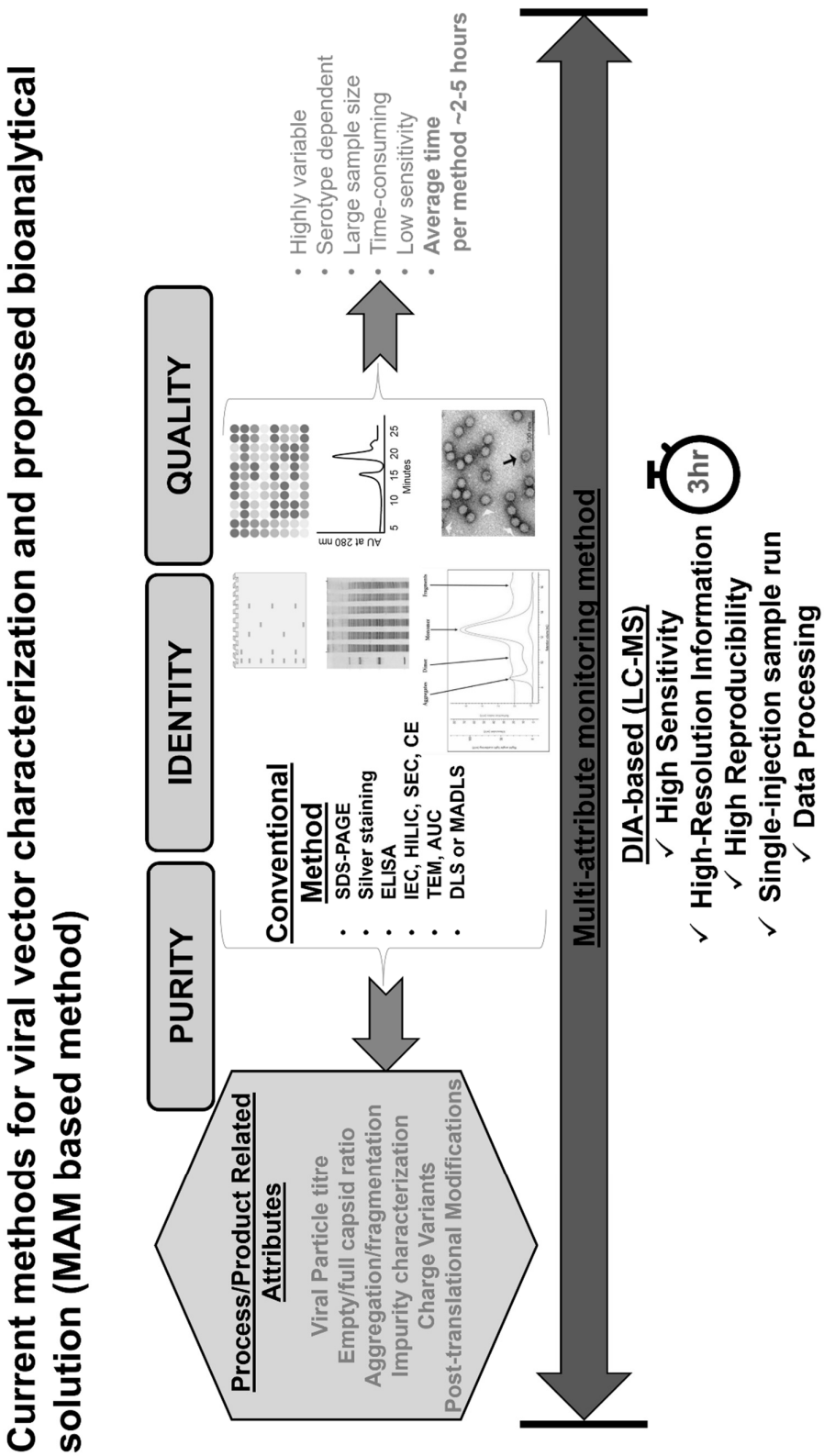

Current methods for viral vector characterization and proposed bioanalytical solution (MAM based method)
PURITY
IDENTITY
QUALITY
Process/Product Related Attributes
Viral Particle titre
Empty/full capsid ratio
Aggregation/fragmentation
Impurity characterization
Charge Variants
Post-translational Modifications
Conventional Method
SDS-PAGE
Silver staining
ELISA
IEC, HILIC, SEC, CE
TEM, AUC
DLS or MADLS
Highly variable
Serotype dependent
Large sample size
Time-consuming
Low sensitivity
Average time per method ~2-5 hours
Multi-attribute monitoring method
DIA-based (LC-MS)
✓ High Sensitivity
✓ High-Resolution Information
✓ High Reproducibility
✓ Single-injection sample run
✓ Data Processing
3hr

**Figure 7**

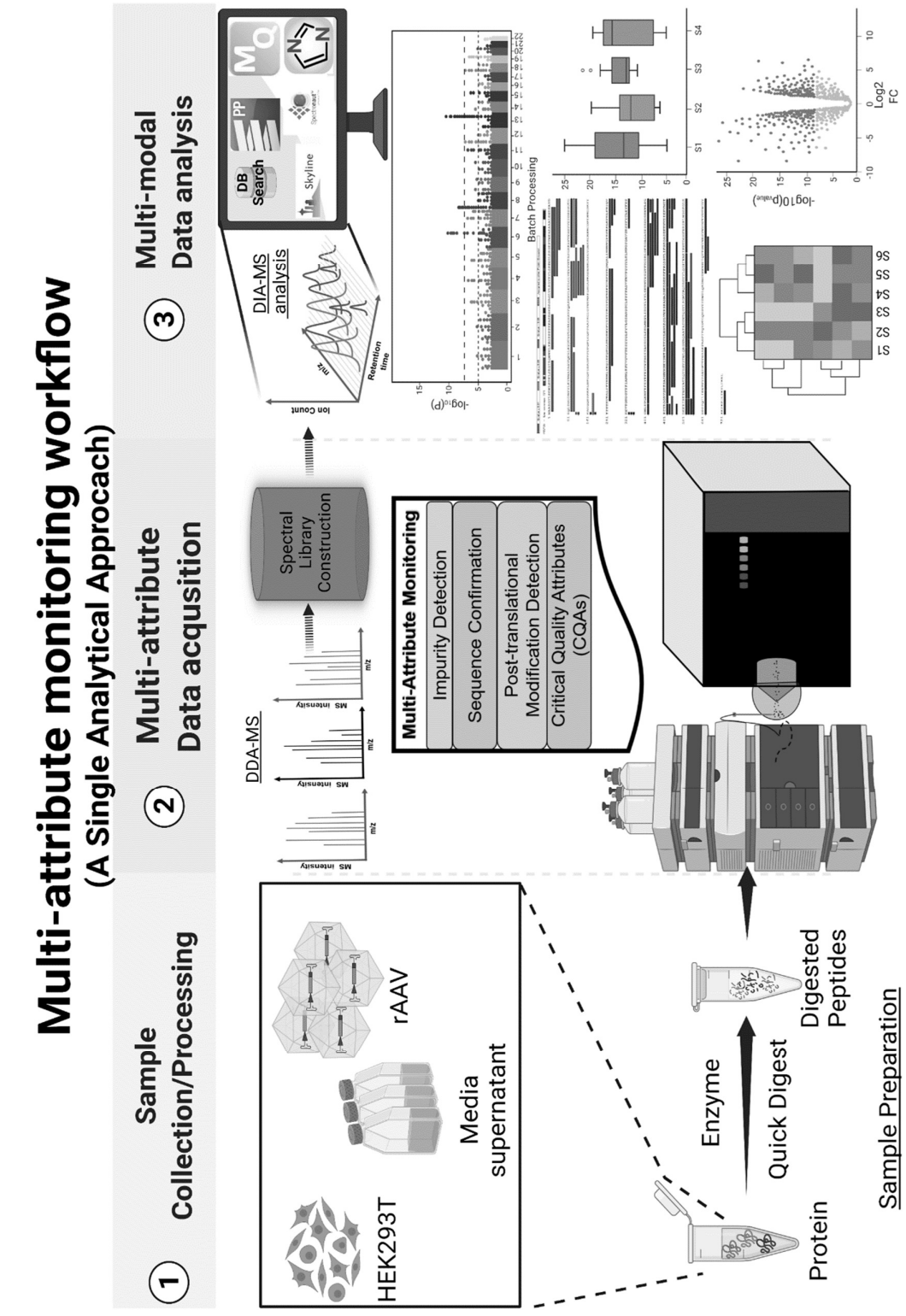
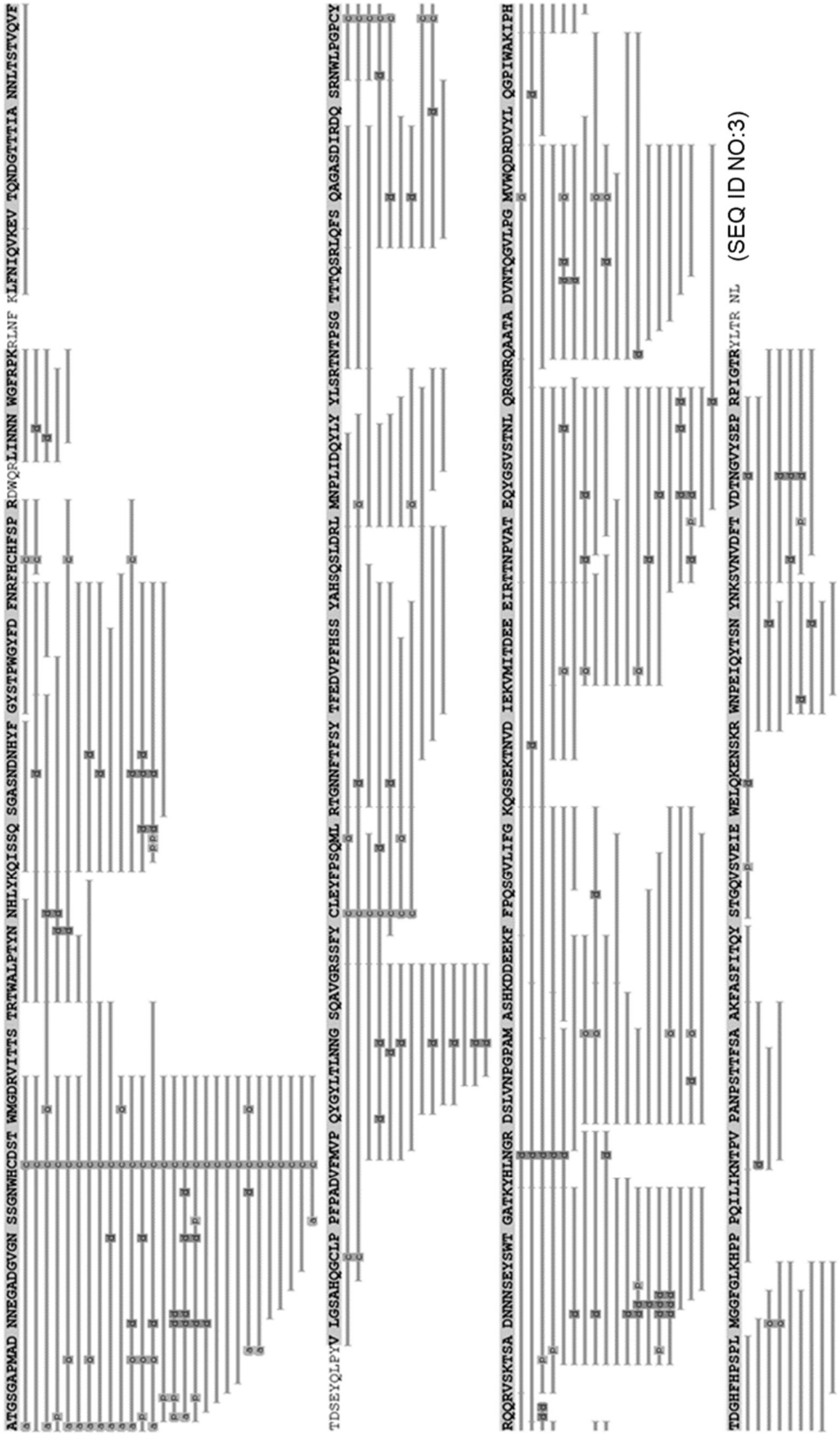

Multi-attribute monitoring workflow
(A Single Analytical Approcach)
1 Sample Collection/Processing
2 Multi-attribute Data acquisition
3 Multi-modal Data analysis
HEK293T
rAAV
Media supernatant
Protein
Enzyme
Quick Digest
Digested Peptides
Sample Preparation
DDA-MS
Spectral Library Construction
Multi-Attribute Monitoring
Impurity Detection
Sequence Confirmation
Post-translational Modification Detection
Critical Quality Attributes (CQAs)
DIA-MS analysis
Ion Count
m/z
Retention time
DB Search
PP
Skyline
Batch Processing
Log2 FC

**Figure 8**




(SEQ ID NO:3)

**Figure 9**

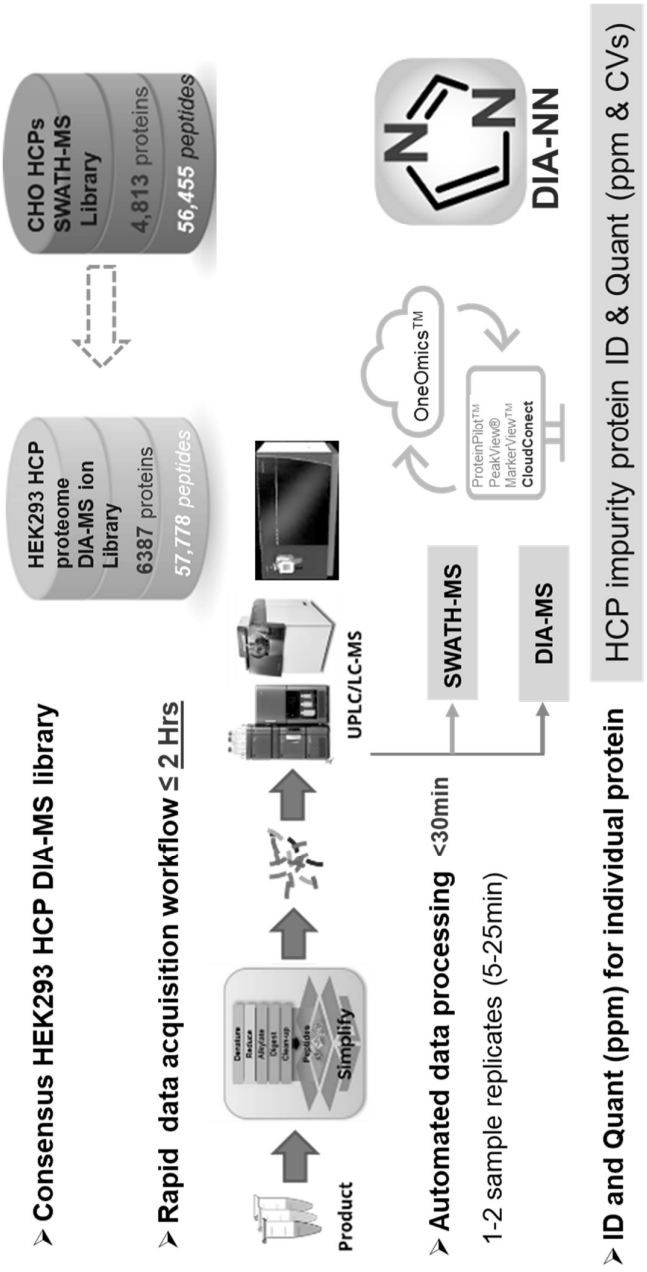

➢ Consensus HEK293 HCP DIA-MS library
➢ Rapid data acquisition workflow ≤ 2 Hrs
➢ Automated data processing <30min
1-2 sample replicates (5-25min)
➢ ID and Quant (ppm) for individual protein
HEK293 HCP proteome DIA-MS ion Library
6387 proteins
57,778 peptides
CHO HCPs SWATH-MS Library
4,813 proteins
56,455 peptides
Product
Simplify
UPLC/LC-MS
SWATH-MS
DIA-MS
OneOmics™
ProteinPilot™
PeakView®
MarkerView™
CloudConect
DIA-NN
HCP impurity protein ID & Quant (ppm & CVs)

**Figure 10**

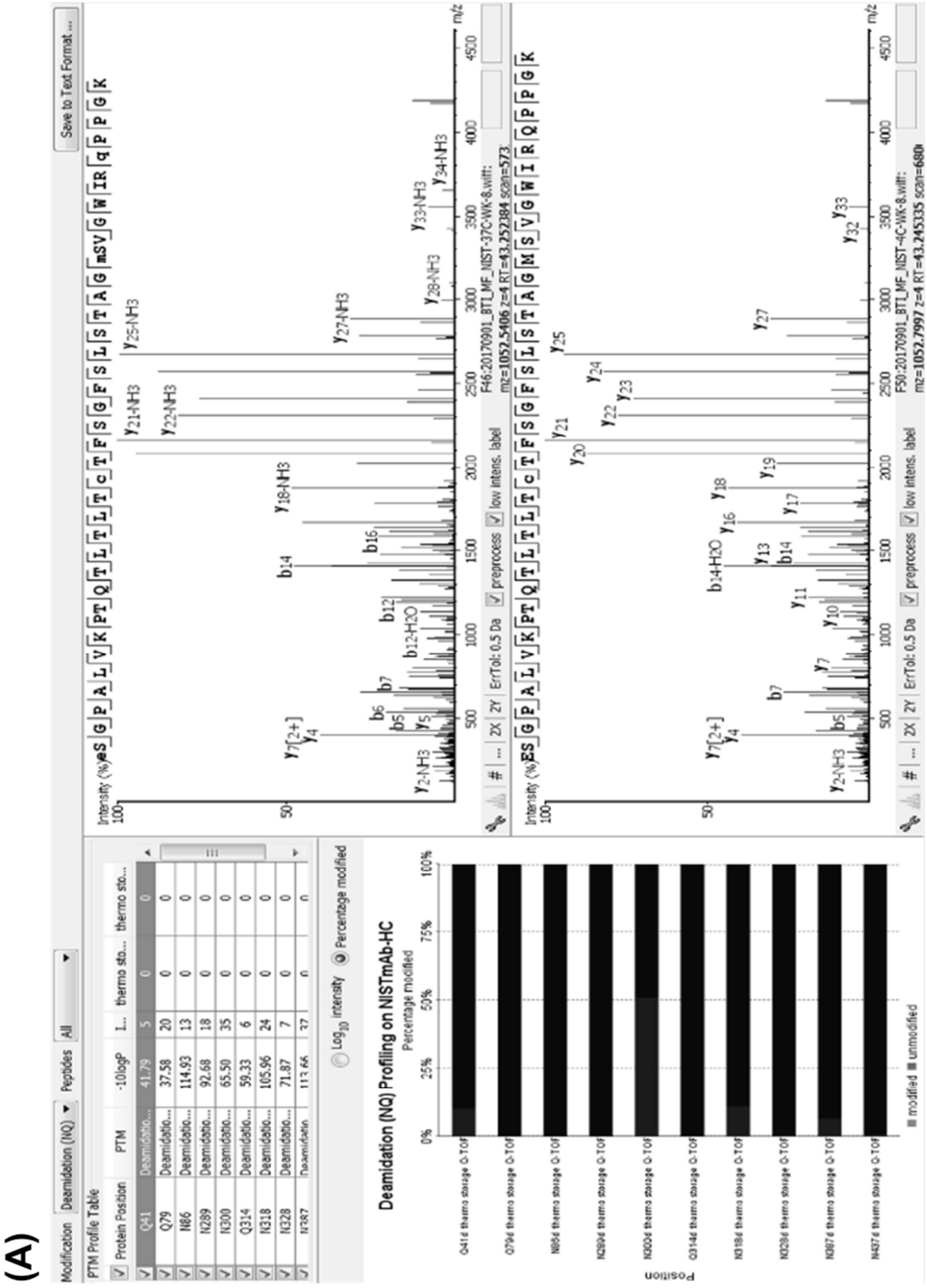



**Figure 11(A) (continued)**

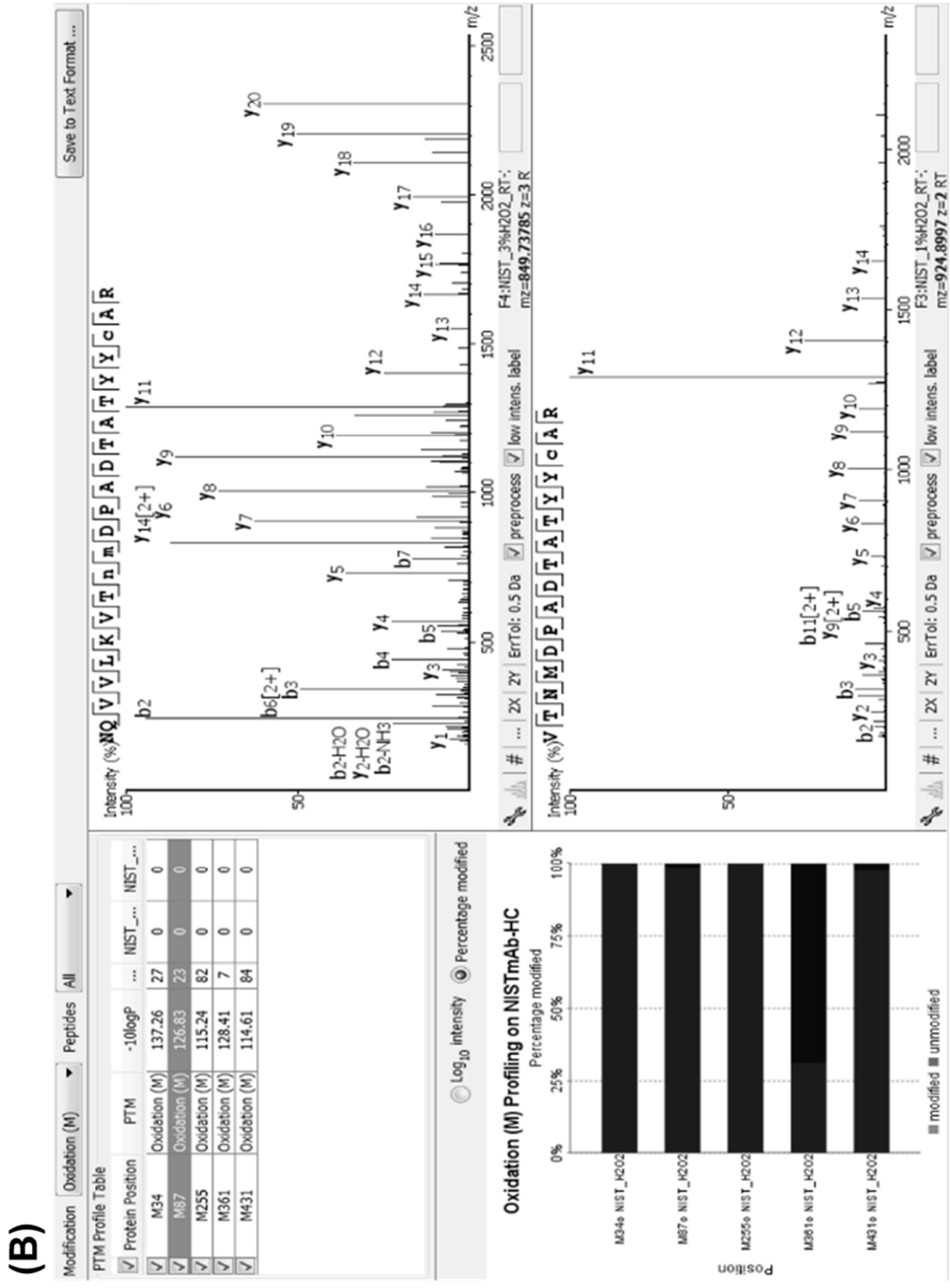



**Figure 11(B) (continued)**

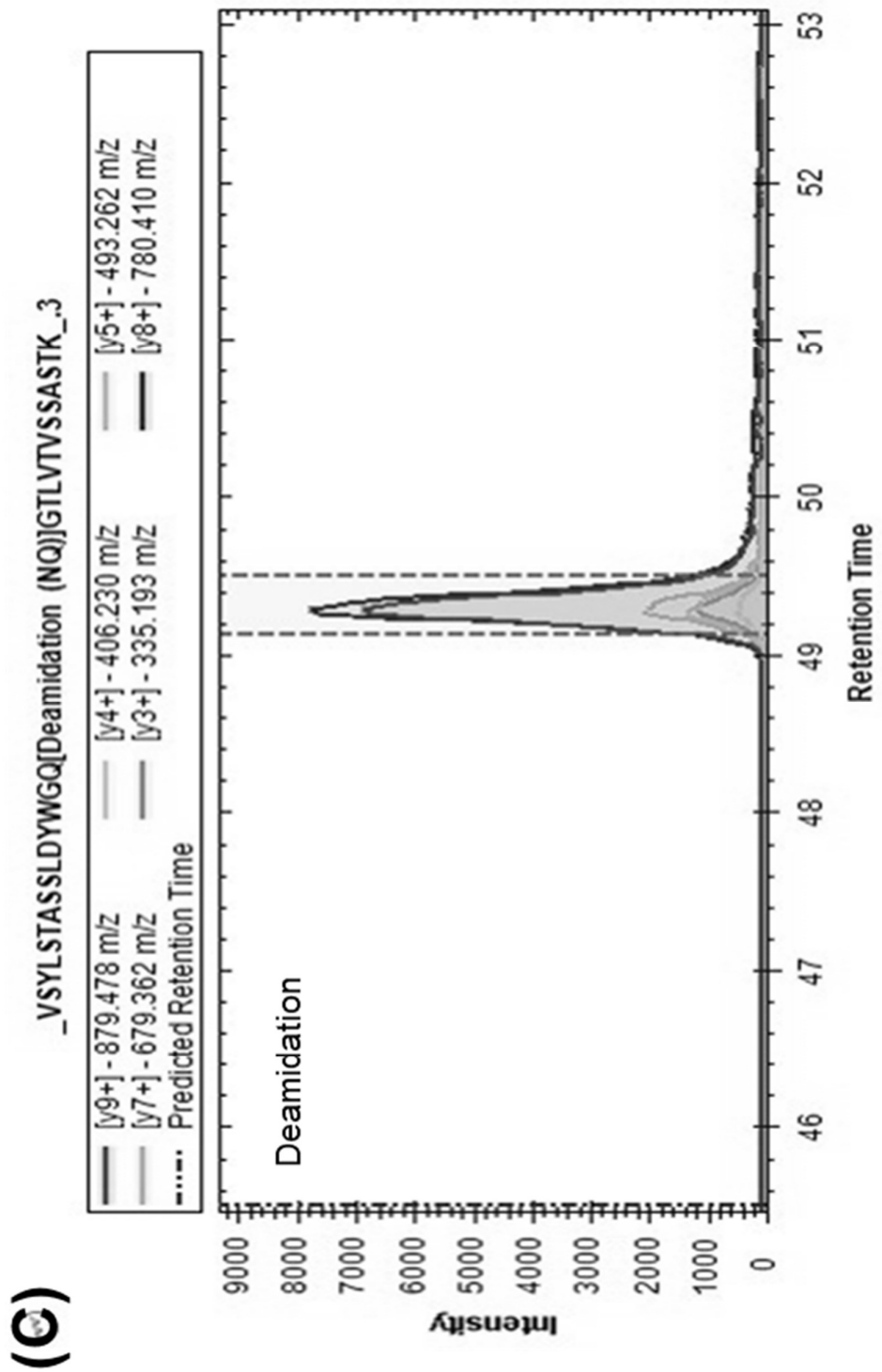



**Figure 11(C) (continued)**

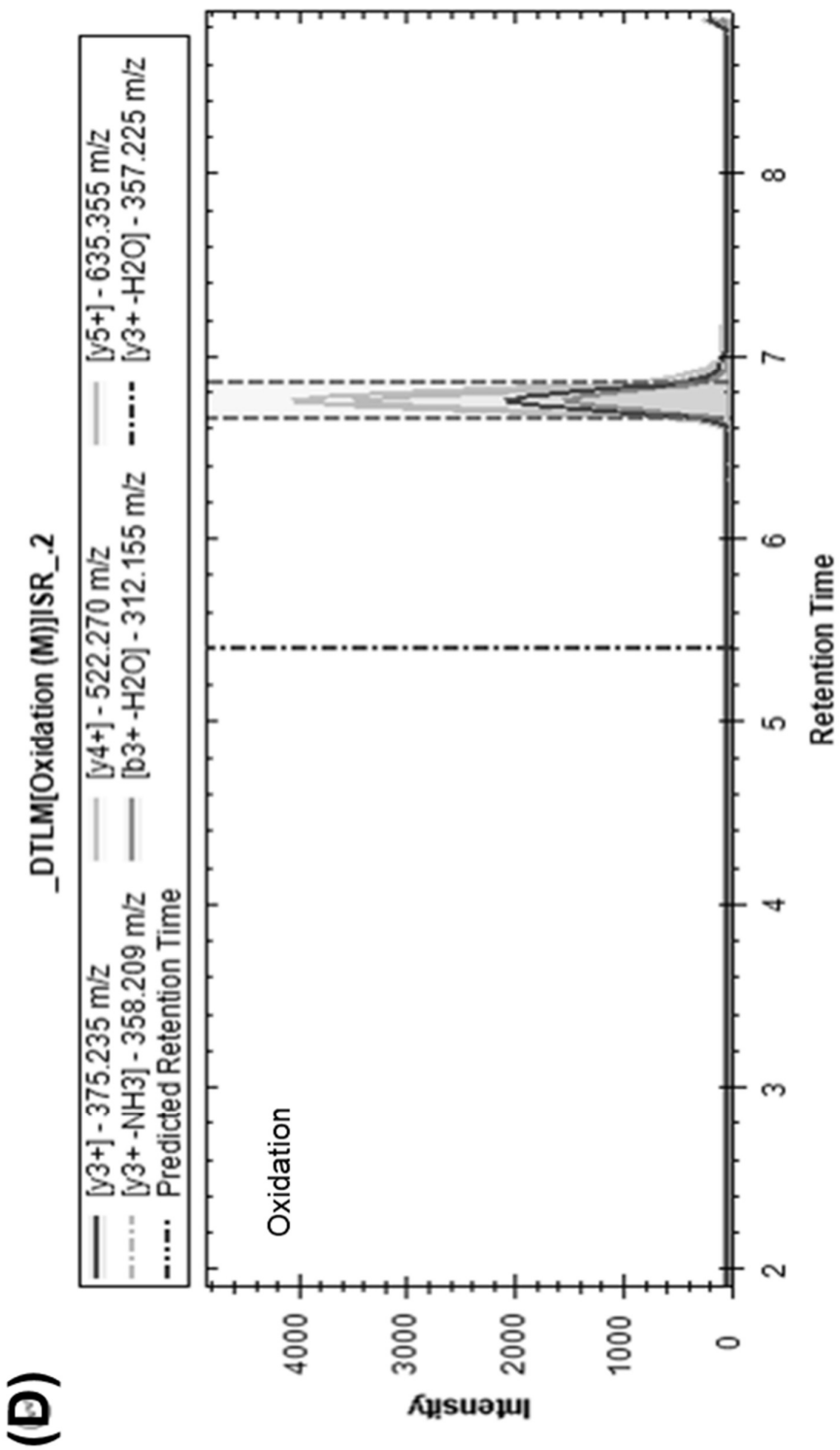



**Figure 11(D) (end)**